Universidade de Aveiro Departamento de Química
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Daniela Filipa da Silva Fonseca

VALORIZAÇÃO DO MEDRONHO (*Arbutus unedo* L.): Da caracterização química ao desenvolvimento de novos produtos alimentares

VALUATION OF STRAWBERRY TREE FRUITS (*Arbutus unedo* L.): From chemical characterization to the development of new food products

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica, especialização Bioquímica Alimentar, realizada sob a orientação científica da Doutora Sílvia M. Rocha, Professora Auxiliar do Departamento de Química da Universidade de Aveiro e do Doutor Armando J. D. Silvestre, Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro.

Aos meus pais.

o júri

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Palavras-chave

Medronho (*Arbutus unedo* L.), compostos bioactivos, compostos lipofílicos, maturação, armazenamento, novos produtos alimentares, inovação

Resumo

O medronheiro (*Arbutus unedo* L.) é uma planta nativa da região Mediterrânica e está presente em todo o território nacional. Em Portugal, o medronho é utilizado maioritariamente na produção de aguardente, licores e compota. Considerando a extensa distribuição do medronho pelo território português e o reduzido número de aplicações, torna-se imperativo valorizar este fruto. Para tal é necessário conhecer a sua composição química e as modificações que ocorrem ao longo do amadurecimento e do armazenamento. Assim, foram estudados alguns parâmetros de qualidade do medronho ao longo da maturação, nomeadamente, o ºBrix, pH, acidez titulável, o conteúdo em fenóis totais e a atividade antioxidante. Paralelamente, foi estabelecido o perfil de compostos lipofílicos do fruto maduro. Com vista a promover o consumo do medronho em fresco foi avaliado o efeito da conservação em frio (≈5ºC) de frutos colhidos em diferentes graus de maturação (≈19 e 21 ºBrix). A fase final desta tese foi focada na inovação e desenvolvimento de novos produtos alimentares com base no uso do medronho.

Ao longo da maturação, observou-se um aumento do ºBrix e do pH, enquanto que a acidez titutável, o conteúdo em fenóis totais e a atividade antioxidante não mostraram nenhuma tendência assinalável. O perfil lipofílico do medronho maduro compreende 41 compostos pertencentes a 5 famílias: triterpenóides, ácidos gordos, esteróis, tocoferóis e álcoois alifáticos de cadeia longa. Os triterpenóides são o grupo maioritário (até 70 % da massa total de extrato após a hidrólise alcalina), com teores entre 3196 a 5494 $mg.kg^{-1}$ peso seco (ps). O conteúdo em fitoesteróis varia entre 217 e 805 $mg.kg^{-1}$ ps e o conteúdo em ácidos gordos varia entre 587 e 2954 $mg.kg^{-1}$ ps, dos quais *ca*. de 50 a 78 % correspondem a ácidos gordos insaturados. O conteúdo em tocoferóis varia entre 21 e 115 $mg.kg^{-1}$ ps e os álcoois gordos de cadeia longa entre 30 e 453 $mg.kg^{-1}$ ps.

O estudo do efeito da conservação do fruto fresco em frio permitiu concluir que o medronho colhido com 21 ºBrix pode ser armazenado até 17 dias enquanto o fruto colhido com 19 ºBrix pode ser armazenado até 20 dias. Na fase ótima de consumo, os frutos colhidos em diferentes estado de maturação apresentam características sensoriais e químicas similares, sendo de realçar que os frutos colhidos em estado de maturação precoce são menos pastosos na boca e apresentam teores significativamente menores de etanol. Estes resultados sugerem que a colheita de frutos com *ca*. 19º Brix, pH 2.7 e acidez titulável de 1 g ácido málico por 100 g de fruto fresco, contribuem para o aumento do tempo de prateleira, mantendo a firmeza do fruto e um reduzido teor de etanol. A fase final desta tese incluiu várias etapas, nomeadamente, a pesquisa sobre hábitos de consumo de medronho pela população portuguesa, a avaliação das oportunidades de mercado para este tipo de produto, e a conceção de alguns produtos que estivessem em linha com as atuais tendências de mercado. Assim, foram concebidos e testadas várias formulações de bolachas, iogurtes, barras de cereais e bombons.

Em conclusão, os resultados obtidos são relevantes no que diz respeito à caracterização química sumária do medronho da região da Beira Serra, com especial destaque para os compostos lipofílicos. A presença de compostos com reconhecidos efeitos benéficos para a saúde humana, nomeadamente os ácidos gordos ω-3 e ω-6, fitoesteróis e os triterpenóides, pode ser um fator determinante para a valorização do medronho. A avaliação do impacto da maturação e da conservação em frio sobre os frutos frescos podem ser usados como uma ferramenta de suporte à decisão por produtores de medronho, com vista a atingir novos consumidores e novos mercados. A fase final do trabalho, também permite confirmar que este produto tem inúmeras aplicações na área alimentar, as quais estão em linha com as actuais tendências de mercado.

Keywords

Arbutus unedo L., bioactive compounds, lipophilic compounds, maturation, new food products, innovation

Abstract

The strawberry tree (*A. unedo* L.) is native from the Mediterranean region and it is implanted all over the Portuguese territory. In Portugal, *A. unedo* fruits have been used in the production of spirit, liqueurs and jam. Considering the wide distribution through Portuguese territory and the limited number of applications, it becomes crucial to value *A. unedo* fruits. For this purpose it is necessary to understand the chemical composition of *A. unedo* fruits, particularly during maturation and storage. Therefore, some quality parameters of *A. unedo* fruits were studied throughout maturation, particularly ºBrix, pH, titratable acidity, total phenolic content and antioxidant activity. In addition, the lipophilic extractives profile was assessed in ripen fruits. To promote the consumption of *A. unedo* fresh fruits it was studied the effect of cold storage (≈ 5 ºC) using fruits from two different ripeness degrees (19 and 21 ºBrix). The final phase of this thesis was focused on innovation and development of new food products based on *A. unedo* fruits.

This study allowed us to conclude that throughout maturation, the ºBrix content and pH increased, whereas titratable acidity, total phenolic content and the antioxidant activity exhibited no remarkable tendency. Moreover, 41 lipophilic compounds were identified in *A. unedo* fruits, which belong to 5 chemical families: triterpenoids, fatty acids, sterols, tocopherols and long chain aliphatic alcohols. Triterpenoids represent the major group of identified compounds, (*ca.* of 70 % of the total amount after alkaline hydrolysis), accounting for between 3196-5494 $mg.kg^{-1}$ dry weight (dw)). Sterols account for between 217-805 $mg.kg^{-1}$ dw and fatty acids content varies between 587-2954 $mg.kg^{-1}$ dw (of which 50 to 78 % corresponded to unsaturated fatty acids). Tocopherols content range between 21-115 $mg.kg^{-1}$ dw and the long-chain aliphatic alcohols are present in a broader range of 30-453 $mg.kg^{-1}$ dw.

Regarding the effect of cold storage using fruits from two different ripeness degrees, this study shows that the ripen-harvested fruits (21 ºBrix) can be stored up to 17 days while the unripen-harvested fruits (19 ºBrix) can be stored up to 20 days. At the best eating-point, berries from both sampling moments presented similar sensory and chemical characteristics. However, the unripen-harvested fruits were less pasty in the mouth and had significantly lower levels of ethanol. These results suggest that the fruits harvested with *ca*. of 19 ºBrix, pH 2.7 and titratable acidity of 1 g malic acid *per* 100 g fresh fruit, contribute to increase their shelf-life while maintaining the firmness and reduced ethanol content. The final phase of this thesis included several stages, namely research on consumer habits of *A. unedo* fruits by the Portuguese population, the assessment of market opportunities for this type of product, and the design of some novel products that are in line with current market trends. Thus, they were conceived and tested over several formulations of cookies, yoghurts, cereal bars and chocolates.

In conclusion, the results obtained are relevant regarding the general chemical characterization of the Serra da Beira region strawberry tree fruits, with emphasis to the lipophilic compounds. The presence of the compounds with reported health benefits, particularly ω-3 and ω-6 fatty acids, phytosterols and triterpenoids, can be a crucial factor on the valuation of *A. unedo* fruits. Assessing the impact of maturation and cold storage on fresh fruits can be used as a decision tool to support *A. unedo* fruits producers, in order to reach new consumers and new markets. The last phase of this work also confirms that this fruit has numerous applications in the food area, which are in line with the current market trends.

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List of Abbreviations/Acronyms

A. unedo	*Arbutus unedo* L.
AH	After alkaline hydrolysis
BH	Before alkaline hydrolysis
dw	Dry weight
$DPPH^{\cdot}$	2,2-diphenyl-1-picrylhydrazyl radical
EC_{50}	Efficient concentration where 50 % of its maximal effect is observed
FID	Flame ionization detector
GAE	Gallic Acid Equivalents
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
m/z	Mass-to-charge ratio
TA	Titratable acidity
TMS	Trimethylsilyl
TPC	Total phenolic content
w/w	weight/weight

CHAPTER I -Introduction

1.1 Overview

Arbutus unedo L. (*A. unedo*), also known as strawberry tree, is an evergreen shrub native from the Mediterranean region. In Portugal it is implanted throughout all the territory and is mainly used to produce a spirituous drink known as "Aguardente de Medronho". *A. unedo* has been employed for curative aims since ancient times; however, this fruit is rarely eaten as fresh fruit and is not largely exploited by the food industry. Nowadays there is a growing interest in the consumption of foods that provide health benefits. Hence, understanding the chemical composition of *A. unedo* fruits, namely the lipophilic components, reported as promoting health benefits may encourage their consumption and industrial usage. This chapter comprises the current knowledge about (1) the botany and geographical distribution of *A. unedo*, including in Portugal, (2) the main applications, (3) the chemical composition with special focus on the lipophilic components, (4) the storage strategies to improve fruit factors related to lifetime and quality and finally (5) the development of new food products.

1.2 *Arbutus unedo* L.

1.2.1 Botany and geographical distribution

A. unedo, also known as strawberry tree, belongs to the *Ericaceae* family and is a Mediterranean plant, with an extension along the Atlantic coast of Europe.[1–4] This species is also found in the United States, Australia and North Africa.[2,4,5] In Portugal, strawberry tree is implanted throughout the country but there are some regions where it has more relevance, namely in the north center and in the south (Algarve) (Figure 1).[6] In addition to the endemic trees spread all over the territory, this species may be found in large plantations, e.g. in Perocabeço, Oleiros and Pampilhosa da Serra, with an extension of 15 and 36 hectares respectively.[7,8]

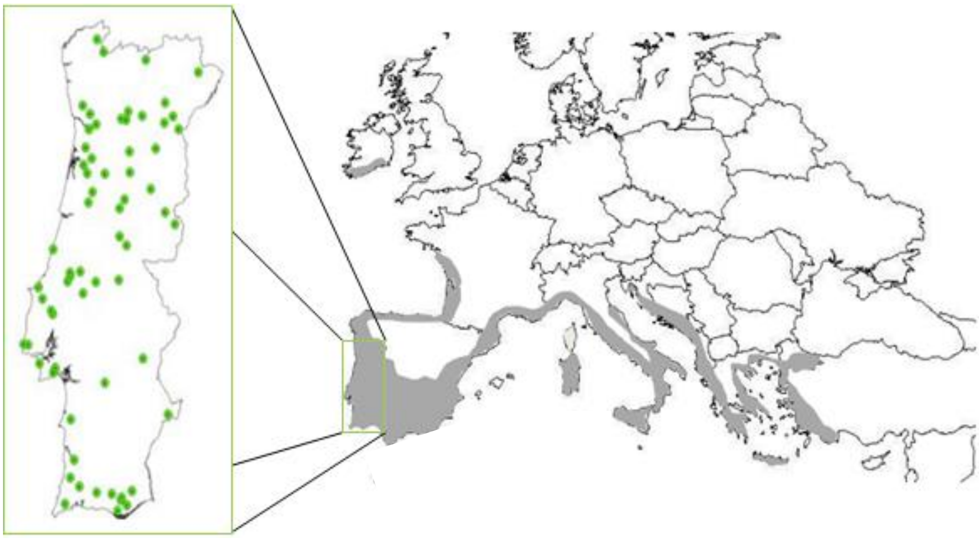


Figure 1 – Distribution of *A. unedo* in Europe and in Portugal (adapted from [2,9])

The strawberry tree can grow from 8 m above the sea level, in Ireland, up to 1200 m in Portugal. The geographical limits of *A. unedo* fruits distribution (northern and eastern boundaries of the Mediterranean) is determined apparently by winter temperature, once these plants only regenerate and flourish in regions in which the average temperature is above 4,5 ºC in January. *A. unedo*'s habitat is characterized by the absence of shading from taller trees, associated with evergreen shrubs of about the same nature. Occasionally it can be found deep in the woods, appearing as a tall tree instead of a common shrub. This species grows on bare, rocky and poor soils, although it is not possible to establish any soils and habitat profile for *A. unedo*. Nevertheless, it is recognized that in Ireland it grows in carboniferous regions. In Spain and in the Mediterranean coast of France it shows preference for non-calcareous rocks while in the south-west coast of France it also grows in sandy areas overlain by peat.[1,4] In Portugal the strawberry tree grows in carboniferous and shale soils and it co-habits with holmoak, cork and pine in the forest.[6]

A. unedo is an evergreen shrub or small tree with 2-3 m or less and rarely exceeding 12 m in height. The shrub has a short trunk with thick foliage and depicts a gray-brown fissured bark, revealing the reddish young bark beneath (Figure 2 (a)). Its shiny and glossy leaves (Figure 2 (b)) are evergreen, alternate and more or less serrate, with variable size (about 8 x 3 cm) and shape but usually oblong-lanceolate. *A. unedo* flowers (Figure 2 (c)) are hermaphrodite, small (about 5 cm), bell-shaped and white, slightly colored with pink or green. *A. unedo* flowering and fructification occurs at the same time.[4,5,10,11] Flowering takes place every year throughout the winter, during September to December. The fruits (Figure 2 (d)) are spherical berries with an average diameter of 2 cm, with a few seeds inside, soft yellow pulp and covered with conical hair-like spikes.[4,5,10] Strawberry tree fruits take a year to ripen and during ripening the green berries become yellow to reddish until it reaches a fully red color.[11]

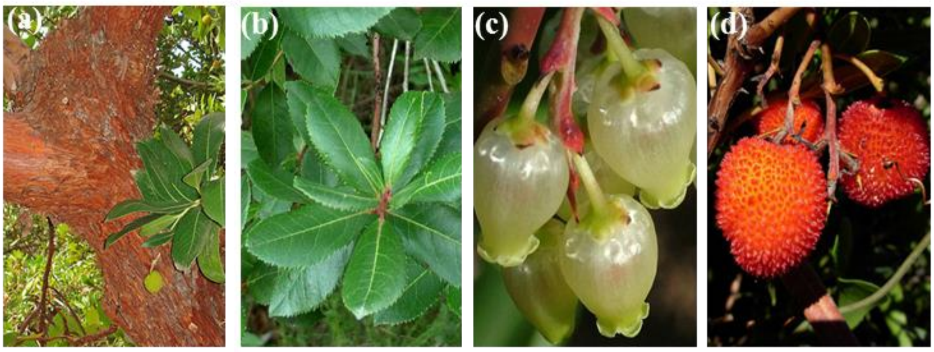


Figure 2 – *A. unedo* (a) bark, (b) leaves, (c) flowers and (d) fruit (ref. [12]).

At an advanced stage of maturity the fruits present a dark red color on the surface and a yellow-orange pulp. At this point the fruits can start fermenting in the tree, reaching an alcoholic content of about 0.02-0.5 %.[6,13] The word '*unedo*' means 'only eat one' and suggests that the fruit is delicious (and alcoholic) and people only need to eat one.[5,6]

1.2.2 Major applications

A. unedo applications have been associated with a long tradition in socio-cultural and microeconomic aspects.[14] The current market for these fruits is oriented to produce alcoholic distillates, such as *A. unedo* spirit and liqueurs and also for jellies and jams. *A. unedo* is also marketed as a dietary supplement (Figure 3).[6,15]

Figure 3 – *A. unedo* fruit products available in the market: (a) spirit, (b) liqueur, (c) jam and (d) dietary supplement (ref. [16–19])

In Portugal, the most common use is the production of "Aguardente de Medronho", a spirit in the Algarve region, which is an important local economic booster, an estimated annual production of 15,000 L in Monchique.[20]

The exploitation of strawberry tree fruits for human use can be traced back to ancient times, where they were used in folk medicine. Strawberry tree fruits are being applied in phytotherapy to treat and/or prevent cardiovascular, dermatological, gastrointestinal and urological disorders.[21,22] Nowadays strawberry tree fruits are not extensively exploited by food industry, which is warranted by the restricted number of applications. However, new potential uses have been studied, being already suggested applications in yoghurts, pastry and other breakfast products.[11,23] Nevertheless, there has been a growing interest to characterize this species, developing breeding programs (to obtain cultures with high quality fruits) and in the promotion of extensive farming.[10,24]

1.2.3 General chemical composition of *A. unedo* fruits

Today foods are intended not only to provide necessary nutrients but also to contribute directly to human health, preventing nutrition related diseases and improving physical well-being. In this perspective, the study of the chemical composition of *A. unedo* fruits, in order to infer about its nutritional value, is important to recognize and to improve the value and applicability of these berries. During the last years, there has been a great interest in the secondary metabolites of foods such as lipophilic or phenolic components due to their importance in human wellness. These compounds showed ability to reduce the risk and likewise to prevent particular diseases (such as obesity, Alzheimer and depression).[25–29] The presence of lipophilic and phenolic compounds in strawberry tree fruits, already described by some authors, might value and provide new applications for them.

The general chemical composition of ripen fruits is given in Table 1. [10,13,30–35]

Table 1- General composition and energetic value of *A. unedo* fruits [10,13,30–35]

Composition (fresh fruits)		Range (Min-Max)
Energy ($kcal.20g^{-1}$)		12.30-27.19
Macronutrients	Carbohydrates ($g.20g^{-1}$)	2.71-6.54
	Lipids ($g.20g^{-1}$)	0.06-0.17
	Protein ($g.20g^{-1}$)	0.11-0.26
Micronutrients	Vitamin C ($mg.20g^{-1}$)	24.4-52.54
	Mineral content ($mg.20g^{-1}$)	29.32-93.36
	β-carotene ($mg.20g^{-1}$)	0.005-0.176
	Lycopene ($mg.20g^{-1}$)	0.020-0.065
Moisture (%)		42.78-72.59
Total fiber ($g.20g^{-1}$)		2.01-4.45
Soluble fiber		0.42-0.84
Insoluble fiber		1.52-3.75
Total phenolic content ($mg\ GAE.20g^{-1}$)		89-394.6
Soluble solids (°Brix)		16.50-31.68
Acidity (%)		0.3-1.59
pH		3.17-4.6
Organic acids[1] ($mg.\ 20g^{-1}$)		69.01-130.25

[1](oxalic + malic + fumaric acids)

The chemical composition will be expressed per 20 g (*ca.* 4-5 fruits) representing the average amount consumed by an individual. For each parameter a range value is present which depends on the harvest, location, environmental conditions (water availability, sunlight exposition and wind) and genetic factors.[30–32]

Carbohydrates represent the major component of *A. unedo* fruits, reaching up 32 % of the total weight **(**Table 1**)**. As sugar content of the majority of fruits ranges between 3.2 and 20 %, the strawberry tree fruits content can be considered high.[31,36] Some authors have already identified and quantified the soluble sugars present in these fruits, namely glucose (1.8-12.14 % dry weight (dw)), fructose (24.21-27.8 % dw), sucrose (1.8-4.2 % dw) and maltose (1.1 % dw).[31,37,38] Fructose represents about double than glucose (ratio glucose/fructose between 0.4 and 0.6) and sucrose and maltose appear in much smaller amounts. Enzymatic hydrolysis of sucrose during ripening can probably explain higher levels of glucose and fructose in *A. unedo* fruits. Additionally, since fructose is the sweetest of all naturally occurring carbohydrates it can explain the intense and pleasant sweet taste of ripe fruits.[31] Soluble solids content (expressed by ºBrix) is used to estimate sugar concentration and maturity state. The ºBrix content of *A. unedo* fruits ranges between 16.50 and 31.68 (Table 1); according to these values, and comparing to other berries (blueberry, bilberry, raspberry, black currant, among others), only the sour cherry ºBrix is of about the same range. All the others showed lower levels of this parameter.[39]

The protein content of *A. unedo* fruits ranges between 0.581 and 1.187 % (weight/weight (w/w)) (Table 1).[31] In the general, *N*-containing compounds are present in fruits in low amounts, 0.1-1.5 %; from these 35-75 % correspond to protein. So, the protein content found in this species is in agreement with that found in the literature for the majority of the fruits.[36] Strawberry tree fruits present between 0.06 and 0.17 $g.20\ g^{-1}$ fresh weight of lipids.[31] The lipid content of fruits is generally of 0.1-0.5 % fresh weight.[36] Thus *A. unedo* fruits lipids content is in agreement with that usually reported for fruits. The strawberry tree fruit presents moisture content between 46.82 and 71.89 % (Table 1) and the differences observed can be explained by the wind, sunlight exposure and water availability, factors responsible for desiccation. [31] The moisture content of *A. unedo* (42.78-72.59 %) is lower than that of the majority of the fruits, which ranges between 75-95 %.[31,36] Also, considering the data available on other berries moisture (87 % average) it is possible to conclude that *A. unedo* fruits have a relative lower water content.[39]

The content of vitamin C on *A. unedo*, an average 38.47 mg. 20 g^{-1} fresh weight, is of the same range of blackcurrant (in average), and higher than blueberry, raspberry, strawberry and other small fruits.[39] These value can also be compared to that present in peaches, apples and plums.[40] Among the micronutrients, β-carotene and lycopene are the minor compounds with less than 0.2 and 0.1 mg. 20 g^{-1} fresh weight respectively. The total fiber content of strawberry tree fruits varies between 2.01 and 4.45 g. 20 g^{-1} fresh weight. Apples and strawberries present 0.4 and 0.44 g dietary fiber per 20 g fresh weight respectively, pears and kiwis 0.6 and 0.68, peaches and bananas, 0.38 and 0.34 and oranges and mango 0.36.[41] Comparing the fiber content of strawberry tree fruits with those commonly consumed fruits is possible to infer that *A. unedo* fruits have a higher fiber content, with 3 to 6 times much fiber than kiwi.

pH and titratable acidity (TA) are parameters commonly measured to give an overview of the maturity state at harvest. TA indicates the total amount of organic acids in the fruit.[36] *A. unedo* fruits presents a pH of about 3.21-4.6, similar than that reported for other berries (such as red currant, gooseberry, blueberry and blackcurrant) which ranges between 2.60 and 4.10.[39]

The total phenolic content (TPC) of strawberry tree fruits ranged between 89 and 394.6 mg Gallic Acid Equivalents (GAE).20 g^{-1} fresh weight.[31–33,35] Comparing these values with other berries (red currant, gooseberry, blueberry, and blackcurrant) it is possible to conclude that the TPC of strawberry tree fruits is higher than many fruits considered to be rich in polyphenols, such as red currant (74.4-108 mg GAE.20 g^{-1} fresh weight), gooseberry (38.2-126 mg GAE.20 g^{-1} fresh weight), blueberry (21.2-173.6 mg GAE.20 g^{-1} fresh weight) and blackcurrant (99.6-282 mg GAE.20 g^{-1} fresh weight).[39] It is reported the presence of phenolic acids, flavonoids (flavones, flavan-3-ols, and anthocyanidins), ellagic acids derivatives and proanthocyanidins in *A. unedo* fruits.[11,33,35,37,40,42–44] Regarding phenolic acids, Ayaz *et al.* [37] quantified gallic, protocatechuic, gentisic, *p*-hydroxybenzoic, vanillic and *m*-anisic acids in strawberry tree fruits and reached a total amount of 13.73 mg.g^{-1} dw. Flavan-3-ols were also quantified and the results showed that the flavan-3-ols content was of about 7.3 mg.20 g^{-1} fresh weight and more specifically, catechin content was about 0.83 mg and that of gallocatechin was about 0.73 mg.20 g^{-1} fresh weight.[33,40] The major anthocyanin was cyanidin-3-galactoside and that the total anthocyanin content (0.15 mg.20 g^{-1} fresh weight) is comparable to apples or pears, sour cherry, pistachio nuts and red plums.[40] Guimarães *et al.* [33] suggested that cyanidin-3-glucoside is the major anthocyanin present in *A. unedo* fruits. Pallauf *et al.* [40] found that this fruit is one of the major sources of procyanidins, with an average abundance of 5.49 mg.20 g^{-1} edible fruit. Also, Pallauf *et al.* [40] determined the ellagic acid derivatives content as 0.31 mg.20 g^{-1} fresh weight and concluded that the major compound is ellagic acid glucoside

The total phenolic content (TPC) is a parameter evaluated by various authors and because phenolic compounds show radical-scavenging properties, the measurement of the antioxidant activity is of great importance as well. This parameter may be expressed as EC_{50} (the effective concentration of sample required to scavenge 50 % of $DPPH^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl radical) free radicals) and the published data showed that EC_{50} ranged between 0.25 and 0.79 mg strawberry tree extract per mL of $DPPH^{\cdot}$ ([]=6×10^{-5} M), comparable to those of blueberries, blackberries and strawberries.[38,42,45] The influence of ripening (unripe, intermediate and ripe stage) on TPC and antioxidant activity of *A. unedo* fruits was studied demonstrating that: (1) the TPC was higher in the intermediate and the ripe stage with 21.0 and 12.1 mg GAE.g^{-1} dw fruit respectively; (2) the lowest phenolic content was reported for the unripe stage (6.3 mg GAE.g^{-1} dw fruit) and (3) the antioxidant activity increased during ripening (($DPPH^{\cdot}$ assay) - EC_{50} of unripe fruits was 0.58±0.03, intermediate 0.37±0.02 and ripe 0.25±0.02 mg extract per mL $DPPH^{\cdot}$). Oliveira *et al.* [45] also reported that the anthocyanin content increased during ripening and since these compounds act as antioxidants these results can support that phenolics are related to an increase on the antioxidant activity.

In summary, a wide variability in nutrient composition of *A. unedo* fruits, as a result of different years and location of harvest, has been reported. Despite the variability in nutrient composition these berries are considered good sources of vitamin C, dietary fiber and polyphenols: the consumption of 20 g of strawberry tree fruits provides *ca.* 41 and 8.5 % of the recommended dietary intake vitamin C and dietary fiber respectively.[31] The phytochemical research approach is considered effective in discovering bioactive profile of fruits. Because of the importance of lipophilic compounds in human health, the next chapters will describe this family in more detail and report the already existing data concerning the composition of *A. unedo* fruits.

1.2.4 Lipophilic compounds of *A. unedo* fruits

Lipophilic compounds constitute a very heterogeneous group of compounds that share the same property: insolubility in water.[36] The number of functions that these naturally occurring compounds exert in nature is very broad: they have structural functions in the cells, act as fuel, on stress and hormonal response, in the inflammatory and autoimmune processes and are important nutrients (sources of essential fatty acids and vitamins). Lipids, even in a minor quantity, deserve a particular importance because they exert main roles in the flavor, palatability and texture of foods, being responsible for the organoleptic quality.[36,46,47] Additionally, some lipids demonstrated to have important physiological effects, reducing the susceptibility to some diseases, which is the reason why the scientists focus so much attention in this group of compounds. The lipophilic composition of fruits generally consists of glyco- and phospholipids, carotenoids, triterpenoids, waxes and phytosterols.[46] *A. unedo* fruits comprises 0.299-0.779 % fresh weight of lipids.[31] The lipophilic assessment in strawberry tree fruits was made regarding the fatty acids composition (in the whole fruit and in the seeds), and also on carotenoids, tocopherols (on the seed's oil) and triterpenoids content.

Fatty acids

Fatty acids are long chain aliphatic carboxylic acids, that might be saturated or unsaturated (Figure 4). In plants they are mostly found esterified with glycerol.[25,47]

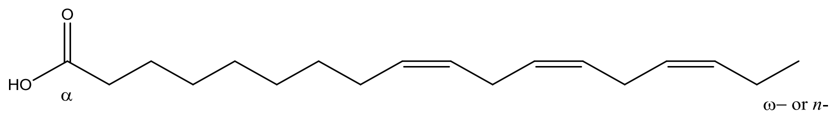


Figure 4 - General structure of a polyunsaturated fatty acid (α-linoleic acid, C18:3 $^{(\Delta 9,\,12,\,15)}$) with the respective numbering

The double bonds of nearly all naturally occurring unsaturated fatty acids are in the *cis* configuration (*trans* fatty acids are obtained from fermentation in the rumen of dairy animals and from dairy products and meat). Unsaturation of fatty acids determines their physical properties: unsaturation forces a kink (or several) into the hydrocarbon chain and these fatty acids cannot pack as tightly as the fully saturated ones. As a consequence, their interactions with each other are weaker and they remain liquid at room temperature. Polyunsaturated fatty acids such as linoleic ($18{:}2^{(\Delta 9,\ 12)}$) an ω-6, and α-linolenic acid ($18{:}3^{(\Delta 9,\ 12,\ 15)}$), an ω-3, are essential nutrients that must be obtained from the diet because the human body lacks saturases enzymes that might biosynthesise them.[25,47]

The fatty acids content of the seeds (essential oil) and of the entire strawberry tree fruit was already studied and is summarized in Table 2.

Table 2 - Fatty acid composition of *A. unedo*

Fatty acids	Abundance range[a] Fruit	Seeds	Ref.
C6:0	0.04 ± 0.00	-	38
C8:0	0.04 ± 0.00	-	38,48
C10:0	0.04 ± 0.00	-	38,48
C12:0	0.65 ± 0.05	-	38,48
C13:0	0.07 ± 0.00	-	38
C14:0	1.34 ± 0.15	0.04-0.07	38,45
C15:0	0.10 ± 0.00	0.03-0.04	38,45,48
C16:0	8.20 ± 0.25	5.92-6.73	38,45,48
C16:1n7	0.11 ± 0.01	0.09-0.11	38,45
C16:1n9	-	0.05-0.09	45
C17:0	0.30 ± 0.01	-	38
C18:0	4.00 ± 0.17	3.72-4.67	38,45,48
C18:1n9	**21.01 ± 0.04**	**26.75-29.38**	38,45,48
C18:2n6	**21.50 ± 0.06**	**18.84-20.14**	38,45
C20:0	0.61 ± 0.05	0.22-0.24	38,45
C20:3n3+ C21:0	0.12 ± 0.01	-	38
C18:3n6	-	0.08-0.10	45
C18:3n3	**36.51 ± 0.64**	**36.90-43.07**	38,45
C20:1n9	0.27 ± 0.02	0.02-0.03	38,45
C20:2n6	-	0.05-0.05	45
C22:1n9	-	0.05-0.09	45
C22:0	0.81 ± 0.03	-	38
C23:0	2.68 ± 0.10	-	38
C24:0	1.45 ± 0.17	0.04-0.08	38,45
Total PUFA[b]	**58.28 ± 0.54**	**52.47-62.01**	38,45
Total MUFA[c]	21.39 ± 0.03	27.00-33.49	38,45
Total SFA[d]	20.32 ± 0.57	10.04-12.64	38,45

[a], results are expressed as mean of relative percentage of each fatty acid. [b], Polyunsaturated fatty acids. [c], Monounsaturated fatty acids. [d], Saturated fatty acids.

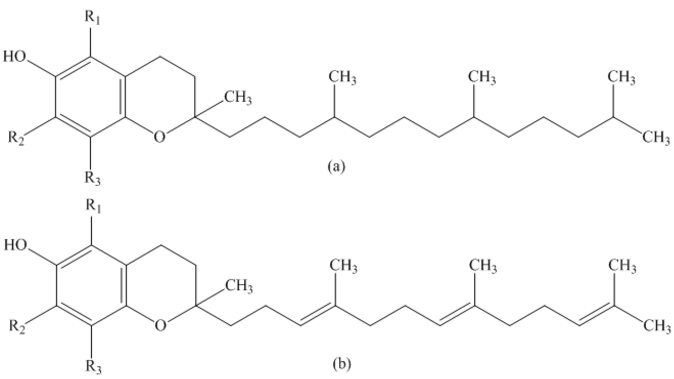
The major fatty acids found in strawberry tree fruits and seeds were polyunsaturated fatty acids, namely α-linolenic ($C18:3^{(\Delta 9, 12, 15)}$) and linoleic acid ($C18:2^{(\Delta 9, 12)}$). Oleic acid ($C18:1^{(\Delta 9)}$) was also one of the main compounds found, in the whole fruit and in the seeds, together with palmitic acid (C16:0), stearic acid (C18:0) and tricosanoic (C23:0) acid. All other fatty acids appeared in quantities below 1.75 %. So, the major fraction of fatty acids in *A. unedo* fruits is composed by the polyunsaturated fatty acids, followed by the monounsaturated fatty acids and in last, the saturated fatty acids.[38,45]

Vidrih *et al*.[32] determined the content of palmitic, stearic, oleic, linoleic and α-linolenic acids in *A. unedo* fruits and found that (1) linoleic acid was the prevailing compound (accounting for *ca.* 18.5 mg.20 g^{-1} fresh weight), followed by α-linolenic acid (*ca.* 17 mg.20 g^{-1} fresh weight), oleic (*ca.* 8 mg.20 g^{-1} fresh weight) and palmitic acid (*ca.* 1 mg.20 g^{-1} fresh weight), (2) stearic acid was found in trace levels and that (3) polyunsaturated fatty acids represented the major fraction. The adequate intakes for linoleic and α-linolenic acids ($g.day^{-1}$) are 16 and 1.6 respectively, for healthy adults.[49] Considering the aforementioned results it is possible to conclude that 20 g of fruits contribute to the intake of 0.12 % and 1.04 % of linoleic and α-linolenic acids respectively. Oliveira *et al*.[45] studied the profile of fatty acids in the seeds during ripening and the results showed that, over time, the content of polyunsaturated fatty acids slightly increased, however, monounsaturated fatty acids showed to decrease and the saturated fatty acids were kept almost constant during ripening. Also, Vidrih *et al*.[25] reported a favorable ratio of ω-3/ω-6 fatty acids, i.e. 0.06. The imbalance of ω-6/ω-3, characteristic in the food supply of Western diet, may change the physiological state to a prothrombotic, proconstrictive and proinflammatory state. These physiological effects are due to the increased consumption of ω-6 fatty acids which improves eicosanoids production, promoting the formation of thrombus and atheromas and contributing to inflammatory disorders.[50]

An understanding of the effect of lipids on health is important to add value, promote the consumption and develop new applications based on *A. unedo* fruits. For that reason, will then be described the potential health benefits of the fatty acids reported in strawberry tree fruits. In a three week study it was assessed the influence of polyunsaturated fatty acids (ω-3 series (eicosapentaenoic ($20:5^{(\Delta 5, 8, 11, 14, 17)}$) and docosahexaenoic acids ($22:6^{(\Delta 4, 7, 10, 13, 16, 19)}$)), supplementation of 2.8 $g.day^{-1}$) during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women. The results suggested that polyunsaturated fatty acids enhanced weight loss and that docosahexaenoic acid seemed to be the active component.[51] Pomegranate oil (with fatty acids such as linoleic, docosanoic (C22:0) and tetracosanoic acids (C24:0)) was shown to stimulate keratinocyte proliferation in cell culture, suggesting that it can be involved in the regeneration of epidermis, improving skin health.[52] Also, epidemiological studies have demonstrated that dietary consumption of ω-3 such as eicosapentaenoic acid and docosahexaenoic acid was inversely related to the risk of impaired overall cognitive function (memory function, cognitive flexibility) and speed of cognitive process and incidence of depression as well.[53,54] Polyunsaturated fatty acids have been also linked to bone metabolism modulation, enhancing the activity of osteoblasts (responsible for mineralization) and inhibiting the activity of osteoclasts (involved in bone resorption) *in vitro*.[55] Despite the reported beneficial roles of lipids more clinical studies are required to fully elucidate their role on health regarding mechanisms of action and daily intake.

Vitamin E

Vitamin E is a fat-soluble vitamin encompassing eight vitamers of two different classes: tocopherols and tocotrienols (Figure 5).[49]



	R_1	R_2	R_3
α-tocopherol/tocotrienol	CH_3	CH_3	CH_3
β-tocopherol/tocotrienol	CH_3	H	CH_3
γ-tocopherol/tocotrienol	H	CH_3	CH_3
δ-tocopherol/tocotrienol	H	H	CH_3

Figure 5 –The structures of the various forms of (a) tocopherols and (b) tocotrienols

These compounds are structurally similar: they have a phenolic functional group on a chromanol/chromane ring and an attached phytyl side chain. Tocopherols (Figure 5) contain a chromanol ring and an attached isoprene derived side chain with 16 carbons. Tocotrienols (Figure 5) present an unsaturated phytyl side chain. Each compound of the tocopherols/tocotrienols class differs in the number and location of the methyl groups on the chromanol ring.[36,49]

Pallauf *et al.*[40] determined the vitamin E content (as α-tocopherol) of *A. unedo* fruits, considering a full ripening stage, and the results revealed 0.005 mg.20 g^{-1} edible portion. The recommended daily intake of vitamin E is 15 mg (vitamin E recommendations are expressed as α-tocopherol).[49] Considering these results it is therefore, possible to infer that the consumption of 20 g of the studied strawberry tree fruits contributes to the intake of 0.03 % of the recommended dietary intake for vitamin E. Oliveira *et al*.[45] identified and quantified six tocopherols and tocotrienols in the crude oil obtained from the seeds of *A. unedo*, along three stages of maturation (unripe, intermediate and ripe). Higher vitamin E amounts were found on the unripe fruits (1368.6 $mg.kg^{-1}$ oil) and the content decreased as maturation occurred. The major constituent found was γ-tocotrienol. α-Tocotrienol and δ-tocopherol contents decreased at such extent, that the amount present in the ripe fruits was not detectable. When fully ripe the strawberry-tree fruit seeds analyzed contained an amount of 556 mg vitamin $E.kg^{-1}$ of oil, of which 32.16±1.55 mg corresponded to α-tocopherol.[45]

The main function of vitamin E is the ability to prevent oxidation however some other functions have been demonstrated.[49] Daily supplementation with 268 or 567 mg α –tocopherol, in a group of individuals with confirmed heart disease, reduced the rate of both nonfatal and total heart attacks.[56] Also, daily vitamin E supplementation (600–900 mg α-tocopherol) by individuals with type 2 diabetes mellitus decreased oxidative damage and improved metabolic control.[57]

Triterpenoids and Carotenoids

Triterpenoids (C_{30}) and carotenoids (C_{40}) are compounds of the terpenoids family, a family of natural products derived from isoprene (C_5). Back in 1997, four triterpenoids were isolated and identified in *A. unedo* fruits for the first time: lupeol, α- and β-amyrin and olean-l2-en-3β,23-diol (Figure 6).[58]

A more complex and detailed characterization of the triterpenoids fraction of strawberry tree fruits was achieved later by the same authors.[48] Besides the aforementioned compounds there were detected: lupenone, amyrone, ursolic aldehyde, α-amyrenone, uvaol, and ursolic and oleanolic acids. [48]

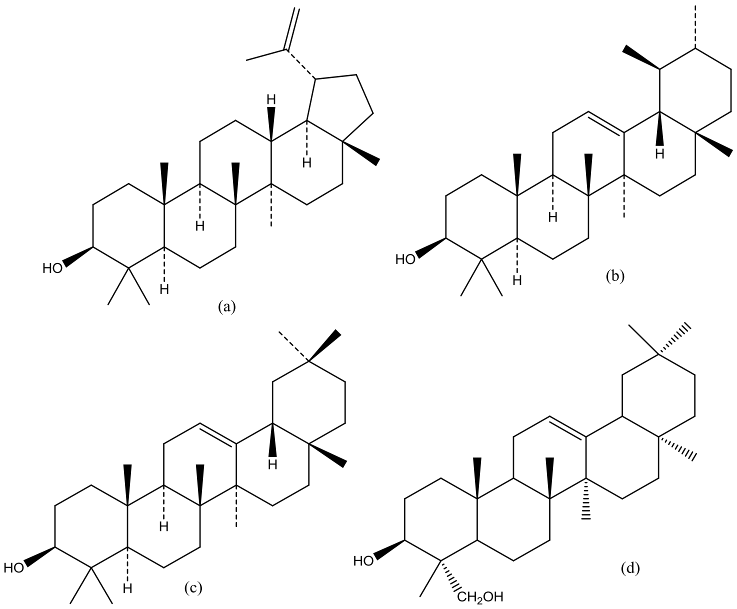


Figure 6 - Triterpenoids identified in *A. unedo* fruits: (a) lupeol, (b) α-amyrin, (c) β-amyrin and (d) olean-l2-en-3β,23-diol

Triterpenoids have shown a wide range of biological activities such as a hypocholesterolemic effect, anti-inflammatory and antitumor among many others.[27] Lupeol has shown anti-inflammatory activity in acute and chronic inflammation when administered orally or intraperitoneally in a dose of 25–200 $mg.kg^{-1}$ in rats and mice.[59] β-amyrin has been shown to exhibit *in vitro* anti-microbial and anti-fungal activity.[60] β- and α-amyrin revealed anti- inflammatory effect in rats and also showed to inhibit plaquelet aggregation *in vitro*, with β -amyrin being more potent that aspirin in inhibiting collagen-induced platelet aggregation.[61,62] Furthermore, since β- and α-amyrin are involved in the biosynthesis of other biologically active compounds, their presence in *A. unedo* fruits makes them a promising source of functional products.[63]

Carotenoids (C_{40}) are widespread in fruits and also found in fungi and bacteria. These compounds play a role in photosynthesis, as accessory light-harvesting pigments, effectively extending the range of light absorbed by the photosynthetic apparatus.[47] Carotenoids possess an extended system of conjugated double bonds that makes them important antioxidant molecules in humans, quenching singlet oxygen and scavenging peroxyl radicals. Carotenoids allow free-radical addition reactions and hydrogen abstraction from positions allylic to this conjugation.[47,49] Furthermore, in a number of fruits, like citrus, peaches and melons, their presence is the main factor determining color.

[47,49] The carotenoids content in *A. unedo* ripen fruits was determined by measuring lycopene, β-carotene, lutein and zeaxanthine (Figure 7).[11,31,40]

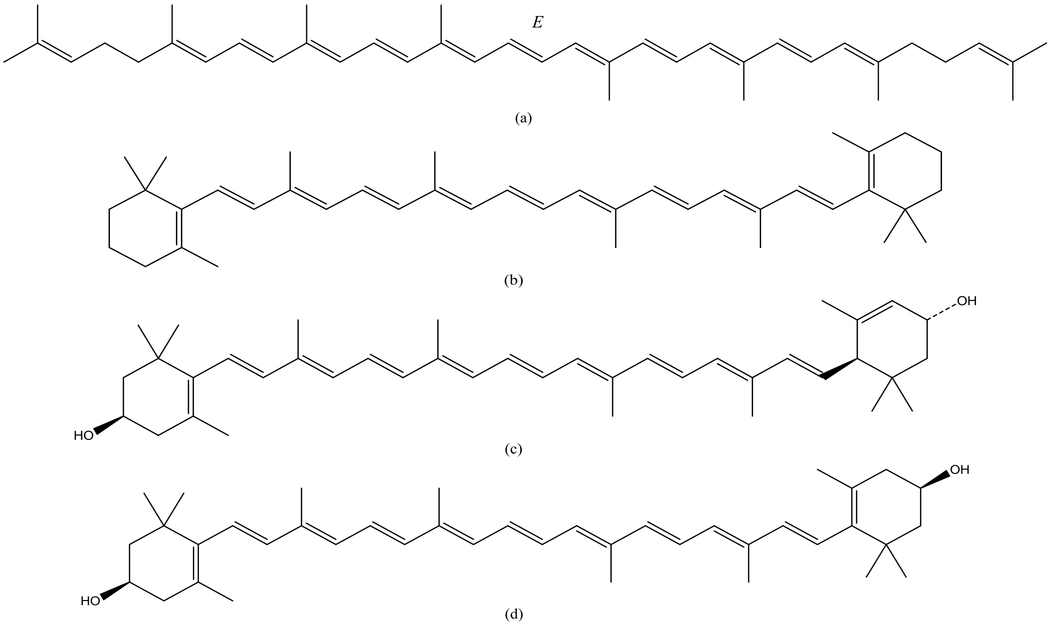


Figure 7 - Carotenoids of *A. unedo* fruits: (a) lycopene, (b) β-carotene (c) lutein and (d) zeaxanthine

Strawberry tree fruits showed to have up to 0.180 mg.20 g^{-1} fresh weight of β-carotene and up to 0.05 mg.20 g^{-1} fresh weight of lycopene, while the lutein + zeaxanthine content was *ca.* 0.01 mg.20 g^{-1} fresh weight.[31,40] β-carotene content was also quantified using fruits of two ripen stages - unripen and ripen - and the results showed that unripen fruits have *ca.* 8 mg.20 g^{-1} dw while the ripen fruits account for *ca.* 14 mg.20 g^{-1} dw respectively.[11] Considering a water content between 42.78 and 72.59 % (already published data, see Table 1), β-carotene ranged between 2-5 and 4-9 mg.20 g^{-1} fresh weight for unripen and ripen fruits respectively.

Recommendations for vitamin A are expressed as retinol activity equivalents (The retinol activity equivalents equivalency of β-carotene is as follows: 1 retinol activity equivalents = 12 µg β-carotene). The daily requirements for vitamin A for adult men and women are 900 and 700 µg retinol activity equivalents respectively. Accordingly to this, the daily requirements regarding β-carotene intake are about 10.8 and 8.4 mg for men and women respectively. Then, it is possible to assume that 20 g of *A. unedo* fruits contribute to the intake of *ca.* 60 and 70 % of the requirements for men and women. Carotenoids function as antioxidants and are thought to be protective against some diseases.[47,49] β-carotene, when included in the diet, originates the A group of vitamins, important in cellular differentiation and vision. Epidemiological studies have shown that individuals with high intake of fruits and vegetables rich in carotenoids have a lower incidence of diseases such as cardiovascular diseases, cancer, cataracts and age-related macular degeneration.[49]

Sterols

Sterols are characterized by a four ring core structure called steroid nucleus. Cholesterol is the most common example. Many sterols are found in plant tissues named as phytosterols. These sterols are characterized by an extra one-carbon or two-carbon substituents on the side chain. [47,49] In literature is reported the presence of 5-α-cholestane, cholestan-3-one, cholesterol, stigmasterol and stigmast-4-en-3-one in strawberry tree fruits (Figure 8).

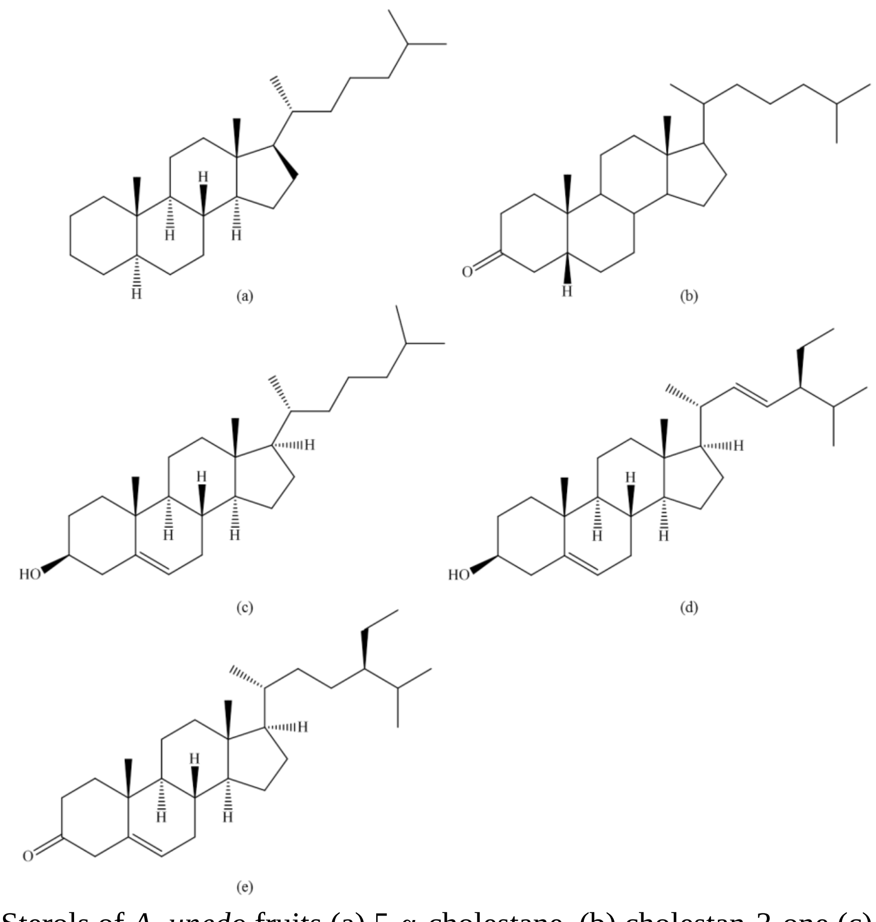


Figure 8 – Sterols of *A. unedo* fruits (a) 5-α-cholestane, (b) cholestan-3-one,(c) cholesterol, (d) stigmasterol, (e) stigmast-4-en-3-one

Phytosterols are considered biologically active and have an important role in human health because they inhibit cholesterol absorption.[25] The role of phytosterols in human health is mainly associated with non-pharmacological lipid-altering therapies, reducing exogenous cholesterol absorption by displacement in the intestinal lumen. As a result, both total cholesterol and HDL are reduced. Consumption of 1.8-2.0 g of phytosterols per day has been shown to lower cholesterol absorption.[64] In *A. unedo* fruits sterols were identified but quantification was not assessed. Thus, it is not possible to infer about the contribution of this fruit consumption in health. However, the presence of phytosterols is important in order to add value to *A. unedo* fruits.

1.3 Storage management of fresh fruits

A. unedo fruits are not usually consumed as fresh fruits. Thus, in order to add value and increase their consumption is crucial do understand the effect of storage on the quality of fresh fruits. To supply fresh fruits in the marketplace it is necessary to consider some factors related to plant metabolism, harvesting and pre- and post-harvesting conditions: these features influence the conditions and time of storage and final quality of the product as well.[65] It is known that the majority of the fruits reach their best eating quality when allowed to ripen on the plant. Any fruit harvested too early or too late is more susceptible to physiological disorders, shorter storage-life and lower quality than the fruits picked at the proper maturity.[65] Nevertheless, due to current market challenges, fruits need to be harvested before ripen. Thus, post harvesting practices such as cold storage, ethylene management or modified/controlled atmosphere among others, are required to maintain product quality and improve long term storage. In this thesis it is presented an overview of the factors regarding cold storage of fruits because this is an easy, conventional and the most commonly practice adopted for storage. To maintain quality during cold storage it is necessary to control (1) the metabolic rate, which results in undesirable changes in color, texture, flavor and nutritional status; (2) water loss, that results in shriveling, softening and loss of crispness and (3) freezing injuries.[65–67]

To reduce the metabolic rates low temperatures and ventilation are usually adopted. Temperature control is a fundamental postharvest tool to increase the quality and the storage life. Lowering the temperatures slows down the metabolism, reduces breathing rates, water losses and also the sensitivity to ethylene. When market's deliveries delay cannot be avoided, the ripe fruits should be cooled to their minimum safe temperature but allowing to reduce the risk of freezing injury. The ideal storage temperature is different depending on the products: it can be as low as -1ºC and reach about 18ºC. Ventilation is necessary to remove the breathing products. If carbon dioxide is not removed from around the product, fermentation occurs. Therefore, the oxygen depletion and high levels of carbon dioxide result in cell death. However, these changes can also be purposely made to increase the storage life of the product. Reducing the oxygen levels slows down respiration until the point when respiration rates increase dramatically (anaerobic compensation). Management of relative humidity (90-95 %) of the air surrounding fruits it's important to prevent water losses. Water losses results in shriveling, flaccidness, soft texture and loss of nutritional value so, it is crucial to maintain high humidity in order to prevent these effects. The risk of freezing injury can be avoided if producers are aware of the susceptibility of a given commodity and the storage temperature. The lowest safe storage temperature is not the same for all fruits and therefore, storage of all commodities can result in decay.[36,65,68]

Despite the post-harvest techniques adopted there are other important factors to consider, the maturity indices: mandatory quality standards for most fresh fruit to ensure minimum acceptability of their flavor quality to consumers - defined as soluble solids concentration, TA and others. These elements are imperative for determining when the fruits should be harvested in order to provide marketing flexibility and to ensure acceptable eating quality to the consumer. [65,66]

There are not specific recommendations for appropriate post-harvesting conditions relative to *A. unedo* fruits: the safe ranges of gases applied, ventilation, safe temperatures, sensibility to ethylene or compatibility with other products in storage. So far, only Guerreiro *et al.* [13] studied the effect of two parameters, temperature and film covers of packages, on the storage ability of strawberry tree fruits (ripe, 22°Brix). They concluded that fruits stored at 0 and 3ºC, during 15 days, preserved the most attractive sensory properties. Guerreiro *et al.*[13] also concluded that at 0ºC these fruits preserved the highest antioxidant capacity. Two different film covers were tested: low density polyethylene films of 10 μm thickness and polyethylene films punctured with holes of 10 mm diameter spaced 50 mm. The results showed that low density polyethylene films were better than polyethylene ones because the first one promoted lower water losses.[13]

1.4 Innovation and development of new food products

New food products are products that have never been produced or commercialized or that have already been produced but are introduced into a new geographic area or into a new position in the market. Line extensions, reformulation of package or image, new flavors or new nutritional profiles of existing products, as well as cost optimization and quality improvement are also seen as new products.[67,69] From this definition, two approaches may be implemented: the incremental innovations and the radical ones. The radical innovations are creative products, addressing new demands previously unrecognized by consumers. Innovative products result from adaptation, refinement and enhancement of already existing products. They provide new benefits or improvements to the existing ones creating new opportunities in the market and increasing competitiveness. The incremental innovations are naturally driven by the product's life cycle (Figure 9).[67,69,70]

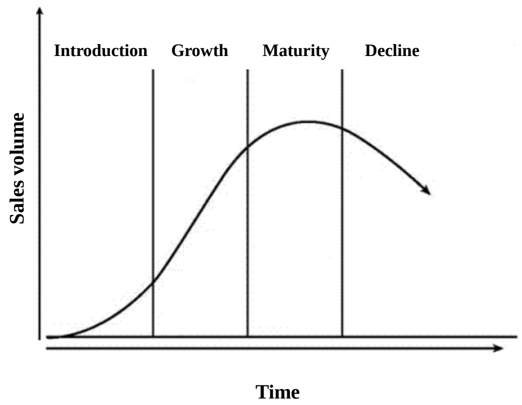


Figure 9 - Product's life cycle

Hence, the potential to introduce new successful products into the market determines which products have long lives and also manufacturer's sustainability.[70] Today there are lots of products in the market, trying to handle requirements of all consumers. The development of new food products is a basic activity in food companies. Though, since market is full of products, develop new ones and improve brand building is a tricky goal. It is necessary to capture consumer's needs, together with the companies' interests, to update and to maintain the selling's and the economic viability. The strategy surrounding the development and the marketing of a new product defines if the product is going to be more competitive and if it can attract the consumer's attention. [69]

1.4.1 Current market trends

In the last decades consumer's demands in the field of food have considerably changed. In particular, five groups of market trends were reached by The Brazil Food Trends 2020 project[71]: sensoriality and pleasure, healthiness and wellness, convenience and practicity, reliability and quality and sustainability and ethics (Figure 10).

Figure 10 - Examples of product's characteristics regarding each one of the five groups of trends reached by Brazil Food Trends 2020 Project. [71]

In 2014 it was expected that clinical nutrition, high quality and sustainable products were among the top food trends. The market research also predicted that reducing waste and regaining consumer trust were among the top market trends to look out for.[72] Clinical nutrition is being used as a profitable platform to improve wellness, so allergen concerns, antioxidant content, among others, will continue to increase, prompting innovative solutions. Likewise, healthy lifestyles are here to stay and a whole new generation of superfruits will emerge. Consumers are looking for 'back to basics' pleasures, like simple cooking, home-cooked food. Moreover, high quality and distinct and gourmet products that hold small scale appeal, but big potential, are targeting niche markets. Encompassing flavors include personalization and elevation of sensory experience. Figure 11 shows the relationship between factors determining consumer's concerns and encouraging the actual market trends.[71,73–75]

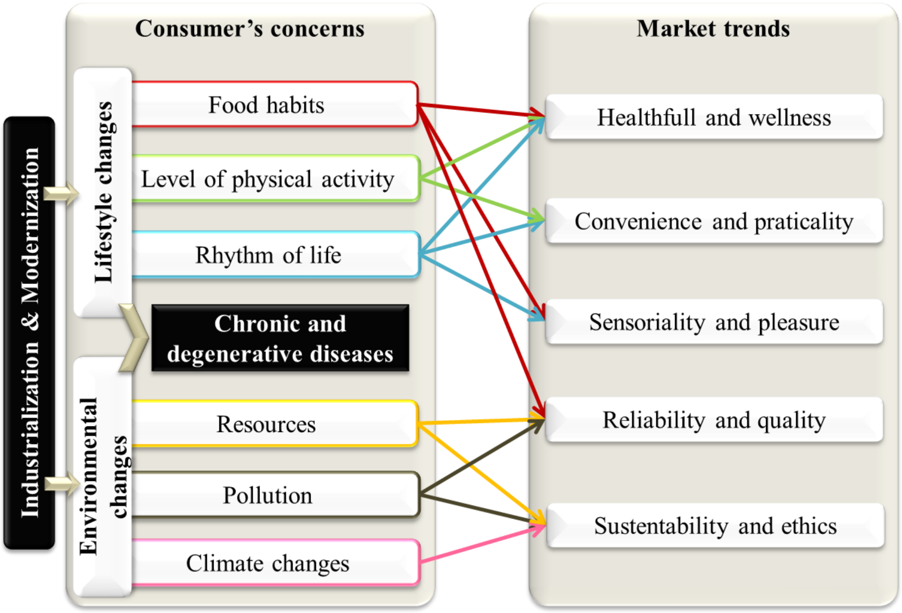


Figure 11 - Relationship between consumers concerns and the actual market trends.[71]

Industrialization and modernization provided growing and aging of population, greater accesses to health care, to information and also to technology. The growing, urbanization and aging of population raised the demand for foods, especially processed or industrialized: with economic development individuals migrated to urban areas in search for better opportunities and as a consequence, they no longer produced their own food. However, negative consequences emerge from this change in lifestyle: (1) higher pollution levels, affecting climate and ultimately, resources and (2) a modern lifestyle, characterized by a low level of exercise, by a stressful rhythm of life and high consumption of industrialized-processed foods was adopted.[71]

The aforementioned factors leaded to a higher incidence of chronic and degenerative diseases (such as obesity, cardiovascular diseases and diabetes), which are between the top 10 major causes of death worldwide.[72] Nevertheless, better education raised consumer's awareness of the consequences of modernization and industrialization. Thus, nowadays, consumers decide more appropriately which food products are best suited for them to consume and they search foods with specific nutritional characteristics.[75]

1.4.2 Innovation and development strategies

The development of new products goes through several stages as seen in Figure 12.

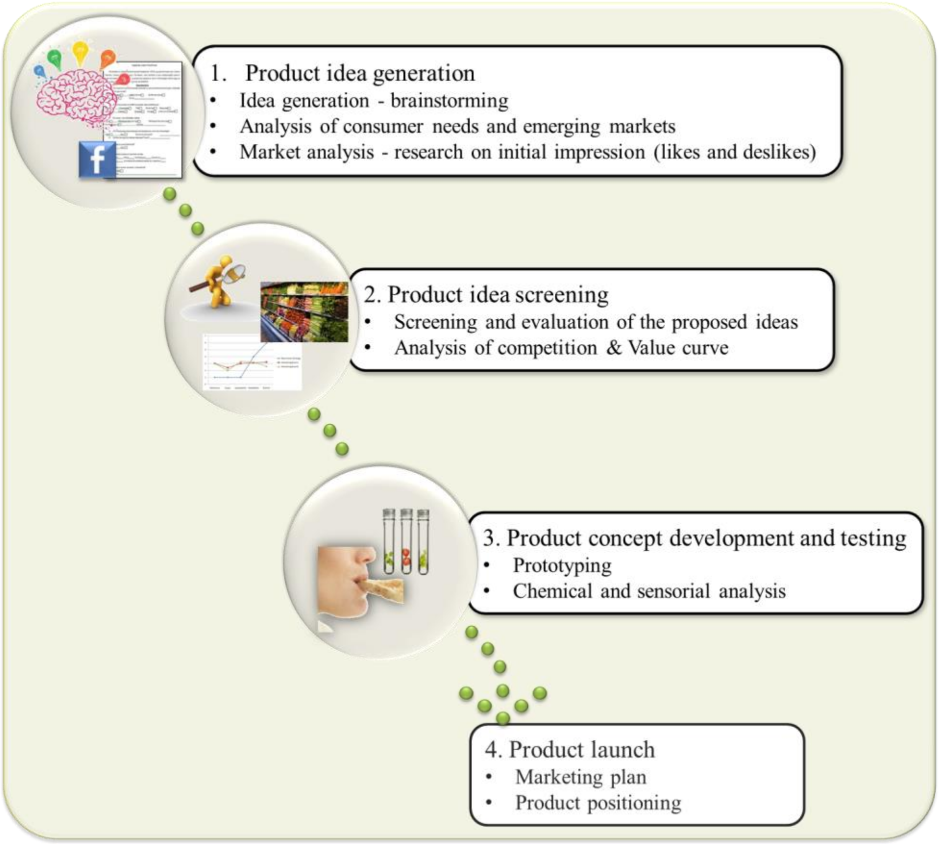


Figure 12 - Schematic representation of the steps associated with the process of developing new food products.[67,69,70]

Numerous steps are taken in each of these phases: ideas generation, concept engineering, business strategy, product viability and also the marketing, environmental and social evaluation. The basic steps of the new product development process are listed as sequential stages but in reality some of them are repeated over and over, making the process cyclical instead of linear. [67,69,70,76] To build up the whole base of the project it is essential to have both creativity and knowledge about the actual needs and opportunities.[67,69]

The methodology of developing a new product was divided into several steps until the goal originally proposed was reached. The product idea generation aims at gathering as much information as possible from potential consumers. Product idea generation involves brainstorming, analysis of consumer's needs and emerging markets, as well as market analysis. After meeting the relevant information collected through the previous steps related to the market analysis as well as the consumer's needs and the brainstorming, the generated ideas are screened: the ideas are studied to see if they were compatible with market suitability and technical possibility. Then, the prototypes are made and chemical and sensory analysis performed. In conclusion, because the consumers constantly look for new products for nutritional purposes, satisfaction or also interest, they demand innovation in the food industry, it is necessary to adopt strategies based on changing technology and consumer habits to survive in the actual crowded markets.[67,69]

1.5 Objectives of the present study

The main objective of the present thesis is the valuation of *A. unedo* fruits, and a strategy that included fruits chemical characterization and the development of new food products was implemented.

Only liqueurs, spirit and jam based on *A. unedo* fruits are available in the market.[6,15] In Portugal the fresh fruits are also not usually found in the market. So, as a starting-point it would be important to sell them as fresh fruits because commercialization would allow the consumers to know the product. This step will also bring fresh insights into the consumer's opinion and make possible the success of novel products based on *A. unedo* fruits.

To fulfill the general objective the following specific objectives were defined:

1. Study of the classical parameters for quality determination during maturation of *A. unedo* fruits
2. Phytochemical research approach focused on lipophilic composition of ripen fruits
3. Valuation of *A. unedo* fruits based on:
    a. Evaluation of the effect of cold storage on the quality of fresh fruits harvested at different maturation stages
    b. Development of new food products

CHAPTER II – Experimental section

The experimental design of the work developed during this thesis is presented in Figure 13.

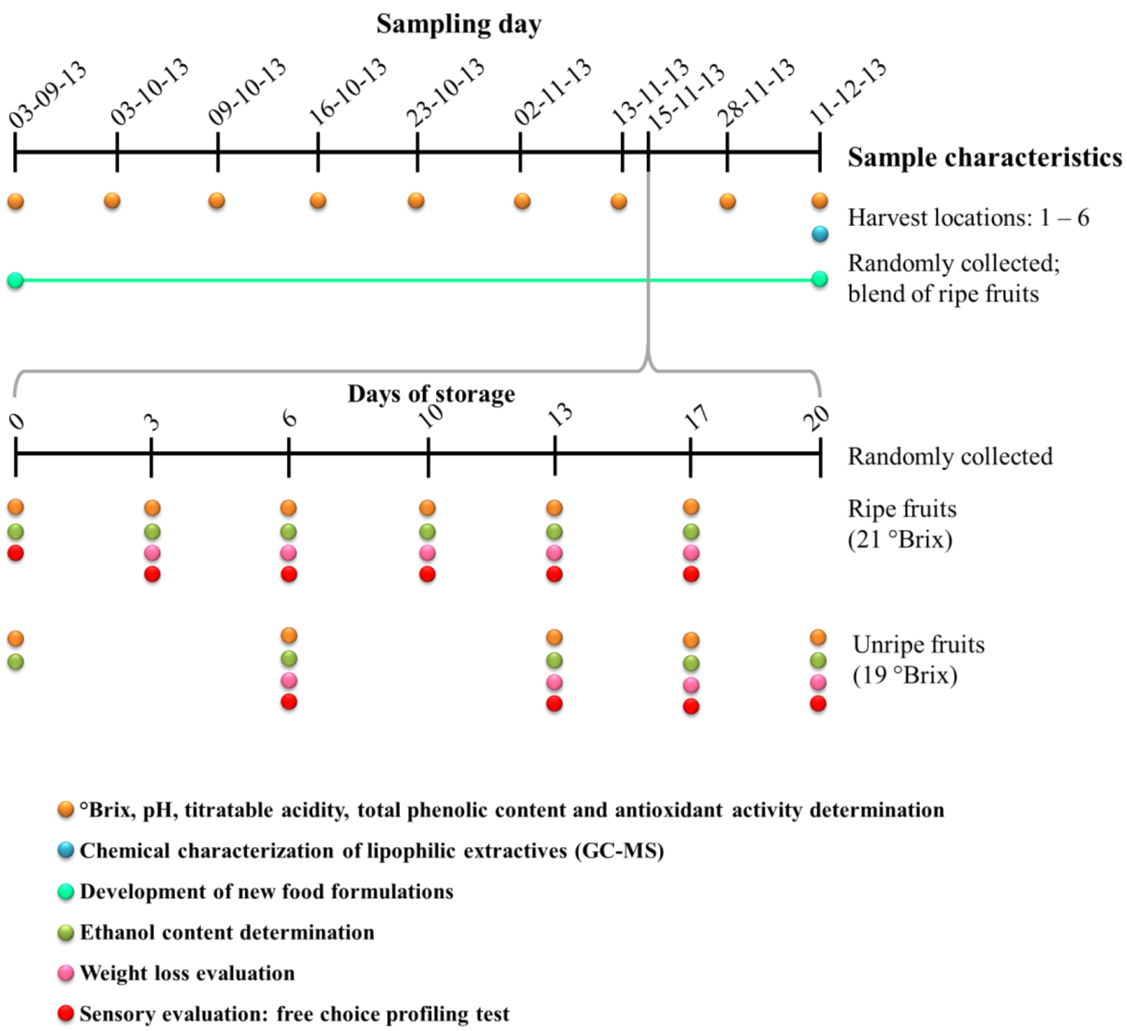


Figure 13 –Experimental design of the work developed during this thesis

To study the classical parameters for quality determination during maturation of *A. unedo* fruits there were used fruits of 6 locations harvested at 9 sampling days: September 3rd, October 3rd, October 9th, October 16th, October 23rd, November 2nd, November 13th, November 28th and December 11th. The phytochemical research focused on the lipophilic composition using fruits collected at each one of the 6 harvesting locations at the last harvesting day, December 11th.

The evaluation of the effect of cold storage on the quality of fresh fruits harvested at November 15th, at two different maturation stages (19 and 21 ºBrix). The fruits were randomly collected from the different locations, stored and the chemical and sensory determinations were made after 0, 3, 6, 10, 13, 17 and 20 days of storage. To the development of new food products there were randomly collected ripen fruits from all locations, from September to December.

2.1 Sampling

A. unedo fruits used in all experiments were collected in São Pedro de Alva, in the Serra da Beira region, Portugal. The samples were supplied by Medronhalva Lda. To study the classical parameters for quality determination during maturation, the fruits were harvested from 6 locations, over four months (from September to December) and stored frozen (-20ºC) until analysis. There were obtained 9 samples along maturation from each one of the harvesting location. The characteristics of the sample locations are described below (Table 3), the satellite view is in Figure 14 and a photographic recording of the fruits sampling is in Appendix 1.

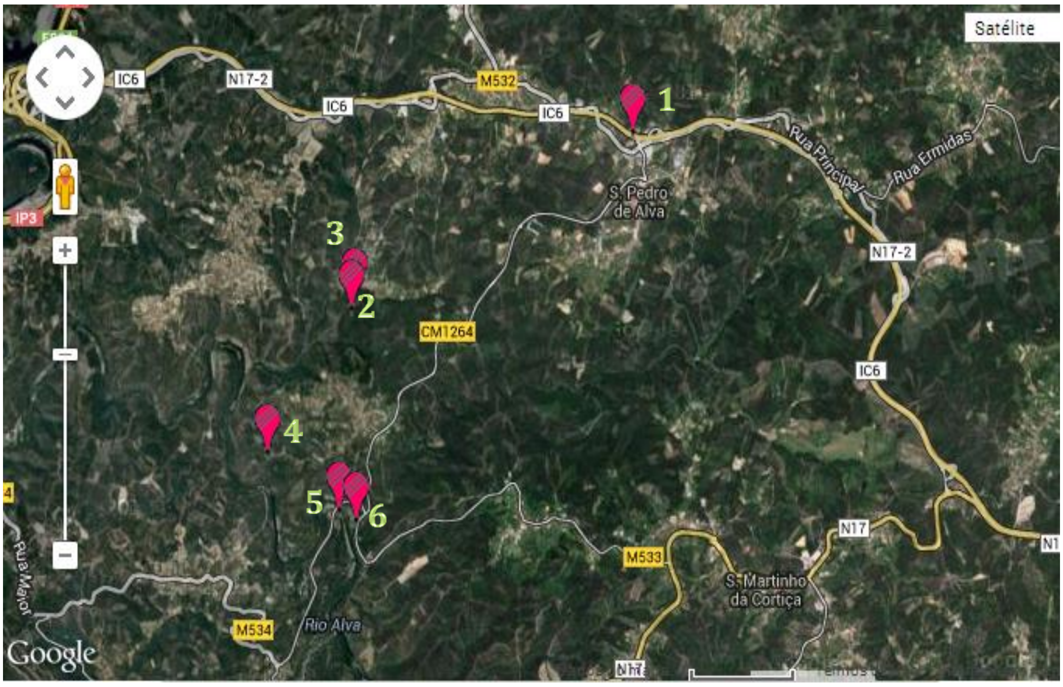


Figure 14 – Satellite view of the sampling locations

Table 3 - Characteristics of sample locations

	Harvest Locations					
	1 – São Pedro de Alva	**2 - Faval**	**3 – Costa da Nogueira**	**4 - Cheirada**	**5 - Roda**	**6 - Vimieiro**
Latitude	40.306	40.293	40.294	40.281	40.277	40.276
Longitude	-8.1668	-8.1978	-8.1975	-8.2072	-8.1993	-8.1973
Altitude	200.76 m	125.00 m	123.22m	100.00m	75.000 m	75.000 m
Orientation	Northeast	West	North	South-west	Northeast	Northwest
Soil type	Shale, with about 10 cm of clay and organic matter	Shale layer with high organic matter and clay	Shale, with considerable layer of organic matter and clay	Shale, with large numbers of stone rolled (pebbles) and clay	Rolling stone (pebble) with some clay on the (parent rock) shale	Shale, with a considerable layer of organic matter and clay
Sunlight exposure	Reduced due to canopy trees around	Moderate due to the proximity of some trees	High with direct exposure to sunlight	High with direct exposure to sunlight	Considerable, given the absence of trees or other cover nearby	High, with direct exposure to sunlight
Irrigation level	Low	Low, although with some water retention capacity at ground level	Low, although with some water retention capacity at ground level	Low, although with some water retention capacity at ground level	Low, although the soil can retain some moisture. Note: Alva River runs about 30 meters from this place	Low, although the soil can retain some moisture. Note: Alva River runs about 30 meters from this place

To evaluate the effect of cold storage on *A. unedo* fruits, the samples were harvested at November 15th, randomly collected (blend of the different locations) from São Pedro de Alva region. Fruits of two different ripening stages: ripen (≈21 ºBrix) and unripen (≈19 ºBrix) were separately collected (Figure 15). Berries were placed into two layers in plastic covers with perforations, and stored at ≈5°C in the dark.

Figure 15 – *A. unedo* fruits at harvest: (a) ripen and (b) unripen samples

To assess the lipophilic profile of strawberry tree fruits the samples collected at the last harvesting date (11th December) from locations 1 to 6 were used.

To the development of new food products ripen fruits were randomly collected (blend of the different locations) from São Pedro de Alva, Serra da Beira region, Portugal, and stored frozen (-20ºC) until use.

2.2 Quality evaluation of *A. unedo* fruits during maturation

To evaluate the quality parameters of *A. unedo* fruits during maturation there were used 9 samples collected through maturation from each one of the 6 harvesting locations of the Serra da Beira region described above (Figure 14, Table 3).

2.2.1 °Brix, pH and titratable acidity determination

The experiments were performed as described by Ruiz-Rodríguez *et al.*, 2001.[31] The °Brix was determined using a hand-held refractometer (Atago) directly in the fruits pulp. pH was measured using a Crison micropH 2000 pH-meter over an homogenized sample (obtained from the fruits manually crushed) 1/4 (w/v) in distilled water. TA was determined by titration with 0.1 M NaOH until pH of 8.1 was reached. TA was expressed as g malic acid.100 g^{-1} fresh weight.

2.2.2 Total phenolic content and antioxidant activity determination

Phenolic compounds extraction

A. unedo fruit samples were freeze-dried and ground prior to extraction. Each sample was submitted to a Soxhlet extraction with dichloromethane for 6 h to remove the lipophilic fraction.[77] The solid residue was suspended (mass/volume 1:100) in a methanol/water/acetic acid mixture, 49.5:49.5:1 at room temperature for 24 h under constant stirring to extract the phenolic compounds. The suspension was then filtered, methanol removed by low-pressure evaporation, and the extract freeze-dried.[78]

Total phenolic content determination

The TPC of the samples was determined by the Folin-Ciocalteu method as described by Singleton.[79] The method is based on the addition of 500 μL of distilled water to 125 μL of the diluted sample (the previously mentioned phenolic extract). The mixture is kept for 5 min and then 1.25 mL of Na_2CO_3 (75 $g.L^{-1}$) and 1 mL of distilled water are added. The solutions were kept for 30 min and then the absorbance was measured at λ=760 nm. All the measurements were made in triplicate, using three aliquots of each extract and the average value was calculated in each case. The calibration curve was performed using gallic acid as standard in a concentration range between 50 and 250 $mg.L^{-1}$. The results were expressed as mg of GAE.100 g^{-1} fresh weight. [78]

Antioxidant activity determination

The antioxidant activity was determined by the DPPH· method. Each extract was serially diluted in the extract to water: methanol ratio 1:2; 1:4; 1:8; 1:16 from an initial solution of 50 mg extract diluted in 10 mL water: methanol (50:50). From this diluted samples 0.1 mL was added to 3.9 ml of DPPH· solution. The mixture was kept for 30 min in the dark. The absorbance was measured at 515 nm, in a Perkin Elmer Lambda 35 UV/Vis Spectrometer and compared with a control sample. All measurements were made in triplicate, using three aliquots of each extract and the average value was calculated in each case. The antioxidant activity was expressed as EC_{50}, mg of dw extract that reduces DPPH· in 50 %.[78] EC_{50} was calculated by: (Abs control – Abs sample)/Abs control *100 and were determined from the plotted graphs of EC_{50} against the concentration of the extracts.

2.3 Evaluation of the effect of cold storage on the quality of *A. unedo* fruits harvested at different maturation stages

To evaluate the effect of cold storage there were used fruits of two different ripening stages: unripen and ripen (19 and 21 ºBrix respectively). The fruits were placed into two layers in plastic covers with perforations, and stored at ≈5°C in the dark. The parameters evaluated are shown in Figure 13. The key parameters - ºBrix, pH, titratable acidity, total phenolic content and antioxidant activity - are already described above.

2.3.1 Weight loss evaluation

This method was based on a method described by Ruiz-Rodríguez *et al.*, 2001.[31] Weight loss was evaluated measuring the initial and final fruit's weight. The fruit containers were measured once every three or four days, accordingly to the storage day (Figure 13), and this parameter was expressed as a percentage of the initial weight.

2.3.2 Ethanol content determination

Ethanol content was determined by headspace solid phase microextraction (HS-SPME) technique coupled with gas chromatography-flame ionization detection (GC-FID). This procedure was based on a method described by Cardeal *et al.*, 2005.[80] For the HS-SPME method a Divinylbenzene/Carboxen /Polydimethylsiloxane -DVB/CAR/PDMS-fiber, 50/30µm film thickness, from Supelco (Bellefonte, PA, USA) was used. For headspace sampling, 10 g of the fruits manually crushed was inserted into a 20.0 ml Pyrex glass vials. The vial was capped with Teflon-lined rubber septum aluminium caps. After that the fiber was introduced for 10 min in the vial thermostated at 40°C. Then, the fiber was withdrawn into the needle and inserted into the GC injector port for desorption. The analysis was carried out with a Perkin Elmer Clarus 400 gas chromatograph equipped with a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 µm film thickness) and a flame ionization detector. The splitless mode was used (1 min). The injector was kept at 220 °C. Hydrogen was the carrier gas (17 $mL.min^{-1}$). The oven initial temperature, 35ºC, was held at for 1 min. The temperature was raised to 50ºC at 5ºC min^{-1} and finally to 220ºC at 25ºC min^{-1}. The FID was kept at 270 °C. A blank of the column was carried out before the sample analysis and the fiber was cleaned by exposing it for 5 min at 250ºC. The results were expressed as area of ethanol/fruit mass ratio (A/m).

2.3.3 Sensory analysis

The sensory evaluation was performed by an untrained panel (10 tasters similar to consumers). The ripen samples were tested at the harvesting day and after 3, 6, 10, 13 and 17 days of storage (Figure 13). At day 20, the fruits presented an unpleasant appearance, indicative of decay, determining the end of storage time at day 17. Regarding the unripen samples, sensory evaluation was performed after 6, 13, 17 and 20 days of storage (Figure 13). After this period, the unripen fruits presented an unpleasant appearance and 20^{th} day was defined as the last day of storage. To each taster there were presented four fruits, placed above a white paper. All panel members helped defining the sensory profile of *A. unedo* fruits and the free choice profiling test is presented in Figure 16.

Panel members were asked to evaluate the appearance, aroma, texture, sweetness, acidity, firmness, flavor and overall liking through a free choice profiling test based on a intensity scale from 1 to 5 (1 (the less perceptible quantification of a defined parameter) and 5 (the most intense perception regarding a defined parameter).

The Graph Pad Prism Software Version 6.04 Trial (GraphPad Software, Inc.) was used to calculate the mean values and standard deviations and to evaluate statistical significance of differences carried by One-Way Analysis of Variance (ANOVA), followed by Tukey's test for multiple comparisons and a significance level of about 5 %. All results are expressed as mean values ± standard deviation.

Free Choice Profiling

Ficha de Prova do Medronho

Data___-___-___ Código da amostra:________

Aparência

% da cor vermelho na superfície do fruto

0%		25%		50%		75%		100%
1	-	2	-	3	-	4	-	5

Intensidade da Cor

Vermelho		Vermelho escuro
1	-	2

Castanho ☐

Outra (Qual?)________

Cor da polpa do fruto

Amarelo esverdeado				Amarelo pálido				Amarelo alaranjado
1	-	2	-	3	-	4	-	5

Odor

Odor característico	Etanol
☐	☐

Outro:__________

Pouco intenso								Muito intenso
1	-	2	-	3	-	4	-	5

Consistência aparente

Firmeza

Muito firme								Muito Mole
1	-	2	-	3	-	4	-	5

Consistência na boca

Pouco arenoso								Muito arenoso
1	-	2	-	3	-	4	-	5

Figure 16 - Free choice profiling test performed during the study of the effect of cold storage on *A. unedo* fruits

Firmeza na boca

Muito firme 1 - 2 - 3 - 4 - 5 Pouco firme

Pouco pastoso 1 - 2 - 3 - 4 - 5 Muito pastoso

Sabor

Pouco doce 1 - 2 - 3 - 4 - 5 Muito doce

Pouco ácido 1 - 2 - 3 - 4 - 5 Muito ácido

Nada adstringente 1 - 2 - 3 - 4 - 5 Muito adstringente

Pouco intenso 1 - 2 - 3 - 4 - 5 Muito intenso

Persistência na boca

________seg

Classificação Global

Má		Medíocre		Aceitável		Boa		Muito boa
1	-	2	-	3	-	4	-	5

Figure 16 (cont)- Free choice profiling test performed during the study of the effect of cold storage on *A. unedo* fruits

2.4 Chemical characterization of the lipophilic extractives from *A. unedo* fruits

To determine the lipophilic profile of *A. unedo* fruits the samples harvested at December 11th were selected (Figure 13). The fruits were freeze-dried and ground prior to extraction. Each sample was submitted to a Soxhlet extraction with dichloromethane for 6 h and the solvent was evaporated to dryness at low pressure obtaining a dichloromethane extract. The dried extracts were weighed and the results are expressed as percentages of dry biomass material.[77]

2.4.1 Alkaline hydrolysis

The procedure was based on a method previously reported by Freire *et al*., 2002.[77] The *A. unedo* fruit dichloromethane extracts were divided into two different groups: before alkaline hydrolysis (BH) and after alkaline hydrolysis (AH). The alkaline hydrolysis reaction was performed to detect indirectly esterified compounds, e.g., triglycerides, steryl esters, etc. About 20 mg of each dichloromethane extract was dissolved in 10 mL of 1 M NaOH in 50 % aqueous methanol. The mixture was heated at 100 °C, under a nitrogen atmosphere, for 1 h. The reaction mixture was cooled, acidified with 1 M HCl to pH ≈ 2, and then extracted three times with dichloromethane, and the solvent was evaporated to dryness.

2.4.2 *Gas chromatography-mass spectrometry* (GC-MS) analysis

Before GC-MS analysis, nearly 20 mg of each dried sample was converted into trimethylsilyl (TMS) derivatives. Each sample was dissolved in 250 μL of pyridine containing 1 mg of tetracosane (internal standard), and compounds with hydroxyl and carboxyl groups were converted into TMS ethers and esters, respectively, by adding 250 μL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide and 50 μL of trimethylchlorosilane. The mixture was maintained at 70 °C for 30 min.

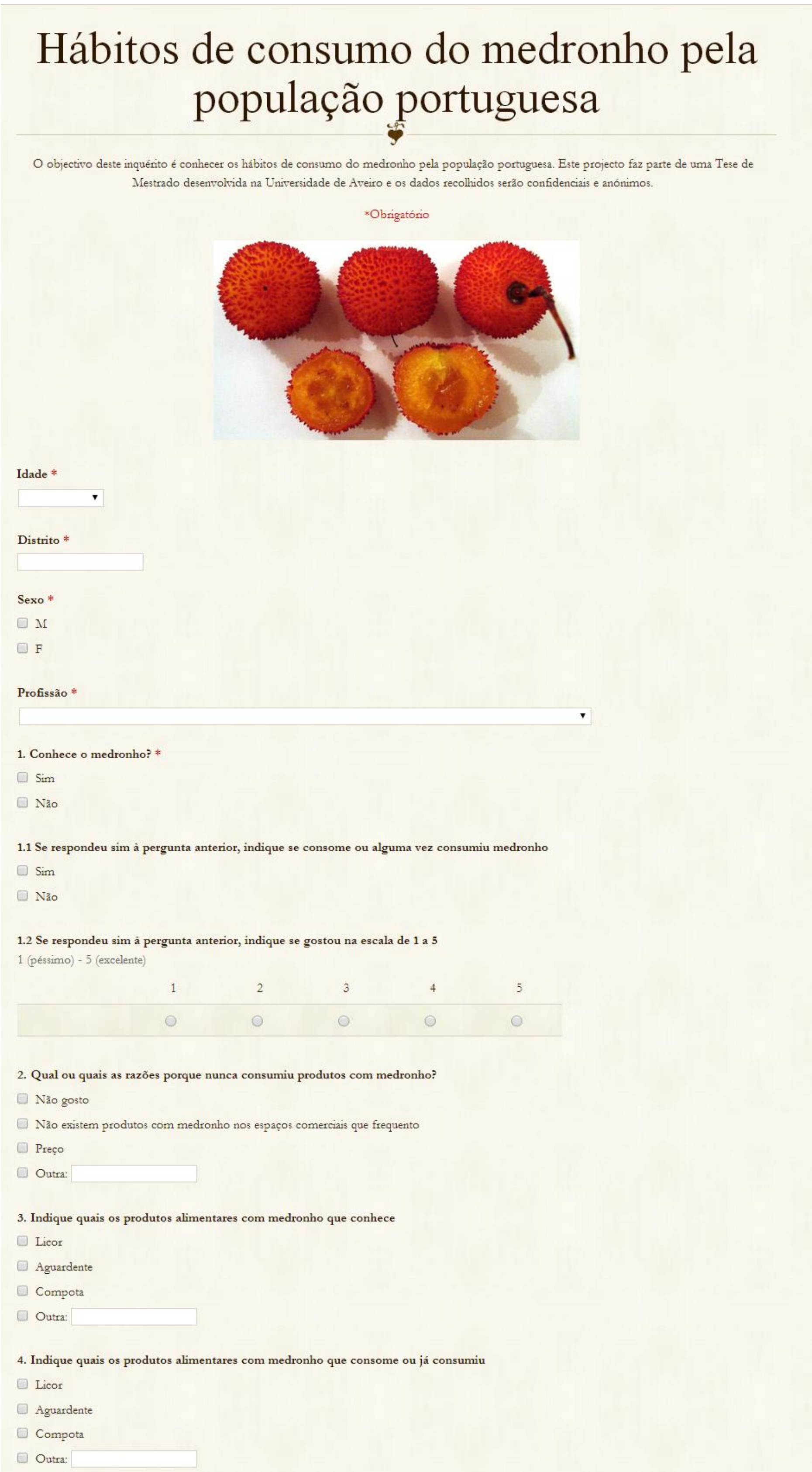
GC-MS analyses were performed using a Trace gas chromatograph (2000 series) equipped with a Thermo Scientific DSQ II mass spectrometer (Waltham, MA). Separation of compounds was carried out in a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness) using helium as the carrier gas (35 $cm.s^{-1}$). The chromatographic conditions were as follows: initial temperature, 80°C for 5 min; temperature rate, 4°C min^{-1} up to 260°C, 2 °C min^{-1} up to 285°C, which was maintained for 8 min; injector temperature, 250 °C; transfer-line temperature, 290 °C; split ratio, 1:33. The mass spectrometer was operated in the electron impact (EI) mode with an energy of 70 eV, and data were collected at a rate of 1 $scan.s^{-1}$ over a range of mass-to-charge ratio (*m/z*) 33-700. The ion source was kept at 250 °C. To detect the presence of esterified structures, dichloromethane extracts were also analyzed in a DB-1 J&W short capillary column (15m × 0.32 mm inner diameter, 0.25 μm film thickness); the chromatographic conditions were as follows: initial temperature, 100 °C for 3 min; temperature gradient, 5 °C min^{-1}; final temperature, 340 °C for 12 min; injector temperature, 290 °C; transfer-line temperature, 290 °C; split ratio, 1:33. Chromatographic peaks were identified by comparing their mass spectra with the equipment mass spectral library (Wiley-NIST Mass Spectral Library, 1999) and with literature data (as specifically adressed in the Results and Discussion section) and, when needed, by injection of standard samples. In some cases, identification was also confirmed on the basis of characteristic retention times (RTs) under the described experimental conditions. For quantitative analysis, GC-MS was calibrated with pure reference compounds, representative of the major lipophilic extractive components (namely, hexadecanoic acid, nonadecan-1-ol, cholesterol, ursolic, and oleanolic and betulinic acids) relative to tetracosane (the internal standard). For tocopherol the response factor of sterols was used. The respective response factors were calculated as the average of four GC- MS runs. Two aliquots of each extract were analyzed BH, and another two AH. Each aliquot was injected in duplicate. The compound contents are expressed as $mg.kg^{-1}$ of dw of plant biomass, mean values ± standard deviation.

2.5 Development of new food products based on *A. unedo* fruits

2.5.1 Market opportunities

2.5.1.1 Searching for information about the consumption of *A. unedo* fruits in Portugal

To search for information about the knowledge and consumption of *A. unedo* fruits the Portuguese consumers were interviewed – via internet (social networking site) and also via postal. The survey drawn up to this purpose is presented in Figure 17. For each consumer the following information was gathered: (1) age, sex and professional activity; (2) the knowledge of *A. unedo* fruits and rate of fruit's taste, on a scale from 1 (bad) to 5 (excellent); (3) to those surveyed that had never consumed strawberry tree fruits were asked what was the reason; in conclusion it was asked (4) what food products based on *A. unedo* fruits did people knew and which were consumed. The technical record arising from this survey is in Appendix 2.



Hábitos de consumo do medronho pela população portuguesa

O objectivo deste inquérito é conhecer os hábitos de consumo do medronho pela população portuguesa. Este projecto faz parte de uma Tese de Mestrado desenvolvida na Universidade de Aveiro e os dados recolhidos serão confidenciais e anónimos.

*Obrigatório

Idade *

Distrito *

Sexo *

- M
- F

Profissão *

1. Conhece o medronho? *

- Sim
- Não

1.1 Se respondeu sim à pergunta anterior, indique se consome ou alguma vez consumiu medronho

- Sim
- Não

1.2 Se respondeu sim à pergunta anterior, indique se gostou na escala de 1 a 5

1 (péssimo) - 5 (excelente)

1	2	3	4	5
○	○	○	○	○

2. Qual ou quais as razões porque nunca consumiu produtos com medronho?

- Não gosto
- Não existem produtos com medronho nos espaços comerciais que frequento
- Preço
- Outra:

3. Indique quais os produtos alimentares com medronho que conhece

- Licor
- Aguardente
- Compota
- Outra:

4. Indique quais os produtos alimentares com medronho que consome ou já consumiu

- Licor
- Aguardente
- Compota
- Outra:

Figure 17 - Survey conducted within the framework of consumption habits of *A. unedo* fruits by the Portuguese consumers

2.5.1.2 SWOT analysis and ideas generation: brainstorming

A SWOT analysis was carried out by a group of persons with simple knowledge on *A. unedo* fruits. There were defined the strengths, weaknesses, opportunities and threats (SWOT analysis) of *A. unedo* fruits.

Brainstorming was also carried out by the same group of persons aforementioned and the discussion was encouraged by the following questions: (1) What does the appearance of *A. unedo* fruits reminds you? Describe *A. unedo* fruits; and (2) Accordingly to the actual trends which new products based on *A. unedo* fruits would you suggest?

2.5.2 Product concept development

Product concept was developed from consumer research and brainstorming with consideration of the technical aspects of the products by the same group of persons aforementioned. To build up the product concept the following products were created: cookies, cereal bars, yoghurt and chocolates. The prototypes were home-made; the full recipes are subject of secrecy and will not be part of a public document. In Figure 18 is presented the production plan adopted during this thesis.

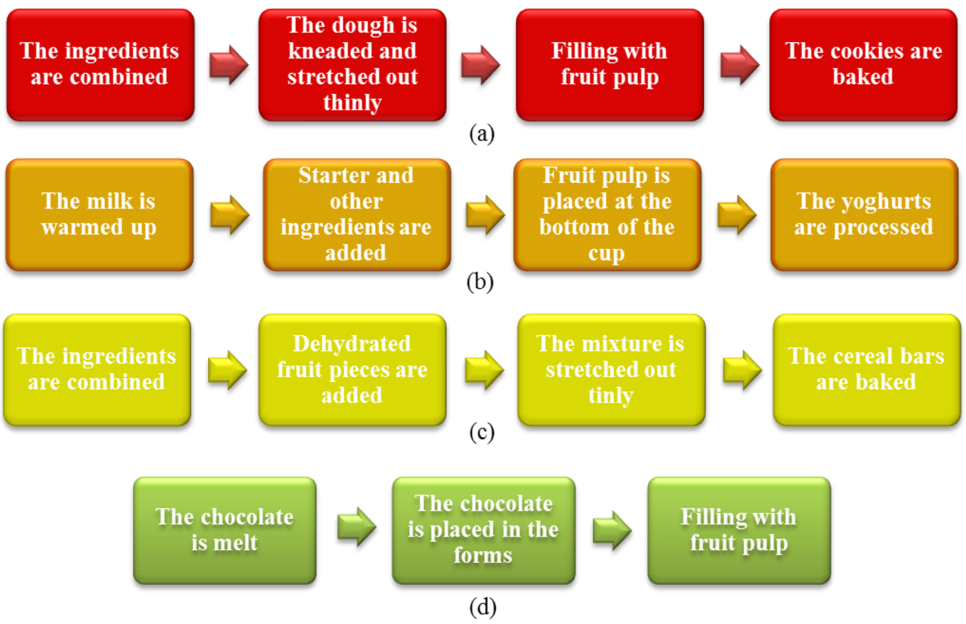


Figure 18 – Production chart for the products made with *A. unedo* fruits (a) cookies, (b) yoghurt, (c) cereal bars and (d) chocolates.

CHAPTER III – Results and Discussion

3.1 Quality evaluation of *A. unedo* fruits during maturation

3.1.1 °Brix, pH and titratable acidity determination

Fruit sugar content might be controlled by using ºBrix. This index is also used as a maturity indicator useful for harvest scheduling.[65] The ºBrix of strawberry tree fruits (harvested from six locations, during maturation in Serra da Beira region) is presented in Figure 19.

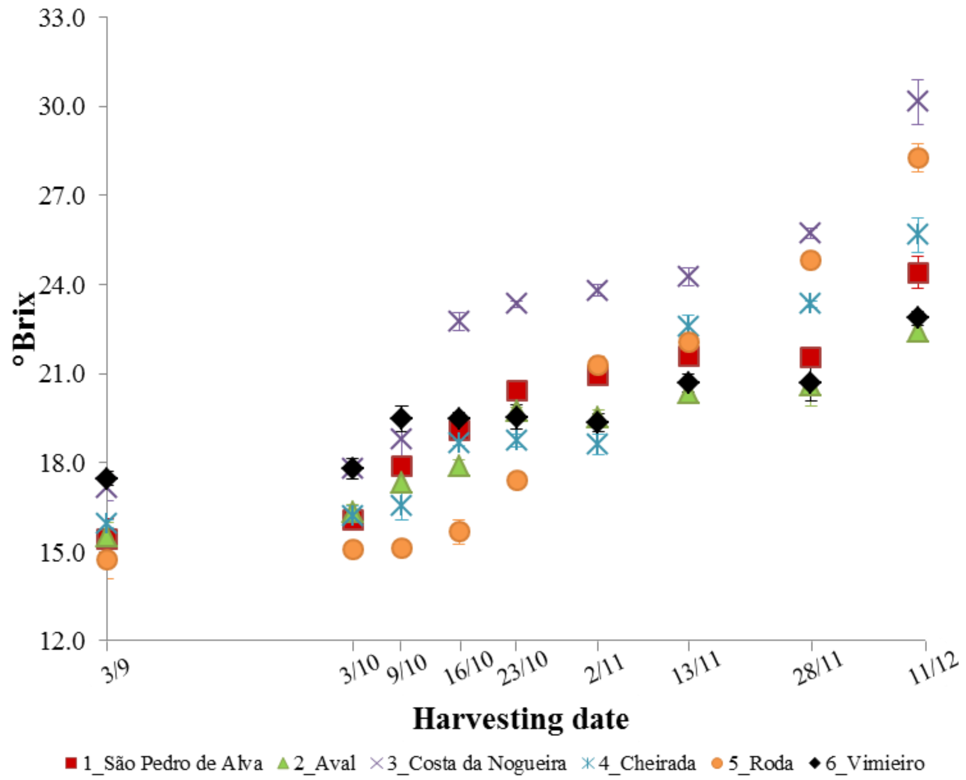


Figure 19 - ºBrix content along maturation in *A. unedo* fruits harvested from six different locations

An increase in ºBrix was observed for the samples harvested in the six locations, throughout maturation. At the first harvest, in September, fruits presented ºBrix content between 14.73 and 17.40. At the last harvest, in December, it ranged between 22.40 and 30.13. Locations 3 and 5 were the ones where the highest sugar content occurred, at the end of the harvesting season. Considering that the last harvested fruits, at December 11th, correspond to ripen fruits (because all berries present a uniform scarlet color), it is possible to conclude that these results are in global accordance with the ones reported in literature (for ripen fruits) which showed that the ºBrix content ranged from 16.50 to 31.68.[10,13,32] To the best of our knowledge, this is the first study regarding the influence of ripening on ºBrix content and Portuguese fruits. These results are thus especially important to characterize Serra da Beira fruits, understand the changes along the season and to provide an overview of the maturity and harvest scheduling.[65]

pH values of the strawberry tree fruits harvested in six locations, during ripening, are displayed in Figure 20.

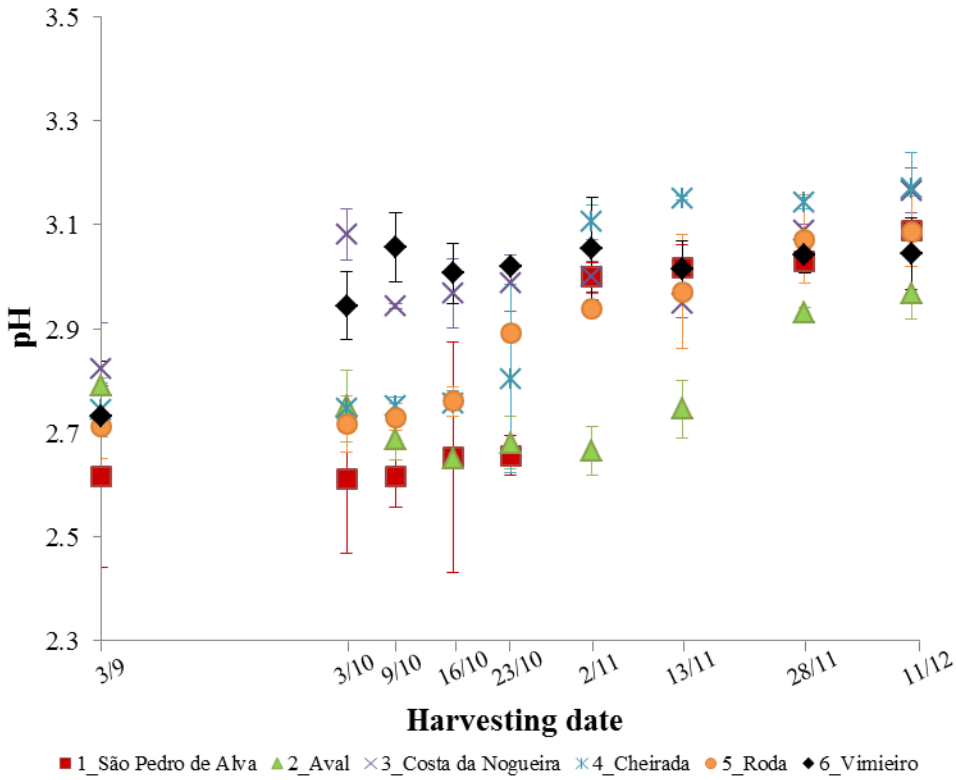


Figure 20 - pH content along maturation in *A. unedo* fruits harvested from six different locations

pH proved to increase through maturation. The pH values, initially at 2.62, 2.79, 2.74 and 2.71, respectively for location 1, 2, 4 and 5, remained constant until October 23rd, November 13th, November 2nd and October 23rd respectively. Thereafter, an increase was observed, until the last harvesting day. Regarding location 3 and 6, pH values, initially at 2.82 and 2.73 respectively, increase from the first to the second harvesting and subsequently remained constant along maturation. The fruits harvested in September had pH ranging between 2.62 and 2.82, while in December it ranged between 2.97 and 3.17.

Such results on *A. unedo* fruits showed lower pH values than those reported in literature for ripen fruits (3.21≤pH≤4.6).[30,31] No studies have been conducted on this parameter, either on Portuguese fruits or during maturation.

Titratable acidity (TA) contents of *A. unedo* fruits during ripening are shown in Figure 21.

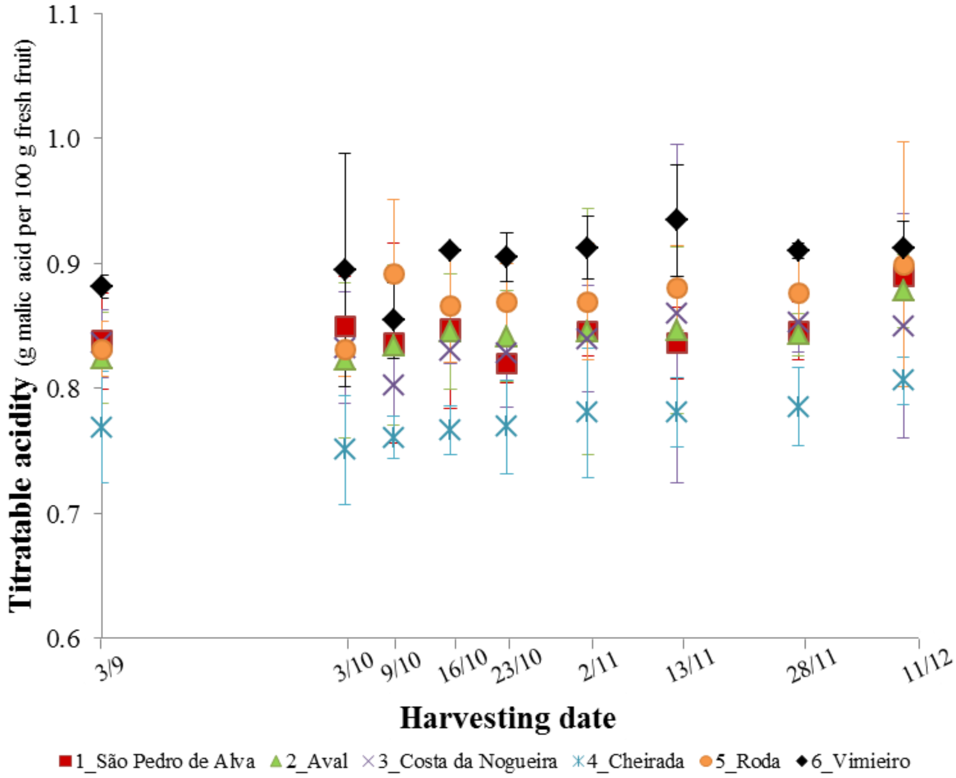


Figure 21 -Titratable acidity along maturation in *A. unedo* fruits harvested from six different locations

As maturation was occurring, TA of the first-harvested fruits was between 0.77 and 0.88; during last harvesting period, TA ranges between 0.81 and 0.91 g of malic acid.100 g^{-1} fresh fruit. TA of the last-harvested fruits is in accordance with the values reported for ripened fruits, which ranges between 0.40 and 1.59 g malic acid.100 g^{-1} fresh fruit. [10,30,34] TA revealed positive lower correlations with pH ($0.1<r^2<0.3$), except for location 4 (r^2=0.7) where in general the pH goes up and TA goes down during maturation.

It is important to highlight that flavor is determined by the balance between acids and sugars. Organic acids are the second contributors (after sugars) and the ºBrix/TA ratio is considered an index of quality and used to determine harvesting.[81] Thus, this study can be used as a decision tool to support *A. unedo* fruits producers since it helps to establish the best harvesting moment. Considering the last harvesting date, December 11th, average sugar/acid ratios of *A. unedo* fruits were 27.42, 25.51, 35.46, 31.83, 31.43, and 25.06 for locations 1 to 6 respectively. Location 1, 2 and 6 have the lowest ºBrix/TA ratio while locations 3, 4 and 5 show the highest. Among these results, location 3 may be the one whose crop is more readily accepted by consumers, considering its higher sugar content. Previous studies showed that ripen strawberry tree fruits from Turkey and Croatia are sweeter than the Portuguese berries, reaching up to a 50.06 sugars/acids ratio.

In literature it is reported that the ºBrix content of some fruits, such as grapes, increases with greater light exposure and in this study, locations 3 and 5 are both highly exposed at sunlight, which can explain the results obtained.[82] Locations 4 and 6 are also highly exposed at sunlight: location 4 presents the fruits with the third highest ºBrix; location 6 presented one of the highest ºBrix content at the beginning of the study. Regarding the pH, in literature is reported that this parameter declines as sunlight exposure increases, being negatively correlated with radiation and positively correlated with water availability.[82,83] The pH of *A. unedo* fruits is higher at the last harvesting day than at the first harvesting day. In general, this is in accordance with that reported in literature since as maturation goes on, we move from fall to winter, when the sunlight exposure is reduced (regardless of sunlight exposure reported in Table 3) and weather becomes wettest. Although the fruits were collected from locations with different characteristics, no particular correlation was observed between location/characteristic and pH. In literature, TA of fruits generally declines as sunlight exposure increases, being negatively correlated with radiation and positively correlated with water availability.[82,83] In the same away as aforementioned to pH, although the fruits were collected from locations with different characteristics, no correlation was observed between location/characteristic and TA.

3.1.2 Total phenolic content and antioxidant activity determination

The results regarding the phenolic content of *A. unedo* fruits during maturation are shown in Figure 22.

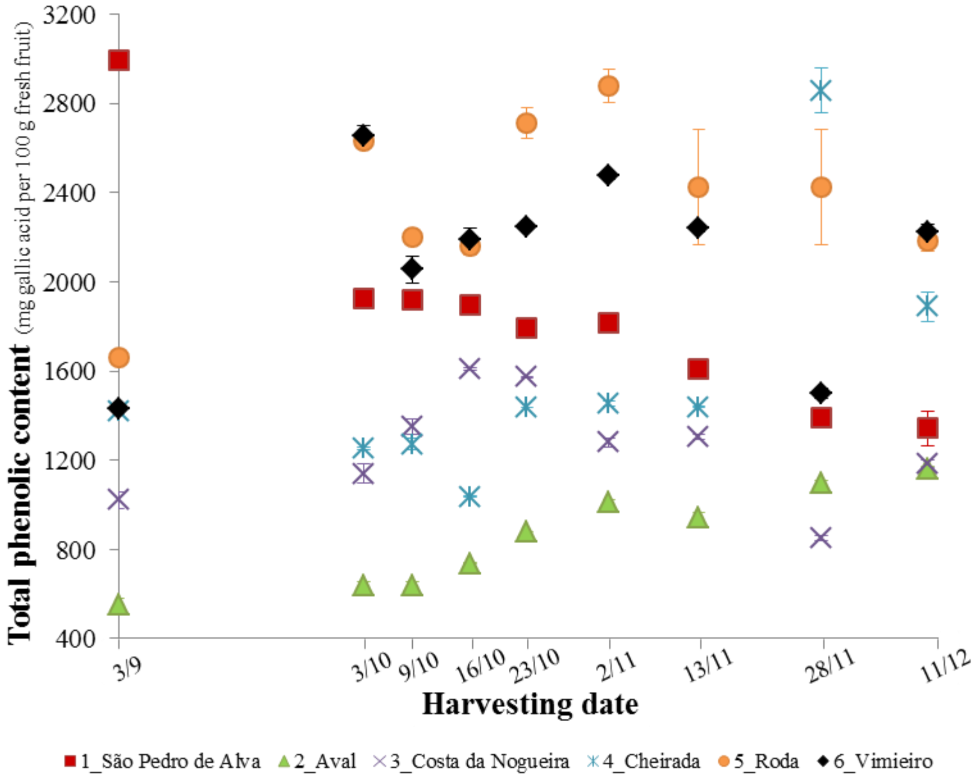


Figure 22 - Total phenolic content along maturation in *A. unedo* fruits harvested from six different locations

TPC exhibited a high variability throughout all maturation and location-wise. TPC of fruits harvested in September ranged between 554 and 2990 mg GAE.100 g^{-1} of fresh fruits. For those harvested in December, TPC varied between 1160 and 2222 mg GAE.100 g^{-1} of fresh fruits. The highest content of TPC in ripen fruits, considering the last harvesting at December 11th, was reached on location 6 (the lowest results having been obtained from fruits of location 1). The strawberry tree fruits of the last-harvesting, December 11th, showed higher values than those reported in literature for ripen fruits (between 590 and 1973 mg GAE.100 g^{-1} of fresh fruits). [31–33] A high phenolic content variability is visible during maturation regardless the location. Interestingly, fruits from location 1 were the only ones whose TPC decreased over time. This tendency (i.e. TPC decreasing during ripening) has already been reported for Portuguese strawberry-tree fruits, by Alarcão-E-Silva *et al.*, [11], who studied fruits of two different stages of ripeness and assessed total phenols, tanins and anthocyanins content. The results obtained showed that, despite increasing anthocyanin content, tannins decreased over ripening (which also was the case for TPC).

Phenolic compounds show radical-scavenging properties and play an important role in human nutrition. In this perspective, the measurement of the antioxidant activity is of great importance, as well. The antioxidant activity is due to the existence of various molecules present in the fruit, including phenolic compounds.[26,84] Antioxidant activity of *A. unedo* fruits during maturation, in six different locations, is given in Figure 23.

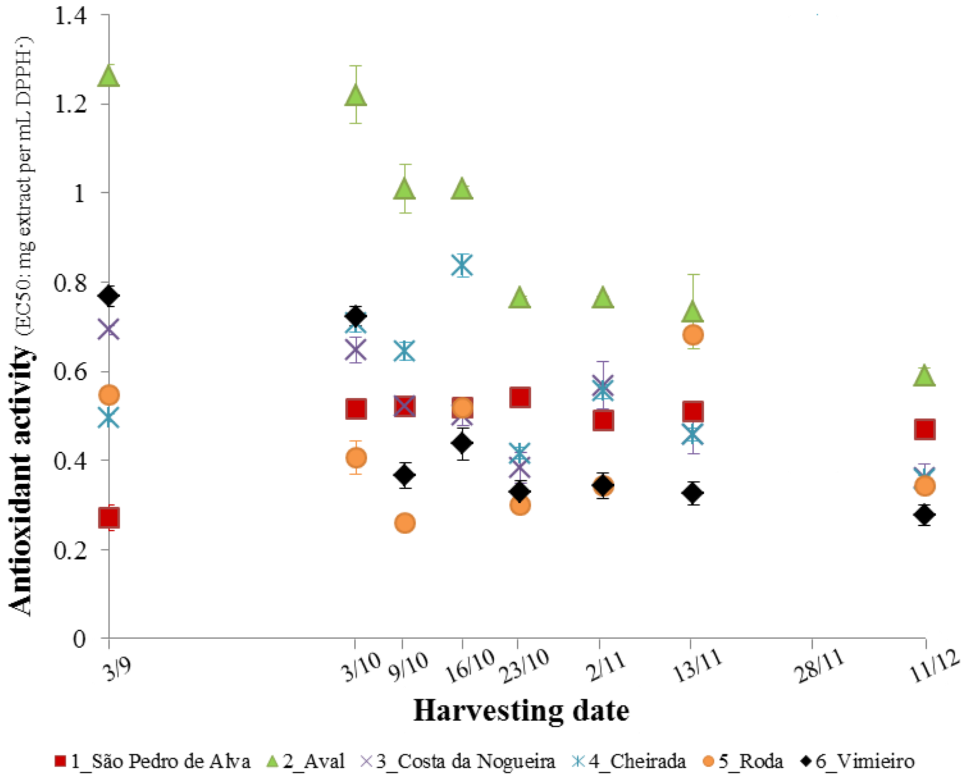


Figure 23 -Antioxidant activity along maturation in *A. unedo* fruits harvested from six different locations (samples at November 28th were not considered)

Similarly to TPC, antioxidant activity is quite variable, depending on location and maturation stage. Regarding the antioxidant activity of strawberry-tree fruits, the lowest value was reached by the samples harvested in September, ranging between 0.269 and 1.26 mg (extract.mL^{-1} DPPH·).In the last-harvested fruits, EC_{50} ranged between 0.277 and 0.588 mg (extract.mL^{-1} DPPH·). Previous studies showed that the antioxidant activity of ripe *A. unedo* fruits (expressed as EC_{50}) ranges between 0.25-0.79 mg extract.mL^{-1} DPPH·.[38,42,45]

In this study, the antioxidant activity of the ripen fruits, considering the last-harvesting, is within the range reported for ripen fruits.

Only one work concerning Portuguese fruits was developed regarding how ripening stage influences the antioxidant activity of *A. unedo*. Oliveira *et al.*, [45] considered three ripening stages (unripen, intermediate and ripen) and the results showed that the antioxidant activity increased during ripening ((DPPH· assay) - EC_{50} of unripe fruits was 0.58±0.03, intermediate 0.37±0.02 and ripe 0.25±0.02 mg extract.mL^{-1} DPPH·). Comparing the aforementioned data, regarding unripe and ripe fruits (since the intermediate stage is not comparable) to the results obtained in this thesis (concerning the first and the last day of storage) it is possible to conclude that the present results are within the range reported. It was observed no strong positive correlation between antioxidant activity and TPC, except for location 1, 2 and 3, which reflects that phenolic compounds are not exclusive in determining the scavenging properties in the fruit.

The TPC of *A. unedo* may be a result from various factors (such as type of soil, microclimatic conditions, geographic position, site, age and vegetational stage of plants).[44] In literature is reported that sun-exposed grape clusters had almost 10 times greater concentration of flavonols than shaded clusters.[82] Also, the water status can affect phenolic content: it was reported an increase in anthocyanins in berries of vines subjected to a water deficit.[85] We observe that the highest TPC values are reached at location 1, 5 and 6 at the first harvesting days and at location 4, 5 and 6 at the last harvesting days. The lowest phenolic content at the beginning of the study was reached at location 2 and 3 and at the end of the study, location 1, 2 and 3 presented the lowest phenolic content. In this study it was not possible to directly correlate all the different location characteristics to the phenolics variability. Regarding antioxidant activity of *A. unedo* fruits there was also no clear correlation of location/characteristic and the variation through maturation.

A general description of the ripen strawberry tree fruits (considering the last harvesting, December 11th) studied here was performed and the results obtained are shown in Table 4 and compared with literature.

ºBrix content of ripen strawberry tree fruits showed values similar to those reported in literature, considering Portuguese fruits and from other European origins. [10,32,34] TA for

Portuguese fruits was assessed for the first time and the results are within the range reported for the fruits harvested out of Portugal. [10,32,34] pH values are lower than the ones already reported for *A. unedo* fruits.[10,30,31] TPC, on the other hand, reached higher values that those already published, not only considering Portuguese samples but also the European ones.[31,32] The observed differences in these parameters can be justified by different environmental conditions, genetic variability and location.[44] Overall, the strawberry-tree fruits studied along this thesis revealed a chemical profile that can be useful to characterize this species of Serra da Beira region.

Table 4 - General characterization of *A. unedo* ripen fruits

Location	ºBrix	Titratable Acidity[a]	pH	Total Phenolic Content[b]	Antioxidant activity[c]
1	24.4±0.5	0.89±0.02	3.08±0.02	1342±76	0.469±0.02
2	22.4±0.3	0.88±0.02	2.97±0.05	1160±13	0.589±0.01
3	30.1±0.8	0.85±0.09	3.17±0.04	1185±18	0.359±0.03
4	25.7±0.6	0.81±0.10	3.17±0.07	1889±66	0.356±0.01
5	28.3±0.5	0.89±0.09	3.09±0.07	2183±48	0.343±0.06
6	22.9±0.2	0.91±0.02	3.05±0.07	2222±35	0.278±0.02
Literature (Portuguese)[ref]	≈22[13]	-	-	≈445-941[35]	0.25-0.79[38,42,45]
Literature (elsewhere)[ref]	16.50-31.68[10,32,34]	0.4-1.59[10,30,32,34]	3.21-4.6[10,30,31]	590-1973[31,32]	-

[a,] titratable acidity expressed as g malic acid per 100 g fresh weight, [b,] total phenolic content expressed as mg gallic acid per 100 g fresh weight, [c,] antioxidant activity expressed as EC_{50}: mg extract per mL DPPH·.

3.2 Evaluation of the effect of cold storage on the quality of *A. unedo* fruits harvested at different maturation stages

Fruits of two different ripening stages were used in this section, unripen and ripen (≈19 and 21 ºBrix respectively), to evaluate the effect and impact of cold storage. The ripen-harvested samples were tested (chemical and sensory parameters) at the harvesting day and after 3, 6, 10, 13 and 17 days of storage (Figure 13). At day 20, the fruits presented an unpleasant appearance (brownish color), indicative of decay, determining the end of storage time at day 17. Regarding the unripen-harvested samples, chemical evaluation was performed at the harvesting day and then, at the same day wherein sensory evaluation was performed, after 6, 13, 17 and 20 days of storage (Figure 13). Day 20 was defined as the last day of storage also based on visual inspection of the fruits (brownish color detected at day 23).

3.2.1 °Brix, pH and titratable acidity determination

In this study, regarding ºBrix content, an increasing trend takes place during storage time (Figure 24).

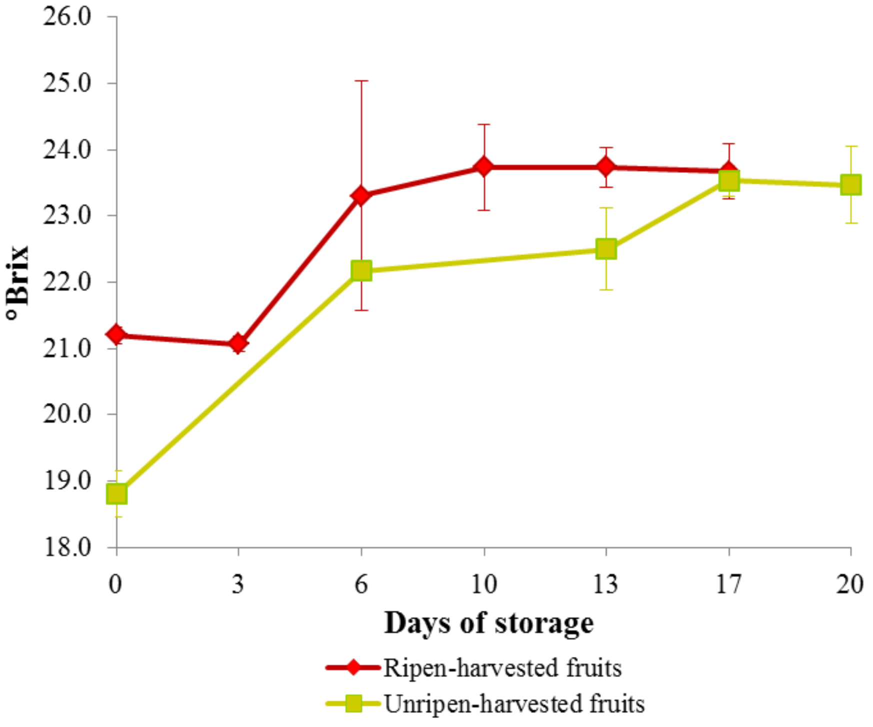


Figure 24 - ºBrix of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

In ripen-harvested samples ºBrix content remains constant from day 0 to day 3 (no statistical significance (p>0.05); it is observed an increase from day 3 to day 6 and thereafter, ºBrix remains constant until the end of storage (p>0.05). Regarding the unripen-harvested samples it is observed that there is a significant increase from day 0 to day 6 (p<0.05); ºBrix content remains constant (p>0.05) from day 6 until day 13, and from day 17 to 20. Despite the increase observed from day 13 to day 17, at the 20^{th} day of storage the unripen-harvested fruits show no statistical difference (p>0.05) when compared to the samples at day 13. At the day of harvesting, ripen-harvested samples presented 21.2±0.12 ºBrix and unripen-harvested samples 18.8±0.35 °Brix. During storage, the °Brix content increased up to 23.73±0.42 and 23.53±0.58 in ripen and unripen-harvested samples, respectively. The group-wise difference (at the beginning of storage) was about 12 % ºBrix whereas by the end of storage the difference was not statistically different (p>0.05).

pH is found to increase significantly during storage in unripen-harvested fruits (from day 6 to day 13), whereas in ripen-harvested fruits the fluctuations observed are not statistically significant (p>0.05) (Figure 25).

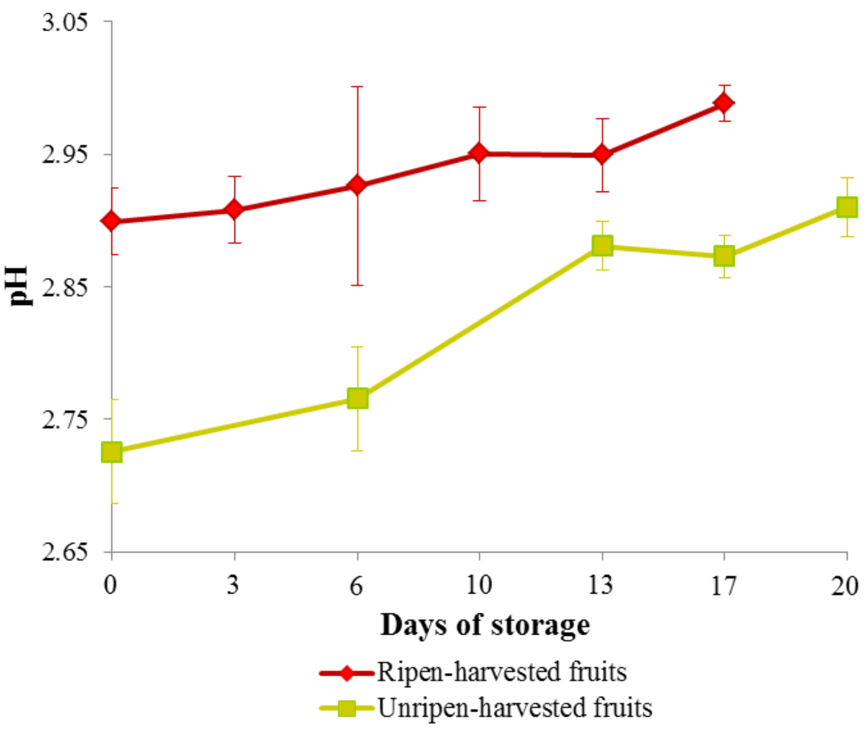


Figure 25 - pH of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

By comparing the pH values it is visible that the different ripening stages at the harvesting moment had no major influence on *A. unedo* fruits pH after storage (p>0.05).

TA, on the other hand, showed a decreasing tendency during storage (Figure 26).

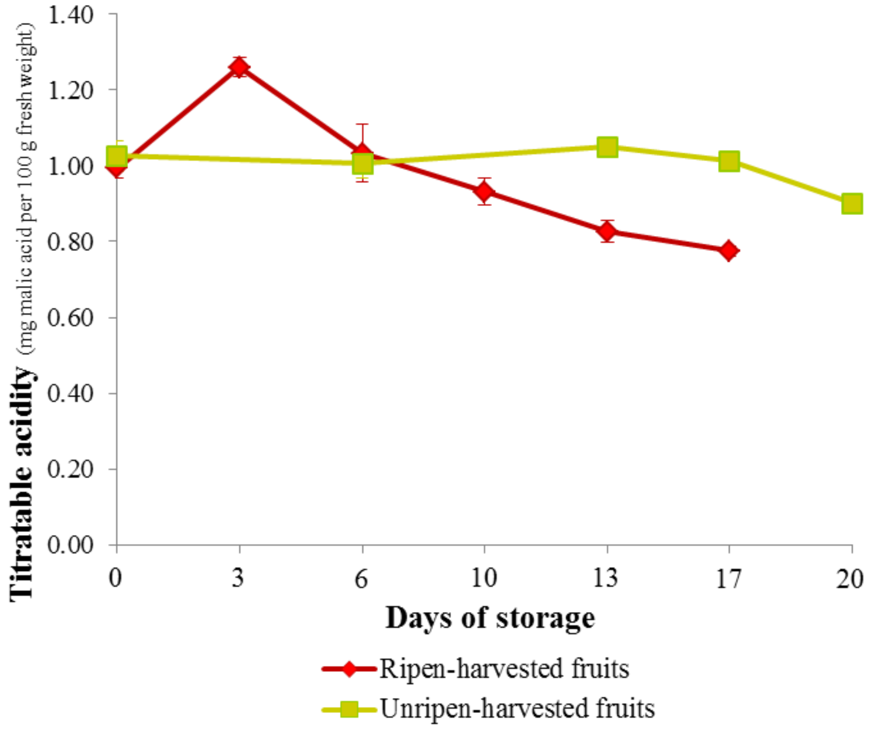


Figure 26 - Titratable acidity of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

For ripen samples, decrease in this parameter is significantly different after day 3 (p-<0.05), while in unripen samples significant changes occur after day 17 (p<0.05). TA revealed positive correlations with pH both for ripen and unripen-harvested samples: in general, the pH tends to go up and TA tends to go down during storage. By comparing the results obtained it is possible to conclude that the different ripening stages at the harvesting moment had no major influence on *A. unedo* fruits TA after storage (p-value>0.05).

3.2.2 Total phenolic content and antioxidant activity determination

An overall view of TPC during storage of *A. unedo* fruits is shown in Figure 27.

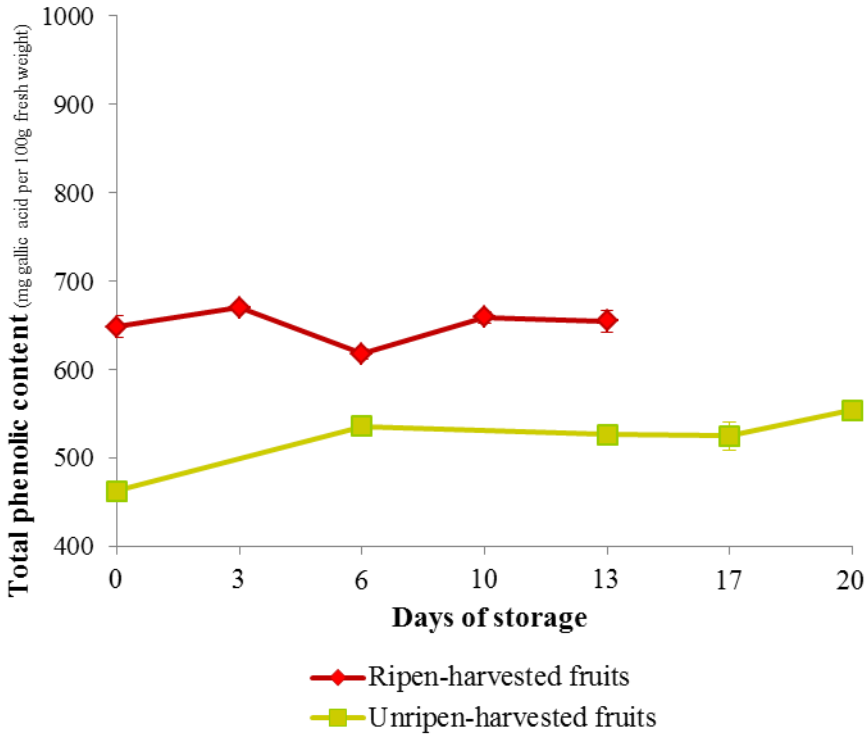


Figure 27 - Total phenolic content of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

Phenolic content in ripen-harvested samples remained constant (no statistical significance (p>0.05)) from day 0 to day 13; however, it was observed a very large increase (of about 40 %) on this parameter at the last day of storage (day 17), and for that reason this value was removed from the Figure 27. The phenolic content at day 17 is far from the other values (p<0.05) which means that this value is an outlier. Regarding the unripen-harvested samples, TPC remains constant (p>0.05) from day 6 until the last day of storage. The ripen and unripen-harvested samples presented significantly different phenolic content (p<0.05) at the end of storage time.

An overall view of antioxidant activity of *A. unedo* fruits during storage is shown in Figure 28.

The DPPH· assay results on ripen-harvested samples show that EC_{50} increases over time, while the unripen-harvested samples have such parameter decreased.

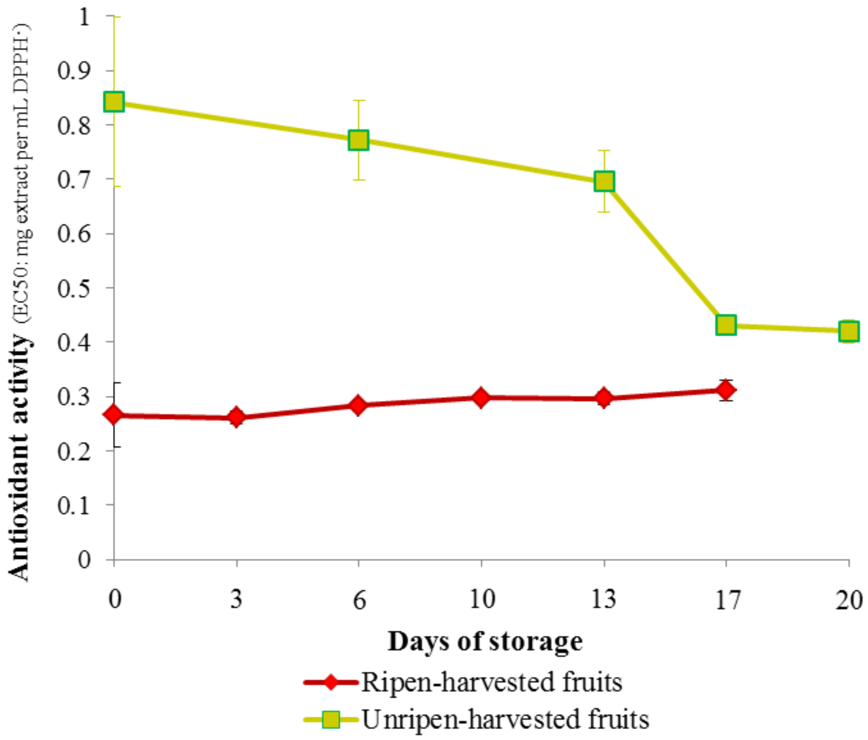


Figure 28 - Antioxidant activity of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

The ripen-harvested samples presented a higher antioxidant activity than the unripen-harvested samples, from the beginning until the end of storage time. The antioxidant activity of the ripen-harvested samples slightly decreases during storage while the unripen-harvested samples have such parameter increased about two times.

3.2.3 Weight loss evaluation

An overall view of weight loss on *A. unedo* fruits during storage is shown in Figure 29.

It is observed weight loss for both ripen and unripen-harvested fruits have an increasing tendency over storage time. Weight is found to increase significantly during storage in the ripen-harvested fruits after day 10, whereas in the unripen-harvest fruits the increase is significant only until the 13th day of storage; thereafter, weight loss is not statistically significant (p>0.05) (Figure 29). It is important to emphasize that both samplings had a linear trend concerning storage time and weight losses, with r^2=0.96 for the ripen-harvested fruits and r^2=0.98 for the unripen-harvested samples.

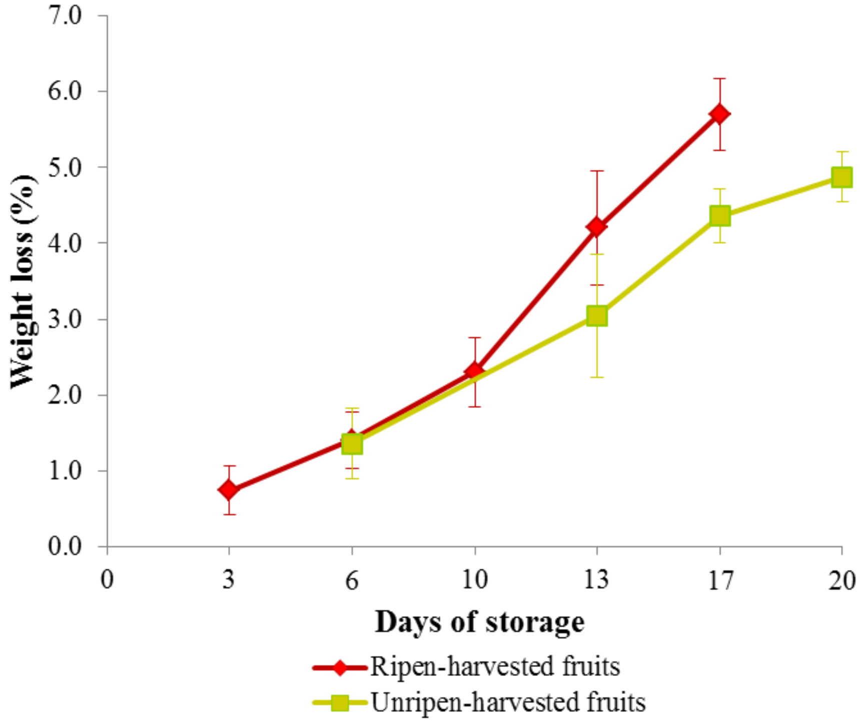


Figure 29 –Weight loss (% - expressed as percentage of the initial weight) of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

This feature could be possibility used to predict water losses on *A. unedo* fruits regarding longer storage periods.

Weight loss is indicative of the fruit's water losses. Additionally, water losses are related to shrivelling, flaccidness and loss of nutritional value. Consequently, a reduced weight loss is related to an increased shelf-life and to a product of superior quality. Figure 29 shows that weight loss reaches maximum values, 5.7 and 4.8 %, after 17 and 20 days of storage, for ripen and unripen-harvested samples respectively. By comparing these parameters we conclude that the unripen-harvested samples lost less weight than the ripen-harvested fruits (p<0.05) and that harvesting fruits at different maturity stages influences weight loss.

3.2.4 Ethanol content determination

The ethanol/mass ratio during storage is shown in Figure 30.

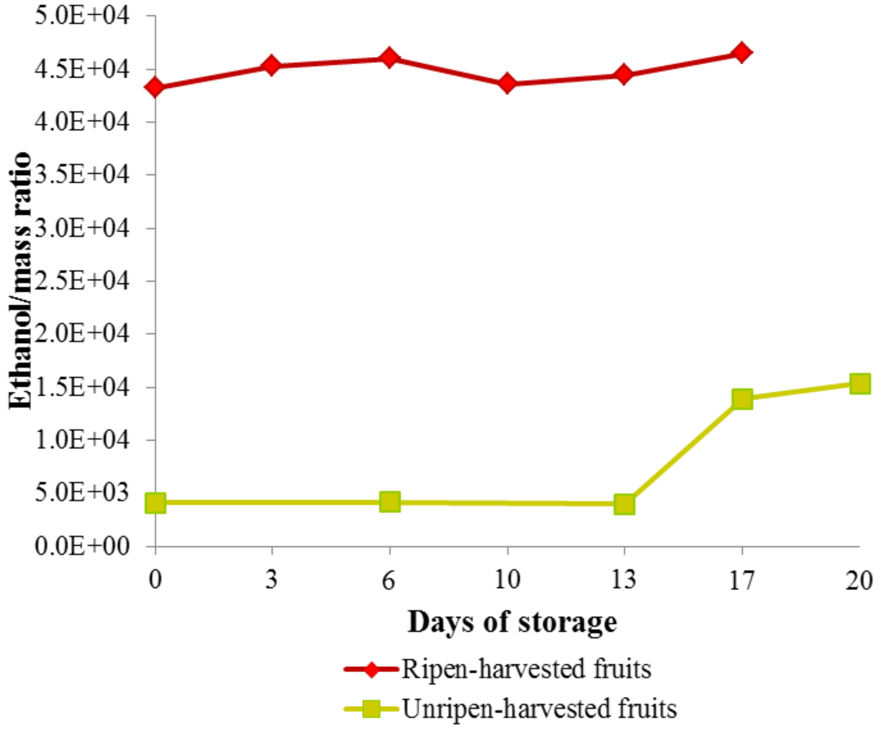


Figure 30 - Ethanol content (as ethanol/mass ratio) of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

Regarding ripen-harvested samples, it is possible to detect some ethanol content fluctuation: ethanol/mass ratio increases from day 0 until day 6, and from day 10 until the end of the storage time; however, a decrease is detected from day 6 to day 10 ($p<0.05$). On unripen-harvested samples some fluctuation was detected, as well: the ethanol content does not show significant differences during the first 13 days ($p>0.05$); up to day 13 ethanol/mass ratio increases significantly ($p<0.05$). After the 17th day, however, ethanol content significantly increased in *A. unedo* fruits. The ethanol/mass ratio in *A. unedo* fruits is about 3 times higher for ripen than for the unripen-harvested fruits. Because of the restrictions applied to the consumption of ethanol (children and pregnant women for example), the unripen-harvested fruits are more suitable for consumption than those already ripen. In general, it is possible to infer that the maturation stage at harvesting has a major impact on the ethanol content of *A. unedo* fruits. However, the ºBrix content, pH and the antioxidant activity of *A. unedo* fruits were similar at the end of storage time, regardless of the different maturation stage at harvesting.

3.2.5 Sensory analysis

The sensory analysis was performed by an untrained-taste panel (with a free-choice profiling test showed in Figure 16), regarding the following parameters: appearance, odour, consistency, flavour and overall liking. Classification was based on a intensity scale (1 (less) -5 (more)). The sensory evaluation was performed at harvesting day for ripen fruits and after 3, 6, 13 and 17 days of storage. For the unripen-harvested samples, as they were harvested unripen and not-ready-to-eat, the sensory analysis began when the berries (1) gained an appearance similar to the ripen-harvested samples and (2) exhibited a ºBrix value into the same range as the ripen-harvested fruits. The sensory analysis of the unripen-harvested samples thus comprises the 6^{th}, 13^{th}, 17^{th} and 20^{th} days of storage. The end of the analysis was determined when, at day 20 for ripen fruits and at day 23 for unripen-harvested fruits, a brownish color was detected by visual inspection.

Appearance

In order to evaluate the visual appearance of *A. unedo* fruits the untrained-taste panel were asked to indicate: (1) the percentage of red on the surface of the fruits (from 0 to 100 % (intensity scale 1 to 5)); (2) the cover colour intensity (red or dark red (intensity scale 1 or 2) and (3) the pulp colour (from green yellow to orange yellow (intensity scale 1 to 5)). All these parameters are indicative of ripeness. Concerning appearance, all samples showed an increasing tendency during storage as regards colour intensity over storage, more specifically: fruit's pulp and cover (Figure 31).

The % of red on the surface remains constant (p<0.05) through storage time to both ripen and unripen-harvested samples. Pulp colour and cover intensity showed an increasing tendency, however not statistically significant (p<0.05). It is also observed that despite the different maturation degrees at harvest, the fruits presented similar characteristics at the end of the storage, showing that it does not have a major influence on *A. unedo* fruits appearance.

These parameters are indicative of the ripening of *A. unedo* fruits, given that, as ripening occurs, the conical swellings assume a darker red and the pulp becomes yellowish, with tones of orange.

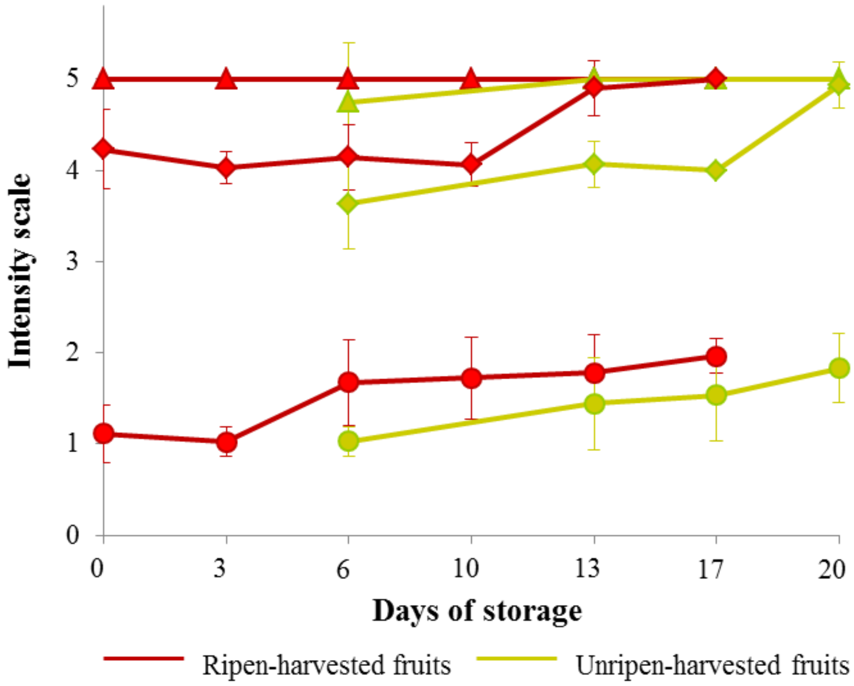


Figure 31 – Appearance of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC. ▲ % red on the surface, ♦ pulp colour, • cover colour intensity.

In that case, it is possible to conclude that the berries did ripen during storage. No detection occurred of brown colouring in the fruits, indicating that they all were suitable for consumption.

Odour

Regarding *A. unedo* fruits odour it was evaluated the scent (characteristic or ethanolic (intensity scale 1-2)) and intensity (from low to high (intensity scale 1-5)) (Figure 32).

During storage only the characteristic scent of the fruits was detected. The odour of both fruit samples did not show variations ($p<0.05$) during storage. Odour was considered of low intensity during the study except for the 20^{th} day of storage of the unripen samples. At this point (i.e. the last testing of the collected unripen samples), there is an indication of a slight increase, however not significant, in sample odour. In general, odour characteristics at the end of storage are similar for both ripen and unripen-harvested fruits and are not influenced by harvesting at different maturation stages.

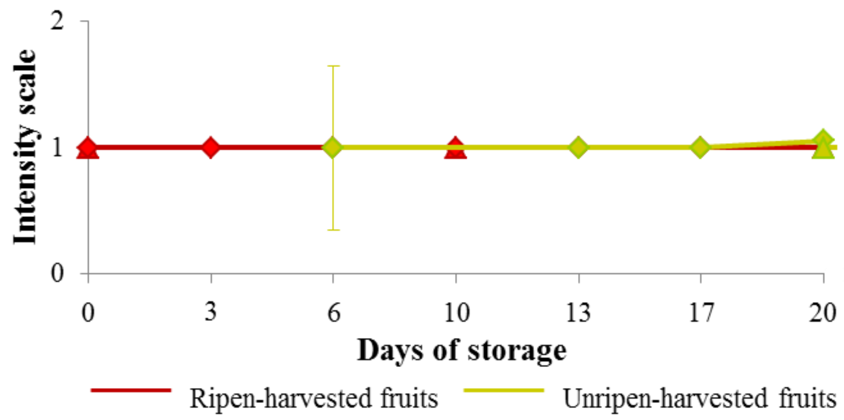


Figure 32 - Odour of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC. ▲ scent, ♦ odor intensity.

Apparent Firmness

Apparent firmness was assessed by touch. Tasters were asked to evaluate firmness from low to high (intensity scale 1 to 5). Results show a decreasing tendency regarding the apparent firmness of the *A. unedo* fruits (Figure 33).

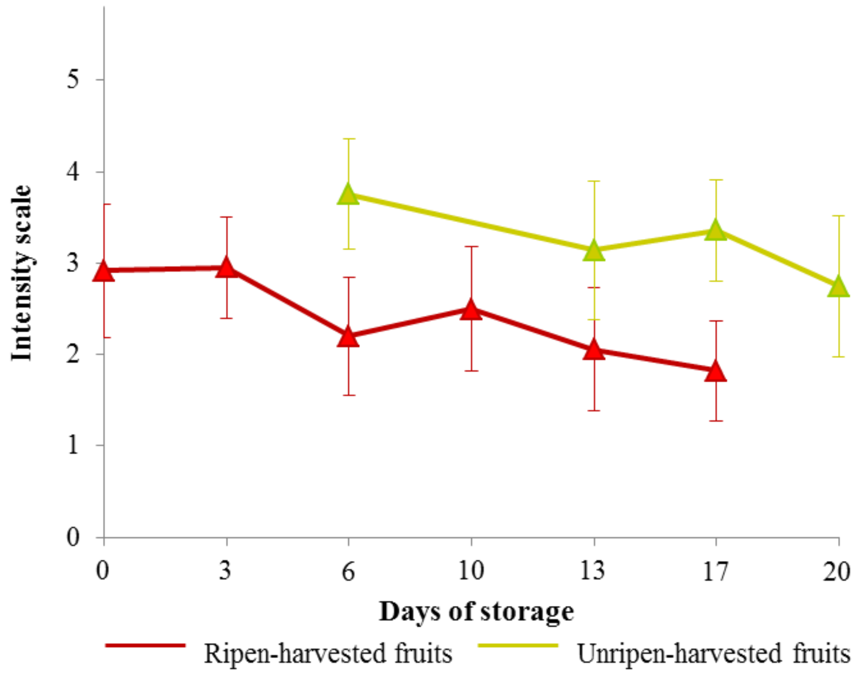


Figure 33 - Apparent firmness of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

Apparent firmness is of crucial importance, because the soft fruits can be assessed as of inferior quality (which might be a rejection factor when consumer-ready). At the end of storage time, ripen and unripen-harvested samples presented similar characteristics (p<0.05) regardless of the maturity degree at harvest.

Flavour

Flavour assessment involved the analysis of a set of parameters: firmness (in the mouth), acidity, