Universidade de Aveiro Departamento de Química
2019

Luana Fernandes

Efeito de várias tecnologias pós-colheita na qualidade e segurança de flores comestíveis

Effect of several post-harvest technologies on the quality and food safety of edible flowers

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciência e Tecnologia Alimentar e Nutrição, realizada sob a orientação científica do Doutor Jorge Manuel Alexandre Saraiva, Investigador Auxiliar do Departamento de Química da Universidade de Aveiro, da Professora Doutora Elsa Cristina Dantas Ramalhosa, Professora Adjunta da Escola Superior Agrária do Instituto Politécnico de Bragança e da Professora Doutora Susana Isabel Pereira Casal Vicente, Professora Auxiliar do Departamento de Ciências Químicas da Faculdade de Farmácia da Universidade do Porto.

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Esta tese é dedicada

à minha MÃE e aos meus AVÓS....

o júri

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Prof. Doutor Jorge Manuel Alexandre Saraiva
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palavras-chave Flores comestíveis, tecnologias pós-colheita, segurança, qualidade.

resumo

As flores comestíveis são usadas na alimentação desde a antiguidade. Atualmente, o seu consumo tem aumentado em países menos tradicionais nessa prática, impulsionado pelo crescimento e desenvolvimento do mercado gastronómico e pelos *chef*s de cozinha, que começam a usar flores comestíveis nos seus pratos, pela sua beleza e sabor. No entanto, trata-se de um produto muito perecível, cuja qualidade e segurança alimentar precisam ser garantidas. Neste sentido, com o presente trabalho pretendeu-se testar algumas tecnologias pós-colheita (separadamente e em combinação) em diferentes espécies de flores comestíveis, de acordo com a disponibilidade sazonal, com o intuito de aumentar o tempo de prateleira das mesmas. Para tal, procedeu-se à caracterização físico-química de algumas flores comestíveis ao longo da floração e foram conduzidos estudos ao nível da aplicação de altas pressões hidrostáticas, desidratação (desidratação osmótica, cristalização, liofilização, secagem por ar quente e secagem à temperatura ambiente (sombra)), de revestimentos comestíveis (alginato) e congelação. Posteriormente, avaliou-se o efeito dessas tecnologias sobre algumas propriedades físico-químicas e biológicas das flores, após tratamento e ao longo do armazenamento. Numa fase final, foram ainda realizados estudos organoléticos envolvendo consumidores e *chef*s de cozinha, com o intuito de avaliar a aceitabilidade e preferência.

Os resultados indicaram que a água foi o maior macronutriente encontrado nas flores estudadas. No entanto, outros componentes como proteínas, fibras e hidratos de carbono, bem como, compostos bioativos (carotenóides, ácidos gordos, vitamina E, flavonóides, antocianinas), compostos voláteis e açúcares livres foram também detetados. Em algumas situações, verificou-se a existência de diferenças significativas entre diferentes espécies de flores e estágios de floração. Adicionalmente, também se detetaram algumas relações entre as propriedades sensoriais e alguns compostos voláteis e bioativos presentes nas flores.

Quanto à aplicação de tecnologias pós-colheita, as altas pressões hidrostáticas mostraram ser uma tecnologia promissora para aumentar o tempo de prateleira dos amores-perfeitos, ao contrário da borragem e camélia que mostraram ser mais suscetíveis às altas pressões. Pelo contrário, a aplicação da desidratação osmótica a amores-perfeitos não originou os efeitos desejados, devido à presença de papilas superhidrofóbicas. Por outro lado, a cristalização mostrou ser uma tecnologia promissora para a preservação dos mesmos. No que se refere à centáurea, a secagem à sombra mostrou ser o método mais promissor, uma vez que se obtiveram as maiores concentrações de compostos bioativos. Já em relação, às pétalas de borragem, a liofilização mostrou ser um método promissor para produzir flores secas para infusões.

A aplicação de baixas temperaturas foi também testada, nomeadamente a congelação em cubos de gelo e de forma individual, tendo-se observado, de um modo geral, que as flores em cubos de gelo mantiveram uma aparência similar às frescas por mais tempo do que as congeladas naturalmente.

No que respeita ao tratamento com revestimentos comestíveis, testou-se o alginato em amores-perfeitos. Este tratamento exibiu bons resultados, tendo-se observado que as flores revestidas e embaladas aumentaram o tempo de prateleira por mais 7 dias do que as frescas.

Testou-se ainda a eficácia da aplicação combinada de processos de conservação, designadamente a aplicação das altas pressões hidrostáticas como pré-tratamento da desidratação osmótica, e a aplicação simultânea dos dois tratamentos a amores-perfeitos. Os resultados indicaram que a combinação de altas pressões hidrostáticas com desidratação osmótica em amores-perfeitos resultou em flores mais frágeis. No entanto, a aplicação de altas pressões hidrostáticas como pré-tratamento à desidratação osmótica pode ser uma tecnologia promissora para esta espécie de flores.

Por último, submeteram-se alguns *chef*s de cozinha e consumidores a uma prova organolética de amores-perfeitos sujeitos a diferentes tecnologias pós-colheita. Os *chef*s e consumidores apresentaram diferentes perfis sensoriais, sendo que ambos gostaram mais dos amores-perfeitos tratados com altas pressões hidrostáticas do que os revestidos com alginato e cristalizados. No entanto, os consumidores também classificaram com boas pontuações os amores-perfeitos sujeitos aos dois últimos processos.

Em conclusão, os resultados deste trabalho permitiram ilustrar o potencial de diversas tecnologias pós-colheita na conservação de algumas flores comestíveis, sem comprometer as suas propriedades físico-químicas e biológicas e com grande possibilidade de serem aceites pelos potenciais utilizadores.

keywords Edible flowers, pos-harvest technologies, preservation, safety, quality.

abstract

Edible flowers have been used in food since ancient times. Currently, their consumption has increased in less traditional countries in what regards this practice, as a result of the growth and development of gastronomic market and by the gourmet chefs, who begin to use edible flowers in their dishes, due to their beauty and taste. However, it is a very perishable product, whose quality and safety need to be guaranteed. In this order, the main objective of the present work was to test several post-harvest technologies (separately and combined) to different edible flowers species, according to their seasonal availability, with the purpose of increasing their shelf life. Several studies were carried out in order to perform the physico-chemical characterization of some edible flowers during flowering involving: high hydrostatic pressures (HHP), dehydration (osmotic dehydration (OD), crystallization, freeze drying, hot air convective drying and drying at room temperature (shade)), edible coatings and freezing. Afterwards, the effect of these technologies in some physico-chemical and biological properties of flowers was evaluated, after treatments and during storage. At the end, sensory studies were carried out involving consumers and chefs, with the purpose of evaluating the acceptability and preference.

The results indicated that water was the main macronutrient found in the studied flowers; however, other components such as proteins, fibers and carbohydrates, as well as, bioactive compounds (carotenoids, fatty acids, vitamin E, flavonoids, anthocyanins), volatile compounds and free sugars were also detected and quantified. In some situations, significant differences between different flower species and flowering stages were observed. Furthermore, in some situations, some relationships were found between volatiles and bioactive compounds with the sensory perception of the flowers.

Regarding the application of post-harvest technologies, the high hydrostatic pressures showed to be a promising technology to increase the shelf life of pansies, while borage and camellia showed to be more susceptible to the high pressures. On the other hand, the application of osmotic dehydration to pansies did not produce the desired effects due to the presence of superhydrophobic papillae; however, crystallization proved to be a promising technology for their preservation. Regarding centaurea, shade drying was the most promising method, since the highest concentrations of bioactive compounds were obtained. Concerning borage petals, freeze-drying showed to be a promising method to produce dried flowers for infusions. The application of low temperatures was also tested, namely freezing (in ice cubes and in their natural form), and it was generally observed that the flowers in ice cubes maintained a similar appearance to fresh ones for a longer period of time than naturally frozen.

With regard to treatment with edible coatings, the alginate was tested in pansies. This treatment gave good results, having the coated and packed flowers increased the shelf life by 7 days more than the fresh ones.
It was also tested the effectiveness of the combined application of preservation processes, namely the application of high hydrostatic pressures as a pre-treatment to osmotic dehydration, and the simultaneous application of the two treatments to pansies. The results indicated that the combination of high hydrostatic pressures with osmotic dehydration to pansies resulted in more fragile flowers. However, the application of high hydrostatic pressures as pre-treatment to osmotic dehydration may be a promising technology for this flower species.
Finally, some chefs and consumers were submitted to an organoleptic test of pansies subjected to different post-harvest technologies. The chefs and consumers presented different sensory profiles, both of which liked more the pansies treated with high hydrostatic pressures than those coated with alginate and crystallized. However, consumers also rated the pansies subjected to the last two processes with good scores. In conclusion, the results of this work allowed demonstrating the potential of several post-harvest technologies in the preservation of some edible flowers, without compromising their physico-chemical and biological properties and with great possibility of being accepted by the potential users.

List of publications and communications resultant from the PhD project

Publications indexed to Journal Citation Reports of ISI Web of Knowledge

Fernandes, L., Casal, S., Pereira, J.A., Saraiva, J., Ramalhosa, E., 2017. Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health. *Journal of Food Composition and Analysis*, 60, 38-50. (http://dx.doi.org/10.1016/j.jfca.2017.03.017). (JCR® Impact Factor (2017): 2.956).

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Fernandes, L., Casal, S., Pereira, J.A., Saraiva, J., Ramalhosa, E. An overview of the edible flower market. Submitted.

Fernandes, L., Pereira, J.A., Saraiva, J.A., Ramalhosa, E., Casal S. Borage, Camellia, Centaurea and Pansies: Nutritional, fatty acids, free sugars, vitamin E, carotenoids and organic acids characterization. Submitted.

Fernandes, L., Pereira, J.A., Saraiva, J.A., Ramalhosa, E., Casal S. Nutritional and nutraceutical compositions of *Borage officinalis* and *Centaurea cyanus* during flower development. Submitted.

Fernandes, L., Casal, S., Pereira, J.A., Ermelinda P., Saraiva, J.A., Ramalhosa, E. Edible flowers in ice cubes and frozen: effect on microbial and antioxidant quality during storage. Submitted.

Fernandes, L., Casal, S., Pereira, J.A., Ramalhosa, E., Saraiva, J.A. Osmotic dehydration, high hydrostatic pressure application and their combination on the appearance, weight loss and water activity of pansies (*Viola × wittrockiana*). Submitted.

Fernandes, L., Casal S., Pereira, J.A., Saraiva, J.A., Ramalhosa, E. Sensory analysis of pansies subjected to different post-harvest technologies: high hydrostatic pressure, alginate coating and crystallization. Submitted.

Oral communications in scientific events

Fernandes, L., Casal, S., Pereira, J.A., Pereira, E., Ramalhosa, E., Saraiva, J. "Effect of high hydrostatic pressure (HHP) on the quality of four edible flowers: *Viola* × *wittrockiana*, *Centaurea cyanus*, *Borago officinalis* and *Camellia japonic*a", VIII Congresso Ibérico de Ciências Hortícolas, from 7 to 10 June 2017, Coimbra (Portugal),

Fernandes, L. Casal, S., Pereira, J.A., Ramalhosa, E., Saraiva, J. "Efeito de altas pressões hidrostáticas (HHP) em flores comestíveis", 2º Workshop de I&D Científico e Tecnológico no Sector Agroalimentar da ESAV, Escola Superior de Tecnologia e Gestão de Viseu, 22 May 2017, Viseu (Portugal).

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Fernandes L., Ramalhosa. E., Pereira, E., Pereira, J.A., Casal, S.I.P., Saraiva, J.A., Effect of high hydrostatic pressure (HHP) on bioactivity and microbial quality of four edible flowers: *Viola* × *wittrockiana*, *Centaurea cyanus*, *Borago officinalis* and *Camellia japonica*. 56th EHPRG Meeting, from 2 to 7 September 2018, Aveiro (Portugal).

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Fernandes L., Casal, S.I.P., Pereira, J.A., Saraiva, J.A., Ramalhosa. E. Effect of alginate coating on the quality of pansies (*Viola x wittrockiana*) during storage. 31st EFoST International Conference Food Science and Technology Challenges for the 21st Century – Research to Progress Society, from 13 to 16 November 2017, Sitges (Spain).

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Fernandes, L., Casal, S.I.P., Pereira, J.A., Baptista, P. Saraiva, J.A., Ramalhosa, E. Efeito da desidratação osmótica nas características físico-químicas de amores-perfeitos

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Fernandes, L., Ramalhosa, E., Pereira, J.A., Casal, S.I.P., Saraiva, J.A., "Evaluation of the effect of high pressure on the quality of edible flowers: *Viola* × *wittrockiana*", Bio.IbericoAmérica 2016, from 5 to 8 June 2016, Salamanca (Spain),

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Acronyms and abbreviations

ANOVA (Analysis of variance)

a_w (water activity)

Cy-3-glu (Cyanidin-3-glucoside)

BHT (3,5-di-*tert*-butyl-4-hydroxytoluene)

Dw (Dried Weight)

DPPH (2,2-diphenyl-1-picrylhydrazil)

EC_{50} (Extract Concentration that provides 50% of antioxidant activity)

FAOSTAT (Statistics Division of Food and Agriculture Organization)

FAME (Fatty Acid Methyl Esters)

FID (Flame Ionization Detector)

Fw (Fresh weight)

GAE (Galic acid equivalent)

GC/MS (Gas chromatography coupled to a mass spectrometry detector)

HHP (High Hydrostatic Pressure)

HPLC (High Performance Liquid Chromatography)

L*, a*, b*,C*, h* (Colour Parameters)

MRS (Man, Rogosa and Sharpe)

MS (Mass spectrometry)

NIST (National Institute of Standards and Technology)

OD (Osmotic dehydration)

PCAg (Plate Count Agar)

PCA (Principal Component Analysis)

QE (Quercetin equivalent)

RBC (Rose Bengal Chloramphenicol)

RSM (Response Surface Methodology)

SPSS (Statistical Package for the Social Sciences)

TA (Titratable acididy)

TAE (Tannic acid equivalent

TPC (Total phenolic content)

TRC (Total Reducing Capacity)

ΔE (Color Difference)

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CHAPTER 1

1.1.

Objectives

The main objective of this thesis was to evaluate the effect of different post-harvest technologies (high hydrostatic pressure (HHP), dehydration, freezing, edible coatings) and their combination in the quality and safety of some edible flowers (ex. pansies (*Viola×wittrockiana*), borage (*Borago officinalis*), cornflowers (*Centaurea cyanus*), camellia (*Camellia japonica*), dandelion (*Taraxacum officinale*), kalachoe (*Kalanchoe blossfeldiana*) and globe amaranth (*Gomphrena globosa*)) in order to increase their preservation and shelf-life.

Thus, the specific objectives of this thesis were to:

1. Study the physico-chemical and biological characteristics of some edible flowers at different flowering stages;
2. Analyze the association between volatile compounds, sensory perception and bioactive compounds of some edible flowers;
3. Study the effect of several post-harvest technologies in physico-chemical and biological properties of different edible flowers, including HHP, drying (hot-air convective drying, shade drying, freeze-drying, osmotic dehydration and crystallization), freezing, edible coating (alginate) and their combination;
4. Evaluate organoleptic characteristics of some flowers subjected to several post-harvest tecnhologies.

To achieve these objectives, the present PhD thesis is organized in three main parts, each part divided in chapters.

PART I – Objectives and Introduction

This part is subdivided in two chapters (Chapters 1 and 2). In the first chapter the objectives are presented. In the second chapter, an overview of the current knowledge on edible flowers in human nutrition, including acceptability, nutritional, antioxidant and antimicrobial activities, as well as their effects on human health is explored.

Furthermore, a review on pos-harvest technologies applied to edible flowers is presented.

PART II – Description and discussion of the experimental work performed

This part is subdivided in three chapters (Chapters 3 to 5), corresponding to accepted or submitted papers, which contain the results obtained in this thesis and presented in accordance with the guidelines of each scientific journal. In Chapter 3, the physico-chemical characterization and sensory properties of different edible flowers at distinct flowering stages are discussed. In Chapter 4, several post-harvest technologies applied to edible flowers are presented and discussed. In Chapter 5, sensory analyses of flowers subjected to different technologies are discussed**.**

PART III – Conclusions

Finally, in Chapter 6 the general discussion and conclusions together with indications of future work, are presented.

CHAPTER 2

2.1. Introduction

Edible flowers are used as food since olden days. Nowadays, their consumption has increased in less traditional countries in this habit, such as Portugal, induced by the growth and development of the food market and the chefs. These are starting to begin to use edible flowers in their dishes, due to their beauty and flavour.

This section is subdivided in two more sections: Section 2.2. is a literature review about nutritional, antioxidant and antimicrobial properties, as well as, on the effects of edible flowers on human health; and Section 2.3 that corresponds to a literature review about post-harvest technologies applied to edible flowers.

2.2.

Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health

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Abstract

Edible flowers have been traditionally used for human consumption in various cultures. They improve the appearance, taste and aesthetic value of food, aspects that consumers appreciate, justifying the increasing trend of fresh top quality flowers' sales worldwide. However, consumers also demand foods with beneficial health properties, in addition to the nutrients they contain, looking for functional qualities such as antioxidant and antimicrobial properties. This review summarizes the data of more than 100 studies performed until now on edible flowers, focusing on nutritional, antioxidant and antimicrobial activities, as well as health effects. This review provides valuable information on edible flowers in order to better characterize them and to increase their popularization among the food industry and consumers.

Keywords: Edible flowers; *Brassica oleracea*; *Viola* × *wittrockiana; Rose* spp.; Food composition data; Food analysis; Antioxidant and antimicrobial properties; Minerals; Phenolic acids; Flavonoids

Introduction

Edible flowers have been used in culinary arts for flavor and garnish for hundreds of years. Early reports indicate that Romans used flowers in cooking, as did Chinese, Middle Eastern and Indian cultures. During Queen Victoria's reign, edible flowers were popular, as well as in North America and Europe (Newman and O'Connor, 2013). Presently, edible flowers are regaining popularity, as evidenced by the increasing number of edible flower cookbooks, culinary magazine articles and dedicated television segments (Barash, 1993; Creasy, 1999; Jacobs, 1999; Roberts, 2014; Rusnak, 1999). Despite being a niche market, attention to this kind of product has been raised by the recent highlights on their potential as a source of nutrients as well as a source of several bioactive compounds, supporting an increased worldwide demand (Patel and Naik, 2010).

Many flowers are edible but proper identification is essential because some are poisonous. Popular edible flowers include chrysanthemum, daylily, lilac, mint, nasturtium, pansy, rose, tulip and violet (University of Kentucky, 2012). Until now, there are no official lists of edible and non-edible flowers emitted by any international body, including Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), Food and Drug Administration (FDA), or European Food Safety Authority (EFSA). However, the European Regulation (EC) N.º 258/97, concerning novel foods and novel food ingredients, provides some information on the safety of these flowers. Accordingly, there are not any legal requirements for edible flowers marketing. Nevertheless, foodborne-disease outbreaks involving edible flowers have already been reported in the Rapid Alert System for Food and Feed (RASFF) (Table 2.2.1). The main problems are associated with the presence of unauthorized chemical compounds such as dimethoate and sulphite, and/or pathogens such as *Salmonella* spp. (RASFF). This fact highlights for the necessity to take adequate measures for a safe cultivation and preservation, and therefore increased knowledge on these food products, from producers to food processors and consumers. Therefore, the aim of this review is to assemble the current knowledge on the edible flowers most studied until now and normally consumed in fresh or used in infusions. Thus, it is reported some of the most important aspects related to those edible flowers, including acceptability, nutritional, antioxidant and antimicrobial activities, effects on human health and safety issues. Nutritional composition, mainly macronutrients and micronutrients, is discussed and summarized in tables. The whole ranges of nutrient and

minerals contents reported in the original papers are provided. Due to values reporting, in the studies reviewed, on fresh and dry weight basis, we decided to convert fresh weight to dry weight-based values. Antioxidant methods applied to edible flowers and the values obtained are also one of the focus of the present review, as well as, the identification of individual phenolic compounds (flavonoids and phenolic acids). Antimicrobial activities against some pathogenic microorganisms, as well as the effects on human health, are subjects also discussed in the present review.

Table 2.2.1 - Outbreaks attributed to some flowers and reported in the Rapid Alert System for Food and Feed (RASFF).

Flower	Country of origin	Detected problem	States notification	Year
Tilia tomentosa flowers	Albania	Dimethoate	Italy	2014
Hibiscus flowers	Egypt	Infested with insects and moulds, rodent excrements	Poland	2008
Cinnamon and cinnamon tree flowers (*Cinnamomum verum*)	Sri Lanka	Sulphite unauthorised	Spain	2005
Fresh edible flower	Thailand	*Salmonella* Mbandaka	Finland	2005
Dried marigold flowers/*Calendula officinalis*	Egypt	*Salmonella* Hadar	Poland	2004

Source: RASFF Portal, Rapid Alert System for Food and Feed. https.//webgate.ec.europa.eu/rasff-window/portal.

Edible flowers: general characteristics and acceptability

There is a wide range of flowers, but only some are edible. Therefore, their proper identification is essential. Table 2.2.2 describes some edible flowers used for culinary purposes. According to Lu et al. (2016), edible flowers are obtained from 97 families, 100 genera and 180 species worldwide. Edible flowers are part of the cuisine of many countries. Examples include *Hemerocallis disticha* (Tai and Chen, 2000) and *Prunus mume* blossoms in China (Shi et al., 2009), *Carica papaya*, *Gmelina arboria*, *Phlogacanthus thyrsiformis*, *Dendrocnide sinuate*, *Justicia adhatoda*, *Oroxylum indicum* and *Nyctanthus arbortristis* in India (Deka and Nath, 2014), *Antigonon leptopus*, *Bougainvillea hybrida*, *Cassia siamea*, *Clitorea ternatea*, *Cosmos sulphureus* and *Malvaviscus arboreus* in Thailand (Kaisoon et al., 2011), *Cucurbita pepo*, *Erythrina americana* and *Erythrina caribaea* in Mexico (Sotelo et al., 2007) and *Sambucus nigra* inflorescences in Central Europe (Kopec, 2004).

The most frequent way to consume edible flowers is in fresh but they can also be consumed dried, in cocktails (in ice cubes), canned in sugar and preserved in distillates (Mlcek and Rop, 2011). Edible flowers are usually used to add color, fragrance and flavor to food such as salads, soups, entrees, desserts and drinks (Mlcek and Rop, 2011; Rop et al., 2012; Kelley et al., 2001a). In this order, edible flowers have attracted the curiosity of some researchers and professional chefs, but there is still high potential to improve their usage and knowledge (Basso and Papalia, 2014).

Pansy (*Viola×wittrockiana*), centaurea (*Centaurea cyanus*), borago (*Borago officinalis*), rose (*Rose* spp.), nasturtiums (*Tropaeolum majus*) and hibiscus (*Hibiscus rosa-sinensis*) are examples of edible flowers that are normally used to garnish dishes. Nevertheless, others are more known by consumers as vegetables, such as artichoke (*Cynara scolymus*), broccolis and cauliflower (*Brassica oleracea*), even though these are inflorescences. Moreover, some herb flowers are edible, namely: alliums (leeks, chives, garlic), thyme (*Thymus vulgaris*), summer savory (*Satureja hortensis*), marjoram (*Origanum majorana*), mint (*Mentha* spp.) and common sage (*Salvia officinalis*), which are used to improve the flavor of dishes. Flowers of some fruit trees can also be used in cuisine as syrups such as elderberry blossoms (*Sambucus* spp.) and citrus blossoms (orange, lemon, lime, grapefruit, kumquat).

In general, edible flowers are eaten whole, but depending on the flower species, only some parts should be consumed. For example, only the petals of Tulipa, Chrysanthemum, Rosa spp. or the flower buds of daisies (*Bellis perenis*) or garden nasturtium (*T. majus*) are consumed. Furthermore, in some flowers it is necessary to remove some parts due to their bitterness such as the white portions of the roses and chrysanthemums petals base.

Kelley et al. (2001b) observed that acceptability of edible flowers by consumers and professional Chefs, varied in accordance to some attributes and flower specie among both groups. In this study the opinion and preferences regarding three edible-flower species (viola, borage and nasturtium) in what regards three attributes, namely taste, fragrance and visual appeal, were registered. Regarding viola, consumers and chefs expressed similar perceptions in what concerns fragrance and visual appeal; however, the first group preferred taste and were more likely to purchase and use viola than the second. In a similar way, consumers also liked more borage than chefs, except for fragrance. In contrast, in nasturtium the three attributes evaluated were rated higher by the chefs. In this way, the decision to purchase and taste a flower can be different for

both participant groups. Moreover, within the group of consumers some variables may influence purchase and use of edible flowers. Consumers from USA with higher college education, female, larger households, and highest annual income were more likely to purchase and use edible flowers (Kelley et al., 2002a). Furthermore, Kelley et al. (2002b) also found that, according to the variables described above, consumers have different attitudes and behavior in relation to how they like to consume the edible flowers (garnish, meal and salad) and the way edible flowers were obtained (grown organically or if they grew the flowers themselves). Generally, participants preferred to purchase edible flowers to eat as garnish, followed by salad and meal, independently of the variables mentioned. Concerning the way edible flowers are obtained, people less than 50 years old, with college/technical graduation and with two or more adults in the household, were more likely to grow their edible flowers. Older people (more than 50 years), female, without university studies, single-family household and with one or more children, were more likely to purchase edible flowers if grown organically. Some properties of edible flowers can also influence consumers at the time of purchase. Kelley et al. (2001a) reported that color was the most influential factor when consumers have to decide which package they would purchase. Price was the next most important factor and container size was the least important. Furthermore, consumers preferred a mix of flower colors over single colors, and some specific color contrasts were preferred over others. Consumers usually preferred most yellow and orange colors, while blue and combinations of other colors were less favored. For example, consumers preferred containers of edible flowers with more than one color of nasturtium and/or containers of nasturtiums with additional types of flowers (Kelley et al., 2001a).

In conclusion, acceptability of edible flowers depends on a number of factors, namely: social group (chefs versus consumers), species of flowers and their characteristics (taste, texture and appearance), personal characteristics of consumers (education, gender, annual income) and the packaging for sale (composition of flowers, size and price).

Table 2.2.2 - Characteristics and biological activities reported in the literature of some edible flowers.

Common name	Scientific name	Flavor	Edible parts	Cooking style	Biological Activities	Reference
Chives	*Allium schoenoprasum*	Mild onion	All parts of the plant	Salads, cooked vegetables, casseroles, cheese dishes, eggs, potatoes, or cream cheese	Bladder and kidney infections, cleanse the blood, lower blood pressure and cholesterol, build up resistance to infection, respiratory disorders, assist digestive tract and urinary system, natural antibiotic.	(Roberts, 2000; Grzeszczuk et al., 2011)
Begonia	*Begonia* × *tuberhybrida*	Slightly lemon	Leaves, flowers, and stems	Salads and garnishes	Nondescript, antiphlogistic, antispasmodic, ophthalmic, gastric.	(Mlcek & Rop, 2011)
Borage	*Borago officinalis*	Crisp, cucumber	Blossoms and leaves	Cakes decoration, pastries, and desserts	Antispasmodic, antihypertensive, antipyretic, aphrodisiac, demulcent, diuretic and is also considered useful to treat asthma, bronchitis, cramps, diarrhea, palpitations and kidney ailments	(Roberts, 2000; Gilani et al., 2007)
Calendula, pot marigold	*Calendula officinalis*	Slightly bitter	Petals	Use petals in salads, soups, butter, rice, stews, poultry, or in tea	Anti-inflammatory, regulates menstruation, aids gastric disturbances, colitis, fevers and infections problems, eczema, oily skin and psoriasis.	(Mlcek & Rop, 2011; Hamad et al., 2011; Muley et al., 2009; Roberts, 2000; Jauron et al., 2013)
Cornflower, Bachelor's button, hurtsickle or cyani flower	*Centaurea cyanus*	Slightly sweet to spicy, clove-like	Petals	Garnish, tea, the petals are also a natural food colorant	Soothing, antioxidant, and used in ocular inflammation.	(Jauron et al., 2013; Garback et al., 1999)
Chrysanthemum	*Chrysanthemum* spp.	Slightly to very bitter	Remove the bitter flower base and use petals only	Tea	For the treatment of constipation, vertigo, hypertensive symptoms and several infectious diseases such as pneumonia, colitis, stomatitis,carbuncle and fever.	(Mlcek & Rop, 2011; Sassi et al., 2008)

Daylilies	*Hemerocallis* spp.	Slightly sweet, combination of asparagus and zucchini	Buds, flowers, petals, remove the stamens	Salads or garnishes	To treat aching muscles, and strains, antipyretic, oral disinfectant.	(Tai & Chen, 2000; , Mlcek & Rop, 2011; Roberts, 2000; Jauron et al., 2013)
Bee balm/bergamot	*Monarda didyma*	Citrus, minty	Flowers, leaves	Salads, garnishes, bee balm tea	Helps to calm digestive problems, colic, nausea, bloated distended stomach, flatulence and belching.	(Roberts, 2000)
Rose	*Rosa* spp.	Sweet and aromatic	Petals, remove the bitter white portion of the petals	Salads or make jelly	Anticancer, diuretic, laxative, ophthalmic, antirheumatic, kidneys.	(Mlcek & Rop, 2011; Jauron et al., 2013)
Common Lilac	*Syringa vulgaris*	Flowerish, lemony taste, floral, pungent	Blossoms, petals	Great in salads and crystallized with egg whites and sugar.	Reduces fever and to get rid of internal parasites.	(Mlcek & Rop, 2011; Jauron et al., 2013)
Nasturtium	*Tropaeolum majus*	Peppery, zesty taste that can be a substitute for mustard	Blossoms, leaves, whole flowers	Salads or cure in vinegar, garnish	Disinfectant, wound-healing, antibiotic, expectorant to relieve chest conditions, antiscorbutic and anticancer activity.	(Mlcek & Rop, 2011; Garzón & Wrolstad, 2009; Jauron et al., 2013)
Tulip	*Tulipa* spp.	Sweet lettuce, fresh baby peas, or cucumber-like	Petals (Some people are strongly allergic)	Salads	Antipyretic, anticancer, laxative, expectorant, depurative.	(Mlcek & Rop, 2011)
Pansies	*Viola* × *wittrockiana*	Perfumed taste, sweet	Whole flower	Garnish, salad and tea	Phytotherapy, skin	(Mlcek & Rop, 2011; Jauron et al., 2013)

Properties of edible flowers

Nutritional value

Few studies were carried out on the nutritional composition of edible flowers, but most of them reported that the content of common components is not different from the composition of other plant organs (Mlcek and Rop, 2011). Mlcek and Rop (2011) reported that, from the nutritional point of view, flowers can be divided in pollen, nectar, petals and other parts. Pollen is a source of proteins and carbohydrates, saturated and unsaturated lipids, carotenoids and flavonoids. Nectar contains a balanced mixture of sugars (fructose, glucose and sucrose), together with free amino acids, proteins, inorganic ions, lipids, organic acids, phenolic substances, alkaloids and terpenoids, among others (Mlcek and Rop, 2011). Petals and other parts of flowers are rich in vitamins, minerals and antioxidants (Mlcek and Rop, 2011).

On edible flowers, water is the main constituent, varying between 70%–95% (Table 2.2.3). So, at the same time that blossoms add visual delight, pleasing aroma and delightful taste, other edible flowers are normally consumed in higher amounts as vegetables (ex. *B. oleracea* – broccoli and cauliflower), having lower energetic value. Nevertheless, a high range of values has been reported, from 75 to 465 kJ/100 g fresh weight, probably due to the high values of total carbohydrates in some cases. Indeed, carbohydrates are the most abundant macronutrient in edible flowers, ranging between 42.4 and 90.2 g/100 g dry weight for *E. caribaea* and *Rosa micrantha*, respectively, followed by the other parameters (proteins, ash and lipids) (Table 2.2.3). However, lower amounts of carbohydrates were detected in *B. oleracea* var. *italic* (10.0 g/100 g dry weight). This variation may probably be due to differences on the prevailing tissues between species (ex. in cauliflower the edible part is represented by the proliferation of meristematic and parenchima tissues, while in others the tegumental tissues prevail), or/ and to the use of different methods on total carbohydrates calculation, including or not the fibers. Few studies have detailed the reducing sugars, namely on *A. schoenoprasum* (10.6 g/100 g dry weight), *R. micrantha* (9.6 g/100 g dry weight) and *Yucca filifera* (53.8 g/100 g dry weight). Concerning fiber contents, a range between 6.1 and 55.4 g/100 g dry weight was observed for *A. schoenoprasum* and *Spilanthes oleracea*/*Tagetes erecta*, respectively. This large range on fiber contents may be due to the application of different methods on the determination of this component, including crude fiber and total dietary fiber. As referred for carbohydrates, also for protein, a high range of values was found, varying between 2.0 and 52.3 g/100 g dry weight for

Begonia boliviensis and *B. oleracea* var. *italica*, respectively. Lipids were present in flowers, ranging between 1.3 to 6.1 g/100 g dry weight for *R. micrantha* (petals) and *Madhuca indica*, respectively. Minerals (ash) are the components with the highest variability on the total content (varying between 2.6 and 15.9 g/100 g dry weight), as well as individually. Potassium, phosphorus, calcium and magnesium are the major components (Table 2.2.4) but the range of values was high, varying between 1.30 × 10^3–4.06 ×10^3 mg/100 g dry weight for potassium (*B. boliviensis* and *Tagetes patula*, respectively), 8–548 mg/100 g dry weight for phosphorus (*Viola×wittrockiana* and *C. cyanus*, respectively), 17–486 mg/100 g dry weight for calcium (*Brassica oleracea* var. *botrytis* and *Viola×wittrockiana*, respectively) and 106–219 mg/100 g dry weight for magnesium (*B. boliviensis* and *T. patula*, respectively). Furthermore, edible flowers are richer in potassium than in sodium, which is of benefit for cardiovascular diseases prevention. Considering the Dietary Reference Intakes (DRIs) for magnesium (375 mg/day), phosphorus (700 mg/day) and potassium (2000 mg/day) for an adult (Regulation (EU) N.º 1169/2011), some edible flowers can help to supply these daily requirements. For example, 46 g of dry *Fuchsia × hybrida*, 34 g of dry *Chrysanthemum parthenium* and 12 g of dry *T. patula*, can contribute to meet 25% of the daily requirements of magnesium, phosphourus and potassium, respectively, in healthy adults (Rop et al., 2012), being these flowers generally consumed fresh in salads. Other minerals determined in edible flowers are only present in low amounts (Table 2.2.4). Observing Table 2.2.4, different values for a specific mineral are reported for the same edible flowers. For example, calcium values on *Viola × wittrockiana* of 30 and 486 mg/100 g dry weight have been determined. Although soil composition is known to influence markedly the mineral content of vegetable in general, the differences are too high to be just attributed to this factor. So, more analytical surveys based on established methodologies must be performed in order to better understand some of the differences observed until now.

Table 2.2.3 – Nutritional composition reported in the literature of some edible flowers.

Common name	Flower	Part of flower	Nutritional composition (g/100g dry weight)							
			Moisture[a]	TC[b]	Fiber	Protein	Fat	Ash	Energy[c]	Reference (recalculated)
Agave	*Agave salmiana*	Whole	87.4	62.1	12.7	16.4	2.8	5.8	-	(Sotelo et al., 2007)
Chives	*Allium schoenoprasum*	Whole	80.0	50.0	6.1	15.3	3.4	3.8	243	(Grzeszczuk et al., 2011)
Aloe vera	*Aloe vera*	Whole	89.5	56.8	13.8	16.4	4.2	8.6	-	(Sotelo et al., 2007)
Snapdragon	*Antirrhinum majus*	Whole	87.4	-	-	3.8	-	-	-	(Rop et al., 2012)
Texas madrone	*Arbutus xalapensis*	Whole	89.7	66.7	10.4	11.3	3.9	6.9	-	(Sotelo et al., 2007)
Begonia	*Begonia boliviensis*	Whole	85.8	-	-	2.0	-	-	-	(Rop et al., 2012)
Cauliflower	*Brassica oleracea* var. *botrytis*	Whole	93.4	43.6	21.7	18.0	2.9	13.9	75	(Vieira, 2013)
Broccoli	*Brassica oleracea* var. *italica*	Whole	92.6	10.0	28.0	52.3	2.0	15.4	84	(Vieira, 2013)
Pot marigold	*Calendula officinalis*	Petals	89.3	62.1	13.1	13.6	3.6	7.7	151	(Vieira, 2013)
Cornflower	*Centaurea cyanus*	Petals	90.3	-	-	6.9	-	-	-	(Rop et al., 2012)
Paris daisy	*Chrysanthemum frutescens*	Petals	90.4	-	-	7.2	-	-	-	(Rop et al., 2012)
Feverfew	*Chrysanthemum parthenium*	Petals	90.1	-	-	6.9	-	-	-	(Rop et al., 2012)
Pumpkin	*Cucurbita pepo*	Whole	93.1	47.1	10.5	21.9	5.0	15.9	-	(Sotelo et al., 2007)
Cardoon	*Cynara cscolumus*	Whole	78.9	60.9	16.6	14.7	2.8	5.9	289	(Vieira, 2013)
Carnation	*Dianthus caryophyllus*	Petals	88.5	-	-	6.0	-	-	-	(Rop et al., 2012)
Coral tree	*Erythrina americana*	Whole	86.6	44.5	17.3	26.2	2.3	9.6	-	(Sotelo et al., 2007)
Erythrina	*Erythrina caribaea*	Whole	88.5	42.4	17.7	27.4	1.5	10.1	-	(Sotelo et al., 2007)
Sun spurge	*Euphorbia radians*	Whole	90.1	47.9	12.6	25.1	4.9	9.4	-	(Sotelo et al., 2007)
Fuchsia	*Fuchsia × hybrida*	Whole	91.6	-	-	2.9	-	-	-	(Rop et al., 2012)
Busy Lizzie	*Impatiens walleriana*	Whole	85.3	-	-	3.1	-	-	-	(Rop et al., 2012)
Mahua	*Madhuca indica*	Whole	73.6	86.0	-	5.3	6.1	2.6	465	(Patel & Naik, 2010)
Rugosa rose	*Rosa micrantha*	Petals	71.6	90.2	-	4.3	1.3	4.2	465	(Guimarães et al. 2010)
Tea Rose	*Rosa odorata*	Petals	89.9	-	-	2.6	-	-	-	(Rop et al.. 2012)
Sechuan button	*Spilanthes oleracea*	Whole	81.7	74.3	55.4	15.6	2.2	7.9	121	(Navarro-González et al., 2015)
Mexican marigold	*Tagetes erecta*	Whole	83.4	85.2	55.4	7.9	1.9	4.8	117	(Navarro-González et al., 2015)
French marigold	*Tagetes patula*	Whole	90.6	-	-	3.1	-	-	-	(Rop et al., 2012)
Garden nasturtium	*Tropaeolum majus*	Whole	89.3	66.9	42.2	18.6	3.1	5.9	88	(Navarro-González et al., 2015)
	Tropaeolum majus	Whole	88.7	-	-	4.2	-	-	-	(Rop et al., 2012)
	Tropaeolum majus	Whole	90.6	48.1	29.7	14.4	3.6	7.3	109	(Vieira, 2013)
Pansies	*Viola × wittrockiana*	Whole	90.0	-	-	6.7	-	-	-	(Rop et al. 2012)
	Viola × wittrockiana	Whole	87.2	64.5	9.3	16.8	5.0	4.4	197	(Vieira, 2013)
Yucca	*Yucca filifera*	Whole	88.1	-	8.5	25.9	2.1	9.7	-	(Sotelo et al., 2007)

[a]Expressed in %; [b]TC- Total carbohydrates; [c]Expressed in kJ/100g fresh weight.

Table 2.2.4 – Mineral composition found in the literature for some edible flowers.

Flower	Moisture (%)	Mineral composition [mg/100g dry weight]												Reference [recalculated]
		Ca	Cu	Fe	K	Mg	Mn	Mo	Na	P	S	Sr	Zn	
Antirrhinum majus	87.4	283	1.3	3.5	2.27×10^3	136	4.5	0.67	70	331	-	-	7.0	(Rop et al., 2012)
Begonia boliviensis	85.8	246	1.4	1.9	1.30×10^3	106	3.1	0.44	66	142	-	-	3.2	(Rop et al., 2012)
Brassica oleracea var. *botrytis*	93.4	17	-	-	-	-	0.2	-	2	58	-	-	-	(Vieira,2013)
Brassica oleracea var. *italica*	92.6	80	-	-	-	-	35.1	-	26	78	-	-	-	(Vieira,2013)
Calendula officinalis	89.3	41	-	-	-	-	0.1	-	1	11	-	-	-	(Vieira,2013)
Centaurea cyanus	90.3	253	0.9	7.1	3.66×10^3	142	2.4	0.50	76	548	-	-	7.8	(Rop et al., 2012)
Chrysanthemum frutescens	90.4	270	2.3	5.4	2.74×10^3	110	8.2	0.31	93	447	-	-	5.7	(Rop et al., 2012)
Chrysanthemum parthenium	90.1	346	2.4	5.9	3.65×10^3	198	7.4	0.31	115	508	-	-	6.0	(Rop et al., 2012)
Crocus sativus	89.7	139	-	16.0	1.40×10^3	113	-	-	10	279	-	-	-	(Serrano-Díaz et al., 2013)
Cynara scolymus	78.9	84	-	-	-	-	0.3	-	60	42	-	-	-	(Vieira, 2013)
Dianthus caryophyllus	88.5	426	2.5	8.5	3.07×10^3	161	6.5	0.48	99	460	-	-	6.2	(Rop et al., 2012)
Fuchsia × hybrida	91.6	286	3.2	9.7	2.35×10^3	204	5.0	0.85	150	257	-	-	13.7	(Rop et al., 2012)
Impatiens walleriana	85.3	275	0.9	4.9	1.92×10^3	138	4.1	0.26	64	260	-	-	5.9	(Rop et al., 2012)
Rosa odorata	90.0	273	2.3	3.5	1.95×10^3	141	3.4	0.63	76	223	-	-	4.5	(Rop et al., 2012)
Tagetes patula	90.3	370	1.2	9.3	4.06×10^3	219	8.4	0.39	122	510	-	-	14.2	(Rop et al., 2012)
Tropaeolum majus	88.7	299	1.0	5.7	2.18×10^3	132	5.2	0.26	78	427	-	-	8.0	(Rop et al., 2012)
Tropaeolum majus	90.6	28	-	-	-	-	0.2	-	1	10	-	-	-	(Vieira, 2013)
Viola × wittrockiana	90.6	486	1.95	7.3	3.96×10^3	190	7.9	0.84	132	514	-	-	11.5	(Rop et al., 2012)
Viola × wittrockiana	90.1	30	-	-	-	-	0.1	-	1	8	-	-	-	(Vieira, 2013)

Antioxidant activity and individual compounds

A substantial number of studies have been made on the antioxidant activity and bioactive compounds of edible flowers (Tables 2.2.5 and 2.2.6). Extraction of flowers antioxidants has been done by several procedures, including different solvents, times, temperatures and extraction methods (Table 2.2.5). Maceration and Soxhlet extraction have been the most used methods (Mojzer et al., 2016). The selection of solvent largely depends on the specific nature of the bioactive compound being measured. Methanol and methanolic solutions have been the solvents used more frequently. However, other solvents, such as ethanol, acetone, isopropanol, ether, water, tetrahydrofuran and solvents mixture have also been used. Temperature is other parameter that can exert some influence on compounds extraction. By observing Table 2.2.5, room temperature is the most used. Nevertheless, slight higher temperatures such as, 35, 37, 60 and 100 °C, as well as, low temperatures (4 °C) have been applied. When high temperatures are applied, small extraction times are used, while at low temperatures, extractions of more than 12 h are needed.

Regarding the analytical methods, the most common were in vitro and used free radicals, namely, DPPH, ABTS, oxygen radical (in ORAC method), superoxide anion, nitric oxide and hydroxyl radical.

The range of values obtained for the antioxidant activity vary widely among flowers. However, it is difficult to compare the antioxidant activity results among flowers because even though authors used the same method, different standards were used, being the results expressed in different units. Furthermore, some authors evaluated only one fraction of the extracts (soluble or insoluble), such as Xiong et al. (2014), Kaisoon et al. (2012) and Fu et al. (2009), and others evaluated the whole extract, which makes it difficult to compare results between different studies. Furthermore, within studies that had analyzed more than one flower, it was possible to verify that there was a high range of antioxidant activity between flowers. Li et al. (2014), for example, evaluated 51 edible flowers with values between 0.17–178 μmol Fe(II)/g FW for the Ferric Reducing Ability of Plasma (FRAP), and 0.19–73.9 μmol Trolox/g FW of Trolox equivalent antioxidant capacity (TEAC), while Kaisoon et al. (2012) studied 12 edible flowers from Thailand and obtained ranges of 31–87% of DPPH free radical scavenging activity and 7.30–62.0 mmol $FeSO_4$/100 g DW for FRAP.

Most studies showed that there is a high correlation between antioxidant capacity and total polyphenolic content, indicating that phenolic compounds could be the main

contributors for the antioxidant capacity (Li et al., 2014; Mao et al., 2006). Among them, flowers'antioxidant activity seems to be mainly due to the presence of flavonoids, phenolic acids, anthocyanins and alkaloids, making their individual quantification essential to understand the true bioactivity potential. As stated in Table 2.2.6, different species have different phenolic compounds, being flavonoids and organic acids the most reported. Recently, Lu et al. (2016) performed a review on phytochemicals in edible flowers.

Flavonoids are synthesized by a branched pathway that yields both colorless compounds such as flavonols, and colored pigments such as anthocyanins and the polymeric phlobaphenes and proanthocyanidins (Koes et al., 2005). One of the most important classes of flavonoids is the anthocyanins, which contribute to the red and blue color of the flowers. The anthocyanins found in edible flowers are described in Table 2.2.6, being cyanidin, delphinidin and pelargonidin the most reported; however, in Viola species are also found malvidin, peonidin and petunidin (Skowyra et al., 2014; Gamsjaeger et al., 2011; Zhang et al., 2012). In the flavonoids group it can also be found flavonols, such as quercetin, kaempferol, myricetin and rutin; flavones as apigenin and luteolin; and flavan-3-ols like catechins and epicatechins. Among the phenolic acids there are two sub-groups, hydroxybenzoic and hydroxycinnamic acids. The most common hydroxybenzoic acids in flowers are vanillic, chlorogenic, protocatechuic and syringic acids, while regarding hydroxycinnamic acids are ferulic, caffeic and p-coumaric acids. However, some phenolic acids are more specific for a particular flower specie, such as, carnosic and rosmarinic acids in *Rosmarinus officinalis* (Baño et al., 2003) and lithospermic acid in *Ocimum basilicum* (Javanmardi et al., 2002).

In this way, flowers have interesting antioxidants and their fresh consumption as well as their extracts can bring benefits to human health.

Table 2.2.5 - Extraction conditions, bioactive compounds and antioxidant activity methods and range of contents for some edible flowers.

Flower	Extraction method	Antioxidant activity method and bioactive compounds	Range of contents	Reference
10 common edible flowers	Maceration Acetone [80%]/ 5 min/ room temperature	Soluble free phenolic fraction DPPH radical scavenging activity ABTS radical scavenging activity ORAC FRAP Total phenolic content Total flavonoids content	Soluble free phenolic fraction Approx. 50-1.03×10^3 µmol TE/g DW[a] Approx. 100-2.06 ×10^3µmol TE/g DW[a] 225-900 µmol TE/g DW Approx. 200-2.64×10^3 µmol Fe^{2+}/g DW[a] 0.31-235 mg CAE/g DW 7.67-89.4 mg RE/g DW	(Xiong et al., 2014)
12 edible flowers from Thailand	Maceration Methanol:HCl [100:1, v/v]/ 12 h/ 35 °C	Soluble fraction DPPH free radical scavenging activity FRAP Total phenolic content Total flavonoid content	Soluble fraction 31-87% inhibition 7.30-62.0 mmol $FeSO_4$/100 g DW 37-89 mg GAE/g DW 11.4-68 mg RE/g DW	(Kaisoon et al., 2012)
12 edible flowers	Maceration Methanol / 24 h /25 °C	DPPH free radical scavenging activity Total phenolic content Total flavonoid content	4.21-6.96 g AAE/kg FW 1.23-2.27 g GAE/kg FW 2.53-5.28 g rutin/kg FW	(Rop et al., 2012)
24 edible flowers	Maceration Methanol/1 day/ room temperature	ABTS radical scavenging activity	0.7-0.01%	(Wetwitayaklung et al., 2008)
51 edible and wild flowers	Maceration Tetrahydrofuran/ 30 min/ 37 °C	FRAP TEAC Total phenolic content	0.17-178 µmol Fe[II]/g FW 0.19-73.9 µmol Trolox/g FW 0.13- 11.5 mg GAE/g FW	(Li et al., 2014)
8 edible flowers	Maceration Ethanol/ 48h /room temperature	DPPH FRAP ABTS Total phenolic content, Total flavonoids content Total anthocyanins content	1.4-175 µg/mL [EC_{50}] 6.3- 83.8 µM Fe[II]/g extract 11.4- 198 µg/mL [EC_{50}] 30.4-228 mg CAE/g extract 12.7- 64.2 mg QE/g extract 0.3-5.4 mg/g of extract.	(Loizzo et al., 2016)

12 edible flowers	Maceration Methanol 80% [v/v]/12 h/ 4 °C	FRAP Total anthocyanins content	0.55-70.4 mmol $FeSO_4$/100 g FW 0.47-14.9 mg c-3-gE/100 g FW	(Benvenuti et al., 2016)
23 edible flowers	Maceration Deionized water /20 min/100 °C	DPPH FRAP ABTS Total phenolic content Total flavonoid content	21.1-599 µmol Trolox/g DW 8.08-914 µmol Trolox/g DW 46.5-2.08×10^3 µmol Trolox/g DW 4.83-222 mg GAE/g DW 0.45-71.5 mg RE/g DW	(Chen et al., 2015)
Agave durangensis	Maceration 60% ethanol / 24 h/ room temperature	DPPH free radical scavenging activity Total flavonoid content Total antioxidant capacity Iron reducing power	0.875 µg/mL [EC_{50}] 1.21×10^3 µg/g dry extract 4.65 mg AAE 98.6 µg/mL [EC_{50}]	(Barriada-Bernal et al., 2014)
Borago officinalis	Soxhlet Methanol or acetone / 5 h/ 60°C Infusion Water/ 1 h/ 100°C	DPPH free radical scavenging activity Total phenolic content β-carotene bleaching assay Reducing power assays	Approx. 20-90% [Conc=1000 µg/mL][a] 50.4 to 64.1 mg GAE/g 57.8- 95.6% Approx. 0.4-2.2 [Conc=1000 µg/mL][a]	(Aliakbarlu et al., 2012)
Calendula officinalis	Maceration Methanol [80%] or ethanol [96%] or isopropanol [100%] or ethanol [60%]/14 h/17-22 °C	DPPH free radical scavenging activity FRAP Total phenolic content Total flavonoid content	Approx. 1.5-3 mmol Trolox/g[a] Approx. 0.25-2.0 mmol Fe[II]/g[a] Approx.120-150 mg GAE/100 mL[a] Approx.40-100 mg QE /100 mL[a]	(Butnariu et al., 2012)
Daylily	Maceration 70% methanol/ overnight/ room temperature	DPPH radical scavenging activity Reducing power Superoxide anion scavenging activity Ascorbic acid β-carotene Individual phenolic compounds [catechin]	At different stages of maturation 63.3- 94.6% [Conc=150 µg/mL] Approx. 0.2-0.68 [Conc= 80µg/mL][a] 61.5-95.7% [Conc=160 µg/mL] 16.3-36.1 mg AA/100 g FW 1.69-1.97 mg β-carotene /100 g FW 65.0-112 mg CE/100 g FW	(Mao et al., 2006)
Daylily	Maceration Water or ethanol/ overnight/ room temperature	DPPH free radical scavenging activity Total phenolic compound Thiocyanate method	Approx. 30-90 % [Conc= 80µg/mL][a] 41.2-160 [mg GAE/g dry extract]	(Fu et al., 2009)

		Reducing capacity Superoxide anion scavenging activity Chelating activity on Fe^{2+} ions	50.6-82.1 % [at 20 µg of extract] Approx. 0.05-0.28 [Conc=20 µg/mL][a] Approx. 20-95 % [Conc= 80µg/mL][a] Approx. 5-100 % [Conc= 80µg/mL][a]	
Edible flowers from Thailand	Maceration Acidified ethanol/ 2 h/room temperature [25 °C]	FRAP ORAC CAA Total phenolic content	99.9-329 µmol Fe^{2+}/g DW 215-492 µmol TE/g DW 413-966 µmol QE/g DW 102-213 mg GAE/g DW	(Kaisoon et al., 2012)
Edible flowers from Thailand	Maceration Methanol/ overnight / room temperature	Antioxidant index Carotenoids Tannins Total phenolics content Total xanthophylls content	2.85-13.0 1.29-3.82 mg/100g DW 17.7 mg TA/100g DW 98.4-12.3 mg pyrocatechol/ 100 g DW 4.24-5.81 mg/100 g DW	(Chanwitheesuk et al., 2005)
Sophora viciifolia [flower]	Maceration 85% methanol/ 48 h/ room temperature	Different fractions DPPH free radical scavenging activity ABTS radical scavenging activity FRAP Total phenolics content Total flavonoid content Reducing power	10.4-142 µg/mL [EC_{50}] 3.5-40.8 µg/mL [EC_{50}] 0.88-6.70 mmol Fe^{2+}/g dry extract 29.3-144 mg GAE/g dry extract 53.3-237 mg RE/g dry extract 13.9-69.8 µg/mL [EC_{50}]	(Tai et al., 2011)
Pyrus pashia	Maceration 85% ethanol / 48h/ 50 °C	DPPH FRAP Total phenolics content Total flavonoid content	109 µg/mL [EC_{50}] 193 µM Fe^{2+}/g dry extract 7.78 mg GAE/g dry extract 3.87×10^3 mg RE/g dry extract	(He et al., 2015)
Prunus mume [flowers]	Maceration Ethanol/water [30:70, v/v] /1 h/ 60 °C	DPPH free radical scavenging activity FRAP ABTS radical-scavenging activity Total phenolics content Scavenging ability of OH^-	43.1 mg/mL [EC_{50}] 2.94 mmol/L/mg extract [EC_{50}] 169 µg/mL [EC_{50}] 150 mg GAE/g DW 6.20 µg/mL [EC_{50}]	(Shi et al., 2009)
Hibiscus flowers	Maceration		Different solvents [ethanol/ water]	(Mak et al., 2013)

	Water and ethanol [99.7%] / 24 h/ room temperature	DPPH free radical scavenging activity FRAP Total phenolic content Total flavonoid content Total flavonols Total tannins Total anthocyanins	83.1/ 97.4 % 2.35×10^3 /2.88×10^3 µmol Fe [II]/100 g DW 4.60×10^3/ 5.44×10^3 mg GAE/100 g DW 2.15×10^3/2.77×10^3 mg CE/100 g DW 572/330 mg QE/100 g DW 2.85×10^3/4.42×10^3 mg CE/100 g DW 155/206 mg c-3-gE/100 g DW	
Tropaeolum majus	Maceration Aqueous acetone [30:70 v/v]/ overnight /1 °C	DPPH free radical scavenging activity ABTS radical scavenging activity Total phenolic content Anthocyanin Ascorbic acid content	91.9 µm TE/g FW 458 µmol TE/g FW 406 mg GAE/100 g FW 72 mg pgd 3-glu /100 g FW 71.5 mg AAE/100 g FW	(Garzón &Wrolstad, 2009)
Trifolium pratense and *Trifolium repens*	Maceration Ethanol /48 h/ room temperature	DPPH ABTS FRAP	10.3-34.0 µg/mL [EC_{50}] 21.4-150 µg/mL [EC_{50}] 44.2 µM Fe^{2+}/g dry extract	(Tundis et al., 2015)
Paeonia Section *Moutan*	Maceration Flavonoids extraction: methanol [70%] / 24 h/ 4 °C Polyphenolics fraction extraction: aqueous methanol [70%] /24 h/4 °C	DPPH free radical scavenging activity Total Flavonoid Content Total phenolic content ABTS radical scavenging activity HRSA FRAP	32.7-18.7 % 0.01-13.2 % 3.85-11.4 mg GAE/100 mg DW 1.19- 3.58 mmol Trolox/g DW 0.56-2.27 mmol CAE/10 g DW 1.04-3.03 mmol Trolox/g DW	(Li et al., 2009)
Passiflora foetida	Soxhlet Petroleum ether and ethanol Maceration Water/24 h/ room temperature	DPPH free radical scavenging activity ABTS radical-scavenging activity Total phenolic content Tannin content Metal chelating activity Hydroxyl radical scavenging activity Nitric oxide radical scavenging activity β-carotene bleaching assay	Aprox. 641-769% [EC_{50}][a] 3.68×10^3-3.99×10^3 µmol/g DM 4.8-5.7 % Aprox. 0.5-1.1 % [a] 5.78×10^3-6.75×10^3 mg EDTA eq /g extract 62.5-65.5% 127-206%	(Sasikala et al., 2011)

			Aprox. 10.5-12.3%[a]	
Petals of 48 cultivars of Zhongyuan tree peony	Maceration Methanol acidified with HCl [0.1%]/ 24 h/4 °C	DPPH free radical scavenging activity FRAP Total phenolic content Total anthocyanin content Total flavone and flavonol content	7.66-31.4 mg AAE/g FW 4.80-17.9 AAE/g FW 3.97-21.7 mg GAE/g FW 0-289 mg cyanidin /100 g FW 0.598-2.31 g rutin/100 g FW	(Fan et al., 2012)
Rosa hybrida cv. Noblered	Maceration 1% trifluoroacetic acid [TFA] [v/v] in methanol/2 days /4 °C	DPPH free radical scavenging activity Anthocyanins isolation and identification	76.5% [Conc = 50 µg/mL]	(Lee et al., 2011)
Rose cultivars	Hydrophilic antioxidants were extracted by acetone and lipophilic antioxidants by hexane	TEAC Total anthocyanin content	11.1-21.1 µM TE/g DW 0.23-0.70 Abs 520 nm /g DW	(Friedman et al., 2010)
Viola × *wittrockiana*	Not chemically pretreated before the Raman spectroscopic analysis	Carotenoid Anthocyanins Flavonoid	All identified by Raman spectroscopy	(Skowyra et al., 2014)

[a]Estimated graphically. Aprox. – Approximately; ABTS - 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; AA - acid ascorbic; AAE - acid ascorbic equivalent; c-3-gE - cyanidin-3-glucoside equivalents; CAA - Cellular antioxidant activity; CAE – chlorogenic acid equivalents; CE - catechin equivalents; Conc – Concentration; DPPH - 2,2-diphenyl-1-picrylhydrazyl; DW - dry weight; EC_{50} - the extract concentration that gives half-maximal response; EDTA - ethylenediamine tetraacetic acid; FRAP - Ferric reducing antioxidant power; FW - Fresh weight; GAE – Gallic acid equivalents; HRSA - Hydrogen peroxide radical scavenging assay; ORAC - Oxygen Radical Absorbance Capacity; pgd 3-glu - pelargonidin 3-glucoside; QE - quercetin equivalent; RE - rutin equivalents; TA - tannic acid; TE - Trolox equivalent; TEAC - Trolox equivalent antioxidant capacity; Abs - absorbance

Table 2.2.6 - Some individual phenolic compounds present in edible flowers.

Compounds	Edible flowers	Origin	Individual compounds	Reference
Flavonoids	*Agave durangensis* Gentry	Mexico	Quercetin-3-*O*-glycoside, kaempferol-3,7-*O*-diglucoside, quercetin-3-*O*-glycoside	(Barriada-Bernal et al., 2014)
	Alcea rosea L.	Japan	Delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, malvidin 3-*O*-glucoside	(Hosaka et al., 2012)
	Allium schoenoprasum L.	Norway	Cyanidin 3-*O*-β-glucoside, cyanidin 3- acetylglucoside, cyanidin 3-glucoside	(Fossen et al., 2000)
	Anethum graveolens L.	Taiwan	(+)-Catechin, (−)-epicatechin, quercetin, luteolin, kaempferol, apigenin	(Shyu et al., 2009)
	Bellis perennis L.	Poland	Quercitin, apigenin, kaempferol, isorhamnetin	(Nazaruk & Gudej, 2001)
	Bellis perennis L.	Poland	Isorhamnetin 3-*O*-β-d-galactopyranoside, isorhamnetin 3-*O*-β-d-(6″-acetyl) galactopyranoside, kaempferol 3-*O*-β-d-glucopyranoside	(Gudej & Nazaruk, 2001)
	Bellis perennis L.	Poland	Apigenin 7-*O*-β-D-glucuronide, apigenin 7-*O*-β-D-glucoside	(Nazaruk & Gudej, 2000)
	Bellis perennis L.	-----	Cyanidin 3-*O*-(4″-*O*-(malonil)-2″ *O*-(β d-glucuronil)-β-d-glucopiranoside), cyanidin 3-*O*-(2″-*O*-(β-d-glucuronil)-β-d-glucopiranoside), cyanidin 3-*O*-(6″-*O*-(malonyl)-2″-*O*-(β-d-glucuronyl)-β-d-glucopyranoside)	(Toki et al., 1991)
	Centaurea cyanus L.	-----	Pelargonidin 3-(6″-succinyl glucoside)-5-glucoside	(Takeda et al., 1988)
	Chrysanthemum morifolium Ramat.	China	Luteolin-7-*O*-6-malonilglucoside, quercetin-3-*O*-glucoside, diosmetin-7-*O*-glucuronide	(Lin & Harnly, 2010)
	Cichorium intybus	Denmark	Delphinidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-malonyl-β-D-glucoside), delphinidin 3,5-di-*O*-β-D-glucoside	(Nørbæk et al., 2002)
	Hibiscus rosa-sinensis L.	-----	Cyanidin-3-sophoroside	(Nakamura et al., 1990)
	Hylocereus undatus Britton & Rose	China	Kaempferol-3-*O*-β-D-rubinobioside, isorhamnetin-3-*O*-β-D-rubinobioside	(Yi et al., 2012)
	Paeonia Section *Moutan*	China	Quercetin 3,7-di-*O*-glucoside, kaempferol 3,7-di-*O*-glucoside, isorhamnetin 3,7-di-*O*-glucoside, luteolin 7-*O*-glucoside	(Li et al., 2009)
	Pelargonium ×	-----	Pelargonidin 5-diglucosides, pelargonidin 3-glucoside-5-(6-acetyl),	(Mitchell et al., 1998)

	domesticurn L.H. Bail.		cyanidin, peonidin, delphinidin, petunidin, malvidin	
	Rosa (120 species)	Japan, United Kingdom	Kaempferol glucosides, quercetin glucosides, cyanidin glucosides, peonidin glucosides	(Mikanagi et al., 2000)
	Rosa (200 varieties and some species)	Netherlands	Cyanidin, pelargonidin, quercetin, kaempferol	(Vries et al., 1974)
	Rosa chinensis Jacq.	China	Kaempferol arabinoside, pelargonidin 3,5-di-*O*-glucoside, quercetin 3-*O*-rhamnoside	(Cai et al., 2005)
	Rosa damascena Mill., *Rosa bourboniana* L. and *Rosa brunonii* Lindl.	India	Quercetin, kaempferol, myricetin	(Kumar et al., 2009)
	Rosa hybrida cv. Noblered	Chorea	Cyanidin-3,5-di-*O*-glucoside, pelargonidin-3,5-di-*O*-glucoside	(Lee et al., 2011)
	Rosa spp.	-----	Cyanidin 3,5-di-*O*-glucoside, cyanidin 3,5-di-*O*-glycoside, pelargonidin 3,5-di-*O*-glucoside, quercetin 3-*O*-arabinoside, kaempferol 4′-*O*-glucoside, kaempferol 3-*O*-rutinoside	(Sumere et al., 1993)
	Sophora viciifolia Hance	China	Luteolin, quercetin, vicenin	(Tai et al., 2011)
	Taraxacum officinale F.H. Wigg	France	Luteolin 7-glucoside, luteolin 7-diglucosides	(Williams et al., 1996)
	Trifolium pratense L.		Genistin 6″-*O*-malonate, formononetin 7-*O*-β-D-glucoside 6″-*O*-malonate, biochanin A 7-*O*-β-D-glucoside 6″-*O*-malonate	(Lin et al., 2000)
	Tropaeolum majus L.	Colombia	Derivatives of delphinidin, derivatives of cyanidin, pelargonidin 3-sophoroside	(Garzón and Wrolstad, 2009)
	Tropaeolum majus L., *Tagetes erecta* L., *Spilanthes oleracea* L.	Spain	Kaempferol-3-*O*-acetil-soporoside, quercetin-*O*-acetilhexoxide, isorhamnetin-3-*O*-hexoside	(Navarro-González et al., 2015)
	Viola × *wittrockiana*	Spain	Quercetin, delphinidin, petunidin, kaempferol	(Skowyra et al., 2014)
	Viola × *wittrockiana*	Germany	Kaempferol, quercetin, luteolin, peonidin, malvidin, pelargodin	(Gamsjaeger et al., 2011)
	Viola tricolor L.	Hungary	Rutin, violanthin	(Vukics et al., 2008)
	10 Species	China	Rutin, quercetin, apigenin, kaempferol	(Xiong et al., 2014)
	10 Species	Czech Republic	Rutin, catechin, quercetin	(Kucekova et al., 2013)
	12 Species	Thailand	Rutin, myricetin, quercetin, apigenin, kaempferol	(Kaisoon et al., 2011)

	48 Species of Zhongyuan	China	Peonidin 3,5-di-*O*-glucoside, pelargonidin 3,5-di-*O*-glucoside, quercetin di-hexoside, luteolin hexoside, apigenin pento-hexoside	(Fan et al., 2012)
Phenolic acids	*Althaea rosea* var. *nigra*	Poland	Ferulic, vanillic, syringic, *p*-coumaric, *p*-hydroxybenzoic, caffeic acids	(Dudek et al., 2006)
	Anethum graveolens L.	Taiwan	Gallic, gentisic, chlorogenic, caffeic, *p*-coumaric, benzoic, sinapic, *p*-anisic acids	(Shyu et al., 2009)
	Chrysanthemum morifolium Ramat.	China	Clorogenic, 5-sinapoylquinic, caffeic acids	(Lin & Harnly, 2010)
	Ocimum basilicum L.	Iran	Rosmarinic, lithospermic, vanillic, *p*-coumaric, hydroxybenzoic, syringic, ferulic, protocatechuic, caffeic, gentisic acids	(Nováková et al., 2010)
	Prunus mume Siebold & Zucc.	China	3-*O*-caffeyolquinic, 5-*O*-caffeyolquinic, 4-*O*-caffeyolquinic acids	(Javanmardi et al., 2002)
	Rosa damascena Mill., *Rosa bourboniana* L. and *Rosa brunonii* Lindl.	India	Gallic acid	(Shi et al., 2009)
	Rosmarinus officinalis L.	Spain	Carnosic, 12-*O*-methylcarnosic, rosmarinic acids	(Kumar et al., 2009)
	Viola tricolor L.	Germany	Caffeic, chlorogenic acids	(Baño et al., 2003)
	10 Species	Czech Republic	Gallic, *p*-coumaric, ferulic, caffeic, sinapic, cinamic acids	(Gonçalves et al., 2012)
	12 Species	Thailand	Gallic, protocatechuic, *p*-hydroxy benzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic acids	(Kucekova et al., 2013)
	51 Edible flowers	China	Gallic, protocatechuic, homogentisic acids	(Kaisoon et al., 2011)

Antimicrobial activity

Antimicrobial activity is sometimes associated to several edible flowers species due to the presence of inhibitory substances for certain microorganisms. Alzoreky and Nakahara (2003) showed that buffered methanol and acetone extracts of *Azadirachta indica* (Meliaceae family) flowers inhibited the growth of *Bacillus cereus*, not being referred the specific compounds responsible for this activity. Also, aqueous and ethanolic hibiscus flower extracts (*Hibiscus rosa-sinensis*) were reported to possess antibacterial activity against various Gram-positive and Gram-negative food-borne bacterial pathogens, probably due to the presence of polyphenols, flavonoids and tannins, as suggested by the authors (Mak et al., 2013). Essential oils and aqueous extracts of *Chrysanthemum* (Shunying et al., 2005; Sassi et al., 2008), ethanolic extracts of *C. officinalis* (Hamad et al., 2011), and organic and aqueous extracts of *Allium rose* (Sassi et al., 2008), showed marked antimicrobial properties due to the presence of camphor (Shunying et al., 2005), tannins (Sassi et al., 2008), flavonoids (Sassi et al., 2008), alkaloids (Sassi et al., 2008), and phenols (Najjaa et al., 2011).

One study performed with methanolic extracts of *Sesbania grandiflora* flower (consumed in India) suggested that phenolic extracts (mainly rutin) of this edible flower had inhibitory effect against *Staphylococcus aureus, Shigella flexneri, Salmonella typhi, Escherichia coli* and *Vibrio cholera* (China et al., 2012). Infusions of *Sesbania grandiflora, Senna siamea* and *Telosma minor* (Thai traditional flowers) showed antimicrobial properties against *Bacillus cereus, E. coli* and *Staphylococcus aureus* due to the presence of flavonoids (Krasaekoopt and Kongkarnchanatip, 2005). *Tamarix gallica* flowers also showed appreciable antibacterial properties against human pathogen strains, such as *Micrococcus luteus* (strongest activity), *E. coli* (lowest activity) and *Candida* (moderate activity) (Ksouri et al., 2009). These results suggested that methanolic extracts of *T. gallica* were more efficient to inhibit bacterial growth than fungal one, probably in relation to their active molecules detected such as: syringic, *p*-coumaric and gallic acids, and to the presence of (+)-catechin that conferred to these extracts their strong antioxidant properties (Ksouri et al., 2009).

Other study done with methanolic extracts of *Rose rugosa* petals showed antimicrobial activity against eight bacteria (i.e. *Staphylococcus epidermidis, S. aureus, Bacillus subtilis, M. luteus, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*) and two yeast strains (*Candida albicans, C. parapsilosis*) (Nowak et al., 2014); however, it was not possible to define explicitly which group of compounds

was responsible for this activity. *Jasminum sambac* (methanolic extract) showed effect against *Streptococcus mutans* and *Streptococcus sanguinis* (Tsai et al., 2008). *Alpinia galangal* flowers consumed raw or made into pickles in Asian cuisine (Yang and Eilerman, 1999), showed a broad spectrum of antimicrobial activity against Gram-positive bacteria, but exhibited little to no antimicrobial efficacy against Gram negative bacteria, when extracted with organic solvents (hexane or ethanol) (Hsu et al., 2010). Solutions of petals of Rose prepared in various solvents (petroleum, ether, alcohol, water) showed antimicrobial activity against 10 bacterial strains, such as *E. coli*, *Streptococcus pneumoniae* and *Salmonella typhimurium* (Hirulkar and Agrawal, 2010). Furthermore, alcoholic rose petal extracts showed higher inhibition zones for *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* when compared to the maximum concentration of the antibiotic streptomycin (Hirulkar and Agrawal, 2010). Thus, extracts of edible flowers show antimicrobial activity, and so they can be used as an alternative to synthetic antimicrobial drugs (Chehregani et al., 2007).

Effects of edible flowers consumption on human health

The interest in edible flowers is probably continuously increasing due to their potential health effects that are related with their chemical composition. Lu et al. (2016) describe in detail the health benefits of edible flowers. Some of the biological effects of the most common edible flowers are summarized in Table 2.2.2. In particular, the hexane fraction of *R. rugosa* is able to inhibit lipid peroxidation and to prevent oxidative damage, as well as, to promote free radicals scavenging (Park et al., 2009). Furthermore, *n*-hexane soluble and the nonsaponifiable lipid fractions of the edible flower extract of chrysanthemum (*Chrysanthemum morifolium*) reduced 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced acute inflammation in mice, due to the presence of triterpenes (Ukiya et al., 2001). Anthocyanin (delphinidin 3-sambubioside), isolated from the dried calices of Hibiscus, exhibited anti-inflammatory properties and reduced the amounts of several LPS induced inflammatory mediators (Sogo et al., 2015). Extracts of *C. officinalis* flowers exhibited potent anti-HIV activity in *in vitro* MTT (methylthiazolyldiphenyl-tetrazolium bromide)-based assay (Muley et al., 2009). Ethanolic extract from *Chrysanthemum indicum* showed anti-inflammatory activity in both acute and chronic irritant contact dermatitis *in vivo*, because the production of IL-1β and TNF-α (proinflammatory cytokines) was inhibited, with the subsequent blockade of leukocyte accumulation

(Lee et al., 2009). Furthermore, natural phenolic compounds (ferulic, gallic, coumaric acids and rutin) contained in *A. schoenoprasum* flowers were reported to be able to inhibit cell proliferation and thus potentially useful for the treatment and prevention of tumour diseases (Lee et al., 2011b; López-García et al., 2013). Edible flowers contain numerous phytochemicals such as: anthocyanins (Chang et al., 2005), flavonoids (Xie et al., 2009), rhein (Duraipandiyan et al., 2012) (isolated from ethyl acetate extract of *Cassia*) with significantly anti-proliferative effect activity, against some cancers such as: *Hibiscus* on liver cancer (Chang et al., 2005); *Chrysanthemum* on human colon and brain cancers (Xie et al., 2009; Yang et al., 2011); and *Cassia fistula* on colon cancer (Duraipandiyan et al., 2012). Furthermore, edible flowers were reported to have effect on weight control in animal and cell models, as well as protective effects on liver fibrosis, namely: *Hibiscus sabdariffa* (Kim et al., 2007; Liu et al., 2006; Huang et al., 2015) and pomegranate flower (Xu et al., 2009). All these studies show the great potential that edible flowers may have in human health.

Harvest

Edible flowers should be harvested in the cool of the day during the peak of bloom because they are in their best flavor. Only flowers free of insect and disease problems should be selected (University of Kentucky, 2012). The collection of unopened blossoms (except daylilies) and wilted or faded flowers should be avoided because they may have a bitter or unappealing flavor (Newman and O'Connor, 2013). Flowers that have been sprayed with pesticides or that occur along roadsides, or flowers from plants that have been fertilized with untreated manure must never be collected for eating purposes because they can be toxic or cause illness due to the presence of pathogens. Moreover, purchase of flowers from florists, garden centers or nurseries must also not be done because they probably use in flowers production some chemical products (synthetic fertilizers and pesticides), which can be dangerous to human health. Only organic cultivation is adequate. Concerning flowers' parts, the stems, sepals, pistils and stamens of most flowers should be removed prior to use. Pollen may detract flower's flavor and may cause allergies in some people. The sepals should be removed from all flowers due to their sourness, except violas, pansies and johnny jump-ups whose sepals are more tasteful. In many flowers (including rose, lavender, tulip, calendula, and chrysanthemum) only the petals are edible (Table 2.2.2).

Post-harvest technologies

Edible flowers are extremely perishable and very delicate. After harvest, flowers are susceptible to petal abscission, discoloration, wilting, dehydration and tissue browning. In this way, refrigeration of edible flowers before packaging for small trips, by keeping them in cold storage between 4 and 6 °C, can increase their durability from 2 to 5 days after harvest (Kou et al., 2012). Nowadays, to protect flowers from desiccation and to preserve their frail structure, they are typically packaged in small, rigid, plastic (or plastic wrapped) packages (Kelley et al., 2001c).

In this way, it becomes essential to develop techniques to aid quality retention and extend shelf life of edible flowers. Until now no guidelines have been established for storage of edible flowers and scarce information has been published that identifies quality limiting factors and preservation methods. Only three studies have been made so far: one on the effect of storage temperature (Kelley et al., 2003), other about the controlled release of 1-methylcyclopropene and the use of modified atmosphere packaging (MAP) (Kou et al., 2012) and the last on the effect of different types of packaging, namely polyvinyl chloride (PVC) or polypropylene (PP), with or without modified atmosphere on flowers' quality (Friedman et al., 2007). Kelley et al. (2003) concluded that viola, pansy and nasturtium flowers can be stored from 0 to 2.5 °C for 2 weeks preserving a perfect visual quality, whereas borage requires lower temperatures (−2.5 °C) to keep acceptable after 2 weeks of storage. So, these results showed each flower species has different storage temperature requirements. Kou et al. (2012) reported that modified atmosphere packaging significantly reduced weight loss for both edible carnations and snapdragons and aided in maintaining their visual quality. On the other hand, treatment with 1-methylcyclopropene (controlled release strips) in conjunction with MAP further helped to maintain fresh appearance, reduce wilting and extend the shelf life of both types of edible flowers and prevented abscission in snapdragon. Friedman et al. (2007) detected that packing of flowers in transparent polyethylene terephthalate (PET) boxes was better than wrapping the trays with PVC, because the shelf-life was increased (additional 2 days) without losing commercial quality or antioxidant capacity.

All studies pointed out that it is necessary to find ways to extend the shelf life of edible flowers because it would bring significant economic benefits, including less wasted product and reduced shipping costs with the possibility of ground transport,

making them a much more appealing choice for restaurants and enabling edible flower growers to expand their market (Kou et al., 2012).

Beyond an appropriate packaging, extension of shelf life using processing technologies that minimally affect the sensory and texture of the products would be of great interest for this market. High hydrostatic pressure (HHP) appears as a good alternative, which has been applied to several types of foods and in particular to some vegetables and plants such as *Stevia rebaudiana* (Carbonell-Capella et al., 2013), *Brassica napus* (Clariana et al., 2011), *Camellia sinensis* (Jun et al., 2011), baby lettuce, spinach, asparagus and cauliflowers (Arroyo et al., 1999). Its application to edible flowers could contribute for the production of high quality products with enhanced microbial safety combined with shelf-life increase. However, to our knowledge, only one study was conducted on edible flowers, namely *Echinacea purpurea* flowers, based on the application of 600 MPa for 3 and 5 min (Chen et al., 2010). This study showed that HHP is an effective pasteurization process treatment, able to reduce the microbial-contamination load, while not adversely altering the chemical and bioactive functions of *E. purpurea* constituents. Therefore, HHP treatment may be one complementary option for edible flowers' preservation, but more studies are needed to fully understand its potentialities.

Conclusions

In general, edible flowers are already well accepted by consumers and chefs. Nevertheless, not all flowers are edible, being important to identify correctly each species and to know which parts of the flowers should be consumed. Some variability on edible flowers nutritional composition has been observed. Inconsistent data have been reported for some minor components, particularly minerals, requiring more studies to understand flowers nutritional potential. Furthermore, flower contains a great variety of natural bioactive compounds, such as phenolic acids, flavonoids, anthocyanin among other phenolic compounds, with already proven antioxidant and antimicrobial properties. Despite the potential health benefits associated to edible flowers consumption, particular attention must be given to their harvest and preservation, due to their high perishability. The most common postharvest technologies include refrigeration and MAP but the shelf life is still short, increasing products losses. As the global market requests for increased shelf life and safety, it is necessary to study potentialities of new food-processing technologies such as HHP, in

order to maintain the quality of the product longer, increase producers' incomes and reduce wastes.

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2.3.

Post-harvest technologies applied to edible flowers - a review
Edible flowers preservation

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Abstract

This review intends to summarize the current knowledge on the post-harvest technologies applied to edible flowers, to help producers to increase their market share and to inform consumers on the technologies that are available to maintain edible flowers' quality and safety. Emerging post-harvest technologies as High Hydrostatic Pressure (HHP) or irradiation have given good results. Freeze- or vacuum-drying has shown to be highly effective in the preservation of flowers' bioactive compounds in comparison with classical drying approaches. While osmotic dehydration is already in use, the application of edible coatings and films can be a healthier alternative, without increasing solute contents.

Keywords: Drying; edible flowers; high hydrostatic pressure; postharvest technologies; shelflife

Introduction

Flowers have held an eminent place in art, religion, health and culinary since ancient times. Particularly, the popularity of edible flowers has increased since the late 1980s. The market of edible flowers is becoming more important due to the increased number of recipe books, magazine articles, and websites on the theme, as well as to the growth of research on their nutritional and bioactive potential. [1,2] However, the market for edible flowers still receives less attention than that of other products, such as vegetables and fruits, because the production of edible flowers is still low and it is still a niche market.

Edible flowers are highly perishable, with short shelf life of 2-5 days after harvest [3], with early petal abscission and discoloration, flower wilt, dehydration and tissue browning. Compared with other types of flowers, edible flowers are more vulnerable than cut flowers used for decoration purposes, because their stems are cut very short and they are stored without additional water supply. Although some publications recommend that flowers should be harvested in the same day that they will be consumed, this advice limits their commercial viability.[4] Until now, no guidelines have been established for storage of edible flowers and few detailed studies have been done on the factors that limit their quality.[3] Presently, most edible flowers are sold fresh, packaged in small, rigid plastic (or plastic wrapped) packages and placed next to fresh herbs in refrigerated sections.[5] Pansy, borage, rose, centaurea, nasturtiums, begonia, carnation and hibiscus are examples of edible flowers that are normally used to garnish dishes. The most common postharvest methods applied to these edible flowers are refrigeration, drying, canning in sugar, and preservation in distillates. Nevertheless, other edible flowers are more known by consumers as vegetables, such as artichoke, broccoli and cauliflower, even though these are inflorescences. The food industry preserves these species at low temperatures and through suitable packaging, transportation and maintenance of storage atmosphere.

However, this industry is very interested in improving marketability of edible flowers, not only as fresh but also as processed products. In this sense, the application of new food preservation technologies able to increase the shelf life of edible flowers will bring important economic benefits, beyond allowing the preservation of the product quality for longer periods of time. So, the aim is to review the current knowledge on the most advanced post-harvest technologies applied to edible flowers, in order to help producers to increase their market share, and allow their transportation over wider

geographical areas, showing also to the scientific community the fields that require further studies. Simultaneously, the present review also aims to inform consumers on the technologies that are available to preserve edible flowers' quality and safety.

Edible flowers

Until now, no official lists of edible flowers have been published by any international body, but according to Lu et al.[6], there are 97 families, 100 genera, and 180 species of edible flowers worldwide. Several edible flowers usually eaten by consumers are not recognized as flowers, as artichoke (*Cynara scolymus*), broccoli and cauliflower (*Brassica oleracea*), being considered vegetables. Also, several flowers usually used for ornamental purposes have edible parts, including pansy (*Viola×wittrockiana*), centaurea (*Centaurea cyanus*), borage (*Borago officinalis*), rose (*Rose* spp.), nasturtiums (*Tropaeolum majus*) and hibiscus (*Hibiscus rosa-sinensis*). Flowers of some fruit trees can also be used in cuisine, such as elderberry blossoms (*Sambucus* spp.) and citrus blossoms (orange, lemon, lime, grapefruit, kumquat). Moreover, some herb flowers are also edible, namely alliums (leeks, chives, garlic), thyme (*Thymus vulgaris*), summer savory (*Satureja hortensis*), marjoram (*Origanum majorana*), mint (*Mentha* spp.) and common sage (*Salvia officinalis*).

The importance of improving edible flowers market

Edible flowers can be part of a diversification plan for market gardeners, especially organic growers, since most of them cannot survive by growing only edible flowers. So, edible flowers are usually grown in conjunction with other cultures, such as cut flowers and herbs, to complement growers' incomes and create opportunities for value-added products. Nowadays, the price varies with the flower and state in which it is sold. For example, the price of 20 fresh pansies (20 flowers) is 6.80 euros in the Spanish company "Flores frescas" (0.34 euros/unit) and of six fresh calendulas is 2.46 euros in the New Zealand company "Kahikatea Farm" (0.41 euros/unit). Furthermore, flowers subjected to post-harvest treatment are generally sold at a higher price than fresh (e.g.,12 crystallized violas cost 19.70 euros in the English company "Meadowsweet flower", corresponding to 1.6 euros/unit).

Around the world there are a lot of companies that are dedicated to edible flowers selling, such as Fleurs et saveurs, Ervas finas, Meadowsweet flower, BloomBites, and Green farm, whose production has been increasing. In addition, there are some

campaigns that promote the consumption and production of edible flowers. For example, “The Herbs of Brussels” in Belgium; “Food safety: Edible flowers” in Thailand; “Look & Taste” in Netherlands and “Essai fleurs comestibles: Transfert et communication” in France. In this sense, edible flowers have become a culinary trend, referred to in international culinary magazines, such as Bon appetit “How to use edible flowers in salads, cocktails, and more” [7] and Food and Wine “The Edible Flower”. [8] The clients of edible flowers are gourmet restaurants and their associated food service operations, and grocery stores. So, to eat edible flowers is a new trend, described as one of the “six trends of food and drinks in summer”. [9] Consequently, the consumption of edible flowers is expanding around the world. This market needs to increase the production, to guarantee better quality and to have more variety of flowers, as well as with longer shelf life.

Compared to other kinds of flowers, edible flowers are more vulnerable to postharvest quality loss than cut flowers, because their stems are cut very short and they are stored without additional water supply. Nowadays, edible flowers are often packed in containers and must be used within 2-5 days after harvest, which requires air transportation to reach most regions before the end of their shelf life. Thus, to find new ways of increasing their shelf life is a big challenge.

Post-harvest technologies

The increasing application of edible flowers in various food commodities demands for new technological approaches to improve their distribution and marketing efficiency as fresh products or minimally processed products. Prolonging post-harvest storage, while preserving the whole quality of edible flowers, will benefit their industrial development, as well as consumers' health. Therefore, the food industry is investing considerable resources to develop new technologies that can maintain all properties and quality of edible flowers, and to meet consumers’ expectations. This section describes some conventional techniques used in edible flowers’ preservation, as well as emerging nonthermal methods (ex: high hydrostatic pressure and irradiation) and packaging approaches. As some edible flowers, such as artichoke, broccoli and cauliflower (*B. oleracea*), are not frequently recognized as edible flowers by the consumers, they will be discussed separately from the other edible flowers.

Low temperature storage

Temperature is one of the most important environmental factors limiting the shelf life of fruits, vegetables and herbs. [10] Until this moment, the main technologies used by the industry of edible flowers are cold storage (refrigeration and freezing) and hot-air drying, as will be detailed below.

Cold storage delays flower senescence and quality deterioration during storage. Storage at low temperatures is associated with an increase of flowers' shelf life, because there is a reduction of respiration and internal breakdown of tissues by enzymes, reduction of water loss and wilting, slower growth of microorganisms, and reduction of ethylene production. [11]

Several studies dealing with freezing and refrigerated storage have been conducted, with their most important details and conclusions assembled in Tables 2.3.1. and 2.3.2.. Regarding Table 2.3.3., relative to edible flowers not including artichoke and Brassica species, good results have been obtained with temperatures at -2.5 °C (ex. borage), 2.5 °C (heartsease, nasturtium, pansies and pumpkin) and 5 °C (pumpkin) for up to two weeks. It must be emphasized that temperatures lower than -2.5 °C have not been tested until now in this type of edible flowers. However, the effect of temperature on each flower must be studied in an independent way because, for example, no good results were obtained for the scarlet runner bean flowers when temperatures between -2.5 and 20 ºC were applied for two weeks. Furthermore, the parameter most evaluated until now has been the visual quality/appearance. On the other hand, more parameters have been determined in artichoke, broccoli and cauliflower when stored at low temperatures, such as vitamin C, phenolics, weight loss, lipid peroxidation, total carotenoids and β-carotene, among others. In general, refrigeration is also an efficient approach for artichoke, broccoli and cauliflower. For example, a temperature at 2 °C does not accelerate the deterioration and lipid peroxidation of broccoli buds when compared to 13 or 23 °C. [12] Furthermore, fast cooling after harvest has been tested in broccoli, with the aim to reduce the metabolic activity that can result in deterioration. [13] Among the different methods of rapid cooling that have been applied to broccoli, hydro-cooling alone, or combined with wrapping with perforated film, has given good results, with lower weight losses, firmness maintenance, and color retention. [13,14]

Table 2.3.1.– Post-harvest technologies applied to edible flowers.

Post-harvest technologies	Edible flowers	Treatment/storage conditions	Reference
Low temperature	Borage (*Borago officinalis*)	Polyethylene bags, -2.5 to 20°C	[5]
	Heartsease (*Viola tricolor*)	Polyethylene bags, at -2.5, 0, 2.5, 5, 10, 20 °C	[5]
	Nasturtium (*Tropaeolum majus*)		
	Pansies (*Viola×wittrockiana*)		
	Pumpkin (*Curcubita pepo*)	2.5 and 5 °C, 2 weeks	[4]
	Runner bean (*Phaseolus coccineus*)	Polyethylene bags at -2.5, 0, 2.5, 5, 10, 20 °C	[5]
Drying methods	Black locust (*Robinia pseudoacacia*)	Sun (3 days, 35°C), hot-air (60°C), freeze (-80°C for 12h) and microwave-vacuum (1500 W, 70 kPa) drying	[19]
	Carnation (*Dianthus chinensis*) (red and pink)	Freezing (-35°C, 2 and 4h) + vacuum drying (27, 37 and 47°C, 0.004-0.007 kPa)	[23]
	Daylily (*Hemerocallis disticha*)	Hot-air (50°C) and freeze-drying	[20]
	Marigold (*Tagetes erecta*)	Freeze (FD) (48 h), hot air (HA) (60°C for 4 h) and combined far-infrared radiation with hot-air convective (FIR-HA) drying	[21]
	Purple coneflower (*Echinacea purpurea*)	Freeze (- 55 °C for 4 days), vacuum microwave with full vacuum (1 kW for 47 min) and air (70°C for 13 h; 40°C for 55h; 25°C for 1 week) drying	[24]
		Vacuum freeze (VFD), cool wind (CWD) (30°C), and hot air (HA) (40, 55 or 70°C) drying	[22]
	Rose (*Rose* spp.) (red and pink)	Freezing (-35°C, 2 and 4h) + vacuum drying (27, 37 and 47°C, 0.004-0.007 kPa)	[23]
High Hydrostatic Pressure (HHP)	Borage *(Borago officinalis)* Camellia japonica *(Camellia japonica)* Centaurea *(Centaurea cyanus)* Pansies (*Viola × wittrockiana*)	75, 150 and 450 MPa, 5 and 10 min 75 MPa, 1 and 5 min 100 MPa, 5 min 75, 100, 200 and 300 MPa, 5 min 75 MPa, 5 and 10 min	[37]

		Purple coneflower *(Echinacea purpurea)*	600 MPa, 2 and 5 min	[38]
Irradiation	Ionising	Borage (*Borago officinalis*) Carnation (*Dianthus chinensis*)	^{60}Co (0, 0.3, 0.6, 0.8 and 1.0 kGy)	[57]
		Heartsease (*Viola tricolor)*	^{60}Co (0.5, 0.8 and 1 kGy), room temperature	[59]
		Nasturtium (*Tropaeolum majus*)	^{60}Co (0.5, 0.8 and 1 kGy), room temperature	[58]
		Sweet alyssum (*Lobularia maritima*)	^{60}Co (0, 0.3, 0.6, 0.8 and 1.0 kGy)	[57]
		Wood violet (*Viola odorata***)**	^{60}Co (0, 0.3, 0.6, 0.8 and 1.0 kGy)	[57]

↑: Increased; ↓: Decreased.

In artichoke, vitamin C content decreased (approx. 28-34%) after 14 days of storage under the assayed temperatures (0, 2, 5, 7 and 10 °C), while chlorogenic, 1,4-dicaffeoylquinic and 4,5-dicaffeoylquinic acids contents increased in the internal bracts after storage, particularly at 2, 5 and 7 °C. [15]

Regarding freezing, this has been tested mostly with cauliflower and broccoli. It was observed that the freezing process itself did not cause vitamin C loss. [16] However, during storage, a small reduction on vitamin C content in broccoli (15-18%) and cauliflower (6-13%) were observed, slightly lower at -30 ºC than at -20 ºC. [16] When freezing cauliflower, it seems to be advantageous to previously immerse it in boiling water for 6 min instead blanching at 95-98 °C (approx. 3 min) in order to retain the vitamin C, antioxidant activity, polyphenols, total carotenoids and β-carotene. [17] Another study in cauliflower concluded that long-term freezing storage (12 months) did not significantly affect the total aliphatic and indole glucosinolates in this flower. [18]

In summary, different temperatures have different effects on the quality and appearance of each type of edible flowers, with the possibility to increase the shelf life of some of them by decreasing the storage temperature. However, more studies should be done in the future for specific applications and over a higher range of temperatures.

Drying

Drying is an important process for handling foods to prolong their shelf life, as well as, to inhibit enzymatic degradation, prevent the growth of microorganisms and reduce weight for cheaper transport and storage. There are many different drying methods, some of them already used in edible flowers, such as hot-air drying, freeze drying, vacuum microwave drying, cool wind-drying, sun drying and osmotic drying, as well as those involving combinations of these (Table 2.3.1). Among them, drying by application of heat (ex. hot-air drying and sun drying) is a classical approach, as for tea petal preparation, but it has some drawbacks, such as undesirable biochemical and nutritional changes in the processed product that may affect its overall quality.

Most of the studies in edible flowers have tested different methods of drying and have evaluated their effects on flowers' quality (Table 2.3.1). Black locust flowers submitted to freeze drying had higher antioxidant activity (DPPH radical scavenging, reducing power and hydroxyl radical scavenging ability) when compared to sun drying, hot-air drying and vacuum-microwave drying. [19] When comparing freeze

drying with hot-air drying, the first process resulted in a lower loss of carotenoids in daylilies [20] and the highest levels of lutein and lycopene in marigold. [21] Freeze drying also retained more bioactive compounds (caffeic acid derivatives and total phenolics) in purple coneflower.[22] Regarding red rose and carnations, higher vacuum-drying temperatures resulted in stiffer flowers and a greater color change. [23] Nevertheless, generally good results have been obtained with freeze drying.

Sun-dried black locust flowers had the worst antioxidant activities and phenolic content [19], suggesting that sun drying may cause loss of important bioactive compounds. The use of high temperatures (ex. 70 °C) in hot-air convective drying may also cause losses of caffeic acid derivatives [22,24] and total phenolics [22] in purple coneflowers. However, in marigold the highest β-carotene content was obtained when applying hot air convective drying (60 °C, 4 h). [21]

Concerning vacuum-microwave drying, its ability to retain the color of purple coneflower and caffeic acid derivatives content when compared to conventional air drying has been reported by Kim et al. [24] Black locust flowers subjected to vacuum-microwave drying also had high total phenolic content and iron-chelating ability, showing vacuum-microwave drying to be a more economical method than freeze drying because it only requires minutes instead of hours or days. [19] This makes it an interesting technology to be studied more in the future. Combining far-infrared radiation with hot-air convection drying allowed the color preservation of marigolds, and maintained the highest values of phenolic compounds, as well as of lutein and lycopene, when compared to untreated flowers (fresh). [21]

Regarding broccoli, cauliflower and artichoke, hot air drying has only been applied to broccoli inflorescences (Table 2.3.2). Broccoli heads treated with hot air at 48 °C for 3 h presented an important delay in their senescence at 20 °C and contributed to the maintenance of an overall better quality (retention of chlorophyll content and higher contents of sugars, proteins and antioxidants). [25] Another drying process that has been applied to broccoli and cauliflower is osmotic dehydration, which is a water removal process involving soaking foods in a hypertonic solution. It is also used as a pre-treatment before other processes, to improve the quality of the final product and to reduce the water activity inhibiting the microbial growth. [26] Until now, no studies have been done on the effect of osmotic dehydration on edible flowers, but some works have been conducted in inflorescences, such as broccoli [27] and cauliflower. [28,29] The osmotic solutions used in those works were sweet (ex: trehalose [27] and

sucrose [28]), salty (ex: sodium chloride), [28,29] and in combination. [28] Until now, the studies done on cauliflower investigated the optimum conditions of osmotic dehydration, considering the quality of the final product. Jayaraman et al. [28] reported that the optimum treatment consisted in soaking in 3% salt and 6% sucrose for 12–16 h at 4 °C, giving a shelf life increase from 3 to 12 months at ambient temperature when packaged in paper-foil-polythene laminate. On the other hand, Vijayanand et al. [29] reported a much faster process, using 12% (w/w) salt at 80 °C for 5 min (twice). The concentration of the osmotic solution, temperature, agitation, osmotic solutes and food pieces size are factors that affect the osmotic process. In broccoli, the only study on osmotic dehydration performed until now evaluated the effectiveness of osmotic dehydration alone or assisted with ultrasound (method applied to increase the mass transfer rate). [27] Compared with the normal osmotic dehydration during 2 h, an ultrasound-assisted dehydration for a shorter time (30 min) increased the water loss, caused higher accumulation of trehalose and decreased the mobility of water in the broccoli cell tissue; however, when applying an ultrasound treatment time of 40 min, opposite results were observed. Thus, these data showed the important role of the time treatment in ultrasound-assisted osmotic dehydration process, being necessary to perform more studies in the future. In conclusion, drying methods can affect the quality of edible flowers, but these changes can be minimized by the appropriate design and choice of the drying process based on specific flowers properties, technological availability and economic impact.

Edible films and coatings

Edible films and coatings are distinct methods used by the food industry. A film can be defined as a thin skin formed and then applied to the product (e.g., through casing with a biopolymer solution prepared separately from the food that is later applied to), while coating is a suspension or an emulsion applied directly on the surface of the food, leading to the subsequent formation of a coating. [30] However, both are generally based on biological materials, such as proteins, lipids and polysaccharides. Presently, both methods are already used in fruits and vegetables, acting mainly as a barrier against gas transport and microorganism growth. [31] Regarding edible coatings, no studies have been done on edible flowers and a few works have been published on artichoke and brassicas . Some polysaccharides have been included in edible coating formulations like alginate [32], chitosan [33,34] and carboxymethyl-cellulose. [31]

Table 2.3.2. – Post-harvest technologies applied to artichoke, broccoli and cauliflower.

Post-harvest technologies	Edible flowers	Treatment/storage conditions	Reference
Low temperature	Artichoke (*Cynara scolymus*)	0, 2, 5, 7 and 10°C for 14 days	[15]
		-20°C and -30°C for 30 and 50 min, respectively The frozen products, packed in polythene bags, were stored for 12 months at both temperatures.	[16]
		Cooled room (2 °C with 95% RH), top-iced (4 kg of flaked ice then placed in 2 °C storage), or hydro-cooled (12 min using water at 1 °C) for 14 days	[13]
	Broccoli (*Brassica oleracea* var. *italica*)	First experiment: Four treatments: - hydro-cooling alone - hydro-cooling combined with wrap - non-cooling and no wrap - non-cooling but with wrap Storage conditions 3, 10 and 17 days at 1 °C and placed afterwards at 13 °C. Second experiment: 1 or 5 °C, 95% relative humidity for a period of 10 days.	[14]
		2, 13, and 23 °C over 144 h	[12]
	Cauliflower (*Brassica oleracea* var. *botrytis*)	-20°C and -30 °C for 30 and 50 min, respectively. The frozen products, packed in polythene bags, were stored for 12 months at both temperatures.	[16]
		Traditional technology: blanching (95–98 °C, aprox. 3 min) + freezing (-20 and -30 °C) Modified technology: cooking (boiling water, 6 min) + freezing (-20 and -30 °C)	[17]

		Storage for: 0, 4, 8, 12 months at -20 or -30 °C. -24 °C for 3, 6, 12 months	[18]
Drying methods	Broccoli (*Brassica oleracea* var. *italica*)	Hot-air (37, 42, 45, 48, 50 °C for 1 or 3 h) Storage conditions: at 20 °C for 4 days	[25]
		Osmotic dehydration: 40% (w/w) syrups of trehalose, 35°C, 2 h Ultrasound assistant osmotic dehydration for 10, 20, 30 and 40 min	[27]
	Cauliflower (*Brassica oleracea* var. *botrytis*)	Osmotic dehydration: salt and sucrose (cane sugar), alone and in combination (optimum condition: 3% salt and 6% sucrose for 12-16 h at 4 °C)	[28]
		Osmotic dehydration: salt concentration (5-25%), temperature (40-90°C), ratio of brine to material (2-4, w/w) and time (5-180 min) (optimum condition: 12% (w/w) brine, 80°C, 5 min in 2 times)	[29]
Edible coating	Artichoke (*Cynara scolymus*)	Dipping: citric acid and calcium chloride solution Coating: Sodium alginate with citric acid After, packaging in: - multilayer-film (All-PE) - biodegradable monolayer film (NVT2) - oriented polypropylene film (OPP) **Storage conditions:** 4 °C for 6 days	[32]
	Broccoli (*Brassica oleracea* var. *italica*)	Chitosan coating	[33]
		Chitosan and chitosan enriched with bioactive compounds (bee pollen, ethanolic extract of propolis, pomegranate dried extract and	[34]

			resveratrol) and essential oils (tea tree, rosemary, clove, lemon, oreganum, calendula and aloe vera) coatings	
			Chitosan or carboxymethyl-cellulose (CM) with or without a previous application of a mild heat shock of 1.5 min at 50 °C	[31]
Edible film		Cauliflower (*Brassica oleracea* var. *botrytis*)	Methyl cellulose-based edible films of various compositions	[35]
High Hydrostatic Pressure (HHP)		Broccoli (*Brassica oleracea* var. *italica*)	180 MPa, −16 °C and 210 MPa, −20 °C	[39]
			50–500 MPa, 15–60 °C between 4-90 min	[40]
			100–500 MPa, 20–40 °C ,15 or 35 min	[41]
			Blanching + 210 MPa at −20.5 °C or 210 MPa, liquid N_2	[43]
			600 MPa, 25 and 75 °C, 10, 20 and 40 min.	[44]
			400 and 600 MPa, 2 min	[45]
			0.1–600 MPa, 25–45 °C, 30 min	[73]
			0.1 and 800 MPa, 20 °C, 10 min	[42]
		Cauliflower (*Brassica oleracea* var. *botrytis*)	400 MPa, 5 °C, 30 min.	[47]
			200 MPa, 5 min	[46]
			200, 300, 350 and 400 MPa, 5 °C, 30 min	[48]
Irradiation	UV	Broccoli (*Brassica oleracea* var. *italica*)	UV-B (4.4, 8.8 and 13.1 kJ m^{-2})	[53]
			UV-C (4, 7, 10 and 14 kJ m^{-2})	[51]
			UV-C (8 kJ m^{-2})	[54]

↑: Increased; ↓: Decreased.

Concerning alginate coating, its application on artichoke showed the best results in terms of shelf life (increased microbial stability) in comparison with dipping in citric acid/calcium chloride solution. [32]

Furthermore, these authors also stated the importance of choosing a correct packaging material, with better results (higher microbial stability and increased shelf life) obtained with the biodegradable monolayer film (NVT2) than multilayer-film (All-PE) and oriented polypropylene film (OPP). The effectiveness of chitosan to improve the microbiological and sensory quality of fresh cut broccoli was evaluated by Moreira et al. [33], regarded as a viable alternative to control the microbiota present in minimally processed broccoli because the growth of total coliforms, psychrotrophic and mesophilic aerobes was substantially inhibited during the whole storage period tested. Furthermore, coated broccoli samples had acceptable scores in all sensory parameters examined until the end of storage, without appearance of undesirable odors and with higher quality levels than control samples. A similar study [34] also showed that chitosan coating enriched with essential oils (tea tree, rosemary, clove, lemon, oreganum, calendula and aloe vera) and bioactive compounds (bee pollen, ethanolic extract of propolis, pomegranate dried extract and resveratrol) had significant antibacterial properties. Again, the application of chitosan coatings alone or enriched did not introduce negative effects on the sensory attributes of minimally processed broccoli. [34] Ansorena et al. [31] also studied the effect of chitosan and carboxymethyl-cellulose coatings on several quality parameters (weight loss, texture, color, microbial load, ascorbic acid, total chlorophyll, browning potential and sensory quality) of fresh-cut broccoli during refrigerated storage, and explored if there was any additional benefit in a combined treatment of mild heat shock followed by edible coating. They concluded that chitosan coating effectively maintained quality attributes and extended shelf life of minimally processed broccoli, but chitosan coating after a mild heat shock showed the best performance for long-term refrigerated storage of minimally processed broccoli. Thus, chitosan and alginate coatings, alone or enriched with biopreservatives or combined with other technologies can be a good post-harvest technology to increase the shelf life and improve the quality of edible flowers. However, in the future more studies must be done on edible coatings to better understand the effect of other materials and to evaluate their role on other edible flowers species.

Concerning edible films, only a study in cauliflower has been published, with the aim to evaluate the effects of methyl cellulose-based edible films with variable amounts of stearic, ascorbic and citric acids, on oxygen (O_2) permeability. [35] The authors observed that films containing ascorbic acid or citric acid applied to cauliflower slowed down browning reactions, as well as the polyphenoloxidase activity and vitamin C losses when compared to uncoated ones and with films without antioxidants. [35] Also here, studies involving other types of edible flowers are required.

High Hydrostatic Pressure

High hydrostatic pressure (HHP) is an emerging nonthermal food processing method that subjects liquid or solid foods, with or without packaging, to pressures between 50 and 1000 MPa. [36] HHP has shown considerable potential as an alternative technology to heat treatments, in terms of assuring safety and quality attributes in minimally processed food products. Some studies of HHP treatments in edible flowers have been done, evaluating the effect on physical (e.g., color and texture) and nutritional characteristics, as well as on microbial and enzymatic inactivation. Among edible flowers, only two studies have been done (Table 2.3..1). On the other hand, much more work on the HHP application to broccoli and cauliflower have been performed (Table 2.3.2).

Regarding edible flowers (Table 2.3.1), these have different cellular structures, which cause a different behavior when submitted to HHP. For example, borage and camellia showed an unacceptable appearance (loss of structure and firmness) after HHP application, while centaurea presented good appearance at 100/5 MPa/min; however, the shelf life did not increase. [37] On contrary, pansies submitted at 75/5 or 75/10 MPa/min maintained good appearance over 20 days of storage at 4 °C, as well as, HHP induced the production of bioactive compounds. This phenomenon might be associated with structural alteration of the cells provoked by the HHPs, yielding a higher amount of extracted metabolites or a physiological response of the flower to stress conditions at higher pressurization levels. [37] The effect of HHP on the retention and bioactivity of natural phytochemicals present in *Echinacea purpurea*, as well as the microbial load, were studied by Chen et al. [38] They reported that HHP significantly reduced the microbial contamination in flowers without affecting the

retention of phytochemical such as chicoric, caftaric and chlorogenic acids, and total alkamides.

On broccoli (Table 2.3..2), the effect of HHP in enzymatic inactivation has been studied by some authors. [39-42] A pressure of 210 MPa at -20 °C was insufficient to inactivate peroxidase and polyphenoloxidase [39], whereas pectinmethylesterase, β-galactosidase and α-rabinofuranosidase were inactivated by HHP. [42] Furthermore, the application of HHP may induce cell permeabilization, favoring glucosinolate conversion and hydrolysis of health promoting products. [40] Concerning the effect of HHP treatment on physical attributes, Fernández et al. [43] concluded that blanched and high-pressure treated broccoli followed by freezing presented better texture than conventional frozen ones, without great changes in color and flavor. Furthermore, Butz et al. [44] reported that no detectable effects on green color (chlorophyll *a* and *b*) were observed after long treatments at 600 MPa and 75 °C. Regarding the effect of HHP on antioxidant activity, pressures at 400 and 600 MPa did not affect the antioxidant properties of broccoli when compared to raw. [45]

On cauliflower, the effect of HHP on folate bioavailability (monoglutamate form) was examined by Melse-Boonstra et al. [46] They detected a 2-3 fold increase of monoglutamate folate form in cauliflower, but also a substantial loss of total folates. In addition, blanching before or after HHP led to great losses on monoglutamate folate content, perhaps due to direct solubilization in the water. Préstamo and Arroyo [47] reported lower structural changes in cauliflower treated at 400 MPa for 30 min at 5 °C than in spinach, since in the latter, more cell membrane damage occurred, with greater loss of nutrients. The resistance of microorganisms subjected to HHP was studied in cauliflower by Arroyo et al. [48] A pressure of 300 MPa, 5 °C for 30 min was sufficient to decrease the viable aerobic mesophiles below the detection limit.

In summary, HHP can be a promising technology on edible flowers and inflorescences, to maintain their quality for long periods of time. However, each flower shows a different behavior to pressure, making it necessary to perform further studies in order to better understand the effects of HHP on each type of flower.

Irradiation

Irradiation applied to plant cells has been a topic of extensive research. [49,50] This technology is a physical process used to inhibit or destroy undesirable microorganisms without involving antimicrobial additives or products of microbial metabolism as

preservative factors. However, irradiation processes can be based on different mechanisms, namely, nonionizing radiation (ex: less energetic UV radiation) and ionizing radiation (ex. gamma rays, electron beams and X-rays).

Ultraviolet radiation

Recently, sub-lethal doses of ultraviolet (UV) have been assayed as a possible postharvest technology. This technology, based on the concept of hormesis, establishes that it is possible to obtain a beneficial effect on the application of a low or sublethal dose of an agent capable of inducing physical or chemical stress. [51] UV light can be divided into three types: UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm). [52] With the exception of broccoli inflorescence (Table 2.3.2), no studies have examined the effect of the postharvest application of UV radiation on edible flowers. Broccoli irradiated with UV-B (peak emission at 312 nm) showed an effective inhibition of chlorophyll degradation during storage, suggesting that the effect could be due to the suppression of chlorophyll-degrading enzyme activities. [53] Similar results have been obtained in broccoli irradiated with UV-C (peak emission at 254 nm) [51], with a lower activity of chlorophyll peroxidase and chlorophyllase when compared to control. Thus, UV-C treatments could be a useful nonchemical method to delay chlorophyll degradation, reduce tissue damage and disruption, as well as, to maintain antioxidant capacity. [51] In a similar way, treated broccoli florets with UV-C showed higher phenolic and ascorbic acid contents, antioxidant activity and soluble sugars, as well as, a reduced number of bacterial and mold populations than control (samples not subjected to UV-C treatment, loosely covered with the same PVC film and stored under the same conditions as the irradiated ones). [54] To explain these results, the authors suggest that the activity of phenylalanine ammonia lyase might have been enhanced, a key regulatory enzyme of the phenylpropanoid metabolism, explaining the higher values of phenols. Furthermore, the UV-C treatment induced a lower rate of decrement in ascorbic acid than the control. Thus, UV treatments can be applied as a postharvest technology to broccoli, since they reduce tissue damage and microbial load, while maintaining the nutritional quality. Nevertheless, it is necessary to test the effect of these UV treatments and to adjust the doses for other edible flowers, because each flower will have a specific behavior when facing UV radiation.

Ionizing radiation

Ionizing energy can be used as a postharvest treatment to delay ripening or senescence of plant foods, although a severe legal control must be followed. According to the Codex General Standard for Irradiated Foods [55], ionizing radiation for food processing is limited to high-energy photons (gamma rays from the radionuclides ^{60}Co or ^{137}Cs; X-rays generated from machine sources operated at or below an energy level of 5 MeV; electrons generated from machine sources operated at or below an energy level of 10 MeV). It is a process recognized as a safe technology by several authorities such as World Health Organization (WHO), International Atomic Energy Agency (IAEA) and Food Agriculture Organization (FAO). [56] Irradiation is able to extend the shelf life of perishable products, to improve hygienic quality, to perform disinfestation of insects and to guarantee food safety. [56] Presently, spices, herbs and dry vegetable seasonings are irradiated in various countries as a way of preservation. [56]

Until now, few studies have focused on the effect of irradiation on the quality and composition of edible flowers. Koike et al. [57] (Table 2.3.1) evaluated the physical tolerance to gamma-rays of some edible flowers and reported that *Borago officinalis* is not tolerant to a dose of 0.3 kGy (dose necessary to eliminate insects). In contrast, *Dianthus chinensis*, *Viola tricolor*, *Viola odorata* and *Lobularia maritima* showed tolerance to gamma rays doses up to 1 kGy. So, flower tolerance to ionizing irradiation varies from species to species. Furthermore, high doses of gamma irradiation caused petal withering, browning and injury in edible flowers. [57] Concerning the effects of ionizing irradiation on the antioxidant activity and phenolic composition of edible flowers, only two studies have been published. One of them tested nasturtium flowers, and the authors concluded that antioxidant activity did not decrease significantly by irradiation; on the contrary, some irradiated samples (1 kGy) showed higher antioxidant activity than the corresponding control (0 kGy). [58] The other study done in *V. tricolor* showed that, in general, gamma-irradiated samples gave higher amounts of phenolic compounds independently of the applied dose, and the antioxidant activity was also higher in the irradiated samples when compared to the control (0 kGy). [59] No explanation was given to this behavior; however we can suppose that the secondary metabolism was enhanced by irradiation. Furthermore, these results only report to *V. tricolor*, make it necessary to perform other studies for other flowers' species.

In conclusion, ionizing irradiation may be applied in doses that do not cause changes in the visual appearance, antioxidant activity and bioactive compounds of edible flowers. However, further studies on the effect of irradiation in disinfestation and reduction of microbial loadings of edible flowers must be performed.

Packaging alternatives

Plants use the carbon dioxide (CO_2) from the environment to produce sugars and oxygen (O_2), which can later be utilized as a source of energy for plant growth. The high perishability of edible flowers is generally proportional to their respiration rates. [4,60] According to Jones [61], flowering plants have a relative high rate of respiration. After harvest, some factors contribute to induce changes in respiration rates of edible flowers, such as temperature [62], time of harvest [63] and packaging.

The principal roles of packaging, in the case of edible flowers, are to protect them from desiccation and to preserve their frail structure, as well as to isolate them from the external environment and to reduce their exposure to pathogens and contaminants. Currently, fresh ornamental edible flowers (ex: pansies, borage, centaurea) are often packed in clam-shell containers and must be used within two to five days after harvest. In this case, there is no atmosphere control at the beginning; however, in other situations the atmosphere that surrounds the product may be changed such as in controlled atmosphere and modified atmosphere packaging. Fresh edible flowers of vegetables (ex: broccoli, artichokes) must be used within 10 and 14 days for broccoli or 2 to 3 weeks for artichokes at 0°C and 95-100% relative humidity after harvest [64], but when they are packaged the shelf life may be prolonged as reported in the next section.

Without atmosphere control

Until now, there are no studies done on edible flowers focused on the role of different plastic films on flowers properties, without changing atmosphere at the beginning. So far, the works carried out have focused on artichoke, broccoli and cauliflower (Table 2.3.3).

Table 2.3.3 – Packaging strategies for artichoke, broccoli and cauliflower.

Type	Edible flowers	Packaging	Conditions	References
Packaging strategies	Artichoke (*Cynara scolymus*)	Perforated polypropylene (Control) Low density polyethylene (LDPE) Polyvinylchloride (PVC) Three microperforated polypropylene films (PP)	LDPE (PO_2*: 2.1 PCO_2*: 4.6 20 µm) PVC (PO_2: 3.7 PCO_2: 8.4 12 µm) PP1 (PO_2: 1.8 PCO_2: 2.8 35 µm) PP2 (PO_2 : 2.3 PCO_2: 3.5 35 µm) PP3 (PO_2: 3.9 PCO_2: 5.1 35 µm) **Storage conditions:** 5 °C for 8 days	[65]
		Polypropylene films: Non-perforated Microperforated Macroperforated	Ordinary atmosphere **Storage conditions:** 4 °C; 90–95% relative humidity (RH), for 3, 6, 9, 13 and 16 days	[66]
		Perforated plastic (PP)	**Storage conditions:** (I) unwashed raw materials, stored at room temperature (II) unwashed raw materials, stored in a cooling chamber without ozone insufflations (III) immersion in ozonized tap water, stored in a cooling chamber without ozone insufflation (IV) immersion in ozonized tap water, stored in a cooling chamber with ozone insufflation for 3 days and without ozone insufflation for the last 4 days (V) immersion in ozonized tap water, stored in in cooling chamber with ozone insufflation for 7 days.	[67,68]

	PET trays (23×17.5×2 cm) and packaged into a semi-permeable polyolefin film	Thickness: 19 mm; O_2 permeability: 3700 cm^3/m^2/24 h; Carbon dioxide permeability: 11100 cm^3/m^2/24 h. **Storage conditions:** 4 °C, 90-95% RH, 0 (production day), 4, 7 and 11 days.	[69, 70]
Broccoli (*Brassica oleracea* var. *italica*)	Polypropylene films: Macro-perforated (Ma-P) Microperforated (Mi-P) Non-perforated (No-P)	Ma-P (PO_2: 0.082 PCO_2: 0.18 20 µm) Mi-P (PO_2: 0.127 PCO_2: 1.27 20 µm) No-P (PO_2: 0.082 PCO_2: 0.18 25 µm) **Storage conditions:** 1 °C, 28 days	[66]
	Polyethylene bags with no holes (M0), two microholes (M1) and four macroholes (M2)	**Storage conditions:** 4 or 20°C	[75]
	Perforated PP	**Storage conditions**: 5°C, 75% relative humidity (RH), cold room for 4 days	[71]
	PP film with 10 pin holes Plastic perforated trays	**Storage conditions:** ambient (15 ± 1 °C, 55 ± 2% RH), and refrigerated (4 ± 0.5 °C, 50 ± 2% RH) storage for 144 h	[72]
	PP LDPE PVC	PP PO_2:0.066 PCO_2: 0.25 35 µm LDPE PO_2:0.56 PCO_2: >2.3 23µm (with an ethylene absorbing sachet) PVC PO_2: 2.9 PCO_2: >8.8 10 µm **Storage conditions:** (I) 7 days at 10 °C (II) 3 days at 4 °C, followed by 4 days at 10 °C.	[73]
Cauliflower (*Brassica oleracea* var. *botrytis*)	PVC LDPE Special LDPE adapted for microwave oven use	PVC 14 µm LDPE 11, 15 and 20 µm Special LDPE 11 µm **Storage conditions:** 7 days, 1.5 °C, 95% RH	[74]

			Shelf life simulation: additional 2.5 days at 20 °C	
		Perforated PVC Non-perforated PVC Microperforated oriented PP	(A) Perforated PVC 12 µm (B) Non-perforated PVC PO_2 1.3 12 µm (C) Microperforated oriented PP PO_2 2.3 36 µm **Storage conditions:** 4 or 8 °C for 20 days	[75]
Controlled atmosphere (CA)	Broccoli (*Brassica oleracea* var. *italica*)	----	**CA conditions:** 2% O_2 + 6% CO_2. Control = air **Storage conditions**: 4 °C for 2, 4, 5 and 6 weeks	[81]
		----	**CA conditions:** 10% O_2 + 5% CO_2 **Application of 1-methylcyclopropene (1-MCP)** **Control:** air, under storage conditions **Storage conditions:** 1-2 °C, 85–90% relative humidity for 2, 6, 13, 20 and 27 days.+20 °C, 2 and 4 days	[82]
	Cauliflower (*Brassica oleracea* var. *botrytis*)	---	**CA:** 3% O_2 + 5% CO_2 **Ambient air:** ~20.5% O_2, 0.03% CO_2 **Storage conditions:** 0 °C for 0, 14, 28, 42 and 56 days	[83]

* PO_2 - O_2 permeability; PCO_2 - CO_2 permeability, expressed in ($\times 10^{-10}$ mol·s^{-1}·m^{-2}·Pa^{-1}); ↑: Increased; ↓: Decreased

Artichoke's packing in different films such as perforated polypropylene (PP, control), polyvinylchloride (PVC), low density polyethylene (LDPE) and microperforated polypropylene (PP) films, caused different effects on flower quality, but all were beneficial because of the weight loss reduction in comparison with control. [65] However, artichoke packed in LDPE, PVC and PP showed lower vitamin C (addition of ascorbic acid and dehydroascorbic acid) contents compared to the control. This may be due to the higher CO_2 levels found in the packages with these films than control, which decreased the dehydroascorbic acid content. Concerning different types of packaging, those with low CO_2 values (PVC and LDPE films) increased the content of phenolic compounds when compared to packages with higher CO_2 levels (PP1, PP2 and PP3 films). These authors observed that the phenolic profile changed with storage, with a large increase of 1,5-dicaffeoylquinic acid (diCQA)+3,5-diCQA linked to the phenylpropanoid pathway. The authors concluded that LDPE was the best film to be used for maintaining artichokes' quality. Furthermore, microperforated and nonperforated films reduced microbial growth and enhance the total polyphenol content, especially for the heads treated with the antibrowning solution, namely 1.0% ascorbic acid and 0.2% citric acid, when compared to those not treated with the antibrowning solution and packaged in the microperforated film. [66] On the other hand, storage under ozonized atmosphere for 3-4 days improved microbiological quality, maintained nutritional quality and enhanced artichoke's polyphenol content. [67,68] Even though, ozone treatments induce an increase of respiration rate (producing a significantly higher O_2 decline), an undesired effect, in some flowers' species it can be successfully applied without significant metabolic consequences. So, the exposure to ozone should be analyzed for each flower because they have different sensitivity.

Similar studies have been done in broccoli and cauliflower, but with different film materials. Regarding broccoli, those packaged in microperforated and non-perforated films had prolonged storability up to 28 days with high quality attributes and health-promoting compounds (total antioxidant activity, ascorbic acid and total phenolic compounds), while unwrapped broccoli (control) could only be stored for 5 days. [69] Similar results have been reported by Jia et al. [70], who showed that the shelf life of broccoli was extended, and its postharvest deterioration was reduced when stored at 4 and 20 °C, packaged in polyethylene bags (40 μm thick, 20 cm ×30 cm) without holes (M1) and with two microholes (750 μm in diameter, one on each side of the bag) (M2), when compared to control (open boxes). Concretely, the polyethylene bags

extended the shelf life of broccoli florets from 10 days (control) to 28.5 days (M1) and 19.1 days (M2) at 4°C, and from 2.5 days (control) to 7.2 days (M1) and 5.6 days (M2), at 20°C.

Furthermore, the contents of chlorophyll and ascorbic acid were maintained when using PP film packages (two holes, each of 0.3 µm diameter) during 4 days of storage. [68] Nath et al. [72] verified that broccoli packaged in PP film bags, with 10 pin holes, stored at 4 °C, were able to retain the maximum of phytochemicals during storage for up to 144 h. These results suggest that perforation of the packaging is a solution to control the atmosphere inside it, as the holes are a way of steering a continued transport of O_2 into the packaging. At the same time CO_2 can get out of the packaging, decreasing the rate of atmosphere modification. However, the effect of perforation in quality and shelf life of flowers depend on whether the size of the holes are adapted to the flower's specie, the type of packaging film and storage temperature. Another study with the same flower, reported that broccoli packaged in LDPE that contained an ethylene absorber (5% O_2, 7% CO_2) stored for 7 days at 10 °C (condition I) and 3 days at 4 °C followed by 4 days at 10°C (condition II) were the samples with the appearance most similar to fresh broccoli. [73] In cauliflower, Artés and Martínez [74] concluded that the weight loss was considerably lower for all LDPE films (11, 15 and 20 µm) than for PVC film, and among the LDPE films the best results were obtained by using 11 µm LDPE. These results were probably due to the lower water vapor transmission rate of the various LDPE films than that in the PVC film assayed. [74] Furthermore, among three types of films, namely, perforated PVC, nonperforated PVC and microperforated oriented PP for packaging minimally processed cauliflower, Simón et al. [75] verified that the three films allowed an acceptable cauliflower appearance without off-odor over 20 days of storage at 4 or 8 °C. In particular, the atmosphere generated within nonperforated PVC film reduced the microbial counts but increased cauliflower yellowing, compared with the perforated PVC film.

According to Friedman et al. [78] (Table 2.3.4), different flowers show a different behavior during the same storage conditions, with the packaged flowers in PET boxes better preserved than wrapping the trays with PVC. In general, packaging with different films improved the physical protection and also reduced the spread of pathogenic organisms, but some materials are more suitable for certain edible flowers. It is necessary to study each in particular. Edible carnations and snapdragons placed in PP trays showed O_2 decline and accumulation of CO_2 and ethylene in both flowers.

Flowers stored in these conditions presented a significantly reduced weight loss, aided in maintaining their visual quality and fresh appearance, reduced wilting, and extended the shelf life and prevented abscission in snapdragon. [3]

In conclusion, until now different plastic films were tested in edible flowers and they had different effects on their shelf life and microbial and nutritional quality. The use of some films can be a way to extend the shelf life and maintain the quality attributes of edible flowers; however, it is always necessary to perform experiments to evaluate the most appropriate for each situation.

With atmosphere control

Controlled (CA) and modified atmosphere packaging (MAP) are technologies used by the industry for extending the shelf life of foods, especially fruits and vegetables]. [79] These technologies are able to reduce the respiration rate of the product, reduce microbial growth and retard enzymatic spoilage by changing the surrounding gaseous environment of the food. [72] In Tables 2.3.3 and 2.3.4 are described the studies performed until now on CA in broccoli and cauliflower, as well as on MAP in edible flowers, respectively.

A controlled atmosphere storage is a commercial system in which the gas concentrations of O2, CO_2 and nitrogen, as well as the temperature and humidity of a storage room are regulated. [80] Until now, few studies have been performed on CA storage of edible flowers, focusing mainly on broccoli and cauliflower. Storage of broccoli florets for 4 weeks or more under a CA 2% O_2 + 6% CO_2 extended the shelf life and improved retention of green color and chlorophyll, when compared with florets stored in air, all at 4 °C, because low O_2 and high CO_2 levels reduce the respiration rate. [81] Broccoli stored under a CA of 10% O_2 and 5% CO_2 maintained the visual quality and reduced the loss of health promoting compounds such as, phenolics, carotenoids and vitamin C, when compared with air storage, all at 2 °C. [82] Cauliflower stored in CA (3% O_2 + 5% CO_2) also resulted in a lower weight loss, a slower decline in the lightness values, and no significant differences on the hue angle values, when compared with cauliflower stored in air. [83]

MAP is a technique of sealing actively respiring food product in polymeric film packages with modified O_2 and CO_2 levels within the package. [84] In edible flowers, only one study using this methodology has been done, namely on nasturtium packaged

in CO_2 (3-5%) and O_2 (10-13%), with good results due to flowers' quality improvement evaluated by visual appearance. [78]

In the last decades, 1-methylcyclopropene (1-MCP) has been added to the list of options for extending the shelf life and quality of plant products [82], because it is a nontoxic antagonist of ethylene, which binds and blocks ethylene receptors, protecting flower from ethylene effects such as, petal senescence and/or petal abscission. 1-MCP combined with MAP, or even CA, has been used in edible flowers such as edible carnations, snapdragons and broccoli. 1-MCP helped to maintain the fresh appearance, reduce wilting and extend the shelf life of carnations and snapdragons, as well as to prevent abscission in the last flower. [3] However, in broccoli, the 1-MCP samples showed a higher decrease in chlorophyll pigments than CA (10% O_2 + 5 % CO_2) at the end of storage, being the latest technology suitable to extend broccoli quality during storage and shelf life. [82] Concretely, CA increased the total phenolic content during cold storage until 13 days, which may be due to the stress caused by the controlled atmosphere storage. It also reduced the loss of chlorophylls; the carotenoids remained constant until the end of storage; and the antioxidant activity showed a smaller decrease in comparison to other samples (control and 1-MCP).

In summary, storage conditions and packaging methods have significant effect on biochemical characteristics of edible flowers. However, each flower has a different behavior, so it is necessary to perform more studies for each flower species at different storage conditions.

Practical uses of edible flowers after the application of post-harvest technologies

Most of the postharvest technologies applied to edible flowers had the main objectives of increasing their shelf life and maintaining their physic-chemical properties, as well as, making the edible flowers market more competitive. Postharvest technologies, such as low temperatures, edible coatings and packaging, might result in products with similar characteristic to fresh flowers. This would allow flowers to be sold as "ready to eat" products, to include in salads, soups and desserts. Dried edible flowers are already a product sold in some stores, such as: Petite Ingredient (Australia), Maddocks Farm Organics (United Kingdom) and Ervas Finas (Portugal). Dried flowers are also sold as ingredients to make teas, embellish drinks, cocktails and to be included in bakery products.

Table 2.3.4 –Modified atmosphere packaging for edible flowers.

Type	Edible flowers	Packaging	Conditions	References
Modified athmosphere (MAP)	Nasturtium (*Tropaeolum majus*) Begonia (*Begonia semperflorens* and *Begonia elatior)* Rose (*Rose* spp.)	PVC PP with or without modified atmosphere Transparent polyethylene terephthalate (PET)	3-5% CO_2+10-13% O_2 **Storage conditions:** 2 ℃ and 4-5 ℃	[78]
	Carnation (*Dianthus caryophyllus*) Snapdragon (*Antirrhinum majus)*	PP 1-Methylcyclopropene + PP (1-MCP+PP) Current commercial packaging (COM) (plastic clamshell containers)	**Storage conditions:** 5 ℃ for 14 days	[3]

↑: Increased; ↓: Decreased

The irradiation of edible flowers allows the food industry to ensure that the product is microbiologically safe, because irradiation is a technology that controls microbes and other organisms that cause foodborne diseases. Irradiated edible flowers can be used, as fresh and ingredients in prepared foods, as already happens with the herbs and spices**.** Regarding HHP, there are already some food companies that use HHP in some edible flowers like broccoli and cauliflower in the formulation of smoothies and ready-to-eat meals. For example, the Juicy Line-Fruity Line® in Holland manufactures juices and smoothies of broccoli-apple-lemon and broccoli-orange-lemon.

Conclusion and future trends

In conclusion, various post-harvest technologies are available and may be used on edible flowers in order to extend their shelf life, while maintaining their quality. In particular, emerging post-harvest technologies as HHP or irradiation show promising results for increasing the shelf life of minimally processed edible flowers. Freeze drying or vacuum drying have shown to be highly effective in the preservation of flowers' bioactive compounds, in comparison with classical drying approaches, but they require great economical inputs. While osmotic dehydration is already in use, the deposition or application of edible films and coatings can constitute a healthier alternative, without increasing the nutritional impact with sugars or salt, while also better preserving the characteristics of the fresh products.

The edible flower industry must be aware of the post-harvest technologies available and should be prepared to adopt those that are more appropriate to their products. This review assembled the most important technologies tested and applied, showing that most studies are still restricted to broccoli and cauliflower, with continued research and development needed worldwide to find better ways of increasing the stability and shelf life of other edible flowers, as these behave in different manners.

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CHAPTER 3

3.1.

Physico-chemical and biological characterization of edible flowers

This section focuses on the nutritional (moisture, protein, fat, carbohydrates and ash contents, and energy values) and nutraceutical compositions (fatty acids, tocopherols, sugars and carotenoids) of four edible flowers (camellia, pansies, borage and centaurea). The influence of flowering stages in the nutritional and nutraceutical compositions of pansies, centaurea and borage flowers is also discussed. Furthermore, this section also discussed possible associations between volatile compounds, sensory perception and bioactive compounds of five edible flowers (borage, calendula, cosmos, johnny jump up and pansies).

3.1.1.

Borage, Camellia, Centaurea and Pansies: Nutritional, fatty acids, free sugars, vitamin E, carotenoids and organic acids characterization.

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Abstract

Edible flowers represent a new niche market in the food industry, but detailed studies on their chemical composition to support choices based on potential bioactivity rather than color and taste are still reduced. So, the aim of the present study was to evaluate some nutritional properties of four edible flowers, namely: borage, centaurea, camellia and pansies (white, yellow and red). In general terms, significant differences between them were observed. As expected, water was the main constituent (>76%, fresh weight), followed by carbohydrates and total dietary fiber. Linoleic and palmitic acids were the major fatty acids found in borage and in red and yellow pansies, while in camellia it was the arachidic acid, and in white pansies were behenic and arachidic acids, but always in very reduced amounts. Concerning vitamin E, α-tocopherol was the major component (0.67-2.21 mg/100 g dw). Carotenoids contents varied between 5.8 and 181.4 mg β-carotene/100g dw in centaurea and borage, respectively, being particularly rich in lutein. Malic acid was the major organic acid (1.84-4.92 g/100g dw), except in centaurea, where succinic acid was the major. Fructose, glucose and sucrose were detected in all flowers. So, these results can contribute to increase the

knowledge in these edible flowers and consequently increase their popularity among consumers and food industry.

Keywords: Edible flowers, Fatty acids, Free sugars, Vitamin E, Carotenoids, Organic acids.

Introduction

The consumption of flowers has been known since old civilizations around the world, but it is still regarded as an uncommon product in modern cuisines, being used either for adding new colors, textures and flavors in cuisine or for their potential health benefits. Globally, the nutritional composition of edible flowers is not different from that of other plant organs (Mlcek and Rop, 2011), but recent works on edible flowers have focused in the analysis of bioactive compounds, such as carotenoids, flavonoids and anthocyanins, as well as in antioxidant activity (Loizzo et al., 2016; Lu et al., 2016; Navarro-González et al., 2015; Petrova et al. 2016; Rachkeeree et al., 2018), because of the impact of these phytochemicals' on human health and in their preventive actions against diseases. Until now, some works in the nutritional and mineral compositions of centaurea and pansies had already been done by Rop et al. (2012) and Vieira (2013), as well as on the antioxidant properties of borage (Aliakbarlu and Tajik, 2012) and pansies (Skowyra et al., 2014; Gamsjaeger et al., 2011; González-Barrio et al., 2018). However, further studies are needed to cover the full range of edible flowers, as well as on the diverse bioactive components that could enhance their consumption. So, the aim of this study was to determine the macronutrients composition, energetic value, fatty acids, free sugars, vitamin E, carotenoids and organic acids profiles of four species of edible flowers, namely: borage (*Borago officinalis*), camellia (*Camellia japonica*), centaurea (*Centaurea cyanus*) and pansies (*Viola×wittrockiana)* (white, yellow and red). Thus, this study intends to provide more information about these four edible flowers, in order to contribute to their popularization and consequently support advices for their increased use in human nutrition.

Material and Methods

Standards and reagents

All reagents were of analytical, chromatographic or spectroscopic grade. HPLC grade *n*-hexane was purchased from Merck (Darmstadt, Germany) and 1,4-dioxane from Sigma (Madrid, Spain). Methanol and KOH were acquired from Panreac (Barcelona, Spain). Boron trifluoride in methanol (14%), butylated hydroxytoluene (BHT) and ascorbic acid were obtained from Sigma. The other reagents were supplied by Merck (Darmstadt, Germany) or Sigma–Aldrich (St. Louis, USA). Concerning standards, tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) were purchased from Calbiochem (La Jolla, USA) and Sigma–Aldrich (St. Louis, USA). The internal standard for vitamin E quantification was tocol (2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol), obtained from Matreya LLC (State College, USA). A 1 μg/mL solution was prepared in *n*-hexane and kept at −20 °C. Triundecanoin was used as the internal standard for fat estimation, based on the total fatty acid amounts, and was purchased from Sigma. A 10 mg/mL solution was prepared in *n*-hexane. A certified fatty acids methyl ester (FAME) reference standard mixture (37 fatty acids from C4 to C24) from Supelco (Bellefonte, USA) was used for the calibration of the FID signals. Concerning carotenoids, organic acids and sugars standards, all of them were obtained from Sigma–Aldrich (St. Louis, USA).

Samples

Blue borage (*Borago officinalis*), camellia (*Camellia japonica*), blue centaurea (*Centaurea cyanus*) and pansies (*Viola×wittrockiana*) (white, yellow and red pansies), at full ripening state (Figure 3.1.1.1), were collected at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança, Portugal. After harvest, the fresh flowers were immediately transported to the laboratory under refrigeration.

Nutritional composition

The nutritional composition (moisture, ash, fat, carbohydrates and dietary fiber) of each flower were determined by following the AOAC procedures (1995) and expressed in g/100g fresh weight (fw). Moisture content was determined by drying the sample to a constant weight at 105 ^{o}C; ash content was measured by calcination at 550 ^{o}C for a minimum of 2 hours, until achieving white ashes. The protein content of the

samples was estimated by the macro-Kjeldahl method, with a conversion factor of 6.25, following Sotelo et al. (2007) and Rop et al. (2012). Total lipids were determined by extracting a known mass of powdered sample with petroleum ether with butylated hydroxytoluene (BHT), using a Soxhlet apparatus. Dietary fiber was determined by an enzymatic-gravimetric method based on the AOAC official method No. 985.29 (AOAC, 2003). Carbohydrates were calculated by difference. Energy value was calculated according to Equation 1:

$$Energy\left(\frac{kcal}{100\ g\ fw}\right) = 4 \times [(protein) + (carbohydrates)] + 9 \times [lipids] + 2 \times [dietary\ fiber](\frac{g}{100\ g\ fw})$$

(Eq. 1)

Extraction of lipid components for further analysis

A 250 mg freeze dried sample was weighted and two internal standards solutions were added: tocol (20 µL; 1 mg/mL) for vitamin E quantification and triundecanoin (200 µL; 1 mg/mL) for total fatty acids quantification, followed by two antioxidants - BHT (20 µL, 10 mg/mL in methanol), and ascorbic acid (50 mg). Propan-2-ol (1.6 mL) and cyclohexane (2.0 mL) were added for lipid extraction. The lipid extraction conditions applied were those reported by Cruz et al. (2013).

Fatty acids

The fatty acid profiles were determined with a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, Netherlands) equipped with a split–splitless injector, a Chrompack CP-9050 autosampler and a flame ionization detector (FID). Helium was used as carrier gas at an internal pressure of 180 kPa. The temperatures of the detector and injector were 250 and 270 °C, respectively. Separation was achieved on a 100-m × 0.25-mm i.d. Select-FAME column (0.19-µm film; Agilent, Santa Clara, USA). The oven temperature was programmed at 180 °C for a 35 min hold, and then programmed to increase to 250 °C at a rate of 3 °C/min. The total analysis time was 60 min. The slip ratio was 1:50 and the injected volume was 1.2 µL. Fatty acids identification (from C11:0 to C22:6) was accomplished by comparing the relative retention times of FAME peaks with standards from diversified suppliers, from literature data, and confirmed by GC-MS on a Agilent chromatograph 7890A with a 5977B MSD (MS source 230ºC; MS QUAD 150; aux 280ºC; m/z 30-800) using the NIST/EPA/NIH Mass Spectral Library (NIST 14). For quantification purposes the FID peaks were

corrected using response factors obtained with standard FAME solutions. The fatty acids results were calculated on a relative percentage basis.

Free sugars

Free sugars were determined by following the procedure mentioned by Barros et al. (2010), with slight modifications. One gram of dried sample powder was extracted with 40 mL of 80% aqueous ethanol (v/v) at 80 ºC for 30 min. The resulting suspension was filtered with pump vacuum (KNF LABOPORT, Darmstadt, Germany). The supernatant was concentrated at 45 ºC with rotary evaporator (Stuart, RE300DB, Stone, UK) and defatted three times with 10 ml of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 3 mL and filtered through 0.2 μm nylon filters from Whatman.

Sugars were analyzed in a Jasco integrated high performance liquid chromatographic system (Tokyo, Japan), equipped with a PU-980 intelligent pump and a refractive index detector (Gilson, USA). Separation was achieved with a SUPELCOGEL Ca column (30 cm × 7.8 mm I.D., Supelco, USA), operating at 80 °C with ultra-pure water at a flow rate of 0.5 mL/min as eluent. Sugar identification was made by comparing the retention times of sample peaks with standards. Quantification was performed by the external standard method with individual calibration curves for each sugar at concentrations ranging from 0.5 to 10 mg/mL. The results were expressed on g/100 g (dry matter).

Vitamin E and carotenoids

Vitamin E and carotenoids composition was determined according to Cruz and Casal (2018). The separation was achieved by normal-phase HPLC. The liquid chromatograph consisted of a Jasco integrated system (Easton, USA) equipped with an autosampler (AS-2057 Plus), a PU-980 intelligent pump, and a multiwavelenght DAD (MD-910, recorded at 450 nm), connected to a fluorescence detector (FD) (FP-2020 Plus; $\lambda_{excitation}$ = 290 nm and $\lambda_{emission}$ = 330 nm). The chromatographic separation was achieved on a Luna Silica (100 mm × 3 mm; 3 μm) (Phenomenex, USA), operating at constant room temperature (23 °C), with a gradient of *n*-hexane and 1,4-dioxane at a flow rate of 1.0 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Tocopherols and tocotrienols (α-, β-, γ-, and δ-) were acquired from Supelco (USA) and Larodan AB (Sweden). Carotenoids standards

(all-*trans*-β-carotene and lutein) were from Sigma Aldrich (USA). Identified tocopherols and carotenoids were quantified by the internal standard method, being reported on a dry mass basis.

Simultaneously, the total carotenoid contents were determined according to the method used by Aquino-Bolaños et al. (2013). One gram of frozen-dried powder of each sample was extracted twice with 20 mL acetone:hexane solution (1:1, v/v). Both extracts were put into a separation funnel, being added 200 mL of distilled water to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to eliminate any residual water, being the remaining solution filtered and completed to 100 mL with hexane. Carotenoids content was determined by reading the absorbance at 450 nm and comparing the results with a β-carotene calibration curve (0.22–8.8 μg/mL). Results were expressed in mg β-carotene/100 g dry matter.

Organic acids

The organic acids in edible flowers were estimated by gas chromatography, after methylation, following the methods reported by Sharma et al. (2016) and Kumar et al. (2017), using a column HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm thickness ultra-inert capillary column, Agilent Technologies) instead the DB-5ms column mentioned by the authors. Individual standards of citric, levulinic, fumaric, succinic, malic, salicylic, hydroxycinnamic, malonic, oxalic, tartaric and benzoic acids, all from Sigma Aldrich (Germany), were derivatized under sample conditions. Quantification was based on individual calibration curves, using specific m/z for each compound, as detailed in Kumar et al. (2017).

Statistical analysis

The statistical analysis was performed on SPSS software, Version No. 18.0 (SPSS Inc., Chicago, USA). The normality of the data was verified by Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch were carried out to determine if there were significant differences ($p<0.05$) between samples, depending on the existence or not of homogeneity of variances. Additionally, if significant differences were detected between treatments, a post hoc analysis was performed, namely: Tukey's honestly significant difference test (if variances in the different groups were identical) or Games-Howell test (if they were not). The homogeneity of the variances was tested by Levene's test.

Results and Discussion

Nutritional composition

The nutritional composition of the four edible flowers is shown in Table 3.1.1.1. In general, the four flowers differed significantly in their nutritional compositions and energetic values. Even though water was the main constituent (76.7-91.3 g/100 g fw), carbohydrates (3.15 – 8.04 g/100 g fw) and total dietary fiber (1.50-15.7 g/100 g fw) were the most abundant macronutrients in all flowers, followed by proteins (0.76-3.04 g/100 g fw). Fat was the less abundant macronutrient, ranging between 0.31 and 1.31 g/100 g fw for camellia and yellow pansies. All edible flowers showed low energetic values (31–61 kcal/100 g fw), with statistically differences between them ($p<0.05$).

Borage had the highest mineral (2.05 g/100 g fw) and protein contents (3.04 g/100 g fw), but the lowest carbohydrates amounts (3.15 g/100 g fw), explaining its low energy value (38.2 kcal/100 g fw). On contrary, camellia presented the lowest ash (0.37 g/100 g fw), protein (0.76 g/100 g fw) and lipids (0.31 g/100 g fw) contents, being the least caloric one. Centaurea also presented one of the lowest protein contents (1.60 g/100 g fw, respectively), as well as the lowest carbohydrates value (3.93 g/100 g fw); however, it had the highest content of total dietary fiber (15.7 g/100 g fw), approx. nine times more than the white pansies that had the lowest amount (1.50 g/100 g fw), also contributing for its higher caloric content. Between pansies of different colors, significant differences were detected. White pansies showed significantly higher values of moisture (91.3 g/100 g fw) than other flowers, while yellow pansies presented the highest lipid content (1.31 g/100g fw) and red pansies the highest carbohydrate content (8.04 g/100 g fw). White pansies presented the lowest dietary fiber content. Regarding energetic values, white pansies had the lowest value (31 kcal/100 g fw), increasing to 52 kcal/100 g fw in the red ones.

Figure 3.1.1.1 - Edible flowers studied in the present work: borage, camellia, centaurea and pansies (white, yellow, red).

Table 3.1.1.1 - Nutritional composition (g/100 g fw) of borage, camellia, centaurea and pansies.

Components	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola×wittrockiana*)		
				White	Yellow	Red
Moisture	86.6 ± 1.8^{b}	$87.7\pm1.3^{b,c}$	76.7 ± 0.6^{a}	91.3 ± 2.1^{c}	86.5 ± 0.7^{b}	85.1 ± 0.3^{b}
Ash	2.05 ± 0.11^{c}	0.37 ± 0.01^{a}	1.20 ± 0.11^{b}	0.92 ± 0.08^{b}	1.10 ± 0.19^{b}	0.94 ± 0.13^{b}
Protein	3.04 ± 0.10^{d}	0.76 ± 0.20^{a}	1.60 ± 0.06^{b}	2.03 ± 0.06^{c}	2.06 ± 0.03^{c}	1.36 ± 0.03^{b}
Lipid	$0.66\pm0.15^{a,b}$	0.31 ± 0.07^{a}	0.80 ± 0.03^{b}	$0.45\pm0.01^{a,b}$	1.31 ± 0.09^{c}	$0.67\pm0.03^{a,b}$
Carbohydrates	3.2 ± 1.7^{a}	$4.1\pm1.3^{a,b}$	3.9 ± 0.4^{a}	3.9 ± 1.9^{a}	$4.8\pm0.8^{a,b}$	8.0 ± 0.4^{b}
Total dietary fiber	$4.74\pm0.10^{b,c}$	6.71 ± 1.76^{c}	15.7 ± 0.5^{d}	1.50 ± 0.09^{a}	4.32 ± 0.02^{b}	3.79 ± 0.23^{b}
Energy*	$38\pm7^{a,b}$	$36\pm4^{a,b}$	61 ± 1^{c}	31 ± 8^{a}	$48\pm4^{b,c}$	$52\pm1^{b,c}$

Mean±Standard deviation. fw – fresh weight. Values with the same letter in the same row are not statistically different ($p>0.05$). *Results are expressed in kcal/100 g fw.

Fatty acids

The results obtained for the individual fatty acids of the studied edible flowers are shown in Table 3.1.1.2. Each flower showed a different profile of fatty acids. Twenty one fatty acids were identified and quantified, being borage the flower with the highest number of fatty acids detected. Linoleic acid (C18:2n6) and palmitic acid (C16:0) were the major fatty acids found in borage, and in red and yellow pansies, ranging from 3.3 to 27.2% in camellia and yellow pansies, respectively; and palmitic acid between 1.5 to 25.9% in white pansies and centaurea, respectively. The same trend in borage was reported by Ramandi et al. (2011), for whom the major fatty acids were palmitic (39.4%), linoleic (26.4%) and oleic (21.1%) acids, although they detected higher percentages than those of the present study. Centaurea and red pansies also showed high percentages of oleic acid (C18:1) (25.9 and 14.0%, respectively). The results found in the present work for centaurea flowers were not in accordance with Pires et al. (2018), who reported that the main fatty acids were eicosapentaenoic acid (C20:5n3; 26.9%) and linolenic acid (C18:3n3; 18.8%), while in the present study were palmitic acid (C16:0; 25.9%) and oleic acid (C18:1; 25.9%); however, the mentioned authors had only analyzed petals and not all flower. Camellia presented high percentages of arachidic acid (C20:0, 42.54%) as the main fatty acid, followed by palmitoleic acid (C16:1, 23.7%), while in white pansies was the behenic acid (C22:0, 24.6%), closely followed by the arachidic acid (C20:0, 24.3%). Pansies with different colors showed a different profile of fatty acids, being the yellow pansies those with the highest number of different fatty acids. Globally, polyunsaturated fatty acids (PUFA) were predominant in borage and yellow pansies, while the remaining flowers showed higher concentrations of saturated fatty acids (SFA). Monounsaturated fatty acids (MUFA) were detected in minor amounts in all flowers, ranging from 6 to 27%.

As regards PUFA/SFA ratios, all flowers were above 0.45, the minimum value recommended by the Health Department (HMSO, 1994). Furthermore, in the flowers where n-6 and n-3 fatty acids were detected, the ratio of both was calculated, being lower than 4.0, which is recommended for the human diet (Guil et al., 1996). High ratios of PUFA/SFA and low n-6/n-3 ratios are associated to health benefits, such as, decrease of the "bad cholesterol" in mice blood (Liu et al., 2016), control markers of metabolic disorders, including obesity, insulin resistance, inflammation, and lipid profiles (Liu et al. 2013) and reduce the risk of cancer, cardiovascular and inflammatory diseases.

Table 3.1.1.2 – Fatty acids composition (relative %) in borage, camellia, centaurea and

Fatty acids	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola ×wittrockiana*) White	Yellow	Red
SFA						
C12:0	0.46±0.07^{a}	0.14±0.12^{a}	0.62±0.17^{a}	0.56±0.06^{a}	7.65±0.33^{c}	6.24±0.89^{b}
C14:0	0.67±0.14^{a}	0.43±0.09^{a}	0.71±0.16^{a}	2.10±0.82^{a}	10.47±0.44^{b}	19.30±2.16^{c}
C15:0	0.25±0.04^{b}	nd	nd	0.05±0.08^{a}	0.13±0.01^{a}	0.22±0.08^{b}
C16:0	17.11±0.66^{b}	nd	25.88±4.39^{d}	1.51±0.13^{a}	20.39±0.68^{b,c}	23.12±1.38^{c,d}
C17:0	0.87±0.15^{c}	0.10±0.11^{a}	0.59±0.34^{b,c}	0.30±0.30^{a,b}	0.54±0.08^{b,c}	0.76±0.24^{c}
C18:0	4.80±0.36^{c}	0.86±0.24^{a}	7.16±1.74^{d}	0.86±0.15^{a}	2.97±0.16^{b}	4.35±0.32^{c}
C20:0	1.80±0.23^{a}	42.54±2.56^{c}	2.50±0.72^{a}	24.31±0.81^{b}	0.55±0.02^{a}	0.65±0.04^{a}
C22:0	2.49±0.38^{d}	0.11±0.18^{a}	1.45±0.49^{c}	24.57±0.81e	0.93±0.08^{b,c}	0.64±0.14^{a,b}
C24:0	2.58±0.34^{c}	0.38±0.30^{a}	1.55±0.64^{b}	0.23±0.28^{a}	1.24±0.39^{b}	1.71±0.54^{b}
Total SFA	31	45	40	54	45	57
MUFA						
C16:1	0.19±0.07^{a}	23.67±0.96^{c}	0.32±0.19^{a}	19.17±0.60^{b}	0.32±0.09^{a}	0.37±0.23^{a}
C18:1	9.90±8.77^{a}	nd	25.87±17.06^{b}	nd	5.17±4.11^{a}	14.02±7.01^{a,b}
C20:1	0.32±0.02^{c}	0.45±0.04^{d}	nd	0.46±0.05^{d}	0.06±0.03^{a,b}	0.12±0.11^{b}
C22:1	1.79±0.19^{c}	1.25±0.14^{b,c}	0.88±0.81^{a,b}	nd	0.27±0.21^{a}	0.38±0.32^{a}
C24:1n9	1.95±0.26^{b}	0.10±0.13^{a}	nd	0.09±0.0.22^{a}	nd	0.22±0.26^{a}
Total MUFA	14	25	27	20	6	15
PUFA						
C18:2n6	21.23±2.22^{c}	3.34±0.63^{a}	12.77±2.91^{b}	4.52±0.60^{a}	27.17±1.77^{d}	20.68±1.87^{c}
C18:3n6	9.64±1.37^{b}	7.41±2.88^{b}	nd	3.10±0.48^{a}	nd	nd
C18:3n3	12.89±1.48^{b}	nd	19.64±5.61^{c}	nd	21.15±1.43^{c}	5.92±0.87^{a}
C18:4n3	6.50±0.79^{b}	14.48±1.37^{c}	nd	16.78±0.78^{d}	0.87±0.11^{a}	1.27±0.22^{a}
C20:2n6	nd	0.11±0.18^{b}	nd	nd	0.08±0.01^{a}	nd
C22:3	nd	1.14±0.08^{b}	nd	0.62±0.05^{a}	nd	nd
C22:4n6	4.54±0.64^{c}	1.89±0.37^{b}	0.07±0.07^{a}	0.75±0.66^{a}	0.05±0.05^{a}	nd
Total PUFA	55	30	32	26	49	28
PUFA/SFA	1.77	0.68	0.82	1.10	0.47	0.49
n-6/n-3	1.8	0.8	0.7	0.5	1.2	2.9

Mean±Standard deviation. nd - not detect. Values with the same letter in the same row are not statistically different (p>0.05).

Tocopherols

Vitamin E composition of the four edible flowers species is shown in Table 3.1.1.3. Four tocopherols (α-, β-, γ- and δ-tocopherol) and two tocotrienols (β- and γ-tocotrienol) were identified and quantified in almost all flowers. However, significant differences between them were observed. In general, the major component in all samples was α-tocopherol, ranging between 0.67 and 22.21 mg/100 g dw for red and yellow pansies, respectively. γ-tocopherol was the second most abundant compound detected, except in centaurea, which was the β-tocopherol. Regarding tocopherols, yellow pansies presented the highest total content (24.89 mg/100 g dw), mainly due to the presence of α-tocopherol. Pires et al. (2017) only detected α- and γ- tocopherols in centaurea petals, while in the present study β- and δ- tocopherols were also identified. Furthermore, a values two times lower than ours was determined for α-tocopherol (0.55 mg/100 g dw), while a similar value of γ-tocopherol (0.29 mg/100g dw) was determined (Pires et al., 2017). Regarding tocotrienols, they were not detected in centaurea and red pansies. Borage and camellia flowers showed higher amounts of β-tocotrienol, followed by γ-tocotrienol. Despite not being regarded as great source of tocopherols, because their lipid content is also very low, they can contribute to supply this vitamin to the organism, even though the daily recommended dose for tocopherols in adults is 300 mg/day (EFSA, 2008), meaning that a large quantity of edible flowers would have to be ingested per day.

Carotenoids

Carotenoids are lipophilic pigments that accumulate in flowers, being responsible for color, which attract pollinators. Flower petals have a wide range of carotenoids levels, depending on the plant species or cultivar (Ohmiya, 2013). The total and some individual carotenoids are shown in Table 3.1.1.4. The studied edible flowers presented significantly ($p<0.05$) different carotenoids contents, ranging between 5.8 and 181.4 mg β-carotene equivalent/100g dw in centaurea and borage, respectively. Two pigments were identified and quantified in all samples, namely lutein and β-carotene. With the exception of camellia, lutein was always in higher contents than β-carotene, the first ranging between 0.42 to 43.73 mg of β-carotene equivalent/100 g dw (camelia and borage, respectively). According to literature, other edible flowers also showed lutein as the main xanthophyll, such as chrysanthemum (1.18-30.7

mg/100g dw) (Park et al., 2015), snapdragon (1.41 mg/100 g dw) (González-Barrio et al., 2018), garden nasturtium (35.0-45.0 mg/100g) (Niizu and Rodriguez-Amaya, 2005), marigold (106.2 mg/100 g fw) (Tinoi et al., 2006) and crem (24.3 mg/100 g dw) (Bona et al., 2017) flowers. Our results are within the values reported by González-Barrio et al. (2018) for carotenoids in pansies, who detected lutein in the highest content (5.11 mg/100g dw), followed by β-carotene (4.15 mg/100g dw). Even though, white and yellow pansies had a higher proportion of lutein than the red ones, the last have 1.9 and 5.0 times more carotenoids than the yellow and white pansies, respectively. Our results were in line with those reported for *Tagetes erecta* and *Calendula cyanus* flowers, where lutein content increased from the yellow to the dark orange flowers (Gregory et al., 1986; Pintea et al., 2003). As expected, red and yellow pansies showed the highest values of total carotenoids (109.2 and 58.0 mg β-carotene/100g dw, respectively) because these pigments are responsible for the red, yellow and orange colors in plants. On contrary, purple and blue colors are due to other pigments, such as anthocyanins. So, centaurea (5.8 mg β-carotene /100 g dw) and camelia (24.7 mg β-carotene/100 g dw) showed the lowest values of total carotenoids, due to the blue and rose color of their petals.

Organic acids

The organic acids profile of the four edible flowers is detailed in Table 3.1.1.5. Statistical differences were observed between samples. Eight organic acids were identified in almost all flowers. Malic acid was the major organic acid found in the studied edible flowers, except in centaurea. The presence of high quantities of succinic (3.62 g/100 g dw), malic (1.84 g/100 g dw) and citric (1.88 g/100 g dw) acids in centaurea flowers were not reported by Pires et al. (2017). This result may be due to the sample analyzed was only petals and not all flower, as in the present study. The second major organic acid detected in borage, camellia, yellow and white pansies was levulinic acid (0.63-4.12 g/100 g dw), while for centaurea was citric acid (1.88 g/100 g dw) and for red pansies was hydroxycinnamic acid (0.82 g/100 g dw). So, edible flowers presented distinct organic acids profiles. Comparing flowers, centaurea and white pansies (9.28 and 10.20 g/100 g dw, respectively) showed the highest amounts of organic acids, while camellia, followed by red pansies, presented the lowest contents (4.15 and 5.61 g/100 g dw, respectively).

Table 3.1.1.3 – Vitamin E in borage, camellia, centaurea and pansies (mg/100 g dw).

Tocols	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola×wittrockiana*)		
				White	Yellow	Red
α-tocopherol	2.21±0.06^{b}	9.27±0.42^{c}	1.24±0.01^{a}	8.64±0.66^{c}	22.21±0.35^{d}	0.67±0.07^{a}
β-tocopherol	0.29±0.01^{b}	0.27±0.01^{b}	0.66±0.07^{c}	0.66±0.02^{c}	0.64±0.02^{c}	0.17±0.01^{a}
γ-tocopherol	0.43±0.01^{b}	1.39±0.21^{c}	0.28±0.01^{a,b}	1.41±0.13^{c}	1.58±0.05^{c}	0.22±0.01^{a}
δ-tocopherol	0.24±0.01^{a}	nd	0.26±0.01^{a}	0.57±0.03^{c}	0.46±0.01^{b}	nd
Total tocopherols	3.17	10.92	2.43	11.28	24.89	1.07
β-tocotrienol	0.28±0.01^{b}	0.23±0.01^{a}	nd	0.36±0.01^{d}	0.33±0.01^{c}	nd
γ-tocotrienol	0.19±0.01^{a}	0.21±0.01^{b}	nd	nd	nd	nd
Total tocotrienols	0.48	0.44	---	0.36	0.33	--

nd - not detected; Mean±Standard deviation. Values with the same letter in the same row are not statistically different (p>0.05).

Table 3.1.1.4 – Total carotenoids (mg β-carotene/100g dw) and individual carotenoids (mg β-carotene equivalent/100 g dw) in borage, camellia, centaurea and pansies.

	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola×wittrockiana*)		
				White	Yellow	Red
Total carotenoids	181.4±13.9^{e}	24.7±4.1^{b}	5.8±1.0^{a}	21.6±1.0^{b}	58.0±3.6^{c}	109.2±2.2^{d}
β-carotene	8.50±0.61^{d}	0.59±0.49^{a,b}	0.04±0.01^{a}	1.11±0.04^{b}	2.12±0.19^{c}	0.18±0.01^{a}
Lutein	43.73±2.24^{d}	0.42±0.33^{a}	1.08±0.03^{a}	3.65±0.23^{b}	9.99±1.96^{c}	1.18±0.07^{a}

Mean±Standard deviation. Values with the same letter in the same column are not statistically different (p>0.05)

Table 3.1.1.5 – Organic acids and sugars composition in borage, camellia, centaurea and pansies (g/100 g dw).

Organic acids	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola×wittrockiana*)		
				White	Yellow	Red
Citric acid	0.93 ± 0.02^{d}	0.41 ± 0.01^{b}	1.88 ± 0.08^{e}	0.22 ± 0.02^{a}	0.52 ± 0.01^{c}	0.38 ± 0.06^{b}
Levulinic acid	1.17 ± 0.07^{c}	0.63 ± 0.05^{a}	0.86 ± 0.06^{b}	4.12 ± 0.06^{d}	0.91 ± 0.04^{b}	0.59 ± 0.03^{a}
Fumaric acid	0.87 ± 0.01^{d}	0.008 ± 0.001^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{c}	0.03 ± 0.01^{c}	$0.011\pm0.001^{a,b}$
Succinic acid	0.49 ± 0.01^{c}	$0.14\pm0.001^{a,b}$	3.62 ± 0.15^{d}	$0.12\pm0.01^{a,b}$	0.23 ± 0.01^{b}	0.11 ± 0.003^{a}
Malic acid	2.88 ± 0.03^{b}	2.82 ± 0.04^{b}	1.84 ± 0.09^{a}	4.92 ± 0.03^{e}	4.44 ± 0.10^{d}	3.59 ± 0.05^{c}
Salicylic acid	0.01 ± 0.01^{a}	0.013 ± 0.001^{a}	0.02 ± 0.01^{a}	0.10 ± 0.008^{b}	$0.11\pm0.01^{b,c}$	0.11 ± 0.01^{c}
Hydroxycinnamic acid	$0.22\pm0.01^{a,b}$	0.13 ± 0.01^{a}	0.35 ± 0.3^{b}	$0.26\pm0.03^{a,b}$	0.59 ± 0.02^{c}	0.82 ± 0.18^{d}
Malonic acid	0.05 ± 0.01^{a}	nd	0.70 ± 0.07^{d}	0.43 ± 0.10^{c}	0.35 ± 0.02^{b}	nd
Total organic acids	6.63	4.15	9.28	10.20	7.16	5.61
Sucrose	3.86 ± 0.75^{d}	$2.35\pm0.09^{c,d}$	$1.33\pm0.06^{a,b}$	$2.26\pm0.34^{c,d}$	0.49 ± 0.09^{a}	$1.91\pm0.50^{b,c}$
Glucose	5.96 ± 0.75^{c}	13.1 ± 0.76^{d}	1.74 ± 0.81^{a}	11.8 ± 1.45^{d}	$3.74\pm0.27^{a,b}$	4.32 ± 0.04^{b}
Fructose	6.97 ± 0.38^{b}	1.74 ± 0.07^{a}	2.71 ± 0.10^{a}	13.8 ± 1.86^{c}	$4.30\pm0.13^{a,b}$	$4.13\pm0.96^{a,b}$
Total Sugars	16.8	17.2	20.6	27.9	8.53	10.4

Mean±Standard deviation. Values with the same letter in the same row are not statistically different ($p>0.05$)

Free sugars

Free sugars composition of the four edible flowers is presented in Table 3.1.1.5. In the edible flowers studied, three free sugars were identified, namely: sucrose, glucose and fructose, varying between 0.49-3.86, 1.74-16.6, and 1.74-13.8 g/100g dw, respectively. Borage was the flower that presented the highest sucrose content, while white pansies were those with the highest glucose and fructose concentrations. On contrary, yellow pansies showed the lowest value in sucrose and glucose. Regarding pansies of different colors, white pansies presented higher values in all detected free sugars than the other two colors (red and yellow). Our results are in line with Pires et al. (2017) for centaurea petals, who also detected these three sugars; however, the values reported for fructose (0.65 g/100 g dw), sucrose (0.38 g/100 g dw) and glucose (0.47 g/100g dw) were lower than ours. The difference in values may be because in our study the whole flower was analyzed, whereas in the aforementioned study, only petals were evaluated.

Conclusion

The present study provided valuable information in the nutritional composition of four edible flowers, as well as in some bioactive compounds. Different nutritional compositions and fatty acids, free sugars, vitamin E, carotenoids and organic acids profiles were observed. PUFA were predominant in borage and yellow pansies, while the remaining flowers showed higher percentages of SFA, complemented with interesting amounts of α-tocopherol, but all in low amounts due to the very low lipid contents. Borage has the highest protein contents in the group, while centaurea has remarkable fiber content, a neglected nutrient in human health. All edible flowers presented sucrose, glucose and fructose. With exception of camellia, lutein contents were always higher than β-carotene, having red pansies and borage the highest contents of total carotenoids. This represents a very important attribute particularly due to lutein protective effects on eye health. Regarding organic acids, all flowers presented high contents of malic acid, except centaurea (with the major contribution of succinic acid). In summary, edible flowers are a valuable nutrients source, so they could be explored for other applications in food industry and gastronomy. Their diverse chemical profiles could be explored for nutraceutical purposes, as the high fiber in centaurea and of the lutein content in borage and yellow pansies.

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3.1.2.

Nutritional and nutraceutical compositions of *Borage officinalis* and *Centaurea cyanus* during flower development.

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Abstract

The present work intends, for the first time, to detail the nutritional and nutraceutical compositions of two edible flowers, borage and centaurea, at three flowering stages, as well as in petals. Water was the main macronutrient, followed by total dietary fiber (3.8-17.2% fresh weight). The two flowers showed statistically ($p<0.05$) different nutritional and nutraceutical profiles, although in both were quantified polyunsaturated fatty acids, with 32 to 55% of total fatty acids (mainly linoleic and α-linolenic acids), free sugars (3.9-28.9 % dry weight as fructose, glucose and sucrose), tocopherols (with the major contribution of α-tocopherol from 1.24 to 2.75 mg/100g dw), carotenoids (0.2-181,4 mg/100g dw; mainly as lutein) and organic acids (6.1-14.4 g/100g dw, with malic, succinic and citric acids as the main). Concerning flowering, significant ($p<0.05$) differences were found for some components, particularly carotenoids, but the major ones were observed in the petals, with lower amounts of all components except tocopherols in Centaurea, and organic acids on both. The present study shows that each edible flower, including their different parts, has a unique composition and nutritional value and this diversity and abundance of healthy compounds could be better explored by the food industry.

Keywords: Edible flowers; Flowering development; Nutritional composition; Fatty acids; Tocopherols; Free sugars; Vitamin E; Carotenoids; Organic acids.

Introduction

Edible flowers commercial value for the food industry is increasing, mostly due to the world renewed interest for natural products, new ingredients with potential health benefits, and the aesthetic benefits of their use in food and beverages (colors, textures and flavor).

Centaurea (*Centaurea cyanus*), also called blue cornflower or bachelor's button, is a plant native of Europe. Its flowers have no fragrance, but they have a sweet-to-spicy clove-like flavor. Its petals are ideal for mixing with other flowers to make attractive confetti for sprinkling over salads, to colorful garnish or to be used as an ingredient in tea (Chaitanya, 2014). Dried flowers are associated to medicinal properties, such as, anti-inflammatory; skin cleansing; regulation of digestion, kidney, gall bladder, liver and menstrual disorder, and increase immunity (Chaitanya, 2014; Pirvu et al., 2012).

Borage (*Borago officinallis*) is an annual herb, native of some parts of Mediterranean region, which is cultivated for medicinal and culinary uses, but mostly for borage seed oil (Asadi-Samani et al., 2014). Its flowers are blue and rarely appear white or rose colored (Asadi-Samani et al., 2014). Petals have a cucumber taste and the stamens add a hint of sweetness. Its flowers are used in vegetable dishes and fruit salads, to garnish soups or decorate desserts, as well as in ice cubes (Husti et al., 2013).

The period of flower development from mature bud to open flower encompasses one of the most active growth periods in all plant development (Reid, 2005). During these stages, a variety of physiological, biochemical and structural processes occur (Collier, 1997; Chen et al., 2012; Çirak et al., 2007). The chemical changes that happen during flower development affect its nutritional and beneficial health properties. So, consumers may eat flowers with different compositions and nutritional values, according to the part of flower or stage of development. Hence, understanding the changes in the chemical and nutritional compositions during flower development could help to enhance their quality and bioactive potential, and thus increasing edible flowers' commercial value. However, publications on the chemical and nutrition characterization of edible flowers during flowering development are limited. A few detailed studies have focused mainly on protein, anthocyanins, sugars and enzymes in rose species (*Rosa hybrida*, *Rosa damascena* and *Rosa bourboniana*) (Dafny-Yelin et

al., 2005; Sood et al., 2006); bioactive compounds of rosemary (*Rosmarinus officinalis)* (Del Baño et al., 2003); carbohydrates and anthocyanin pigments in nasturtium flowers (*Tropaeolum majus*) (Silva, 2012); chemical composition of *Opuntia ficus-indica* and *Opuntia stricta* flowers (Ammar et al., 2014); and physiological and biochemical changes in carnation (*Dianthus chinensis*) (Dar et al., 2014). Thus, the present study was conducted to determine the evolution in nutritional (macronutrients composition and energetic value) and chemical composition (fatty acids, free sugars, vitamin E, carotenoids and organic acids) of two edible flowers, centaurea (*Centaurea cyanus*) and borage (*Borago officinalis*), in different plant parts (flower and petals), along their flowering development (three stages).

Material and methods

Plant material

Blue flowers of both species (borage and centaurea) were collected at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança (Bragança, Portugal). After harvest, flowers at different stages of development were immediately transported to the laboratory under refrigeration. Flower development was divided into three stages (Figure 3.1.2.1), namely: Stage 1 (tight bud stage) - closed bud flower, showing some petal color at the tip of the bud end; Stage 2 (mature bud stage) - closed bud flower, showing the color of the petals in whole surface; Stage 3 (fully open) - complete opened flower, without symptoms of senescence. Furthermore, petals were also picked, because they are often the sole parts used for culinary purposes.

Flowers were evaluated for their moisture content before being freeze dried. All the chemical analyses were performed in the freeze dried samples, with the results presented on a dry weight or converted to fresh weight.

Nutritional composition

The nutritional composition (moisture, ash, fat, carbohydrates and dietary fiber) of each sample was analysed using AOAC procedures (1995) and expressed as g/100g fresh weight (FW). Moisture content was determined by drying the sample to a constant weight at 105 °C; ash content was measured by calcination at 550 ºC for at least 2h, until achieving white ashes. Protein content of the samples was estimated by the macro-Kjeldahl method, with a conversion factor of 6.25, according to Sotelo et al. (2007) and Rop et al. (2012). Lipids were determined by extracting a known mass of

powdered sample with petroleum ether with 0.01% BHT (2,6-di-*tert*-butyl-4-methylphenol) to prevent oxidation, using a Soxhlet apparatus. Dietary fiber was determined by an enzymatic-gravimetric method based on AOAC official method No. 985.29 (AOAC, 2003). Total carbohydrates were calculated by difference.

Energy was calculated according to equation 1:

$$Energy\ \left(\frac{kcal}{100g\ fw}\right) = [(4 \times (protein + carbohydrates)) + (9 \times lipids) + (2 \times dietary\ fiber)](\frac{g}{100g\ fw}) \quad \text{(Eq. 1)}$$

Analysis of lipid micro components

Lipid compounds extraction

Lipid extraction for compositional analysis was performed according to the method described by Cruz et al. (2013), with slight modifications. A 250 mg portion of lyophilized sample was weighted for a plastic tube, followed by the addition of two internal standards solutions: tocol (20 µL; 1 mg/mL) for vitamin E quantification, and triundecanoin (200 µL; 1 mg/mL), for total fatty acids quantification, followed by two antioxidants - BHT (20 µL, 10 mg/mL in methanol), and ascorbic acid (50 mg), and glass pearls. Propan-2-ol (1.6 mL) and cyclohexane (2.0 mL) were added for lipid extraction. The mixture was agitated briefly by vortexing and kept overnight under refrigeration (4 ºC). The non-lipid material was removed by washing with 2.5 mL of 1 % NaCl solution (m/v), with agitation by vortexing and centrifugation (3000 rpm, 5 min; Heraeus Sepatech, Germany). The organic phases were dehumidified with anhydrous sodium sulfate and divided into two portions. Half of the total extract volume was transferred to Pyrex derivatization tubes for fatty acid analysis. The solvent was evaporated under a nitrogen stream (60 °C). Hydrolysis was performed with 1.5 mL of KOH (0.5 M in methanol) at 100 °C (10 min) in a dry heating block (Stuart SBH200D/3, Staffordshire, UK). After reaching room temperature, methylation was completed by the addition of 1.5 mL of BF_3 (14 % in methanol) and heated for further 30 min at 100 °C. After cooling, 1 % NaCl (m/v) (2.5 mL) and *n*-hexane (2×2 mL) were added and the mixture was mixed by vortexing, followed by centrifugation (3000 rpm, 5 min). The supernatant was transferred to a tube with anhydrous sodium sulphate, followed by centrifugation at 5000 rpm for 5 min. A supernatant portion (2 mL) was directly transferred to a clear glass vial (Supelco,

Sigma, Bellfonte, USA), the solvent was evaporated under a nitrogen stream (60 °C), resuspended with heptane (1 mL) and the solution mixed in a vortex. This vial was positioned in the gas chromatograph autosampler for fatty acid analysis, as detailed below. The remaining lipid extract (1/2) was transferred to an amber glass vial. The solvent was evaporated under a gentle nitrogen stream (40 °C), resuspended in about 600 µL *n*-hexane (HPLC grade) and placed in the HPLC autosampler for vitamin E and carotenoids quantification.

Fatty acids

Fatty acids were analysed in a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, Netherlands) equipped with a split-splitless injector, a Chrompack CP-9050 autosampler and a flame ionization detector (FID). The temperatures of the injector and detector were 250 and 270 °C, respectively. Separation was achieved on a 100-m × 0.25-mm i.d. Select-FAME column (0.19-µm film; Agilent, Santa Clara, USA). Helium was used as carrier gas at an internal pressure of 180 kPa. The column temperature was 180 °C, for a 35 min hold, and then programmed to increase to 250 °C at a rate of 3 °C/min. The total analysis time was 60 min. The slip ratio was 1:50 and the injected volume was 1.2 µL. Fatty acids identification (from C11:0 to C22:6) was accomplished by comparing the relative retention times of FAME peaks with standards from diversified suppliers, from literature data, and confirmed by GC-MS on a Agilent chromatograph 7890A with a 5977B MSD (MS source - 230ºC; MS QUAD 150; aux 280ºC; m/z 30-800) using the NIST/EPA/NIH Mass Spectral Library (NIST 14). For quantification purposes the FID peaks were corrected using response factors obtained with standard FAME solutions. The fatty acids results were calculated on a relative percentage basis.

Vitamin E and carotenoids

Separation was achieved by normal-phase HPLC, based on the method described by Cruz and Casal (2018). The liquid chromatograph consisted of a Jasco integrated system (Easton, USA) equipped with an autosampler (AS-2057 Plus), a PU-980 intelligent pump, and a multiwavelenght DAD (MD-910, recording at 450 nm for carotenoids, connected to a fluorescence detector (FD) (FP-2020 Plus; $\lambda_{excitation}$ = 290 nm and $\lambda_{emission}$ = 330 nm) for vitamin E. The chromatographic separation was achieved on a Luna Silica (100 mm × 3 mm; 3 µm) (Phenomenex, USA), operating at

constant room temperature (23 °C), with a gradient of *n*-hexane and 1,4-dioxane at a flow rate of 1.0 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Tocopherols and tocotrienols (α-, β-, γ-, and δ-) were acquired from Supelco (USA) and Larodan AB (Sweden). Carotenoids standards (all-*trans*-β-carotene and lutein) were from Sigma Aldrich (USA). Identified tocopherols and carotenoids were quantified by the internal standard method, being reported on a dry mass basis.

The total carotenoid contents were estimated according to the method described by Aquino-Bolaños et al. (2013). One gram of frozen-dried powder of each sample was extracted twice with 20 mL acetone:hexane solution (1:1, v/v). Both extracts were put into a separation funnel, being added 200 mL of distilled water to eliminate acetone. The acetone-free phase was mixed with anhydrous sodium sulphate (5g) to eliminate any residual water, being the remaining solution filtered and completed to 100 mL with hexane. Carotenoids content was determined by reading the absorbance at 450 nm and comparing the results with a β-carotene calibration curve (0.22–8.8 μg/mL). Results were expressed as mg β-carotene equivalent/100 g dw.

Organic acids

The organic acids in edible flowers were determined by gas chromatography, after methylation, following the procedure described by Sharma et al. (2016) and Kumar et al (2017). Organic acids were extracted from dried powdered flowers by adding 0.5 N HCl and methanol (0.5 mL each), after addition of an accurate amount of internal standard solution (adipic acid, Sigma). The samples were shaken for 3 h, followed by centrifugation at 12,000 rpm for 10 min. To the supernatant, 300 μL of methanol and 100 μL of 50% sulphuric acid (v/v) were added, followed by overnight incubation at 60 °C. The mixture was cooled down to 25 °C, and 800 μL of chloroform and 400 μL of distilled water were added to it, followed by vortexing for 1 min. The lower chloroform layer was used to quantify methylated organic acids by GC-MS (7890A gas chromatograph coupled to a 5977 B mass selective detector, both from Agilent Technologies, USA). To determine organic acids contents, 2 μL of sample (lower chloroform layer) was injected into a GC-MS system. The GC conditions were the following: helium was used as carrier gas, the starting column temperature was 50 °C, held for 1 min, which was raised to 125 °C at 25 °C/min, followed by an additional enhancement to 300 °C at 10 °C/min, held for 15 min. The injection temperature was

250 °C, the injection mode was split (1:4), gas flow in the column was 1.7 mL/min, and analytical column HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm thickness ultra-inert capillary column, Agilent Technologies) was used. MS conditions: Ion source temperature was set at 200 °C and interface temperature was 280 °C, solvent cut time was 3 min. Mass spectra were obtained by electron ionization (EI) at 70 eV, in a full scan mode, with a spectrum range of ion mass captured between 25 and 400 m/z and an average of 3.5 scans/s (sample rate of 2). The mass spectra were evaluated using Enhanced ChemStation software (Version F.01.03.2357, Agilent Technologies). Individual standards of citric, levulinic, fumaric, succinic, malic, salicylic, hydroxycinnamic, malonic, oxalic, tartaric and benzoic acids, all from Sigma Aldrich (Germany), were derivatized under sample conditions. Quantification was based on individual calibration curves, using specific m/z for each compound, as detailed in Kumar et al (2017).

Sugars

Free sugars were extracted by following the procedure described by Barros et al. (2010), with slight modifications. Dried sample powder (1.0 g) was extracted with 40 mL of 80% aqueous ethanol (v/v) at 80 ºC for 30 min. The resulting suspension was filtered with a vacuum pump (KNF LABOPORT, Darmstadt, Germany). The supernatant was concentrated at 45 ºC in a rotary evaporator (Stuart, RE300DB, Stone, UK) and defatted three times with 10 mL of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 3 mL and filtered through 0.2 μm Nylon filters from Whatman.

Sugars were analyzed in a Jasco integrated high performance liquid chromatographic system (Tokyo, Japan), equipped with a PU-980 intelligent pump and a refractive index detector (Gilson, USA). Separation was achieved with a SUPELCOGEL Ca column (30 cm × 7.8 mm I.D., Supelco, USA), operating at 80 °C with ultra-pure water at a flow rate of 0.5 mL/min as eluent. Sugar identification was made by comparing the retention times of sample peaks with standards. Quantification was performed by the external standard method with individual calibration curves for each sugar at concentrations ranging from 0.5 to 10 mg/mL. The results were expressed as g/100 g (dry matter).

Statistical analysis

The statistical analysis was performed on SPSS software, Version No. 18.0 (SPSS Inc., Chicago, USA). The normality of the data was verified by Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch were carried out to determine if there were significant differences ($p<0.05$) between samples, depending on the existence or not of homogeneity of variances, respectively. Additionally, if significant differences were detected between treatments, a post hoc analysis was performed, namely: Tukey's honestly significant difference test (if variances in the different groups were identical) or Games-Howell test (if they were not). The homogeneity of the variances was tested by Levene's test.

Results and Discussion

Nutritional Composition

The nutritional compositions of borage and centaurea at three flowering stages are detailed in Table 3.1.2.1. Water was the main constituent in both flowers at all stages (73.7 - 90.5 g/100 g fw), followed by total dietary fiber (3.84 – 17.2 g/100g fw), while lipids were the minor constituents (0.41 - 1.13 g/100g fw).

When comparing both flowers, borage always presented statistically higher ($p<0.05$) moisture contents (> 85 g/100g fw) than centaurea (> 73 g/100g fw), as well as ash values (1.74-2.05 vs. 1.20 -1.56 g/100g fw, respectively). On the other hand, centaurea always presented statistically higher amounts of total dietary fiber (14.8 – 17.2 g/100g fw) than borage (4.74 – 6.74 g/100g fw) as well as of energy value (centaurea 60.7 – 69.0 kcal/100g fw vs. borage 35.6 – 41.4 kcal/100g fw). In general, these nutritional values were similar to some vegetables, as asparagus (moisture 79.34-92.20, proteins 3.62-4.83, fat 0.33-0.90 g/100g fw) (Ferrara et al., 2011), white cabbage (moisture 91.8, proteins 1.4, fat 0.4, dietary fiber 2.4, carbohydrates 3.5 g/100 g fw) and cucumber (moisture 95.1, proteins 1.4, fat 0.6, dietary fiber 0.7, carbohydrates 1.7 g/100 g fw) (INSA, 2010).

During flowering stage (from tight bud to fully open flower), the nutritional composition trends of both flowers were different. Borage showed an increase in protein, while in centaurea a decrease in ash and protein contents was observed. Despite these variations, lipids, carbohydrates and global caloric contents remained almost constant. These different patterns are consistent with literature results for other flowers, as the two *Opuntia* species at four stages of development reported by Ammar

et al. (2014): the flowers of *Opuntia ficus-indica* showed an increase in fat and carbohydrates and a decrease in the fiber contents (three first stages of flowering), while in *Opuntia stricta* the opposite was detected (Ammar et al., 2014). So, each flower, at each state of flowering, shows a unique composition and nutritional value.

Petals alone presented the highest moisture content and the lower energy value when compared with the whole flower, on both flowers. In borage, the separation of the petals from the flower represents a highly significant reduction in protein, lipids and total dietary fiber, while in centaurea only minor alteration were observed. For the highest protein contents, borage should be consumed as fully opened flower, while in centaurea the tight bud should be preferred. For increased fiber content, tight buds should be chosen. In general terms, both flowers can be included in low-calorie diets.

Flower	Development stages			
	Tight bud stage	Mature bud stage	Fully open	Petals
Borage				
Centaurea				

Figure 3.1.2.1 - Petals and three flowering stages of borage (*Borago officinalis*) and centaurea (*Centaurea cyanus*)

Table 3.1.2.1 – Nutritional composition of borage and centaurea at three flowering stages plus isolated petals (g/100 g fw)

Nutritional composition	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
Moisture	$86.9\pm0.4^{a,B}$	$85.3\pm1.8^{a,B}$	$86.6\pm1.8^{a,B}$	$90.5\pm0.4^{b,B}$	$73.7\pm2.2^{a,A}$	$76.3\pm1.2^{a,A}$	$76.7\pm0.6^{a,A}$	$80.5\pm0.7^{b,A}$
Ash	$2.02\pm0.14^{c,B}$	$1.74\pm0.04^{b,B}$	$2.05\pm0.11^{c,B}$	$0.88\pm0.04^{a,A}$	$1.56\pm0.06^{c,A}$	$1.43\pm0.11^{b,c,A}$	$1.20\pm0.11^{a,b,A}$	$1.11\pm0.04^{a,A}$
Protein	$2.24\pm0.09^{b,A}$	$2.62\pm0.10^{c,B}$	$3.04\pm0.10^{d,B}$	$0.89\pm0.02^{a,A}$	$2.89\pm0.55^{b,A}$	$1.78\pm0.28^{a,A}$	$1.60\pm0.06^{a,A}$	$1.66\pm0.24^{a,B}$
Lipid	$0.68\pm0.16^{a,A}$	$0.56\pm0.02^{a,A}$	$0.66\pm0.15^{a,A}$	$0.41\pm0.08^{a,A}$	$0.89\pm0.08^{a,A}$	$1.13\pm0.20^{a,B}$	$0.80\pm0.03^{a,A}$	$0.86\pm0.16^{a,B}$
Carbohydrates	$1.78\pm0.48^{a,A}$	$3.11\pm1.78^{a,A}$	$3.15\pm1.73^{a,A}$	$3.49\pm0.44^{a,B}$	$3.77\pm1.84^{a,A}$	$4.52\pm1.28^{a,A}$	$3.93\pm0.43^{a,A}$	$1.12\pm0.84^{a,A}$
Total dietary fiber	$6.28\pm0.16^{c,A}$	$6.74\pm0.05^{d,A}$	$4.74\pm0.10^{b,A}$	$3.84\pm0.03^{a,A}$	$17.2\pm1.4^{b,B}$	$14.8\pm0.8^{a,B}$	$15.7\pm0.5^{a,b,B}$	$14.8\pm0.7^{a,B}$
Energy*	$36\pm2^{a,A}$	$41\pm7^{a,A}$	$38\pm7^{a,A}$	$28\pm2^{a,A}$	$69\pm3^{c,B}$	$65\pm1^{b,c,B}$	$61\pm1^{b,B}$	$48\pm1^{a,B}$

Values are expressed as: Mean±Standard deviation. fw- fresh weight. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$). * Energy are expressed as g in kcal/100 g fw.

Fatty acids

The fatty acids details of borage and centaurea at three flowering stages are shown in Table 3.1.2.2, presented as individual and grouped as total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), together with the ratios of PUFA/SFA and n-6/n-3. Nineteen fatty acids were identified and quantified. The major fatty acid found in borage at all stages was linoleic acid (C18:2n6) (19.1-23.7%), followed by palmitic acid (17.1-18.0%). In centaurea, oleic acid (C18:1) was the major fatty acid in tight buds (26.4%), while in mature buds and fully open flowers the palmitic acid (C16:0) was the main one (30.0 and 25.9%, respectively) and in petals α-linolenic acid (C18:3n3) was the major one (27.7%). However, palmitic (C16:0), oleic (C18:1), and α-linolenic (C18:3n3) acids did not show statistical differences along centaurea development. Furthermore, this flower didn't show γ-linolenic (C18:3n6) and eicosenoic (C20:1) acids in their fatty acids composition, as detected in borage. During the three stages of flowering, borage didn't show statistical differences in the major fatty acids, with the exception of α-linolenic (C18:3n3) that increased from 10.4 to 12.9%. In all stages of development, PUFAs predominated over SFA in borage, followed by MUFA, due to the abundance of C18:2 and C18:3. On contrary, in centaurea it was observed that SFA>PUFA>MUFA, due to the contribution of palmitic acid, although the SFA values were not so different from PUFA values. Furthermore, it is recommended for the human diet, that the PUFA/SFA ratios should be higher than 0.45 and n-6/n-3 ratios lower than 4.0 (Guil et al., 1996), which was observed in almost all stages of flowering, except in borage petals (PUFA/SFA = 0.40) and mature buds in centaurea (PUFA/SFA = 0.20). So, in general terms both edible flowers at all flowering stages and petals have healthy lipid profiles.

Vitamin E

Free vitamin E was evaluated in all flowering stages, being detailed in Table 3.1.2.3. α-tocopherol was the major compound in all samples, followed by γ-tocopherol in borage and β-tocopherol in centaurea. This richness in α-tocopherol is important from an health point of view since it represents the primary defense against lipid peroxidation (Bartoli et al., 1997), and is similar to the one found in other floral species, such as: calendula (Pires et al., 2017; Miguel et al., 2016); centaurea (Pires et al., 2017); rose species (Pires et al., 2017, Guimarães et al., 2010), capper (Tlili et al.,

2009) and lilium (Arrom and Munné-Bosch, 2010). In almost all flower development stages, borage showed higher tocopherols contents than centaurea; however, centaurea presented the highest value of α-tocopherol in petals (3.47 mg/100 g dw), as well as higher β-tocopherol values than borage (0.41-1.01 versus 0.29-0.33 g/100 g dw). No tocotrienols were detected in centaurea, while borage showed β- and γ-tocotrienols in their composition in reduced amounts. In centaurea flowers, Pires et al. (2017) also described the presence of α- and γ- tocopherols.

Along flower development, the content of total tocopherols decreased in both flowers, between 3.7 to 3.2 mg/100g dw (borage) and 3.0 to 2.4 mg/100 g dw (centaurea). However, borage and centaurea showed significant differences in the individual tocols. In borage, α- and β-tocopherols decreased (from 2.75 to 2.21 and 0.33 to 0.29 mg/100 g dw, respectively) from tight bud to fully open stage, while γ-tocopherol increased (from 0.34 to 0.43 mg/100 g dw). In centaurea, the α-tocopherol remained constant, while β- and γ-tocopherols decreased (from 1.01 to 0.66 and 0.32 to 0.28 mg/100 g dw), showing that different flowers had distinctive trends in vitamin E composition along flower development.

Tlili et al. (2009), when analyzing caper of different Tunisian regions, found that buds presented higher α- and γ- tocopherols values than flowers. An increase in α-tocopherol contents during the progression of senescence in chrysanthemum petals, followed by a decline in advanced stages (stage 4 - about 50% of full browning of the petal and evidence of wilting, and stage 5 - browning involves 50- 100% of the petal), have been reported by Bartoli et al. (1997), as well as, in lilium floral organs, α- and γ-tocopherols increased with the progression of tepal senescence between stages I (closed flowers, with fully compacted organs and green tepals) and V (flowers with dehydrated tepals, just before abscission) (Arrom and Munné-Bosch, 2010). It must be stated that centaurea petals were richer in α- and γ-tocopherols than the whole flower in each state of flowering.

Table 3.1.2.2 – Fatty acid composition (relative %) of borage and centaurea at three flowering

Fatty acids (%)	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
SFA								
C12:0	$0.5\pm0.2^{a,b,A}$	$0.6\pm0.2^{b,A}$	$0.5\pm0.1^{a,b,A}$	$0.3\pm0.1^{a,A}$	$0.7\pm0.2^{a,A}$	$0.9\pm0.1^{a,B}$	$0.6\pm0.2^{a,A}$	$0.8\pm04^{a,B}$
C14:0	$0.5\pm0.3^{a,A}$	$0.8\pm0.3^{a,A}$	$0.7\pm0.1^{a,A}$	$2.1\pm0.1^{b,B}$	$0.7\pm0.3^{a,A}$	$1.0\pm0.5^{a,A}$	$0.7\pm0.2^{a,A}$	$0.7\pm0.3^{a,A}$
C15:0	$0.2\pm0.1^{a,A}$	$0.2\pm0.1^{a,A}$	0.2 ± 0.1^{a}	$0.4\pm0.1^{b,B}$	$0.1\pm0.1^{a,A}$	$0.2\pm0.2^{a,A}$	nd	$0.2\pm0.2^{a,A}$
C16:0	$18.0\pm0.8^{a,A}$	$17.8\pm0.9^{a,A}$	$17.1\pm0.7^{a,A}$	$17.2\pm0.1^{a,A}$	$24.8\pm4.5^{a,B}$	$30.0\pm1.2^{a,B}$	$25.9\pm4.4^{a,B}$	$25.2\pm4.2^{a,B}$
C17:0	$0.9\pm0.5^{a,B}$	$1.0\pm0.1^{a,A}$	$0.9\pm0.2^{a,A}$	$1.3\pm0.1^{a,A}$	$0.4\pm0.1^{a,A}$	$1.2\pm0.3^{b,c,A}$	$0.6\pm0.3^{a,b,A}$	$1.3\pm0.6^{c,B}$
C18:0	$4.9\pm0.4^{a,A}$	$3.8\pm1.8^{a,A}$	$4.8\pm0.4^{a,A}$	$5.0\pm0.1^{a,A}$	$5.8\pm1.1^{a,A}$	$8.6\pm1.7^{a,b,B}$	$7.2\pm1.7^{a,B}$	$10.6\pm3.2^{b,B}$
C20:0	$1.5\pm0.8^{a,A}$	$1.8\pm0.2^{a,A}$	$1.8\pm0.2^{a,A}$	$1.8\pm0.1^{a,A}$	$2.5\pm0.8^{a,A}$	$2.9\pm0.4^{a,B}$	$2.5\pm0.7^{a,B}$	$2.9\pm1.1^{a,B}$
C22:0	$2.4\pm0.7^{a,B}$	$2.6\pm0.3^{a,B}$	$2.5\pm0.4^{a,A}$	$3.2\pm0.4^{a,B}$	$1.2\pm0.4^{a,A}$	$2.0\pm0.3^{a,A}$	$1.4\pm0.5^{a,B}$	$1.6\pm0.5^{a,A}$
C24:0	$2.4\pm1.3^{a,A}$	$2.6\pm0.2^{a,B}$	$2.6\pm0.3^{a,B}$	$3.9\pm0.2^{b,B}$	$1.3\pm0.5^{a,A}$	$2.0\pm0.3^{a,A}$	$1.6\pm0.6^{a,A}$	$1.3\pm0.4^{a,A}$
Total SFA	31.0	31.0	31.0	35.0	37.0	49.0	40.0	45.0
MUFA								
C16:1	$0.1\pm0.1^{a,A}$	0.3 ± 0.2^{a}	$0.2\pm0.1^{a,A}$	0.2 ± 0.1^{a}	$0.2\pm0.1^{a,A}$	nd	$0.3\pm0.2^{a,B}$	nd
C18:1	$12.3\pm1.5^{a,A}$	$10.3\pm7.7^{a,A}$	$9.9\pm8.8^{a,A}$	$6.7\pm1.2^{a,A}$	$26.4\pm15.3^{a,B}$	$9.04\pm3.45^{a,A}$	$25.9\pm17.1^{a,A}$	$19.8\pm18.5^{a,B}$
C20:1	0.4 ± 0.2^{a}	0.6 ± 0.4^{a}	0.3 ± 0.0^{a}	0.5 ± 0.1^{a}	nd	nd	nd	nd
C22:1	$2.5\pm0.9^{a,b,B}$	$2.1\pm0.5^{a,B}$	$1.8\pm0.2^{a,B}$	$3.2\pm0.4^{b,B}$	$0.7\pm0.5^{a,A}$	$0.75\pm0.66^{a,A}$	$0.9\pm0.8^{a,A}$	$0.5\pm0.4^{a,A}$
C24:1n9	$2.6\pm0.8^{a,B}$	2.2 ± 0.3^{a}	2.0 ± 0.3^{a}	3.5 ± 0.3^{b}	0.1 ± 0.1^{A}	nd	nd	nd
Total MUFA	18.0	16.0	14.0	14.0	27.0	10.0	27.0	20.0
PUFA								
C18:2n6	$19.1\pm1.2^{a,B}$	$21.3\pm1.5^{a,b,A}$	$21.2\pm2.2^{a,b,B}$	$23.7\pm0.8^{b,B}$	$15.6\pm2.4^{b,A}$	$20.7\pm3.0^{c,A}$	$12.8\pm2.9^{a,b,A}$	$10.6\pm0.7^{a,A}$
C18:3n6	7.0 ± 1.4^{a}	9.1 ± 1.2^{b}	9.6 ± 1.4^{b}	6.1 ± 0.2^{a}	nd	nd	nd	nd
C18:3n3	$10.4\pm1.7^{b,A}$	$12.5\pm1.3^{b,c,A}$	$13.0\pm1.5^{c,A}$	$8.2\pm0.7^{a,A}$	$19.4\pm6.7^{a,B}$	$20.3\pm2.2^{a,B}$	$19.6\pm5.6^{a,B}$	$27.7\pm7.8^{a,B}$
C18:4n3	$5.9\pm1.1^{a,b}$	$6.2\pm0.8^{b,B}$	6.5 ± 0.8^{b}	$4.7\pm0.3^{a,B}$	nd	$0.5\pm0.6^{a,A}$	nd	$1.8\pm0.7^{b,A}$
C22:4n6	8.5 ± 1.5^{b}	4.2 ± 0.5^{a}	$4.5\pm0.6^{a,B}$	8.4 ± 00.3^{b}	nd	nd	0.1 ± 0.1^{A}	nd
Total PUFA	51.0	53.0	55.0	51.0	35.0	42.0	32.0	35.0
PUFA/SFA	0.58	0.52	0.45	0.40	0.73	0.20	0.68	0.44
n6/n3	2.13	1.86	1.83	2.96	0.80	1.00	0.65	0.36

Values are expressed as: Mean±Standard deviation. Nd - not detected. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$)

Table 3.1.2.3 – Vitamin E contents (mg/100 g dw) in borage and centaurea at three flowering stages.

Tocols (mg/100g)	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
α-tocopherol	$2.75\pm0.16^{c,B}$	$2.24\pm0.05^{a,A}$	$2.21\pm0.06^{a,B}$	$2.47\pm0.11^{b,A}$	$1.32\pm0.20^{a,b,A}$	$1.78\pm0.39^{b,A}$	$1.24\pm0.01^{a,A}$	$3.47\pm0.29^{c,B}$
β-tocopherol	$0.33\pm0.01^{b,A}$	$0.30\pm0.01^{a,A}$	$0.29\pm0.01^{a,A}$	$0.33\pm0.01^{b,A}$	$1.01\pm0.07^{c,B}$	$0.61\pm0.06^{b,B}$	$0.66\pm007^{b,B}$	$0.41\pm0.02^{a,B}$
γ-tocopherol	$0.34\pm0.01^{a,B}$	$0.43\pm0.01^{c,B}$	$0.43\pm0.01^{c,B}$	$0.38\pm0.02^{b,A}$	$0.32\pm0.01^{b,A}$	$0.34\pm0.04^{b,A}$	$0.28\pm0.01^{a,A}$	$0.39\pm0.01^{c,A}$
δ-tocoferol	$0.24\pm0.01^{a,A}$	0.25 ± 0.01^{b}	$0.24\pm0.01^{a,A}$	$0.24\pm0.01^{a,b,A}$	$0.35\pm0.01^{b,B}$	nd	$0.26\pm0.01^{a,A}$	$0.34\pm0.03^{b,B}$
Total tocopherol	3.7	3.2	3.2	3.4	3.0	2.7	2.4	4.6
β-tocotrienol	0.23 ± 0.01^{b}	0.28 ± 0.01^{c}	0.28 ± 0.01^{c}	0.22 ± 0.01^{a}	nd	nd	nd	nd
γ-tocotrienol	0.23 ± 0.01^{b}	0.20 ± 0.01^{a}	0.19 ± 0.01^{a}	0.25 ± 0.01^{c}	nd	nd	nd	nd
Total tocotrienol	0.5	0.5	0.5	0.5	nd	nd	nd	nd

Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$).

Table 3.1.2.4 - Total and individual carotenoids in borage and centaurea at three flowering stages and petals.

Flowers	Flowering stages	Total carotenoids (mg β-carotene/100 g dw)	β-carotene (mg β-carotene equivalent/100 g dw)	Lutein (mg β-carotene equivalent/100 g dw)
Borage	Tight bud	$23.5\pm4.2^{b,B}$	$0.45\pm0.03^{b,B}$	$13.04\pm0.61^{b,B}$
	Mature bud	$23.8\pm0.1^{b,B}$	$1.04\pm0.07^{c,B}$	$14.95\pm0.32^{c,B}$
	Fully open	$181.4\pm13.9^{c,B}$	$8.50\pm0.61^{d,B}$	$43.73\pm2.24^{d,B}$
	(Petals)	$3.3\pm0.5^{a,A}$	$0.04\pm0.01^{a,A}$	$0.73\pm0.15^{a,A}$
Centaurea	Tight bud	$0.2\pm0.2^{a,A}$	$0.002\pm0.001^{a,A}$	$0.03\pm0.01^{a,A}$
	Mature bud	$7.8\pm0.3^{c,A}$	$0.041\pm0.005^{b,A}$	$1.16\pm0.06^{c,A}$
	Fully open	$5.8\pm1.0^{b,A}$	$0.036\pm0.003^{b,A}$	$1.08\pm0.03^{c,A}$
	(Petals)	$4.8\pm0.2^{b,B}$	$0.091\pm0.039^{c,B}$	$0.69\pm0.21^{b,A}$

Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$)

Organic acids

The organic acids profiles of borage and centaurea during flowering development is presented in Table 3.1.2.5. Eight organic acids were detected, with distinct profiles on both flowers. In borage and centaurea, the total contents of organic acids ranged between 6.1-9.2 and 8.6-14.4 g/100 g dw, respectively. The major organic acid in borage was malic acid (2.09 – 4.66 g/100 g dw), followed by citric (0.52-1.73 g/100 g dw) and levulinic (0.69–1.58 g/100 g dw) acids. On contrary, in centaurea the major organic acid was succinic acid (1.89-9.68 g/100 g dw), followed by malic (1.47-2.17 g/100g dw) and citric (1.09-2.76 g/100 g dw) acids. Petals had the highest total organic acid contents, with different patterns of both flowers, but similar to those presented in flower development, with malic acid being prevalent in borage petals (50.6%) and succinic acid in centaurea petals (67.2%). Pires et al. (2017) also obtained different organic acids profiles for dahlia, rose, calendula and centaurea, for which the major organic acids were: malic acid (0.74 g/100g dw), quinic and malic acids (1.52 and 1.23 g/100g dw, respectively), succinic acid (1.77 g/100 g dw), and oxalic acid (0.18 g/100g dw), respectively. Furthermore, Pires et al. (2017), when analyzing dried centaurea petals (commercial sample), did not detect any of our major organic acids, only reporting oxalic and shiquimic acids.

In both flowers, from tight bud to fully open stage, citric acid decreased (from 1.73 to 0.92 and 2.76 to 1.88 g/100 g dw in borage and centaurea, respectively). In borage, levulinic and malic acids increased during flower development (0.69 to 1.17 and 2.09 to 2.88 g/100 g dw, respectively). In centaurea, succinic acid increased from tight bud (1.89 g/100 g dw) to fully open (3.62 g/100 g dw) stages, while malic acid decreased from 2.17 to 1.84 g/100 g dw. Borage petals showed higher values of all organic acids quantified except citric acid. In centaurea petals only succinic, hydroxycinnamic and malonic acids increased with flower development. So, consumers can have edible flowers as a new source of different organic acids, depending on the stage of flowering, as well as, the part of the flower to be consumed (petals or whole flower).

Furthermore, to malic and citric acids, which are important components in centaurea and borage flowers, have been reported that they have protective effects on myocardial ischemia/reperfusion injury (Tang et al., 2013) and antimicrobial activity against some pathogenic microorganisms (Eswaranandam, 2004).

Sugars

The free sugars composition of borage and centaurea at different flowering stages are shown in Table 3.1.2.5. Three free sugars were identified, namely, sucrose, glucose and fructose. Fructose was the major sugar in both flowers at all flowering stages (1.57-10.4 g/100 g dw), except in centaurea petals, in which glucose predominated (7.30 g/100 g dw). Similar results were reported for other flowers by Guimarães et al. (2010) and Pires et al. (2017), who detected fructose as the main sugar in *Rosa micrantha*, dahlia and centaurea petals. Borage presented higher values of all free sugars, being probably associated with its sweet accent. Concerning different flowering stages and part of flower, petals showed the highest values of free sugars in both flowers, except for sucrose in centaurea. Different functions in flower development have been attributed to sugars, namely, energy source (Moalem-Beno et al., 1997), osmotic regulators (Bieleski, 1993) and precursors of metabolic processes. During flower development (from tight bud to fully open), the sucrose and glucose remained constant in borage, while fructose increased from 5.72 to 6.97 g/100 g dw. In centaurea, sucrose also remained constant, while glucose and fructose increased (from 0.91 to 1.74 and 1.57 to 2.71 g/100 g dw).

Table 3.1.2.5 – Soluble sugars and organic acids composition in borage and centaurea at three flowering stages (g/100 g dw)

Organic acids	**Borage**				**Centaurea**			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
Citric acid	$1.73\pm0.05^{d,A}$	$0.99\pm0.01^{c,A}$	$0.92\pm0.02^{b,A}$	$0.52\pm0.02^{a,A}$	$2.76\pm0.04^{d,B}$	$2.20\pm0.12^{c,B}$	$1.88\pm0.08^{b,B}$	$1.09\pm0.06^{a,B}$
Levulinic acid	$0.69\pm0.03^{a,A}$	$1.20\pm0.07^{b,B}$	$1.17\pm0.07^{b,B}$	$1.58\pm0.15^{c,B}$	$0.77\pm0.03^{c,B}$	$0.70\pm0.01^{b,A}$	$0.86\pm0.06^{d,A}$	$0.53\pm0.03^{a,A}$
Fumaric acid	$0.75\pm0.06^{a,B}$	$0.91\pm0.07^{b,B}$	$0.87\pm0.01^{b,B}$	$1.30\pm0.08^{c,B}$	$0.018\pm0.001^{b,A}$	$0.017\pm0.001^{b,A}$	$0.017\pm0.001^{b,A}$	$0.013\pm0.001^{a,A}$
Succinic acid	$0.56\pm0.01^{b,A}$	$0.51\pm0.01^{a,A}$	$0.49\pm0.01^{a,A}$	$0.55\pm0.03^{b,A}$	$1.89\pm0.09^{a,B}$	$2.94\pm0.16^{b,B}$	$3.62\pm0.15^{c,B}$	$9.68\pm0.21^{d,B}$
Malic acid	$2.09\pm0.05^{a,A}$	$2.94\pm0.10^{b,B}$	$2.88\pm0.03^{b,B}$	$4.66\pm0.29^{c,B}$	$2.17\pm0.03^{c,B}$	$2.09\pm0.23^{c,A}$	$1.84\pm0.09^{b,A}$	$1.47\pm0.09^{a,A}$
Salicylic acid	$0.010\pm0.001^{a,A}$	$0.012\pm0.001^{c,A}$	$0.011\pm0.001^{b,A}$	$0.010\pm0.001^{a,A}$	$0.019\pm0.001^{b,B}$	$0.019\pm0.001^{b,B}$	$0.017\pm0.001^{a,B}$	$0.019\pm0.001^{b,B}$
Hydroxycinnamic acid	$0.24\pm0.01^{a,B}$	$0.22\pm0.01^{a,A}$	$0.22\pm0.01^{a,A}$	$0.49\pm0.06^{b,A}$	$0.21\pm0.02^{a,A}$	$0.27\pm0.03^{b,B}$	$0.35\pm0.02^{c,B}$	$0.49\pm0.05^{d,A}$
Malonic acid	$0.055\pm0.006^{a,A}$	$0.057\pm0.002^{a,A}$	$0.053\pm0.002^{a,A}$	$0.064\pm0.003^{b,A}$	$0.77\pm0.04^{a,B}$	$0.73\pm0.03^{a,B}$	$0.70\pm0.07^{a,B}$	$1.13\pm0.02^{b,B}$
Total	6.1	6.8	6.6	9.2	8.6	9.0	9.3	14.4
Soluble sugars								
Sucrose	$3.55\pm0.53^{a,B}$	$4.92\pm0.67^{a,B}$	$3.86\pm0.75^{a,B}$	$9.60\pm1.67^{b,B}$	$1.45\pm0.15^{a,A}$	$1.30\pm0.05^{a,A}$	$1.33\pm0.06^{a,A}$	$1.18\pm0.45^{a,A}$
Glucose	$5.12\pm0.44^{a,B}$	$6.94\pm0.71^{b,B}$	$5.96\pm0.75^{a,b,B}$	$8.85\pm0.15^{c,B}$	$0.91\pm0.10^{a,A}$	$2.10\pm0.04^{b,A}$	$1.74\pm0.07^{b,A}$	$7.30\pm0.46^{c,A}$
Fructose	$5.72\pm0.36^{a,B}$	$7.86\pm0.87^{b,B}$	$6.97\pm0.38^{b,B}$	$10.4\pm0.30^{c,B}$	$1.57\pm0.12^{a,A}$	$2.49\pm0.04^{b,A}$	$2.71\pm0.10^{b,A}$	$3.43\pm0.40^{b,A}$
Total	14.4	19.7	16.8	28.9	3.9	5.9	7.5	11.9

Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$).

Conclusions

In summary, the present work describes, for the first time, the chemical and nutritional composition of two edible flowers, centaurea and borage, at three flowering stages and petals. Both flowers have very low caloric content (28 to 69 kcal/100 g fw), due to their high-water amounts (> 73%). In general, centaurea presented the highest values of energy and total dietary fiber, while borage showed the highest protein, PUFA and total carotenoids contents. During flowering, few changes in the compounds analyzed were observed in both flowers, being the major differences detected between flower and petals. In borage petals the lowest values of protein and total carotenoids were observed.

In conclusion, both edible flowers showed a nutritional composition and compounds with functional properties. Despite the lower amounts of some compounds, the daily consumption of edible flowers may contribute to supply some macronutrients, vitamins and organic acids to the organism. So, these characteristics may encourage the use of edible flowers (whole flower and petals) for human consumption.

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3.1.3.

Nutritional and nutraceutical composition of pansies (*Viola×wittrockiana*) during flower development

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Abstract

Edible flowers consumption and use are an increasing food trend worldwide, although information concerning their nutritional composition and nutraceutical value is still scarce. Thus, the aim of this study was to contribute to the popularization of pansies (*Viola×wittrockiana*), through the analysis of the nutritional and nutraceutical features of pansies with different colors (white, yellow and red) and flowering stages. Both flower type and flowering stage influenced the flower composition. When completely open, white and yellow pansies had the highest contents of protein and fat, while red pansies had the highest content in carbohydrates. During flower development, there was an increase in protein and fat contents in white and yellow pansies, whereas in red pansies the values maintained constant. Regarding the fatty acids profiles, linoleic acid was always predominant, followed by the palmitic and linolenic acids, but during flower development, linolenic acid increased in white and yellow pansies, remaining constant in the red ones. Red pansies presented the highest contents in total carotenoids and monomeric anthocyanins; however, white and yellow pansies showed an increase in the values of total reducing capacity (total phenols), hydrolysable tannins, flavonoids, monomeric anthocyanins and antioxidant activity from the bud to completely open flower stage. Our results underline the nutritional differences

between pansies with different colors at distinct stages of development and their potential health benefits, suggesting that they can be used as ingredient to improve the nutritional properties of foods.

Keywords: Pansies; Flowering stages; Nutritional composition; Fatty acids; Bioactive compounds; Antioxidant activity.

Introduction

The consumption and use of edible flowers has increased in recent years. Supermarkets are beginning to sell flowers and gourmet chefs to use them on their dishes, drinks and deserts. Furthermore, the number of scientific papers regarding this topic has increased compared to the past (Rop et al., 2012; Loizzo et al., 2016). Even though edible flowers can be considered food sources, they have not been sufficiently exploited from the nutritional and health points of view.

Pansies (*Viola×wittrockiana*) from Violaceae family, represents one of the most popular edible flowers. Petals come in a myriad of rainbow pastel colors, often with two or three colors on the same flower (Lim, 2014). The whole flower and buds are edible, although they can have a mild, fresh flavor or a more prominent wintergreen taste, depending on the part of flower (the whole flower tastes stronger than the petals alone). They are added to salads or used as a garnish and to embellish desserts (frosted cakes, sorbets and iced drinks), as well as, crystallized and eaten as a sweet treat. However, in past, pansies have also been used as multipurpose medicinal agents, with some laxative, depurative, expectorant, emetic, alterative, anti-inflammatory, diuretic, sedative, antioxidant and antiseptic properties (Tang et al., 2010). Some of those biological activities can be attributed to their phenolic compounds and carotenoids (Skowyra et al., 2014; Vukics et al., 2008; Gamsjaeger et al., 2011; Rop et al., 2012). Until now, few studies on the physicochemical characterization of pansies have been reported, with some data on antioxidant activity (Carazo et al., 2005, Vukics et al., 2008), mineral composition (Rop et al., 2012), and carotenoids and flavonoids (Gamsjaeger et al., 2011). According to our knowledge, only one work has investigated compositional differences associated with petal colors (red, yellow and violet) (Skowyra et al., 2014). However, no one has characterized pansies at their different flowering stages, as already studied for safflower (*Carthamus tinctorius* L.) (Salem et al., 2011), *Acacia cyclops* (Kotze et al., 2010), and roses species (Dafny-

Yelin et al., 2005, Schmitzer et al., 2010, Sood et al., 2006), focusing on volatiles, phenolics, antioxidant activity and proteins changes during flower development.

This study was conducted to contribute to the popularization of edible flowers as a new and prospective source for the food industry, as well as a promising product for human nutrition. So, the aim of this research was to increase the knowledge in the microscopic structure of petals and in their physicochemical and biological properties (dimensions, aw, pH, flowers' color and weight, nutritional composition, individual fatty acids, carotenoids, flavonoids, hydrolysable tannins, monomeric anthocyanins, total reducing capacity, DPPH radical scavenging activity and reducing power) of pansies with different colors (white, yellow and red) at different flowering stages (bud, half open and flower completely open). Thus, this work aims to understand if there are significant differences between pansies of different colors and at distinctive flowering stages that could results in diverse nutritional impacts for the consumer and different applications for the food or supplements industries.

Material and methods

Samples

Pansies (*Viola×wittrockiana*) of three different colors (white, yellow and red) were obtained from the greenhouse of the School of Agriculture, Polytechnic Institute of Bragança (Portugal). Flowers were harvested at three flowering stages (bud, half-opened flower and completely open flower), being the plants of each colour randomly chosen (Figure 3.1.3.1). After harvest, the flowers were transported to the laboratory. Some analyses were done in the fresh flowers, while others were performed later in flowers preserved by lyophilization (Scanvac, Coolsafe, Lynge, Denmark), ground to homogenous powder and kept at room temperature protected from light.

Microscopic analysis of pansies petals

Fresh petals of different colors were dehydrated by passing them through increasing concentrations of ethanol (70, 80, 90, and 100%) for 4h each and then placed in paraffin to fix. Thin sections of the petals were then cut into random transversal sections, mounted in water and observed on a light microscope equipped with a camera (Fotocamere Leica DFC49012, Heerbrugg, Switzerland). Petals' thickness was measured in different points (minimum 36 times) on three flowers.

Physicochemical analysis

Weight, dimensions, colour, pH and a_w

Ten samples of each flowering stage and colour were weighed in a digital balance (Kern, Balingen, Germany). Axial dimensions, length and width were measured with a digital caliper (0-150 mm) (Powerfix, Leeds, UK).

pH was measured according to the method described by Aquino-Bolaños et al. (2013) and AOAC method 920.149, with some modifications. Briefly, 1g of each sample was mixed with 50 mL of distilled water, boiled for 1 hour, filtered, and the pH measured with a potentiometer (Hanna HI8417, Amorim, Portugal).

Water activity (a_w) was determined in a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland).

Nutritional composition

The nutritional composition (moisture, ash, fat, carbohydrates and dietary fibre) of each sample were analysed following the AOAC procedures (1990), and expressed in g/100 g fresh weight (fw). Moisture content was determined by drying the sample to constant weight at 105 °C; ash content was measured by calcination at 550 °C during at least 2 h, until achieving white ashes. Protein content of the samples was estimated by the macro-Kjeldahl method, with a conversion factor of 6.25, according to Sotelo et al. (2007) and Rop et al. (2012). Lipids were determined by extracting a known weight of powdered sample with petroleum ether with 0.01% BHT (2,6-di-tert-butyl-4-methylphenol) to prevent oxidation, using a Soxhlet apparatus. These samples were preserved for the fatty acid analysis as detailed below. Dietary fiber was determined by an enzymatic-gravimetric method based on AOAC official method No. 985.29 (AOAC, 2003). Carbohydrates were calculated by mass difference. Energy was calculated according to Equation 1:

$$\boldsymbol{Energy}\left(\frac{\boldsymbol{kcal}}{\boldsymbol{100g\ fw}}\right) =$$

$$[(\mathbf{4} \times (\boldsymbol{protein} + \boldsymbol{carbohydrates})) + (\mathbf{9} \times \boldsymbol{lipids}) + (\mathbf{2} \times \boldsymbol{dietary\ fiber})] \quad \text{(Eq. 1)}$$

Nutraceutical composition

Fatty acids

As reported in the nutritional composition section, the lipid fraction had BHT and was stored at -20 °C for fatty acid analysis. Fatty acid methyl esters were obtained by cold hydrolysis with methanolic potassium hydroxide 2M, according to ISO 12966-2 (2011). Fatty acids were determined by gas chromatography (Chrompack, CP-9001 model, Netherlands) with flame ionization detection (GC-FID). Fatty acids separation was carried out on a Select FAME (100 m × 0.25 mm × 0.25 microm) (Agilent, USA) column. Helium was used as carrier gas at a pressure of 190 kPa. The temperatures of the injector and detector were 250 and 260 ºC, respectively. The collection and processing of the data were performed by the CP Maitre Chromatography Data System program, Version 2.5 (Chrompack International B.V., Middelburg, Netherlands). The identification of the chromatographic peaks was confirmed by GC-MS using a similar column on an Agilent chromatograph 7890A, with an MSD 5977B detector (MS source 230ºC; MS Quadropole 150ºC; auxiliary temperature 280ºC and detection in the full scan mode with a m/z of 30 to 800), using analytical standards and the NIST 14 Mass Spectral Library.

Carotenoids

Carotenoids contents were determined according to the method used by Fernandes et al. (2018). One gram of freeze-dried powder samples was extracted twice with 20 mL acetone:hexane solution (1:1, v/v). Both extracts were combined in a separation funnel, being added 200 mL of distilled water to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to eliminate any residual water, being the remaining solution filtered and completed to 100 mL with hexane. Total carotenoids content was determined by reading the absorbance at 450 nm and comparing the results to a β-carotene calibration curve (0.22–8.8 μg/mL). Results were expressed in μg β-carotene equivalent/100 g dw.

Monomeric anthocyanins, total flavonoids, hydrolysable tannins and total reducing capacity

The extraction performed was based on the method described by Li et al. (2014) with slight modifications. Freeze-dried powders (1 g) of each sample were extracted with

50 mL of water:acetone (6:4, v/v) at 37 ºC for 30 min, under agitation (IKA, RCT Model B, Staufen, Germany) at 1000 rpm. The water:acetone extracts were filtered and placed in a rotary evaporator (Stuart, RE300DB, Stone, UK) to remove the acetone. Then, all extracts were frozen and placed in the freeze drier (Coolsafe, Lynge, Denmark) for 2 days. The extracts obtained were redissolved with the same solvent (water:acetone) to a concentration of 50 mg extract/mL and covered with aluminum foil under freezing until further analysis.

The total monomeric anthocyanins, total flavonoids and hydrolysable tannins contents, as well as, the total reducing capacity (TRC) of the edible flowers extracts, were quantified following the methodologies used by Fernandes et al. (2018). All measurements were performed in triplicate. The results for monomeric anthocyanins were expressed in µg cyanidin-3-glucoside/g dry weight (µg Cy 3-glu/g dw), flavonoids in mg of quercetin equivalent/g dry weight (mg QE/g dw), hydrolysable tannins in mg of tannic acid equivalent/g dry weight (mg TAE/g dw) and TRC in mg gallic acid equivalent/g dry weight (mg GAE/g dw).

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activities of the extracts were determined by the procedure described by Fernandes et al. (2018). Pansies extracts diluted solutions (300 µL) were added to 2.7 mL of the DPPH methanolic solution (6.09×10^{-5} mol/L). After 1 hour in the dark, at room temperature, absorbance was read at 517 nm. Antioxidant activity was expressed by the percentage of scavenging effect, according to Equation 2:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \quad \text{(Eq. 2)}$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The extract concentration providing 50% of DPPH radical scavenging effect (EC_{50}) was calculated from the graph of the DPPH radical scavenging effect percentage *versus* extract concentration.

Reducing power

The reducing power of each extract was determined by the procedure described by Fernandes et al. (2018). To 1.0 mL of pansies extract solutions, at different

concentrations, were added 2.5 mL of phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ 1% (m/v). After shaking, the mixtures were incubated at 50 °C for 20 minutes after which 2.5 mL of 10% trichloroacetic acid (m/v) was added with further stirring. A volume of 2.5 mL of the mixture was transferred to another test tube, to which 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1% (m/v) were added. The absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ *versus* concentration, the EC_{50} values were determined corresponding to the extract concentration that gave an absorbance of 0.5.

Statistical analysis

The SPSS Statistical software, v. 18.0 (SPSS Inc., Chicago, IL), was used for the statistical treatment of the data. The normality of the data was verified by Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch were carried out to determine if there were significant differences ($p<0.05$) between samples, depending on the existence or not of homogeneity of variances, respectively. Additionally, if significant differences were detected between treatments, a *post hoc* analysis was performed, namely, the Tukey's honestly significant difference test (if variances in the different groups were identical) or Games-Howell test (if they were not). The homogeneity of the variances was tested by Levene's test.

Results and discussion

Microscopic analysis of pansies petals

Petals of *Viola×wittrockiana* completely open flowers were examined in relation to their structure (Figure 3.1.3.2). Conical papillae in the adaxial epidermis of all petals (A) and parenchyma tissues (B) were found. By observing in more detail this structure, it was detected that the walls of the papillae cells were thin, covered by a layer of cuticle forming parallel striate. The parenchyma tissues was composed by irregular branched cells, with large intercellular air spaces between them. Furthermore, in parenchyma tissues were found stomata (C), vascular bundles located in the middle of the petal (D) and epidermis cells with folded walls (E). No structural differences were observed between the three colours.

Flowering stages		
Bud	Half open	Completely open
		
		
		

Figure 3.1.3.1 - Flowering stages (bud, half open flower and completely open flower) of pansies (*Viola×wittrockiana*) with three colors (white, yellow and red).

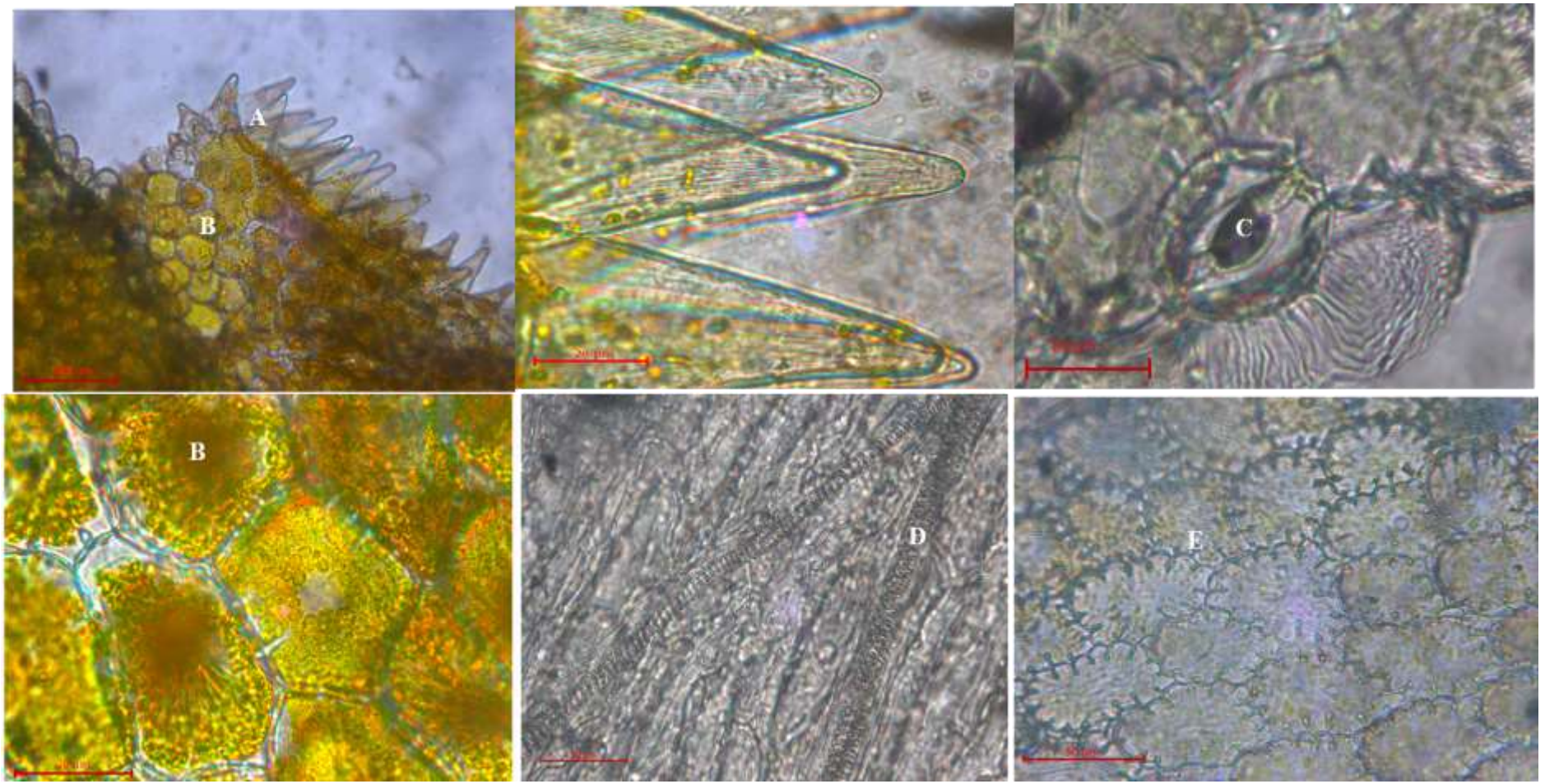

Figure 3.1.3.2 - Structure of pansies petals: A – longitudinal section of the petal with the conical papillae in the adaxial epidermis (A) and parenchyma tissues (B); C - fragment of the epidermis with a stomata; D - branched vascular bundles in the petal mesophyll; E - epidermis cells with folded walls.

Physical analysis

Weights, lengths, widths, pH and water activity (a_w) values for pansies with three colours during flower development are presented in Table 3.1.3.1. As expected, the weight, width and length at the first stage of flowering (bud) showed the lowest values and reached their maximum values when the flowers were completely open. The increase was much visible from the stage of half open flower to completely open flower than from bud to half open flower. With few exceptions, when comparing the three colours, no statistical differences in the weight and dimensions for all flowering stages were detected, suggesting that *Viola×wittrockiana* flowers of different colours have similar dimensions. Damalas et al. (2014) reported lower values of length (18.8 mm) and width (15.2 mm) for *Viola arvensis* flowers; however, this is another species of viola. Regarding pH and a_w, few significant differences between flowering stages, as well as, between pansies of different colours, were detected, but without any trend.

Nutritional composition

The nutritional composition of pansies of different colors at distinct flowering stages is described in Table 3.1.3.2. Water was the main constituent in pansies, ranging between 85.0 and 91.3 g/100 g fw, as has been described by other authors for edible flowers (Rop et al., 2012). Carbohydrates were the second most abundant macronutrients in all samples (3.94 to 8.78 g/100g fw), followed by total dietary fiber (1.50 to 4.66 g/100g fw), proteins (0.95 to 2.06 g /100 g fw) and ash (0.92 to 1.16 g/100 g fw). Fat was the less abundant macronutrient, ranging between 0.37 and 1.31 g/100 g fw, in white bud and the completely open yellow flower, respectively. The caloric energy values varied between 31 to 52 kcal/100g fw, for completely open white flower, and bud and completely open red flower, respectively. Our range of results converted to dry weight (carbohydrates 42.0-55.6 g/100g dw; fiber 17.2-43.2 g/100g dw; protein 9.15-23.17 g/100g dw; fat 4.48-5.21 g/100g dw; ash 6.3-10.5 g/100g dw) is in accordance with the ones described by González-Barrio et al. (2018), who reported similar values of macronutrients (carbohydrates 47.7 g/100g dw; protein 15.4 g/100g dw; fat 3.22 g/100g dw; ash 8.11 g/100g dw) for pansy flowers, as well as by Rop et al. (2012), who detected 6.7 g/100 g dw of protein. Vieira (2013) reported higher protein (16.8 g/100 g dw) and carbohydrates (64.5 g/100g dw) contents, and lower fiber (9.3 g/100g dw) values for pansies.

Concerning flowering stages, no consistent trends were observed between pansies with different colors. An increase in protein and fat contents was observed in white and yellow pansies on the last stage of flower development (completely open flower) while dietary fiber decreased, while in red pansies an increase in carbohydrates content was detected. Barros et al. (2011) also found that there wasn't a similar trend in all macronutrients along the development of *Crataegus monogyna* flower. In fact, they stated that moisture and protein contents decreased, while ash, carbohydrates and fat increased when comparing buds with flowers. A similar trend for protein was found in roses, namely, from the transition of stage 1 (small bud with petals still covered by sepals) to stage 6 (fully open flower at anthesis) that was accompanied by a decrease in protein content of petals (203.2 to 88.6 g/100 g dw) (Dafny-Yelin et al., 2005). So, considerable variations in the nutritional composition may be observed during the development of different flowers, including pansies. For nutritional purposes, generally the completely open flowers presented the highest protein content, but the lowest total dietary fiber. Nevertheless, all flowers at different flowering stages showed low energy contents, without significant differences between them, being suitable for low calorie diets.

Table 3.1.3.1 – Physico-chemical characterization of pansies of three different colors at different flowering stages*

Parameters	Color	Flowering stage		
		Bud	Half open	Completely open
Weight (g)	White	$0.15\pm0.05^{a,A,B}$	$0.20\pm0.05^{a,A}$	$0.62\pm0.12^{b,A}$
	Yellow	$0.18\pm0.04^{a,B}$	$0.20\pm0.07^{a,A}$	$0.63\pm0.15^{b,A}$
	Red	$0.12\pm0.03^{a,A}$	$0.20\pm0.04^{a,A}$	$0.68\pm0.18^{b,A}$
Width (mm)	White	$7.94\pm2.01^{a,A}$	$18.33\pm5.01^{b,B}$	$53.12\pm5.64^{c,B}$
	Yellow	$7.86\pm1.57^{a,A}$	$14.97\pm3.30^{b,A,B}$	$45.98\pm11.42^{c,A}$
	Red	$7.79\pm1.37^{a,A}$	$13.84\pm3.08^{b,A}$	$49.84\pm6.30^{c,A,B}$
Length (mm)	White	$15.86\pm3.61^{a,A}$	$19.28\pm3.79^{a,A}$	$58.38\pm6.18^{b,B}$
	Yellow	$21.02\pm2.17^{a,B}$	$20.46\pm4.43^{a,A}$	$48.24\pm5.45^{b,A}$
	Red	$18.39\pm1.52^{a,A,B}$	$22.00\pm3.22^{a,A}$	$53.69\pm5.48^{b,A,B}$
pH	White	$6.15\pm0.07^{b,A,B}$	$6.10\pm0.08^{a,b,A}$	$6.03\pm0.09^{a,A}$
	Yellow	$6.18\pm0.10^{a,B}$	$6.15\pm0.05^{a,A}$	$6.21\pm0.07^{a,B}$
	Red	$6.08\pm0.06^{a,A}$	$6.32\pm0.13^{b,B}$	$6.01\pm0.08^{a,A}$
a_w	White	$0.985\pm0.001^{a,B}$	$0.985\pm0.001^{a,A}$	$0.982\pm0.001^{a,B}$
	Yellow	$0.984\pm0.001^{b,A,B}$	$0.983\pm0.001^{a,b,A}$	$0.978\pm0.001^{a,A}$
	Red	$0.982\pm0.001^{a,A}$	$0.984\pm0.001^{b,A}$	$0.984\pm0.001^{a,b,B}$

*Values are expressed as mean±standard deviation Lowercase letters compared different flowering stages in the same color flower. Uppercase letters compared the same flowering stage in flowers of different colors.

Table 3.1.3.2 - Nutritional composition of pansies with three different colors and at different flowering stages.

Color	Flowering stage	Moisture	Protein	Fat	Carbohydrates*	Total dietary fiber	Ash	Energy
		(g/100g fw)						(kcal/100g fw)
White	Bud	85.6±0.3^{a,A}	1.05±0.01^{a,A}	0.37±0.02^{a,A}	7.7±0.1^{a,A}	4.5±0.1^{b,A}	1.03±0.07^{a,A}	47±1^{a,A}
	Half open	89.5±7.0^{a,A}	1.10±0.13^{a,A}	0.36±0.09^{a,A}	4.2±1.3^{a,A}	4.5±0.7^{b,A}	1.00±0.03^{a,A}	31±1^{a,A}
	Completely open	91.3±2.1^{a,B}	2.03±0.06^{b,B}	0.45±0.01^{a,A}	3.9±1.9^{a,A}	1.5±0.1^{a,A}	0.92±0.08^{a,A}	31±8^{a,A}
Yellow	Bud	85.6±0.3^{a,A}	0.95±0.14^{a,A}	0.54±0.04^{a,B}	7.1±0.1^{a,A}	4.6±0.1^{b,A}	1.16±0.09^{a,A}	46±2^{a,A}
	Half open	85.3±1.5^{a,A}	0.96±0.01^{a,A}	0.47±0.04^{a,A}	8.8±1.6^{a,A}	4.0±0.1^{a,A}	1.16±0.08^{a,A}	51±6^{a,A}
	Completely open	86.5±0.7^{a,A,B}	2.06±0.03^{b,B}	1.31±0.09^{b,B}	4.8±0.7^{a,A}	4.3±0.1^{b,C}	1.10±0.19^{a,A}	48±4^{a,A}
Red	Bud	85.0±1.7^{a,A}	1.46±0.05^{b,B}	0.75±0.09^{a,C}	7.7±1.9^{a,A}	4.4±0.1^{b,A}	1.08±0.14^{a,A}	52±8^{a,A}
	Half open	85.3±1.8^{a,A}	1.19±0.06^{a,A}	0.80±0.04^{a,B}	6.6±2.3^{a,A}	4.7±0.1^{b,A}	1.02±0.12^{a,A}	48±9^{a,A}
	Completely open	85.1±0.3^{a,A}	1.36±0.03^{b,A}	0.67±0.03^{a,A}	8.0±0.4^{a,A}	3.8±0.2^{a,B}	0.94±0.13^{a,A}	52±1^{a,A}

Values are expressed as: Mean±Standard deviation. fw: fresh weight. Lowercase letters compared different flowering stages in the same color flower. Uppercase letters compared the same flowering stage in flowers of different colors. *Dietary fiber is not included.

Nutraceutical composition

Fatty acids

The fatty acid composition of pansies at different flowering stages and colors is presented in Table 3.1.3.3. Eighteen fatty acids were identified in pansies. The predominant fatty acids were linoleic acid (C18:2n6), followed by palmitic acid (C16:0) and linolenic acid (C18:3n3). Similar results were reported by other authors (Pires et al., 2017; Guimarães et al., 2010). The highest relative amounts of linoleic acid (C18:2n6) were detected in red buds (51.0%), of linolenic acid (C18:3n3) in completely open yellow flowers (23.0%) and of palmitic acid (C16:0) in completely open red flowers (17.4%). The first two fatty acids are essential fatty acids, as they cannot be synthesized by the human organism due to the lack of desaturase enzymes required for their production. Moreover, both were mentioned to reduce some heart disease risk factors, as triglycerides blood pressure and cholesterol profile (Shidfar et al., 2008; Ramel et al., 2010; Miyoshi et al., 2014; Miura et al., 2008; Singer et al., 1990). Furthermore, myristic (C14:0) acid was also detected in high contents in red and yellow pansies, as well as heptadecanoic acid (C17:0) in white pansies. When considering the overall fatty acids profile for pansies, it was found that this flower showed higher values of PUFA and SFA than MUFA. In general, all pansies at the three flowering stages showed PUFA/SFA ratios higher than 0.45 (ranging from 0.46 to 2.13) and n-6/n-3 ratios lower than 4.0 (varying between 0.9 and 4.0), which are recommended for the human diet (HMSO, 1994), helping to ensure that flowers are considered a healthy food.

Concerning flowering stages, significant differences were found (Table 3.1.3.3). In general, the main fatty acids detected in pansies showed different trends during flower development. Linolenic acid (C18:3n3) increased 32.4 and 66.4% in white and yellow pansies, respectively, while palmitic acid (C16:0) increased in red pansies from 14.8% to 17.4% and linoleic acid decreased from 51.0 to 18.7%. Similar trends were observed by other authors in other flower species, particularly, between bud and flower stages of *Crataegus monogyna*, an increase in linolenic acid (26.8 to 29.5%), and a decrease in linoleic acid (15.6 to 14.2%) were observed (Barros et al. 2011). Moreover, in two species of *Opuntia* flowers, from vegetative to full flowering stages, an increase in palmitic (from 38.2 to 43.0% for *Opuntia ficus-indica*; 48.9 to 59.5% for *Opuntia stricta*) and linolenic acids (from 3.7 to 6.2% for *Opuntia ficus-indica*) were reported (Ammar et al., 2014). In general terms, MUFA percentage decreased

from bud to completely open flower in all color pansies (28.4, 66.1 and 31.2% in white, yellow and red pansies, respectively); however, in red pansies SFA increased significantly from 29.9 to 65.5% and PUFA decreased from 63.7 to 30.0%.

Total reducing capacity, total carotenoids, hydrolysable tannins, total flavonoids and monomeric anthocyanins

Total reducing capacity, total carotenoids, hydrolysable tannins, total flavonoids and monomeric anthocyanins contents of pansies with three different colors and at distinct flowering stages are described in Table 3.1.3.4. Quantitative differences in these compounds during flower development and between pansies with different colors have been observed. Regarding the color of the flower, the major differences in values were detected in carotenoids and monomeric anthocyanins, with red pansies showing the highest values of both types of compounds (873-1300 μg β-carotene/g dw) and monomeric anthocyanins (303-402 μg Cy-3 glu/g dw). Regarding total carotenoids, the yellow pansies showed always higher values than white pansies. According to Park et al. (2015), most yellow accents in flowers result from the presence of carotenoids (especially, xanthophylls), whereas anthocyanins are responsible for the most red, blue, and purple colored petals. However, these authors found a "Kastelli" cultivar of chrysanthemum with red colored petals and high concentrations of carotenoids, probably because this cultivar might accumulate reddish carotenoids that are absent in yellow petals. Regarding anthocyanins, Skowyra et al. (2014) reported similar results to ours, mentioning that violanin was the major anthocyanin in the three different colored pansies (red, yellow and violet), highlighting its higher content in red (11.40 mg/g freeze-dried weight) in comparison to yellow (4.69 mg/g freeze-dried weight) petals.

In white, yellow and red pansies, other bioactive compounds showed similar values, namely the TRC, ranging from 5.3 to 21.8 mg GAE/g dw (yellow bud and completely open white flower, respectively); hydrolysable tannins between 19.2 and 55.7 mg TAE/g dw (completely open red and white flowers, respectively) and flavonoids between 40.7 and 124.5 mg QE/g dw (white bud and completely open white flower, respectively).

Concerning flowering stages, significant differences in pansies with different colors were observed. White and yellow pansies showed much similar behaviors than red ones, increasing the values of TRC, hydrolysable tannins and flavonoids from bud to

completely open flower stage. On contrary, in red pansies, opposite trends were observed. The increase observed in TRC values might be related to the accumulation of phenolic compounds during the full-flowering stage that may also be related to ecological functions, such as the intensification of antifungal defenses and the attraction of pollinators (Langenheim, 1994). Our results are in line with those of Bagdonaite et al. (2012), who reported that the bioactive compounds of *Hypericum perforatum* flowers revealed significant differences between two developmental stages (budding and full-flowering stages), being the growth and development of the reproductive parts followed by an increase in the contents of bioactive compounds. Red pansies showed the highest values of TRC (13.8 mg GAE/g dw), hydrolysable tannins (30.1 mg TAE/ g dw) and flavonoids (68.4 mg QE/g dw) in the bud stage, slight decreasing until the completely open flower stage. Regarding carotenoids and monomeric anthocyanins, the highest contents (1300 µg β-carotene/g dw and 402 µg Cy-3 glu/g dw, respectively) were obtained in completely open flowers. These different trends and contents of bioactive compounds during the development of flowers of the same specie with different colors were also found in other flower species as *Carthamus tinctorius* (Salem et al., 2011). For example, in orange flowers of *Carthamus tinctorius* the phenolic compounds increased during the flower development, while in yellow and red flowers they decrease (Salem et al., 2011). However, in all pansies during flower development, the content of total monomeric anthocyanins increased from the bud to completely open flower stage (6.3 to 35.9, 1.5 to 22.9 and 303 to 402 µg Cy-3 glu/g dw in white, yellow and red pansies, respectively). Similar results were found in petals of eight cultivars of *Rosa×hybrida* (Schmitzer et al., 2010). On contrary, Sood et al. (2006) reported an increase in total anthocyanins content in flowers of *Rose damascene* and *Rose bourboniana* at the first stages of flower development, followed by a decrease in the half and fully opened flowers. So, the contents of anthocyanins do not follow a consistent trend (Sakata and Uemoto, 1976; Sood et al., 2006).

Table 3.1.3.3 – Fatty acids composition (g fatty acid/100g fatty acids) of the oils extracted from pansies of different colors at distinct flowering stages.

Fatty acid	White			Yellow			Red		
	Bud	Half open	Completely open	Bud	Half open	Completely open	Bud	Half open	Completely open
SFA									
C12:0	2.0±2.5^{a,A}	1.1±0.3^{a,A}	1.1±0.3^{a,A}	3.2±0.7^{a,A}	4.2±0.9^{a,B}	16.1±5.4^{b,B}	2.2±0.3^{a,A}	4.9±3.2^{a,B}	23.4±0.9^{b,C}
C14:0	2.2±1.4^{a,A}	6.9±4.0^{b,A}	5.0±0.9^{a,b,A}	13.8±6.4^{a,B}	14.8±5.8^{a,B}	11.8±5.6^{a,B}	5.0±0.7^{a,A}	8.6±1.5^{b,A,B}	17.8±0.4^{c,C}
C15:0	0.4±0.4^{a,A}	0.2±0.1^{a,A}	0.2±0.3^{a,A}	0.3±0.2^{a,A}	0.5±0.4^{a,A}	0.2±0.2^{a,A}	nd	0.4±0.4^{a,A}	0.3±0.1^{a,A}
C16:0	15.0±2.1^{a,A}	15.4±1.9^{a,A}	16.6±3.7^{a,A}	16.6±0.3^{a,A}	17.0±2.3^{a,A}	16.5±1.0^{a,A}	14.8±1.1^{a,A}	14.7±2.1^{a,A}	17.4±0.6^{b,A}
C17:0	12.3±9.4^{a,B}	8.9±2.9^{a,A,B}	14.9±3.1^{a,B}	2.6±0.7^{c,A}	1.4±0.2^{b,A}	0.5±0.2^{a,A}	1.2±0.3^{a,A}	12.0±9.8^{b,B}	1.0±0.6^{a,A}
C18:0	3.0±0.4^{a,b,A}	4.5±1.7^{b,A}	2.4±1.2^{a,A}	3.2±1.6^{a,A}	4.2±0.8^{a,A}	2.9±0.5^{a,A}	3.0±0.2^{a,A}	3.8±0.5^{b,A}	3.6±0.2^{b,B}
C20:0	0.6±0.6^{a,A}	1.2±0.4^{b,A}	nd	1.0±0.5^{a,b,A}	1.3±0.2^{b,A}	0.5±0.4^{a,A}	1.0±0.2^{a,A}	0.6±0.7^{a,A}	0.4±0.2^{a,A}
C22:0	1.6±0.4^{b,A}	2.1±0.8^{b,A}	0.6±0.5^{a,A}	1.8±0.5^{b,A}	2.0±0.5^{b,A}	1.0±0.2^{a,A}	1.3±0.2^{b,A}	1.2±0.7^{a,b,A}	0.7±0.1^{a,A}
C24:0	2.7±3.6^{a,A}	2.3±1.2^{a,A}	1.5±0.6^{a,A}	2.1±1.2^{a,A}	2.0±1.1^{a,A}	1.4±1.2^{a,A}	1.4±0.4^{a,b,A}	2.4±1.2^{b,A}	1.0±0.1^{a,A}
MUFA									
C16:1n7	nd	0.6±0.3^{A}	nd	0.3±0.2^{a,A}	0.2±0.2^{a,A}	0.3±0.2^{a}	0.3±0.1^{A}	nd	nd
C18:1n9	6.8±1.6^{a,b,A}	7.8±1.5^{b,B}	5.3±1.4^{a,B}	10.8±3.4^{b,B}	11.5±2.5^{b,C}	3.6±0.7^{a,A}	6.2±2.0^{b,A}	3.6±0.6^{a,A}	4.4±0.5^{a,b,A,B}
PUFA									
C18:2n6	37.4±5.8^{a,B}	33.6±1.5^{a,A}	33.2±3.8^{a,B}	29.1±1.6^{a,A}	25.6±11.3^{a,A}	21.9±1.9^{a,A,B}	51.0±3.9^{c,C}	32.8±5.6^{b,A}	18.7±0.7^{a,A}
C18:3n3	14.6±1.7^{a,A}	14.3±0.5^{a,A}	19.3±3.6^{b,B}	13.8±3.0^{a,A}	15.4±2.2^{a,A}	23.0±1.5^{b,C}	12.7±3.1^{a,A}	14.1±3.0^{a,A}	11.0±0.3^{a,A}
C18:4n3	0.2±0.4^{A}	nd	nd	1.1±1.2^{a,A}	nd	0.4±0.3^{a,A}	nd	0.8±1.0^{a}	0.4±0.3^{a,A}
SFA	39.9	42.7	42.2	44.5	47.3	50.8	29.9	48.6	65.5
MUFA	7.4	9.1	5.3	11.5	11.7	3.9	6.4	3.6	4.4
PUFA	52.7	48.2	52.5	44.0	41.0	45.3	63.7	47.7	30.0
PUFA/SFA	1.32	1.13	1.24	0.99	0.87	0.89	2.13	0.98	0.46
n6/n3	2.6	2.3	1.7	2.1	1.7	0.9	4.0	2.3	1.7

ND - not detected; Mean ± standard deviation. (n=3). Lowercase letters compared different flowering stages for the same color flower. Uppercase letters compared flowers with different colors at the same flowering stage.

Table 3.1.3.4 - Nutraceutical composition of dried pansies of three different colors at different flowering stages

Color	**Flowering stage**	**TRC** (mg GAE/g dw)	**Total carotenoids** (µg β-carotene /g dw)	**Hydrolyzable tannins** (mg TAE/g dw)	**Flavonoids** (mg QE/g dw)	**Monomeric anthocyanins** (µg Cy-3 glu/g dw)	**DPPH** EC_{50} (mg/ml)	**Reducing Power** EC_{50} (mg/ml)
White	Bud	$7.3\pm0.6^{a,B}$	$404\pm30^{c,A}$	$21.5\pm1.4^{a,A}$	$40.7\pm1.6^{a,A}$	$6.3\pm0.3^{a,A}$	$0.43\pm0.01^{b,B}$	$0.92\pm0.02^{c,B}$
	Half open	$13.0\pm0.6^{b,B}$	$278\pm8^{b,A}$	$35.5\pm2.5^{b,B}$	$77.7\pm1.0^{b,C}$	$11.1\pm0.7^{b,A}$	$0.38\pm0.02^{a,B}$	$0.84\pm0.01^{b,B}$
	Completely open	$21.8\pm1.1^{c,C}$	$132\pm6^{a,A}$	$55.7\pm5.0^{c,C}$	$124.5\pm4.0^{c,C}$	$35.9\pm2.9^{c,A}$	$0.38\pm0.01^{a,C}$	$0.67\pm0.01^{a,C}$
Yellow	Bud	$5.3\pm0.8^{a,A}$	$1073\pm28^{c,B}$	$26.5\pm1.2^{a,A,B}$	$47.3\pm1.0^{b,B}$	$1.5\pm0.1^{a,A}$	$0.48\pm0.01^{c,C}$	$1.71\pm0.02^{c,C}$
	Half open	$7.6\pm0.5^{b,A}$	$804\pm30^{b,B}$	$23.7\pm2.7^{a,A}$	$42.2\pm0.6^{a,A}$	$3.1\pm0.6^{b,A}$	$0.30\pm0.01^{b,A}$	$0.99\pm0.01^{b,C}$
	Completely open	$13.3\pm1.1^{c,B}$	$576\pm36^{a,B}$	$42.8\pm4.0^{b,B}$	$82.5\pm0.7^{c,B}$	$22.9\pm0.8^{c,A}$	$0.20\pm0.01^{a,A}$	$0.58\pm0.01^{a.A}$
Red	Bud	$13.8\pm1.2^{b,C}$	$1133\pm36^{b,C}$	$30.1\pm4.7^{b,B}$	$68.4\pm5.0^{b,C}$	$303\pm24^{a,B}$	$0.17\pm0.01^{a,A}$	$0.48\pm0.01^{a,A}$
	Half open	$12.5\pm0.5^{a,b,B}$	$873\pm12^{a,C}$	$20.4\pm0.5^{a,A}$	$51.5\pm1.1^{a,B}$	$353\pm44^{a,b,B}$	$0.34\pm0.02^{c,A}$	$0.73\pm0.01^{c,A}$
	Completely open	$11.6\pm1.1^{a,A}$	$1300\pm26^{c,B}$	$19.2\pm1.6^{a,A}$	$47.9\pm4.0^{a,A}$	$402\pm42^{b,B}$	$0.26\pm0.01^{b,B}$	$0.64\pm0.01^{b,B}$

*Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages for the same colour flower. Uppercase letters compared flowers of different colours at the same flowering stage.

Antioxidant activity

The antioxidant activity of pansies was determined in flowers with different colors and at three flowering stages, using the DPPH radical scavenging activity and reducing power assays (Table 3.1.3.4). Bud red pansies showed the lowest EC_{50} values (0.17 mg/mL for DPPH and 0.48 mg/mL for reducing power), indicative of a higher antioxidant activity. This might be related to the fact that the buds of red pansies had a high content of phenols (predicted by TRC assay), major compounds responsible for the antioxidant activity of plant materials (Zhao et al., 2014). Yellow and white pansies showed an increase in the antioxidant activity trend from the bud to completely open stage (lower EC_{50} values), accompanied by an increase in total phenols content. Comparing pansies of different colors, no trends were observed. In completely open flowers, yellow pansies showed the highest antioxidant activity, followed by red and white. Different results have been reported by Skowyra et al. (2014), who detected higher values of antioxidant activity for red pansies than yellow ones, independently of the solution used in the extraction.

Conclusion

The present work demonstrated that there are significant changes in the nutritional and nutraceutical compositions of pansies with different colors during flower development. In white, yellow and red pansies, water was the main macronutrient, followed by carbohydrates, proteins and ash, being appropriate to low calorie diets. During flower development, it was observed an increase in protein and fat contents in white and yellow pansies, whereas in red pansies the values remained constant. PUFA and SFA predominated, mainly due to the contribution of linoleic, linolenic and palmitic acids. Red pansies showed always the highest total carotenoids and monomeric anthocyanins contents; however, in white and yellow pansies the TRC, hydrolysable tannins, flavonoids and monomeric anthocyanins increased from the bud to completely open flower stage. So, this study helped to increase the knowledge in pansies flowering behavior, although more studies are necessary to improve the information on other edible flowers already consumed.

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3.1.4.

Borage, calendula, cosmos, Johnny Jump up and pansy flowers: volatiles, bioactive compounds and sensory perception

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Abstract

The aim of the present work was to study the main volatile and bioactive compounds (monomeric anthocyanins, hydrolysable tannins, total flavonoids and total reducing capacity) of five edible flowers: borage (*Borage officinalis*), calendula (*Calendula arvensis*), cosmos (*Cosmos bipinnatus*), Johnny Jump up (*Viola tricolor*) and pansies (*Viola* × *wittrockiana*), together with their sensory attributes. The sensory analysis (10 panelists) indicated different floral, fruity and herbal odors and taste. From a total of 117 volatile compounds (SPME-GC-MS), esters were most abundant in borage, sesquiterpenes in calendula, and terpenes in cosmos, Johnny Jump up and pansies. Some bioactive and volatile compounds influence the sensory perception. For example, the highest content of total monomeric anthocyanins (cosmos and pansies) was associated with the highest scores of colors intensity, while the floral and green fragrances detected in borage may be due to the presence of ethyl octanoate and 1-hexanol. So, the presence of some volatiles and bioactive compounds affects the sensory perception of the flowers.

Keywords: Edible flowers; Volatile compounds, Sensory analysis; Bioactive compounds

Introduction

Edible flowers are becoming more popular in recent years due to the interest of consumers and professional chefs. Flowers not only look great, but also add color, aroma and flavor to drinks and dishes. It is known that fragrance/aroma and appearance are attributes that affect consumers' preferences, being important quality factors. Usually, flavors and fragrances of flowers are analyzed through their volatile essential oils [1]. Currently there are some studies that have applied solid-phase microextraction (SPME) method to analyze volatile compounds in fresh flowers [2-5], having a more clear perception of the real flower volatiles by avoiding the interferences of newly formed compounds induced by extraction condition or enzymatic action. Edible flowers have a complex flavor, without single compound that accounts for a distinctively flavor. The characteristic flavor of a flower is mainly due to the association of several volatile constituents, which are mostly made up of terpenes, esters, alcohols, carbonyls and alkane compounds [2,4,6]. However, there are others nonvolatile chemical constituents, such as phenolic compounds, sugars and organic acids, that have a variable impact on the volatility of aroma compounds and taste and consequently on their sensory perception [7]. Thus, the present work had two objectives. The first one was to quantify the main bioactive compounds (monomeric anthocyanins, flavonoids, total reducing capacity (TRC) and hydrolysable tannins) and volatile compounds by headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME-GC-MS) present in five common fresh edible flowers (borage, pansies, Johnny Jump up, calendula and cosmos), together with their organoleptic appreciation. The second objective was to determine possible relationships between the volatile and phenolic compounds identified in the five flowers with the tasters' sensory perception.

Materials and Methods

Samples

White borage (*Borage officinalis*), yellow calendula (*Calendula arvensis),* purple cosmos (*Cosmos bipinnatus),* purple Johnny Jump up (*Viola tricolor*) and red pansies (*Viola* × *wittrockiana*) flowers were obtained from a Portuguese store, located in the Northeast of Portugal, that sells edible flowers. In Figure 3.1.4.1 the five studied

flowers are presented. Around 20 g of each flower was used to perform the analyses described below.

Volatile compounds

Headspace solid-phase microextraction (HS-SPME) fibers

For the HS-SPME a fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 μm) was selected, based on a preliminary assay conducted with other fiber (PDMS; 100 μm), both from Supelco (Bellefonte, USA). The selection of the fiber was based on the highest qualitative (number of volatiles extracted) and quantitative data (peak areas) obtained for a sample (data not shown).

HS-SPME

The HS-SPME was carried out according to the methodology applied by Almeida et al. [8], with some modifications. Firstly, fresh petals of borage, calendula and cosmos (0.20-0.30 g), whole pansy flowers and four flowers of Johnny Jump up (0.70 – 1.00 g) (cut a few mm below the calix, being the pedicels wrapped in aluminum foil to minimize water loss) were placed in 50 ml vials (except pansies in 100 ml vials). Then, 4-methyl-2-pentanol was added as internal standard (10 ppm in methanol), being the vials immediately sealed with a polypropylene cap with silicon septum. The volatiles were released at room temperature (± 25 °C) during 5 min. After that, the 30/50 μm DVB/CAR/PDMS fiber was exposed during 30 min. at room temperature for volatiles adsorption, and then inserted into the injection port of the GC system for thermal desorption and reconditioning (10 min at 280 ºC). For each sample of flower the HS-SPME analysis was performed in quintuplicate.

Gas chromatography - mass spectrometry (GC-MS) analysis

A Shimadzu GC-2010 Plus gas chromatographer equipped with a mass spectrometer Shimadzu GC/MS-QP2010 SE detector was used for volatiles determination. A TRB-5MS (30 m × 0.25 mm × 0.25 μm) column (Teknokroma, Spain) was used. The injector was set at 220 °C and the manual injections were made in splitless mode, with helium (Praxair, Portugal) at a linear velocity of 30 cm/s and a total flow of 24.4 mL/min as mobile phase. The oven temperatures were the following: 40 °C (1 min); 2 °C/min until 220 °C (30 min). The ionization source was maintained at 250 °C with ionization energy of 70 eV, and with an ionization current of 0.1 kV. All mass spectra

were acquired by electron ionization in the m/z 35-500 range. The full scan MS spectra fragments were compared with those obtained from a database (NIST 11), and with those of commercial standards acquired from diverse producers (see Table 3.1.4.1). For qualitative purposes, the areas of the chromatographic peaks were determined integrating the re-constructed chromatogram from the full scan chromatogram using for each compound the ion base (m/z intensity 100 %). For semi-quantification purposes, volatile amounts were calculated by the ratio of each individual base ion peak area to the area of the internal standard base ion peak area and converted to mass equivalents on the basis on the internal standard mass added.

Bioactive compounds

Extraction

Fresh samples were extracted with water:acetone (6:4; v/v), at 37 ºC, for 30 min under agitation (IKA, RCT Model B, Staufen, Germany) at a frequency of 1000 rpm, following the methodology used by Li et al. [9]. The extracts were filtered, concentrated in the rotary evaporator (Stuart RE3022C, Staffordshire, United Kingdom), frozen at -18ºC and lyophilized (48h, Coolsafe, Lynge, Denmark). The obtained extracts were redissolved in water:acetone (6:4; v/v) to a concentration of 50 mg extract/mL and preserved under freezing until further analysis. Each extraction treatment was performed in triplicate.

Monomeric anthocyanins, total flavonoids, hydrolysable tannins and total reducing capacity

The total monomeric anthocyanins, total flavonoids and hydrolysable tannins contents, as well as the total reducing capacity (TRC) of the edible flowers extracts were determined following the methodologies used by Fernandes et al. [10]. All measurements were performed in triplicate. The results for monomeric anthocyanins were expressed in mg cyanidin-3-glucoside/g dried weight (mg Cy 3-glu/g DW), flavonoids in mg of quercetin equivalent/g dried weight (mg QE/g DW), hydrolysable tannins in mg of tannic acid equivalent/g dried weight (mg TAE/g DW) and TRC in mg gallic acid equivalent/g dried weight (mg GAE/g DW).

Sensory analysis

For sensory analysis, ten tasters (6 females and 4 males, from 29 to 45 years), from the Polytechnic Institute of Bragança, Portugal (including teachers, students and other staff) agreed to participate in the evaluation sessions. The panel is subject to periodic training and updates in sensory analysis, especially for the evaluation of olive oils, cheeses and table olives. In order to evaluate the sensory profile of the five flowers under study in the present work, the flowers were examined without any condiments, bread, crackers, etc. After a careful evaluation of the perceived flavors (minimum three flowers of each species/ panelist), the tasters were asked to fill out a questionnaire aimed at determining the performances of the edible flowers. Different organoleptic characteristics (color, spiciness, sweetness, astringency, bitterness, taste and odor) were included in the evaluation scheme and were expressed in a scale of 1–10.

Statistical analysis

The SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. The normality and variance homogeneity were checked by Shapiro–Wilk and Levene tests, respectively. As the data followed a normal distribution, analyses of variance (ANOVA) or ANOVA Welch were carried out to evaluate if there were significant differences ($p < 0.05$) between samples. ANOVA was applied when homogeneity of variances was observed, while ANOVA Welch was applied for the other cases. Additionally, significant post hoc analyses were performed (Tukey HSD test if variances in the different groups were identical or Games-Howell test if they were not). All analyses were performed in triplicate.

Results and Discussion

Volatile compounds

The volatile composition of the five edible flowers is described in Table 3.1.4.1. In total, 117 volatile compounds were identified, belonging to different chemical classes. Calendula flowers presented the highest number of identified compounds (62), followed by Johnny Jump up (42), pansy (34) and cosmos (29). Borage showed the lowest diversity of compounds identified (24). The volatile compounds were distributed by seven chemical classes, namely alcohols (7); aldehydes (8); aliphatic hydrocarbons (4); esters (25); ketones (4); sesquiterpenes (32); and terpenes (36).

In general terms, the five edible flowers analyzed showed differences in the volatile profiles considering qualitative and quantitative results. Terpenes were the major chemical class in almost all edible flowers studied (values between 55.7 and 1494 µg/ 100 g flower). This was expected because these compounds have important roles in plants, such as pollinator attraction, direct and indirect defense against insects, bacteria, fungi, and in intra/inter-plant signaling [11]. Mazza and Cottrell [6] also observed that terpenes were the most identified volatile compounds (82-91%) for flowers and stems of three *Echinacea* species. Flamini et al. [12] also detected monoterpenes in high proportion, namely 90.5 and 93.3% in whole flowers and petals of *Citrus deliciosa*, respectively. Within the 36 terpenes identified, limonene was detected in all studied flowers. However, the most abundant identified terpene was different for each edible flower. In more detail, for borage and cosmos the main terpene was ρ-cymene; in calendula it was α-thujene followed by α-pinene; in Johnny Jump up it was β-myrecene followed by limonene; and in pansy it was limonene.

Esters were the second chemical class in terms of diversity in borage (16 compounds) and Johnny Jump up (9 compounds) flowers. Between the esters identified, ethyl benzoate was the most abundant in both flowers (832 and 24.0 µg/100 g flower, respectively). It is reported that ethyl benzoate has a pleasant odor that could be described similar to wintergreen or mint, and it is frequently used in pharmacy, cosmetic and food industry [13].

Sesquiterpenes were the second chemical class most abundant in calendula concerning the number of the compounds quantified, being 28 compounds detected, but seven of those were just tentatively identified as sesquiterpene-like compounds. α-Caryophyllene (118 µg/100 g flower) was the major sesquiterpene, followed by δ-cadinene (31.5 µg/100 g flower), β-caryophyllene (26.4 µg/100 g flower) and γ-muurolene (22.6 µg/100 g flower). In the other flowers, β-caryophyllene was always detected, except in borage; and it was the most abundant in cosmos and pansy. On contrary, α-caryophyllene was the most abundant sesquiterpene in Johnny Jump up flowers. This particular compound is one of the 12 most common volatile compounds detected in floral scents. Furthermore, some sesquiterpenes were only detected in one flowers' species, such as: caryophyllene oxide in Johnny Jump up, longifolene in pansies and α-guaiene in cosmos. In calendula, several sesquiterpenes were detected solely in this flower, such as β-bourbonene and α-muurolene.

Some alcohols were also identified, such as 1-hexanol and (*Z*)-3-hexen-1-ol, the most abundant. (Z)-3-Hexen-1-ol is produced in small amounts by the plants and it acts as an attractant to many predatory insects [14].

Aldehydes were present in flowers in small amounts. Nonanal was the most abundant in cosmos and borage, phenylacetaldehyde in Johnny Jump ups and (*E*)-2-octenal in pansies. Nonanal is an attractant for some insects and repellent to others depending on its concentration [15-17].

Ketones and aliphatic hydrocarbons were the compounds with lower representativeness (number of compounds identified) in the edible flowers studied, although Johnny Jump up and pansies were the ones that presented the major number of aliphatic hydrocarbons and ketones, respectively. Among ketones, 6-methyl-5-hepten-2-one was the only compound identified in three flowers (cosmos, Johnny Jump ups and pansies) but at low levels. This compound is formed from the degradation of carotenoids and reported to contribute to off-flavors [18]. Dodecane was the aliphatic hydrocarbon present in all studied flowers, except in borage. Regarding other compounds, veratrole was detected in Johnny Jump up and ρ-xylene in cosmos and pansies

Edible flowers	
Borage (*Borago officinalis* L.)	
Johnny Jump up (*Viola tricolor*)	

Pansies
(*Viola* × *wittrockiana*)

Calendula
(*Calendula arvensis*)

Cosmos
(*Cosmos bipinnatus*)

Figure 3.1.4.1 – Visual appearance of the five edible flowers.

Table 3.1.4.1- Volatile profile of five edible flowers, expressed in µg/100 g of flower (mean ± standard deviation).

Chemical class	Compound	Sensory description	LRI[a]	LRI lit[b]	QI (m/z)[c]	ID[d]	Edible flowers* (µg/100 g of flower)				
							Borage	Calendula	Cosmos	Johnny Jump up	Pansy
Alcohols	(*Z*)-3-Hexen-1-ol	Moldy, earthy	867	859	67	S/MS	n.d.	32.8±14.5	n.d.	14.1±3.0	n.d.
	1-Hexanol	Fruity, floral, herbal, sweet	871	870	56	S/MS	85.5±22.9	n.d.	n.d.	6.5±1.7	2.83±2.89
	1-Octen-3-ol	Herbal, spicy carrot	980	979	57	S/MS	67.8±8.0	n.d.	n.d.	n.d.	n.d
	2-Ethyl-1-hexanol	Citrus, green, rose,	1032	1033	57	MS	29.4±5.4	n.d.	n.d.	n.d.	n.d.
	(*E*)-2-Octen-1-ol	Green	1071	1066	57	MS	n.d.	n.d.	n.d.	n.d.	0.53±0.31
	1-Octanol	Floral, herbal, green, fatty	1073	1068	41	S/MS	n.d.	n.d.	n.d.	n.d.	0.53±0.30
	2-Phenylethanol	Floral, spicy, honey, lilac, rose	1107	110	91	MS	n.d.	n.d.	n.d.	7.0±2.2	n.d.
	Σ of alcohols						183	32.8	n.d.	27.6	3.89
Aldehydes	Hexanal	Fruity, herbal, grassy	798	801	44	S/MS	n.d.	7.94±5.17	n.d.	n.d.	n.d.
	(*E*)-2-Hexenal	Green, apple-like	859	859	41	S/MS	n.d.	3.06±0.84	n.d.	0.76±0.20	n.d.
	Benzaldehyde	Fruity, woody almond, burnt sugar	958	960	77	S/MS	n.d.	n.d.	n.d.	3.83±1.49	n.d.
	Decanal	Floral, , green, fatty, lemon, orange peel	1204	1203	43	S/MS	n.d.	0.42±0.16	n.d.	n.d.	0.16±0.06
	Phenylacetaldehyde	Floral, hyacinth	1041	1042	91	S/MS	n.d.	n.d.	n.d.	18.8±10.8	n.d.
	(*E*)-2-Octenal	Fresh cut grass	1058	1054	41	MS	n.d.	n.d.	n.d.	n.d.	4.09±1.60
	Nonanal	Citrus, floral, fruity, lavender, melon	1102	1102	57	S/MS	3.58±0.60	1.98±0.78	10.0±2.97	n.d.	1.80±0.85
	Octanal	Fruity, floral,	1073	1068	41	S/MS	n.d.	n.d.	n.d.	0.19±0.05	1.57±0.60

	Σ of aldehydes	citrus, fatty,					3.58	13.4	10	23.6	7.62
Aliphatic Hydrocarbons	Undecane	---	1103	1100	57	MS	n.d.	n.d.	n.d.	0.39±0.24	n.d.
	Dodecane	Alkane, fusel	1201	1200	57	MS	n.d.	0.91±0.26	1.81±0.25	0.39±0.05	0.85±0.38
	Tetradecane	Alkane	1401	1400	57	MS	n.d.	n.d.	1.16±0.41	1.09±0.18	0.54±0.11
	Pentadecane	Alkane	1499	1500	57	MS	n.d.	n.d.	n.d.	0.20±0.05	n.d.
	Σ of aliphatic hydrocarbon							0.91	2.97	2.07	1.39
Esters	Butyl 2-methybutanoate	Green	799	804	71	MS	n.d.	n.d.	n.d.	2.32±1.37	n.d.
	Ethyl butanoate	Fruity	853	854	57	MS	477±71	n.d.	n.d.	n.d.	n.d.
	Methyl 2-methylbutanoate	Fruity	853	854	57	S/MS	84.8±9.9	n.d.	n.d.	n.d.	n.d.
	Ethyl isovalerate	Fruity	858	858	88	MS	n.d.	n.d.	n.d.	n.d.	8.99±4.43
	Ethyl pentanoate	Fruity	902	901	88	MS	24.9±3.8	n.d.	n.d.	n.d.	n.d.
	Ethyl 2 methylbutanoate	Acidic	942	948	55	MS	24.1±4.8	n.d.	n.d.	n.d.	n.d.
	Ethyl hexanoate	Fruity	999	997	88	MS	101±10	9.43±2.01	1.41±0.13	1.03±0.16	n.d.
	Hexenyl acetate	Green	1007	1005	43	S/MS	n.d.	n.d.	n.d.	3.08±0.94	n.d.
	Hexyl acetate	Fruity, herbal, citrus, green,, spicy	1016	1009	43	S/MS	n.d.	n.d.	7.91±0.66	n.d.	85.2±47.8
	Methyl benzoate	Wintergreen, almod floral	1091	1090	105	MS	19.3±7.3	n.d.	n.d.	n.d.	n.d.
	Diethyl malonate	Fruity	1091	1090	115	MS	13.8±4.3	n.d.	n.d.	n.d.	n.d.
	Ethyl heptanoate	Fruity	1098	1093	88	MS	34.1±6.5	n.d.	n.d.	0.59±0.13	n.d.
	Ethyl benzoate	Fruity, chamomile, minty, lavender, melon	1168	1168	105	MS	832±132	n.d.	n.d.	24.0±6.0	n.d.
	Hexenyl butanoate	Green	1187	1186	67	MS	3.41±0.50	n.d.	n.d.	n.d.	n.d.
	Ethyl-(4*E*)-octenoate	Fruity	1189	1186	55	MS	1.59±0.36	n.d.	n.d.	n.d.	n.d.
	Methyl salicylate	Minty, sweet	1189	1191	120	MS	n.d.	n.d.	n.d.	7.63±3.13	2.57±1.01
	Hexyl butanoate	Apple peel	1192	1192	43	MS	9.37±1.16	n.d.	n.d.	n.d.	n.d.

	Ethyl octanoate	Fruity, floral, green, anise, sweet	1197	1197	88	MS	40.8±7.3	4.82±2.45	n.d.	2.09±0.29	n.d.
	Thymol methyl ether	Herbal	1235	1235	149	MS	n.d.	1.07±0.39	1.66±0.19	n.d.	n.d.
	Hexyl 2-methylbutanoate	Green	1240	1236	103	MS	n.d.	n.d.	0.55±0.09	4.22±0.91	n.d.
	Ethyl salicylate	Wintergreen, mint	1267	1269	120	MS	n.d.	n.d.	n.d.	16.9±2.53	1.07±0.25
	Ethyl nonanoate	Waxy	1296	1296	88	MS	49.6±16.0	n.d.	n.d.	n.d.	n.d.
	Ethyl decanoate	Fruity, grape, waxy	1395	1395	88	MS	9.99±2.94	n.d.	n.d.	n.d.	n.d.
	Gerany acetone	Floral	1452	1455	43	MS	n.d.	n.d.	1.78±0.61	n.d.	n.d.
	(*E*)-Ethyl cinnamate	Honey, cinnamon	1460	1460	131	MS	7.93±2.21	n.d.	n.d.	n.d.	n.d.
	Σ of esters						1734	15.3	13	59.5	97.8
Ketones	3-Octanone	Soap, gasoline	986	983	43	S/MS	26.9±6.53	n.d.	n.d.	n.d.	n.d.
	3-Heptanone	Soap	887	890	57	MS	n.d.	n.d.	n.d.	n.d.	13.5±10.9
	6-Methyl 5-hepten-2-one	Blackcurrant, boiled fruit, citrus, pepper, woody	988	986	43	S/MS	n.d.	n.d.	4.82±1.49	0.91±0.31	7.46±2.77
	2-Nonanone	Fruity, green. Baked, fatty	1089	1097	43	S/MS	n.d.	n.d.	n.d.	n.d.	8.79±4.42
	Σ of ketones						26.9	n.d.	4.82	0.91	29.8
Sesquiterpenes	δ-Elemene	Woody	1335	1338	121	MS	n.d.	2.36±0.58	n.d.	n.d.	n.d.
	α-Cubebene	Herbal, wax	1348	1348	161	MS	n.d.	6.31±1.61	n.d.	n.d.	n.d.
	α-Ylangene	-----	1369	1375	105	MS	n.d.	1.77±1.04	n.d.	n.d.	n.d.
	α-Copaene	Woody, spicy	1374	1376	161	MS	n.d.	17.7±3.06	n.d.	n.d.	n.d.
	Sesquiterpene-like compound 1		1384		81	MS	n.d.	1.19±0.38	n.d.	n.d.	n.d.
	β-Bourbonene	Herbal	1381	1385	81	MS	n.d.	21.5±6.5	n.d.	n.d.	n.d.
	β-Copaene	Woody	1387	1391	161	MS	n.d.	9.73±1.77	n.d.	n.d.	n.d.
	β-Cubebene	Citrus, fruity	1387	1391	161	MS	n.d.	3.69±0.92	n.d.	n.d.	n.d.
	Sesquiterpene-like		1392		105	MS	n.d.	3.11±1.58	n.d.	n.d.	n.d.

compound 2										
Longifolene	Woody	1398	1400	161	MS	n.d.	n.d.	n.d.	n.d.	0.20±0.05
α-Gurjunene	Woody, balsamic	1405	1409	204	MS	n.d.	5.90±3.37	n.d.	n.d.	n.d.
α-Caryophyllene	Floral, woody	1415	1419	93	MS	n.d.	118±26	n.d	4.23±0.05	n.d.
Sesquiterpene-like compound 3		1425		120	MS	n.d.	7.70±2.84	n.d.	n.d.	n.d.
(*E*)-α- -bergamotene	Woody, warm, tea	1434	1434	93	MS	n.d.	0.66±0.30	n.d.	n.d.	n.d.
α-Guaiene	Woody, spicy	1440	1439	105	MS	n.d.	n.d.	0.40±0.08	n.d.	n.d.
Aromadendrene	Woody	1441	1441	161	MS	n.d.	0.37±036	n.d.	n.d.	n.d.
Sesquiterpene-like compound 4		1443		161	MS	n.d.	3.11±1.86	n.d.	n.d.	n.d.
β- Caryophyllene	Woody, spicy, sweet	1449	1454	93	S/MS	n.d.	26.4±13.9	2.30±0.27	0.72±0.19	1.92±1.20
Sesquiterpene-like compound 5		1470		161	MS	n.d.	2.13±0.42	n.d.	n.d.	n.d.
Sesquiterpene-like compound 6		1474		161	MS	n.d.	9.82±2.03	n.d.	n.d.	n.d.
γ-Muurolene	Citrus, fruit	1477	1479	161	MS	n.d.	22.6±8.2	1.06±0.57	n.d.	n.d.
Alloromadendrene	Woody	1481	1483	105	MS	n.d.	5.86±2.56	n.d.	n.d.	n.d.
Germacrene D	Woody, spicy	1486	1485	161	MS	n.d.	2.73 ±0.80	n.d.	n.d.	n.d.
α-Muurolene	Woody	1496	1500	105	MS	n.d.	13.5±1.8	n.d.	n.d.	n.d.
(*E*,*E*)-α-Farnesene	Woody, citrus, sweet	1507	1505	41	S/MS	n.d.	n.d.	n.d.	2.36±0.50	0.31±0.09
Sesquiterpene-like compound 7		1513		161	MS	n.d	0.55±0.19	n.d.	n.d.	n.d.
Calamenene	Herbal, spicy	1518	1518	159	MS	n.d.	10.8±2.4	n.d.	n.d.	n.d.
δ-Cadinene	Thyme, medicine, woody	1521	1523	161	MS	n.d.	31.5±4.4	1.88±1.45	0.08±0.07	n.d.
α-Cadinene	Woody	1534	1538	105	MS	n.d.	6.46±1.20	1.44±1.55	n.d.	n.d.
α-Calacorene	Woody	1538	1542	157	MS	n.d.	1.15±0.25	n.d.	n.d.	n.d.
Caryophyllene oxide	Woody	1576	1583	41	MS	n.d.	n.d.	n.d.	0.17±0.05	n.d.
Epi-α-cadinol	Herbal	1635	1640	161	MS	n.d.	0.70±0.42	n.d.	n.d.	n.d.

	Σ sesquiterpenes						n.d.	338	7.08	7.56	2.43
Terpenes	Styrene	Balsamic, gasoline	890	889	104	MS	n.d.	7.72±2.16	13.7±2.0	n.d.	n.d.
	Camphene	Camphor	949	954	93	MS	n.d.	n.d.	n.d.	0.49±0.15	n.d.
	α- Thujene	Woody, green, herbal	929	930	93	MS	n.d.	788±97	n.d.	1.92±0.48	n.d.
	α-Pinene	Fruity, green, woody, camphor, citrus, pine	934	939	93	S/MS	n.d.	268±33	n.d.	19.6±3.9	9.47±4.34
	β-Pinene	Woody	976	979	93	S/MS	n.d.	n.d.	n.d.	11.4±2.3	3.69±1.47
	Sabinene	Pepper, turpentine, woody	975	975	93	MS	n.d.	98.6±8.0	2.91±2.87	5.17±0.82	n.d.
	β-Myrecene	Balsamic, fruity, lemon, spicy, sweet	992	990	41	MS	n.d.	21.8±8.3	3.10±1.24	106±20	7.32±2.65
	α-Phellandrene	Flowery, citrus, sweet	1001	1002	93	MS	n.d.	66.6±14.7	n.d.	n.d.	n.d.
	α-Terpinene	Anise, floral, fruity, minty, oily, peach	1015	1017	121	MS	n.d.	11.8±1.2	n.d.	0.64±0.19	n.d.
	ρ-Cymene	Balsamic, citrus, fruity, herbaceous, lemon, spicy	1023	1024	119	MS	42.1±6.6	n.d.	36.0±6.6	3.86±0.38	10.4±4.3
	Limonene	Citrus, fruity, minty, orange, peely	1028	1029	68	S/MS	9.11±1.39	40.7±2.5	5.83±0.61	56.9±6.2	188±122
	(*E*)-β-Ocimene	Sweet, tropical fruits	1051	1050	93	MS	n.d.	9.29±2.25	n.d.	n.d.	n.d.
	β-Ocimene	Flowery, sweet	1052	1050	93	MS	n.d.	n.d.	7.70±6.02	10.1±1.8	7.89±4.14
	γ-Terpinene	Fruity, lime	1059	1059	93	MS	4..46±0.97	n.d.	n.d.	1.55±0.21	n.d.
	(*Z*)-Sabinene hydrate	Balsamic	1067	1070	93	MS	n.d.	0.76±0.30	n.d.	n.d.	n.d.

	(E)-Sabinene hydrate	Woody, balsamic	1060	1068	93	MS	n.d.	n.d.	6.17±1.22	n.d.	n.d.
	ρ-Cymenene	Citrus, terpenic woody spicy	1088	1088	117	MS	n.d.	111±7	19.4±12.0	n.d.	n.d.
	Linalool	Floral, freesia	1098	1096	71	S/MS	n.d.	0.66±0.19	0.91±0.15	n.d.	n.d.
	δ-Terpinene	gasoline, turpentine	1106	1059	93	MS	n.d.	37.5±1.0	n.d.	n.d.	n.d.
	1,3,8-*p*-Menthatriene	Turpentine	1109	1110	91	MS	n.d.	n.d.	6.87±4.20	n.d.	n.d.
	β-Thujone	Thujonic	1112	1114	41	MS	n.d.	1.73±1.68	n.d.	n.d.	n.d.
	Perillene	Woody	1117	1114	69	MS	n.d.	3.65±1.19	n.d.	n.d.	n.d.
	Alloocimene	Herbal	1130	1132	121	MS	n.d.	1.57±0.29	n.d.	1.42±0.20	1.24±0.64
	(-)-Champor	Camphor, medicine	1141	1139	95	S/MS	n.d.	n.d.	1.14±0.50	n.d.	4.48±1.24
	Neo-Allo-ocimene	Sweet, Herbal	1143	1144	121	MS	n.d.	1.23±0.22	3.34±2.22	1.48±0.21	1.47±0.77
	L-Menthone	Minty	1151	1152	112	MS	n.d.	n.d.	n.d.	n.d.	0.53±0.19
	Neomenthol	Mentholic, minty sweet	1163	1165	71	MS	n.d.	n.d.	n.d.	n.d.	2.44±0.92
	3-Thujen-2-one	----	1170	1171	108	MS	n.d.	4.56±2.29	n.d.	n.d.	n.d.
	Menthol	Peppermint, mentholic	1171	1171	71	S/MS	n.d.	n.d.	1.16±0.41	1.34±0.89	24.6±16.0
	Terpin-4-ol	Fruity, herbaceous, licorice, musty, spicy, sweet, terpenic, woody	1174	1177	148	MS	n.d.	1.08±0.24	n.d.	n.d.	n.d.
	Isomenthol	Mentholic	1180	1182	71	MS	n.d.	n.d.	n.d.	n.d.	0.75±0.25
	Terpinolene	Fruity, herbal, pine, sweet, woody	1083	1088	93	MS	n.d.	2.85±0.10	n.d.	0.78±0.11	n.d.
	Neoisomenthol	Menthol	1186	1186	71	MS	n.d.	n.d.	n.d.	n.d.	0.77±0.23
	Estragole	licorice, anise	1194	1195	148	MS	n.d.	0.54±0.07	n.d.	n.d.	n.d.
	(*Z*)-Cadina-1(6),4-diene	----	1460	1463	161	MS	n.d.	12.0±3.7	0.59±0.43	n.d.	n.d.
	(*E*)-Cadina-1,4-diene	----	1528	1534	119	MS	n.d	1.88±0.56	n.d.	n.d	n.d.

	Σ of terpenes					55.7	1494	109	223	263
Other compounds	ρ-Xylene	Geranium	872	872	91	n.d.	n.d.	17.8±2.7	n.d.	18.4±12.1
	Veratrole	----	1146	1148	138	n.d.	n.d.	n.d.	0.29±0.11	n.d.
	Σ of others compounds					n.d.	n.d.	17.8	0.29	18.4

*Values are from semi-quantification using 4-methyl-2-pentanol as internal standard ; n.d.—not detected. [a] LRI—Linear retention index obtained. [b] LRI Lit—Linear retention index reported in literature [37]. [c] Quantification ions. [d]Identification method (S – identified with standard; MS – identified by comparing mass spectrum with database NIST 11).

Total reducing capacity, hydrolysable tannins, total flavonoids and monomeric anthocyanins

Table 3.1.4.2 shows the total reducing capacity (TRC), hydrolysable tannins, total flavonoids and monomeric anthocyanins contents determined in the five studied edible flowers. Significant differences among them ($p<0.05$) were observed. Pansies showed the highest values of TRC (18.0 mg GAE/g DW) and flavonoids (98.8 mg QE/g DW), while cosmos presented the highest contents of hydrolysable tannins (82.9 mg TAE/g DW) and monomeric anthocyanins (4.18 mg Cy 3-glu/g DW). Nevertheless, Johnny Jump ups, pansies and borage also presented high values of hydrolysable tannins, not being significantly different from cosmos. On contrary, calendula always presented the lowest values of all studied bioactive compounds. Intermediary values of TRC and monomeric anthocyanins were detected in Johnny Jump ups; and of hydrolysable tannins and flavonoids in borage. When expressing our results in fresh weight, cosmos, Johnny Jump ups and pansies presented the highest TRC. Pansies also had the highest content of flavonoids, while cosmos showed the highest contents of hydrolysable tannins and monomeric anthocyanins, followed by pansies. In our work the values were equal to 11.6, 1.39 and 0.91 mg Cy 3-glu/100 g FW for pansies, borage and calendula, respectively. Similar results were reported by Benvenuti et al. [19]. These authors detected the following values for red pansies (12.4 mg Cy 3-glu/100 g FW), borage (1.43 mg Cy 3-glu/100 g FW) and calendula (0.47 mg Cy 3-glu/100 g FW).

Table 3.1.4.2 - Total reducing capacity, monomeric anthocyanins, flavonoids, hydrolysable tannins of five edible flowers expressed in dry weight

Flowers	**Parameters**			
	TRC (mg GAE/g DW)	Hydrolysable tannins (mg TAE/g DW)	Flavonoids (mg QE/g DW)	Total monomeric anthocyanins (mg Cy 3-glu/g DW)
Borage	5.6±0.6[b]	74.7±10.4[b]	40.4±5.5[b]	0.15±0.05[a]
Calendula	1.1±0.2[a]	3.7±1.8[a]	0.35±0.10[a]	0.07±0.01[a]
Cosmos	12.4±1.1[c]	82.9±7.4[b]	44.5±3.3[b]	4.18±0.09[d]
Johnny Jump up	15.8±0.8[d]	79.2±14.8[b]	68.9±8.5[c]	0.64±0.17[b]
Pansy	18.0±1.0[e]	78.4±6.7[b]	98.8±3.5[d]	1.47±0.06[c]

Values are expressed as: Mean±Standard deviation. Values with the same letter in the same column are not statistically different ($p>0.05$).

Sensory analysis

Figure 3.1.4.2 and Table 3.1.4.3 show the sensory profiles of the five studied edible flowers. The radar plots, for odors and tastes detected by the panelists, are shown in Figure 3.1.4.2. The odors detected in the flowers were divided into six classes, namely, floral (ex: carnation, lilies, marigold, orange blossom, orchid, pollen, rose, violet), fruity (ex: banana skin, fig, mandarin, peach, plum), herbal (ex: green grass/leaves), marine (sea air), spicy (vanilla) and wood (cedar, acacia). For the taste, five classes were used, namely floral (lavanda, lilies, petals rose, pollen), fruity (apple, cherries, chestnut, grape seed, banana skin, walnut, cabbage), herbal (green grass/leaves, mint, parsley), sweet (honey) and wood (camphor, cedar). It was observed that each flower had its individual sensory characteristics (Figure 3.1.4.1). Regarding odor, the panelists detected more floral fragrances in Johnny Jump up (fragrances of carnation, lilies, pollen, rose and violet), cosmos (fragrances of acacia, carnation, pollen and violet) and pansies (fragrances of lilies, orchid, rose and violet), while for calendula a wood odor was detected (fragrance of cedar). In borage several fragrances were felt such as rose and violet (floral), green leaves (herbal) and sea air (marine). Concerning taste, pansies and Johnny Jump up flowers showed a fruity flavor. In more detail, pansies tasted more like chestnut and walnut, while Johnny Jump up like cherries and walnut. Cosmos presented a high lavanda taste, followed by parsley, camphor green grass/leaves and mint tastes, a complex mixture of floral, herbal and wood flavors. Regarding calendula, a high mixture of flavors was also detected by the panel, such as parsley, cabbage, grape seed, rose petals, cedar, cherries and banana skin. On the other hand, borage showed the smallest range of flavors, being the cabbage and chestnut flavors the most intense.

Furthermore, each flower was classified according to three sensory attributes including visual appearance, odor and taste (Table 3.1.4.3). Regarding visual appearance, two descriptors were evaluated, color intensity and physical integrity of the plant. Cosmos and pansies were the flowers with the highest scores of colors intensity, while all flowers showed good physical integrity (> 9). According to the panel, all flowers revealed a pleasant odor sensation (> 7.5), with no significant differences between them. On contrary, concerning odor intensity, Johnny Jump up, calendula and cosmos had the highest scores, while borage and pansies had the lowest. Concerning taste, all flowers had a delightful mouthfeel (> 5), although pansies were distinguished from the others with the highest value (8.4). Cosmos, pansies and calendula had the most

persistent flavor. In more detail, calendula and cosmos originated a more bitter, astringent and spicy taste than borage, Johnny Jump up and pansies. In contrast, pansies had the sweetest taste, followed by borage and Johnny Jump up. These results were like those referred by Benvenuti et al. [19], who reported that calendula showed higher values of spiciness and bitterness than borage and pansy, as well as, borage and pansy were sweeter than calendula. Thus, our results showed that the five studied flowers have a high sensory biodiversity. This will allow their valorization because these flowers can make the dishes more attractive and confer a peculiar taste and odor.

Association between sensory attributes and bioactive compounds

Sensory attributes of flowers are dependent on the content of minor components like phenolic and volatile compounds. Furthermore, each single component can contribute to different sensory perceptions. Bioactive compounds, such as phenolics, are plant metabolites and contribute to important organoleptic properties (color, bitterness and astringency) [20]. Concerning flowers' color, anthocyanins play an important role. By observing Tables 3.1.4.2 and 3.1.4.3, the flowers that presented the highest contents of total monomeric anthocyanins, namely cosmos and pansies (4.18 and 1.47 mg Cy 3-glu/g DW, respectively), were those that had the highest scores of colors intensity (8.3 and 8.5, respectively).

Phenolic compounds are responsible for the bitterness and astringency of plants, being tannins more likely to be astringent and flavonoids more bitter [21]. In this order, it was expected that pansies were the most bitter flowers followed by Johnny Jump ups, because they had the highest values of TRC and flavonoids. However, the panel reported low scores of bitterness for both flowers. These results can be due to the sweetness caused by the sugars present in the nectar of the flowers (not analyzed), which may be an efficient masking agent of astringency and bitterness caused by the phenolic compounds [22]. In fact, both flowers were described by the panelists to have a sweet taste (6.4 for pansies and 2.6 for Johnny Jump ups).

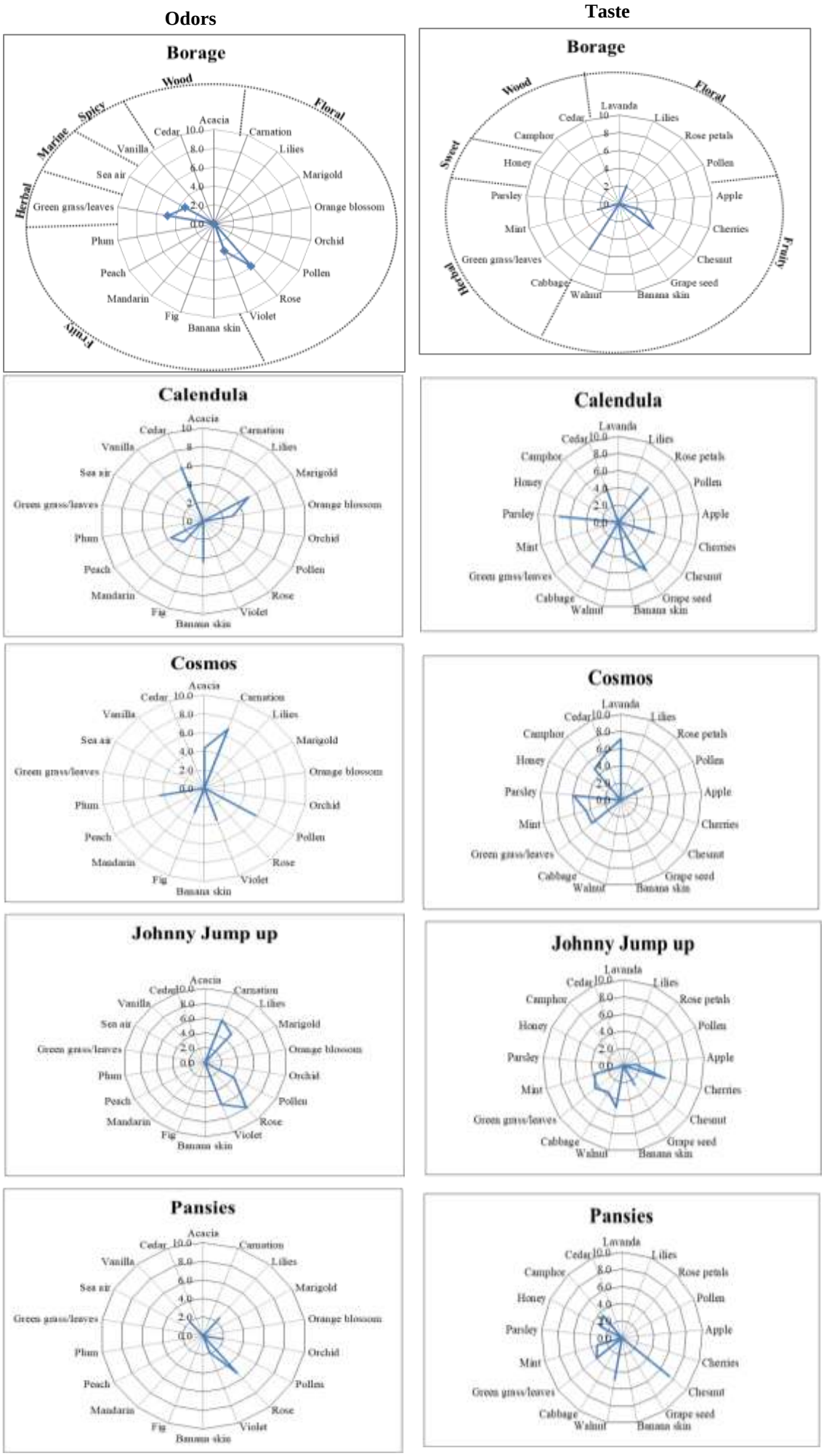

Figure 3.1.4.2 - Odors and tastes detected by the panel of tasters for each edible flower species.

Table 3.1.4.3 - Sensory evaluation of the five edible flowers

Sensory attributes	Edible flowers				
	Borage	**Calendula**	**Cosmos**	**Johnny Jump up**	**Pansies**
Visual appearance					
Colors intensity	6.4±1.1[a] (5.1-7.8)	6.8±0.5[a] (6.3-7.3)	8.3±0.7[b] (7.2-9.4)	7.6±1.1[a,b] (5.0-8.3)	8.5±0.8[b] (7.4-9.7)
Physical integrity of the plant	9.4±0.6[a] (8.5-10)	9.6±0.4[a] (8.9-10.0)	9.3±0.3[a] (9.1-10)	9.5±0.8[a] (7.7-10)	9.5±0.6[a] (8.3-10)
Odor					
Odor sensation	8.0±0.9[a] (6.5-9.2)	7.8±0.5[a] (6.6-8.5)	8.4±0.6[a] (7.5-9.2)	8.6±0.8[a] (7.2-9.9)	7.6±2.8[a] (1.5-9.7)
Odor intensity	6.1±0.9[a] (4.9-7.8)	8.4±0.7[b] (7.7-9.8)	7.2±1.4[a,b] (4.7-9.0)	9.0±0.6[b] (8.1-9.9)	5.3±2.7[a] (2.2-8.6)
Taste					
Mouthfeel	5.8±1.1[a] (4.0-7.2)	6.2±0.7[a,b] (5.4-7.0)	7.5±1.0[b.c] (6.3-9.2)	6.8±1.6 [a,b.c] (4.9-9.9)	8.4±0.6[c] (7.4-9.3)
Persistence	4.2±1.1[a] (2.9-6.4)	6.6±0.8[b,c] (5.3-7.6)	7.8±1.0[c] (6.1-9.2)	5.6±2.0[a,b] (1.5-7.6)	7.5±1.6[b,c] (4.6-9.1)
Bitterness	1.6±0.4 [b] (0.9-2.1)	6.4±1.1 [c] (4.4-7.8)	6.3±1.0[c] (4.6-7.6)	1.5±1.2[a,b] (0.1-3.7)	0.3±0.4[a] (0.0-1.0)
Astringency	1.4±0.7[a] (0.7-2.4)	4.4±1.8[b] (2.0-7.3)	4.2±1.4[b] (1.8-5.9)	1.6±1.3[a] (0.1-4.1)	0.3±0.4[a] (0.0-1.0)
Spiciness	0.3±0.5[a] (0.0-1.5)	2.6±0.9[b,c] (1.2-4.2)	3.6±0.9[c] (2.7-5.3)	0.3±0.5[a] (0.0-1.2)	1.7±0.7[b] (1.0-2.3)
Sweetness	3.5±1.0[b] (2.1-5.1)	0.5±0.4[a] (0.0-0.9)	0.7±0.8[a] (0.0-2.6)	2.6±1.1[b] (0.4-4.5)	6.4±1.0[c] (5.2-7.8)

Mean ± standard error (minimum- maximum).Values with the same letter in the same line are not statistically different ($p>0.05$)

On contrary, for cosmos the panelists did not detect large sweet notes (0.7), being bitterness (6.3) and astringency (4.2) the most detected flavors. These results are in accordance with those obtained for some analyzed bioactive compounds. In fact, cosmos presented the highest values of hydrolysable tannins (82.95 mg TAE/g DW) and intermediary values of flavonoids (44.55 mg QE/g DW). Calendula showed the lowest value of TRC (Table 3.1.4.2), but the panelists detected in this flower a great range of tastes and odors, probably because the intensities of fruity and floral aromas/flavors seem to increase when the level of polyphenols decrease [23].

Association between sensory attributes and volatile compounds

The description of the odor of each isolated volatile compound was obtained from the literature [24-29] and is presented in Table 3.1.4.1. In this section, it was analyzed the possible relationship between volatile compounds obtained by GC-MS and the sensory attributes, assessed by the panelists. Firstly, it is known that the volatile compounds present at higher concentration, are not necessarily the major contributors of odor [28]. In borage flowers a high number of esters associated to fruity fragrances were detected [13, 26, 30]. In fact, the panelists detected some green and floral notes, and a fruity taste (chestnut, cabbage and cherries). Those floral and green fragrances may be due to the presence of some volatile compounds, as ethyl octanoate and 1-hexanol as reported by Śliwińska et al. [30]. Calendula showed woody (cedrus), floral (marigold and orange blossom) and fruity (banana, mandarin and peach) fragrances that were reported by panel (Figure 3.1.4.2). The woody odor of calendula was probably due to the high levels of sesquiterpenes detected, most of them described as contributing to a wood odor (Table 3.1.4.1). Furthermore, some sesquiterpenes (ex: calamenene, β-caryophyllene, α-copaene) give the sensory perception of spicy, so probably it was because of this that the panel detected a spicy taste for this flower (2.6, the second highest). Mandarin and orange blossoms fragrances may be due to the presence of α-pinene. According to the panelist group, cosmos showed floral and fruity odors (Figure 3.1.4.2). The fruity odor was probably due to the high levels of ρ-cymene (41.7 μg/100 g). Regarding Johnny Jump up and pansies, the panel detected floral fragrances for both flowers. β-Ocimene and 1-hexanol are important floral scents in different flowers [31-34] and their odor was described as floral [30], while β-myrecene is described as sweet and fruity. Therefore, these components might play an important role in the sweet, floral aroma of these two samples. Furthermore, the panel detected in

both flowers notes of rose, wherein 2-phenylethanol is one of the principal component of fragrant rose flowers [35, 36]; however, this compound was only detected in Johnny Jump up. The panel also mentioned some mint flavors when they tasted both flowers, probably due to presence of menthol, isomenthol, neomenthol, neoisomenthol and L-menthone compounds.

So, the volatiles produced from flowers vary significantly among species, contributing to the diverse range in fragrances and aromas found in the plant kingdom.

Conclusions

The flowers analyzed showed statistical differences in their sensory attributes (colors intensity, odor intensity, mouthfeel, persistence, bitterness, astringency and sweetness) and in the variety of volatiles detected, presenting calendula the highest number of identified compounds (62), followed by Johnny Jump up (42), pansy (34), cosmos (29) and borage (24). Terpenes were the major chemical class in terms of diversity in almost all edible flowers studied. Regarding bioactive compounds, the highest values of TRC and flavonoids were determined in pansies, and hydrolysable tannins and monomeric anthocyanins in cosmos, having calendula the lowest. Some relationships were found between color, bitterness and astringency with the presence of some bioactive compounds. For example, more anthocyanins gave higher intensities of color (pansies and cosmos), more bitterness to a higher content of flavonoids (cosmos) and more astringency to higher levels of tannins (cosmos); however, regarding taste, it was more difficult to take precise conclusions because some compounds can mask the presence of others. Additional relationships were found between the presence of some volatile compounds and the sensory perception, such as the high number of sesquiterpenes detected in calendula can be associated with the woody notes detected by the panel.

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CHAPTER 4

4.1.

Post-harvest technologies

Several post-harvest technologies were applied to edible flowers, being the results presented and discussed in this section.

4.2.

High Hydrostatic Pressure (HHP)

High Hydrostatic Pressure (HHP) technology has been applied to liquid or solid food, being the product subjected to pressures between 300 and 1000 MPa for few minutes. Generally, this technology does not depreciate the nutritional and sensory characteristics of food, and yet it maintains or extend the shelf life of the products. So, the application of HHP was tested in edible flowers, with the aim to increase their shelf-life and maintain their properties (ex. appearance, nutritional, microbiological and bioactivity).

This section is subdivided in two subsections. First subsection (4.2.1) is a literature review about the effect of HHP treatment on edible flowers' properties, such as physical (e.g. colour and texture), nutritional characteristics, microbial and enzymatic inactivation. The second subsection (4.2.2) contains the results about the effect of HPP on the quality (physico-chemical properties and microbial quality) of four edible flowers: *Viola wittrockiana*, *Centaurea cyanus*, *Borago officinalis* and *Camellia japonica.*

4.2.1.

Effect of high hydrostatic pressure (HHP) treatment on edible flowers' properties (Review)

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Abstract

Edible flowers are increasing worldwide because they can improve the appearance, taste and aesthetic value of food, aspects that the consumer appreciates. However, some of these are highly perishable and have short shelf-life. To overcome these problems, high hydrostatic pressure (HHP) food processing might be applied, allowing producing high quality food with enhanced safety and increased shelf-life.

The application of HHP to vegetables has been extensively discussed and is already an industrial reality but information on edible flowers is scarce and incomplete. Thus, in order to summarize the current knowledge on potential applications of HHP treatment in edible flowers and to determine the effect of this treatment on physical (e.g. colour and texture) and nutritional characteristics, as well as, on microbial and enzymatic inactivation, a literature review was performed.

It was stated that broccoli and cauliflower (inflorescences, usually not considered by consumers as flowers) have been the most studied, existing few information for other edible flowers. Thus, much more works are needed to better understand the effect and mechanisms behind HHP, and to define the adequate technological conditions for each flower.

Keywords: High Hydrostatic Pressure Treatment; Edible flowers; Physicochemical characteristics; Enzyme activity; Microbial inactivation

Introduction

Flowers have traditionally been used in cooking in various cultures. Nowadays, sales of fresh, top quality flowers for human consumption are increasing worldwide, because they can increase and improve the appearance, taste and aesthetic value of food, aspects that the consumer appreciates (Newman and O'Connor, 2013). However, consumers also demand foods with beneficial properties for their health, having some edible flowers nutritional, antioxidant and antimicrobial properties in accordance with the consumers' wishes. Some edible flowers are already consumed on a daily basis, but they are not seen as flowers by consumers, such as: artichoke, white and green cauliflower, Romanesco broccoli and broccoli.

According to EUROSTAT, imports of flowers had increased in quantity and economic value, namely, in 2014 a total of 414,580 tonnes, corresponding to €1.59 billion, were imported by the EU (Altmann 2015). However, all information in the floriculture sector is related to cut and ornamental flowers, with no further details about edible flowers. Although, statistical data doesn't exist about the market of edible flowers, it is evident that they have become more popular, due to the increased number of books recipes, magazine articles and websites on the theme, as well as the growth of research on their nutritional potential (Kelley et al. 2003). In some countries, edible flowers are now being promoted as a healthy food. The Thai Health Promotion Foundation has initiated in 2005 a campaign under the name 'Food Safety: Edible Flowers" to encourage restaurants to incorporate flowers into their menus and to incentivize producers to grow organic blooms (Wongwattanasathien et al. 2010). Furthermore, until now few studies on consumers' preferences in relation to edible flowers have been done; however, Kelley et al. (2001) observed that important characteristics for consumers are color combinations of flowers, container size and price.

Edible flowers are usually highly perishable, with a shelf life of days, being quite difficult to expand this niche market. The most frequent way to consume edible flowers is in fresh, dried, in cocktails (in ice cubes), canned in sugar and preserved in distillates (Newman and O'Connor, 2013). Therefore, extension of food shelf life using mild processing technologies that minimally affect the sensory and texture of the products would be of great interest for this market. Furthermore, it meets the

consumers' demands, who are looking for minimally processed foods, more similar to fresh products and without the presence of additives (Yaldagard et al. 2008).

High hydrostatic pressure (HHP) technology is gaining popularity, not only because of its food preservation capacity but also due to the advantages associated with the process and obtained products. In Table 4.2.1.1., it is presented the main advantages and limitations of this technology, as well as, its role on edible flowers. In general terms, the HHP may increase edible flowers' safety and extend their shelf-life by using low temperatures able to retain flowers' properties, compounds and freshness. However, this technology shall be always tested for the studied product. This technology has been applied to several animal origin products such as fish, hams, minced beef muscle, minced pork and milk (Kadam et al. 2012), as well as to vegetable origin products mainly fruits, such as guacamole, fruit juices and purees, among others (Oey et al. 2008; Cano et al. 1997; Butz et al. 1997; Palou et al. 2002).

As scarce information exists on the effect of HHP on edible flowers, the aim of this review was to summarize the potential applications of HHP treatment to edible flowers, to know its effects in their quality and safety, particularly regarding sensory characteristics, essential nutrients, bioactive compounds, enzymes and microbial load.

Table 4.2.1.1- Advantages and limitations of the HHP when applied to edible flowers (Sources: Kadam et al. 2012; Yaldagard et al. 2008)

	Advantages	Limitations
Process	- Treatment uniformity - Independent of product size and geometry - Food processing at ambient temperature or even lower temperatures - The equipment is simple and easily operated - Processing is isostatic - Reduction of process time and temperature - Environment friendly (requires only electric energy and there are no waste products) - Chemical additives reduction	- The equipment is expensive - Requires airtight and flexible packages - Most foods need low temperature storage and distribution after treatment - The HHP treatment unit immediately becomes the rate limiting step in the processing operation
Edible flowers	- Extension of shelf-life retain flowers' quality and natural freshness - May cause changes in product functionality leading to novel foods (for example: with antioxidant properties) - Positive consumer appeal No evidence of toxicity - No breakage of covalent bonds (maintaining the natural flavour) - Increased safety due to microorganisms inactivation - New product development possibilities (for example: HPP keeps the antimutagenic components in cauliflower and broccoli) - Maintenance of the bioactive substances	- Might affect porous integrity some enzymes and bacterial spores present on edible flowers may be very resistant to pressure - Some colour changes (browning) may occur

Foodborne outbreaks on edible flowers

The number of foodborne outbreaks attributed to edible flowers is limited because the market is not large and there aren't any legal requirements for edible flowers marketing. Nevertheless, outbreaks involving edible flowers have already been reported in the Rapid Alert System for Food and Feed (RASFF), such as for: *Tilia tomentosa*, *Hibiscus*, *Cinnamomum verum* (cinnamon) and *Calendula officinalis*. The main problems are associated with the presence of unauthorized chemical compounds and/or pathogens such as *Salmonella* spp. Furthermore, Simpson (1996) reported that consumption of raw broccoli and cauliflower in the USA has been associated with incidents of *Listeria monocytogenes*. The foodborne outbreaks resulted by these pathogens can be decreased by HHP, since it is referred to as a technology able to eliminate or inactivate microorganisms.

Industrial applications of HHP technology

HHP is now an industrial reality, although it is still under research (Bermúdez-Aguirre and Barbosa-Cánovas, 2011). Today there are some pressurized vegetable products, including edible flowers, that show the advantages of this nonthermal technology in their final quality. At the end of 2007, about 120 industrial HP processing plants had been installed all over the world, with an estimated production of 150,000 tons/year (Wan et al. 2009). According to data from Hiperbaric, S.A., in 2012, vegetable products account for 28% of the total percentage utilization of HHP equipment. This technology has been applied to the development of several vegetable products, such as avocado, fruit juices and smoothies, salads, creams and soups, as well as sauerkraut (Hiperbaric). The use of pressure in the range of 200-800 MPa has been adopted for selective commercial applications by the vegetable products industry, namely, for microbial inactivation in raw products, enzyme inactivation, enhancement of green color (Wan et al. 2009), keep the original taste and color, allow the creation of highest quality premium range of products, maintain the nutritional and functional properties of the products, and development of organic and preservative free products.

Nowadays, there are already some food companies that use HHP in some edible flowers like broccolis and cauliflower in the formulation of smoothies and ready to eat meals. For example, the Juicy Line-Fruity Line® in Holland, manufacturing juices and smoothies of broccoli-apple-lemon and broccoli-orange-lemon (Bello et al. 2014).

Effects of HHP treatment on edible flowers

Sensory characteristics

Some studies have been conducted on edible flowers in order to evaluate the sensory changes observed after HHP treatments, regarding parameters such as structure, texture, appearance, flavour and colour (Tables 4.2.1.2 and 4.2.1.3).

Even though in general the HHP treatment does not induce high changes in aroma, flavour and other sensory characteristics, its effects will depend on several factors such as pressure, temperature and time (Hogan et al. 2005). If very high pressures are used, colour changes (browning) may occur together with a "cooked" aspect, proteins denaturation and softening, among other changes. Nevertheless, it has been referred that high pressures (at low or moderate temperatures) have a limited effect on pigments (for example, chlorophyll, carotenoids, anthocyanins, etc.); however, changes during storage due to incomplete inactivation of enzymes and microorganisms can result in undesired chemical reactions, including colour modifications (Oey et al. 2008).

Regarding textural changes, these are related with the pressure itself or to modifications that occur in the cell wall polymers due to enzymatic and non-enzymatic reactions. The application of HHP promotes cell disruption, thereby allowing some substrates, enzymes and ions that are located in different cell compartments to be released and interact with each other (Oey et al. 2008). On the contrary, flavour does not seem to be changed by the HHP process, since the structure of the small flavour molecules is not directly affected by high pressure (Oey et al. 2008).

The effect of HHP treatment in the texture, flavour and colour of some edible flowers has been evaluated in some works. Concerning broccoli, Fernández et al. (2006) concluded that blanched and high-pressure-frozen treated broccoli presented less cell damage, lower drip losses and better texture than conventional frozen ones, because the microstructure and texture were better preserved, probably due to the instantaneous and uniform ice crystallization. Moreover, pressure-shift freezing did not produce major changes in colour and flavour of blanched broccoli samples. Furthermore, Butz et al. (2002) when evaluating the effect of HHP on chlorophyll *a* and *b*, responsible for the green colour of broccoli, did not observe any detectable effect after long treatments at pressure of 600 MPa at 75 °C. Moreover, Weemaes et al. (1999) evaluated the colour of broccoli juice and found that greenness was marginally affected by pressure at the temperature range of 30-40 °C. A pressure treatment at 800 MPa and 50 °C during 50

min resulted in only about 10% greenness loss. Nevertheless, higher temperatures in combination with high pressures would cause noticeable colour changes.

Regarding cauliflower, Arroyo et al. (1999) found that its firmness was preserved at 350 MPa but it underwent slight browning of the outer portions. Flavour was preserved, even after pressurization at 400 MPa. Préstamo and Arroyo (1998) when investigating structural changes in cauliflower and spinach at 400 MPa for 30 min at 5 °C, reported higher structural changes in spinach, since more cell membrane damage occurred, with greater loss of nutrients.

In conclusion, the degree of cells disruption of flowers depends not only on the pressure and temperature applied but also on the flower cell type, each with different behaviours at HHP. As some are more sensitive than others, it is necessary to evaluate each flower individually. Moreover until this moment, no studies had been performed on the role of HHP on sensory characteristics of ornamental edible flowers, such as, pansies, calendula and rose.

Nutrients

Edible flowers are rich in bioactive compounds, fiber and micronutrients, including antioxidants, pigments and vitamins that are referred to have positive effects on consumer's health. Some studies on the effect of HHP application on the nutrients of edible flowers have been performed, being these discussed in following sections.

Pigments and Vitamins

Regarding carotenoids, vitamin A precursors, McInerney et al. (2007) studied the effect of HHP in broccoli juice and their *in vitro* availability, using simulated gastrointestinal digestion. Lutein and β-carotene, quantitatively the major carotenoids in broccoli, were not substantially affected by HHP after applying pressure levels at 400 and 600 MPa, as well as their digestive bioavailability. Concerning clorophylls, their degradation in broccoli juice has been studied after application heat and combined pressure-temperature (Loey et al. 1998), degrading more rapidly the chlorophyll *a* than chlorophyll *b* under all pressure-temperature combinations tested (1-800 MPa/50-120 °C).

For vitamin C, Houska et al. (2006) found that the content of this compound in apple-broccoli juice was dependent on the holding time of pressurisation but it was independent of the pressure level. Moreover, the combination of a pressure at 500 MPa

and a retention time of 10 min only caused a decrease of 15% on vitamin C content. As previously stated, more research is needed to better understand the behaviour of this vitamin when subjected to HHP. The content of vitamin B9 (folate) and/or its degradation products, has been also studied in edible flowers after HHP treatment, in particular in cauliflower (Melse-Boonstra et al. 2002) and broccoli (Verlinde et al. 2008). Bioavailability of folate (monoglutamate form) in cauliflower after HHP treatment was examined by Melse-Boonstra et al. (2002), with a 2-3 fold increase in cauliflower compared to total folate; however, this processing treatment also resulted in a substantial loss of total folate. In addition, blanching before or after the HHP treatment led to great losses on monoglutamate content, perhaps due to direct solubilisation in the water. Nevertheless, for raw broccoli, it was found that thermal treatment at high pressures (25-45 °C, 100-600 MPa) led to significant losses of folate, ranging between 48 and 78% (Verlinde et al. 2008).

In general terms, it can be stated a lack of knowledge on the effect of HHP treatment on other edible flowers beyond broccoli and cauliflower, regarding pigments and vitamins. Moreover, the role of high pressures over anthocyanins is also unknown. These compounds are responsible for the color of edible flowers, which is a very important characteristic for consumers. Even though, the fat content on edible flowers is low (Navarro-González et al. 2015), the effect of HHP on vitamin E is also unknown, being interesting to study these vitamers in the future.

Enzymes

Enzyme activity is an important quality parameter (Hogan et al. 2005) that can induce changes in product characteristics. Enzymes, like other proteins, are stable within a certain pressure-temperature domain, but exceeding these limits disturbs the three-dimensional protein structure, causing unfolding and denaturation of the molecule, and hence, inactivation (Sila et al. 2008). In edible flowers and derived products, the activity of some enzymes such as polyphenol oxidase, peroxidase, pectin methylesterase, polygalacturonase and lipoxygenase (Hogan et al. 2005; Terefe et al. 2014) affects their quality. Pectin methylesterase and polygalacturonase are associated with the breakdown of cell walls which causes a reduction in viscosity and changes in colour and other organoleptic properties (Hogan et al. 2005). Other enzymes such as peroxidase, polyphenol oxidase and lipoxygenase also affect colour and lipid breakdown (Hogan et al. 2005).

Concerning the effect of HHP treatment on enzymes, the polyphenol oxidase, peroxidase and pectin methylesterase have been referred as highly resistant to HHP, although their sensitivity to pressure depends on their origin and environment. On the contrary, polygalacturonase and lipoxygenase are relatively more sensitive to pressure and have been referred as being inactivated by HHP (Terefe et al. 2014). In more detail, Préstamo et al. (2004) measured the peroxidase activity in broccoli and observed that this enzyme is highly resistant to pressure at 400 MPa for 30 min at 5 °C, being this pressure value insufficient to inactivate this enzyme, as well as the polyphenol oxidase. Regarding pectin methylesterase, few studies have been made in edible flowers; however, Houben et al. (2004) when investigating the thermal and pressure stability of broccoli pectin-converting enzymes, in particular pectin methylesterase (PME), β-galactosidase (β-Gal), and α-arabinofuranosidase (α-Af), concluded that PME was clearly more pressure stable than β-Gal and α-Af, showing β-Gal higher pressure stability than α-Af at all pressure levels.

Some authors have also investigated the effect of HHP in myrosinase, present in high quantities in the *Brassica* family. This enzyme is responsible for glucosinolates hydrolysis, originating compounds with beneficial health effects, including anticarcinogenic compounds (Eylen et al. 2008; Eylen et al. 2009; Ghawi et al. 2012); however, the conversion of glucosinolates into active compounds by myrosinase only occurs after cell disruption. Eylen et al. (2008) verified that the application of HHP treatment may induce cell permeabilisation favouring glucosinolate conversion, creating health promoting hydrolysis products. Furthermore, Ludikhuyze et al. (1999) reported that the application of low pressure (<350 MPa) in broccoli juice resulted in retardation of thermal inactivation of myrosinase.

Concerning the resulting compounds from the hydrolysis of glucosinolates by myrosinase, some works have been performed on the sulforaphane amplification content (Houska et al. 2006), glucosinolate conversion (isothiocyanates, nitrile metabolites and products of the indole glucosinolates) (Eylen et al. 2009) and total content of isothiocyanates (Tříska et al. 2007). Regarding glucosinolates that are hydrolized by myrosinase to give sulforaphanes with health properties, Eylen et al. (2009) after treating broccoli at 100-500 MPa and 20-40 °C, found that there was no degradation of glucosinolates after 15 minutes at 20 °C, while after 35 min treatment at high pressures (200 to 300 MPa) an approximately 20% reduction was observed. At 40 °C and high pressures (100-500 MPa) there was clear glucosinolates degradation after

15 min, while after 35 min this effect was even more pronounced, with 63% of glucosinolates being degraded at 300 MPa. At 40 °C, myrosinase was slowly inactived at 300 MPa, whereas at 500 MPa the inactivation occurred faster. This fact explained why at 500 MPa a lower amount of hydrolysed glucosinolates was observed than at 300 MPa. Beyond this, at low pressures (100 MPa), the limiting factor was cellular disintegration, necessary to promote contact between myrosinase and glucosinolates. Houska et al. (2006), when studying broccoli juice, detected that the content of sulforaphane at a high pressure of 500 MPa for 10 min was similar to the frozen sample. On the other hand, Tříska et al. (2007) observed that the HHP treatment caused a decrease in total isothiocyanates content in vegetables, such as cauliflower, when compared to frozen samples, except in broccoli. These results are due to the richness of broccoli in glucoraphanin (aliphatic glucosinolate) and sulforaphane (isothiocyanate), both compounds stable to high pressures (Tříska et al. 2007).

In conclusion, the behaviour of enzymes is variable, showing some of them HHP resistance, being necessary to combine with temperature (heat) to induce their inactivation (Hogan et al. 2005).

Antioxidant activity and bioactive compounds

Edible flowers are considered rich in antioxidant compounds, being important to study the effect of HHP in these constituents and activity (Tables 4.2.1.2 and 4.2.1.3). Few studies have been performed on individual compounds and the effects of HHP on the antioxidant activity of edible flowers has been evaluated by the following several methodologies, namely: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity; ferric reducing antioxidant power (FRAP) and oxygen radical absorption capacity (ORAC) (McInerney et al. 2007; Chen et al. 2010).

McInerney et al. (2007) found that the HHP treatment in broccoli did not affect its antioxidant activity (evaluated by the FRAP assay) after applying pressures at 400 and 600 MPa. Similar results were obtained by Chen et al. (2010), who reported that pressurization at 600 MPa for 2 and 5 min did not change the antioxidant capacity of *Echinacea purpurea* flowers (determined by ORAC and ABTS assays).

Concerning phenolic components, only one study has been performed until now on the effect of HHP on these compounds in edible flowers, being not affected significantly in *Echinacea purpurea* flowers (Chen et al. 2010).

Recently, some studies have been performed on the application of HHP to extract bioactive compounds from edible flowers, avoiding the application of high temperatures. In the extraction of flower buds of *Lonicera japonica*, the samples subjected to HHP provided higher yields, higher extraction selectivity and required less time and lower energy consumption, as well as higher purity of the extracts, than the conventional extraction method (Wen et al. 2015). Furthermore, samples treated with HHP for 2 min exhibited higher yields of chlorogenic acid and cynaroside than other extraction methods involving several hours. So, HHP treatments can be applied as an extraction method of active compounds from plant material (Jun et al. 2011), as well as, to maintain their antioxidant properties. Nevertheless, when optimizing extraction conditions, several factors have to be taken into account such as pressure, time and temperature. For example: the optimal conditions for extracting chlorogenic acid and cynaroside in *Lonicera japonica* were ethanol concentration 60%, extraction pressure at 400 MPa, extraction time for 2 min, extraction temperature at 30 °C and the solid/liquid ratio 1:50 (Wen et al. 2015); and for caffetannic acid in *Chrysanthemum morifolium* were ethanol concentration 40%, pressure at 300 MPa, time for 5.5 min (Xia et al. 2010).

Edible flowers are rich in colored pigments such as anthocyanins, however no study has been performed on the effect of HHP on these compounds until now. On contrary, in juice and fruits some studies have been done so far. For example, the content of anthocyanins in Chinese bayberry juice treated with different pressures (400, 500 and 600 MPa) remained stable during HHP treatment at moderate temperatures (Yu, et al., 2013); anthocyanins in blood orange juice were retained after pressure treatments at 400, 500 and 600 MPa for 15 min at ambient temperature (Torres et al. 2011); strawberries anthocyanins were more rapidly degraded as the pressure increased at constant temperature, but the effect of increasing pressure was smaller than the effect of increasing temperature (Verbeys et al. 2010); and strawberry pulps exhibited no change on monomeric anthocyanins (pelargonidin-3-glucoside, pelargonidin-3-rutoside and cyanidin-3-glucoside) after HHP treatments regardless pressure or treatment time (Cao et al. 2011). So, in general the anthocyanin content of various fruits and juices has been minimally affected by HHP treatment. Even though some results can be extrapolated for edible flowers, they need to be verified in the future because the matrixes are different.

Table 4.2.1.2 - Application of HHP in edible flowers.

Flowers	Conditions (pressure/ temperature/ time)	Flower's portion	Evaluated parameters	Reference
Broccoli	Vacuum packed bags, 180 MPa/−16 °C Vacuum packed bags, 210 MPa/−20 °C	Small pieces (1.5×3 cm)	- Protein content; - Enzymes (peroxidase and poliphenoloxidase); - Micro-structure changes.	Préstamo et al., 2004
	Vacuum packed bags, 50–500 MPa, 15–60 °C between 4-90 min	50 g of randomly mixed small broccoli head pieces (2 cm from top)	- Stability and activity of endogenous myrosinase; - Glucosinolate conversion; - Cell permeability.	Eylen et al., 2008
	Vacuum packed bags, 100–500 MPa/ 20–40 °C /15 or 35 min	Floret part cut in small pieces (about 2.5 cm long) and for each sample 10 g of broccoli pieces	- Glucosinolates, isothiocyanates and indole degradation products contents.	Eylen et al., 2009
	210 MPa/−20.5 °C.	----	- Microstructure; - Drip losses after centrifugation; - Electrical conductivity; - Texture; - Colour; - Sensory analysis.	Fernández et al., 2006
	600 MPa/25 and 75 °C/10-40 min.	-----	- Pigments (chlorophyll *a* and *b*)	Butz et al., 2002
	Vacuum packed bags, 400 and 600 MPa/2min	900 g were divided equally into three samples	- Antioxidant activity; - Total carotenoid content and *in vitro* availability.	McInerney et al., 2007
	Vacuum packed bags, 0.1–600 MPa/25–45 °C/30 min	-----	- Folylpoly-γ-glutamate stability and conversion.	Verlinde et al., 2008
	1-Instantaneous pressure increase	Small pieces (2.5 cm flower	- Headspace components analysis;	Kebede et al.

from 0.1 to 150 MPa; 2- Pressure increase until 600 MPa at a rate of 10 MPa/s; 3-Holding at 600 MPa during 1 min.	and 1 cm stem)	- Identification and linkage to possible process-induced chemical changes.	2013
0.1 and 800 MPa/ 20 °C/ 10 min	Purée (stem and floret organs were first separated, after which demineralized water was added in a 4:3 (w/w) ratio to each of the broccoli parts)	- Stability of Pectin-Converting Enzymes	Houben et al. 2014
400 MPa/5 °C /30 min.	-----	- Structure (cryo-fracture scanning electron microscopy).	Préstamo, & Arroyo, 1998
200, 300, 350 and 400 MPa/ 5 °C/30 min	10g	- Analysis of saprophytic, pathogenic and phytopathogenic microorganisms; - Sensory evaluation; - Peroxidase activity.	Arroyo et al., 1999
200 MPa/5 min	Florets (2-4 cm)	- Total, monoglutamate and polyglutamate folate contents.	Melse-Boonstra et al., 2002
600 MPa/2 and 5 min.	-----	- Chicoric, caftaric and chlorogenic acids; - Alkamides; - Microbiological analysis; - Antioxidant activity; - Cell-based intracellular oxidation; - Nitric oxide inhibition.	Chen, et al., 2010
1-Instantaneous pressure increase from 0.1 to 150 MPa;	Small pieces (2.5 cm flower and 1 cm stem)	- Headspace components analysis; - Identification and linkage to	Kebede et al. 2013

	2- Pressure increase until 600 MPa at a rate of 10 MPa/s; 3-Holding at 600 MPa during 1 min.		possible process-induced chemical changes.	
	0.1 and 800 MPa/ 20 °C/ 10 min	Purée (stem and floret organs were first separated, after which demineralized water was added in a 4:3 (w/w) ratio to each of the broccoli parts)	- Stability of Pectin-Converting Enzymes	Houben et al. 2014
	400 MPa/ 5 °C /30 min.	-----	- Structure (cryo-fracture scanning electron microscopy).	Préstamo, & Arroyo, 1998
Cauliflower	200, 300, 350 and 400 MPa/ 5 °C/30 min	10g	- Analysis of saprophytic, pathogenic and phytopathogenic microorganisms; - Sensory evaluation; - Peroxidase activity.	Arroyo et al., 1999
	200 MPa/5 min	Florets (2-4 cm)	- Total, monoglutamate and polyglutamate folate contents.	Melse-Boonstra et al., 2002
Purple coneflower *(Echinacea purpurea)* (roots and flowers)	600 MPa/2 and 5 min.	-----	- Chicoric, caftaric and chlorogenic acids; - Alkamides; - Microbiological analysis; - Antioxidant activity; - Cell-based intracellular oxidation; - Nitric oxide inhibition.	Chen, et al., 2010

Table 4.2.1.3- Application of HHP in derived products obtained from edible flowers.

Product	**Conditions** (pressure/temperature/time)	**Preparation method**	**Evaluated parameters**	**Reference**
Broccoli juice	0.1 to 850 MPa/30 to 90 °C	Florets were squeezed in a liquidizer	- Colour; - Chlorophyll, pheophytin and pyropheophytin.	Weemaes et al., 1999
	500 MPa/10 min	Chilled broccoli flower was squeezed and the pH of the fresh juice was 6.49.	- Sulforaphane analysis; - Carotenoids and ascorbic acid; - Sensory evaluation; - Challenge tests; - Anti-mutagenic activity.	Houska et al., 2006
	500 MPa/10 min	Broccoli was pressed and the juice was left at rest for a period of 100 min, filtered and filled into bottles, acidified by citric acid to pH 4.	- Antimutagenic activity of juice and its main hydrolysis products (sulforaphane and indole-3-carbinol)	Mandelová, & Totušek, 2007
Broccoli juice Cauliflower juice	500 MPa/10 min	Chilled broccoli and cauliflower flower were squeezed and the pH of the fresh juice was 6.49.	- Total isothiocyanates.	Tříska et al., 2007
Cauliflower Juice	400 and 600 MPa/25 °C/10 min, 600 MPa/50 °C/10 min, 800 MPa/35 °C/10 min.	250 g of edible parts were homogenized in a home mixer with integrated centrifuge	- Peroxidase activity; - Antimutagenic effect.	Butz et al., 1997

Microbial inactivation

The use of HHP has been mentioned as a potential inactivation method of some microorganisms in edible flowers, being the spores the most resistant. For vegetative microorganisms some mechanisms induce their inactivation such as damage of cell membranes, enzymes denaturation and disruption of ribosomes, which may be critical to their survival (Hogan et al. 2005).

The resistance of microorganisms (Gram negative and Gram-positive bacteria, moulds and yeasts) subjected to high pressures was studied in cauliflower by Arroyo et al. (1999). A pressure at 300 MPa, 10 °C for 20 min, was sufficient to completely reduce the population of *Saccharomyces cerevisiae*. Nevertheless, pressures up to 350 MPa were required to decrease the most part of Gram-negative bacteria, moulds, yeasts and *Listeria monocytogenes*, while the remaining Gram-positive bacteria were the most resistant, being pressures at 400 MPa completely unable to reduce their populations.

Chen et al. (2010) reported that after HHP application the microbial load in *Echinacea purpurea* flower heads was inhibited. Furthermore, HHP for either 2 or 5 min reduced the survival of *Escherichia coli* and this reduction persisted for 2 week after processing. Moreover, they reported that yeast, mold, lactic acid bacteria and total coliform counts were significantly suppressed at 1 and 15 days after HHP.

Generally, these results show the great potential of HHP to reduce the microbiological counts in edible flowers, in order to obtain safer products.

Conclusion

As edible flowers are very perishable foods, promising results may be achieved by HHP in order to maintain their quality for long periods of time. Among edible flowers, broccoli and cauliflower has been the most studied, being urgent to analyse other edible flowers namely those used to garnish and give flavour to dishes because HHP can bring either desirable or undesirable consequences. Each flower shows different behaviours to pressure. In this way, it is always needed to perform studies in order to better understand the effects of HHP on each flower and to determine which pressure, temperature, time and other critical process factors are more effective for microbial and enzymatic inactivation, without compromising the physical (e.g. colour and texture), chemical and nutritional characteristics.

Generally, HHP treatment shows some advantages over other technologies already implemented in the food industry and allows for improving the shelf life and quality of this kind of products. Nevertheless, much more studies are needed to better understand the effect and mechanisms behind HHP.

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4.2.2

Effect of high hydrostatic pressure (HHP) on the quality of four edible flowers: *Viola* × *wittrockiana, Centaurea cyanus, Borago officinalis* and *Camellia japonica*

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Abstract

The aim of the study was to evaluate the effect of High Hydrostatic Pressure (HHP) on the appearance, bioactivity and microbial content of four edible flowers along storage. Several treatments at 75 to 450 MPa and holding times (1, 5 and 10 min) were applied. Borage and camellia were unacceptable after all treatments, while centaurea showed good appearance at 100/5 MPa/min; however, the shelf life didn't increase. Pansies treated at 75/5 and 75/10 MPa/min also retained the appearance of fresh flowers. Furthermore, pansies submitted at 75/5 MPa/min maintained good appearance over 20 days of storage at 4 °C, while the untreated remained satisfactory only until 6 days. Even though no significant differences on microbial load were observed between untreated and HHP treated pansies in day 0, HHP induced the production of bioactive compounds, increasing the shelf-life of pansies. So, the HHP treatment is a promising technology for *Viola*×*wittrockiana*.

Keywords: Edible flowers; high hydrostatic pressure; appearance; microbial load; bioactivity; storage.

Introduction

Edible flowers are becoming more popular, as evidenced by the increase in number of cookbooks, culinary magazine articles and television segments dedicated to edible flowers (Mlcek and Rop, 2011). On the other hand, despite still being regarded as a niche market, attention to this kind of product begins to increase due to their interesting potential as a source of nutrients and bioactive compounds (Patel and Naik, 2010; Benvenuti *et al.* 2016; Lu *et al.*, 2016; Loizzo *et al*., 2016), in line with the actual healthy food trends.

Nevertheless, edible flowers are quite perishable and have a very short shelf life. Until this moment, the unique technologies used by the industry are cold storage (Landi *et al.*, 2015), hot air convective drying, freeze-drying and other drying methods (Zheng *et al.*, 2015; Oberoi *et al*., 2007; Ding *et al*., 2012). However, all these methods have drawbacks: cold storage is a short-term food preservation method, hot air convective drying may cause undesirable biochemical and nutritional changes in the processed product that may affect its overall quality, and freeze-drying has high productive costs. Therefore, the food industry is very interested in improving the marketability of edible flowers, not only as fresh but also as processed products. Furthermore, many health conscious people prefer unprocessed (e.g., lotus or marigold flowers) or minimally processed forms (e.g., dried rose petals or saffron powder) rather than supplements (Chen and Wei, 2017).

In this sense, finding new food technologies able to increase the shelf-life of this kind of product will bring important economic benefits. Hence, high hydrostatic pressure (HHP) treatments appear as good alternatives to extend shelf life and keep the original freshness, taste and odour of products (Corbo *et al*., 2009). HHP is an innovative and emerging technology, already in use by the food industry to preserve a wide range of products (Chawla *et al*., 2011; Huang *et al*., 2017). Recently, Fernandes *et al*. (2017) performed a review on the effect of HHP on edible flowers' properties, stating that broccoli and cauliflower, which are inflorescences usually not considered by consumers as flowers, have been the most studied. Much less information exists for other edible flowers. Thus, the aim of the present work was to evaluate the potential of HHP to preserve four edible flowers, namely, pansies, borage, centaurea and camellia, the most sold and known by consumers. So, in the present study several combinations of high pressure (between 75 to 450 MPa) and time (5 and 10 min) were tested. The quality of edible flowers was evaluated in relation to some physicochemical characteristics,

namely: visual appearance, color, water activity (a_w) and weight loss. Afterwards, the best binominal was selected for each flower according to the properties mentioned above, being studied the flowers' behavior during normal storage conditions (4 ºC). Previously, visual scales were developed in order to be used in the future to easily evaluate the appearance of the mentioned flowers.

Materials and methods

Samples

White/violet fresh pansies (*Viola×wittrockiana*), blue centaurea (*Centaurea cyanus*), blue borage (*Borago officinalis*) and rose camellia (*Camellia japonica*) in full ripening state were collected at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança, Portugal. After harvest, the fresh flowers were immediately transported to the laboratory under refrigeration.

High Hydrostatic Pressure treatments

For each HHP treatment, fresh flowers were placed into polyethylene bags (1 flower per bag) and sealed after eliminating the contained air. The bags were placed into a hydrostatic pressure vessel (55 L volume) of a Hiperbaric equipment (Burgos, Spain). Different pressures and holding times have been tested in each flower, since each flower had different behaviour when subjected to HHP. Pansies were treated at 75, 150 and 450 MPa for 5 and 10 min, centaurea at 75, 100, 200 and 300 MPa during 5 min, borage at 75 MPa for 1 and 5 min, and camellia at 75 for 1 and 5 min and 100 MPa for 5 min. All assays have been done at room temperature and each pressure/time combination was performed in triplicate.

Physicochemical characterization

Visual appearance of the edible flowers

Visual scales were firstly established for the fresh flowers by evaluating their appearance along eight days after harvest and storage at 4 °C. Every day, at the same time and conditions, pictures of the flowers were taken, being determined the a_w and *WL*. For pansies, the color and dimensions were also measured. For each flower a scale with different classes was established. In Figure 4.2.2.1, three levels of appearance, namely, excellent, satisfactory and unsatisfactory are represented. The scale used to establish the classes for each flower is presented (Table 4.2.2.1), as well as the pictures

associated to these classes (Figure 4.2.2.1). These scales were also used to classify the samples subjected to HHP treatments in order to evaluate their effect on the visual appearance of the edible flowers.

Color, dimensions, water activity (a_w) and weight loss

The color of pansies and camellias was evaluated with a colorimeter Minolta CR-400 (Osaka, Japan), using the CIE*Lab* scale. *L**, *a** and *b** coordinates, as well as, Chroma (*C**) and Hue Angle (*h**) values, were determined. In order to analyse the color changes due to HHP treatment, the total color difference (*ΔE**) was also calculated according to the following equation: $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta b^*)^2 + (\Delta a^*)^2}$, whereΔ was the difference of the parameters' values after the HHP treatment and before it (fresh sample, day 0). The width and length of pansies were measured with a digital caliper (Powerfix, Berlin, Germany). To evaluate the width and length changes due to the HHP treatments, the differences were calculated according to Equations 1 and 2, respectively:

$$\Delta Length\ (\%) = \frac{Length_{\ Flower\ after\ HHP} - Lenght_{Fresh\ flower}}{Length_{Fresh\ Flower}} \times 100 \quad (1)$$

$$\Delta Width\ (\%) = \frac{Width_{Flower\ after\ HHP} - Width_{Fresh\ flower}}{Width_{Fresh\ Flower}} \times 100 \quad (2)$$

The color, width and length of borage and centaurea flowers weren't measure due to the small size of the petals, which difficult the correct measurement.

Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland).

Weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany).

Weight loss (WL) was determined according to the following equation: $WL = \frac{M_0 - M}{M_0} \times 100$, where M_0 is the initial mass of fresh pansies before HHP treatment, *M* is the mass of pansies after HHP treatment.

Storage

After selection of the best HHP treatment to apply to each flower, namely 75/5 MPa/min and 100 MPa/5 min for pansies and centaurea, respectively, the flowers' quality was also evaluated during refrigerated storage. Fresh (control) and HHP treated pansies and centaurea were stored at 4 °C until presenting unsatisfactory visual appearance. Every day, at the same time, photos of the flowers were taken and the a_w, *WL*, dimensions and color were measured, as described in the previous section. As

unsatisfactory results were observed for borage and camellia HHP treated, even when low pressures were applied, no studies along storage were done for both flowers. As pansies looked suitable after HHP treatment and along storage, the microbial quality and bioactivity of these flowers were evaluated. The methods used are described in the following section.

Bioactivity of pansies

Extraction conditions

The extraction conditions used were those described by Li *et al.* (2014), with slight modifications. Dried flower powder (0.5 g) was extracted with 50 ml of water:acetone (6:4, v/v) at 37 ºC for 30 min under agitation (900 rpm, IKA, RCT Model B, Staufen, Germany). The solution was filtered and the final volume was adjusted to 40 mL.

Flavonoids

Total flavonoid content was determined by the method described by Viuda-Martos et al. (2011). Flavonoids were quantified using a standard curve of quercetin (10-160 µg/mL), being the results expressed in mg of quercetin equivalents/g freeze dried flower (mg QE/g freeze dried flower).

Hydrolysable tannins

The content of hydrolysable tannins was determined by the method described by Elfalleh *et al*. (2012). Different concentrations of tannic acid (0.025 to 1.6 g/L) were used for calibration. Results were expressed in mg of tannic acid equivalents/g freeze dried flower (mg TAE/g freeze dried flower).

Total monomeric anthocyanin

The total monomeric anthocyanin contents in the flower extracts were estimated by the pH differential method, as described by Bchir *et al.* (2012). The monomeric anthocyanin pigment contents (mg Cy 3-glu/g freeze dried flower) were calculated by the following equation: $A \times MW \times DF \times 1000 \times 25/(\varepsilon \times 1 \times M)$ (3)

where $A = (A_{510\ nm} - A_{700\ nm})_{pH\ 1,0} - (A_{510\ nm} - A_{700\ nm})_{pH\ 4.5}$, MW = molecular weight (449.2), DF = dilution factor, ε = molar absorptivity (26900 L/(mol.cm)) and M = initial sample mass. All measurements were performed in triplicate.

Total Phenolics

The total phenolics of each sample were determined by the Folin-Ciocalteu method, described by Falcão et al. (2007). A calibration curve was obtained with gallic acid (0.25 to 5 mg/L) and the results expressed in mg gallic acid equivalents/g freeze dried flower (mg GAE/g freeze dried flower).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity was determined by the procedure described by Delgado *et al.* (2010), using 300 µL of flower extract (diluted 50 fold). Antioxidant activity was expressed by the percentage of scavenging effect according to Eq. 2:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \quad (2)$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The blank was made with the solvent used in the extraction of the samples.

Reducing Power

The reducing power of the extracts was determined by the procedure described by Delgado *et al.* (2010), being the results the absorbance values read at 700 nm.

Microbial quality of pansies

Samples (Fresh and HHP treated) at the beginning of storage (0 days) and after 20 days of storage (4°C) were collected to determine the microbial quality of pansies. Three grams of sample was mixed with 27 mL of sterile peptone water solution and homogenized in a Stomacher. Decimal dilutions were prepared in the same diluent and plated on appropriate media in duplicate. The growth media and incubation conditions were the following for the studied microorganisms: (I) Total mesophilic: Plate Count agar (PCA, Merck, Algés, Portugal) for 2 days at 30 °C; (II) Yeasts and moulds: Rose Bengal Chloramphenicol Agar (RBC agar, Merck, Algés, Portugal) incubated at 27 °C for 5 days; (III) Lactic acid bacteria (LAB): Man, Rogosa and Sharpe Agar (MRS agar, Merck, Algés, Portugal) incubated at 37 °C for 3 days; and (IV) Total coliforms and

Escherichia coli by the SimPlate® method. All counts were expressed as $\log_{10}$ cfu/g fresh sample.

Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with α=0.05. This treatment was carried out using SPSS version 18.0 program (SPSS Inc., Chicago, IL).

Results and discussion

Flowers' characteristics along cold storage

Regarding pansies, borage, centaurea and camellias cold storage (Figure 4.2.2.1), borage was the most sensitive, preserving satisfactory quality during the shortest period (only one day). On contrary, centaurea maintained a satisfactory quality for 12 days. Pansies and camellias showed an intermediate behavior, maintaining a satisfactory quality for 6 days. These results show that the flowers exhibit different behaviors after harvest and storage, probably due to the existence of morphological differences between these flowers.

Effect of HHP on flowers' characteristics

Visual appreciation

The four edible flowers subjected to HHP showed different behaviours. Pansies subjected to the three first pressure treatments combinations (75/5, 75/10 and 150/5 MPa/min) showed the best appearance; however, only the first two binomials maintained a similar appearance to fresh flowers (Figure 4.2.2.1). On contrary, the flowers submitted to the treatment of 150/5 MPa/min seemed more fragile (Figure 4.2.2.2). After application of the other three combinations, the color of the flowers changed and mixed (Figure 4.2.2.2). At a pressure of 450 MPa the flowers swelled for both holding times. When comparing visual appearance after applying HHP with the proposed visual scale, pansies subjected to the first two binomials of pressure/time can be classified as excellent (similar to fresh flowers (0 days)), while flowers submitted at 150/5 MPa/min were only rated as very good. The remaining binomials did not resemble any level of the proposed scale, being visually considered unsatisfactory.

According to the proposed visual scale, the best binomials to be applied in the future to pansies will be 75/5 and 75/10 MPa/min. Camellia and borage flowers treated at low pressure and time (75/1 MPa/min) showed unsatisfactory appearance (Figure 4.2.2.1). So, this technology can't be used to increase their shelf life. Centaurea subjected to the two first pressure treatment combinations (75/5 and 100/5 MPa/min) showed the best appearance, while the flowers submitted to the others binomials seemed damage (Figure. 4.2.2.1 and 4.2.2.2). Until now, some studies have been conducted on other edible flowers (broccolis and cauliflower). For example, cauliflower treated at 350 MPa maintained its firmness but it underwent slight browning of the outer portions (Arroyo *et al*., 1999); while broccoli treated at 600 MPa and 75 °C did not show any detectable effect on chlorophyll *a* and *b*, responsible for the green colour (Butz *et al*., 2002). These results show that broccolis and cauliflower are more HHP resistant than the edible flowers that were studied in the present work. Thus, each type of flower has its own HHP behavior, being necessary to perform experiments in order to find out the best pressure/time binomial.

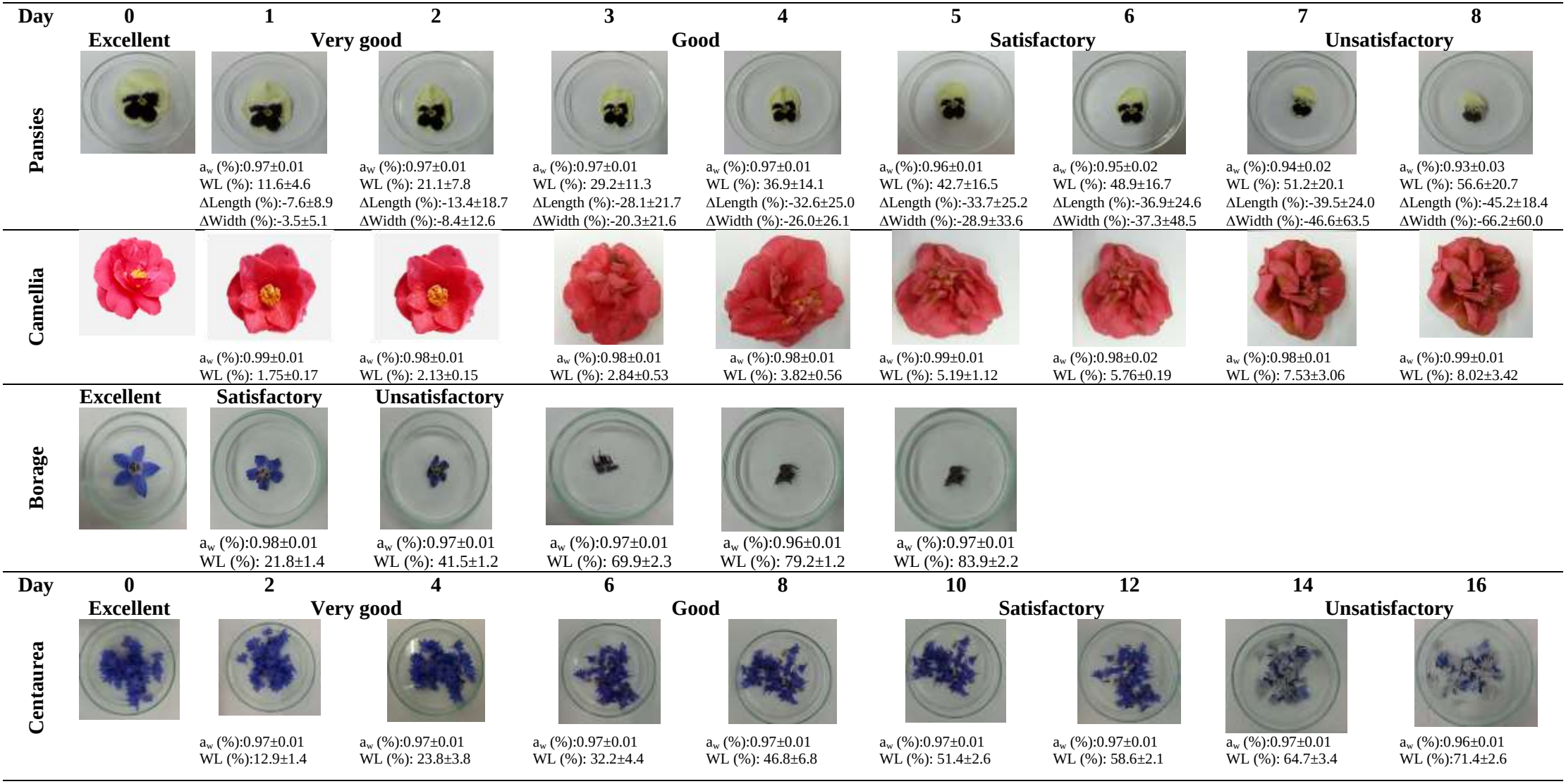

Figure 4.2.2.1 - Visual scales developed to evaluate the appearance of four edible flowers.

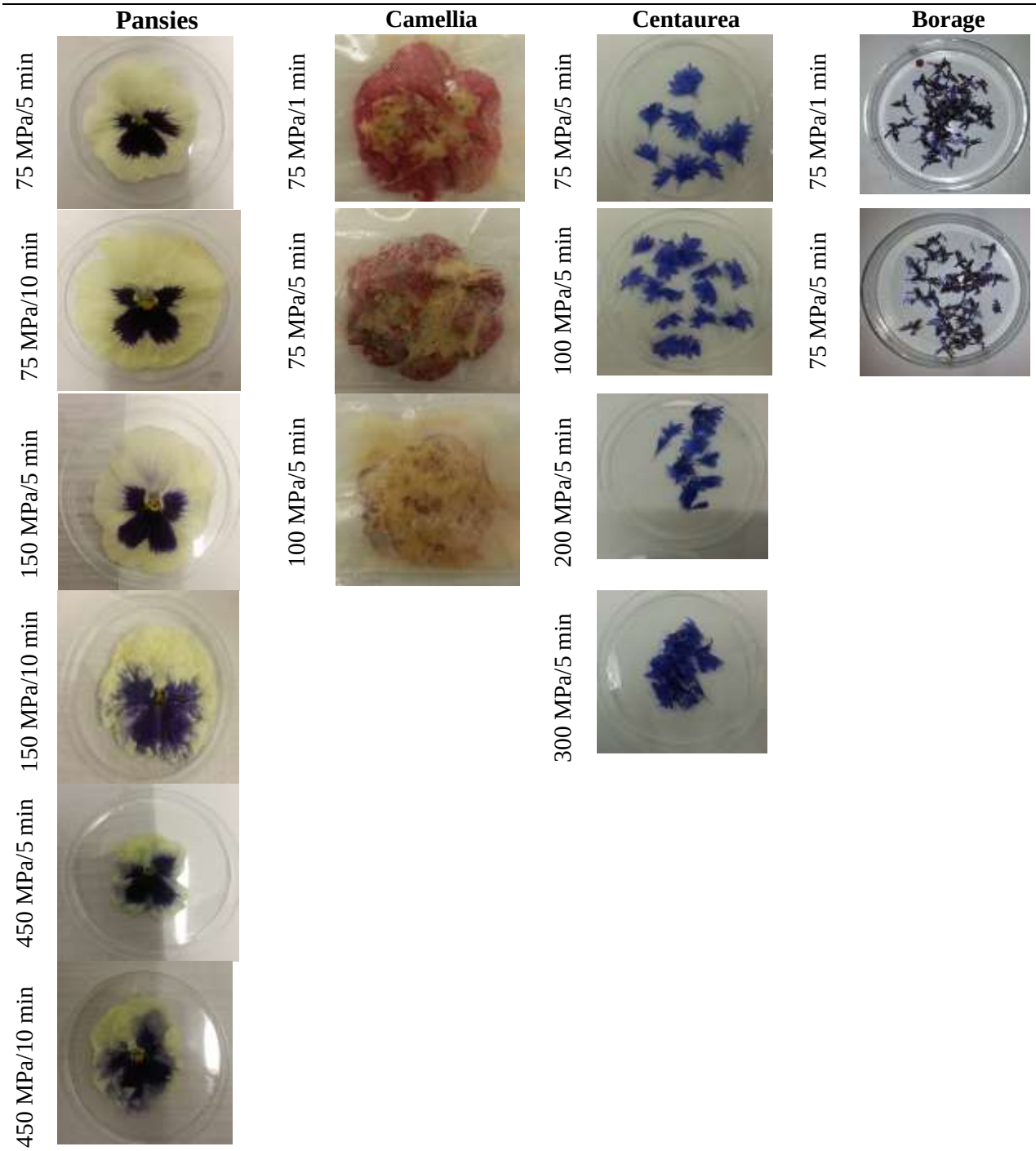

Figure 4.2.2.2 - Visual appearance of pansies, camellia, centaurea and borage after applying HHP treatments.

Color, dimensions, water activity (a_w) and weight loss

Color and dimensions were measured only in pansies (Tables 4.2.2.3 and 4.2.2.4), due to the difficulty of measuring these parameters in centaurea. A_w and *WL* of both flowers are presented in Table 4.2.2.3. As borage and camellia flowers were damaged after HHP treatments, they were not evaluated on these parameters.

When analyzing the color of the white part on pansies, significant differences (p <0.05) were observed in almost color parameters, indicating some effect of HHP treatments on pansies color (Table 4.2.2.2). In general, color parameters did not show a constant trend, explaining the inexistence of significant variations on the ΔE^* parameter between treatments (p=0.060). Nevertheless, all HHP treatments caused visible variations on flower's global color, with ΔE^* of up to 3 units indicating color changes and appreciable to the human eye (Trivellini *et al*. 2014). When observing the results of the violet part, it could be stated that this region was more HHP sensitive than the white part, because all Δ values, independently of the parameter, were much higher than those obtained for the white part. Even though no significant differences on Δa^*, Δb^* and Δc^* were observed between treatments (p=0.082, 0.139, 0.159, respectively), the last three HHP treatments (150/10, 450/5 and 450/10 MPa/min) presented higher ΔE^* values than the other three treatments indicative of higher changes on pansies' overall color. These results suggest that pansies of different colors will behave differently under HHP and thus each variety must be tested individually, with one-color pansies probably being less affected.

Regarding dimensions variations (length and width) of pansies, no significant differences were observed between treatments (p=0.321, 0.537, respectively) probably due to the high standard deviations determined, linked to the difficulty of measuring the pansies' dimensions, as previously explained. Nevertheless, all HHP caused a reduction on the flowers' dimensions, expressed by the negative values of changes of dimensions, being the lowest variation obtained at the lowest binomial: 75/5 MPa/min.

Concerning WL of pansies (Table 4.2.2.3), significant differences were found among the six binominals of pressure/time (p <0.01), varying between 4.3% (75/5 MPa/min) and 13.8% (450/5 MPa/min). The application of higher pressures induced higher changes, more than 10%. Although all the values determined were lower than the *WL* observed along storage, *WL* is undesirable because it will mean a loss of economic revenue and must be minimized. For centaurea no significant differences were detected between the different pressure/time treatments applied, being the *WL* less than 5% (p=0.649).

Water activity (a_w) is one of the most critical factors in determining quality and safety of food because it affects its shelf life, safety, texture, flavor and smell (Jangam & Mujumdar, 2010). However, after application of HHP to pansies, the a_w values

continued to be high, similar to those of fresh flowers (0.980±0.005), and no significant differences between treatments were observed (p=0.458) (Table 4.2.2.3). Centaurea flowers had also high values of a_w after all treatments applied, however, the binomial 75/5 MPa/min showed a significant ($p < 0.01$) lower value (0.955±0.001) than other treatments.

Table 4.2.2.1 – Description of the visual scales established for pansies, camellia, centaurea and borage.

Flower	Class	Description
Pansies	Excellent	Flowers remain their freshness and intense color (corresponding to day 0)
	Very good	Flowers show more fragile petals but continue to have intense color (corresponding to 1 and 2 days); WL average is less than 25%
	Good	Petals ends start to shrivel (corresponding to 3 and 4 days) and WL average may vary between 25-40%
	Satisfactory	Petals are starting to wilt and there is loss of color (corresponding to 5 and 6 days) while WL mean varies between 41-50%
	Unsatisfactory	Petals are shriveled and most of the flower is already wilted (corresponding to 7 and 8 days flowers) and the WL mean is higher than 50%
Camellia	Excellent	Flowers remain their freshness and intense color (corresponding to day 0)
	Very good	Flowers show more fragile petals but continue to have intense color (corresponding to 1 and 2 days); WL average is less than 2.5%
	Good	Petals ends start to shrivel and are brownish (corresponding to 3 and 4 days) and WL average may vary between 2.5-4.0%
	Satisfactory	Petals are starting to wilt and there are more brown petals (corresponding to 5 and 6 days) while WL mean varies between 4.0-7.0%
	Unsatisfactory	Petals are shriveled and most of the flower is already brown (corresponding to 7 and 8 days flowers) and the WL mean is higher than 7.0%.
Borage	Excellent	Flowers remain their freshness and intense color (corresponding to day 0)
	Satisfactory	Ends of the petals are starting to wilt and there is loss of color (corresponding to 1 day), being WL average less than 25%
	Unsatisfactory	Petals are totally shriveled and all flower is already wilted (corresponding to 2 - 5 days flowers). The WL mean is higher than 25%
Centaurea	Excellent	Flowers remain their freshness and intense color (corresponding to day 0)
	Very good	Flowers show more fragile petals but continue to have intense color (corresponding to 2 and 4 days); WL average is less than 25%
	Good	Petals start to shrivel (corresponding to 6 and 8 days); WL average may vary between 25-50%
	Satisfactory	Petals are starting to wilt and there is loss of color (corresponding to 10 and 12 days). The WL mean varies between 50-60%
	Unsatisfactory	Petals are shriveled and loose color, changing to white (corresponding to 14 and 16 days flowers). The WL mean is higher than 60%

Table 4.2.2.2 - Color changes for *L** (*ΔL**), *a** (*Δa**), *b** (*Δb**), *c** (*Δc**), *h* (*Δh**) and total color difference (*ΔE**) of pansies.

Flower's region	Pressure/Time (MPa/ min)	Δ*L**	Δ*a**	Δ*b**	Δ*c**	Δ*h**	Δ*E**
White	75/5	-2.4±3.4^{a,b,c}	-0.7±0.8^{a,b}	2.2±2.4^{a,b}	2.3±2.5^{b}	-1.0±0.6^{a,b}	5.1±0.4^{a}
	75/10	0.5±2.2^{c}	-3.5±2.8^{a}	6.8±3.8^{b}	4.2±4.9^{b}	-6.0±7.4^{a}	8.0±4.6^{a}
	150/5	-1.3±2.4^{b,c}	-0.2±1.2^{a,b}	0.7±2.5^{a,b}	0.3±2.7^{a,b}	-0.3±4.4^{a,b}	3.3±1.8^{a}
	150/10	-8.3±7.1^{a}	1.2±4.1^{b}	-4.3±7.4^{a}	1.3±0.7^{b}	5.0±13.0^{a,b}	10.6±9.6^{a}
	450/5	-7.8±3.2^{a,b}	0.7±0.5^{b}	-4.4±2.3^{a}	-4.3±2.3^{a}	5.9±4.9^{b}	4.1±0.8^{a}
	450/10	-2.5±1.5^{a,b,c}	-0.9±1.0^{a,b}	1.2±2.7^{a,b}	1.5±2.9^{b}	1.5±0.7^{a,b}	9.1±3.7^{a}
Violet	75/5	1.8±0.4^{a}	7.0±6.9^{a}	-5.1±4.7^{a}	8.5±8.1^{a}	-7.7±6.1^{b}	9.8±6.9^{a}
	75/10	9.9±7.0^{a,b}	0.1±8.5^{a}	-5.6±2.1^{a}	3.5±7.5^{a}	-12.4±2.2^{a,b}	14.7±4.3^{a}
	150/5	4.9±3.0^{a}	5.8±5.4^{a}	-9.1±3.3^{a}	9.9±6.3^{a}	-7.7±1.7^{b}	13.1±3.3^{a}
	150/10	16.2±6.0^{b,c}	-4.4±12.4^{a}	-4.4±11.8^{a}	-0.6±17.1^{a}	-12.0±2.3^{a,b}	24.0±1.4^{b}
	450/5	19.6±1.1^{c}	4.5±3.1^{a}	-12.5±4.7^{a}	11.8±5.2^{a}	-17.3±6.1^{a}	24.1±3.3^{b}
	450/10	13.0±4.1^{a}	6.0±3.6^{a}	-9.7±1.9^{a}	10.8±3.6^{a}	-13.1±0.7^{b}	17.5±5.1^{a,b}

Values are expressed as: Mean ± Standard deviation. Values with the same letter in same column are not statistically different ($p>0.05$).

Table 4.2.2.3 - Weight loss, a_w and changes on dimensions of white/violet pansies and centaurea subjected to different combinations of pressure and time.

Flower	Pressure/time (MPa/min)	WL (%)	a_w	Dimensions (%)	
				ΔLength	ΔWidth
Pansies	75/5	4.3±1.3[a]	0.981±0.002[a]	-4.8±2.0[a]	-1.1±2.0[a]
	75/10	8.2±3.4[a,b]	0.980±0.004[a]	-6.1±10.1[a]	-7.0±10.1[a]
	150/5	7.5±0.8[a,b]	0.986±0.002[a]	-8.7±1.4[a]	-5.6±4.3[a]
	150/10	8.9±0.8[b,c]	0.983±0.002[a]	-8.7±1.4[a]	-9.6±4.6[a]
	450/5	13.8±0.3[d]	0.983±0.002[a]	-18.4±13.3[a]	-9.6±7.3[a]
	450/10	13.0±0.5[c,d]	0.983±0.002[a]	-11.6±7.0[a]	-6.0±2.3[a]
Centaurea	75/5	3.4±0.8[a]	0.955±0.001[a]	--------	
	100/5	3.7±1.2[a]	0.974±0.001[b]		
	200/5	3.2±0.7[a]	0.973±0.001[b]		
	300/5	3.8±0.9[a]	0.979±0.001[b]		

Values are expressed as: Mean±Standard deviation. Values with the same letter in same column are not statistically different ($p>0.05$).

Storage behavior - Comparison between HHP treated and untreated pansies and centaurea

Only pansies and centaurea showed good appearance after HHP treatment, showing borage and camellia to be more HP sensitive, being the structure destroyed more quickly even at low pressures. So only, the behavior of pansies and centaurea has been studied along storage. Even though untreated centaurea maintained good appearance until 8 days of storage at 4 °C, centaurea treated at 100/5 MPa/min only presented good aspect for 1 day (Figure 4.2.2.3). So, HHP didn't increase centaurea's shelf-life. Untreated (fresh) pansies maintained good condition until 4 days of storage (4 °C), while pansies treated at 75/5 MPa/min presented good aspect for 20 days (Figure 3). The different behavior of pansies and centaurea may be due to their different epidermis structures, as pansies present superhydrophobic structures called papillae (Schulte *et al.* 2011, Weryszko-Chmielewska and Sulborska 2012), while centaurea doesn't, consisting the centaurea florets of elonged cells, with straight walls (Chiru *et al.*, 2013).

Comparing the a_w and *WL* (Figure 4.2.2.4A and 4.2.2.4B), both HHP treated and untreated pansies had similar behaviors until 8 days of storage. After that period, the a_w of HHP treated samples continued to decrease until 0.564. Regarding dimensions (Figure 4.2.2.4C), untreated samples shrank faster than HHP treated for the same storage period. Concerning color, the overall color difference (ΔE^*) showed some

variability, with a slight increase along storage time, being again more perceivable that the violet part on the untreated and treated samples suffered higher color changes than the white part (Fig.ure 4.2.2.4D). Due to their intense color, anthocyanin's drainage from damaged cells is probable the cause for these observations. Furthermore, when comparing untreated and HHP treated samples of the white or violet parts, it was stated that the HHP treated samples presented slight higher ΔE^* values than the untreated along time, with some exceptions.

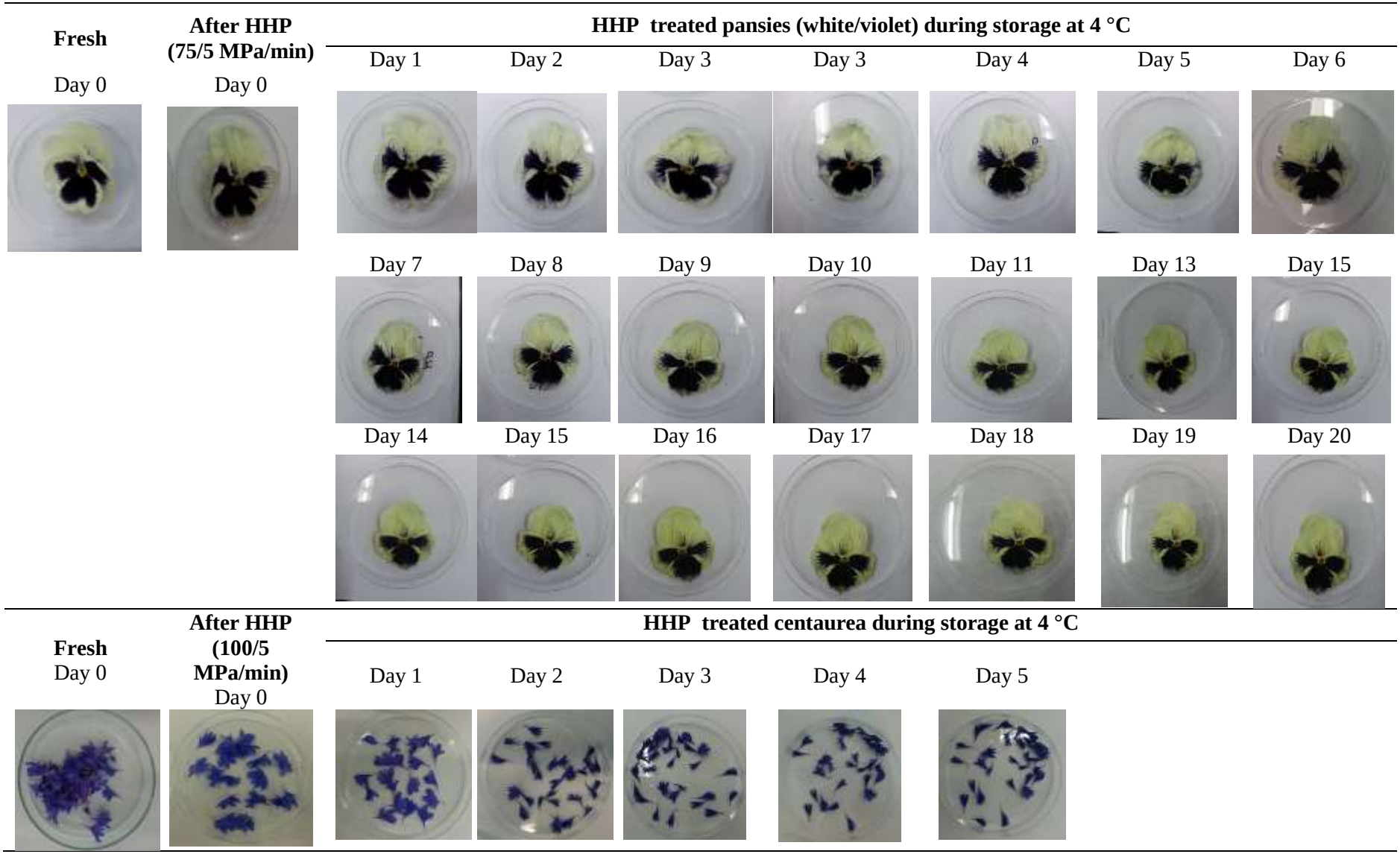

Figure 4.2.2.3 - Visual appearance of untreated and HHP treated pansies (75/5 MPa/min) and centaurea (100/5 MPa/min) along storage.

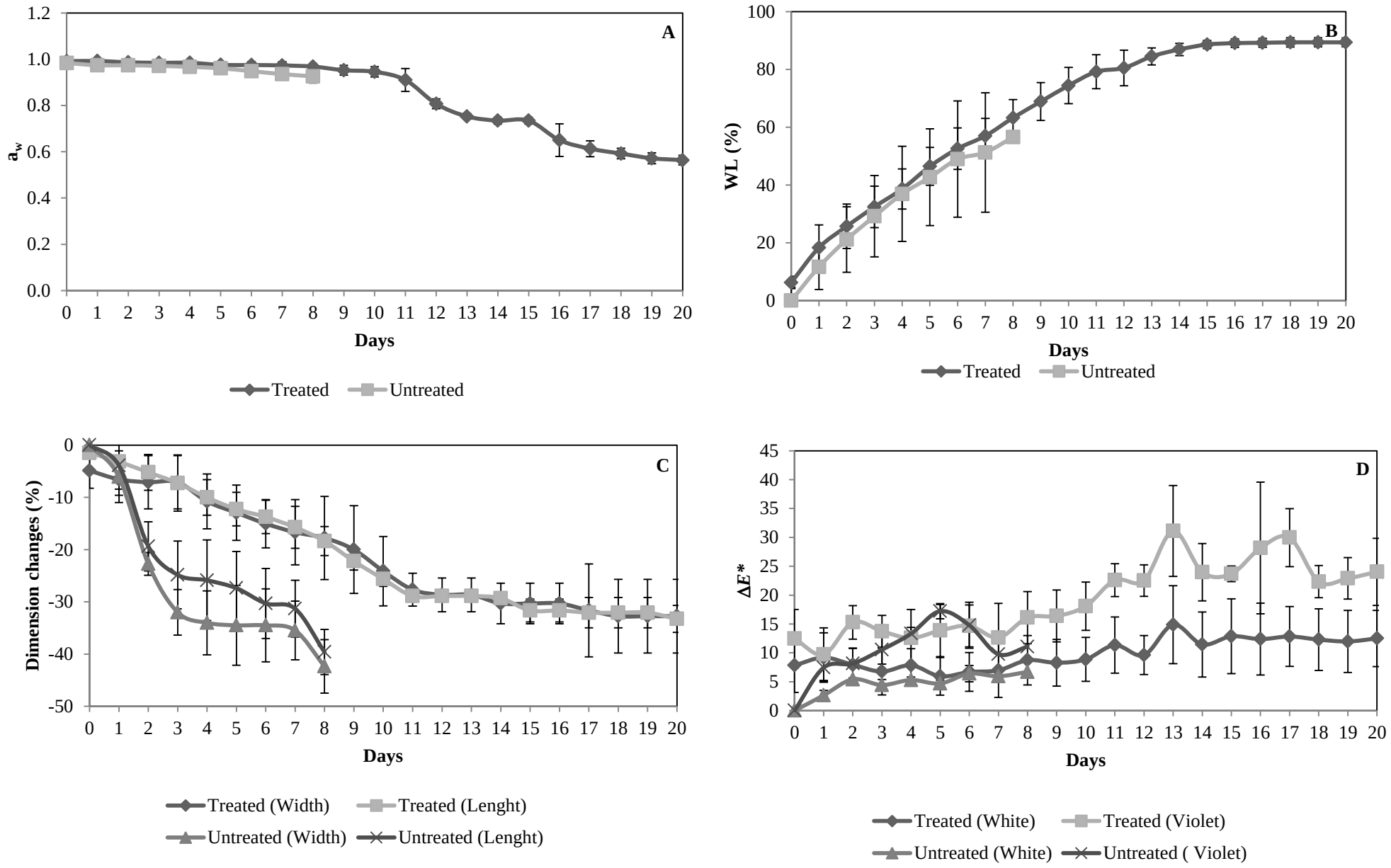

Figure 4.2.2.4 - HHP treated (75/5 MPa/min) and untreated (fresh) pansies during storage for 20 days, for: a_w (A), WL (%) (B), dimension changes (%) (C) and ΔE^* (D).

Bioactivity of pansies

The highest values of total phenolics, hydrolysable tannins and flavonoids were detected in HHP treated pansies in day 0 (Table 4.2.2.4). These higher values determined in pansies pressurized at 75 MPa when compared to untreated, might be associated with structural alteration of the cells provoked by the HHPs, yielding a higher amount of extracted metabolites (Ferrari *et al*., 2011). Other possible explanation is that the higher concentrations of those compounds are a physiological response of the flower to stress conditions at higher pressurization levels (Ortega *et al*., 2013). HHP treated pansies after 20 days of storage showed a decrease in all bioactive compounds contents, as well as in the antioxidant activity measured by the DPPH radical scavenging assay, which can be explained with changes in the activity of enzymes involved in their synthesis, as well as, the presence of oxygen that may cause compounds' oxidation and the occurrence of pressure-induced degradation of polyphenols (Ferrari *et al*., 2011; Ortega *et al*., 2013). On contrary, during storage of untreated pansies (0 to 20 days) the values of these bioactive compounds and antioxidant activity measured by the DPPH assay increased, showing that cold storage might have induced the production of these compounds. When comparing untreated and HHP treated pansies after 20 days of storage, no significant differences were observed for total phenolics, flavonoids, DPPH radical scavenging effect and reducing power. Furthermore, HHP treated pansies after 20 days of storage had higher contents of bioactive compounds (except total monomeric anthocyanins) and antioxidant activity measured by the DPPH assay than fresh flowers (day 0). So, the bioactivity of pansies was not influenced negatively by HHP.

Table 4.2.2.4 – Total phenolics, hydrolysable tannins, flavonoids, total monomeric anthocyanins, DPPH radical scavenging effect and Reducing Power in untreated and HHP treated pansies at 0 and 20 days of storage.

Parameters	HHP untreated		HHP treated	
	0 days	20 days	0 days	20 days
Total phenolics (mg GAE/g freeze dried flower)	12.2 ± 0.5^{a}	19.2 ± 0.9^{b}	27.3 ± 1.0^{c}	19.8 ± 0.4^{b}
Hydrolysable tannins (mg TAE/g freeze dried flower)	26.6 ± 1.3^{a}	48.3 ± 0.6^{c}	51.8 ± 3.1^{c}	37.4 ± 0.7^{b}
Flavonoids (mg QE/g freeze dried flower)	60 ± 6^{a}	134 ± 10^{b}	182 ± 6^{c}	137 ± 10^{b}
Total monomeric anthocyanins (mg Cy 3-glu/g freeze dried flower)	0.09 ± 0.02^{a}	0.18 ± 0.03^{b}	0.18 ± 0.03^{b}	0.08 ± 0.02^{a}
DPPH radical scavenging effect (%)*	12.0 ± 0.5^{a}	24.0 ± 3.9^{b}	32.7 ± 1.1^{c}	22.1 ± 0.3^{b}
Reducing power (Abs_{700nm})	0.35 ± 0.01^{a}	0.34 ± 0.01^{a}	0.35 ± 0.01^{a}	0.36 ± 0.01^{a}

Values are expressed as: Mean±Standard deviation. Values with the same letter in the same row are not statistically different ($p>0.05$).*Percentage relative to a flower extract diluted 50 fold.

Microbial quality of pansies

There were no significant differences between untreated and treated pansies in day 0, except for yeasts counts, indicating that 75 MPa/5 min were not sufficient to cause a significant decrease in microorganisms load (Table 4.2.2.5). However, after 20 days of storage untreated pansies had higher microorganism counts than treated pansies, namely for total aerobic mesophilic count and moulds, suggesting some protection of the HHP treatment. *E.coli* and lactic acid bacteria were not detected in any sample.

Table 4.2.2.5- Mean counts (log cfu/g±standard deviation) of total aerobic mesophilic count, yeasts, moulds, total coliforms, *E. coli*, psychrotrophic bacteria and lactic acid bacteria examined in untreated and HHP treated pansies at 0 and 20 days of storage.

Conditions		Microbial groups						
Samples	Days	Total aerobic mesophilic count	Yeasts	Moulds	Total coliforms	*E. coli*	Psychrotrophic bacteria	Lactic acid bacteria
HHP untreated	0	7.14 ± 0.01^{b}	5.95 ± 0.03^{a}	$<2^{a}$	1.30 ± 0.43^{a}	<1	7.07 ± 0.82^{a}	<2
	20	8.97 ± 0.24^{c}	$6.11\pm0.14^{a,b}$	4.72 ± 1.02^{b}	3.34 ± 0.11^{b}	< 1	9.08 ± 0.29^{b}	<2
HHP treated	0	7.20 ± 0.01^{b}	6.35 ± 0.05^{b}	$<2^{a}$	1.15 ± 0.21^{a}	<1	7.11 ± 0.01^{a}	<2
	20	6.32 ± 0.05^{a}	$6.19\pm0.09^{a,b}$	$<2^{a}$	2.80 ± 0.28^{b}	<1	$9.11\pm0.01^{a,b}$	<2

Values with the same letter in the same column are not statistically different ($p>0.05$).

Conclusion

In summary, borage and camellia flowers subjected to HHP showed unsatisfactory appearance even when low pressures and small times were applied. On contrary, pansies and centaurea flowers submitted at 75 and 100 MPa, during 5 min, showed similar appearance to the fresh flowers. Nevertheless, flowers treated with high pressures showed perceivable changes to the consumer.

During storage (4 °C), HHP treated centaurea rapidly showed an unsatisfactory appearance compared to the fresh samples. On contrary, HHP induced the production of bioactive compounds in pansies and these maintained good appearance until 20 days of storage, even though they were more dried. No significant differences on microbial load were observed between untreated and HHP treated pansies in day 0; however, after 20 days of storage, untreated pansies had higher microorganism counts than HHP treated. So, lower pressures and short holding times may be a promising technology to increase the shelf-life of pansies.

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4.3.

Dehydration

Dehydration is a food preservation method, based on the removal of water from food, preventing the activity of enzymes and growth of bacteria. In food industry different methods of drying are used, namely freeze-drying, hot air convective drying, osmotic drying and sugar crystallization.

The next section focuses on the aplication of different drying methods in two edible flowers, namely pansies and centaurea. Concretely, in the first subsection (4.3.1) it is evaluated the effect of three drying technologies, hot air convective (HA), shade (SD) and freeze drying (FD), on bioactive compounds, antioxidant activity and physico-chemical properties of centaurea petals. The second subsection (4.3.2.) contains the results about the effect of osmotic dehydration on physico-chemical characteristics of pansies (*Viola×wittrockiana*), using different hypertonic solutions (sucrose and sodium chloride). The last section (4.3.3) focuses on the effect of crystallization on the quality (nutritional composition, bioactivity and microbial quality) of pansies during three months of storage, under room temperature.

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4.3.1

Effects of different drying methods on bioactive compounds and antioxidant properties of edible Centaurea (*Centaurea cyanus* L.) petals

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Abstract

The present study aimed to evaluate the effects of hot-air convective drying, shade drying and freeze-drying on the bioactive compounds and antioxidant activity of Centaurea (*Centaurea cyanus* L.) petals, as well as on several of their physicochemical properties. All the dried samples showed different appearances as compared to fresh petals, with lower titratable acidity, lower carotenoids and hydrolysable tannins contents, and greater antioxidant activity. Of the drying methods, shade drying presented the highest values for monomeric anthocyanins, flavonoids, hydrolysable tannins, total reducing capacity and antioxidant activity. By contrast, greater losses were observed as a result of hot-air convective drying. Thus shade drying is a highly promising process that should be considered as a suitable drying method for Centaurea petals.

Keywords: Centaurea petals; Hot-air convective drying; Shade drying; Freeze-drying; Bioactive compounds; Antioxidant activity.

Introduction

The Centaurea (*Centaurea cyanus* L.) flower, also known as blue cornflower or bachelor's button, grows as a wild and common garden plant throughout Europe (CHIRU, 2009). Due to its intense blue flowers it is used as an ornamental plant, for colouring sugar and confectionaries, in teas and salads and to garnish dishes (CHIRU, 2009; CHAITANYA, 2014). Several therapeutic activities have also been attributed to Centaurea flowers, including the treatment of indigestion, gallbladder dysfunction, kidney regulation, menstrual disorder regulation, increasing immunity and for the efficient cleaning of wounds (CHIRU, 2009; LIM, 2014). However, fresh Centaurea flowers are difficult to store since the petals show visible signs of deterioration just a few days after harvest, even under refrigeration. Up to now, no guidelines have been established for the storage of edible flowers, and few studies have been carried out to determine which factors limit their quality (KOU et al., 2012).

Drying is the most common post-harvest method for the preservation of plants (BATRAS, 2015) and fruits (SADLER, 2016). This technology can be used to obtain products that are easily processed, can be stored for long periods and can be used conveniently in the manufacture of formulated foods. Drying methods decrease the water-related activities of plants, and consequently inhibit the growth of microorganisms while decreasing the rate of the biochemical reactions, thus extending the shelf life of the products at room temperature (HAMROUNI-SELLAMI et al., 2013). Furthermore, these processes are more economical with respect to storage and transport costs, since dried flowers occupy less space, weigh less and do not require refrigeration. However, knowledge concerning the application of drying methods to edible flowers remains insufficient (ABASCAL et al., 2005). Several studies have been carried out with marigold flowers (*Tagetes erecta* L.) (SIRIAMORNPUN et al., 2012), purple coneflower (*Echinacea purpurea* (L.) Moench) (LIM, 2014; KIM et al., 2000), roses (*Rosa* × *hybrida* L.), carnations (*Dianthus caryophyllus* L.) (CHEN et al., 2000), daylilies (*Hemerocallis disticha* Donn.) (TAI; CHEN, 2000) and black locust flowers (*Robinia pseudoacacia* L.) (JI et al., 2012), but none have involved Centaurea petals. Thus the aim of this work was to investigate the effects of three different drying methods (hot-air convective drying, shade-drying, and freeze-drying) on the bioactive compounds of Centaurea petals, including the monomeric anthocyanins, carotenoids, flavonoids and hydrolysable tannin contents, as well as on the total reducing capacity

and antioxidant activity (DPPH radical scavenging activity and reducing power), and on several physicochemical properties, in order to identify the best method to preserve the bioactive richness and quality of Centaurea petals.

Material and Methods

Fresh petals

Approximately 1 kg of fully developed fresh Centaurea flowers was collected from different plants at the greenhouse of the School of Agriculture, Polytechnic Institute of Bragança, Portugal. Immediately after harvesting, the fresh flowers were transported to the laboratory under refrigeration and the petals separated from the remaining parts of the flowers.

Drying methods

The fresh petals were dried using three methods, as described below.

1. Hot-air convective drying: The petals were distributed uniformly as thin layers on trays and dried in an oven at 50 °C (Memmert, Schwabach) for 1, 2, 3 or 4 hours. This temperature was chosen because it is commonly found in similar studies on drying flowers and herbs (MAO et al., 2006; BALLADIN; HEADLEY, 1999; CHEN et al., 2000). The relative humidity and temperature were controlled in the different sections of the hot-air convective oven using portable thermo-hygrometers (Hanna Instruments, HI 9564, Woonsocket) and digital thermometers (Hanna Instruments, HI 98509, Woonsocket), respectively. The relative humidity was maintained constant at 4.3% ± 1.2% and the temperature at 49.6 °C ± 2.4 °C throughout the experiments;

2. Shade drying: The Centaurea petals were distributed uniformly as thin layers on trays and dried in the dark for 3 days at room temperature (22 °C), with a relative humidity of 41.0% ± 0.4%;

3. Freeze-drying: The Centaurea petals were frozen at (-18 °C) and then freeze-dried at -120 °C (Scanvac, Coolsafe, Lynge, Denmark) for 24 h.

Each treatment was carried out in triplicate. The drying times were established in order to attain a water activity (aw) below 0.5.

Weight loss, moisture content, water activity, pH and titratable acidity

The weight loss (WL) was determined according to Equation 1:

$$L = \frac{M_0 - M}{M_0} \times 100 \qquad \text{(Eq. 1)}$$

where: M_0 was the initial mass of fresh Centaurea petals before drying and M the mass of Centaurea petals after drying.

The weight was determined using a digital balance (Kern ACJ/ACS, Balingen, Germany) and the moisture content from the weight loss at 105 °C to constant weight (BOLAND; CUNNIFF, 1999). The water activity (a_w) was determined using a portable water-activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). To determine the titratable acidity (TA), a 0.5 g sample was homogenized in 50 mL of distilled water using an Ultra-Turrax homogenizer (IKA, Werke, Germany), filtered (GAITHERSBURG; HORWITZ, 1990), and 10 mL of the filtrate titrated with 0.01 N NaOH, using phenolphthalein as the indicator. The results were expressed as g citric acid/100 g of dry weight (DW).

Carotenoids

The carotenoid content was determined according to the method used by Aquino-Bolaños et al. (2013). One gram of dried petals, subjected to the different drying methods, as well as a fresh sample (control), were extracted twice with 20 mL of an acetone:hexane solution (1:1, v/v). The extracts were each placed in a separation funnel, to which 200 mL of distilled water were added to eliminate the acetone. The acetone-free phase was mixed with 5 g of anhydrous sodium sulphate to eliminate any residual water; and the remaining solution filtered and the volume completed to 100 mL with hexane. The carotenoid content was determined by reading the absorbance at 450 nm, using a calibration curve of β-carotene in hexane (0.22-8.8 μg/mL). The results were expressed in μg β-carotene/g DW.

Preparation of the extracts for the analysis of polar bioactive compounds and antioxidant activity

Dried powdered samples obtained by the different drying methods (1 g), as well as a fresh sample (control), were extracted with 50 mL of water:acetone (6:4; v/v) at 40 °C for 30 min with agitation (IKA, RCT Model B, Staufen, Germany) at a frequency of 1000 rpm (LI et al., 2014). The extracts were filtered, concentrated in a rotary evaporator (Stuart RE3022C, Staffordshire, United Kingdom) (40 °C), frozen and freeze-dried. The extracts obtained were re-dissolved in water:acetone (6:4; v/v) to a

concentration of 50 mg extract/mL, and preserved by freezing until further analysis. Each extraction treatment was carried out in triplicate.

Monomeric anthocyanins, total flavonoids, hydrolysable tannins, and total reducing capacity

The total monomeric anthocyanins, total flavonoids and hydrolysable tannin contents, as well as the total reducing capacity (TRC) of the Centaurea petal extracts (from the control and the dried samples) were determined according to the methodologies used by Fernandes et al. (2017). All determinations were carried out in triplicate. The results for monomeric anthocyanins were expressed in mg cyanidin-3-glucoside/g dry weight (mg Cy 3-glu/g DW), for flavonoids in mg of quercetin equivalent/g DW (mg QE/g DW), for hydrolysable tannins in mg of tannic acid equivalent/g DW (mg TAE/g DW), and for TRC in mg gallic acid equivalent/g DW (mg GAE/g DW).

Determination of Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The DPPH radical-scavenging activity was determined by the procedure described by Fernandes et al. (2017) and the antioxidant activity expressed by the percentage of scavenging effect according to Equation 2:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \qquad \text{Eq. 2}$$

where: A_{DPPH} is the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample.

The blank was prepared with the solution used in the extraction of the samples. The extract concentration providing 50% of DPPH radical-scavenging effect (EC_{50}) was calculated from the graph of DPPH radical-scavenging effect percentage *versus* extract concentration.

Reducing power

he reducing power of the extracts was determined using the procedure described by Delgado et al. (2010) and expressed by the EC_{50} values. These were determined from the graph of Abs700nm *versus* extract concentration, corresponding to the concentration that provided an absorbance of 0.5. Each solution was analysed in triplicate.

Statistical analysis

SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. Analyses of variance (ANOVA) or Welch's ANOVA were carried out to evaluate whether there were significant differences ($p < 0.05$) amongst the samples. In addition, significant post-hoc analyses were carried out (Tukey's HSD test if the variances in the different groups were identical or the Games-Howell post-hoc test if they were not). The homogeneity of the variance was evaluated by Levene's test and the correlations between the variables were determined by Pearson's correlation coefficient. The principal component analysis (PCA) was carried out to differentiate Centaurea petals in the fresh state from those subjected to the three drying methods. The variables considered were the WL, aw, TA, pH, carotenoids, total reducing capacity, hydrolysable tannins, flavonoids and anthocyanin contents, as well as the EC_{50} values obtained from the DPPH and reducing power assays.

Results and Discussion

Visual appearance

Figure 4.3.1.1 shows the visual appearance of the fresh versus the dried Centaurea petals. In general, all the dried petals were darker, shrivelled and smaller than the fresh petals. The petals had a different visual appearance as compared to the fresh ones even after short hot-air convective drying periods (1 or 2 hours). No visual differences were perceived amongst the three drying methods, with all the petals presenting similar colours.

Physicochemical analyses

With respect to water activity (a_w), significant differences were found amongst the drying methods (Table 4.3.1.1). The a_w values of the dried flowers ranged from 0.33 (freeze-drying) to 0.92 (hot-air convective drying for 1h). As expected, these values were lower than that of the fresh sample (0.97); however, no significant differences were observed between fresh petals and those subjected to hot-air convective drying for 1 and 2 hours, with the a_w only being further reduced by longer exposure to hot-air convective drying. According to Barbosa-Cánovas et al. (2003), pathogenic

microorganisms cannot grow at a_w values below 0.86, and yeasts and moulds cannot develop at a_w values below 0.62, but values below these were only reached with hot-air convective drying for 4 hours, shade drying for 72 hours and freeze-drying for 24 hours. Furthermore, a product with an a_w of 0.3 will be more stable in relation to enzymatic activity (BARBOSA-CÁNOVAS et al., 2003), but only the freeze-dried flowers showed a_w values with this order of magnitude. With respect to WL, this was more pronounced as a result of freeze-drying (88.0%), followed by shade drying (77.8%), and hot-air convective drying for 4 hours (73.0%) and 3 hours (72.5%).

Figure 4.3.1.1. Fresh Centaurea petals and those subjected to three drying methods: hot-air convective drying, shade-drying and freeze-drying.

Table 4.3.1.1. - Physicochemical properties of Centaurea petals subjected to different drying methods.

Samples	**Time** (h)	$\mathbf{a_W}$	**Weight Loss** (%)	**Titratable Acidity** (g citric acid/100g DW)
Fresh	--	0.97 ± 0.01^{d}	----	0.69 ± 0.05^{c}
	1	0.92 ± 0.01^{d}	29.8 ± 1.1^{a}	0.24 ± 0.05^{a}
Hot-air convective	2	0.90 ± 0.01^{d}	59.3 ± 1.5^{b}	0.47 ± 0.03^{b}
dried	3	0.67 ± 0.05^{c}	72.5 ± 2.3^{c}	$0.53\pm0.03^{b,c}$
	4	0.47 ± 0.01^{b}	73.0 ± 3.7^{c}	0.66 ± 0.04^{c}
Shade-dried	72	0.47 ± 0.01^{b}	77.8 ± 6.3^{c}	0.43 ± 0.03^{b}
Freeze-dried	24	0.33 ± 0.01^{a}	88.0 ± 0.3^{d}	0.49 ± 0.05^{b}

*Values are expressed as: Mean ± Standard deviation. Values with the same letter in the same column are not statistically different ($p > 0.05$).

Regarding TA, the values were reduced by drying from 0.69 g citric acid/100 g DW (fresh sample) to as low as 0.24 (hot-air convective drying for 1 h). The decrease in acidity as a result of drying could be attributed to conversion of the acids into sugars or some other compounds, or the acids might have been used in the respiration process (PRAJAPATI et al., 2011). The application of longer hot-air convective drying periods caused an increase in acidity (0.24 (1h) to 0.66 (4h) g citric acid/100 g DW), while the shade-dried and freeze-dried samples showed intermediate acidity values (0.43 and 0.49 g citric acid/100 g DW, respectively). No explanation for this variability was found in the literature, indicating that more detailed studies are needed involving the formation of free fatty acids and the transformation of organic acids.

Total carotenoids

A severe loss of total carotenoids was caused in the Centaurea petals by all the drying methods, with significant differences in these losses amongst the drying methods (Table 4.3.1.2). The highest carotenoid contents were found in the hot-air convective dried samples, followed by the shade-dried samples, and the lowest values in the freeze-dried petals. These results were unexpected, and the explanation for the carotenoids losses in the freeze-dried Centaurea petals remains unknown and requires further investigation, as also found for the lycopene content in freeze-dried tomatoes (CHANG et al., 2006).

When comparing the present results with those of Siriamornpun et al. (2012) for marigold flowers, a similar trend was seen, with the higher total carotenoid content being supported by larger amounts of lycopene, β-carotene and lutein in the case of hot-air convective drying than in the case of freeze-drying. Furthermore, in the present study the fresh flowers presented carotenoid values (28.1 µg β-carotene/g DW) approximately three times higher than those of the dried flowers (less than 10 µg β-carotene/g DW). The results of this study suggest that drying, when applied to flowers, may cause carotenoid degradation. Carotenoids are thermally labile (MURATORE et al., 2008) and unstable at low water activity values (LAVELLI et al., 2007).

Monomeric anthocyanins

The total monomeric anthocyanin contents of the Centaurea petals subjected to different drying methods varied significantly according to the method used (Table 4.3.1.2), ranging from 1.90 (hot-air convective drying for 3 h) to 5.11 mg Cy 3-glu/g DW (shade drying), with values for the fresh petals of 3.95 mg Cy 3-glu/g DW. Almost all the hot-

air dried samples presented the lowest monomeric anthocyanin content, probably due to the high temperatures used and high oxygen concentrations involved in this type of drying, leading to rapid degradation of the anthocyanins (PIGA et al., 2003). However, higher values were determined after 4 h than after 3 h for hot-air convective drying. This might be a consequence of the balance achieved between the drying temperature and time or due to the transformation of polyphenols (including anthocyanins) from the bound state to the free state, which may occur at high temperatures after long periods (ZHENG et al., 2015). Apart from the heat, a number of other factors such as light, temperature and storage conditions can also be responsible for the anthocyanin degradation during drying (SHAHIDI; NACZK, 2004). Since shade drying does not imply in the use of high temperatures and exposure to light is limited, these factors may explain the higher preservation of the anthocyanins when using this method.

Total flavonoids

Table 4.3.1.2 shows the total flavonoid contents of the Centaurea petals subjected to the different drying methods. Once again the hot-air dried samples presented the lowest concentrations, those dried with hot air for only 1 h showing the lowest amount (10.04 mg QE/g DW), corresponding to 46% of the content determined in the fresh samples (21.87 mg QE/g DW). These results are in agreement with those of Piga et al. (2003), who found that hot-air drying may lead to a significant decrease in flavonoids. On the other hand the shade-dried petals presented the highest flavonoid concentration (30.06 mg QE/g DW) and freeze-drying caused flavonoid losses of approx. 36% in comparison with the fresh petals.

Table 4.3.1.2 - Monomeric anthocyanins, flavonoids, hydrolysable tannins, total reducing capacity (TRC), total carotenoids and antioxidant activity (DPPH radical scavenging capacity and reducing power) of fresh Centaurea petals and of those subjected to different drying methods.

Samples	**Time** (h)	**Monomeric anthocyanins** (mg Cy 3-glu/g DW)	**Flavonoids** (mg QE/g DW)	**Hydrolysable tannins** (mg TAE/g DW)	**Total Reducing capacity (TRC)** (mg GAE/g DW)	**Total carotenoids** (µg β-carotene/g DW)	**DPPH EC_{50}** (mg extract/ml)	**Reducing power EC_{50}** (mg extract/ml)
Fresh	--	3.95 ± 0.17^{e}	21.87 ± 0.64^{e}	13.67 ± 0.27^{c}	$6.27\pm0.17^{a,b}$	28.1 ± 2.5^{c}	0.92 ± 0.07^{c}	3.65 ± 0.08^{d}
Hot-air	1	2.15 ± 0.05^{b}	10.04 ± 0.42^{a}	$5.44\pm0.94^{a,b}$	6.12 ± 1.13^{a}	5.7 ± 0.2^{b}	0.83 ± 0.03^{c}	1.68 ± 0.02^{c}
	2	3.21 ± 0.02^{d}	17.71 ± 1.68^{d}	6.20 ± 0.76^{b}	9.84 ± 1.00^{c}	7.9 ± 1.2^{b}	0.72 ± 0.03^{b}	1.41 ± 0.03^{b}
	3	1.90 ± 0.05^{a}	$11.32\pm0.62^{a,b}$	3.56 ± 0.36^{a}	$7.34\pm1.02^{a,b}$	6.0 ± 0.8^{b}	0.69 ± 0.03^{b}	1.63 ± 0.21^{c}
	4	2.60 ± 0.07^{c}	$12.96\pm1.43^{b,c}$	$4.78\pm0.58^{a,b}$	$7.93\pm1.02^{a,b,c}$	8.1 ± 1.2^{b}	0.89 ± 0.10^{c}	1.79 ± 0.06^{c}
Shade-dried	72	5.11 ± 0.04^{f}	30.06 ± 2.11^{f}	6.77 ± 2.01^{b}	15.12 ± 2.35^{d}	2.2 ± 0.5^{a}	0.55 ± 0.02^{a}	1.10 ± 0.03^{a}
Freeze-dried	24	2.23 ± 0.05^{b}	13.95 ± 0.68^{c}	6.05 ± 1.94^{b}	$8.43\pm1.09^{b,c}$	0.2 ± 0.1^{a}	0.85 ± 0.05^{c}	1.78 ± 0.09^{c}

Values are expressed as: Mean ± Standard deviation. Values with the same letter in the same column are not statistically different ($p > 0.05$).

Hydrolysable tannins

Significant differences were detected between the different drying methods with respect to the hydrolysable tannin content (Table 4.3.1.2), ranging from 3.56 to 6.77 mg TAE/g DW for hot-air convective drying for 3 h and shade drying, respectively. However, the highest values, at least twice the amount, were observed in the fresh samples (13.67 mg TAE/g DW). These findings were similar to those of Salminen (2003), who reported lower values for hydrolysable tannins in birch leaves (*Betula pubescens* Ehrh.), dried in an oven (60 °C for 12 h) (11.58 mg/g DW) than in those dried in a fume hood at room temperature for 4 days (14.14 mg/g DW) and in the freeze-dried (48 h) leaves (15.35 mg/g DW). Moreover, the room temperature air-dried and freeze-dried samples were not different (SALMINEN, 2003), as also observed in the present work.

Total reducing capacity

Regarding the total reducing capacity (TRC) of Centaurea petals subjected to the different drying methods, the values varied between 6.12 and 15.12 mg GAE/g DW (Table 4.3.1.2). The highest TRC was recorded for petals dried at room temperature (shade-dried), 1.26 times higher than the fresh petals. All the dried samples, with the exception of the hot-air convective samples dried for 1 h, had higher mean values for TRC than the fresh samples, although some cases were not significantly different. This is in agreement with the results of Suvarnakuta et al. (2011), who reported that dried plant materials contained larger amounts of antioxidants, such as polyphenolic compounds, than the fresh plant materials. Dehydration treatments may release bound phytochemicals (e.g., phenolic compounds) from the matrix, making them more accessible to extraction (WOJDYŁO et al., 2014). In addition, the intercellular spaces of the tissues may collapse, liberating more bioactive secondary metabolites such as polyphenolic compounds (DI CESARE et al., 2003; YOUSIF et al., 1999). Furthermore, Suhaj (2006) suggested that some of the bioactive compounds in the fresh samples might be unstable or degraded by enzymatic action, suggesting the use of dried samples. When comparing hot-air convective drying with freeze-drying, no significant differences were observed between the methods, with the exception of 1 h convective drying, results that differed from those of Siriamornpun et al. (2012), Mao et al. (2006) and Zheng et al. (2015) for marigold, daylily and loquat flowers, respectively. These authors found higher values for TRC after freeze-drying than after hot-air convective drying. However, the hot-air drying conditions applied by those authors were different

from those used in the present study, namely, 60 °C for 4 h (SIRIAMORNPUN et al., 2012), 55 °C for 24 h (MAO et al., 2006), and 40 °C or 60 °C for 8 h or 5 h, respectively (ZHENG et al., 2015). Thus higher temperatures and longer drying times were used in these studies as compared to those used in the present work. Since phenolic compounds are sensitive to heat, an increase in temperature may result in a significant loss of these compounds (LIN et al., 2011).

Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

Table 4.3.1.2 shows the DPPH scavenging activities of the Centaurea petals dried by the methods used in the present work. The shade-dried samples presented the highest antioxidant activity, with the lowest EC_{50} values for DPPH (0.55 mg extract/mL), while the fresh, freeze-dried and hot-air dried samples for 1 h and 4 h showed higher EC_{50} values. These results corroborate with the previous analysis, showing that the shade-drying method resulted in petals with higher DPPH radical-scavenging activity than the other drying methods. The present results were similar to those reported by Pinela et al. (2012) for *Tuberaria lignosa* (Sweet) Samp, who observed that infusions of shade-dried samples showed the highest DPPH radical-scavenging activity and reducing power as compared to infusions of freeze-dried and fresh samples. Furthermore, the present study showed that hot-air drying for 2 h and 3 h (0.72 and 0.69 mg extract/mL, respectively) produced samples with higher DPPH radical-scavenging activities than freeze-drying (0.85 mg extract/mL). These results were different from those obtained with daylily flowers, where the water and ethanol extracts from freeze-dried flowers showed higher DPPH scavenging activities than those prepared from hot-air dried samples (MAO et al., 2006), and also for those prepared from marigold, where the freeze-dried and fresh petals showed higher DPPH scavenging activities (67% and 65%, respectively) than those dried with hot air (52.4%), for a 0.1 mg/mL extract solution (SIRIAMORNPUN et al., 2012). However, these different results in relation to the present results can be explained by the different times and temperatures used in both studies. In the first study, the flowers were treated at 55 °C for 24 h (hot-air dried) and for 12 h (freeze-dried), and in the second study at 60 °C for 4 h (hot-air dried) and for 48 h (freeze-dried). Thus the application of different drying conditions and methods significantly influences the DPPH scavenging activities of dried petals.

Reducing Power

With respect to reducing power, significant differences were detected (Table 4.3.1.2). The EC_{50} values ranged between 1.10 and 3.65 mg extract/mL for shade-dried and fresh petals, respectively and hence the shade-dried samples presented the highest reducing power, since they had the lowest EC_{50} value. In addition, the reducing power values of hot-air convective dried petals (1 h, 3 h and 4 h) were not significantly different from those of freeze-dried petals. However, all these EC_{50} values were lower than those of the fresh samples, suggesting that drying increased the antioxidant activity in terms of the reducing power of the petals.

Correlations between total reducing capacity, monomeric anthocyanins, flavonoids, hydrolysable tannins, and antioxidant activity

The Pearson correlation coefficients determined between the TRC, monomeric anthocyanins, flavonoids, hydrolysable tannins and antioxidant activity (EC_{50} values of DPPH and reducing power assays) are presented in Table 4.3.1.3. Significant positive correlations were found between the TRC and monomeric anthocyanins (0.668), as well as between the TRC and the flavonoids (0.737), showing the important role of these compounds in the TRC. As expected, negative correlations were detected between the TRC and the EC_{50} values of DPPH (-0.751) and reducing power (-0.531), since these properties are inversely correlated. The flavonoids also presented a positive correlation with the monomeric anthocyanins (0.965), since anthocyanins are a subclass of flavonoids.

Concerning the antioxidant activity, a significant negative correlation was found between the flavonoids and the EC_{50} DPPH values (-0.502), related to the antioxidant potential of these compounds. Moreover, a significant negative correlation was found between the EC_{50} DPPH and monomeric anthocyanins (-0.402), demonstrating their role in the DPPH free-radical scavenging effect.

Table 4.3.1.3 - Pearson correlation coefficients for the total reducing capacity, monomeric anthocyanins, flavonoids, hydrolysable tannins and EC_{50} values of DPPH and Reducing Power assays.

	Monomeric anthocyanins	**Flavonoids**	**Hydrolysable tannins**	**EC_{50} DPPH**	**EC_{50} Reducing Power**
Total reducing capacity	0.668**	0.737**	-0.065	- 0.751**	-0.531**
Monomeric anthocyanins	___	0.965**	0.510**	- 0.402**	0.087
Flavonoids	___	___	0.491**	- 0.502**	0.042
Hydrolysable tannins	___	___	___	0.302	0.787**
EC_{50} DPPH	___	___	___	___	0.650**

Correlation is significant at $^{**}p < 0.01$

Principal component analysis

The principal component analysis (PCA) was applied to classify the fresh and dried samples into groups. Figure 4.3.1.2 shows the scores of the first two principal components for the Centaurea petals subjected to the three different drying treatments. The first two principal components explained 88.5% of the total variation (PC1 = 55.0% and PC2 = 33.5%, respectively). PC1 was mainly correlated positively to the hydrolysable tannins, carotenoids, EC_{50} values of the reducing power, TA and a_w, and negatively to WL. PC2 was highly correlated to the monomeric anthocyanins, TRC, flavonoids and pH, and negatively to the EC_{50} values of the DPPH assay. Three groups were obtained. Group I was formed by the shade-dried flowers (SD), with high scores in PC2 due to the high values of the monomeric anthocyanin contents, TRC and flavonoids. With respect to the fresh petals (F), corresponding to Group II, these samples presented the highest hydrolysable tannins and carotenoid contents, TA and EC_{50} values of the reducing power, whereas the hot-air dried and freeze-dried samples (Group III) showed the highest WL and EC_{50} values of the DPPH, indicating lower antioxidant activity.

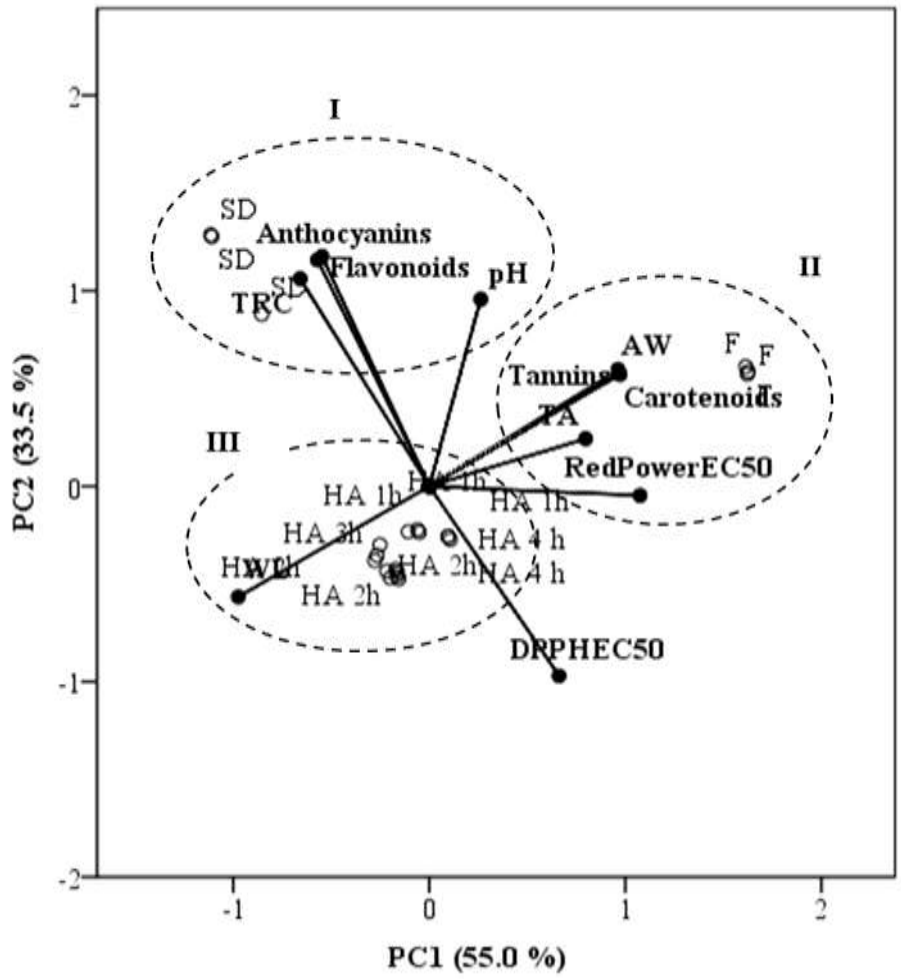

Figure 4.3.1.2 - Principal component analysis plot of Centaurea petals in fresh (F) and dried by the following methods: hot-air convective drying (HA), freeze-drying (FD) and shade-drying (SD). Principal component 1 (PC1), Principal component 2 (PC2), total reducing capacity (TRC), water activity (A_W), weight loss (WL).

Conclusion

The results of this study show that each drying method produces different effects on the physicochemical properties and bioactive compounds of Centaurea petals. In general, drying produced darker, shrivelled and smaller flowers with lower TA values and carotenoid and hydrolysable tannin contents than fresh flowers, while increasing their total reducing capacity and antioxidant activity in terms of reducing power. The highest carotenoid content was identified in hot-air convective dried samples, but this method produced the lowest anthocyanin and flavonoid contents, while the shade-dried samplespresented the highest values for both compound classes. The shade-dried

samples also presented the highest TRC, DPPH radical-scavenging activity and reducing power, and one of the lowest a_w values. In summary, the present study has provided useful information on the industrial drying processes of Centaurea petals, showing the advantages of shade drying at room temperature, which is a sustainable and green technology with low energy costs.

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4.3.2.

Effect of osmotic drying on pansies (*Viola* × *wittrockiana*) physicochemical properties

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Abstract

The objective of this work was to study the effect of osmotic drying in pansies (*Viola* × *wittrockiana*), using different hypertonic solutions (sucrose and sodium chloride) on physicochemical characteristics. The same treatments were applied to lettuce to compare the behavior of flowers with other vegetables. Pansies superhydrophobic surface structure, called papillae, increased the resistance to exchanges with hypertonic solutions. No weight loss was observed after most treatments (sucrose: between 2.2 and 6.8 %; NaCl: between -23.0 % and 1.5 %), a_w maintained high values (> 0,94) and monomeric anthocyanins were preserved (fresh 0.10 and 0.19 mg Cy-3glu/g fresh matter for 20%/1 h in NaCl and 60%/1 h in sucrose) . When applying more drastic conditions, as sodium chloride for more than 1 hour, undesirable textural and color changes were observed. For lettuce, all treatments caused osmotic dehydration, weight loss (ranged between -9.3 to -30.3 % for 80%/1 h in sucrose and 15%/1 h in NaCl) and a reduction in a_w (< 0,97) and carotenoids, causing sodium chloride more damage in visual appearance than sucrose. Therefore, immersion in osmotic solutions can be applied to lettuce but for pansies the desired effect was not achieved due to the morphological structure of pansies' flowers epidermis.

Keywords: *Viola×wittrockiana*; Lettuce; Osmotic dehydration; Carotenoids; Monomeric anthocyanins.

Introduction

Garden pansies (*Viola* × *wittrockiana*) result from extensive hybridizing and selection involving the species of *Viola tricolor*, *Viola lutea*, *Viola altaica* and others (Lim, 2014). Pansies are edible flowers, added to salads or used to garnish desserts (frosted cakes, sorbets and iced drinks). They can also be crystallized and eaten as a sweet delicacy. Furthermore, these flowers are rich in health-promoting compounds, such as anthocyanins, carotenoids, flavonoids, potassium and phosphorus (Vukics et al., 2008; Gamsjaeger et al., 2011; Rop et al., 2012), contributing for a healthy nutrition, while providing protection against cancer and cardiovascular diseases, among others (Lu et al., 2015). However, edible flowers have a limited shelf-life, with petal abscission, discoloration, wilting, dehydration and tissue browning occurring soon after harvest. The most frequent ways to preserve edible flowers include refrigeration, drying or canning in sugar, and preservation in distillates. However, these processes can have a negative impact on their nutritional and sensory quality and appearance. The recent culinary trends demanding for increased availability and shelf life of edible flowers, is essential to find out new technologies or pretreatments able to extend edible flowers shelf life with minimal impact on their sensory and textural properties.

Immersion in osmotic solutions is a common procedure used to dehydrate foods, and it can be regarded as a way to decrease the water activity and maintain the appearance of pansies. However, few studies on the effect of osmotic dehydration have been conducted on edible flowers. This technology has already been applied to broccoli (Xin et al., 2013) and cauliflower (Vijayanand et al., 1995; Jayaraman et al., 1990), edible flowers less known by consumers, using sweet (sucrose or trehalose) and/or salty (ex: sodium chloride) approaches, but no studies on osmotic drying was found for pansies.

The main objective of the present work was to investigate the effect of osmotic dehydration in sucrose (60 and 80%, w/v) and sodium chloride (15%, 20%, 25%, w/v) solutions, at room temperature, on the quality of pansies (*Viola* × *wittrockiana*). Visual appearance, weight loss, a_w and color were evaluated before and after treatments. Simultaneously, the contents of carotenoids and monomeric anthocyanins were determined in the treated flowers. Lettuce, a leafy vegetable with a homogeneous color

distribution, was used as a control, being subjected to the same treatments and evaluations.

Materials and Methods

Samples

Fresh white/violet pansies (*Viola* × *wittrockiana*) were collected at the greenhouse of the School of Agriculture, Polytechnic Institute of Bragança (Bragança, Portugal), being immediately transported to the laboratory under refrigeration. The production mode was organic. Fresh lettuce was bought at a local market in Bragança city that is located in the Northeast of Portugal.

Preparation of osmotic solutions

The osmotic agents, sucrose and sodium chloride, were of commercial grade, purchased from a local market. The different concentrations of 60 and 80% (w/v) of sucrose and 15%, 20% and 25% of sodium chloride were prepared by dissolving the required amounts of sucrose or sodium chloride in distilled water.

Osmotic dehydration

Pansies or lettuce leaves were placed into the vessels containing the different sucrose/sodium chloride solutions, remaining totally submerged. The ratio of raw material to osmotic solution was 1:4. At each sampling time (15 min, 30 min, 1 h and 2 h for sodium chloride / 1 h and 2 h for sucrose), the flowers and lettuce leaves were taken out, gently dried with adsorbent paper and weighed. These conditions were selected after several preliminary tests, where higher times and mechanical agitation were shown to induce degradation. Each treatment/time was performed in triplicate, on both pansies and lettuce.

A portion of each sample, including fresh ones, was immediately analyzed for weight, a_w, color, and microscopy, while other was preserved by freeze-drying for analysis of bioactive compounds, as detailed in next sections.

Physicochemical characterization of samples

Color, water activity (a_w) and weight loss

The color of pansies (white and violet parts) and lettuce were evaluated in three samples of each treatment/time, with a colorimeter Minolta CR-400 (Osaka, Japan), using

CIE*Lab* mode. *L**, *a** and *b** coordinates were measured, where *L** varies between 0 (completely black) and 100 (completely white), *a** -100 (green) to +100 (red), and *b** from -100 (blue) to +100 (yellow). Furthermore, the Chroma (*C**) and Hue Angle (*h**) values were determined. In order to analyse the color variations, the total color difference (*ΔE**) was also calculated, according to the following equation 1:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta b^*)^2 + (\Delta a^*)^2} \qquad \text{Eq. 1}$$

, where Δ was the difference between the parameters' values after the immersion in osmotic solutions and before it (fresh sample).

Water activity (a_w) was determined in a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). This parameter was evaluated on three samples before treatment (control) and after the immersion in the osmotic solutions. Weight measured in a digital balance (Kern ACJ/ACS, Balingen, Germany), is the weight variation expressed in relative percentage of initial weight.

Freeze-dried

Flowers and lettuce treated and untreated were frozen and lyophilized (Scanvac, Coolsafe, Lynge, Denmark) for 2 days. They were ground to a homogenous powder, being afterward stored protected from light and moisture until extraction.

Carotenoids

The carotenoid contents were determined according to the method used by Aquino-Bolaños et al. (2013). One gram of frozen-dried powder of the osmotic dehydrated and fresh pansies and lettuce was extracted twice with 20 ml acetone:hexane solution (1:1, v/v). Both extracts were put into a separation funnel, is added 200 ml of distilled water to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to eliminate any residual water, being the remaining solution filtered and completed to 100 ml with hexane. Carotenoid content was determined by reading the absorbance at 450 nm and comparing the results to a β-carotene calibration curve (0.22–8.8 μg/ml). Results were expressed in μg β-carotene/g fresh matter.

Monomeric anthocyanins

Extraction was based on the method described by Li et al. (2014) with slight modifications. Dried powdered sample (0.5 g) was extracted with 20 ml of methanol, at 37 °C, for 30 min under agitation (IKA, RCT Model B, Staufen, Germany) at a frequency of 900 rpm. The methanol extracts were filtered and concentrated in the rotary evaporator (Stuart RE3022C, Staffordshire, United Kingdom), frozen and lyophilized for 2 days. The extract obtained was re-dissolved with methanol to a concentration of 50 mg extract/ml covered with aluminium foil and preserved under freezing until further analysis.

The total monomeric anthocyanin contents on fresh pansies and lettuce, as well as on the samples immerged in the osmotic solutions, were estimated by the pH differential method, following the methodologies used by Bchir et al. (2012) and Rajasekar et al. (2012). The method consisted of using two buffer systems: potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate at pH 4.5 (0.4 M). Extracts portions were diluted on both buffers, and allowed to stand for 30 min at room temperature. Subsequently, the absorbance readings were made on a UV-Visible spectrophotometer (Thermo, Genesys 10 UV, Waltham, USA) at the wavelengths of 510 and 700 nm, being the absorbance difference (*A*) determined by the equation:

$$A = (A_{510\ nm} - A_{700\ nm})_{\ pH\ 1.0} - (A_{510\ nm} - A_{700\ nm})_{\ pH\ 4.5} \quad \text{Eq. 2}$$

The monomeric anthocyanin pigment concentration was expressed as cyanidin-3-glucoside, determined by the equation 3:

$$\text{Monomeric anthocyanin pigment (mg Cy 3-glu/l)} = A \times MW \times DF \times 1000/(\varepsilon) \quad \text{Eq. 3}$$

where MW= molecular weight (449.2), DF = dilution factor and ε = Molar absorptivity (26,900). All measurements were performed in triplicate. The results were expressed in mg Cy 3-glu/g fresh matter.

Microscopic analysis of pansies petals

Thin sections of petals were cut into random transversal and longitudinal sections, mounted in water and observed on a light microscope equipped with a digital camera (Leica DFC49012, Heerbrugg, Switzerland). The structure of the petals epidermis was analyzed at different points.

Statistical analysis

The SPSS Statistical software, v.18.0 (SPSS Inc., Chicago, IL), was used for the statistical treatment of the data. The normality of the data was verified by the Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch was carried out to determine if there were significant differences ($p < 0.05$) between samples, depending on the existence or not of homogeneity of variances. Additionally, if significant differences were detected between treatments, a *post hoc* analysis was performed, namely: the Tukey's honestly significant difference test, if variances in the different groups were identical, or Games-Howell test if they were not. The homogeneity of the variances was tested by Levene's test.

Results and Discussion

Visual appearance and color

Figure 4.3.2.1 shows the visual appearance of fresh pansies and lettuce before and after treatments with different concentrations and immersion times in osmotic solutions (sucrose and sodium chloride). Flowers and lettuce subjected to the four treatments with sucrose (1h or 2h at 60 and 80%) and lower concentration of sodium chloride (15% for 15 or 30 min) showed similar appearance to fresh samples. However, when higher concentrations of sodium chloride (20 and 25%) or longer immersion times (1 and 2 h) were applied, structural damage was observed, as well as color changes. Furthermore, when pansies were removed from these higher concentrated osmotic salt solutions they shrank, making them more fragile. Lettuce leaves also became more brittle and showed a darkening in color.

The total color difference, ΔE^*, which is a combination of the parameters L^*, a^* and b^* values, is a colorimetric parameter used to characterize the variation of colors in food during different treatments. For pansies and lettuce, higher values of ΔE^* and standard deviations were observed in samples treated with sodium chloride (Figure 4.3.2.2 and 4.3.2.3, respectively). Taking into account that ΔE^* of up to 3 CIE*Lab* units indicates color differences appreciable to the human eye (Trivellini et al., 2014), both samples showed color differences detectable by consumers after treatments. Regarding pansies, a distinct behavior was observed in the two colored parts studied, white and violet. Generally, the white part showed lower values of ΔE^* than the violet one, without significant differences between treatments in the white part, with the exception of the treatment 15% NaCl for 1h. On the contrary, the violet part was more color sensitive,

with the more significant alterations observed with the following treatments: 80% sucrose/2h, 15% NaCl/15 min, 15% NaCl/30 min, and 15% NaCl/2h. Lettuce, probably due to its increased color homogeneity, showed constant values of ΔE^*, without significant differences detected between treatments, except for 60% sucrose/2h.

Weight and a_w variation

Immersion time and sucrose/sodium chloride concentration effects on weight and a_w variation in pansies and lettuce are also given in Figure 4.3.2.2 and 4.3.2.3, respectively. Pansies weight (Figure 4.3.2.2) showed significant weight increases in both osmotic solutions among treatments. It could be expected that, after immersion treatment, pansies should lose weight due to diffusion of water, even though accompanied by simultaneous counter diffusion of solutes from the osmotic solution into the tissue. Furthermore, it is desired to have a high water loss for preservation purposes and a low solids gain, which will lead to high weight variations. These exchanges of solute and water probably didn't happen, because pansies' petals have a structure named papillae (conical epidermal cells) in the epidermis on both sides of all the petals (spurred, lateral and upper) (Weryszko-Chmielewska and Sulborska, 2012) and this structure has been reported to be superhydrophobic (Schulte et al. 2011). In the present work, we observed pansies' petals by microscopy (Fig. 4.3.2.4). The papillae were very well visible, explaining the obtained results. In some situations the increased weight observed in pansies could be due to some sugar or salt dried in the flowers outside. However, when salt was used in different concentrations (15, 20, 25%) during 1 hour, pansies lost weight because probably some cell rupture occurred, contributing to the bad visual appearance (Figure 4.3.2.1). Regarding lettuce, all treatments induced weight loss, occurring effective osmotic dehydration. Comparing different concentrations of sucrose (60% and 80%) at 1 h of immersion, 80% of sucrose was not more effective than at 60%. This is in line with Ponting *et al*. (1966), who stated that above 60% sugar concentration, an additional increase in sugar concentration did not promote further water loss in vegetables. However, it can be observed that lettuce decreased weight by increasing the immersion time (1 and 2 hours) for both sucrose concentrations. Regarding salt treatments, lettuce showed a higher decrease in weight with higher times of immersion (more than 30 min). However, it was observed that the effect was not as evident as when the concentrations of the osmotic solution increased.

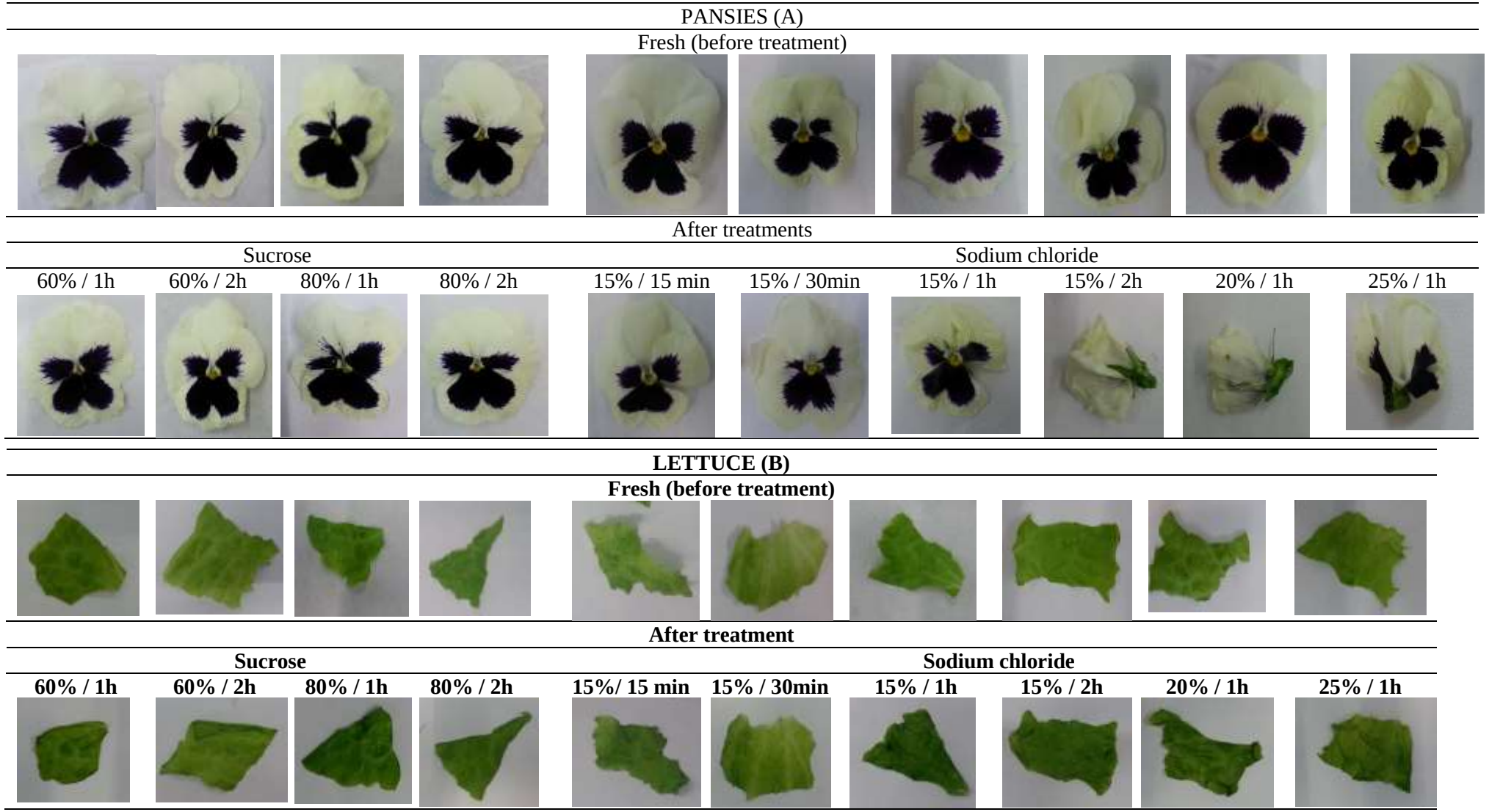

Figure 4.3.2.1- Visual appearance of pansies (A) and lettuce (B) before and after immersion in osmotic solutions with sucrose and sodium chloride.

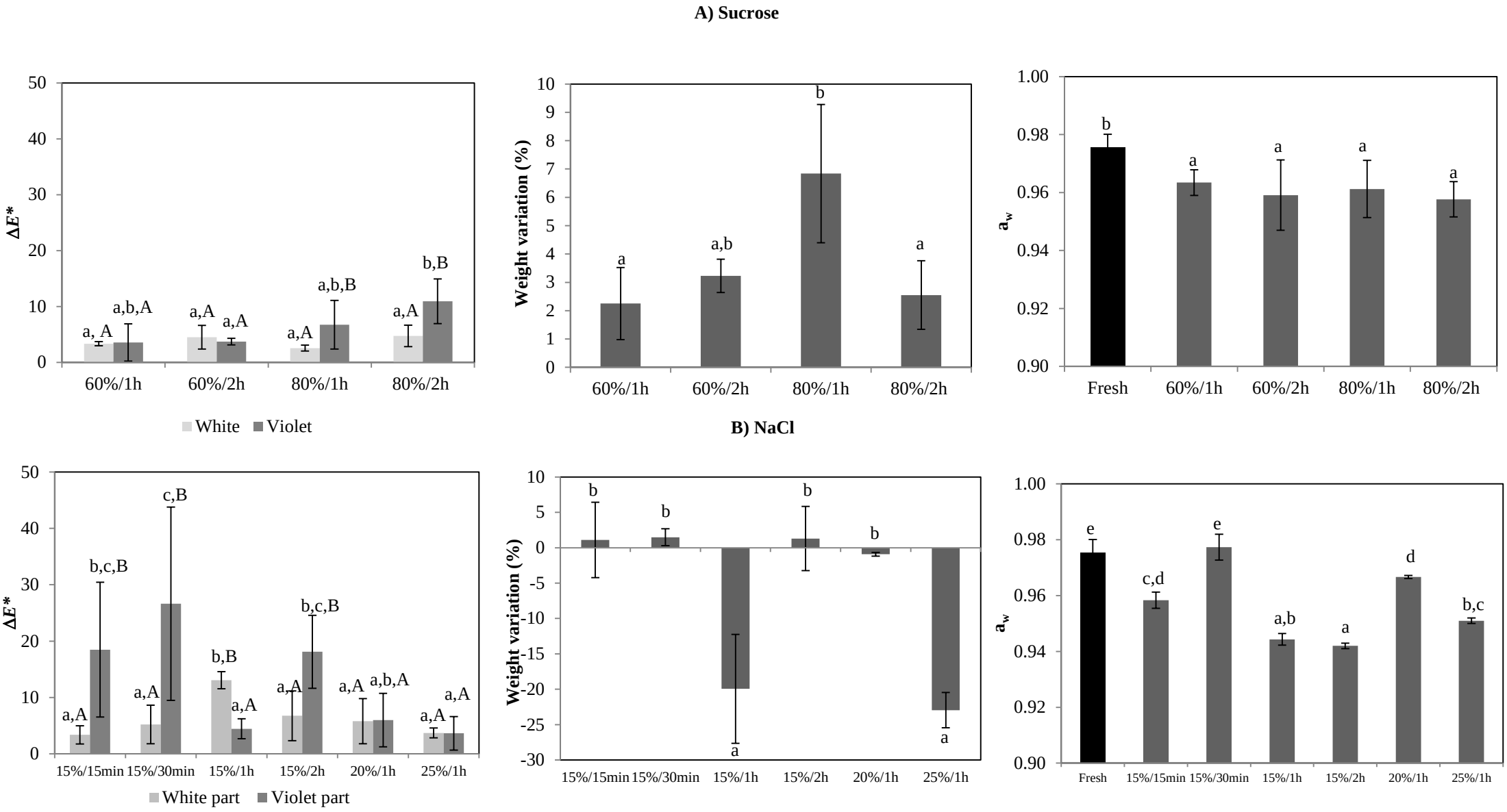

Figure 4.3.2.2 - A_w, weight (%) and ΔE* variation before and after pansies treatments with sucrose (A) and sodium chloride (B) solutions (lower letters compare treatments while caps compare different parts of pansies (white and violet). Values with the same letter are not statistically different ($p>0.05$)).

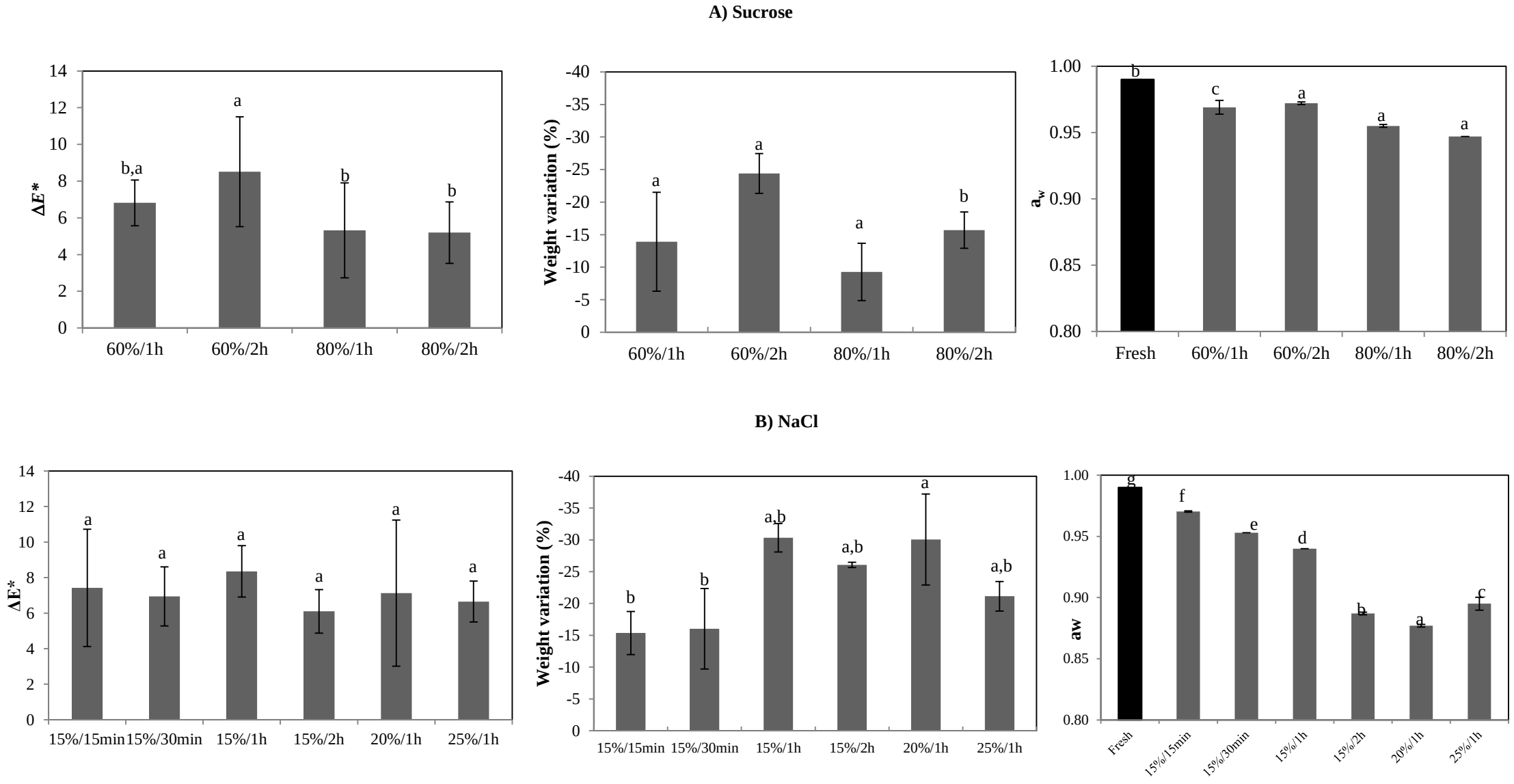

Figure 4.3.2.3- $A_{w,}$ weight variation and ΔE^* contents before and after lettuce treated with sucrose (A) and sodium chloride (B) solutions.

Based on these observations it seems that pansies' structure does not allow the exchange of solutes with osmotic solutions, being therefore not adequate for osmotic drying.

Low water activity reduces the growth of microorganisms and decreases biochemical reactions, important for the preservation of food. Regarding the water activity (a_w) contents in pansies, the highest values were obtained in fresh samples. A decrease in a_w values was observed after all treatments, except with 15% NaCl for 30 min. However, when using sucrose no significant differences were detected between treatments. With NaCl, higher contact times (ex: 1 and 2 h at 15%, w/v) or higher salt concentrations (20 and 25%; w/v) caused an effective decrease on the a_w values. As expected, on lettuce subjected to different osmotic solutions, a_w values also decreased compared to the fresh sample. In general, increased immersion times and concentrations induced a decrease of a_w, with a linear decreasing effect. Furthermore, this decrease was more pronounced when using sodium chloride as an osmotic solution.

Carotenoids

Figure 4.3.2.5 presents the carotenoids contents of pansies (A) and lettuce (B) before and after immersion in osmotic solution. The highest concentrations were observed in the fresh sample for pansies and lettuce (75.7 and 72.8 μg β-carotene /g fresh matter, respectively). In general, a decrease of carotenoids contents was observed with the osmotic treatments. This decrease might be due to partial leaching of these pigments as the osmotic stress increased, due to the breakage of the cell structures (Tadesse et al., 2015), or that carotenoids are susceptible to oxidation, isomerisation and other chemical changes during processing mostly due to their extensive conjugated double-bond systems (Shi and Maguer, 2000). Samples immersed in sucrose showed lower contents of carotenoids than in sodium chloride. In more detail, the effect of different concentrations of osmotic solutions and immersion time on carotenoids content of lettuce and pansies were significant ($p < 0.05$). For pansies, the best conditions to retain the highest amount of carotenoids was 15% sodium chloride with 30 min of immersion, and for lettuce was 15% of sodium chloride for 15 min. For pansies, when using sucrose, no significant differences were obtained between treatments.

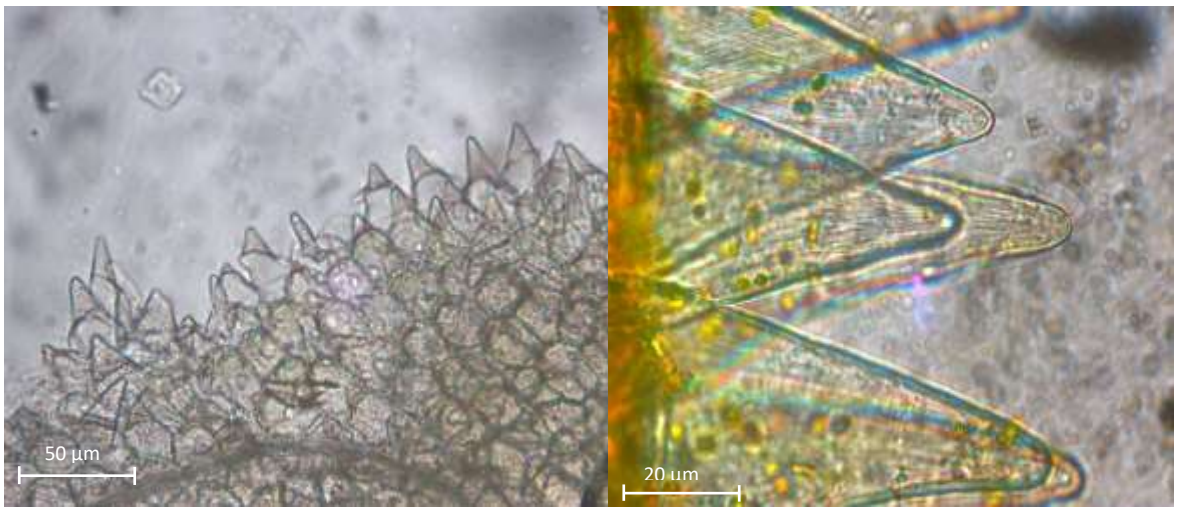

Figure 4.3.2.4 - Conical papillae on the surface of *Viola×wittrociana*.

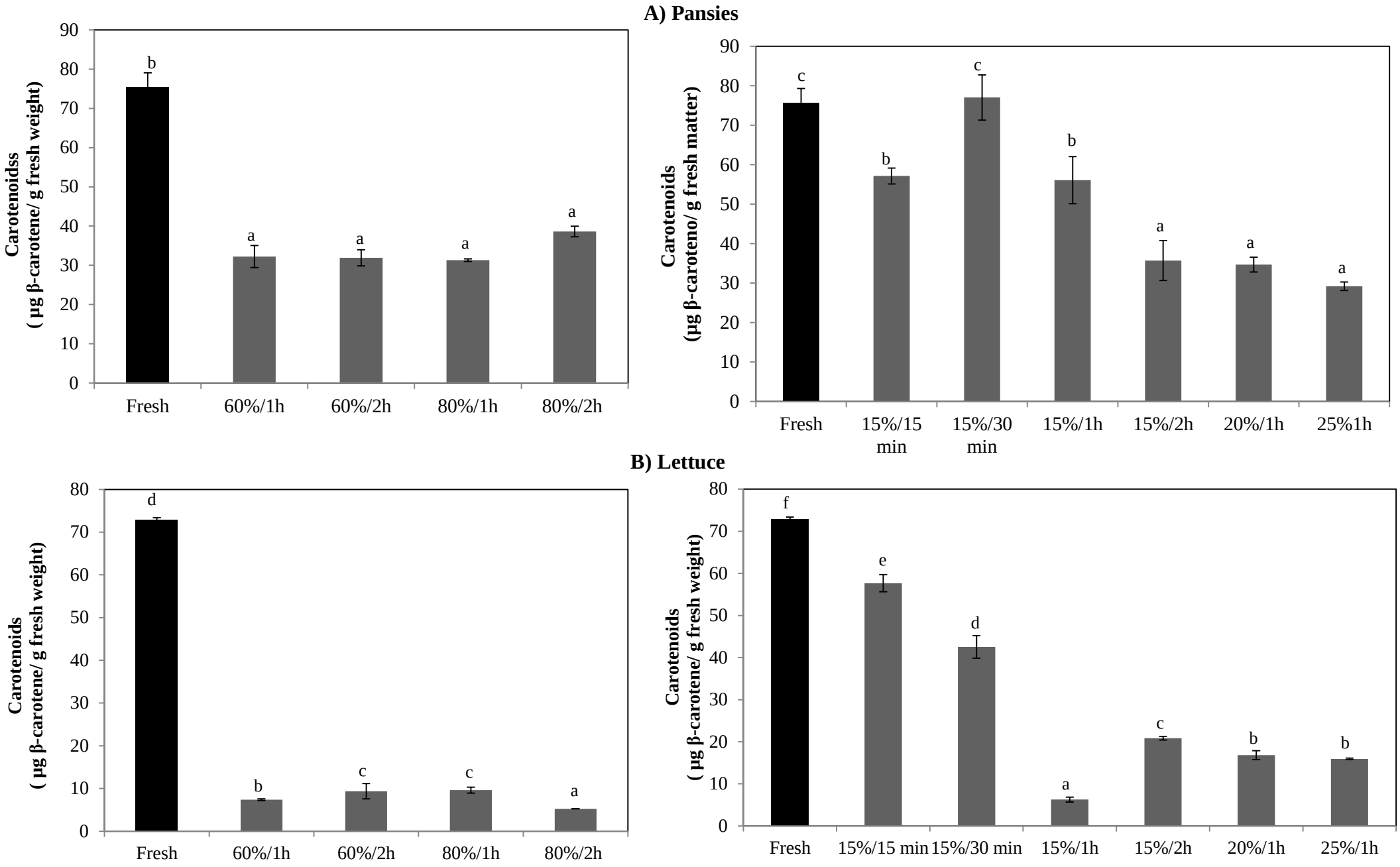

Figure 4.3.2.5 - Carotenoids content (µg β-carotene/g fresh matter) before and after pansies (A) and lettuce (B) treatments with sucrose solutions and sodium chloride.

Monomeric anthocyanins

The total monomeric anthocyanins contents in the fresh and pansies immersed in osmotic solutions are shown in Figure 4.3.2.6A (sucrose) and B (NaCl). Lettuce anthocyanins, if present, were below the detection limit.

Anthocyanins are the main compounds responsible for the wide range of colors in pansies. Some authors report that the anthocyanins present in *Viola* species are formed from malvidin, peonidin and petunidin anthocyanidins (Skowyra *et al*., 2014; Gamsjaeger *et al*., 2011; Zhang *et al*., 2012). From all the treatments, only immersion in 60% sucrose during 1h induced a significant increase of total monomeric anthocyanins compared with fresh samples. A direct interpretation of this result could not be within the expected because anthocyanins are soluble in water and they exist in epidermal and sub-epidermal cells, dissolved in vacuoles or accumulated in vesicles called anthocyanoplasts (Karami et al., 2013). Therefore, they could leak into the osmotic medium through the cuticle and skin ruptures (Karami et al; 2013). Within the other sucrose treatments, no significant differences were observed between assays, not indicating the existence of degradation or leaching of these compounds. Furthermore, the sugar concentrations applied in the present work were not sufficient to increase the pH of the solution, which may raise the percentage of anthocyanins in the colourless carbinol base form that is very unstable, making the pigment more susceptible to degradation by oxygen (Stojanovic and Silva, 2007; Karami et al.; 2013).

For NaCl, only the treatment with 15% and 2h of immersion caused a decrease of the monomeric anthocyanins content when compared to the fresh state. This result might be due to the increase in the contact time of pansies in the sodium chloride solution leading to an increase in osmotic pressure and enhanced water loss. High water loss resulted in a higher loss of anthocyanins because they are water-soluble pigments.

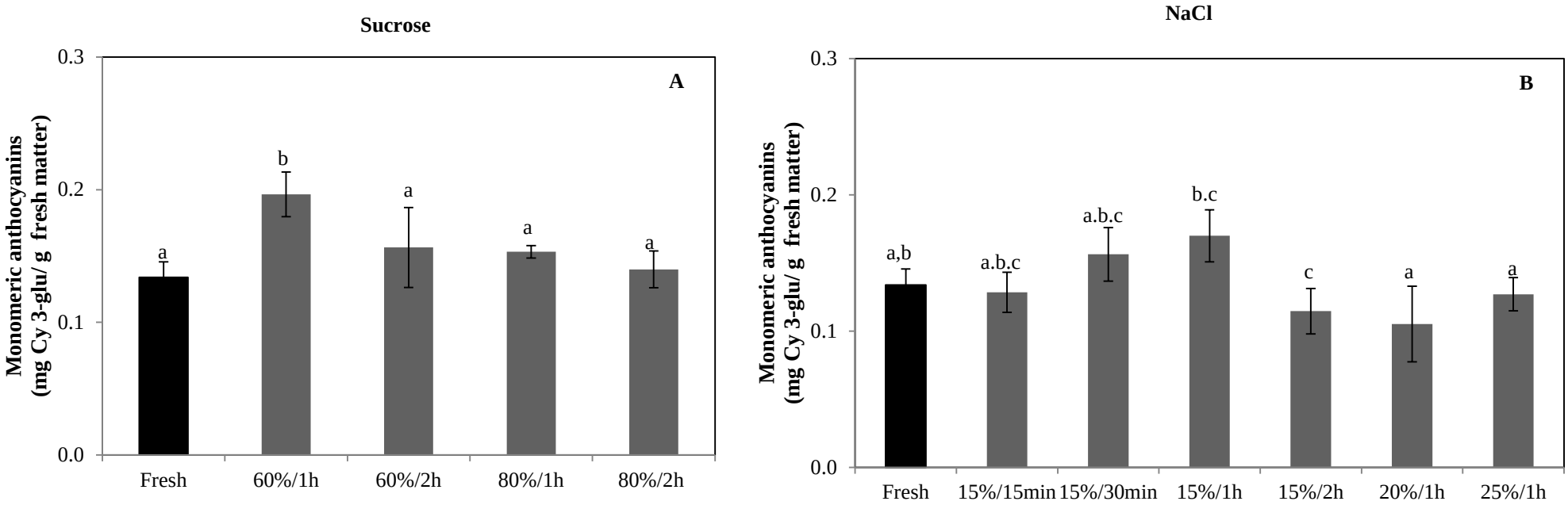

Figure 4.3.2.6 - Monomeric anthocyanins content (mg Cy 3-G/g fresh matter) before and after pansies treatments with sucrose (A) and sodium chloride (B) solutions.

Conclusion

In summary, pansies present a superhydrophobic structure that increases the resistance to osmotic dehydration with both sucrose and sodium chloride solutions. In general, when pansies were submitted to osmotic solutions, an almost general weight gain, a_w maintenance and high monomeric anthocyanins contents were observed. However, high concentrations of sodium chloride induced damage in pansies' structure and color alterations. Comparedto pansies, all treatments applied to lettuce caused effective osmotic dehydration, with weight loss and reduced a_w, as expected when performing the osmotic dehydration of vegetables. So, immersion in osmotic solutions cannot be applied to pansies due to their morphological structure, namely the presence of papillae (conical epidermal cells) that are extremely hydrophobic.

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4.3.3.

Physicochemical, antioxidant and microbial properties of crystallized pansies (*Viola* × *wittrockiana*) during storage.

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Abstract

Edible flowers, such as pansies, have a short shelf-life. Crystallization is a treatment used to prolong the shelf-life of fruits and vegetables. So, the aim of the present work was to investigate the effect of crystallization in the physicochemical, microbial and antioxidant properties of pansies (*Viola×wittrockiana*) during room temperature storage (7, 15, 30, 60 and 90 days). Comparing fresh and crystallized pansies, significant differences were detected. After 90 days of storage, crystallized pansies still kept good visual appearance. A significant decrease in water activity, moisture, ash and protein contents was observed. Some bioactive compounds, such as flavonoids and monomeric anthocyanins, showed a significant decrease after 90 days of storage, while hydrolysable tannins increased. Concerning phenolic compounds, these maintained constant along storage. The microbial load of crystallized pansies during all storage time was lower than fresh ones. So, crystallization can be an effective method for pansies preservation, being some nutritional and bioactive compounds, little affected.

Keywords: Edible flowers; Crystallization; Bioactive compounds; Antioxidant activity; Nutritional composition; Microbial quality; Storage.

Introduction

Garden pansies (*Viola* × *wittrockiana*) are edible flowers that have been used for years in cooking or as decoration in several dishes. Their flavor and colour make them special, not only for their fresh consumption, but also as an ingredient in salads and to garnish desserts (frosted cakes, sorbets and iced drinks). Pansies are perfect cool-season edible flowers, so they will grow in winter and again in spring. In order to have flowers' products available for the food industry all around the year, new technologies must be tested and applied to edible flowers. The market of this product is not only interested in fresh products but also in processed flowers' products, which should maintain the sensory properties as unaltered as possible. In this sense, a process that originates good quality flowers' products could be an option to increase their market share. The most frequent applied processes to preserve pansies include refrigeration, drying and preservation in distillates. Nevertheless, these technologies have undesirable effects, namely, changes in texture and color, non-fresh flavor with a loss of nutritive value, which reduces its economic importance.

Crystallization is a technology that consist in placing fresh or frozen raw materials in a hypertonic solution prepared from sugars, most often from sucrose and glucose (Barat et al. 2002; Korel and Balaban 2006; Witczak et al. 2017). In particular, crystallized flowers consist in coating a flower with egg white (pasteurized), followed by dipping and rolling the flower in a sugar solution, and then sprinkling the upper surface of the flower again with sugar (Clemons, 2006). Crystallization is described as a technology able to extend the shelf-life (due to dehydration), to maintain appropriate taste and nutritive value, and to retain the firmness, the natural aroma and color of the fresh product (Witczak et al. 2017). However, until now this technology was most applied to fruits, such as: orange peel (Witczak et al. 2017), chestnuts (Korel and Balaban, 2006), pineapple (Barat et al., 2002) and plums (Nunes et al., 2008). Some cookbooks mention recipes for this method to be applied to edible flowers (Creasy, 2012, Dupree, 2004; McGee, 2010). Crystallized flowers are fantastic for decorating cakes or desserts and to accompany any sweet dish. Nowadays, there are some companies that sell crystallized flowers, such as Candiflor in Toulousse, Marx Patry in US, Fresh Origins in California, Eat My Flowers in United Kingdom, Meadowsweet flowers in England and Ervas Finas in Portugal. Of our knowledge, until now there is no available scientific literature about the effect of crystallization on quality and shelf life of pansies. In this order, the objective of this study was to evaluate, for the first time, the effect of crystallization on

the quality of pansies during 90 days of storage under room temperature. The quality of pansies was evaluated in relation to some physicochemical characteristics, namely: visual appearance, water activity (a_w) and weight loss, as well as, bioactive compounds (total reducing capacity, flavonoids, monomeric anthocyanins and hydrolysable tannins) and antioxidant activity (DPPH radical scavenging activity and reducing power). Furthermore, the nutritional and microbial qualities were also evaluated.

Material and Methods

Samples

Fresh white pansies (*Viola×wittrockiana*) were collected in full ripening state at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança (Bragança, Portugal). After harvest, fresh flowers were immediately transported to the laboratory under refrigeration.

Crystallization

Crystallization treatment was applied according to some cookbooks. Pansies were painted with egg white (pasteurized) on the front and back of flowers, using a fine brush. Then they were sprinkled with sugar evenly over the wet petals and placed face down on paper. Previously, the immersion of pansies in a sucrose solution was tested, but the flowers became wrinkled and with a fragile texture. Crystalized flowers were maintained under refrigeration (4°C) for about 48 h.

Storage

Crystallized pansies were stored under room temperature, during 90 days. After 7, 15, 30, 60 and 90 days of storage, photos of the flowers were taken and the physico-chemical composition, bioactivity and antioxidant activity were measured, as detailed below. After storage the samples were frozen and freeze-dried (Coolsafe, Lynge, Denmark) to perform the chemical analyses, while for microbial quality evaluation the samples in fresh state were used.

Water activity and weight loss

Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). Weight was measured in a digital balance (Kern

ACJ/ACS, Balingen, Germany) and weight loss (WL) was determined according to Equation 1:

$$WL = \frac{M_0 - M}{M_0} \times 100 \quad \text{Equation 1}$$

where M_0 is the initial mass of crystallized pansies in day 0 and M is the mass of crystallized pansies during storage.

Nutritional composition

The nutritional composition (moisture, ash, total lipids, reducing sugars and carbohydrates) of each sample were analysed using AOAC procedures (1990) and expressed in g/100 g fresh weigh (fw). Moisture content was determined by drying the sample to a constant weight at 105 ^{o}C; ash content was measured by calcination at 550 ^{o}C for at least 2 hours, until achieving white ashes. Protein content of the samples was estimated by the Kjeldahl method, using a conversion factor of 6.25, according to Sotelo et al. (2007) and Rop et al. (2012). Lipids were determined by extracting a known mass of powdered sample with petroleum ether, using a Soxhlet apparatus. Reducing sugars were determined by the dinitrosalicylic acid (DNS) method. Carbohydrates were calculated according to Equation 2:

$$Carbohydrates\ \left(\frac{g}{100g\ fw}\right) = 100 - (moisture + ash + protein + lipids\ ) \quad \text{Eq. 2}$$

Energy was calculated according to equation 3:

$$Energy\ \left(\frac{kcal}{100g\ fw}\right) = \left(4 \times \left[\frac{g(protein)}{100\ g\ fw} + \frac{g(carbohydrates)}{100\ g\ fw}\right]\right) + \left(9 \times \frac{[g(lipids)]}{100\ g\ fw}\right) \quad \text{Eq. 3}$$

Extraction conditions for bioactivity evaluation

The extraction conditions used were those described by Li et al. (2014), with slight modifications. Freeze-dried powder of each sample (1 g) was extracted with 10 ml of water:acetone (6:4, v/v), at 37 ºC for 30 min under agitation (900 rpm, IKA, RCT Model B, Staufen, Germany). The water:acetone extracts were filtered and placed in a rotary evaporator (Stuart, RE300DB, Stone, UK) to remove the solvent. Then, all extracts were frozen and placed in the freeze drier (Coolsafe, Lynge, Denmark) for 2 days. The extracts obtained were redissolved within the same solvent to a concentration of 50 mg extract/mL and covered with aluminium foil under freezing until further analysis.

Bioactive compounds

Total monomeric anthocyanins, total flavonoids and hydrolysable tannin contents, of fresh (0 days) and crystalized pansies (0, 7, 15, 30, 60 and 90 days) were determined following the methodologies used by Fernandes et al. (2018). All measurements were performed in triplicate. The results for monomeric anthocyanins were expressed in μg cyanidin-3-glucoside/g dry weight (μg Cy 3-glu/g dw), flavonoids in mg of quercetin equivalent/g dry weight (mg QE/g dw) and hydrolysable tannins in mg of tannic acid equivalent/g dry weigh (mg TAE/g dw).

Total reducing capacity

The total reducing capacity (TRC) of each sample was determined by the Folin-Ciocalteu method described by Falcão et al. (2017). To 8 mL of the extract solution was added 500 μL of Folin-Ciocalteu reagent. After 3 to 8 minutes, 1.5 mL of saturated sodium carbonate solution was added. After 2 hours the absorbance values were read at 765 nm. The blank and standards were prepared similarly, replacing the sample by the solvent used in the extraction and the standards, respectively. A calibration curve was obtained with gallic acid (0.25 to 5 mg/L) and the results expressed on mg gallic acid equivalent/g dry weight (mg GAE/g dw).

Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity was determined by the procedure described by Delgado et al. (2010) with some modifications. The pansies extract solutions were diluted with the solvent used for the extraction and 300 μL of these solutions were added to 2.7 mL of the DPPH methanolic solution (6.09×10^{-5} mol/L). After 1 hour in the dark at room temperature, the absorbance was read at 517 nm. Antioxidant activity was expressed by the percentage of scavenging effect according to the formula in Equation 4:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \quad \text{Eq. 4}$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The blank was made with the solution used in the extraction of

the samples. The extract concentration providing 50% of DPPH radical scavenging effect (EC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage *versus* extract concentration.

Reducing power

The reducing powers of the extracts were determined by the procedure described by Delgado et al. (2010). To 1.0 mL of extract solutions at different concentrations were added 2.5 mL of phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ 1% (m/v). After shaking, the mixture was incubated at 50 °C for 20 minutes. 2.5 mL of 10% trichloroacetic acid (m/v) was added with further stirring. A volume of 2.5 mL of the mixture was transferred to another test tube, to which 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1% (m/v) were added. The absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ *versus* concentration, the EC_{50} values were determined corresponding to the concentration that gave an absorbance of 0.5.

Microbial quality

In fresh and crystallized pansies (0 days), as well as, crystallized pansies after storage (7 and 90 days) were determined the total aerobic mesophilic, yeast, moulds, total coliforms, *Escherichia coli* and psychrotrophic bacteria counts. Five grams of sample was diluted in 45 mL of glycerol (20%). Samples were placed in sterile stomacher bags and homogenized in a Stomacher 400 (Seward, UK) for 2 min. The homogenates were subjected to serial dilutions with peptone water and then 1 mL of each dilution was pipetted into the surface of Plate Count Agar (PCAg, Merck), Dichloran Glycerol (DG-18, Merck) and Man, Rogosa and Sharpe Agar (MRS-Agar, Merck). The PCAg plates were then incubated for 2 days at 30 °C for total aerobic mesophilic count and 5 days at 10 °C for psychrotrophic bacteria count. Yeast and moulds were determined in DG18-agar plates, incubated at 27 °C for 3-5 days. Total coliforms and *E. coli* were determined according to the SimPlate method. All counts were expressed as log10 cfu/g fresh sample.

Statistical analysis

The statistical analysis was performed on SPSS software, Version No. 18.0 (SPSS Inc., Chicago, USA). The normality of the data was verified by Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch were carried out to determine if there were

significant differences ($p<0.05$) between samples, depending on the existence or not of homogeneity of variances, respectively. Additionally, if significant differences were detected between treatments, a post hoc analysis was performed, namely: Tukey's honestly significant difference test (if variances in the different groups were identical) or Games-Howell test (if they were not). The homogeneity of the variances was tested by Levene's test.

Results and Discussion

Visual appearance, a_w and weight loss

Visual appearance, a_w and weight loss of fresh and crystallized flowers during storage are described in Figure 4.3.3.1 and Table 4.3.3.1. The visual appearance of the flowers is one of the attributes that more attracts the consumer and strongly affects the decision of buying. Furthermore, at the point of purchase, the consumer uses factors associated to appearance to provide an indication of freshness and quality. Regarding Figure 4.3.3.1, crystallized pansies in 7, 15, 30, 60 and 90 days showed similar appearance to that of crystallized ones at day zero. So, storage time didn't influence the visual appearance of crystallized pansies. However, looking the data with more detail, the moisture content decreased from 1.69 to 0.28 g/100 g fw and the a_w from 0.83 to 0.38 at 0 and 90 days of storage. After this period of time, the weight loss was only equal to 12.6 %. Thus, some water loss was observed, but as the sugar layer was so significant, the loss of water was not visible, being pansies' appearance preserved.

Time of storage	Visual appearance
0 day	
7 days	
15 days	
30 days	
60 days	
90 days	

Figure 4.3.3.1 - Visual appearance of crystallized pansies during storage

Table 4.3.3.1 – Water activity (a_w), weight loss (WL) (%), moisture content, macronutrients composition (g/100 g fw) and energetic value (kcal/100 g fw) of fresh and crystallized pansies during storage.

Samples	Storage	a_w	WL	Moisture	Ash	Protein	Lipid	Reducing sugars	Carbohydrates	Energy
Fresh	0 days	0.98 ± 0.01^{d}	---	91.3 ± 2.1^{b}	1.00 ± 0.03^{c}	2.03 ± 0.06^{c}	$0.45\pm0.01^{a,b}$	0.84 ± 0.05^{a}	5.3 ± 2.0^{a}	33 ± 8^{a}
Crystallized	0 day	$0.83\pm0.01^{c,C}$	---	$1.69\pm0.04^{a,C}$	$0.51\pm0.04^{b,B}$	$5.07\pm0.47^{d,C}$	$0.26\pm0.18^{a,A}$	$3.99\pm0.08^{d,C}$	$92.2\pm0.6^{b,A}$	$393\pm1^{b,A}$
	7 days	$0.43\pm0.01^{a,b,A,B}$	6.0 ± 5.3^{a}	$1.40\pm0.20^{a,B,C}$	$0.38\pm0.09^{a,b,A,B}$	$1.49\pm0.14^{b,c,B}$	$0.30\pm0.11^{a,b,A}$	$2.09\pm0.29^{b,A,B}$	$96.4\pm0.2^{c,d,B}$	$394\pm1^{b,A,B}$
	15 days	$0.50\pm0.01^{b,B}$	8.6 ± 6.4^{a}	$1.35\pm0.33^{a,B,C}$	$0.47\pm0.04^{b,B}$	$1.42\pm0.22^{b,c,B}$	$0.73\pm0.12^{b,B}$	$2.38\pm0.02^{c,B}$	$96.0\pm0.5^{c,B}$	$396\pm1^{b,B,C}$
	30 days	$0.50\pm0.01^{b,B}$	10.0 ± 6.8^{a}	$1.05\pm0.22^{a,B}$	$0.48\pm0.10^{b,B}$	$1.55\pm0.08^{b,c,B}$	$0.68\pm0.20^{a,b,A,B}$	$4.08\pm0.39^{d,C}$	$96.2\pm0.7^{c,B}$	$397\pm1^{b,C,D}$
	60 days	$0.35\pm0.01^{a,A}$	11.7 ± 6.8^{a}	$0.52\pm0.10^{a,A}$	$0.25\pm0.10^{a,b,A,B}$	$1.09\pm0.25^{a,b,A,B}$	$0.38\pm0.15^{a,A,B}$	$4.27\pm0.12^{d,C}$	$97.8\pm0.2^{c,d,C}$	$399\pm1^{b,D}$
	90 days	$0.38\pm0.01^{a,A}$	12.6 ± 6.5^{a}	$0.28\pm0.05^{a,A}$	$0.19\pm0.05^{a,A}$	$0.67\pm0.07^{a,A}$	$0.29\pm0.13^{a,A}$	$1.91\pm0.01^{b,A}$	$98.6\pm0.3^{d,C}$	$400\pm1^{b,D}$

Values are expressed as: Mean±Standard deviation. Lowercase letters compare all samples, while uppercase letters only compare crystallized pansies. Values with the same letter in the same column are not statistically different ($p>0.05$)

Nutritional composition

Data on the nutritional composition and energetic value of fresh and crystallized pansies are shown in Table 4.3.3.1. Fresh pansies showed the highest water activity value (0.98), as well as moisture and ash contents (91.3 and 1.00 g/100 g fw, respectively), Carbohydrates were the second most abundant macronutrients in fresh pansies (5.3 g/100g fw), followed by proteins (2.03 g/100g fw). Fresh pansies only presented 0.84 g/100g fw in reducing sugars and an energetic value of 33 kcal/100 g fw, being a good food option for low-calorie diets. Similar results for fresh pansies were reported by other authors, namely: moisture (86.32 g/100 g fw), carbohydrates (6.5 g/100 g fw), proteins (2.1 g/100g fw), lipids (0.44 g/100 g fw), ash (1.1 g/100 g fw) and energy (38 kcal/100 g fw) (González-Barrio et al. 2018); and proteins (0.67 g/100 g fw) (Rop et al. 2012).

Crystallization of pansies with sucrose (0 days) decreased the water activity to 0.83, and the moisture and ash contents to 1.69 and 0.51 g/100 g fw, respectively, because the added sucrose decreased the water availability for microorganisms and contributed to the fresh weight, reducing the water and ash's proportion. In fact, the sugar used was refined and so its mineral content is low, explaining the observed decrease in ash content. On contrary, the reducing sugars and carbohydrates contents in crystalized pansies (0 days) increased to 3.99 and 92.2 g/100g fw, approximately 4.8 and 17 times more than in fresh (not crystalized) due to the addition of sugar to pansies. One part of the sucrose may had suffered hydrolysis, liberating glucose and fructose, which are reducing sugars. Consequently, the energetic value increased 12 times more, corresponding to 393 kcal/100 g fw, when compared to fresh (not crystalized).

Along storage (90 days), the moisture content decreased from 1.69 to 0.28 g/100 g fw because some water might had diffused out from pansies. A final water activity equal to 0.38 has been achieved, indicating that crystallized pansies were microbiologically stable. Furthermore, the lowest ash, protein and reducing sugars contents were observed after 90 days of storage, while the highest carbohydrates content and energetic value were obtained. A similar decrease in moisture content was reported for a pumpkin candy, between 20.1 to 9.3% for 0 to 3 months, respectively (Muzzaffar et al. 2016). Furthermore, a decrease in ash and protein values was also observed in a tomato candy with different percentages of sugar (40, 50 and 60%) during storage (0, 2, 4 and 6 months) (Hasanuzzaman et al., 2014). Concerning reducing sugars, Muzzaffar et al. (2016) observed an increase in these compounds from 2.9 to 3.9% at 0 to 3 months,

respectively, during storage, while in the present work no trend in the reducing sugars of crystallized pansies along 90 days of storage was observed.

Bioactive compounds

Table 4.3.3.2 shows that fresh pansies presented the highest values for all studied bioactive compounds. These results were because crystallized pansies had sugar around flower, which contributed to the dry weight, decreasing the bioactive compounds' concentrations. Comparing crystallized pansies during storage, statistically significant differences in bioactive compound values were detected, except in total reducing capacity (total phenols). However, a similar trend was not detected for the bioactive compounds during storage. In general, flavonoids and monomeric anthocyanins decreased along storage. This could mainly be a result of oxidation, polymerization of some phenolic compounds with proteins or to the disruption in cell structure during processing (Varela-Santos et al., 2012, Kim and Padilla-Zakour 2004). On contrary, hydrolysable tannins increased their values from 1.75 to 3.59 mg TAE/g dw during 90 days of storage. Concerning the total phenols contents (TRC assay) of crystallized pansies, similar values were observed during all storage period, suggesting that crystallization conferred some degree of protection on these compounds. Different results to ours were reported by Muzzaffar et al. (2016), who detected a 10% decrease in phenolic compounds contents during storage (0 days to 3 months) of a pumpkin candy.

Antioxidant activity

The antioxidant activity determined by the DPPH radical scavenging and reducing power assays are reported in Table 4.3.3.2. The results showed that fresh pansies had higher values of antioxidant activity than crystallized pansies. During storage of crystallized pansies, statistically significant differences in the antioxidant activity were observed. An increase in the EC_{50} DPPH value was detected between day 0 and 90 days (12.9 to 18.2 mg extract/mL), indicative of a decrease in the antioxidant power. A similar decrease was observed in DPPH (34.10 to 28.27%) and reducing power (48.08 to 40.26%) assays for a pumpkin candy stored during 0 days to 3 months (Muzzaffar et al. 2016). However, in the present work a slight decrease of EC_{50} reducing power values was observed (24.5 to 22.7 mg extract/mL for 0 and 90 days, respectively), suggesting a higher antioxidant activity.

Table 4.3.3.2 – Total phenols, hydrolysable tannins, flavonoids, total monomeric anthocyanins, DPPH radical scavenging effect and reducing power in fresh and crystallized pansies during storage (n=3).

Samples	Storage	TRC (mg GAE/g dw)	Total flavonoids (mg QE/g dw)	Hydrolysable tannins (mg TAE/g dw)	Monomeric anthocyanins (µg Cy-3 glu/g dw)	EC_{50} DPPH (mg extract/mL)	EC_{50} Reducing Power (mg extract/mL)
Fresh	0 days	5.43 ± 1.25^{b}	115 ± 7.9^{b}	52.3 ± 10.0^{b}	1016 ± 30^{d}	0.34 ± 0.02^{a}	6.60 ± 0.24^{a}
Crystallized	0 day	$0.61\pm0.16^{a,A}$	$4.11\pm0.72^{a,B}$	$1.75\pm0.10^{a,A}$	$40.3\pm12.7^{a,A}$	$12.9\pm0.4^{b,A}$	$24.5\pm0.4^{f,F}$
	7 days	$0.60\pm0.05^{a,A}$	$4.03\pm0.22^{a,B}$	$3.13\pm0.6^{a,B,C}$	$186\pm3^{c,D}$	$18.7\pm1.0^{d,e,C,D}$	$19.8\pm0.1^{c,d,C}$
	15 days	$0.64\pm0.03^{a,A}$	$3.50\pm0.29^{a,A}$	$2.64\pm0.52^{a,B}$	$187\pm3^{c,D}$	$16.1\pm0.5^{c.B}$	$19.4\pm0.1^{c,B}$
	30 days	$0.54\pm0.09^{a,A}$	$2.08\pm0.08^{a,A}$	$5.06\pm0.33^{a,D}$	$196\pm15^{c,D}$	$15.6\pm1.0^{c,B}$	$16.3\pm0.2^{b,A}$
	60 days	$0.62\pm0.06^{a,A}$	$2.45\pm0.15^{a,A}$	$4.39\pm0.66^{a,D}$	$117\pm9^{b,C}$	$19.7\pm0.1^{e,D}$	$20.2\pm0.1^{d,D}$
	90 days	$0.59\pm0.04^{a,A}$	$1.94\pm0.04^{a,A}$	$3.59\pm0.25^{a,C}$	$100\pm2^{b,B}$	$18.2\pm0.5^{d,C}$	$22.7\pm0.2^{e,E}$

Values are expressed as: Mean±Standard deviation. Lowercase letters compare all samples, while uppercase letters only compare crystallized pansies. Values with the same letter in the same column are not statistically different ($p>0.05$)

Microbial quality

The total viable bacterial counts in fresh and crystallized samples (after 7 and 90 days) are shown in Table 4.3.3.3. From the results, it was found that the bacterial load in crystallized pansies was lower than in fresh samples, and it decreased along storage.

Particularly, fresh pansies presented higher values of yeast (5.95 log cfu/g), total coliforms (1.15 log cfu/g) and total aerobic mesophilic microorganisms (4.84 log cfu/g) than crystallized samples; however, it should be noted that the values determined for fresh pansies are still acceptable, taking into account the established limits for salads and vegetables group (Santos et al. 2005)

Furthermore, crystallized samples stored for 7 days showed higher values of total aerobic mesophilic microorganisms than stored for 90 days (4.34 and 3.75 log cfu/g, respectively). This decrease was probably due to the sugar added to pansies that bound the water and reduced the amount of water available for the growth of microorganisms. So, they could not multiply and cause food spoilage (Hasanuzzaman et al. 2014; Muzzaffar et al., 2016), with crystallized pansies remained safe for human consumption during three months of storage.

Table 4.3.3.3 - Mean counts (log10 cfu/g±standard deviation) of total aerobic mesophilic microorganisms, yeasts, moulds, total coliforms, *E. coli* and psychrotrophic bacteria examined in fresh and crystallized pansies during storage for 0, 7 and 90 days.

Conditions							
Samples	Time	Total aerobic mesophilic	Yeasts	Moulds	Total coliforms	*E. coli*	Psychrotrophic bacteria
Fresh	0	4.84 ± 0.18^{c}	5.95 ± 0.03^{b}	<2	1.15 ± 0.21^{b}	<1	<2
Crystallized	7 days	4.34 ± 0.10^{b}	$<2^{a}$	<2	$<1^{a}$	<1	<2
	90 days	3.75 ± 0.11^{a}	$<2^{a}$	<2	$<1^{a}$	<1	<2

Values with the same letter in the same column are not statistically different ($p>0.05$).

Conclusion

In summary, pansies subjected to crystallization showed good appearance for 90 days of storage. However, significant differences in the nutritional composition, bioactive compound contents and antioxidant activity were detected between fresh and crystallized pansies. During storage, pansies showed a decrease in moisture, ash and protein contents, as well as, flavonoids and monomeric anthocyanins. On contrary, hydrolysable tannins increased. Furthermore, crystallized pansies had lower microorganism counts than fresh samples. So, crystallization method not only improved the shelf life of pansies but also maintained their appearance and improved their microbial quality.

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4.4.

Freezing

Freezing is a food preservation method successfully employed for long-term storage of many foods; however, this technology has not been applied frequently to edible flowers. Some new options are possible: to freeze edible flowers or put them in ice cubes. So, in the next section it is described the effect on microbial and antioxidant quality of four edible flowers (borage, pansies, kalanchoe, dandelion) that were frozen and put in ice cubes, during 1 and 3 months of storage (-18°C).

4.4.1.

Edible flowers in ice cubes and frozen: effect on microbial and antioxidant quality during storage

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Submitted

Abstract

Edible flowers are a new gourmet product, but they don't bloom all year. Thus, it is extremely important to find out technologies to guarantee this product for longer time. Four flowers (*Borago officinalis*, *Viola tricolor*, *Kalanchoe blossfeldiana* and *Taraxacum officinale*) were subjected to freezing (in their natural form and in ice cubes) and analyzed in terms of visual appearance, flavonoids, hydrolysable tannins, phenolics, antioxidant activity (DPPH radical scavenging activity and reducing power) and microbial quality, after storage for 1 and 3 months. Flowers in ice cubes showed similar appearance to fresh ones during the 3 months of storage, whereas frozen flowers were only equivalent up to 1 month, with exception of kalanchoe. No trend in bioactive compounds and antioxidant activity was observed between freezing treatment and storage time. Even though, flowers in ice cubes showed good appearance after 3 months of storage, they had the lowest values of bioactive compounds and antioxidant activity. On contrary, when frozen, the content of bioactive compounds maintained or even increased up to 1 month of storage compared to fresh flowers, except for borage. Furthermore, in both freezing treatments, the microorganisms' counts decreased or

maintained when compared to fresh samples, except in dandelion. So, in general, both treatments may allow keeping the flowers after their flowering times.

Keywords: Edible flowers, Frozen, Ice cubes, Antioxidant activity, Microbial quality, Storage.

Introduction

Edible flowers are often eaten fresh in salads or as garnishes because they can affect the sensory characteristics of food by improving color, taste and aesthetic appearance. For example: borage petals have a cucumber taste and its stamens add a hint of sweetness; dandelion has a sweet honey-like flavor; heartsease has a slightly sweet green or grassy flavor and kalanchoe flowers are sweet. However, flowers have short durability and their seasonality explains the necessity to apply preservation technologies. There are some technologies already used by edible flowers producers, such as, refrigeration, drying, crystallization and freezing. Among these methods, freezing is one of the most common ways for maintaining the quality of fresh foods because the respiration rates and enzymatic activities are reduced and the metabolic rates are lowered, extending product shelf life (Garcia and Barrett, 2002). Frozen flowers are suitable to be used in bakery products and ice creams. Another option is to use flowers to make ice cubes, described in several cookbooks (Cohen and Fisher, 2012; Horrelson, 2003). In this form, they will add colorful, elegance and flavor to beverages, such as: cocktails, ice teas, water, juices and alcoholic drinks. However, nutritional changes in frozen foods might occur along the storage time (Rickman et al., 2007). Of our knowledge, no work has been performed until now, on the effect of freezing on edible flowers. So, the aim of this study was to evaluate the effect of freezing in four edible flowers (borage, pansies, kalanchoe and dandelion, as it is and in ice cubes), on their bioactive compounds (flavonoids, hydrolysable tannins, and total phenols determined by the total reducing capacity assay), antioxidant activity (DPPH scavenging activity and reducing power), and microbial quality, during 1 and 3 months of storage (-18°C).

Materials and Methods

Samples

Fresh white borage (*Borago officinalis*), violet heartsease (*Viola tricolor*), yellow kalachoe (*Kalanchoe blossfeldiana*) and dandelion (*Taraxacum officinale*) in full

ripening state were collected at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança, Portugal. After harvest, the fresh flowers were immediately transported to the laboratory under refrigeration.

Preparation of ice cubes and freezing storage conditions

Four edible flowers were placed in an ice cubes tray. Ultrapure water was added to about 1/2 of the way full (4 mL), just enough to cover the flowers. They were placed in the freezer until solidification, and then more 4 mL of water was added until the cubes were completely full and frozen again. Ice cubes were stored under freezing (-18 °C) during 1 and 3 months. Portions of 10 g of each flower were put inside plastic bags and frozen at -18 °C during the same time.

Microbial quality

The microbial quality of the fresh flowers, the water used to produce the ice cubes, the ice cubes with frozen flowers and frozen flowers (in their natural form), were analyzed after 1 and 3 months of storage (-18°C). One gram of each sample was mixed with sterile peptone water solution and homogenized in a Stomacher 400 (Seward, UK). Decimal dilutions were prepared in the same diluent and plated on appropriate media in duplicate. The growth media and incubation conditions were the following for the studied microorganisms: (I) total mesophilic: Plate Count Agar (PCAg, Merck, Algés, Portugal) for 2 days at 30 °C; (II) yeasts and moulds: Rose Bengal Chloramphenicol Agar (RBC agar, Merck, Algés, Portugal) incubated at 27 °C for 5 days; and (III) total coliforms and *Escherichia coli* by the SimPlate® method. All counts were expressed as $\log_{10}$ cfu/g sample.

Extraction conditions for bioactive compounds analysis

The extraction conditions used were those described by Li et al. (2014), with slight modifications. One gram of each flower (fresh or after thawing – frozen flowers and in ice cubes) was extracted with 10 mL of water:acetone (6:4, v/v) at 37 ºC for 30 min under agitation (900 rpm, IKA, RCT Model B, Staufen, Germany). The solution was filtered and the final volume was adjusted to 10 mL. The procedure was done in triplicate.

Bioactive compounds (total flavonoids and hydrolysable tannins)

Total flavonoids and hydrolysable tannins contents of the extracts of fresh, ice cubes and frozen flowers during 1 and 3 months of storage, were determined following the methodologies used by Fernandes et al. (2017). All measurements were performed in triplicate. The results for flavonoids were expressed in mg of quercetin equivalent/g fresh weight (mg QE/g fw) and hydrolysable tannins in mg of tannic acid equivalent/g fresh weight (mg TAE/g fw).

Total reducing capacity

The total reducing capacity (TRC) of each sample was determined by the Folin-Ciocalteu method described by Falcão et al. (2017). To 8 mL of the extract solutions were added 500 μL of Folin-Ciocalteu reagent. After 3 to 8 minutes, 1.5 mL of saturated sodium carbonate solution was added. After 2 hours the absorbance values were read at 765 nm. The blank and standards were prepared similarly, replacing the sample by the solvent used in the extraction and the standards, respectively. A calibration curve was obtained with gallic acid (0.25 to 5 mg/L) and the results expressed on mg gallic acid equivalent/g fresh weight (mg GAE/g fw).

Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity was determined by the procedure described by Delgado et al. (2010), with some modifications. The extract solutions were diluted with the solvent used for the extraction and 300 μL of these solutions were added to 2.7 mL of the DPPH methanolic solution (6.09×10^{-5} mol/L). After 1 hour in the dark at room temperature, absorbance was read at 517 nm. The antioxidant activity was expressed by the percentage of scavenging effect according to Equation 1:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \qquad \text{Equation 1}$$

where A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The blank was made with the solution used in samples' extraction. The extract concentration providing 50% of DPPH radical scavenging effect

(EC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage *versus* extract concentration.

Reducing power

The reducing powers of the extracts were determined by the procedure described by Delgado et al. (2010). To 1.0 mL of the extract solutions at different concentrations were added 2.5 mL of phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ 1% (m/v). After shaking, the mixture was incubated at 50 °C for 20 minutes. 2.5 mL of 10 % trichloroacetic acid (m/v) was added with further stirring. A volume of 2.5 mL of the mixture was transferred to another test tube, to which 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1% (m/v) were added. The absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ *versus* extract concentration, the EC_{50} values were determined corresponding to the extract concentration that gave an absorbance of 0.5.

Results and Discussion

Visual appearance

Figures 4.4.1.1 and 4.4.1.2 show the visual appearance of fresh, in iced cubes and frozen flowers (in their natural form) during storage. Flowers in ice cubes showed good visual appearance until 3 months of storage (Figure 4.4.1.1), probably because the water around the flower protected them for physical and oxidative damage. However, frozen samples only showed good visual appearance until 1 month of storage. After this period, borage and dandelion showed signs of browning, and heartsease presented some loss of texture, being limp, resulting from chilling injury. Furthermore, flowers' freezing is a method that allows the flowers to look fine as long as they stay frozen, but they turn limp and their colors darken, as soon as they thaw (Nicolau and Gostin, 2015). On contrary, frozen kalanchoe had a visual appearance similar to fresh after 3 months of storage, probably because they have more rigid petals.

Storage time	Visual appearance			
	Borage (*Borago officinalis)*	**Dandelion** (*Taraxacum officinale*)	**Heartsease** (*Viola tricolor*)	**Kalanchoe** *(Kalanchoe blossfeldiana)*
Fresh				
1 day				
1 month				

Figure 4.4.1.1 – Visual appearance of borage, dandelion, heartsease and kalanchoe in fresh and in ice cubes during storage (-18 °C)

Storage time	Visual appearance			
	Borage (*Borago officinalis)*	**Dandelion** (*Taraxacum officinale*)	**Heartsease** (*Viola tricolor*)	**Kalanchoe** *(Kalanchoe blossfeldiana)*
Fresh				
1 month				
3 months				

Figure 4.4.1.2 - Visual appearance of borage, dandelion, heartsease and kalanchoe in fresh and frozen in their natural form during storage (-18 °C).

Total reducing capacity, total flavonoids and hydrolysable tannins

The content of bioactive compounds of fresh, and defrosted flowers (in their natural form and in ice cubes) after 1 and 3 months of storage are presented in Figures 4.4.1.3A, B and C. Compounds with antioxidant activity are very important for keeping

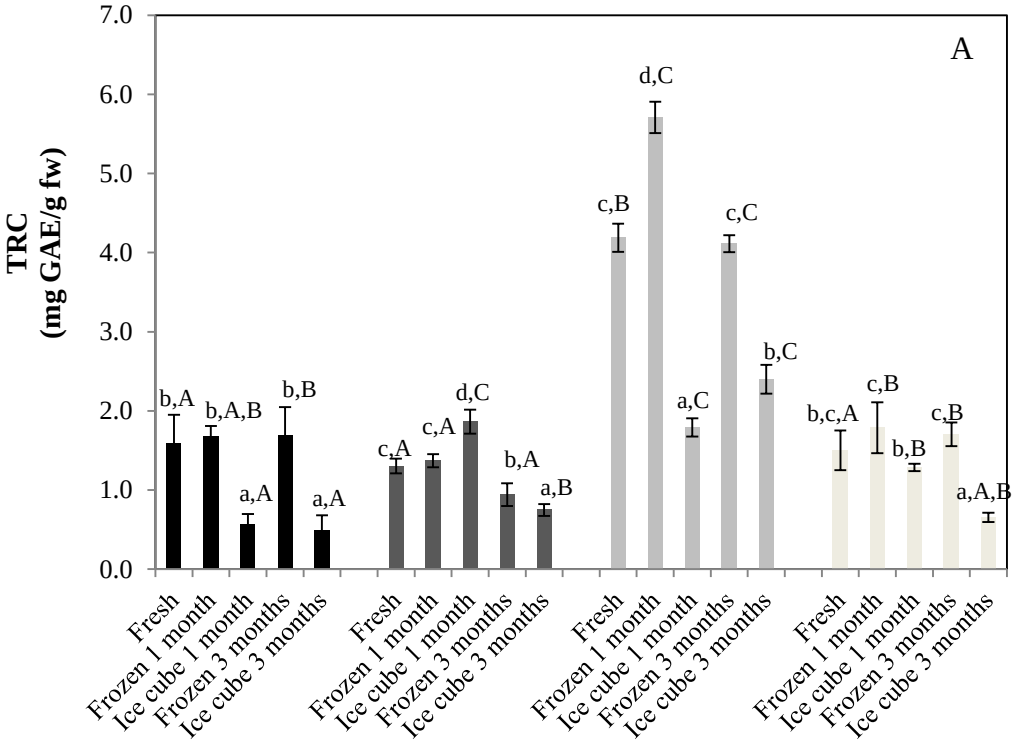
the quality of flowers because these substances delay the process of senescence and decay, which is caused by the action of reactive oxygen radicals on biomembranes (Panavas and Rubinstein, 1998).

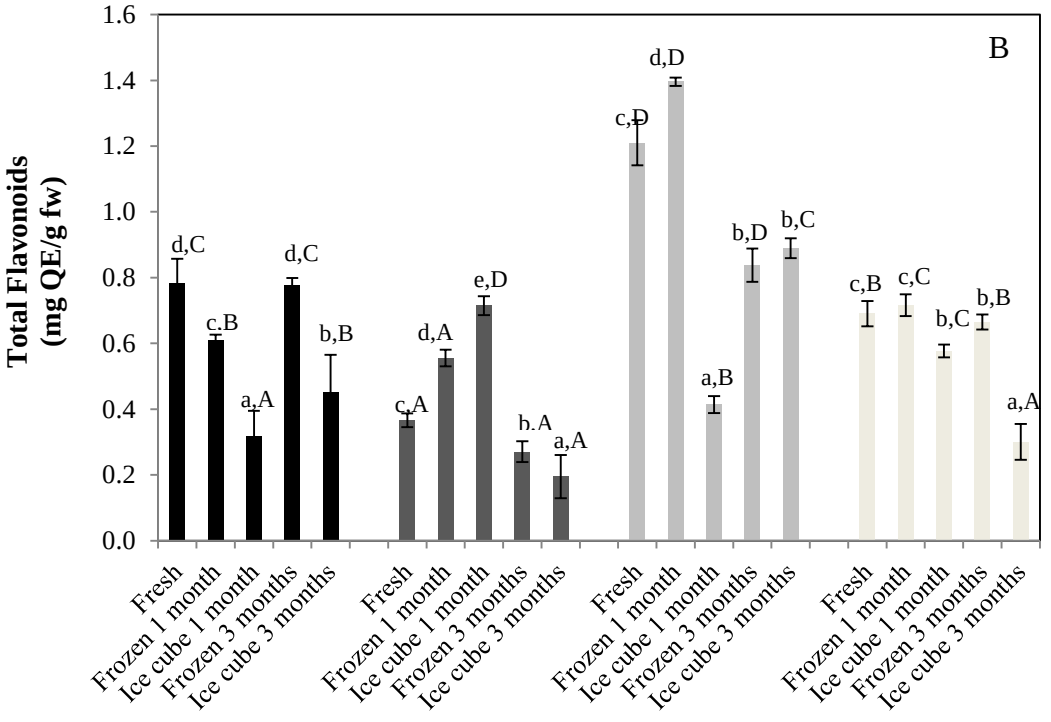
Comparing the four fresh flowers, heartsease showed the highest values of TRC, total flavonoids and hydrolysable tannins. Regarding treatments, freezing and ice cubes modified the amounts of bioactive compounds in all edible flowers. However, no trend was observed between treatments and time of storage. In particular, ice cubes of borage and heartsease, during both times of storage, showed lower TRC and total flavonoids than fresh ones, while for dandelion and kalanchoe this behavior was only observed after 3 months of storage. Is seems plausible that the cellular structures brake with the frozen process, enabling lixiviation of some components into the water during the defrosting process.

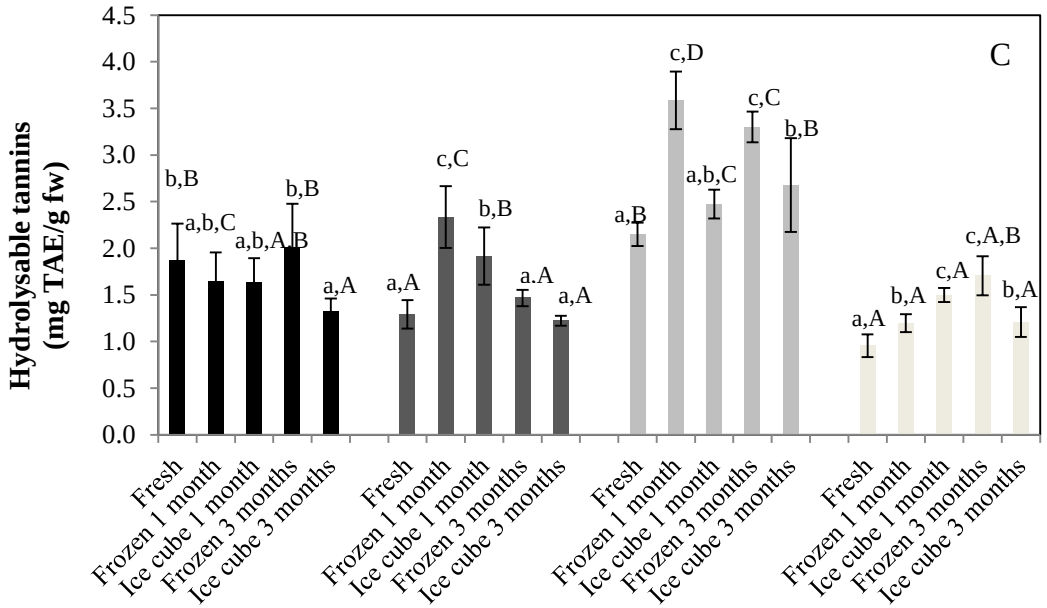
When the flowers were frozen (in their natural form) and stored during 1 to 3 months, a significant decrease in TRC (31 and 28%) and total flavonoid (51 and 40%) contents was observed in dandelion and heartsease, respectively. In frozen kalanchoe (at both storage times), higher or similar values of all bioactive compounds were observed than in fresh status. This could be attributed to some loss of water during the frozen process.

So, in general, flowers in ice cubes after 3 months of storage showed the lowest values of bioactive compounds, with some few exceptions. Furthermore, it was observed that for all frozen flowers the contents of phenolic compounds (TRC, tannins and flavonoids) were kept or even increased during 1 month of storage, except borage. The same tendency was also observed in other studies in plants, such as in fresh and frozen horseradish and lovage leaves, for which an increment of 15 and 0.5 %, respectively, were determined (Tomsone and Kruma, 2014). These results can be explained by the fact that ice crystals formed within the plant matrix can rupture the cell structure, allowing the lixiviation of water soluble cellular components into the drained water (Asami et al., 2003).

Considering a frozen flower in its natural form and that in an ice cube, the first consists of freezing the water contained in the plant cell, while in ice cubes two layers of water will freeze around the flower, protecting it. Therefore, it is expected that more ice crystals will be formed inside the flower tissue during freezing, which may induce a higher degree of cell wall damage (Petzold and Moreno, 2016), releasing more bioactive compounds. However, when analyzing the solution released during thawing, no bioactive compounds were quantified.







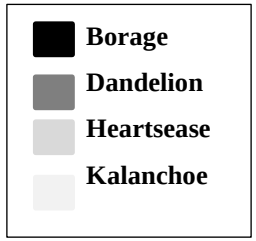


Figure 4.4.1.3 - TRC, total flavonoids and hydrolysable tannins contents in fresh, frozen in their natural form and in ice cubes of four edible flowers during storage (-18 °C). Lowercase letters compared different treatments in the same flower species. Uppercase letters compared flower species subjected to the same treatment.

Antioxidant activity (DPPH radical scavenging activity and reducing power)

The EC_{50} values of DPPH radical scavenging activity and reducing power for fresh, frozen and in ice cubes for the four flowers studied in the present work are shown in Figures 4.4.1.4. As expected, fresh heartsease flowers showed the lowest values of EC_{50} for DPPH radical scavenging activity and reducing power, indicative of higher antioxidant activity, probably associated with the accumulation of phenolic compounds (ex. flavonoids and tannins) as mentioned in the previous section. In general, the four flowers in ice cubes showed the highest values of both EC_{50}, indicative of lower antioxidant activity. Furthermore, frozen borage and dandelion flowers after 1 month of storage showed higher antioxidant potential (lower values of EC_{50} of DPPH) than for other treatments and times. A similar increase of antioxidant activity was detected in frozen dandelion, heartsease and kalanchoe after 1 month of storage for reducing power. In general, each flower showed a different behavior when subjected to different frozen methods (in natural form or in ice cubes), and during storage time.

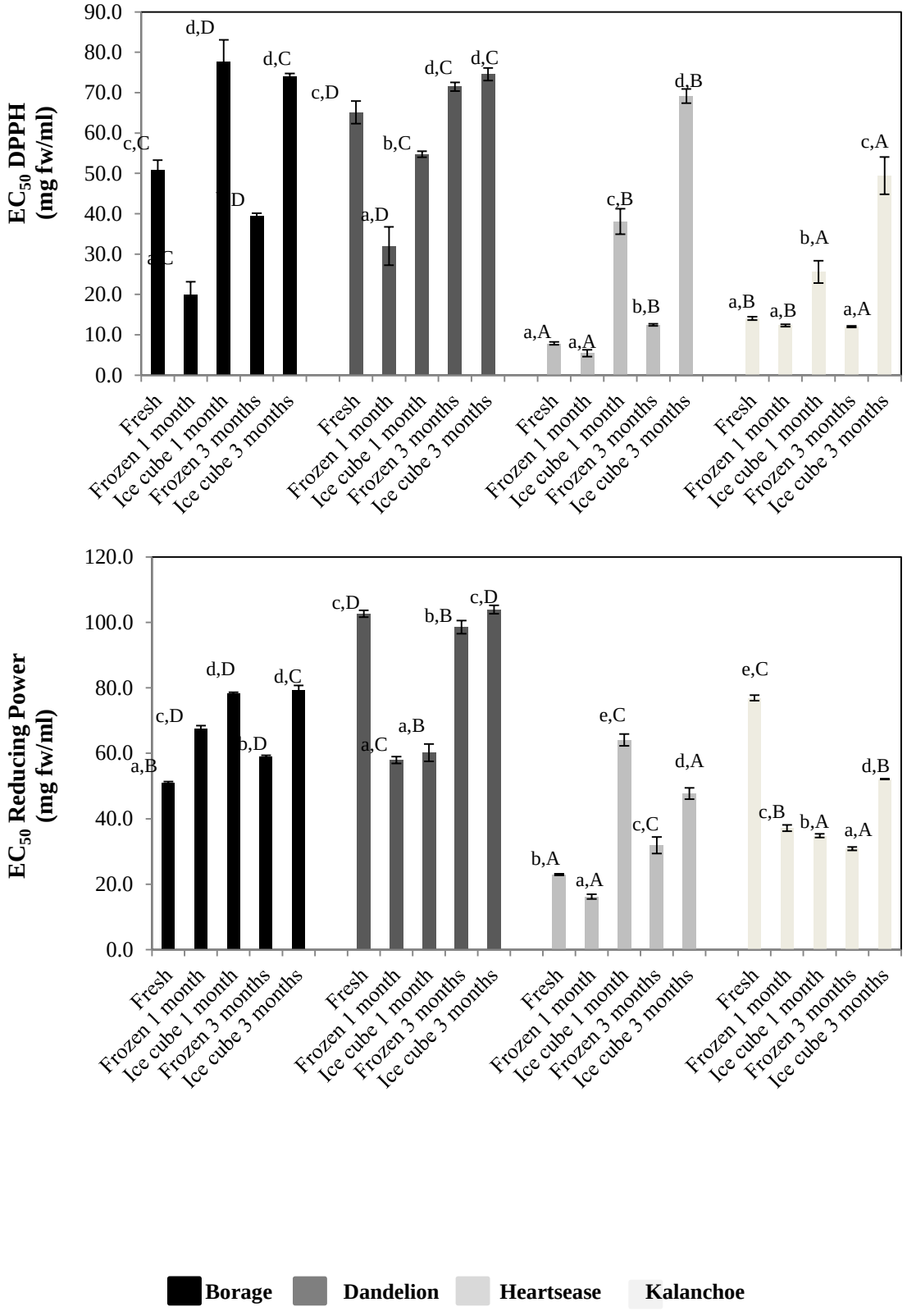


Figure 4.4.1.4 – EC_{50} values of DPPH radical scavenging activity and reducing power for fresh, frozen in their natural form and in ice cubes of four edible flowers during storage (-18 °C). Lowercase letters compared different treatments in the same flower specie. Uppercase letters compared flower species subjected to the same treatment.

Microbial quality

The microbial quality of fresh, in ice cubes and frozen flowers in their natural form is detailed in Table 4.4.1.1. The water used for preparing ice must be free from solids, bacteria, flavours, odours and dissolved minerals must be at the lowest possible level (WHO 1976), and should not contain pathogenic bacteria that could remain viable during storage (Dickens et al. 1985)

The water used for preparing the ice cubes did not contain pathogenic bacteria (*E. coli*) that could remain viable during storage, as well as, none of the microorganisms investigated (Table 4.4.1.1). So, water was not a vehicle of contamination. Regarding fresh flowers, borage showed the lowest microorganisms counts, while kalanchoe had the highest values. In general, both freezing methods decreased or maintained the levels of microorganisms when compared to fresh samples. There was an exception regarding frozen dandelion, with higher counts of total aerobic mesophilic (3 months) and moulds (at both storage times) than fresh flowers, as well as, in ice cubes (at both storage times) for moulds. In general terms, low-temperature conditions had a protective effect against the growth of some spoilage microorganisms and foodborne pathogens. Comparing frozen flowers in ice cubes and in their natural form, the first freezing method resulted in flowers with a lower level of microbial contamination, probably because in ice cubes the flowers were more protected from external contaminations than frozen flowers. Concerning storage time (1 and 3 months) for ice cubes, in most of the flowers no significant differences were detected, except for moulds in dandelion and total aerobic mesophilic in heartsease. In both cases, higher storage times induced higher values of microorganisms. Frozen samples stored for 3 months had similar or higher values of total aerobic mesophilic than those stored during 1 month.

Table 4.4.1.1 - Mean counts ±standard deviation (log cfu/g) of total aerobic mesophilic, yeasts, moulds, total coliforms, *E. coli* and psychrotrophic bacteria examined in water and fresh, in ice cubes and frozen flowers during storage.

Samples	Conditions	Days	Total aerobic mesophilic	Yeasts	Moulds	Total coliforms	*E. coli*	Psychrotrophic bacteria
Water			<2	<2	<2	<1	< 1	<2
Borage	Fresh	0	4.53±0.12^{d}	<2^{a}	<2^{a}	<1	<1	4.14±0.17^{b}
	Ice cube	1 month	<2^{a}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Frozen		4.00±0.01^{b}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Ice cube	3 months	<2^{a}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Frozen		4.15±0.01^{c}	<2^{a}	<2^{a}	<1	<1	2.19±0.24^{a}
Dandelion	Fresh	0	5.69±0.14^{a}	2.48±0.40^{a}	2.23±0.15^{a}	<1	<1	4.68±0.09^{a}
	Ice cube	1 month	5.49±0.10^{a}	2.27±0.20^{a}	2.69±0.18^{b}	<1	<1	4.55±0.07^{a}
	Frozen		5.70±0.03^{a}	2.19±0.24^{a}	3.01±0.13^{c}	<1	<1	4.74±0.06^{a}
	Ice cube	3 months	5.64±0.09^{a}	2.67±0.26^{a}	3.19±0.06^{c}	<1	<1	4.57±0.11^{a}
	Frozen		6.44±0.36^{b}	2.35±0.40^{a}	3.50±0.14^{d}	<1	<1	4.72±0.15^{a}
Heartsease	Fresh	0	5.32±0.04^{d}	<2^{a}	<2^{a}	<1	<1	4.68±0.10^{d}
	Ice cube	1 month	3.20±0.15^{a}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Frozen		4.75±0.06^{c}	<2^{a}	<2^{a}	<1	<1	2.40±0.32^{b}
	Ice cube	3 months	3.56±0.16^{b}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Frozen		4.77±0.09^{c}	<2^{a}	<2^{a}	<1	<1	3.06±0.18^{c}
Kalanchoe	Fresh	0	6.67±0.08^{c}	2.12±0.24^{a}	<2^{a}	<1	<1	5.44±0.03^{b}
	Ice cube	1 month	3.56±0.10^{a}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Frozen		3.53±0.19^{a}	<2^{a}	<2^{a}	<1	<1	2.08±0.15^{a}
	Ice cube	3 months	3.61±0.06^{a}	<2^{a}	<2^{a}	<1	<1	<2^{a}
	Frozen		3.95±0.11^{b}	<2^{a}	<2^{a}	<1	<1	<2^{a}

Values with the same letter in the same column are not statistically different (p>0.05).

Conclusion

Flowers preserved in ice cubes showed good visual appearance up to 3 months, whereas in frozen, in their natural form, it was only perceived up to 1 month, with the exception of kalanchoe. No trend in the studied bioactive compounds and antioxidant activity was observed between freezing treatments and time of storage. However, in general, flowers preserved in ice cubes for 3 months showed the lowest values of bioactive compounds and antioxidant activity. On contrary for flowers frozen in their natural form, the contents of phenolic compounds (TRC, tannins and flavonoids) maintained or increased during one month of storage, except borage. Concerning microbial quality, frozen flowers in their natural form and frozen in ice cubes decreased or maintained the levels of microorganisms when compared to fresh samples, except in dandelion. Thus, our results indicate that to maintain flowers during 3 months, preservation in ice cubes seems to be more appropriate, while for shorter times, as up to one month, freezing alone is also a good alternative. So, both freezing treatments can be regarded as interesting solutions to preserve edible flowers behind their flowering season. Furthermore, new uses and products may be developed for these frozen flowers, such as, new drinks, cocktails, ice creams and bakery products.

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4.5.

Edible coatings

Edible coatings are defined as a thin layer of material, which can be eaten by the consumer and it provides a barrier to moisture, oxygen and solute movement for the food. Nowadays, this emergent technology is applied to many products such as fruits, vegetables and meat, althought never tested in flowers. So, the next section reports the results about the effect of edible coatings (alginate) in pansies (*Viola×wittrockiana*) with different colors and sizes. Furthermore, after application of the edible coatings the influence in some flowers' properties (visual appearance, weight loss, a_w, color, dimensions change, microbial quality, antioxidant activity and bioactive conpounds) were evaluated during storage (4°C).

4.5.1.

Effect of application of edible coating and packaging on the quality of pansies (*Viola×wittrockiana*) of different colors and sizes

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Abstract

The effects of alginate edible coating on the quality of pansies (visual appearance, weight loss (WL), a_w, color and dimensions change) were studied during refrigerated storage (4°C). The role of pansies' color and size, as well as packaging, was also studied. Alginate treatments resulted in a beneficial effect on the visual appearance of pansies under refrigerated storage when compared to the uncoated, delaying their degradation from 3-4 days up to 14 days, depending on the pansies color, probably derived from their different petal thicknesses (83 to 183 µm for yellow and red pansies). The unpackaged coated pansies had different behaviors during storage, associated with their dimensions (the larger coated pansies maintained good visual appearance during longer storage times than the smaller ones). However, the packaged pansies treated with edible coating, showed to have lower WL and shrinkage than the unpackaged, irrespective of the color and size, with physical stability up to 14 days. In summary, the cumulative use of alginate coating with packaging under refrigerated storage may contribute to extent pansies' shelf life.

Keywords: Flowers, alginate coating, cold storage, visual appearance, shelf life.

Introduction

The demand for edible fresh flowers has increased in recent years due to the growing interest of consumers and professional chefs. Pansies (*Viola×wittrockiana*) are attractive edible flowers, with a colorful pallet of options, rich in health-promoting compounds such as anthocyanins, carotenoids, flavonoids, potassium, and phosphorus, and showing interesting in-vitro antioxidant and free radical-scavenging properties (Gamsjaeger et al. 2011; Rop et al. 2012; Vukics et al. 2008). However, edible flowers have a limited shelf-life and are very delicate, being highly susceptible to petal abscission, discoloration, wilting, dehydration and browning soon after harvest. Several strategies have been tentatively used to improve postharvest storage of fresh pansies, including refrigerating, drying or canning in sugar, or preserving in distillates. However, these methods may cause undesirable biochemical and nutritional changes in the final product that may affect its overall quality. Therefore, finding suitable methods for preserving the quality of pansies is extremely important. Until now, few studies on the postharvest preservation of pansies have been done. Kelley et al. (2003) had studied the effect of temperature (-2.5 to 20 °C) on pansies and showed that only the storage at 0 and 2.5 °C maintained the visual quality of pansies for 2 weeks, which decreased drastically when higher temperatures were used. Edible coatings are a recent technology used in fruits and vegetables and reported to have the potential to improve food appearance and prolong their shelf-life (Alboofetileh et al. 2014; Alvarez et al. 2013; Contreras-Oliva et al., 2012; Li et al., 2012). However, the few studies on edible flowers coating performed until now have focused on broccolis (Alvarez et al. 2013; Ansorena, et al. 2011; Moreira et al. 2001), all studies using chitosan as the main polysaccharide. Alginate coating, extensively used on fruits and vegetables, such as lettuce (Tay and Perera, 2004), apple fresh cut (Moldão-Martins et al., 2003), carrot (Amanatidou et al., 2000) and mushroom (Hershko and Nussinovitch, 1998), has never been used in edible flowers. Furthermore, alginate coatings are reported to possess good film-forming properties, producing uniform, transparent and water-soluble films (Lin and Zhao, 2007). They also improve the coating adhesion to the surface of vegetables (Fisher and Wong, 1972), are good oxygen barriers (Conca and Yang, 1993), and reduce the weight loss and natural microflora counts (Amanatidou et al., 2000). Even though alginate is not such a good barrier to water loss as chitosan, it can surpass the

allergy problem that some sensitive persons have to seafood, from which chitosan is extracted.

To our knowledge, until now, there is no available scientific literature about the use of edible coatings for maintaining the quality and extending the shelf life of pansies. In this order, the objective of this study was to evaluate for the first time the effect of alginate-based edible coatings on the quality of pansies during storage under refrigeration (4°C), with and without packaging. The quality of pansies, of different colors and sizes, was evaluated in relation to some physicochemical characteristics, namely, visual appearance, color, water activity, and weight loss.

Material and methods

Samples

Fresh pansies (*Viola* × *wittrockiana*) with different colors (red, white, violet, and yellow) were collected in full ripening state on October 2016 at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança (Bragança, Portugal). The degree of edible flower full ripeness was determined on the basis of flowers' size, opening, and color (Rop et al., 2012). After harvest, fresh flowers were immediately transported to the laboratory under refrigeration.

Edible coatings application

Edible coating treatment was applied according to the method used by Tay and Perera (2004). Pansies were immersed in 0.5% alginate (Panreac Química SA, Barcelona, Spain) from brown algae solution (w/v) (made with sterile distilled water) for 30 min. Residual alginate solution was allowed to drain for 5 min before immersing the samples in 1% $CaCl_2$ solution (w/v) for 30 min to induce spontaneous cross-linking reactions. Surface water was carefully blotted as before, using paper towels.

Storage assays

In the first experiment, fresh and coated pansies of four colors (white, yellow, red, and violet) were stored under refrigeration (4 °C), until presenting unsatisfactory visual appearance. Every day, at the same time, photos of the flowers were taken and the water activity, weight loss, dimensions, and color were measured, as detailed below.

In the second experiment, two factors were studied during pansies' storage, namely, size and the role of packaging. To perform this experiment, pansies of two colors (white and

violet), with three different sizes (small, medium, and large), were used and analyzed in terms of the same parameters mentioned previously. The criterion used to define the size was the following: small: < 3.5 cm; medium: between 3.5 to 4.5 cm; and large: > 4.5 cm (regarding width or length). Pansies with different sizes were divided into two groups: one group was left inside Petri dishes, while the second group was packed in thermosealed plastic bags (polyamide and polyethylene) (Alfa, Spain). Each experiment was performed on six flowers (n = 6).

Color, dimensions, water activity, and weight loss

Flowers' color was evaluated in different parts of the pansies' petals, with a colorimeter Minolta CR-400 (Osaka, Japan), using CIE*Lab* scale, namely, *L**, *a**, and *b** coordinates, where *L** varies between 0 (black) and 100 (white), *a** ($^{-}$green to red^{+}), and *b** ($^{-}$blue to yellow^{+}). Furthermore, the Chroma (*C**) and Hue Angle (*h**) values were determined. In order to analyse color changes due to edible coatings treatment, total color difference (*ΔE**) was also calculated according to the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta b^*)^2 + (\Delta a^*)^2} \quad \text{Eq. 1}$$

where Δ was the difference of the parameters' values after the edible coating treatment and before it (fresh sample, day 0).

Width and length of pansies were measured with a digital caliper (Powerfix, Berlin, Germany). To measure these dimensions the flowers were positioned with the darkest petals down. Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). This parameter was evaluated on three samples before treatment (control) and on all samples after edible coating treatment.

Weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany).

Weight loss (WL) was determined according to equation 2:

$$WL = \frac{M_0 - M}{M_0} \times 100 \quad \text{Eq. 2}$$

where M_0 is the initial mass of fresh pansies before edible coating treatment and *M* is the mass of pansies after edible coating application.

Microscopic analysis of pansies' petals

Fresh petals of different colors were dehydrated by passing them through increasing concentrations of ethanol (70, 80, 90, and 100%) for 4h each and then placed in paraffin

to fix. Thin sections of the petals were then cut into random transversal sections, mounted in water and observed on a light microscope equipped with a camera (Fotocamere Leica DFC49012, Heerbrugg, Switzerland). Petals' thickness was measured in different points (minimum 36 times) on three flowers.

Statistical analysis

The SPSS Statistical software, v. 18.0 (SPSS Inc., Chicago, IL), was used for the statistical treatment of the data. The normality of the data was verified by Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch were carried out to determine if there were significant differences ($p<0.05$) between samples, depending on the existence or not of homogeneity of variances. Additionally, if significant differences were detected between treatments, a *post hoc* analysis was performed, namely, Tukey's honestly significant difference test if variances in the different groups were identical, or Games-Howell test if they were not. The homogeneity of the variances was tested by Levene's test.

Results and Discussion

Effect of edible coating on pansies' characteristics during storage

Visual appearance: Uncoated and coated. Figure 4.5.1.1 shows changes during storage of the uncoated and coated pansies. Yellow uncoated pansies showed good appearance until 3 to 4 days, while red uncoated pansies presented a good appearance until 6 days of storage. After this period, the borders of the petals started to shrivel. When alginate coatings were applied, better results were observed. All pansies (white, yellow, violet, and red) maintained a similar appearance to fresh flowers until 6 days. After 6 days, the darker flowers (violet and red) maintained best appearance than lighter flowers (white and yellow). In fact, the darkest flowers maintained similar appearance to fresh ones until 12 days, in opposition to the lighter ones that shrank after 6 days. These results are very promising because the shelf life almost doubled.

When comparing uncoated and coated pansies, application of edible coatings increased their shelf life irrespective of the pansies' color. Furthermore, edible coatings may behave as a protective barrier and consequently being able to reduce respiration and transpiration rates through flower surfaces, retard microbial growth and color changes (Ansorena et al., 2011; Meneghel et al., 2008). Specifically regarding alginate use,

Nussinovitch and Hershko (1996) observed that alginate coatings served as a barrier to moisture loss in garlic and later Hershko and Nussinovitch (1998) found that coated

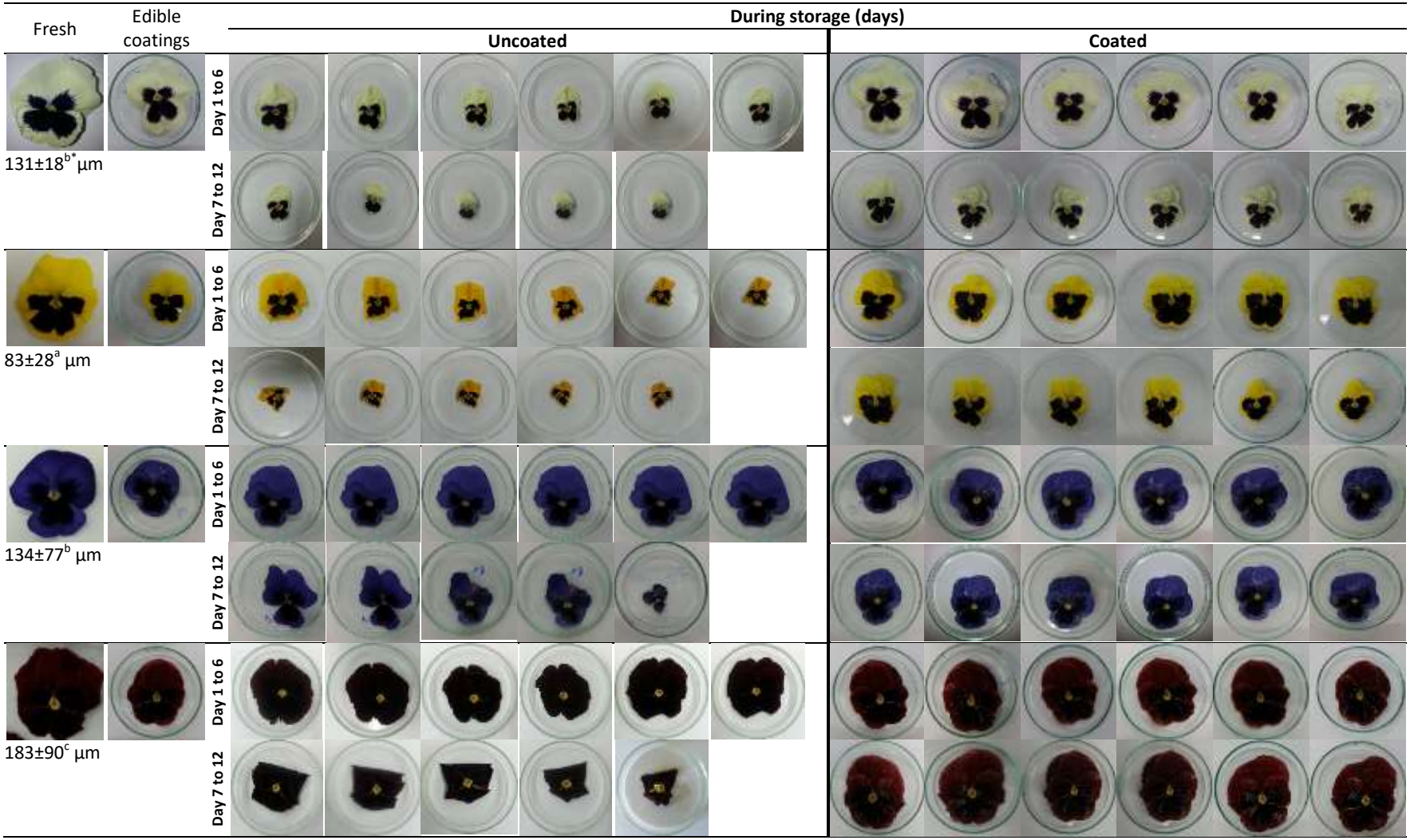

Figure 4.5.1.1 - Visual appearance of pansies (white, yellow, violet, and red) in fresh, uncoated and after applying edible alginate coating, during refrigerated (4 ºC) storage (n=3). Average values of petals' thickness (µm) are also presented. *****Petals' thickness (µm). Values with the same letter are not statistically different ($p>0.05$).

mushrooms have better appearance and color, as well as an advantage in weight variations, in comparison with uncoated mushrooms.

Weight loss, water activity, dimensions, and color.

Weight loss (%), water activity, width (cm), and length (cm) of coated pansies along storage compared to fresh uncoated pansies are shown in Figure 4.5.1.2 A-D. Concerning weight loss (Figure 4.5.1.2A), some differences were found among pansies with different colors: red and violet pansies presented consistently lower WL than yellow and white ones. After 12 days of storage, white and yellow pansies had the highest values of WL, with yellow (68.6%) and white (66.8%) pansies losing almost twice the weight compared to red pansies (32.5%). These severe losses are undesirable because it will mean a loss of economic revenue and has a strong impact on the appearance of the final product, due to shrinkage. It must be again mentioned that by using fresh uncoated pansies as references, negative weight losses were obtained in the first days of storage due to the weight gain imposed by the incorporation of alginate to the surface of the coated pansies.

Even though a decrease in water activity values was observed after application of edible coatings to pansies with different colors during storage, the measured values remained quite similar to those of fresh flowers (0.980±0.005) (Figure 4.5.1.2B).

Regarding width and length, all pansies treated with edible coating reduced their dimensions during storage (Figures 4.5.1.2C and 4.5.1.2D); however, red pansies showed a lower reduction, when compared with the other pansies. After 12 days of storage, yellow and white pansies decreased their size to half, namely, width reductions from 5.0 ± 0.2 to 2.5 ± 0.2 and 5.0 ± 0.2 to 3.0 ± 0.1 cm, respectively, and length reductions from 6.1 ± 0.3 to 2.6 ± 0.4 and from 6.1± 0.3 to 3.2 ± 0.5 cm, respectively.

Total color variation (ΔE^*) of the coated pansies during storage is shown in Table 4.5.1.1. In general, all pansies showed no significant differences ($p<0.05$) up to 12 days of storage. Nevertheless, after the application of edible coatings, pansies treated with alginate (0 days) showed appreciable differences to the human eye compared to fresh pansies (ΔE^* of up to 3 units as suggested by Trivellini et al. (2014)). This can be explained because after application of edible coating the flowers become brighter, as a result of the formation of the film around the flower. In our opinion this fact is not undesirable but in future sensory analyses must be performed in order to evaluate the acceptability or even the preference of consumers.

Table 4.5.1.1 – Total color difference (ΔE^*) of pansies (yellow, white, red, and violet) treated with alginate, during storage in comparison to the uncoated (fresh, t= 0 days) pansies

	Yellow		White			Violet	
Days	Light part	Dark part	White part	Violet part	Red	Light part	Dark part
0	23.9±1.4[a]	21.3±17.1[a]	6.6±2.7[a]	24.6±18.2[a]	12.4±0.8[a]	3.3±0.6[a]	23.7±18.9[b]
3	31.0±5.7[b]	24.2±15.6[a]	6.5±1.1[a]	19.7±3.8[a]	15.0±8.2[a]	15.1±7.2[b]	8.9±3.3[a,b]
5	32.5±5.2[b]	26.5±16.3[a]	6.1±1.4[a]	33.2±18.0[a]	15.4±8.2[a]	6.2±1.0[a]	9.0±5.2[a,b]
9	31.4±2.0[b]	21.8±11.8[a]	7.4±3.2[a]	22.1±12.8[a]	18.1±5.4[a]	14.0±0.2[b]	8.0±1.1[a]
12	32.7±0.9[b]	27.9±17.7[a]	7.6±2.8[a]	31.4±22.5[a]	19.3±6.1[a]	6.8±0.8[a]	10.2±5.4[a,b]

Values are expressed as: mean ± standard deviation. Values with the same letter in same column are not statistically different ($p>0.05$).

Thickness of pansies' petals of different color.

Thickness of the fresh pansies' petals was measured in order to understand if pansies with different colors (white, yellow, violet, and red) had petals with different thickness, explaining in part the results obtained during their storage and shelf-life. To our knowledge, these are the first results pertaining to the thickness of pansies' petals. Our data showed that yellow pansies had significantly ($p<0.05$) thinner petals than other colors (Figure 4.5.1.1), ranging from 47 to 141µm. On contrary, red pansies had thicker petals (ranging from 62 to 357 µm), while white and violet pansies showed petals with intermediate values, without significant differences between them. These results are very interesting and may explain why red pansies showed lower WL, maintained their dimensions, and showed good visual appearance during longer storage times. Their higher thickness may improve their barrier against exchanges with the exterior, since flowers are generally characterized by active metabolism, even during refrigerated storage (Cevallos and Reid, 2000). On contrary, yellow flowers showed the highest weight and dimensions loses, probably because their petals were thinner, leading to lower resistance.

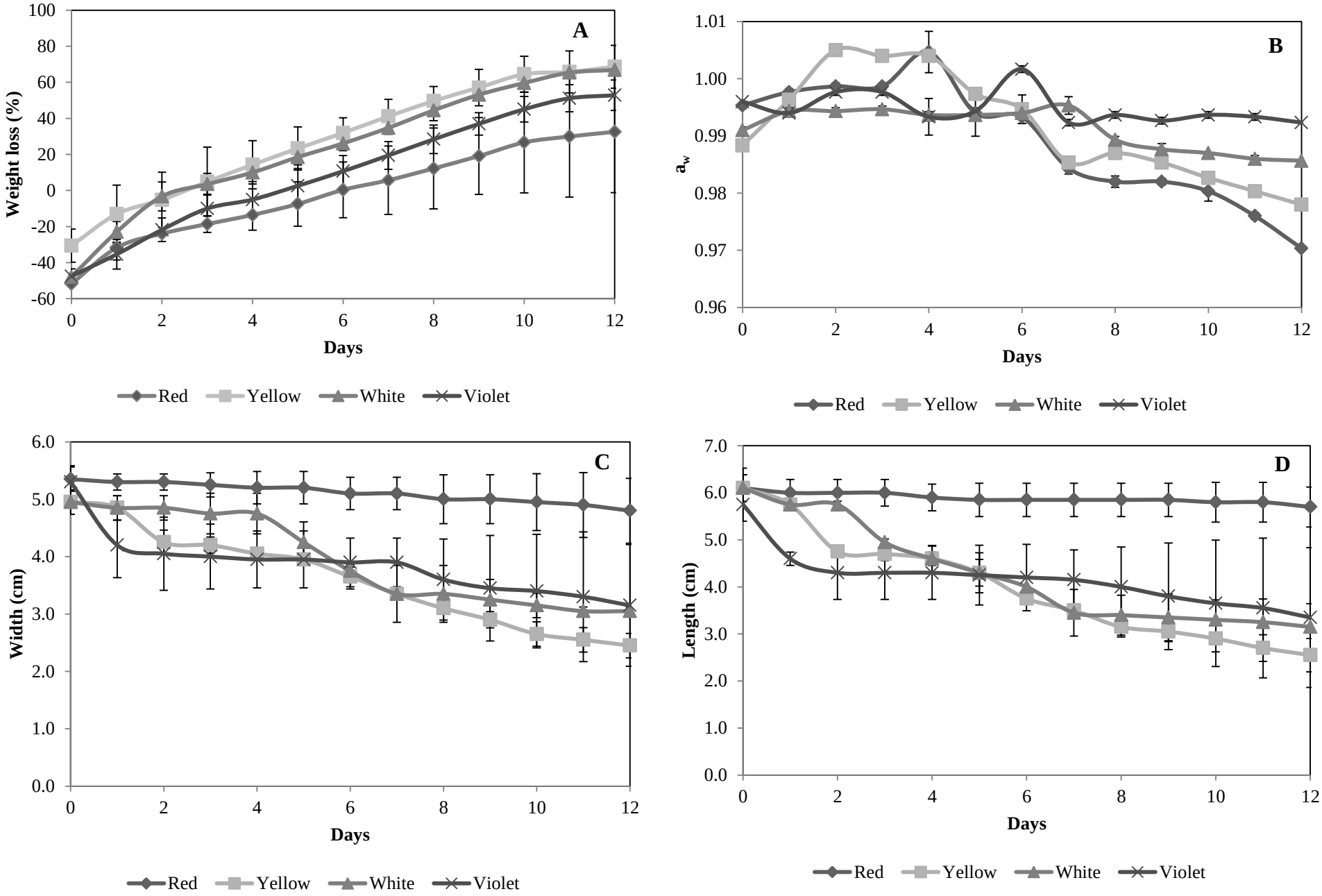

Figure 4.5.1.2 - Treated pansies with edible coatings during refrigerated (4 ºC) storage: (A) weight loss (%), (B) water activity (a_w), (C) width (cm) and (D) length (cm). Values are expressed as: mean ± standard deviation (n=3).

Effect of edible coatings and packaging on pansies with different sizes

In this section, we studied the effect of pansies' size and the role of packaging. White and violet pansies were chosen to perform the experiments because they are the most consumed, while having simultaneously two colors that showed opposite behaviors in the previous study.

Visual appearance.

Figure 4.5.1.3 shows the appearance of the coated pansies of different sizes (large, medium, and small) during storage, with or without plastic packaging. Regarding the packaged coated flowers, its appearance (color and size) kept constant up to 14 days of storage, while the unpackaged coated flowers showed more undesirable changes, shrinking rapidly, in accordance with the previous study. Moreover, fresh flowers of smaller sizes were spoiled easier than the larger ones, while these size-induced differences were not perceived in the packaged flowers. By observing Figure 4.5.1.3, it was clear that after 7 days of storage the smaller flowers were dry and shriveled, while the larger flowers had a better appearance for longer storage periods. Furthermore, violet pansies seemed to keep better appearance than the white pansies, in line with the previous results.

Weight loss, dimensions, water activity, and color.

The WL of coated pansies of different sizes along storage is detailed in Figure 4.5.1.4. (4.5.1.4. A and 4.5.1.4B for white and violet pansies, respectively), with and without packaging. After 14 days of storage, the unpackaged pansies presented the highest WL. Among sizes, smaller pansies were the most affected ones, decreasing the weight in average 82 and 75% in white and violet pansies, respectively. On the contrary, the packaged pansies maintained their weight almost constant during storage, independently of the size and color studied. These results are very promising, showing that packaging applied to the coated pansies will reduce WL, when compared to the unpackaged pansies.

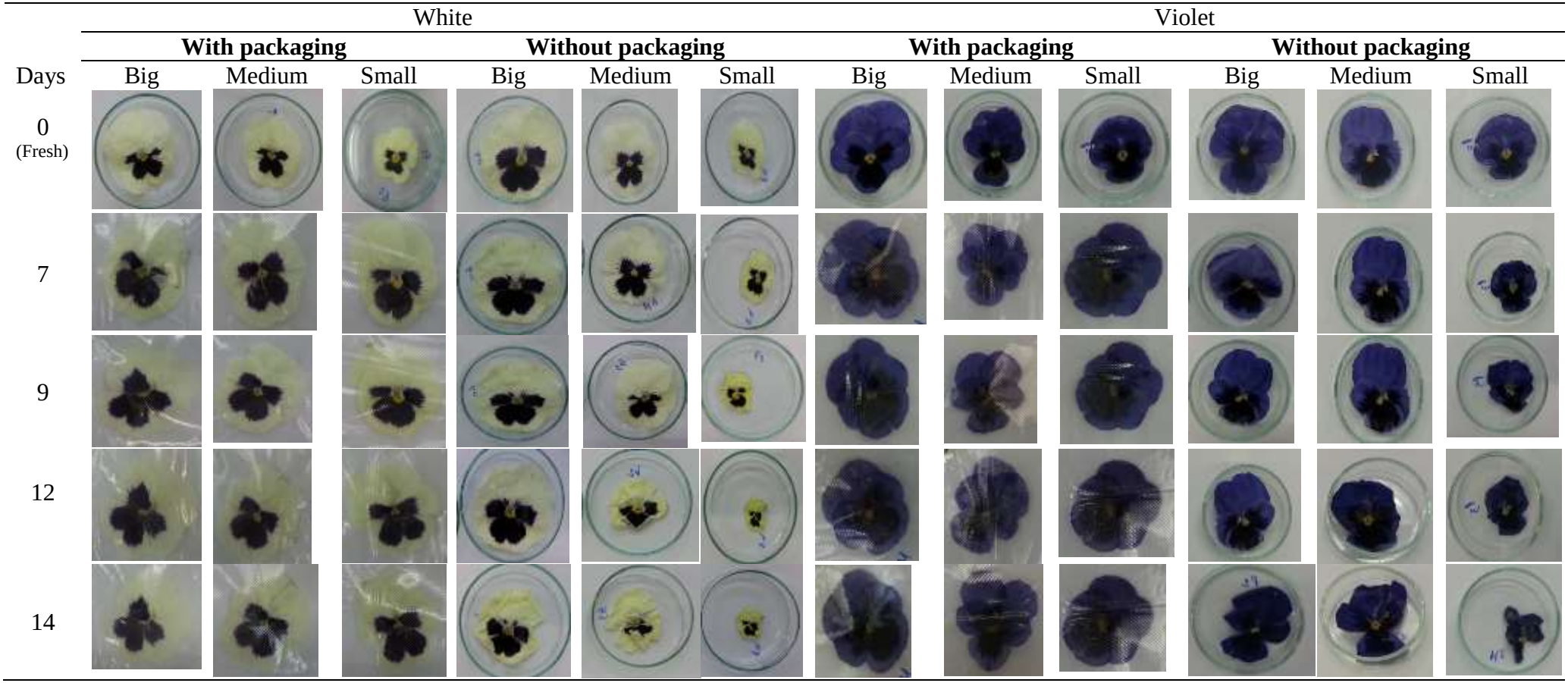

Figure 4.5.1.3 – Visual appearance of pansies (white and violet) of different sizes (small, medium, and large), with or without plastic packaging, before and after applying edible coatings during storage.

Weight loss, dimensions, water activity, and color.

The WL of coated pansies of different sizes along storage is detailed in Figure 4.5.1.4. (4.5.1.4. A and 4.5.1.4B for white and violet pansies, respectively), with and without packaging. After 14 days of storage, the unpackaged pansies presented the highest WL. Among sizes, smaller pansies were the most affected ones, decreasing the weight in average 82 and 75% in white and violet pansies, respectively. On the contrary, the packaged pansies maintained their weight almost constant during storage, independently of the size and color studied. These results are very promising, showing that packaging applied to the coated pansies will reduce WL, when compared to the unpackaged pansies.

Regarding dimensions, the packaged pansies maintained constant their dimensions during the 14 days of storage, while the unpackaged coated pansies showed a significant reduction of the width and length values in both colors studied (Figures 4.5.1.4.C to 4.5.1.4.F). The highest impact was observed in the smaller group, with reduction by half: white pansies averagely decreased in width from 3.7 to 1.5 cm and in length from 3.8 to 1.9 cm.

The water activity values in all cases maintained high ($\geq$ 0.961), slightly higher in the packaged pansies, with values between 0.982-0.988 (medium and large sizes, respectively) than in the unpackaged pansies, with values between 0.961-0.985 (small and large sizes, respectively).

Color changes of the coated and unpackaged pansies during storage are detailed in Table 4.5.1.2. Significant differences (p<0.05) were detected on individual factors (storage day and size) and their interactions. Between sizes, even though some significant differences were determined in some situations, it was not possible to observe any trend. Moreover, measurement of plain color in smaller flowers is more difficult, explaining some high standards deviations obtained. Regarding storage time, the only color parameter for which a consistent and significant decrease along time (0 and 14 days) was observed for the three sizes groups was the hue (h*) value in the light part of both pansies, but this behavior wasn't observed in the dark region. Thus, in general terms, it was concluded that the treatment applied did not affect in great extent the color of pansies in all sizes during storage because no relevant changes were observed on all color parameters.

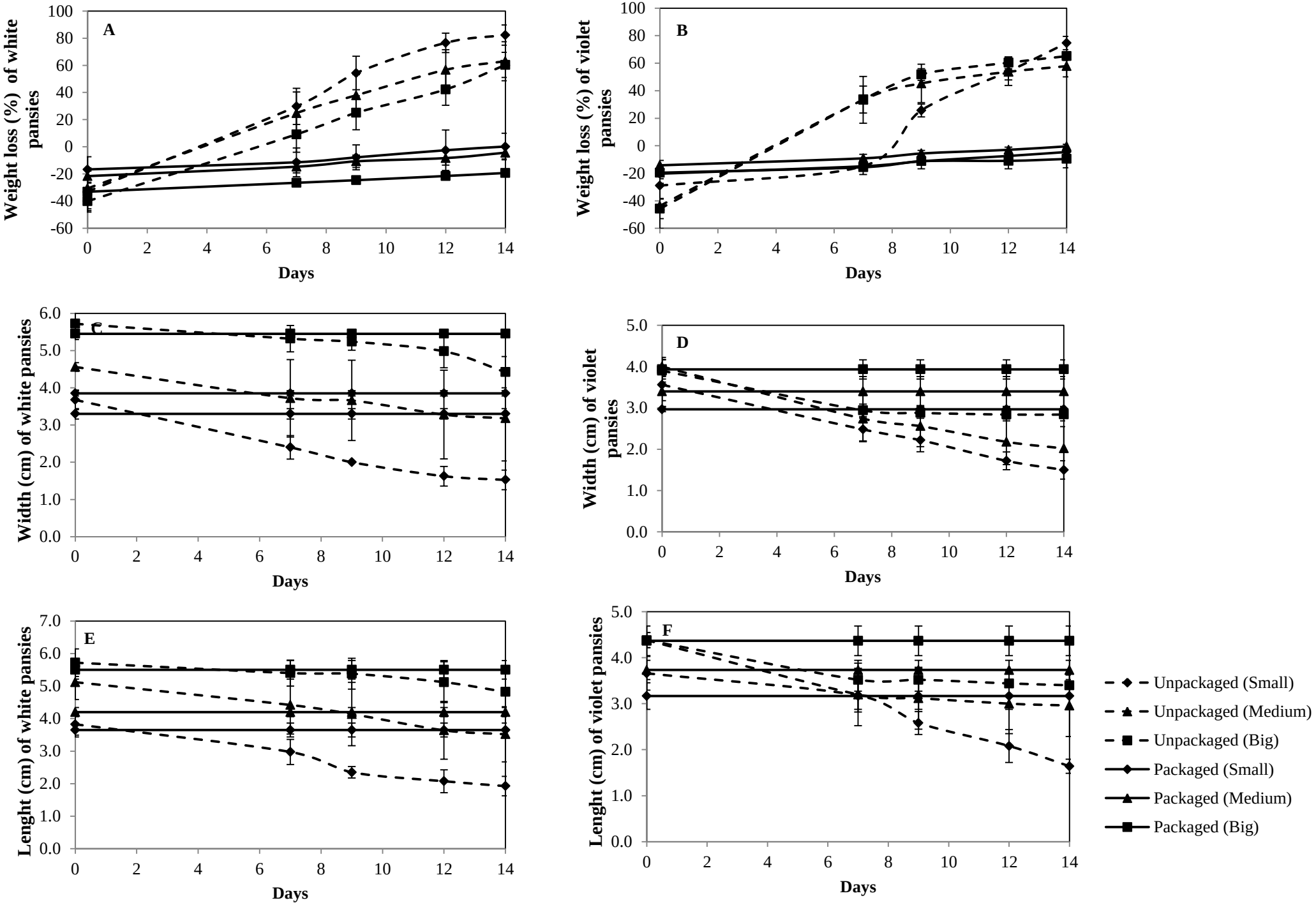

Figure 4.5.1.4 - Packaging effect during refrigerated (4 ºC) storage of the coated pansies of different sizes on: (A) and (B) weight loss (%); (C) and (D) width (cm), and (E) and (F) length (cm), of white and violet pansies, respectively.

Table 4.5.1.2 – Color changes for *L**, *a**, *b**, *C**, and *h** of alginate coated and unpackaged pansies of different sizes, during storage.*

Pansy Region	Days	*L**			*a**			*b**			*C**			*h**		
		Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
White/Violet White	0	81.5±1.9^{a,A}	86.0±2.6^{b,B}	87.0±1.3^{b,A}	-8.3±2.5^{a,A}	-7.2±0.7^{a,b,A}	-6.3±0.7^{b,B}	20.4±7.2^{b,A}	17.4±2.0^{a,b,A}	14.9±1.9^{a,A}	22.0±7.6^{b,A}	18.8±2.1^{a,b,A}	16.2±2.0^{a,A}	112.4±1.2^{a,B}	112.5±0.3^{a,B}	113.1±1.0^{a,B}
	7	84.5±2.1^{a,B}	85.4±1.6^{a,B}	88.3±2.0^{b,B}	-9.1±2.3^{a,A}	-7.2±1.2^{b,A}	-6.8±0.8^{b,B}	25.0±5.8^{b,A}	18.2±3.5^{a,A}	16.0±2.9^{a,A}	26.7±6.2^{b,A}	19.4±3.7^{a,A}	17.4±3.0^{a,A}	110.0±2.1^{a,A}	111.8±0.6^{b,B}	113.4±1.5^{c,B}
	14**	82.4±0.7^{b,A}	73.2±11.8^{a,A}	88.7±0.9^{b,B}	-9.1±2.6^{a,A}	-4.6±3.9^{b,B}	-8.1±0.3^{a,A}	26.3±7.6^{b,A}	16.1±6.7^{a,A}	21.4±1.3^{a,b,B}	27.8±8.0^{b,A}	17.1±7.0^{a,A}	22.9±1.2^{b,B}	109.1±0.7^{b,A}	99.1±19.2^{a,A}	110.0±0.9^{b,A}
	0	37.4±9.2^{b,A}	13.5±1.0^{a,A}	9.1±1.6^{a,A}	2.7±4.6^{a,A}	9.7±2.0^{b,B}	16.8±1.5^{c,C}	15.8±15.7^{b,A}	-7.9±1.0^{a,A}	-12.5±0.5^{a,A}	19.1±12.2^{b,A}	12.5±2.2^{a,A}	21.0±1.5^{b,C}	141.2±125.6^{a,A,B}	320.7±2.2^{b,A}	323.3±1.5^{b,A}
	7	37.0±15.1^{c,A}	23.6±15.0^{b,B}	11.3±0.7^{a,A}	3.0±2.9^{a,A}	5.6±5.1^{a,A}	12.9±1.9^{b,B}	9.5±13.0^{c,A}	-2.2±9.1^{b,A,B}	-10.4±1.0^{a,A,B}	13.8±8.9^{a,b,A}	10.9±4.6^{a,A}	16.6±2.1^{b,B}	208.8±128.9^{a,B}	280.7±94.9^{a,b,A}	320.8±2.0^{b,A}
	14**	49.4±16.0^{b,A}	20.5±8.4^{a,A,B}	21.8±1.0^{a,B}	-1.4±4.9^{a,A}	6.1±2.3^{b,A}	8.1±3.2^{b,A}	13.0±10.3^{b,A}	-1.2±7.9^{a,B}	-8.2±5.0^{a,B}	14.3±9.8^{a,A}	9.4±3.7^{a,A}	12.1±4.7^{a,A}	77.9±37.7^{a,A}	273.9±104.3^{b,A}	278.9±7.6^{b,A}
Violet Light	0	27.5±2.6^{a,A}	32.4±3.5^{a,b,A}	37.4±4.3^{b,A}	26.3±1.2^{a,A}	26.0±3.3^{a,B}	22.1±4.0^{a,A}	-30.5±0.6^{a,A}	-31.7±2.4^{a,A}	-29.1±3.6^{a,A}	40.3±1.2^{a,A}	41.0±4.0^{a,A}	36.6±1.6^{a,A}	310.8±0.7^{b,C}	309.2±1.4^{b,B}	307.0±1.6^{a,B}
	7	32.6±2.1^{a,B}	34.4±4.7^{a,A}	38.3±4.9^{a,A}	24.4±3.7^{a,A}	24.0±0.3^{a,A,B}	21.1±1.5^{a,A}	-29.8±3.8^{a,A}	-30.1±1.3^{a,A}	-27.2±2.0^{a,A}	38.5±5.3^{a,A}	38.5±1.2^{a,A}	34.4±2.5^{a,A}	309.2±0.7^{b,B}	308.5±1.0^{a,b,A,B}	307.9±0.1^{a,B}
	14	33.8±0.5^{a,B}	33.0±1.5^{a,A}	38.1±1.2^{b,A}	24.8±0.1^{c,A}	22.5±1.7^{b,A}	19.3±1.7^{a,A}	-32.2±0.3^{a,A}	-29.4±2.6^{b,A}	-27.7±0.9^{b,A}	40.8±0.2^{c,A}	37.0±3.1^{b,A}	33.8±1.7^{a,A}	307.6±0.2^{b,A}	307.5±0.3^{b,A}	304.8±1.5^{a,A}
Violet Dark	0	27.3±0.6^{b,A}	27.2±4.6^{b,B}	20.7±1.7^{a,A}	6.4±1.8^{a,A}	7.8±0.3^{a,A}	11.9±0.7^{b,A}	-3.8±5.7^{b,A}	-2.5±5.2^{b,B}	-12.0±2.5^{a,B}	8.5±3.8^{a,A}	9.3±1.4^{a,A}	16.9±2.2^{b,A}	323.8±16.9^{a,A}	316.5±0.7^{a,A,B}	315.2±4.5^{a,A}
	7	30.5±0.9^{b,A}	20.5±1.4^{a,A}	21.2±1.6^{a,A}	6.7±0.6^{a,A}	10.2±2.9^{b,A,B}	15.6±0.7^{c,C}	-2.3±1.2^{c,A}	-7.6±6.0^{b,A,B}	-15.0±0.8^{a,A}	7.2±0.8^{a,A}	13.1±5.7^{b,A,B}	27.7±1.7^{c,B}	338.2±2.5^{b,A}	329.2±16.0^{a,b,B}	316.0±0.4^{a,A}
	14	26.6±7.9^{a,A}	22.4±4.5^{a,A,B}	26.9±0.5^{a,B}	6.4±3.8^{a,A}	11.8±1.7^{b,B}	14.1±1.0^{b,B}	-3.3±5.6^{b,A}	-12.5±1.4^{a,A}	-15.5±2.1^{a,A}	8.1±5.4^{a,A}	17.3±2.2^{b,B}	21.0±2.2^{b,B}	323.7±5.9^{b,A}	312.8±1.1^{a,A}	312.5±2.0^{a,A}

*Values are expressed as: mean ± standard deviation. Values with the same uppercase letter in the same column are not statistically different (p>0.05). Values with the same lowercase letter in the same line are not statistically different (p>0.05).

** For pansies of small size the last measure of color was performed at 9 days because of the reduction of the dimensions.

Table 4.5.1.3 – Total color difference (ΔE^*) of the coated and packaged pansies with different sizes after 14 days of storage comparing to fresh ones

Size	White		Violet	
	White part	Violet part	Light part	Dark part
Small	1.3±0.1[a]	4.1±1.5[a]	1.4±0.5[a]	18.3±10.7[b]
Medium	2.2±1.5[a]	4.4±5.9[a]	8.0±1.5[b]	7.2±2.9[a]
Large	8.7±1.0[b]	10.9±0.2[b]	7.0±0.9[b]	5.2±3.0[a]

Values are expressed as: mean ± standard deviation. Values with the same letter in same column are not statistically different ($p>0.05$).

The color differences (ΔE^*) of the coated and packaged pansies after 14 days of storage is shown in Table 4.5.1.3. Among the white pansies, the larger flowers showed significantly higher values of ΔE^* for both parts (white/violet) than the others two sizes. So, more changes of color occurred in larger white pansies. On contrary, for the violet pansies, the smaller flowers were the ones presenting a significantly different behavior when compared with medium and larger flowers. The lowest value of ΔE^* was observed in the light part of the small violet pansies, while its dark parts presented the highest value of ΔE^*, with a high standard deviation derived from the difficulty on the measurements, as explained previously.

Conclusion

Edible coating with alginate seemed to have a beneficial impact on visual appearance of pansies during storage. However, flowers of different sizes and colors have different behaviors. Concerning packaging plus alginate coating, a delay in pansies' WL and shrinkage was observed, being high water activity values maintained, independently the color and size. In summary, pansies treated with alginate and packaged represent a cumulative protection, being a very interesting solution to prolong pansies' shelf life, independently their color and size. However, other studies should be performed such as sensory analyses and acceptability tests of consumers to evaluate if these products will have acceptability on the market.

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4.5.2

Effect of alginate coating on the physico-chemical and microbial quality of pansies (*Viola* × *wittrockiana*) during storage

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Abstract

Edible flowers, such as pansies, are becoming more popular, but they are highly perishable. So, postharvest technologies are needed, being edible coatings a good alternative. Thus, the aim of this study was to evaluate the effect of alginate coating on physico-chemical and microbiological quality of pansies during cold storage (4 ºC for 0, 7, 14, 21 days). Coated pansies maintained good appearance until 14 days of storage, 7 days more than uncoated ones. Flavonoids, hydrolysable tannins and monomeric anthocyanins, as well antioxidant activity, were higher in coated pansies when compared to uncoated ones, on all assayed storage times. Furthermore, after 14 days of storage, uncoated pansies presented microorganism counts higher than coated, namely yeasts and moulds, suggesting an effective barrier protection of the alginate coating treatment. In summary, alginate coating has potential for extending shelf-life and improving physico-chemical and microbiological quality of pansies.

Keywords: *Viola×wittrockiana;* Alginate coating; Antioxidant activity; Bioactive compounds; Microbial load

Introduction

Edible flowers have been eaten for thousands of years, as evidenced in old writings. Nowadays, the demand for edible flowers has increased because consumers search for unique culinary experiences and they want to make a return to earlier lifestyles, in which edible flowers played an important role (1).

Pansies (*Viola×wittrockiana*) are edible flowers with an intense flavor being used in soups, salads and drinks, and to give shape and color to dishes. In addition, pansies contain healthy components such as anthocyanins, carotenoids, flavonoids, potassium and phosphorus, with recognized bioactivity in terms of antioxidant and free radical-scavenging properties (1-3). Nowadays, pansies are marketed fresh, suitably packed in bunches, boxes, *etc*. and sold either directly in farm shops or through various specialized outlets. However, pansies have a limited shelf-life because flowers are susceptible to petal abscission, discoloration, wilting, dehydration and tissue browning soon after harvest. The most common methods used to improve postharvest storage of fresh pansies flowers quality include refrigeration, drying, canning in sugar and preservation in distillates. However, these methods may cause undesirable biochemical and nutritional changes in the processed product that may affect its overall quality.

Edible coatings can be used to protect perishable food products from deterioration by providing a selective barrier to moisture, oxygen and carbon dioxide, delaying dehydration, suppressing respiration, improving textural quality, while helping to retain volatile flavor compounds and reducing microbial growth (4). The use of coatings derived from proteins, lipids and polysaccharides for this purpose, has received increased interest over recent years, particularly regarding the preservation of important characteristics as texture (5). Therefore, the application of edible coatings can be a suitable method for preserving pansies. Thus, the objective of this study was to evaluate the effect of alginate coating on the quality of white pansies during cold storage (4 °C). Thus, the following physicochemical characteristics were evaluated: visual appearance, weight loss, water activity (a_w), pH and acidity, as well as several bioactive compounds (monomeric anthocyanins, flavonoids, carotenoids, total phenolic content and hydrolysable tannins) and antioxidant activity (Reducing power and DPPH radical scavenging activity). Furthermore, pansies' microbial quality was also evaluated.

Materials and Methods

Samples

Fresh white pansies (*Viola* × *wittrockiana*) were collected in full ripening stage at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança (Bragança, Portugal). After harvest, fresh flowers were immediately transported to the laboratory under refrigeration.

Edible coatings

Edible coating treatment was applied according to the method used by Tay and Perera (6). Commercial sodium alginate (Panreac Química SA, Barcelona, Spain) solution was prepared by solubilizing 2.0 g of its powder in 100 mL of water under stirring. Pansies were immersed in the alginate solution for 30 min at room temperature and afterwards allowed to drip off. Then, pansies were immersed in a calcium chloride solution (1%, w/v) for 5 min to induce spontaneous cross-linking reactions. When sodium alginate is put into a solution of calcium ions, the calcium ions replace the sodium ions in the polymer, as each calcium ion can attach to two of the polymer strands. Alginate coating was selected because it has good film-forming properties and it produces uniform, transparent and water-soluble films (7). It also enhances the coating adhesion to the surface of vegetables (8). Furthermore, alginate coatings are good oxygen barriers (9), and reduce the weight loss and the microflora counts (10). Even though alginate is not such a good barrier to water loss as chitosan, alginate will not cause allergy to sensitive persons to seafood, from which chitosan is obtained.

Storage

Approximately 2 kg of fresh and coated pansies were stored under refrigeration (4 °C) during 21 days. After 7, 14 and 21 days of storage, photos of the flowers were taken and some physico-chemical properties were evaluated. A portion (300 g) was frozen and freeze-dried (Scanvac, Coolsafe, Lynge, Denmark) for later evaluation of bioactivity and antioxidant activity, as detailed below.

Physico-chemical analyses

Moisture was determined by weight loss at 105 °C until constant weight (11). Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-

aw, Lachen, Switzerland). Weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany). Weight loss (WL) was determined according to Eq. 1:

$$WL = \frac{M_0 - M}{M_0} \times 100 \quad (1)$$

where M_0 is the initial mass of pansies (fresh or coated) in day 0, M is the mass of pansies after storage.

pH and titratable acidity (TA) were determined following standard methods (12). Briefly, 0.5 g sample was homogenized in 50 mL of distilled water, filtered and the pH measured with a potentiometer (Hanna Instruments, HI8417). TA was measured by titrating 10 mL of this solution with a 0.01 N NaOH solution using phenolphthalein as an indicator. Results were express in g acid citric/100 g of dry weight (DW).

Carotenoids

The carotenoid contents were determined according to the method used by Aquino-Bolaños et al. (13). One gram of freeze-dried powder of uncoated and coated pansies was extracted twice with 20 mL acetone:hexane solution (1:1, v/v). Both extracts were combined in a separation funnel, being added 200 mL of distilled water to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to eliminate any residual water, being the remaining solution filtered and completed to 100 mL with hexane. Total carotenoid content was determined by reading the absorbance at 450 nm and comparing the results to a β-carotene calibration curve (0.22–8.8 μg/mL). Results were expressed in μg β-carotene equivalents /g DW.

Extraction conditions for monomeric anthocyanins and bioactivity determination

Extraction was based on the method described by Li et al. (14) with slight modifications. Freeze-dried powders (1 g) of uncoated and coated pansies were extracted with 50 mL of water:acetone (6:4, v/v) at 37 ºC for 30 min, under agitation (IKA, RCT Model B, Staufen, Germany) at 1000 rpm. The water:acetone extracts were filtered and placed in a rotary evaporator (Stuart, RE300DB, Stone, UK) to remove the solvent. Then, all extracts were frozen and placed in the freeze drier (Coolsafe, Lynge, Denmark) for 2 days. The extracts obtained were redissolved within the same solvent to a concentration of 50 mg extract/mL and covered with aluminium foil under freezing until further analysis.

Monomeric anthocyanins

The total monomeric anthocyanin contents on the extracts of uncoated and coated pansies during storage were estimated by the pH differential method, following the methodologies used by Bchir et al.(15) and Rajasekar et al. (16). The method consisted on using two buffer systems: potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate at pH 4.5 (0.4 M). Extracts portions were diluted on both buffers, and allowed to stand for 30 min at room temperature. Subsequently, the absorbance readings were made on a UV-Visible spectrophotometer (Thermo, Genesys 10 UV, Waltham, USA) at the wavelengths of 510 and 700 nm, being the absorbance difference (*A*) determined by the equation:

$$A = (A_{510\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{510\ nm} - A_{700\ nm})_{pH\ 4.5} \quad (2)$$

The monomeric anthocyanin pigment concentration was expressed on cyanidin-3-glucoside, determined by the equation:

$$\text{Monomeric anthocyanin pigment (mg Cy 3-glu/L)} = A \times MW \times DF \times 1000/(\varepsilon) \quad (3)$$

where MW= molecular weight (449.2), DF = dilution factor and ε = Molar absorptivity (26,900). All measurements were performed in triplicate. The results were expressed in mg of cyanidin-3-glucoside/g fresh weight (mg Cy 3-glu/g FW).

Total flavonoids

The total flavonoid content was determined by the method described by Viuda-Martos et al. (17), with slight modifications. To fresh and coated pansies extracts (1 mL) were added 0.3 mL of $NaNO_2$ (5%, m/v) and, after 5 min, 0.3 mL of $AlCl_3$ (10%, m/v) were mixed. After 6 min, 2 mL of NaOH (1 M) were added. Absorbance was read at 510 nm and flavonoids were quantified using a standard curve of quercetin (10-160 µg/mL). Results were expressed in mg of quercetin equivalent/g fresh weight (mg QE/g FW).

Hydrolysable tannins

The content of hydrolysable tannins was determined by the method described by Elfalleh et al. (18). To one mL of uncoated and coated pansies extracts, 5 mL of 2.5% KIO_3 was added and stirred for 10 s. Absorbance was measured at 550 nm. Different concentrations of tannic acid (0.025 to 1.6 g/L) were used for calibration. Results were expressed in mg of tannic acid equivalent/g fresh weight (mg TAE/g FW).

Total phenolic content

The total phenolic content (TPC) of each sample was determined by the Folin-Ciocalteu method as described by Falcão et al (19). To 8 mL of uncoated and coated pansies extracts solutions were added 500 µL of Folin-Ciocalteu reagent. After 5 min, 1.5 mL of saturated sodium carbonate solution was added. After 2 h the absorbance values were read at 765 nm. A calibration curve was obtained with gallic acid (0.25 to 5 mg/L) and the results expressed in mg gallic acid equivalent/g fresh weight (mg GAE/g FW).

Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity was determined by the procedure described by Delgado et al. (20) with some modifications. A 0.0024 g amount of DPPH was dissolved in 100 mL of methanol to obtain a 6.09 $\times 10^{-5}$ mol/L solution. Pansies extract diluted solutions (300 µL) were added to 2.7 mL of the DPPH methanolic solution. After 1 h in the dark at room temperature, absorbance was read at 517 nm. Antioxidant activity was expressed by the percentage of scavenging effect according to the formula in Eq. 4:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \qquad 4$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The extract concentration providing 50% of DPPH radical scavenging effect (EC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage *versus* extract concentration.

Reducing power

The reducing power of each extract was determined by the procedure described by Delgado et al. (20). To 1.0 mL of uncoated and coated pansies extracts solutions at different concentrations were added 2.5 mL of phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ 1% (m/v). After shaking, the mixtures were incubated at 50 °C for 20 minutes after which 2.5 mL of 10 % trichloroacetic acid (m/v) was added with further stirring. A volume of 2.5 mL of the mixture was transferred to another test tube, to which 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1% (m/v) were added. The

absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ *versus* concentration, the EC_{50} values were determined corresponding to the concentration that gave an absorbance of 0.5.

Microbial quality

Uncoated (3 g in triplicate) and coated (3 g in triplicate) pansies at the beginning of storage (0 days), as well as, after 14 days of cold storage (4 °C) were analyzed for total aerobic mesophilic, yeast and moulds, lactic acid bacteria, total coliforms, *Escherichia coli* and psychrotrophic bacteria counts. All samples were diluted in 27 mL physiological peptone water. Samples were placed in sterile stomacher bags and homogenized in a Stomacher 400 (Seward, UK) for 2 min. The homogenates were subjected to serial dilutions with peptone water and then 1 mL of each dilution was pipetted into the surface of plate count agar (PCAg, Merck, Algés, Portugal), Rose Bengal Chloramphenicol Agar (RBC-Agar, Merck) and Man, Rogosa and Sharpe Agar (MRS-Agar, Merck). The PCAg plates were then incubated for 2 days at 30 °C for total aerobic mesophilic count and 5 days at 10 °C for psychrotrophic bacteria count. Lactic acid bacteria were determined in MRS-Agar plates, at 37 °C for 2 days. Yeast and moulds were determined in RBC-Agar plates, incubated at 27 °C for 3-5 days. Total coliforms and *E. coli* were determined according to the SimPlate method. All counts were expressed as log10 cfu/g fresh sample.

Statistical analysis

SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. Analyses of variance (ANOVA) or ANOVA Welch were carried out to evaluate if there were significant differences ($p < 0.05$) between samples. ANOVA was applied when homogeneity of variances was observed, while ANOVA Welch was applied for the other cases. Additionally, significant post hoc analyses were performed (Tukey HSD test if variances in the different groups were identical or Games-Howell test if they were not). The homogeneity of variance was tested by Levene's test. The correlations between variables were determined by Pearson correlation coefficients. All analyses were performed in triplicate.

Results and Discussion

Visual appearance

The visual appearance of the uncoated and coated pansies during storage (4 °C) is shown in Fig. 4.5.2.1. The uncoated pansies showed good appearance until 7 days, but after this period the petals were shrived and smaller than at 0 days. On the other hand, coated pansies showed good appearance until 14 days, similar to fresh samples. After 21 days, although the majority of coated pansies preserved a good appearance, some began to present brown spots on the petals, as those develop under moist conditions (21).

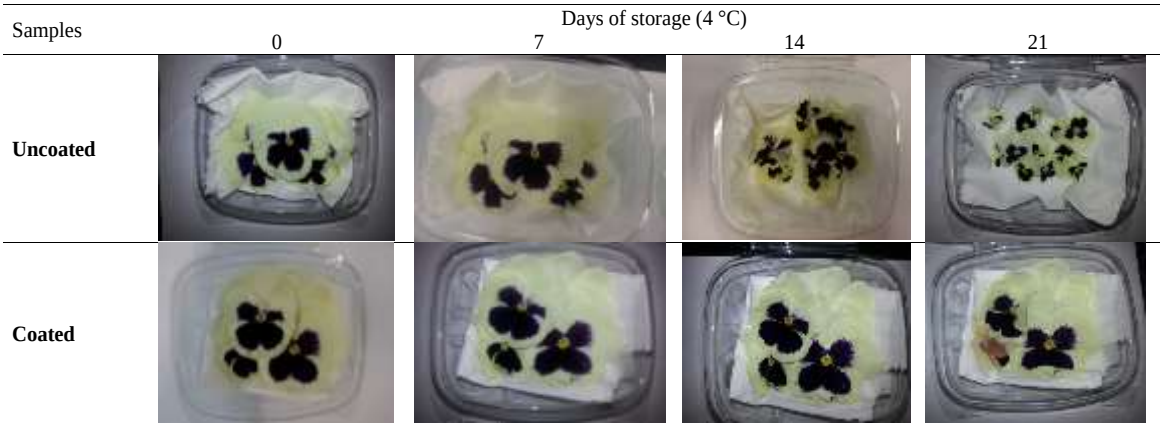

Figure 4.5.2.1 – Visual appearance of uncoated and coated pansies during storage (4 °C)

Weight loss, a_w, pH and titratable acidity

Weight loss increased during cold storage for both uncoated and coated pansies (Table 4.5.2.1), with masses at 21 days of storage corresponding to losses 85.9% for uncoated and 81.8% for coated. Even though coated pansies had always lower mean weight loss values than uncoated ones, there were no statistically differences during the storage period.

Low a_w values are important not only to prevent microbial growth but also to avoid texture degradation and to minimize deteriorative chemical and enzymatic reactions. Coated (0.97) and uncoated (0.91) pansies maintained high values of a_w until 14 days of storage (Table 4.5.2.1), despite some visual differences after 14 days of storage, with uncoated pansies showing drier and more shriveled petals than coated pansies. Only

after 21 days of storage, both samples showed a_w values (0.50 and 0.59 for uncoated and coated pansies, respectively) that are known to prevent pathogenic microorganisms (a_w<0.86) and yeasts and moulds (a_w<0.62) growth (22), resulting in a hurdle to microbial development.

Table 4.5.2.1 – Weight loss, a_w, pH, TA and total carotenoids of uncoated and coated pansies during storage (4 °C)

Properties	Storage days	Uncoated	Coated
Weight loss (%)	0	--	-18.0 ± 5.5^{a}
	7	$43.7\pm6.9^{a,A}$	$29.6\pm6.7^{b,A}$
	14	$74.9\pm6.7^{b,A}$	$66.8\pm5.2^{c,A}$
	21	$85.9\pm3.6^{b,A}$	$81.8\pm2.5^{d,A}$
a_w	0	$0.98\pm0.01^{c,A}$	$1.00\pm0.00^{b,B}$
	7	$0.96\pm0.02^{c,A}$	$0.97\pm0.01^{b,A}$
	14	$0.91\pm0.02^{b,A}$	$0.97\pm0.04^{b,B}$
	21	$0.50\pm0.04^{a,A}$	$0.59\pm0.03^{a,A}$
pH	0	$6.09\pm0.29^{b,B}$	$5.42\pm0.09^{a,A}$
	7	$5.56\pm0.06^{a,A}$	$5.67\pm0.27^{b,A}$
	14	$6.04\pm0.06^{b,B}$	$5.56\pm0.06^{a,b,A}$
	21	$6.00\pm0.08^{b,B}$	$5.48\pm0.07^{a,b,A}$
TA (g citric acid/100g FW)	0	$0.10\pm0.01^{b,A}$	$0.11\pm0.04^{a,A}$
	7	$0.12\pm0.02^{b,B}$	$0.07\pm0.02^{a,A}$
	14	$0.04\pm0.01^{a,A}$	$0.08\pm0.01^{a,B}$
	21	$0.05\pm0.01^{a,A}$	$0.06\pm0.02^{a,A}$
Total carotenoids (mg β-carotene /g FW)	0	$93.0\pm4.3^{c,A}$	$107.4\pm6.4^{c,B}$
	7	$45.0\pm3.6^{b,A}$	$73.5\pm3.8^{b,B}$
	14	$39.7\pm0.4^{a,b,A}$	$69.6\pm4.2^{b,B}$
	21	$33.0\pm1.9^{a,A}$	$31.7\pm3.3^{a,A}$

Lowercase letters -Values with the same letter in the same column are not statistically different ($p>0.05$); Uppercase letters -Values with the same letter in the same line are not statistically different ($p>0.05$)

Regarding pH, some variability was observed, without any special trend, varying the results between 5.56 and 6.09 for uncoated, and 5.42 to 5.67 for coated pansies. The pH of uncoated pansies after 7 days decreased slightly when compared to fresh ones, while for coated pansies the pH increased. This pH increase may be due to the break-up of acids with respiration during storage. However, at 14 and 21 days of storage, the pH values for the coated and uncoated pansies were not significantly different to fresh.

Concerning TA of uncoated pansies, our results suggest that TA decreased after 14 days (Table 4.5.2.1), probably due to the use of organic acids as substrates for the respiratory metabolism in vegetables during postharvest storage (23). After 7 days, an increase on

TA content of uncoated pansies was observed, which was in line with the decrease of pH. On the other hand, no significant differences on TA values of coated pansies were observed along storage. In general, the TA content changed more slowly in coated than in uncoated pansies. So, alginate coating delayed the reduction of TA in pansies. This may be attributed to the modification of endogenous levels of O_2 and CO_2 imposed by the coating presence, inhibiting the respiratory activities and reducing ethylene biosynthesis (24, 25). Still, our results were similar to those reported by Varasteh et al. (25), who reported a reduction of TA during storage (45, 90 and 135 days) in uncoated and coated (chitosan) pomegranate fruits.

Total carotenoids

Total carotenoids decreased during storage on both coated and uncoated pansies (Table 4.5.2.1), from 93.0 to 33.0 (uncoated) and 107.4 to 31.7 (coated) mg β-carotene/g FW, probably due to carotenoids' degradation. After treatment (day 0), 7 and 14 days, coated pansies showed significantly higher ($p<0.05$) values than uncoated pansies (aprox. 1.6 times more). Thus, coating had a positive effect in preserving total carotenoids content until 14 days, probably by reducing oxygen's exposure of the product, since β-carotene is rapidly oxidized when exposed to light and oxygen (26). Similar results were observed with alginate coating and cold preservation of different plum cultivars (27).

However, in our work no significant differences were observed between samples at the end of storage (21 days), being obtained the lowest total carotenoids' content (around 3-fold lower than at the beginning of storage), besides the unsatisfactory visual appearance for both coated and uncoated pansies.

Total phenolic content

Fig. 4.5.2.2A shows the TPC of uncoated and coated pansies extracts, over 21 days of storage at 4 °C. Significant differences among uncoated and coated pansies ($p<0.05$) were observed. Coated pansies always showed higher values of TPC than uncoated ones along the storage period, probably because the alginate edible coating produces an abiotic stress on tissue plants, modifying their metabolism and affecting the production of some secondary metabolites such as phenolics (28, 29).

After 21 days of storage, coated pansies (0.91 mg GAE/g fresh weight) showed a TPC content 3-fold higher than uncoated ones (0.37 mg GAE/g fresh weight). No significant differences on the TPC of uncoated pansies were observed along 21 days of storage

(from 0.27 to 0.37 mg GAE/g fresh weight for 0 and 21 days, respectively). On contrary, the phenolic content in coated pansies decreased initially (from 2.06 to 1.24 mg GAE/g fresh weight for 0 and 7 days, respectively), but after that period the TPC remained relatively constant (from 1.24 to 0.91 mg GAE/g fresh weight, for 7 and 21 days). Similar results were reported by Robles-Sánchez et al. (30), who detected that phenols content also decreased significantly during 12 days in alginate coated fresh-cut Kent mangoes. This initial decrease can be attributed to an increase in the activity of some enzymes that may cause the oxidation of phenolics (31), as well as to chemical degradation that can occur during storage, depending on the available oxygen and exposure to light (32).

Flavonoids

The total flavonoids contents in uncoated and coated pansies are presented in Fig. 4.5.2.2B. In coated pansies a pronounced reduction in total flavonoids was observed during the first 7 days of storage. After that period the decrease in total flavonoids was lower (0.96 to 0.62 mg QE/g fresh weight at 7 and 21 days, respectively). A different behavior was reported in alginate coated fresh-cut Kent mangoes, when a reduction in total flavonoids was observed only after 6 days of storage (30). In the case of uncoated samples, the contents of total flavonoids remained constant until 21 days of storage (0.11 to 0.12 mg QE/g fresh weight at 0 and 21 days, respectively). Furthermore, coated pansies showed always higher flavonoids content than uncoated samples during all storage period, probably because the production of these compounds, which are a class of phenolics, may be promoted in order to protect the plant tissues against biotic and abiotic stresses, as reported previously in section relative to TPC.

Hydrolysable tannins

Fig. 4.5.2.2C shows the changes in the hydrolysable tannins contents of alginate coated and uncoated pansies over 21 days of storage at 4 °C. The hydrolysable tannins contents of coated pansies were always significantly higher than uncoated, being this difference more pronounced at day 0 (5.06 versus 1.68 mg TAE/g fresh weight for coated and uncoated pansies, respectively). In coated pansies the hydrolysable tannins contents decreased significantly from the beginning until 7 days of storage (2.66 mg TAE/g fresh weight, approx. 1.9 fold), remaining constant afterwards. Concerning uncoated pansies,

no significant differences were detected on hydrolysable tannins contents along the storage period, but these were always smaller than coated ones.

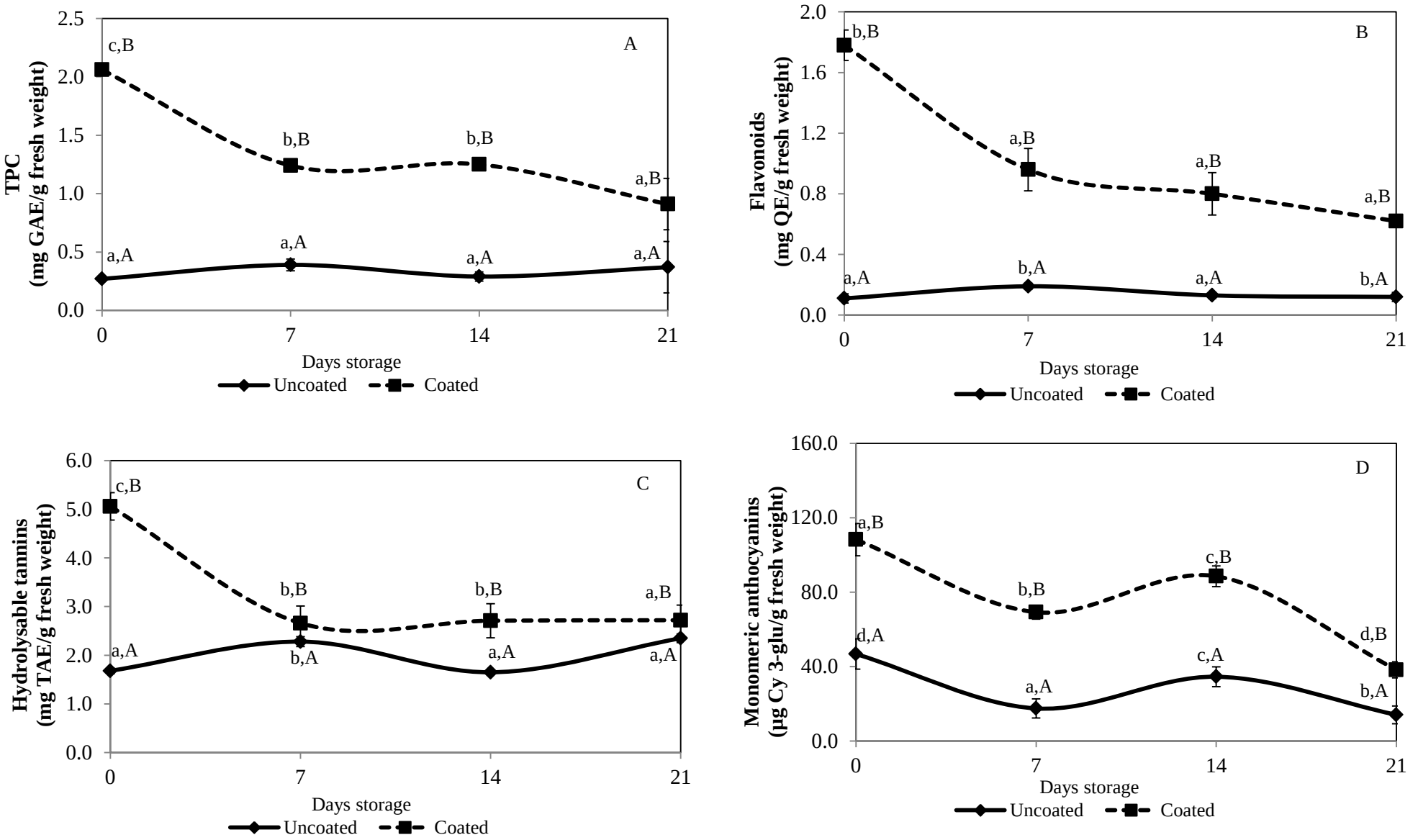

Figure 4.5.2.2 – TPC (A), flavonoids (B), hydrolysable tannins (C) and monomeric anthocyanins (D) contents in uncoated and coated pansies during storage (4 °C)

Monomeric anthocyanins

The amount of monomeric anthocyanins in pansies stored during 21 days is represented in Fig. 4.5.2.2D. Again, coated pansies showed always higher values of monomeric anthocyanins than uncoated pansies, with the lowest anthocyanins contents being observed after 21 days of storage for both samples, 14.1 and 38.3 µg Cy 3-glu/g fresh weight for uncoated and coated pansies, respectively. This reduction of anthocyanins during storage has been reported for other coated fruits such as peel of litchi fruits coated with 1.0 and 2.0% chitosan during storage at 4 °C (33) and strawberry fruit coated with 0.5–1.0% (w/v) carboxymethyl cellulose (CMC) along storage under refrigerated conditions for 21 days (34). Furthermore, in general terms, the behavior of hydrolysable tannins (Fig. 4.5.2.2C) and monomeric anthocyanins (Fig. 4.5.2.2D) was very similar to flavonoids (Fig. 4.5.2.2B), probably because both are subclasses of flavonoids (35).

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The EC_{50} values of DPPH radical scavenging activity for uncoated and coated pansies are shown in Fig. 4.5.2.3A. As expected, coated samples had always lower EC_{50} values of DPPH radical scavenging activity than uncoated pansies, indicative of higher antioxidant activity, probably associated with the accumulation of phenolic compounds (ex. flavonoids) as mentioned in previous sections and similarly to reported by Reyes and Cisneros-Zevallos (36) and Frusciante et al. (37). Furthermore, until 14 days of storage, the values of EC_{50} of coated samples increased, indicative of an antioxidant activity reduction. This fact may be due to the decrease observed on phenolics contents as stated in Fig. 4.5.2.2A.

Reducing power

Fig. 4.5.2.3B shows the changes in reducing power of uncoated and alginate coated pansies over 21 days of storage at 4 °C. Pansies treated with alginate coating showed significant differences ($p<0.05$) on their reducing power, increasing the EC_{50} values along storage, indicative of a decrease in the antioxidant potential of coated pansies. Regarding uncoated samples, no changes values were observed throughout storage (1.32 and 1.22 µg extract/mL at 0 and 21 days, respectively).

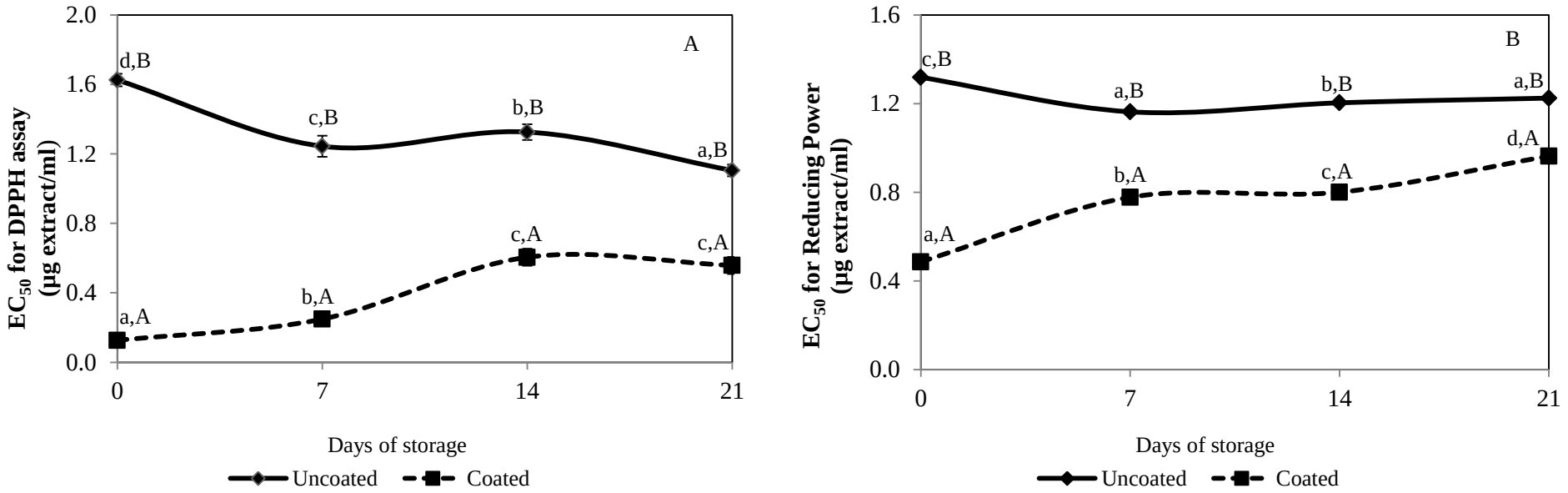

Figure 4.5.2.3 – EC_{50} values for DPPH (A) and reducing power (B) assays for uncoated and coated pansies during storage (4 °C).

As observed in DPPH assay, the EC_{50} values of the reducing power of uncoated pansies were always higher than coated pansies. So, our results show that alginate coating increases the antioxidant potential of pansies.

Correlations between monomeric anthocyanins, flavonoids, hydrolysable tannins, total phenolic content, DPPH radical scavenging activity and reducing power

Table 4.5.2.2 shows the correlations among monomeric anthocyanins, flavonoids, hydrolysable tannins, total phenolic content, DPPH radical scavenging activity and reducing power of uncoated and coated pansies. It was found that the contents of flavonoids and hydrolysable tannins showed significantly positive correlations with total phenolic content, namely 0.911 and 0.965, respectively. These results were expected because flavonoids and hydrolysable tannins are phenolic compounds. Negative correlations of the EC_{50} values of DPPH with monomeric anthocyanins (-0.836), hydrolysable tannins (-0.836), flavonoids (-0.697) and total phenolic content (-0.751) were obtained. These results indicated that bioactive compounds, such as flavonoids, monomeric anthocyanins, hydrolysable tannins and phenolic compounds, have an important role in the antioxidant properties of pansies. A higher content of these compounds implies higher antioxidant activity, corresponding to a lower EC_{50} value. Regarding, the EC_{50} of reducing power assay, a negative correlation was only detected with monomeric anthocyanins (-0.886), showing again the antioxidant potential of these compounds.

Table 4.5.2.2 - Pearson correlation coefficients for total phenolic content, monomeric anthocyanins, flavonoids, hydrolysable tannins and EC_{50} values of DPPH and reducing power assays.

	Monomeric anthocyanins	**Flavonoids**	**Hydrolysable tannins**	**EC_{50} DPPH**	**EC_{50} Reducing Power**
Total phenolic content	-0.951**	0.911**	0.965**	- 0.751**	0.850**
Monomeric anthocyanins	___	-0.876**	-0.958**	- 0.836**	-0.886**
Flavonoids	___	___	0.936**	- 0.697**	0.684**
Hydrolysable tannins	___	___	___	-0.836**	0.794**
EC_{50} DPPH	___	___	___	___	-0.531**

Correlation is significant at **p < 0.01

Microbial quality

The results of microbial quality of uncoated and coated pansies are shown in Table 4.5.2.3. There were no significant differences between uncoated and coated pansies in day 0. Even though no significant differences were observed between both samples along the storage period, after 14 days of storage uncoated pansies showed higher microorganism counts than coated ones, namely yeasts and moulds, suggesting some protection of the alginate coating treatment. *E. coli* and lactic acid bacteria were not detected in any sample.

According to the guidelines of microbiological quality for ready-to-eat foods (38), and including pansies in level 3 (this level applies to foods such as fresh fruits and vegetables, including salad vegetables), our results suggest that coated (0 and 14 days) and uncoated (0 day) pansies were regarded as being of satisfactory and acceptable quality for all microorganisms analyzed. After 14 days of storage, uncoated pansies presented high levels of moulds ($>10^3$), having an unacceptable quality.

In summary, pansies coated with alginate showed good appearance until 14 days of storage, 7 days more than uncoated. Furthermore, after 14 days of storage, coated pansies also showed higher TA, higher values of some bioactive compounds (carotenoids, total phenols, total flavonoids, hydrolysable tannins and monomeric anthocyanins) and antioxidant activity (DPPH and reducing power assays) than uncoated pansies along storage. Furthermore, coated pansies presented a significant reduction of yeasts and moulds counts compared with uncoated pansies after 14 days of storage, suggesting some protection of the alginate coating treatment. So, according to these results, it can be concluded that alginate coated pansies can be stored 14 days at 4 °C, without damages on the appearance and quality. The use of alginate coating in this type of flowers could be considered as a safe and effective treatment. Future research should be focused on evaluating the effect of pansies treated with edible coatings on sensory quality.

Table 4.5.2.3 - Mean counts (log cfu/g ± standard deviation) of total aerobic mesophilic, yeasts, moulds, total coliforms, *E. coli*, psychrotrophic bacteria, and lactic acid bacteria examined in uncoated and coated pansies at 0 and 14 days of storage.

Conditions		**Microbial groups**						
Samples	Days	Total aerobic mesophilic	Yeasts	Moulds	Total coliforms	*E. coli*	Psychrotrophic bacteria	Lactic acid bacteria
Uncoated	0	4.83±0.73^{a}	5.95±0.30^{c}	<2^{a}	1.15±0.22^{a}	<1	<2^{a}	<2
	14	5.48±0.34^{a}	4.20±0.28^{b}	4.42±0.60^{b}	< 1^{a}	< 1	6.40±0.14^{b}	<2
Coated	0	5.12±0.26^{a}	5.34±0.01^{b,c}	<2^{a}	1.30±0.01^{a}	<1	<2^{a}	<2
	14	5.08±0.96^{a}	2.85±0.53^{a}	<2^{a}	< 1^{a}	<1	5.76±0.35^{b}	<2

Values with the same letter in the same column are not statistically different (p>0.05).

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4.6. Combination and application of different methods

This section includes two subsections. The first subsection (4.6.1) focuses on methods combination that helps to establish more effective treatment conditions, originating products more stable and safe (inhibit or retard microbial growth), as well as, with high sensory and nutritive properties. So, osmotic dehydration and high hydrostatic pressure were tested, and the best conditions of each method were combined to create a safe, stable and with longer shelf-life final product. The second subsection (4.6.2) contains the results about the application effect of different preservation methods (drying methods and edible coatings) on quality parameters in borage flowers.

4.6.1.

Osmotic dehydration, high hydrostatic pressure application and their combination on the appearance, weight loss and water activity of pansies (*Viola* × *wittrockiana*) High pressure and osmotic dehydration of pansies.

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Submitted

Abstract

Pansies (*Viola*×*wittrockiana*) are usually preserved by crystallization, cold storage or hot air drying. However, new technologies are needed to increase fresh flowers shelf life and their market share. Thus, the effect of osmotic dehydration (OD), high hydrostatic pressure (HHP) as pre-treatment to OD, and simultaneous application of HHP and OD (HHP+OD), on the appearance of pansies, was for the first time investigated. The best results for OD alone were obtained with 80% (w/v) sucrose for 8h. Application of HHP (75 MPa/5 or 10 min) followed by OD with 80% (w/v) sucrose enabled a reduction of OD to 6h, with flowers maintaining a similar appearance to fresh and water activity values lower than 0.7. On contrary, simultaneous application of HHP+OD resulted on more fragile pansies and texture loss. So, these preliminary results showed that the application of HHP as pretreatment to OD may be a promising post-harvest technology for pansies.

Keywords: edible flower; visual appearance; post-harvest treatments.

Introduction

Edible flowers have been used in the cuisine of different cultures, incorporated into traditional and everyday foods. Presently, they are receiving increased attention from restaurants *chefs*, who used them to add color and flavor to their dishes, while being associated with the concept of "natural" and “healthful”, with an interesting opportunity to increase their market.

Pansies are one of the most commonly used edible flowers in cuisine, added to salads or used to garnish desserts (frosted cakes, sorbets and iced drinks) or crystallized and eaten as a sweet delicacy (Rop *et al*., 2012). Nevertheless, edible flowers are highly perishable, with very short shelf life (Fernandes *et al*., 2017). Until this moment, the unique technologies used by the industry to preserve pansies are cold storage and hot air drying; however, preservation by heat has some drawbacks, such as undesirable biochemical and nutritional changes of the processed product that may affect its overall quality. Crystallization with egg white and sugar is a meticulous process, used only in art confectionary (Miller, 2004). Therefore, the food industry is very interested in improving the marketability of edible flowers, not only as fresh but also as processed products. In this sense, the finding of new food technologies, able to increase the shelf-life of the pansies will bring important economic benefits, beyond allowing the preservation of their quality for longer periods of time.

High hydrostatic pressure (HHP) is an innovative, emerging technology, already in use by the industry to preserve solid and liquid foods of high added value. It is one of the most promising emerging food preservation technologies, being able to destroy microorganisms without the use of heat. In the particular case of pansies, as studied previously (Fernandes *et al*., 2017), HHP alone (75/5 MPa/min) was able to grant an economical extension to flowers shelf life (20 days of storage at 4 °C). However, HHP combined with other methods has not been studied yet. On the other hand, osmotic dehydration (OD) is widely used for the partial removal of water from plant tissues by immersion in a hypertonic (osmotic) solution, allowing the production of more stable products by decreasing their water activity (a_w), as occurs with crystallization. So, HHP treatments combined with OD appear as good alternatives to create new products with distinctive final characteristics. HHP can also be used as a pretreatment to drying to accelerate diffusive transport within foods because HHP changes the cell structure and the tissue architecture, making cells more permeable (Eshiatghi *et al.*, 1994). The

combination of HHP and OD has already been used with success in some products, namely: pineapple (Rastogi and Niranjan, 1998); potato (Rastogi *et al*., 2000 a, b; Sopanangku et al., 2002); turkey breast (Villacís *et al*., 2008); strawberries (Nuñez-Mancilla *et al*., 2013); cherry tomatoes (Dermesonlouoglou *et al*., 2008) and red paprika (Ade-Omowaye *et al*., 2012).

Thus, the objective of this study was to investigate for the first time the effect of OD, HHP as pre-treatment to OD and the combination of HHP+OD, on the appearance of pansies (*Viola×wittrockiana*).

Material and methods

Samples

White/violet fresh pansies (*Viola×wittrockiana*) in full development state were collected in the greenhouse of School of Agriculture, Polytechnic Institute of Bragança, Portugal. After harvest, the fresh flowers were immediately transported to the laboratory under refrigeration.

Osmotic dehydration

Sucrose (osmotic agent) was of food grade, purchased at a local market. Two concentrations of sucrose (40 and 80%, w/v) were prepared by dissolving the required amounts of sucrose in distilled water. Pansies flowers were placed into vessels (50 ml) containing the different sucrose solutions, remaining totally submerged. At each sampling time (12, 24 and 48 h for 40% sucrose; 3, 6, 8, 9, 12, 24 and 48 h for 80% sucrose), flowers were taken out, gently dried with adsorbent paper and weighed. Each treatment/time was performed in triplicate.

High Hydrostatic Pressure before osmotic dehydration

For each HHP treatment, fresh flowers were placed in polyethylene bags, being these sealed after eliminating air from the inside. Then, they were placed into the hydrostatic pressure vessel. The HHP treatments were carried out in a Hiperbaric equipment (Burgos, Spain), with 55 L of vessel volume. Pansies were treated at 75 MPa, during two different holding times (5 and 10 min) at room temperature. The chosen pressure was determined in preliminary studies performed by our research group (Fernandes *et al.*, 2017), in which higher pressures were also tested. However, the application of higher pressures damaged the pansies. After HHP treatment, pansies were osmotic

dehydrated in 80% (w/v) sucrose, with immersion times of 2, 4, 6 and 8 h. These conditions were established taking into account the results obtained in the previous section. Each treatment/time was performed in triplicate.

Simultaneous application of osmotic dehydration under high hydrostatic pressure

Fresh flowers were placed in polyethylene bags with 80% (w/v) sucrose, and then sealed and subjected to HHP (75 MPa, 5 and 10 min). After the HHP treatments, the flowers remained inside the bags with the osmotic solution for 4 h more.

Quality parameters

The quality parameters evaluated included visual appearance, water activity and weight loss. The visual appearance regarding pansies' color, loss of brightness, darkness and shrinkage was evaluated after each treatment. Water activity (a_w) of whole flower (the state that will be sold in the future) was determined in a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland).

The weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany), being the weight loss (WL) determined according to Equation 1:

$$WL = \frac{M_0 - M}{M_0} \times 100 \quad \text{Eq. 1}$$

where M_0 is the initial mass of fresh pansies before treatment and M is the mass of pansies after treatment.

Statistical analysis

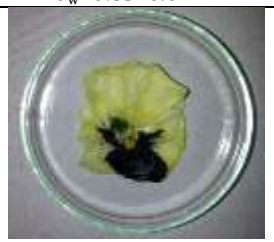
SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. The normality of the data was verified by the Shapiro-Wilk test. The analyses of variance (ANOVA) or ANOVA Welch were carried out to evaluate if there were significant differences ($p < 0.05$) between samples. Additionally, significant post hoc analyses were performed (Tukey HSD test if variances in the different groups were identical or Games-Howell test if they were not). The homogeneity of variance was tested by Levene's test.

Results and Discussion

Osmotic dehydration

Firstly, two sucrose concentrations and different immersion times were studied to define the best combination (% sucrose and time), taking into account the flowers' appearance and the beginning of mass exchanges between the flowers and the osmotic solution (occurrence of WL). In fact, the last topic is important because pansies' petals have a superhydrophobic surface, due to the presence of particular structures called papillae (Weryszko-Chmielewska and Sulborska, 2012; Schulte et al., 2011), which hinders the occurrence of exchanges between the osmotic solution and the flower, making difficult the occurrence of OD.

After applying two immersion times (12 and 24 h) on 40% (w/v) sucrose (Figure 4.6.1.1), the flowers' weight increased (negative WLs) instead of decreasing, which could be due to the presence of sugar on the surface of the flowers. After 48 h WL was equal to 1.4%; however, this value was not significant different from the previous due to the high standard deviations. Nevertheless, the visual appearance was unsatisfactory probably due to some cell rupture (Figure 4.6.1.1). Thus, it was concluded that with 40% (w/v) sucrose a longer immersion time was needed to observe OD but this would affect negatively the visual appearance. In this order, a higher sucrose concentration (80%, w/v) was tested (Figure 4.6.1.1). At this concentration, and until 8 hours of immersion, pansies remained with "fresh-like" appearance. After this time, pansies petals began to shrunk. Furthermore, only after 12 h of immersion, OD occurred (WL with a positive value equal to 3.2±6.3%). It was also observed that all samples subjected to OD still had high values of water activity, between 0.80 and 0.95, which would hinder their preservation. Thus, since one of the aims of the present study was to maintain the appearance of fresh flowers, it was considered that pansies' immersion in 80% (w/v) sucrose along 2, 4, 6 and 8 h, would be the conditions to be applied in the following studies in combination with HHP, as higher times irreversibly affected the visual appearance of pansies. Even though for 2, 4, 6 and 8 h a low OD was observed, it was expected that the combination of OD with HHP might increase WL, without damaging the flowers further.

Sucrose concentration (%, w/v)	Time (hours) 3h	6h	8h	9h	12h	24h	48h
40	----	----	----	----	 WL=-4.8±10.1[a] a_w=0.85±0.04 [a]	 WL=-14.1±2.5 [a] a_w=0.93±0.01[b]	 WL=1.4±16.8 [a] a_w=0.84±0.06 [a]
80	 WL=-21.8±6.1[a] a_w=0.95±0.01[b]	 WL=-12.9±15.5 [a,b] a_w=0.89±0.02 [a,b]	 WL=-23.2±4.6 [a] a_w=0.89±0.03[a, b]	 WL=-13.8±10.3 [a,b] a_w=0.91±0.01 [b]	 WL=3.2±6.3 [a,b,c] a_w=0.88±0.02 [a,b]	 WL=13.6±7.6 [b,c] a_w=0.89±0.01 [a,b]	 WL=19.2±14.6 [c] a_w=0.80±0.08 [a]

Values are expressed as: Mean±Standard deviation. Values with the same letter in the same line are not statistically different (p>0.05).

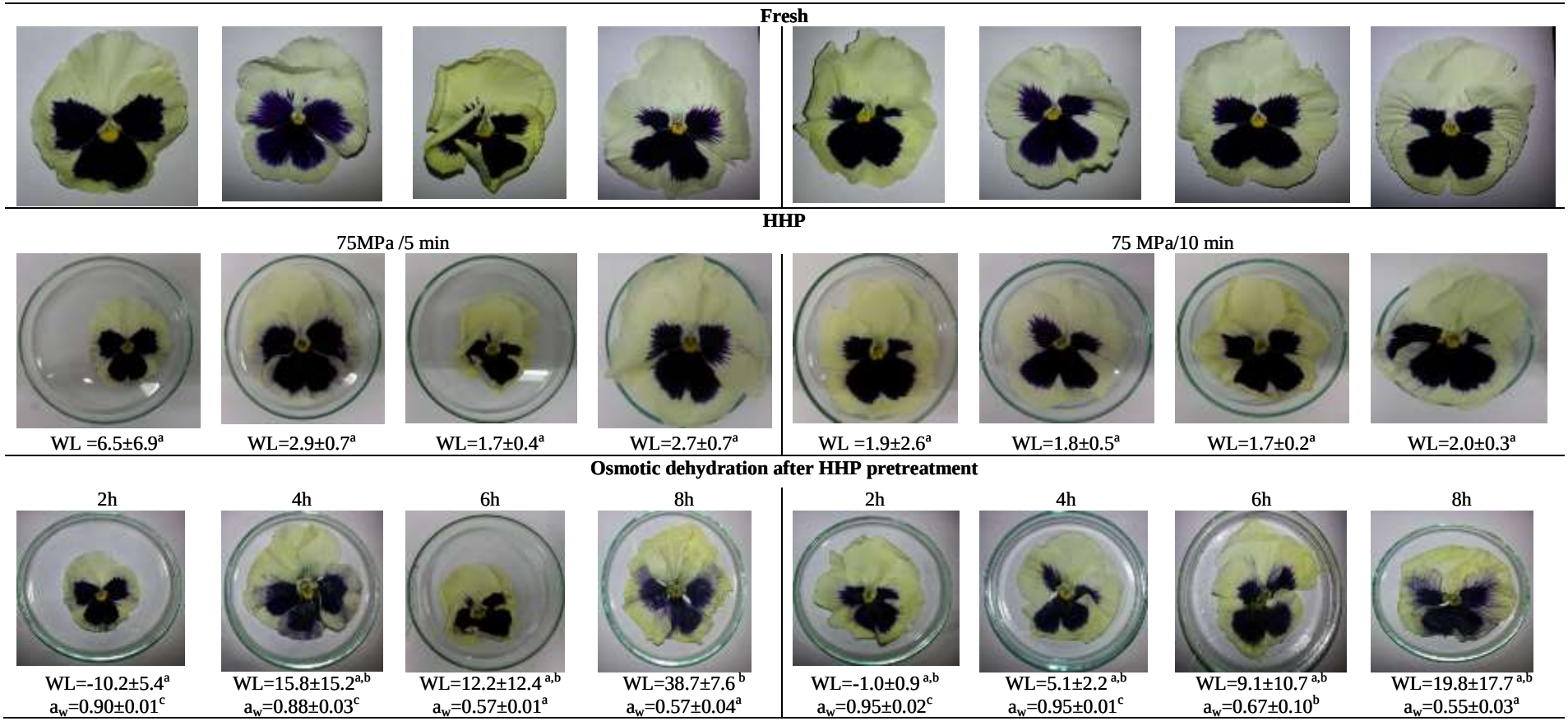
Figure 4.6.1.1 - Visual appearance of pansies after immersion in sucrose solutions (40 and 80% (w/v)) along time (WL = weight loss (%) and a_w = water activity).

High Hydrostatic Pressure as pretreatment to osmotic dehydration

Pansies subjected to HHP (5 and 10 min) maintained good appearance in comparison to fresh ones (Figure 4.6.1.2), although a small WL was already observed (<7%). After the HHP pretreatments, pansies were immersed in 80% sucrose (w/v), along different immersion times (2, 4, 6 and 8 h), as explained in the previous section. By observing Figure 4.6.1.2, pansies pretreated at 75 MPa for 5 or 10 min, followed by immersion in 80% (w/v) sucrose until 6 h, remained with satisfactory appearance. After that time, the color of the violet parts began to expand to the white ones.

After both HHP pre-treatments (75MPa/5 or 10 min) and 4 h of immersion, WL was already observed, a sign of effective exchanges of solutes and water. However, the a_w values only decreased with at least 6 h of immersion. So, comparing the application of HHP before OD with OD alone, it was observed that a smaller immersion time in the osmotic solutions was necessary to achieve mass transfer, quantified by WL, as well as to decrease the a_w from 0.95 to 0.6. Nevertheless, this value was achieved for 8 h of OD, having the pansies an undesirable aspect. With 6h of OD the flowers preserved their appearance and the a_w was already reduced (approx. 0.6). These results are very important because they are the first reported for pansies. They are also in line with other authors, who had mentioned that HHP increased the mass transfer rates in pineapple (Rastogi and Niranjan, 1998) and cherry tomatoes (Dermesonlouoglou *et al.*, 2008).

So, applying HHP as pre-treatment to OD can be a promising solution to maintain the appearance of pansies and to increase their shelf-life due to the lower a_w values achieved.



Values are expressed as: Mean±Standard deviation. Values with the same letter in the same line are not statistically different (p>0.05).

Figure 4.6.1.2 - Visual appearance of pansies subjected to two HHP treatments (75MPa/5 min and 75MPa/10 min) (A) and after immersion in 80% sucrose (w/v) along different immersion times (B).

Simultaneous application of osmotic dehydration with high hydrostatic pressure

Afterwards, simultaneous application of OD and HHP was applied to pansies. Even though at both times of pressure (5 and 10 min) pansies fragility increased (Figure 4.6.1.3), immersion in 80% (w/v) sucrose for 4 h wasn´t sufficient to induce OD, with no WL observed. So, the combination of the two treatments at the same time does not seem to be adequate for pansies. Similar results were reported by Nuñez-Mancilla *et al*. (2013) for strawberries, where changes in chromatic coordinates were observed, causing noticeable modifications of surface fruit colour, which was an undesirable effect.

HHP combined with OD

75 MPa/5min - 80%/4h	75 MPa/10 min - 80%/4h
	
WL=-23.4±7.4[a]	WL=-10.6±11.9[a]
a_w=0.92±0.02[a]	a_w=0.90±0.01[a]

Values are expressed as: Mean±Standard deviation. Values with the same letter in the same line are not statistically different (p>0.05).

Figure 4.6.1.3 - High hydrostatic pressure treatments (75MPa/5 min and 75MPa/10 min) combined with osmotic dehydration (80% sucrose (w/v)).

Conclusions

In conclusion, it was observed that pansies subjected only to OD did not lose weight easily and when OD began to occur, the appearance of pansies was already

unsatisfactory. On contrary, pansies pre-treated with HHP followed by OD, showed a considerable decrease in a_w values and shorter immersion times were necessary to be observed weight loss. The HHP pre-treatment at 75 MPa/5 or 10 min, followed by immersion in 80% (w/v) sucrose for 6 h, resulted in pansies with fresh appearance and low a_w (< 0.7), while the simultaneous application of OD+HHP was not a successful approach, originating pansies with unsatisfactory appearance. Thus, the combination of HHP pretreatment with OD can be a promising technology to be applied to pansies to increase their shelf-life.

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The effect of different post-harvest treatments on the quality of borage (*Borago officinalis*) petals

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Abstract

Background. Borage is an edible flower with a very limited shelf-life (approx. 1 day). After harvest, flowers dry and shrink rapidly and become darker. Extending the shelf life of borage will make it more appealing for commercialization and it will enable borage growers to expand their market. The aim of the present work is to evaluate the effect of three post-harvest technologies.

Material and methods. Freeze-drying, hot air convective drying and alginate edible coating were applied to borage petals, and the visual appearance, water activity (a_w) and weight loss was evaluated.

Results. Hot air-dried samples had an unsatisfactory visual appearance. Freeze dried flowers were less shrunken and dried while showing the lowest *aw* (0.25 ±0.01). Alginate coated flowers had a good visual appearance, like fresh flowers, which was maintained during refrigerated storage (for 5 days), four days longer than those which were uncoated. Nevertheless, the flowers became fragile and it was difficult to handle them without causing damage.

Conclusion. Freeze drying may be applied to produce dried borage flowers for infusions, while alginate coating is a promising treatment to increase shelf-life subject to further development.

Keywords: borage, drying, alginate edible coating, quality, storage.

Introduction

Borage (*Borago officinalis*) is a medicinal and culinary herb native of the Mediterranean region, although presently it is commercially cultivated mainly for its seed oil (Hafid et al., 2002). Edible flowers, such as borage flowers, are increasingly attracting interest from gourmet chefs and consumers. The borage flower has five petals, which have a triangular-pointed shape (Ramandi et al., 2011), and one of two colors: blue (wild variety) or white (Montaner et al., 2001). Their cucumber taste with a hint of sweetness from the stamens, make them an interesting choice to garnish salads and summer fruit drinks. However, edible flowers have a limited shelf life, with senescence progressing rapidly after harvest; protein content falls, protease activity and respiration rate increase, and the fluidity of lipids membrane declines (Silva et al., 2003). These phenomena in the flower's metabolism are accompanied by morphological and biochemical deterioration; petal abscission and discoloration, wilting, dehydration and tissue browning (Serek and Reid, 2000). Generally, the majority of edible flowers must be used within 2 to 5 days of harvest (Kou et al., 2012), although this time period varies for each flower, and borage has a particularly short shelf-life (approx. 1 day). Therefore, it is necessary to find technologies able to maintain quality of flowers over an extended period of time. The effect of temperature during the storage of borage flowers has been studied by Kelley et al. (2003), showing that flowers stored at 0–5°C were marketable after 1 week, and stored at –2.5°C were still marketable after 2 weeks. Regarding the application of hot air convective drying, freeze-drying and edible coatings to this flower, to our knowledge no study has been performed until now. Thus, the objective of our work was to evaluate the effect of three post-harvest technologies (hot air drying, freeze-drying and edible coating) on the visual appearance of borage in order to increase its shelf-life. Water activity (a_w) and weight loss were measured. Furthermore, a visual scale was developed in order to be used in the future to easily evaluate the appearance of this edible flower.

Material and methods

Samples

Fully-developed fresh borage petals (*Borago officinalis*) were collected from the greenhouse of the School of Agriculture, Polytechnic Institute of Bragança, Portugal. Full development was established based on the flower's size, degree of opening and colour (Rop et al., 2012). After harvest, the fresh flowers were immediately transported to the laboratory under refrigeration.

Post-harvest treatments

Hot air convective drying

Borage petals were distributed uniformly in a thin layer on trays and dried in a hot air convective oven (Memmert, Schwabach) at 50 °C for 60, 90, 105 and 120 minutes. This temperature was chosen because it is a common temperature used in other drying studies of flowers and herbs (Balladin and Headley, 1999; Chen et al. 2000; Mao et al., 2006). Relative humidity and temperature values were measured at different places of the hot air convective oven with portable thermo-hygrometers (Hanna Instruments, HI 9564, Woonsocket) and digital thermometers (Hanna Instruments, HI 98509, Woonsocket), respectively. The relative humidity at 4.3 ±1.2% and temperature 49.6 ±2.4°C were kept constant throughout all experiments.

Freeze-drying

Petals were kept frozen at −20 °C for a period of 24 h and lyophilized in a freeze-dryer (Scanvac, Coolsafe, Lynge, Denmark) for 24 h.

Edible coatings

The edible coating treatment was applied according to the method used by Tay and Perera (2004). Borage petals were immersed in a 0.5% alginate solution (made with sterile distilled water) for 30 min. Residual alginate was allowed to drain for 5 min before the samples were immersed in a 1% $CaCl_2$ solution (w/v) for 30 min to induce spontaneous cross-linking reactions. Surface water was carefully blotted using paper towels.

Storage of fresh and coated flowers

As the flowers subjected to hot air convective drying and freeze drying had unsatisfactory appearance, they were discarded. Conversely, the alginate coated ones exhibited good visual appearance and so they were stored under refrigeration (4 °C) in plastic containers, until they presenting unsatisfactory visual appearance. Every day, at the same time, photos of the flowers were taken and the water activity and weight loss were measured. At the same time uncoated fresh flowers were also subjected to the same storage conditions (control).

Water activity (a_w), weight loss, color and petal width

Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). Weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany) and weight loss (WL) was determined according to Equation 1:

$$WL = \frac{M_0 - M}{M_0} \times 100 \quad \text{Eq. 1}$$

where M_0 and M are the masses of the flowers before and after treatment, respectively.

The color was evaluated with a colorimeter Minolta CR-400 (Osaka, Japan), using the CIE*Lab* scale. *L**, *a** and *b** coordinates, as well as, Chroma (*C**) and Hue Angle (*h**) values, were determined. The width of borage petals were measured with a digital caliper (Powerfix, Berlin, Germany).

Statistical analysis

All statistical tests were performed by SPSS Statistical software, v. 18.0 (SPSS Inc., Chicago, IL). The level of significance was set at $p < 0.05$. The evaluation of statistical significance was determined by ANOVA, followed by Tukey's HSD *Post-hoc* test, since data normality was observed and the variances of the groups were identical. The normality and variance homogeneity were evaluated by the Kolmogorov-Smirnov and Levene's tests, respectively.

Results and Discussion

Proposal of a visual scale for borage

A visual scale to be used in the future to evaluate the appearance of borage was developed and is shown in Figure 4.6.2.1. Based on these observations, a classification based on three categories classes is suggested:

Excellent = flowers retain their freshness and intense color

Satisfactory = flowers begin to wither and shrink

Unsatisfactory = petals are very wilted and dried and there is loss of color.

Based on the assayed samples, only day zero flowers were classified as excellent and satisfactory appearance was observed only on day 1. From day 2 to 5 all flowers were classified as unsatisfactory. These results confirmed the short shelf-life of borage at 4 °C; approximately one day.

The effect of treatments on the characteristics of borage

Visual appearance, color and petal width

Figures 4.6.2.2 and 4.6.2.3 show the visual appearance of borage flowers after the three treatments. With all drying treatments (Figure 4.6.2.2), the flowers were found to be shrunken and dried, with an unsatisfactory appearance. Still, freeze-dried samples had a better appearance than hot air dried ones. They were less shrunken, which was confirmed by measuring petal width (3.42 mm for hot air convective drying for 120 min and 5.36 mm for freeze dried versus 26.76 mm for fresh). Furthermore, the loss of the flowers' blue color after both drying treatments was observed (b^* equal to 4.71 for hot air convective dried and –6.39 for freeze dried compared to –24.19 for fresh). As has been suggested, freeze dried flowers can be used in infusions and it could be interesting to study their compositional changes in terms of bioactive compounds, in order to determine if their bioactivity is retained with this preservation method, as well as the expected shelf life.

Regarding Figure 4.6.2.3, on the appearance of borage flowers' treated with alginate edible coating, on day 0 they retained their fresh-like appearance. During storage, alginate coated flowers maintained better appearance than fresh ones. In more detail, after 3 and 5 days, the coated flowers were found to be less dried and with a more intense color than fresh samples (Figures 4.6.2.1 and 4.6.2.3). Nevertheless, it must be stated that the coated flowers were quite fragile, being difficult to handle after treatment.

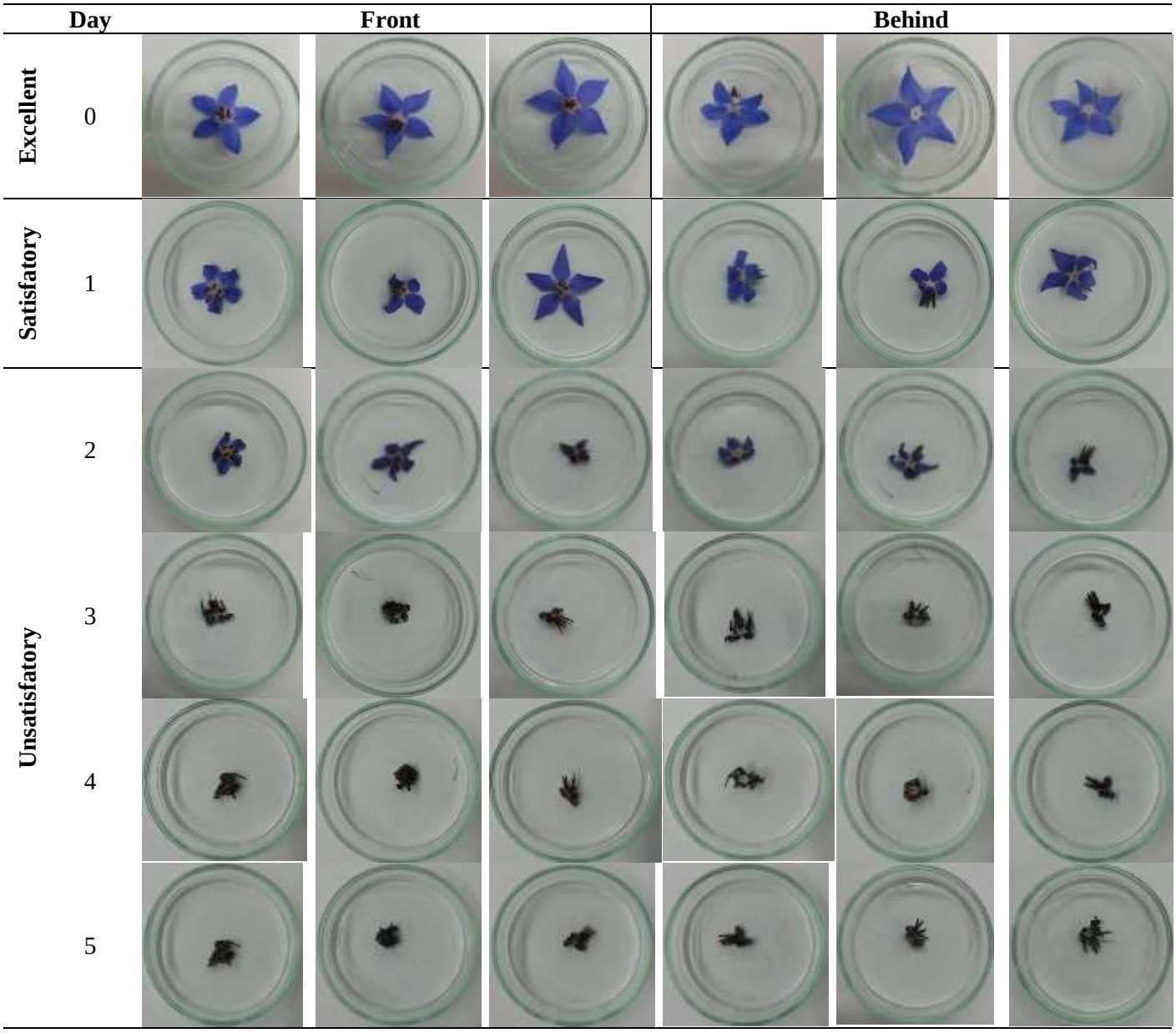

Figure 4.6.2.1 - Visual scale developed to evaluate the appearance of borage flowers stored at 4 ºC.

Water activity (a_w) and weight loss

Water activity has its most useful application in controlling the growth of bacteria, yeasts and moulds. For edible flowers, as with other kind of fresh foods, water activity (a_w) reduction can be used as a tool to increase shelf life during storage. According to Yan et al. (2008), a water activity of nearly 0.6 inhibits the growth of most microorganisms. Only flowers subjected to freeze-drying for 24 h and hot air convection drying for 105 and 120 min achieved a_w values lower than 0.6. However, borage flowers were not visually appealing, particularly those which had undergone air convective drying. On the other hand, all alginate coated flowers were found to have very high values of water activity (between 0.98–0.99), which did not change during storage, indicative of their increased susceptibility to microbial growth.

Concerning WL, both drying methods caused a drastic decrease in the weight of the flowers, due to water loss, although it was more pronounced in freeze-dried sample (91.7%), followed by the sample which had undergone hot air convective drying for 120 min (87.3%), but it had a strong impact on the appearance of the final product. The alginate coated borage flowers showed a negative WL on the first day of storage, due to the weight gain caused by the incorporation of alginate onto the surface of the flowers. After 3, 5 and 7 days of storage, the alginate coated borage showed WLs equal to 54.7, 76.0 and 86.9%, indicating that the flowers were wilting in the same way as the fresh ones, despite their protective coating, indicating that it does not act as a barrier to water loss.

TREATMENTS					
Fresh	**Hot air convective drying**				**Freeze drying**
	60 min	**90 min**	**105 min**	**120 min**	**24 h**
					
a_w :0.96±0.01[c] *L**: 56.37±5.11[c] *a**: 15.49±3.63[b] *b**: -24.19±7.28[a] *C*:* 28.74±8.04[b] *h**: 303.20±2.78[a] Petal width, mm:26.76±4.42[b]	a_w:0.90±0.01[c] WL, %: 69.2±0.8[a]	a_w:0.78±0.17[c] WL,%: 80.3±5.8[b]	a_w:0.51±0.01[b] WL ,%:86.9 ±0.1[b,c]	a_w:0.44±0.02[b] WL, %: 87.3±0.8 [b,c] *L**: 45.30±1.12[b] *a**: 5.10±0.27[a] *b**: 4.71±0.53[c] *C*:* 6.97±0.17[a] *h**: 42.69±4.71[b] Petal width, mm:3.42±0.34[a]	a_w:0.25±0.01[a] WL,%: 91.7±0.3[c] *L**: 32.41±0.20[a] *a**: 5.90±0.30[a] *b**: -6.39±1.68[b] *C*:* 8.74±1.39[a] *h**: 309.58±6.50[b] Petal width, mm:5.36±0.87[a]

Figure 4.6.2.2 - Borage in fresh and after being subjected to two drying methods: hot air convective drying and freeze drying.

	Along storage (days)			
	0	**3**	**5**	**7**
Edible coating				
	a_w:0.99±0.01[b] WL, %: -74.5±38.9[a]	a_w:0.98±0.17[a] WL,%: 54.7±16.5[b]	a_w:0.99±0.01[a,b] WL, %:76.0 ±14.3[b,c]	a_w:0.99±0.01[a,b] WL, %:86.9 ±6.0[c]

Figure 4.6.2.3 - Visual appearance of borage during storage (4 °C) after applying an edible coating.

Conclusions

In summary, the present work showed that borage petals are highly perishable, with only one day of shelf-life at 4ºC. When applying drying methods to borage petals, those subjected to hot air convective drying (50ºC; 60-120 min) and freeze drying did not present a fresh-like appearance. In fact, hot air convective drying applied to borage petals caused them to shrunk and lose their characteristic blue color. On the other hand, freeze-dried petals maintained better appearance than did hot air convective dried petals, so they can be used for applications which require dried flowers, such as infusions, but not for garnishing dishes. On other hand, alginate coated borage showed good appearance after 5 days of storage, 4 days more than fresh flowers; however, the petals were fragile and maintained high values of a_w. Alginate coating of borage could be a promising technology to increase its shelf-life. Nevertheless, in the future it is necessary to improve this technology in order to increase flowers' resistance and to allow them to be more easily handled.

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CHAPTER 5

5.1

Global assessment of the best post-harvest technologies to apply to edible flowers

Next section contains the results obtained after performing an online survey about habits and perceptions/knowledge of edible flowers and post-harvest technologies. Furthermore, it also includes organoleptic studies (preference and acceptability tests) done to final products subjected the best post-harvest treatments, by gourmets chefs and consumers. Sensory analysis was only performed in pansies treated with HHP, alginate coated and crystallized. However, other flowers and technologies, such as ice cubes and drying methods (shade drying) showed previously good results, but were not included in the sensory analysis. The reason was because these products would have to be included as an ingredient of other food (ex: ice cubes in drinks and dried flowers in infusions or bakery products), which would make the sensory analysis much more difficult and complex.

5.2.

Sensory analysis of pansies subjected to different post-harvest technologies: high hydrostatic pressure, alginate coating and crystallization

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Submitted

Abstract

The market of edible flowers is increasing, although it is a product with high perishability. New post-harvest technologies can be used to increase the shelf-life and to maintain the quality of edible flowers. However, when using new treatments, it is necessary to know if consumers and chefs will accept or not the final product. Thus, the objectives of this study were firstly to investigate consumers' perceptions and knowledge about edible flowers and post-harvest technologies through an online survey. Secondly, the effects of some post-harvest technologies, namely, high hydrostatic pressure (HHP), alginate coating and crystallization, on sensory characteristics of pansies were evaluated by gourmet chefs and consumers. The results showed that most of the participants of the online survey (94.5%) recognised edible flowers as food component and had a positive opinion about them (73.5%). However, only 54.0% had already tasted them at restaurant and at home. Refrigeration, freezing and crystallization were the post-harvest technologies most known and mentioned to be able to increase edible flowers' shelf-life. Most of the participants were willing to pay a little more (1 or 3 euros more) to acquire a product with a longer shelf-life. The sensory profiles of gourmet chefs and consumers were different. The first ones liked more the HHP treated pansies than alginate coated and crystallized ones. The second ones also liked more the

HHP treated pansies but they also high rated the alginate coated and crystallized pansies, with high overall acceptance (≥7). Regarding purchasing intentions, the gourmet chefs would purchase more the HHP pansies, but they would only pay ≤5 € for a package with 12 flowers. On contrary, more than 60% of consumers would be willing to buy flowers subjected to the three treatments. In summary, the present work may provide important information to help the market/producers of edible flowers in the selection of the most promising post-harvest technologies to apply to pansies, taking into account consumers' and chefs' perceptions.

Keywords: Edible flowers; post-harvest technologies; online survey; sensory analysis; chefs; consumers.

Introduction

Floriphagia is the consumption of flowers as a food (Lara-Cortés et al., 2013), and it is an old practice which has now been again appearing. Edible flowers have gained popularity as a creative and innovative ingredient in the culinary world because they provide flavor, aroma and color, when added to foods. Gourmet chefs and consumers use flowers in culinary as solids (in dishes as roasts, salads, soups, cakes and jellies), liquids (alcoholic beverages, teas and cold drinks made from flowers), and in the form of flavorings (olive oils, other oils and vinegars) (Guiné et al., 2017). However, the idea of eating flowers is still viewed with suspicion by some consumers because it involves a kind of neophobia (the reluctance to try novel foods) or sometimes they have difficulty to know which flowers can be ingested without having toxicity problems, as well as, they don't know how to use them in dishes or where to buy (Benvenuti et al., 2016).

Sensory analysis is an experimental method of food analysis, which provides information about the degree of acceptance of a food and it is also widely used for the determination of overall quality. Regarding edible flowers, there is little information on organoleptic appreciation by consumers or gourmet chefs. Only studies involving twelve species of edible flowers (Benvenuti et al., 2016), *Allium* species (D'Antuono and Manco, 2013), daylilies (Pollard et al., 2004), and viola, borage and nasturtium (Kelley et al., 2001) were already done. Furthermore, there are two more studies in consumers' attitudes of edible flowers (Chen and Wei, 2017; Rodrigues et al., 2017). Furthermore, there is no study about sensory analysis of edible flowers subjected to new post-harvest technologies. Until now, some new post-harvest technologies have been

tested in edible flowers with good results, such as high hydrostatic pressure (Fernandes et al., 2017) and edible coating (Fernandes et al., 2018a,b) in pansies, as well as crystallization. So, it is important to evaluate consumer and gourmet chefs acceptance and perception of organoleptic attributes because those technologies can have some effects on flavor, taste and texture in edible flowers. Furthermore, opinions of consumers will help the edible flowers producers to improve the characteristics that should be included in the final product and to understand the importance of some sensory characteristics that affect consumers in their purchase decision. So, the objectives of this work were to determine the sensory profile and consumers acceptability of pansies (*Viola* × *wittrockiana*) subjected to three post-harvest technologies (high hydrostatic pressure, alginate coating and crystallization) by consumers and chefs. Furthermore, an online survey was done previously to understand the knowledge of the population on edible flowers and post-harvest technologies.

Materials and Methods

Online survey

The questionnaire was placed on social networks websites or send by email. The sample was randomly selected as potential consumers/users of edible flowers and consisted of 422 participants. Twelve questions were developed to assess the knowledge of participants on edible flowers and post-harvest technologies, as well as on the habits of the respondents about consuming edible flowers. The first questions were about demographic information (gender, age, educational level and geographical origin). To evaluate consumers' knowledge on the use of flowers as food ingredient, perceptions of shelf-life, and opinions about their use, habits of consumption, characteristics more valorized and which words are more associated to edible flowers, some questions were formulated. Furthermore, to evaluate the perception of participants on post-harvest technologies, five questions more were added, regarding their knowledge on some technologies, which technologies could be used on flowers, if these technologies can increase shelf-life of flowers, if they would be willing to taste flowers treated with post-harvest technologies and what added value of the price they would be willing to pay.

Data were collected between June and July 2018. All ethical issues were followed when designing and applying the questionnaire, as well as, the confidentiality of the answers obtained was totally guaranteed.

Samples

Fresh pansies (*Viola×wittrockiana*) were bought in a store of edible flowers and they were immediately transported to the laboratory under refrigeration.

Post-harvest Technologies

High Hydrostatic Pressure (HHP)

Fresh pansies were placed into polyethylene bags and treated at 75 MPa during 5 and 10 min, in a hydrostatic pressure vessel (55 L of volume) of a Hiperbaric equipment (Burgos, Spain), according to the best results obtained by Fernandes et al. (2017).

Alginate coating

Fresh pansies were immersed in 0.5% (w/v) alginate solution (Panreac Química SA, Barcelona, Spain), prepared in sterile distilled water, for 30 min, according to the method described by Fernandes et al. (2018a,b). The residual alginate solution was left to drain for 5 min before immersion in 1% $CaCl_2$ solution (w/v) for 30 min, to induce spontaneous cross-linking reactions. Surface water was carefully blotted as before, using paper towels.

Crystallization

Fresh pansies were painted with pasteurized egg white on the front and back of the flowers, using a fine brush. Then, they were sprinkled with sugar evenly over the wet petals, being the flowers placed down on paper. Flowers were stored under refrigeration (4°C) for about 48h. After this period, the flowers were stored at room temperature (approx. 20°C).

Sensory analysis

A sensory analysis was conducted to investigate the perceptions and acceptability of pansies subjected to the three post-harvest technologies described before, by two customer segments. The two segments were defined as professional chefs (gourmet chefs) and common consumers, both important product users. Eight Portuguese gourmet chefs were recruited and selected on the basis that they already use or will possibly use edible flowers in their restaurants, as well as, 30 consumers (24 females and 6 males), were recruited among the university community (students, teachers and other staff) from

the Polytechnic Institute of Bragança (Portugal). The sensory analyses were carried out in July 2018. Three flowers of each treatment were presented to the panelists on a glass dish. The evaluators were provided with plain water. After being introduced to the objective of the study and the testing procedure, the chefs/consumers rated each sample on a 9-point hedonic scale, from "dislike extremely" to "like extremely". When they didn't feel the attribute, this was classified with zero. Five different organoleptic characteristics (visual appearance, flavor, taste (bitterness, astringency, spiciness and sweetness), texture and overall acceptance) were included in the evaluation survey. Furthermore, the following questions were also asked: if they normally buy edible flowers; if they answered negatively, it was asked, why not?; if answered positively, it was asked what kind of flowers do they buy; if they would buy this new product; which price they were willing to pay and what kind of dishes this new product would/might be included. Finally, they are asked to say what they liked and disliked more on the product.

Statistical analysis

SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. To compare groups with different demographic characteristics (gender, age, geographic origin and educational level), the Chi-Squared test was performed. To be able to perform the statistical analysis, due to the low number of responses of some of the groups, class joining was performed. For example, the educational classes were divided into 2 classes namely, college/technical degree and graduate, as well as, the age (<30, 31-40, 41-50, >50 years). In sensory data analysis, Pearson's correlation coefficients were determined.

Results and Discussion

Online survey

Demographic information of participants

The demographic questions are described in Table 5.1.1.1 and they included the age, gender, educational level and geographical origin. Of the 422 participants, 78.7% were female and 21.3% male. The age of most of the participants was between 20 and 40 (74.6%). Regarding education, the majority of the participants had a bachelor's degree (40.3%), followed by master's degree (28.0%). Table 5.1.1.1 shows that 62.1% (n=262)

of the participants were from the North of Portugal, 15.9% (n= 67) from the Centre and 13.0% (n=55) from other countries.

Table 5.1.1.1 -Demographic information of the participants on the online survey about edible flowers and post-harvest technologies.

Variables	**n (%)**
Gender	
Female	332 (78.7)
Male	90 (21.3)
Age (years)	
<20	4 (0.9)
20-30	169 (40.0)
31-40	146 (34.6)
41-50	64 (15.2)
51-60	30 (7.1)
>60	9 (2.1)
Education	
Basic Education	6 (1.4)
Secondary Education	80 (19.0)
Technical/ Professional degree	22 (5.2)
Bachelor's degree	170 (40.3)
Master's degree	118 (28.0)
Doctorate and post doctorate degrees	26 (6.2)
Geographical Origin	
North of Portugal	262 (62.1)
Centre of Portugal	67 (15.9)
South of Portugal	32 (7.6)
Islands (Azores and Madeira)	6 (1.4)
Other countries	55 (13.0)

Consumers' knowledge and habits about edible flowers' consumption

Most of the participants expressed knowledge on the use of flowers as food component (94.5 %, n=399) and had a positive opinion about them (73.5 %, n=310) (Table 5.1.1.2). However, only half of the respondents (54.0 %, n = 228) of the participants had already eaten edible flowers. The group that never ate flowers (46.0 %) reported that the main causes were because they can't easily find this product for sale (61.3 %) and did not know this product (17.0 %). However, 93.8 % of the group that never ate flowers was interested in tasting them.

The group of people that had already tasted flowers said that they had consumed the flowers in fresh (89.1 %), at restaurants (58.7 %) or home (36.1 %). Salads (57.0 %), desserts (40.4 %) and appetizers (36.8 %) were the dishes where the flowers were most

consumed. Between the words purposed to define edible flowers, the most associated were attractive/appealing (54.0 %), decoration (51.4 %), colorful (50.0 %), innovative (47.9%) and exotic (46.9 %). So, edible flowers for participants are associated to words that are related to the form of utilization (decoration), sensory properties (colorful), liking (appellative/attractive) and elements that denote new/unfamiliar food category (exotic and innovative). However, a small percentage of participants associated edible flowers to negative words such as strange (6.9 %), toxic (1.4 %) and repulsive (0.7 %). The main reason to consume edible flowers was for dishes decoration (49.3 %). However, organoleptic (37.9 %), medicinal (37.4 %) and nutritional (36.7 %) properties and to be considered a healthy product (36.7 %), were also selected reasons by the participants. Furthermore, the visual appearance (81.0 %) and taste (59.7 %) were the main characteristics valued in edible flowers. The odor (33.6 %), flower's species (14.2 %) and size (6.2 %) were properties less valorized by the participants of this survey

Consumers' knowledge and opinion about post-harvest technologies

Most of the participants thought that the shelf-life of flowers after harvest was up to 2 days (43.8%) and 5 days (43.8 %) (Table 5.1.1.2). However, almost all agreed (96.2 %) that post-harvest technologies can be used to increase the shelf-life of edible flowers. From the participants, most of them know refrigeration (77.0 %), freezing (61.4 %), crystallization (44.5 %) and hot air dehydration (39.8 %). On contrary, participants' awareness about radiation (16.4 %), edible coating (15.9 %), HHP (14.5 %) and ohmic heating (6.2 %) was low; most of them probably are 'unfamiliar' with these new post-harvest technologies. So, when asked "Which are the post-harvest technologies that can be applied to increase shelf-life of edible flowers?", most of the participants reported those they knew best, such as refrigeration (57.0%), freezing (40.5 %), crystallization (36.5 %) and hot air dehydration (32.3 %). Most of the participants showed willingness to taste flowers treated with post-harvest technologies (88.4 %) and pay more for flowers with a longer shelf-life (80.3 %) (Table 5.1.1.2). However, the added-price that they would be willing to pay were 1 euro more (35.1 %), 3 euros more (33.4 %) and 5 euros more (11.8 %).

Table 5.1.1.2 – Participants' answers to the online survey about edible flowers and post-harvest technologies

Variables	n (%)
n	422
Recognize flowers as food	
Yes	399 (94.5%)
No	23 (5.5%)
Opinion on edible flowers	
Positive	310 (73.5%)
Neutral	109 (25.8%)
Negative	3 (0.7%)
Tasted flowers before	
Yes	228 (54%)
No	194 (46%)
Why did not taste (n=194)	
It is not easily find for sale	119 (61.3%)
Don´t know this product	33 (17.0%)
Don't like it	17 (8.8%)
Expensive	3 (1.5%)
Others reasons	22 (11.3%)
Have not tasted, but would like to try (n=194)	
Yes	180 (93.8%)
No	12 (6.3%)
Place where they had tasted edible flowers (n=230)*	
Restaurant	135 (58.7%)
Home	83 (36.1%)
Events (ex: party, conference)	61 (26.5%)
Bakery	21 (9.1%)
Others (work, school, field)	10 (2.4%)
How was the flower tasted (n=229)*	
Fresh	204 (89.1%)
Dried	57 (24.9%)
Crystallized	39 (17.0%)
Preserved with syrup	1 (0.4%)
Dishes where they had tasted edible flowers (n=228)*	
Salads	130 (57.0%)
Desserts	92 (40.4%)
Appetizers	84 (36.8%)
Meat dishes	73 (32.0%)
Drinks	44 (19.3%)
Fish dishes	40 (17.5%)

Alone	32 (14.0%)
Others (soups, cereals)	6 (2.6%)
Words that they associate to edible flowers (n=422)	
Attractive/appealing	228(54.0%)
Decoration	217(51.4%)
Colorful	211 (50.0%)
Innovative	202(47.9%)
Exotic	198(46.9%)
Natural	167(39.6%)
Healthy	158(37.4%)
Smelling	122 (28.9%)
Vegetable	97 (23.0%)
Strange	29 (6.9%)
Toxic	6 (1.4 %)
Repulsion	3 (0.7%)
Others	3 (0.7%)
Reason to eat edible flowers (n= 422)	
Decoration	208 (49.3%)
Organoleptic characteristics (e.g. flavor, taste)	160 (37.9%)
Medical properties	158 (37.4%)
Nutritional properties	155 (36.7%)
Be a healthy product	155 (36.7%)
Antioxidants properties	141(33.4%)
New trend, fashion	110 (26.1%)
Others	6 (1.4 %)
Characteristics valued in edible flowers (n=422)	
Visual appearance	342 (81.0%)
Taste	252 (59.7%)
Odor	142 (33.6%)
Flower's specie	60 (14.2%)
Size flower	26 (6.2%)
Post- harvest	
Opinion on the shelf-life of flowers (n=422)	
Until 2 days	185 (43.8%)
3 to 5 days	185 (43.8%)
6 to 7 days	38 (9%)
8 to 10 days	8 (1.9%)
11 to 15 days	3 (0.7%)
Up to 16 days	3 (0.7%)
Post-harvest technologies can increase shelf-life (n=422)	
Yes	406 (96.2%)
No	16 (3.8%)
Willingness to try flowers after application of post-harvest technologies (n=422)	
Yes	373 (88.4 %)
No	42 (10.0%)

Without answer	7 (1.6%)	
Post-harvest technologies	They know (n=422)	Can be used to increase the shelf-life of flowers (n=400)
Refrigeration	325 (77.0%)	228 (57.0%)
Freezing	259 (61.4%)	162 (40.5%)
Crystallization	188 (44.5%)	146 (36.5%)
Hot air dehydration	168 (39.8%)	129 (32.3%)
Freeze-drying	128 (30.3%)	96 (24.0%)
Osmotic dehydration	108 (25.6%)	76 (19.0%)
Radiation	69 (16.4%)	21 (5.3%)
Edible coating	67 (15.9%)	61 (15.3%)
HHP	61 (14.5%)	46 (11.5%)
Ohmic heating	26 (6.2%)	7 (1.8%)
Didn't know	---	4 (1.0%)
Others	---	3 (0.8%)
Added-price that they will be willing to pay (n=422)		
More 1€	148 (35.1%)	
More 3€	141 (33.4%)	
≥ 5€	50 (11.8%)	
I would not pay more	83 (19.7%)	

* The number of respondents was 228, but it was a question where multiple choices were allowable.

Relationships between demographic characteristics and attitudes/knowledge on edible flowers/post-harvest technologies

The participants' responses were divided into groups to test the existence of significant differences in edible flowers and post-harvest attitudes and knowledge based on gender, age, educational level and geographical origin. Concerning the topic "Have already tasted edible flowers?", no significant differences between gender, educational level and geographical origin were detected (p>0.05). However, it was found a statistically significant difference (p-value <0.01) with age. Younger participants (<30 years old) have tasted less edible flowers than the other age groups.

Concerning the questions "Can post-harvest technologies increase the shelf-life of flowers?" and "Are you willing to pay added-price for edible flowers with high shelf-life?", no significant differences between all demographic characteristics were detected (p>0.05).

Regarding knowledge on post-harvest technologies, statistically significant differences between educational level and age were detected (p<0.01 in both cases). On contrary, most of the participants knew one or two post-harvest technologies, independently if they were male or female. On the other hand, participants with ≤30 years old knew

more post-harvest technologies than the others age groups (Table 5.1.1.3). However, most of the participants, independently of the age group, answered that they knew one or two post-harvest technologies. Graduated participants (with master, doctorate and post-doctorate degrees) knew a higher number of post-harvest technologies than people with basic degree (Table 5.1.1.3).

Sensory analysis

Consumers' and gourmet chefs' opinion on edible flowers

Consumers and gourmet chefs were asked about their purchasing habits of edible flowers (Table 5.1.1.4). Most of the interviewed chefs buy edible flowers and they like more to purchase fresh flowers. On contrary, most of the consumers (93.3 %) reported that they do not buy edible flowers because they didn´t know that some of them were edible (33.3 %), and 53.3 % of the consumers reported that it is a product not easy to find. Identical factors were previously mentioned in the online survey for never having tasted flowers. So, probably some consumers don't use or ever tried to buy edible flowers because most grocery or specialty food stores don't have this kind of product. Only one consumer told that he/she didn't like this kind of product, and other that it was expensive. So, differences in the use of edible flowers were observed between consumers and gourmet chefs. However, most of the consumers didn't express a negative opinion about consuming edible flowers as food. They did not mention, for example, that they don't like them or reported other depreciative characteristics.

Table 5.1.1.3 – Effects of gender, education, age and geographical origin on consumers' responses about tasted flowers, post-harvest technologies that can increase shelf-life, knowledge about post-harvest technologies and added-price that they will be willing to pay.

Variables	Tasted flowers before			Post-harvest technologies can increase shelf-life			Knowledge about post-harvest technologies Nº of technologies that were mentioned						Added-price that they will be willing to pay				
	Yes	No	Total	Yes	No	Total	1	2	3	4	≥5	Total	More 1€	More 3€	≥ 5€	I would not pay more	Total
Gender																	
Female	176	156	332	319	12	331	94	67	56	51	64	322	112	110	40	70	332
Male	52	38	90	85	6	91	21	21	17	8	23	90	36	31	10	13	90
Total	228	194	422	404	18	422	115	88	73	59	87	422	148	141	50	83	422
Education																	
College/technical degree	63	45	108	104	4	108	43	30	16	12	7	108	40	30	14	24	108
Graduate	165	149	314	302	12	314	72	58	57	47	80	314	108	111	36	59	314
Total	228	194	422	406	16	422	115	88	73	59	87	422	148	141	50	83	422
Age																	
<30	79	94	173	168	5	173	35	34	23	29	52	173	61	63	23	24	173
31-40	82	64	146	142	4	146	36	39	28	19	24	146	53	46	14	33	146
41-50	32	32	64	58	6	64	29	8	16	4	7	64	23	22	4	15	64
>50	35	4	38	38	1	39	15	7	6	7	4	39	9	10	9	11	39
Total	228	194	422	406	16	422	115	88	73	59	87	422	148	141	50	83	422
Geographical Origin																	
North of Portugal	140	122	262	252	10	262	63	53	43	37	66	262	90	90	32	50	262
Centre of Portugal	36	31	67	64	3	67	21	13	15	12	6	67	23	22	5	17	67
South of Portugal	17	15	32	31	1	32	12	6	9	3	2	32	15	9	2	6	32
Islands (Azores and Madeira)	5	1	6	6	0	6	3	1	0	1	1	6	0	2	0	4	6
Other countries	28	27	55	53	2	55	16	15	6	6	12	55	20	18	11	6	55
Total	226	196	422	406	16	422	115	88	73	59	87	422	148	141	50	83	422

Table 5.1.1.4 – Responses given by gourmet chefs and consumers in relation to their habits on edible flowers' use

Variable (n)	Chefs		Consumers	
Have bought edible flowers before	Yes 7	No 1	Yes 2	No 28
If Yes				
Fresh	6		2	
Dry	2		-	
Crystallized	1		-	
If No				
Don't know		-		10
Don't like		-		1
Difficult to find		-		16
Expensive		-		1
Others		1		-

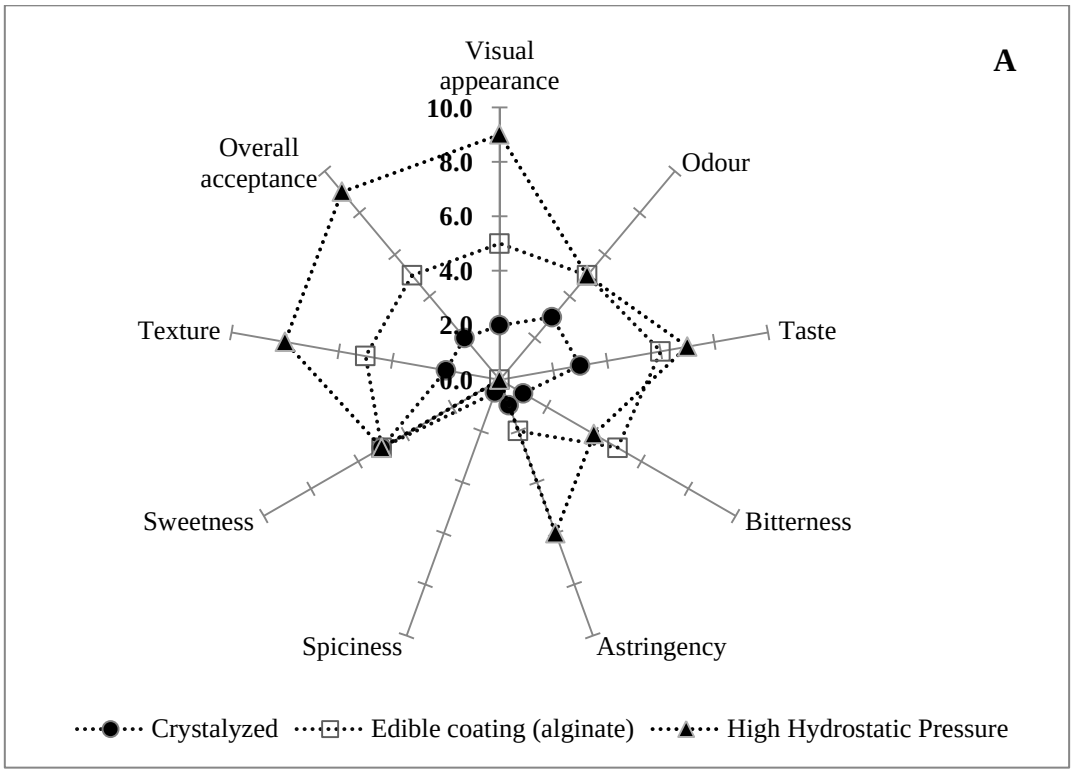
Sensory profile of the gourmet chefs

Figure 5.1.1.1.A shows the sensory profiles for pansies subjected to high hydrostatic pressure, alginate coating and crystalization of the gourmet chefs. Comparing treatments, high hydrostatic pressure was the treatment with the highest scores, followed by alginate coating and crystallization. Regarding visual appearance (a key factor for the successful acceptance of edible flowers), the pansies were rated high (9) when subjected to HHP. Texture and taste also showed high rates (8 and 7, respectively) for HHP pansies, followed by alginate coating. In terms of bitterness, astringency, spiciness, sweetness and odour, most of the chefs mentioned that these properties were quite difficult to detect, giving some of them the classification of zero. So, these attributes were classified with low rates for all treatments. Probably, it may be attributed to the light flavor and taste intensity of fresh flowers, as reported by Benvenuti et al. (2006). Furthermore, the applied treatments do not promote new tastes and flavors to pansies. Concerning flowers' overall acceptance, pansies subjected to HHP (9) had good acceptance by chefs, while alginate and crystalized had low scores, so low acceptance. Concerning the chefs' comments, they referred that the sugar masks the color and the appearance of the flower, as well as, they mentioned that the most prominently flavor was the sweetness of the sugar in the crystallized pansies. Thus, they classified crystallized pansies with low scores. Regarding alginate coated pansies, chefs mentioned that it was visible a bright layer on the flower, a negative attribute for them. On contrary, pansies subjected to HHP treatment seemed to be a new product with

potential to be used in the cuisine by chefs. Chefs referred that pansies treated with HHP looked like the fresh ones, with the same color intensity, texture and visual appearance.

Sensory profile of the consumers

Figure 5.1.1.1.B shows the sensory profiles of pansies subjected to the three post-harvest treatments by consumers. There wasn't a great difference between treatments in relation to visual appearance, texture, odour, taste and overall acceptance. Consumers classified the visual appearance of pansies submitted to the three treatments with "likely moderately and extremely". In more detail, pansies subjected to HHP showed higher values on visual appearance (9), bitterness (5), astringency (5) and texture (8) than other treatments. As expected, crystallized pansies were high rated in sweetness (8) because probably the consumers like the taste of sugar. Spiciness wasn't detected by the consumers in all treatments, because probably pansies don´t have spicy taste as reported by Benvenuti et al. (2016). Furthermore, most of the consumers in the final comments reported that flowers were not very fragrant and had a light taste. In general terms, consumers evaluated the pansies subjected to all treatments with high rates of overall acceptance (≥7), although alginate coated and HHP were distinguished from crystallized ones.



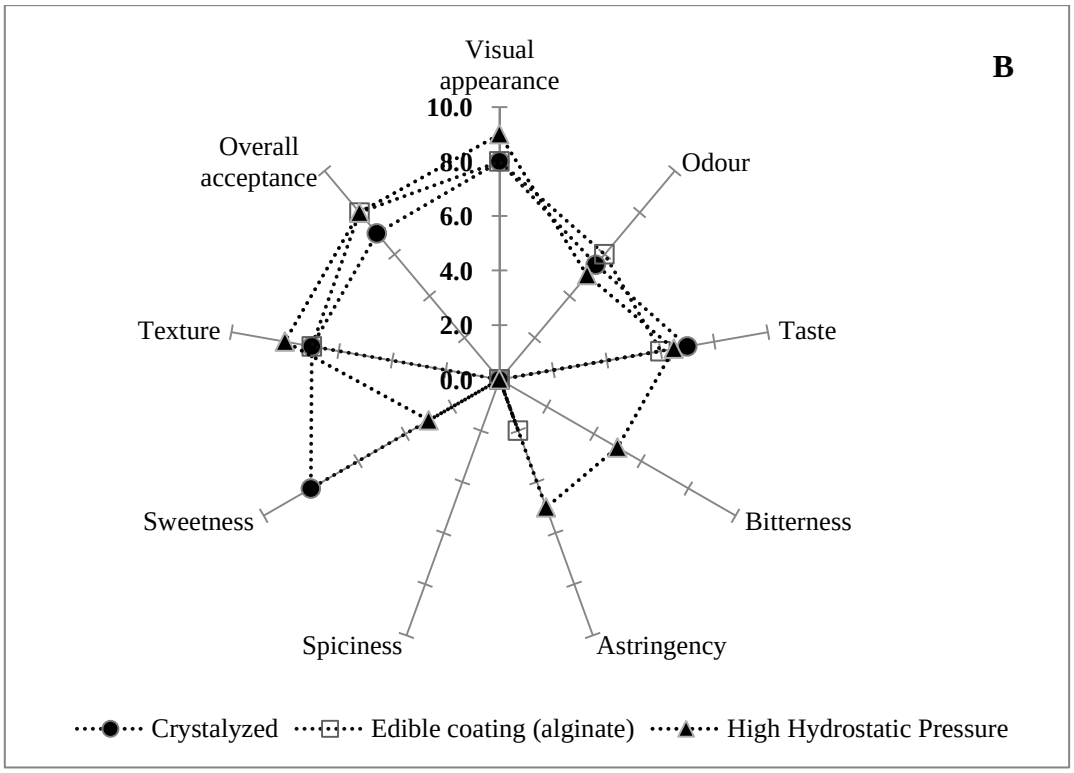


Figure 5.1.1.1 - Sensory profiles (visual appearance, odor, taste, bitterness, astringency, spiciness, sweetness, texture and overall acceptance) of the gourmet chefs (A) and consumers (B) for pansies subjected to the three post-harvest technologies.

Comparison between the sensory profiles of gourmet chefs and consumers

Figure 5.1.1.2 summarizes the results of the hedonic evaluations performed by chefs and consumers for each post-harvest treatment. Concerning crystallized pansies (Figure 5.1.1.2A), a great difference between consumers and chefs was detected. The gourmet chefs classified all attributes of crystallized pansies with low values, while consumers ranked them with high marks, except for spiciness, astringency and bitterness. Regarding alginate coated pansies, both groups classified odour and taste with similar values (Figure 5.1.1.2B). However, consumers reported higher values of texture, visual appearance and overall acceptance than chefs. Differences on consumers and chefs preferences were also detected for viola and borage flowers (Kelley et al., 2001). On contrary, chefs and consumers gave similar scores to pansies treated with HHP (Figure 5.1.1.2C). These similarities in ratings may indicate that no separate marketing strategy is necessary for pansies treated with HHP.

Significant correlations were detected between the overall acceptance with visual appearance, odor, texture and taste for both groups. These correlations were higher for chefs than consumers. For chefs, those correlations were 0.929 for visual appearance, 0.925 for texture, and 0.818 for taste. For consumers, the correlations were 0.720 for visual appearance, 0.460 for odour and 0.782 for taste**.**

Consumers' and chefs' purchasing intentions to buy post-harvest treated pansies

Opinions of chefs and consumers on their intention to buy post-harvest treated pansies, what price would they pay and which will be the more suitable dishes to include pansies subjected to the three different post-harvest technologies, are described in Table 5.1.1.5. All chefs would purchase HHP pansies and 66.7 % of them would pay ≤5€ for a package with 12 flowers. Regarding other post-harvest treatments, only 50 % of chefs showed intention to buy alginate coated pansies, and 37.5 % crystallized pansies. On contrary, more than 60% of consumers would be willing to buy flowers subjected to the three treatments. However, most of the consumers would buy the treated flowers at a price lower than 5 euros. Only a small percentage reported highest price values (>10-15 euros). Concerning the price of crystallized flowers, consumers and chefs were willing to pay lower values than those practiced in the market (ex. a Portuguese enterprise sells a container with 12 flowers for 26.90€, and other enterprise sells a package of 20 flowers for 22.7€) (Ervas Finas®, 2018; Eat My Flowers®, 2018).

There was a significant correlation between the decision of purchasing with the visual appearance and overall acceptance for the consumers group. The relationship between purchase and visual appearance for treated pansies was 0.419 and for overall acceptance 0.338 (p=0.01). This indicated that consumer ratings of visual appearance of flowers were strongly related to their willingness to purchase the flowers. Our results were similar to those reported by Kelley et al. (2001, 2002), who detected that the visual aspect has been indicated as an important factor for the successful use of edible flowers. Furthermore, it was the most valorized characteristic of edible flowers according to the participants of the online survey.

When asked both groups about the more suitable dishes to include treated flowers, a variety of responses were given (Table 5.1.1.5). Most of the consumers and chefs reported that crystallized flowers should be included in desserts or eaten alone. On the other hand, for chefs, alginate coated pansies should be included in fish dishes (4), while for consumers in salads (12) and desserts (9). Concerning, HHP flowers, chefs

preferred fish dishes (6) and desserts (6), and consumers salads (9), drinks (9) and desserts (9).

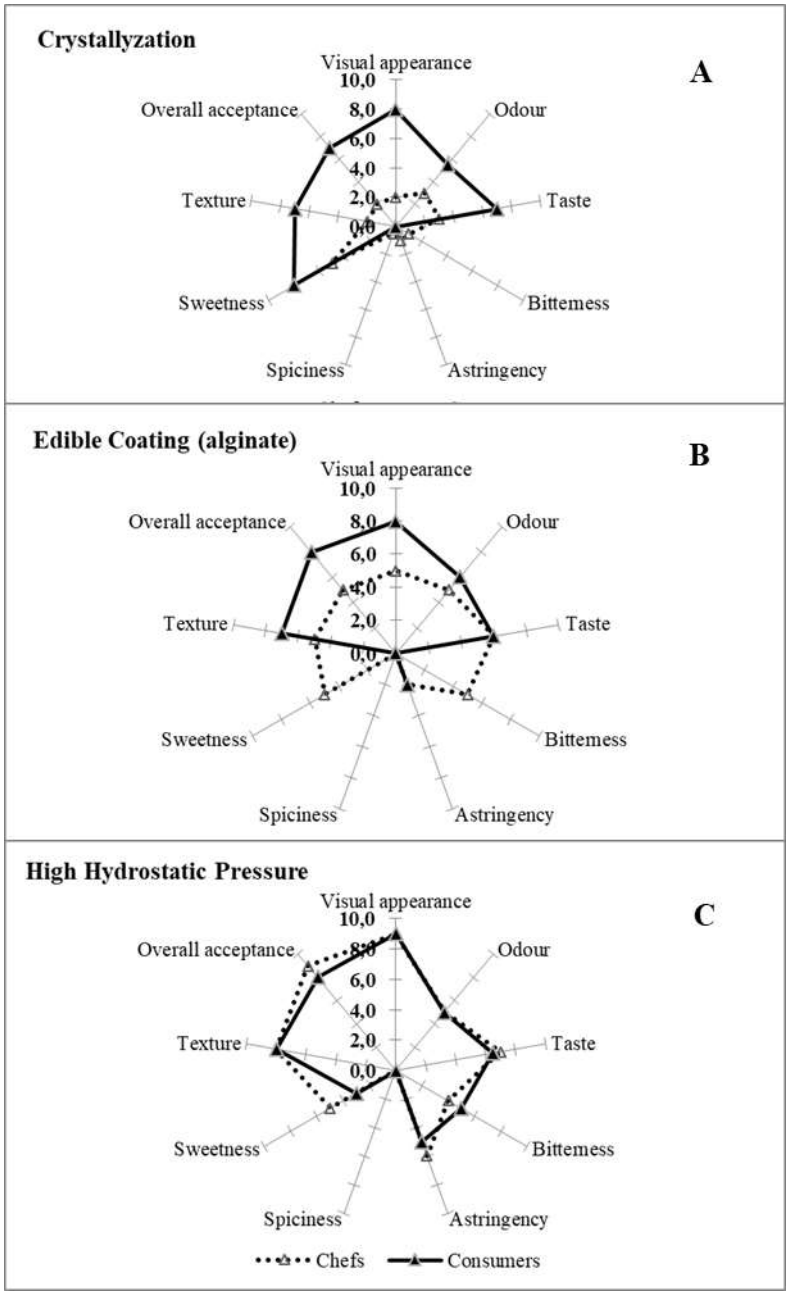


Figure 5.1.1.2 - Sensory perceptions of chefs and consumers for pansies subjected to HHP (A), alginate coating (B) and crystallized (C) (medians).

Table 5.1.1.5 - Opinions of chefs and consumers about their intention to buy, what price would they pay and which will be the more suitable dishes to be included pansies subjected to the three different post-harvest technologies.

Frequencies (%)		Crystallized		Alginate coating		HHP	
		Chefs	Consumers	Chefs	Consumers	Chefs	Consumers
Would buy	Yes	37.5	61.5	50	77.3	100	66.7
	No	62.5	38.5	50	22.7	0	33.3
Price (€)	≤5	66.7	50	50	52.9	75	66.7
	>5-10	33.3	37.5	50	41.2	25	25
	>10-15	-	12.5	-	5.9	-	8.3
Dishes to include (n):							
Salads		1	4	2	12	5	9
Entries		-	8	2	7	4	7
Fish dishes		-	2	4	4	6	2
Meat dishes		-	1	2	2	5	1
Drinks		-	9	1	6	2	9
Desserts		3	14	2	9	6	9
Alone		2	2	-	-	3	-
Other		-	-	-	-	2	-

Conclusion

In summary, most of the participants of the online survey recognized edible flowers as a food component and expressed a positive opinion about them. However, only half of the inquired people had already tasted edible flowers. Furthermore, consumers recognized the importance of post-harvest technologies. Refrigeration, freezing and crystallization were the processes most known and mentioned by the participants. Moreover, more than 79 % were willing to pay more for edible flowers with longer shelf-life. In regard to sensory profiles of gourmet chefs and consumers, these were different: chefs and consumers highlighted the organoleptic properties of pansies treated with HHP. However, in relation to alginate coated and crystallized pansies, differences between both groups were detected. Consumers rated the alginate coated and crystalized pansies with high scores, while chefs rated these pansies with intermediary and low values, respectively. All chefs indicated that they would purchase the pansies treated with HHP, but only 50% would buy the alginate coated and 37.8% the crystallized pansies. On contrary, at least 60% of the consumers reported that they would buy the flowers subjected to the three treatments. In summary, the results obtained in the present study are very useful for the commercialization and marketing of pansies treated with different post-harvest technologies, showing that they might be purchased by consumers

and not only by the gourmet chefs. Furthermore, the first ones liked the pansies subjected to the three methodologies evaluated.

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CHAPTER 6

6.1. General discussion

The consumption of edible flowers has increased in less traditional countries; however, it is a very perishable product, whose food quality and safety need to be guaranteed. Until this moment, edible flowers are usually sold fresh or dried by hot air, being the development of new products, most similar to fresh flowers and with a longer shelf-life, a challenge.

When the following edible flowers, camellia (*Camellia japonica* L.), pansies (*Viola* × *wittrockiana* Gams), borage (*Borago officinalis* L.) and centaurea (*Centaurea cyanus* L.) were physico-chemical and biological characterized, the results obtained allowed to distinguish them from the nutritional point of view (macronutrients and caloric value) and chemical (sugars, organic acids, fatty acids, tocols and carotenoids). After determining the volatile and bioactive compounds profiles, as well as after performing sensory analysis to borage, calendula (*Calendula arvensis* L.), cosmos (*Cosmos bipinnatus* Cav.), Viola (*Viola tricolor* L.) and pansies, relationships were established between these parameters. The results of the sensory analysis indicated that the flowers presented different floral, fruity and herbal odors and taste. One hundred and seventeen (117) volatile compounds were detected, being esters the most abundant in borage, sesquiterpenes in calendula, and terpenes in cosmos, Johnny Jump up and pansies. Regarding bioactive compounds, the highest values of total phenols and flavonoids were determined in pansies, while hydrolysable tannins and monomeric anthocyanins were in cosmos, having calendula the lowest values. Furthermore, some relationships were found between volatiles and bioactive compounds with the sensory perception of the flowers.

Concerning the application of the following post-harvest technologies: high hydrostatic pressures (HHP), dehydration (osmotic dehydration (OD), crystallization, freeze drying, hot air convective drying and drying at room temperature (shade)), edible coatings and freezing, to different edible flowers species, according to their seasonal availability, the following general results were obtained. When applying HHP technology to borage and camellia, these presented unacceptable appearance after all treatments. On contrary, centaurea showed good appearance at 100/5 MPa/min; however, the shelf life didn’t

increase. Pansies treated at 75/5 and 75/10 MPa/min also retained the appearance of fresh flowers. Furthermore, pansies submitted at 75/5 and 75/10 MPa/min maintained good appearance over more 14 days of storage at 4 °C, when compared to the untreated. Even though no significant differences on microbial load were observed between fresh and HHP treated pansies (after treatment), HHP induced the production of bioactive compounds.

Regarding dehydration, this was applied to centaurea and pansies. The effect of hot air convective, shade and freeze drying, on bioactive compounds and antioxidant activity of centaurea petals, as well as on some physico-chemical properties, was evaluated. All dried samples had different appearance to the fresh ones, with lower titratable acidity, carotenoids and hydrolysable tannins contents, but higher antioxidant activity. Among the drying methods, shade dried petals presented the highest values of monomeric anthocyanins, flavonoids, hydrolysable tannins, total reducing capacity and antioxidant activity. On the other hand, higher losses were observed when hot-air convective drying was applied. Thus, shade drying is a very promising process that should be considered as a suitable drying method for centaurea petals. When applying OD and crystallization to pansies, the results indicated that this flower presents a superhydrophobic surface structure, called papillae, which increased the resistance to exchanges with hypertonic solutions. So, no weight loss was observed after most treatments, high values of a_w were observed and the monomeric anthocyanins were preserved. When applying more drastic conditions, as sodium chloride for more than 1 hour, undesirable textural and color changes were detected. Thus, the immersion of pansies in osmotic solutions had an undesired effect, due to the morphological structure of pansies' flowers epidermis. Concerning crystallization, crystallized pansies still kept good visual appearance after 90 days of storage. However, a significant decrease in water activity, moisture, ash and protein contents was observed. Furthermore, a significant decrease in flavonoids and monomeric anthocyanins was detected, while hydrolysable tannins increased. On contrary, the phenolic compounds maintained constant along storage. The microbial load of crystallized pansies during all storage time was lower than fresh ones. Thus, crystallization can be an effective method for pansies preservation, being some nutritional and bioactive compounds, little affected.

The application of low temperatures was also tested, namely in borage, viola, kalanchoe (*Kalanchoe blossfeldiana* Poelln.) and dandelion (*Taraxacum officinale* Wiggers), which were subjected to freezing (in their natural form and in ice cubes). Flowers in ice

cubes retained a similar appearance to fresh ones during 3 months of storage, whereas frozen flowers were only identical up to 1 month. The flowers that were frozen in their natural form maintained or even increased the content of bioactive compounds and antioxidant activity when compared to fresh. On contrary, flowers in ice cubes showed a slight decrease in the values of bioactive compounds and antioxidant activity. Furthermore, both freezing treatments guaranteed the microbial quality.

Concerning edible coatings, alginate coating was tested in pansies. This treatment resulted in a beneficial effect on the visual appearance of pansies under refrigerated storage when compared to uncoated, delaying their degradation from 3-4 days up to 14 days, depending on pansies' color. Then, packaging plus alginate coating was tested. In this case, unpackaged coated pansies presented different behaviors during storage, associated with their dimensions. Larger coated pansies maintained good visual appearance during longer storage times than smaller ones. However, packaged pansies submitted previously to alginate coating, showed to have lower weight losses than unpackaged, independently to the color and size, showing physical stability up to 14 days, contributing to extend pansies shelf life. Afterwards, the effect of alginate coating on physico-chemical and microbiological quality of pansies during cold storage (4 ºC for 0, 7, 14, 21 days) was analyzed. Bioactivity and antioxidant potential were higher in coated pansies than uncoated ones, on all assayed storage times. Furthermore, after 14 days of storage, alginate coated pansies presented microorganism counts lower than uncoated. So, alginate coating has potential for extending shelf-life and improving physico-chemical and microbiological quality of pansies.

Based on the results obtained, the effectiveness of combining post-harvest treatments was tested, namely: HHP as pre-treatment to OD, and the simultaneous application of HHP and OD (HHP+OD). The best results were obtained when it was applied HHP (75 MPa/5 or 10 min) as a pre-treatment to OD (80%, w/v). The treated flowers presented similar appearance to fresh ones and water activity values lower than 0.7. On contrary, simultaneous application of HHP+OD resulted on more fragile pansies, with texture loss.

To borage flowers were applied different post-harvest technologies, such as drying methods (freeze-drying, hot air convective drying) and edible coatings. Hot air-dried samples showed unsatisfactory visual appearance. Freeze dried flowers were less shrunken, showing the lowest a_w. On the other hand, alginate coated flowers showed good visual appearance, like fresh flowers, that was maintained during refrigerated

storage (until 5 days), four more days than uncoated ones. Nevertheless, flowers became fragile and it was difficult to handle them without causing further damages.

Finally, the general population's perceptions and knowledge about edible flowers and post-harvest technologies were investigated through an online survey. Subsequently, some gourmet chefs and consumers sensory evaluated some flowers subjected to different post-harvest technologies. Concerning the online survey, most of the participants recognized edible flowers as a food component and had a positive opinion about them. However, only half of the participants had already tasted them at restaurants or at home. Refrigeration, freezing and crystallization were the post-harvest technologies most known and mentioned by the respondents to be able to increase edible flowers' shelf-life. Regarding sensory analyses, the gourmet chefs and consumers high rated the flowers subjected to HHP. However, consumers also high rated the alginate coated and crystallized pansies, while the gourmet chefs rated them with lower values. 60% of the consumers would be willing to buy flowers subjected to the three treatments. On contrary, the gourmet chefs would purchase more the HHP treated flowers. Furthermore, consumers showed more willingness to pay more for treated flowers than chefs.

6.2. Conclusions and future work

In this PhD thesis, the influence of diverse post-harvest technologies on edible flowers physico-chemical, biological and sensory characteristics and shelf-life, were studied, in order to increase the fundamental knowledge about the effect of these technologies on edible flowers, contributing to an possible future use of these technologies by edible flowers producers.

After analyzing several edible flowers, water was the main constituent, while other components, such as, macronutrients (proteins, fiber, carbohydrates), bioactive compounds (carotenoids, fatty acids, vitamin E, flavonoids, anthocyanins), volatile compounds and free sugars were also detected, but in less amounts. Different flower species and at distinct flowering stages showed different profiles of these compounds. Furthermore, it was stated that some bioactive and volatile compounds influenced the sensory perception.

Concerning the effect of post-harvest technologies on edible flowers, it was verified that each flower species showed a different behavior, namely:

- HHP showed to be a promising technology to increase the shelf-life of pansies (more 14 days than fresh), when flowers were treated with low pressure and short holding times (75 MPa at 5 or 10 min). On the other hand, borage and camellia were more susceptible to HHP treatment, resulting in an unacceptable final product after all treatments. Centaurea showed good appearance at 100/5 MPa/min, but the shelf life didn't increase;
- Shade drying showed to be a highly promising process that should be considered as a suitable drying method for centaurea petals. Shade dried petals presented higher contents of monomeric anthocyanins, flavonoids, hydrolysable tannins, total reducing capacity and antioxidant activity when compared with other drying methods (hot-air convective and freeze drying);
- Osmotic dehydration was a technology that had an undesirable effect on pansies due to the morphological structure of pansies' flowers epidermis. In more detail, the presence of an hydrophobic structure increased the resistance to exchanges with hypertonic solutions. So, osmotic dehydration didn´t occur in pansies even when more drastic conditions were applied, where undesirable textural and color changes were observed;

- Crystallization applied to pansies showed to be an effective preservation method, being some nutritional and bioactive compounds little affected, during storage. Furthermore, the microbial load of crystallized pansies during all storage time was lower than fresh ones.
- Freezing treatments (in their natural form and in ice cubes) applied to borage, violet heartsease, kalachoe and dandelion showed to allow keeping these flowers for a longer period of time. In general, flowers in ice cubes showed similar appearance to fresh ones during three months of storage, whereas frozen flowers were only equivalent up to one month. Furthermore, the bioactive compounds were little affected and the microorganisms' counts decreased or maintained when compared to fresh samples. However, some differences were detected between flower species;
- Alginate coating applied to unpackaged pansies showed different effects according to the size and color of the flowers, probably derived from their different petal thicknesses. However, alginate coating combined with packaging gave cumulative protection to pansies, being a very interesting solution to prolong pansies' shelf life (7 days more than fresh), independently their color and size. Furthermore, alginate coated pansies maintained their physico-chemical and microbiological qualities, showing the potential of this technology;
- Combination of HHP+OD to pansies resulted on more fragile flowers and texture loss. However, the application of HHP as pretreatment to OD may be a promising post-harvest technology for pansies;
- Freeze drying showed to be a potential method to produce dried borage flowers for infusions, while alginate coating was a treatment that can increase their shelf-life but the flowers were fragile.

Since, pansies were the flowers with more good results in some post-harvest technologies, the sensory characteristics of treated flowers by different methods were evaluated by gourmet chefs and consumers. It was observed that sensory profiles of chefs and consumers were different. Chefs liked more the HHP treated pansies than alginate coated and crystallized ones. Consumers also liked more the HHP treated pansies, but they also high rated the alginate coated and crystallized pansies. So, pansies treated with different post-harvest technologies, showed to have high possibility to be purchased by consumers and not only by the gourmet chefs. Furthermore, consumers and chefs are prone to buy these new products.

As a general conclusion, the results obtained in this thesis revealed that some post-harvest technologies can be applied without compromising the physico-chemical characteristics of edible flowers and to improve their shelf-life. However, each flower had a different behavior after application of those new technologies. So, it was concluded that technologies, such as HHP, alginate coating, crystallization and ice cubes, can help to extend the marketing period of some edible flowers extending it to distant markets other than local markets, thereby benefiting the flowers' producers.

From the results obtained in the present work, the following future studies can be proposed:

i) Extend the application of some of the post-harvest technologies tested in this thesis in other edible flowers;

ii) Evaluate the effect of temperature abuse (15 ºC) during storage on physico-chemical and microbiological properties of edible flowers treated with the applied post-harvest technologies;

iii) Study the antimicrobial activity against some bacteria, such as: *Staphylococcus aureus*, *Listeria* sp., *Bacillus cereus*, *Salmonella* sp. after application of post-harvest technologies;

iv) Test other combinations between post-harvest technologies in order to obtain possible synergies;

v) Test other post-harvest technologies (e.g. modified atmosphere packaging, other edible coatings, UV radiation, pulsed light);

vi) Evaluate the commercial acceptance of these new products;

vii) Publicize and promote the commercialization of these foods in gourmet restaurants and food stores;

viii) Promote more studies on the nutraceutical potencial of edible flowers.

ANNEXES

Annex I: Sensory analysis

Name:	
Code:	Date__/__/__

1. Mark with a cross the perceptions of the parameters perceived in the flower received:

Visual appearance		**Odour**	
Scale		**Scale**	
1- Disliked extremely		1- Disliked extremely	
2- Disliked moderately		2- Disliked moderately	
3- Disliked regularly		3- Disliked regularly	
4- Disliked slightly		4- Disliked slightly	
5- I did not like it or dislike it		5- I did not like it or dislike it	
6- Liked slightly		6- Liked slightly	
7- Liked regularly		7- Liked regularly	
8- Liked moderately		8- Liked moderately	
9- Liked extremely		9- Liked extremely	
		Not felt	

Taste (in general)		**Bitter**:	
Scale		**Scale**	
1- Disliked extremely		1- Disliked extremely	
2- Disliked moderately		2- Disliked moderately	
3- Disliked regularly		3- Disliked regularly	
4- Disliked slightly		4- Disliked slightly	
5- I did not like it or dislike it		5- I did not like it or dislike it	
6- Liked slightly		6- Liked slightly	
7- Liked regularly		7- Liked regularly	
8- Liked moderately		8- Liked moderately	
9- Liked extremely		9- Liked extremely	
		Not felt	

Astringency		**Spiciness**	
Scale		**Scale**	
1- Disliked extremely		1- Disliked extremely	
2- Disliked moderately		2- Disliked moderately	
3- Disliked regularly		3- Disliked regularly	
4- Disliked slightly		4- Disliked slightly	
5- I did not like it or dislike it		5- I did not like it or dislike it	

6- Liked slightly		6- Liked slightly	
7- Liked regularly		7- Liked regularly	
8- Liked moderately		8- Liked moderately	
9- Liked extremely		9- Liked extremely	
Not felt		**Not felt**	

Sweetness		**Texture**	
Scale		**Scale**	
1- Disliked extremely		1- Disliked extremely	
2- Disliked moderately		2- Disliked moderately	
3- Disliked regularly		3- Disliked regularly	
4- Disliked slightly		4- Disliked slightly	
5- I did not like it or dislike it		5- I did not like it or dislike it	
6- Liked slightly		6- Liked slightly	
7- Liked regularly		7- Liked regularly	
8- Liked moderately		8- Liked moderately	
9- Liked extremely		9- Liked extremely	
Not felt			

Overall acceptance	
Scale	
1- Disliked extremely	
2- Disliked moderately	
3- Disliked regularly	
4- Disliked slightly	
5- I did not like it or dislike it	
6- Liked slightly	
7- Liked regularly	
8- Liked moderately	
9- Liked extremely	

Please, do not consider the flowers used to make infusions (teas).

2. Please, indicate if you have ever purchased edible flowers?

Yes ☐ No ☐

2.1. If you answered YES in the previous question, please indicate what kind of flowers you bought? After you have answered, go to point 3.

Fresh ☐

Dried ☐

Crystallized with sugar ☐

Other: ☐ Which? _____

2.2. If you answered NO, please state why you have never bought edible flowers:

I did not know the existence of this kind of product ☐

Do not like this type of product ☐

It is too expensive ☐

Can not easily find it for sale ☐

Another reason: ☐ Indicate which: _____

3. Please indicate if you would buy this product?

Yes ☐ No ☐

3.1. If yes, please indicate what price you would be willing to pay for a pack of 12 flowers?

☐ ≤5 € ☐ >5-10 € ☐ >10-15€ ☐ >15-20€ ☐ > 20-30€ ☐ >30-40€ ☐ > 40€

3.2. Please indicate where you would include this type of product:
(may include more than one option)

Salads ☐

Appetizer ☐

Fish dishes ☐

Meat dishes ☐

Drinks ☐

Desserts ☐

Consumed alone ☐

Others: ☐ Examples: _____

4. Please, describe what you liked and disliked more in this product.

Liked:

_____

Disliked:

_____

Annex II: Sensory analysis of pansies subjected to different post-harvest technologies

Name:	
Code:	Date__/__/__

Briefly describe the perceptions of the parameters felt in the flower received:

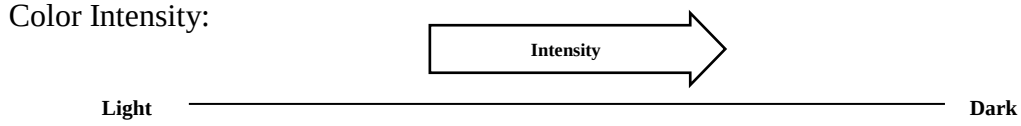
Visual appearance:

Indicate if the flower has:

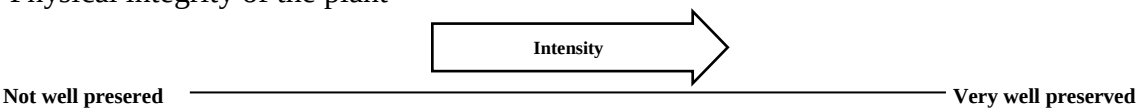
Simple Color: _____ Mixed color: _____

Any visual defect: _____

Color Intensity:

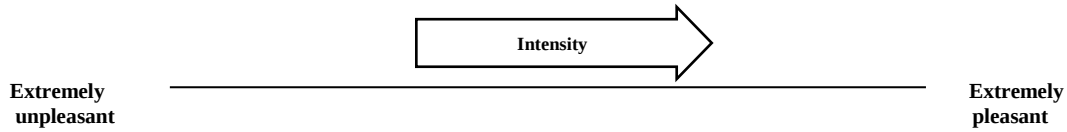


Physical integrity of the plant

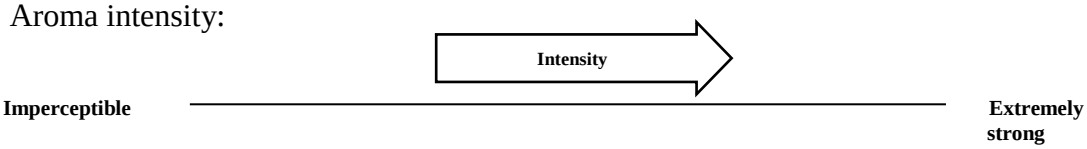


Odour:

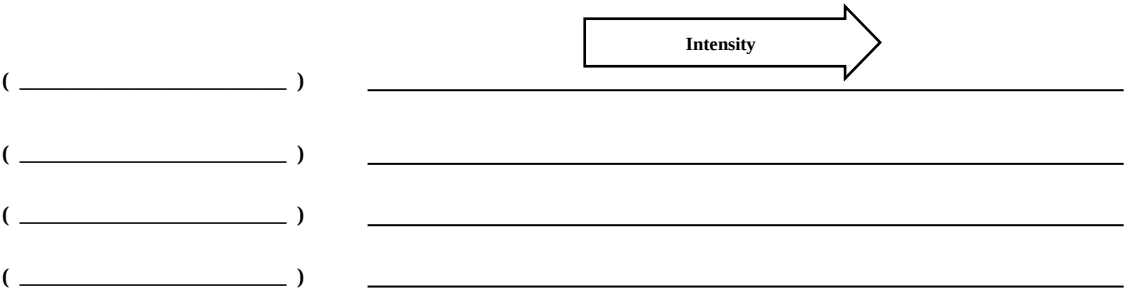
Aroma sensation:



Aroma intensity:

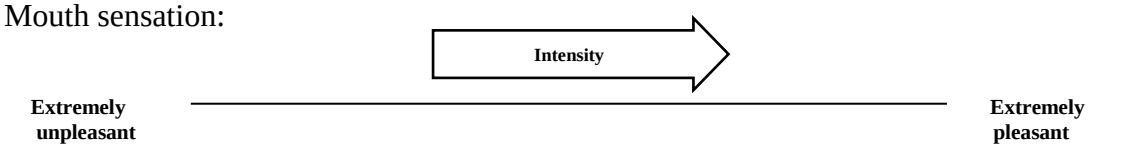


Detected flavours**:**

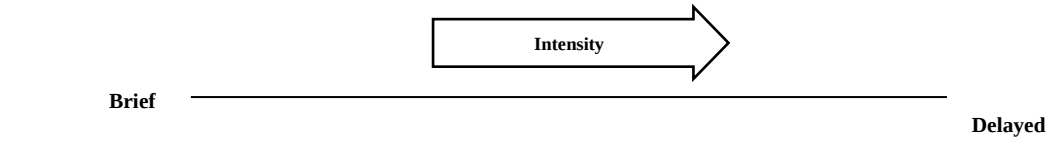


Taste:

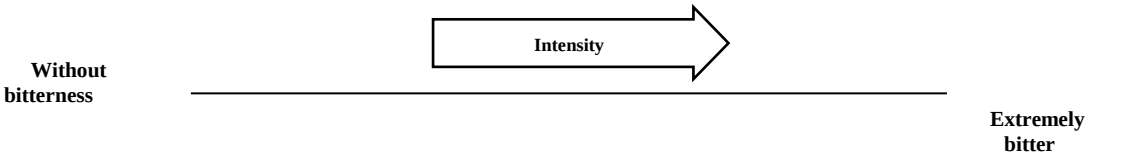
Mouth sensation:



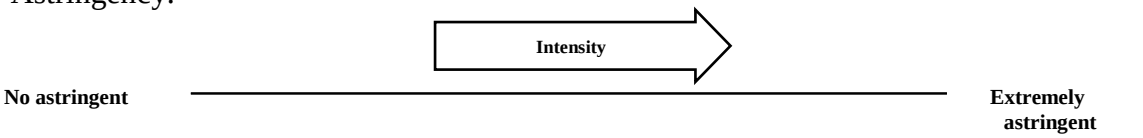
Persistence:



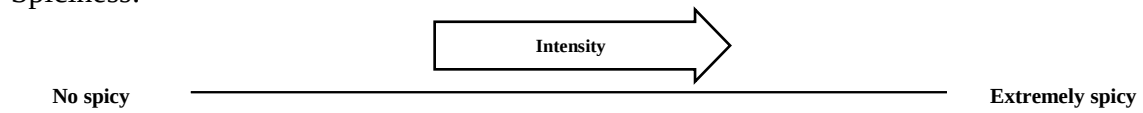
Bitterness:



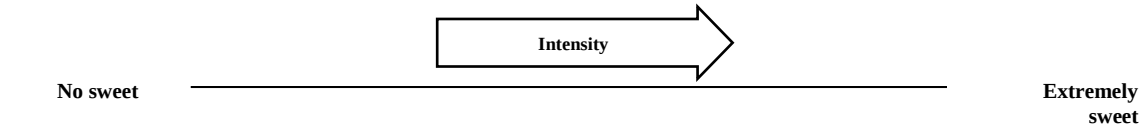
Astringency:



Spiciness:



Sweetness:



Taste identified: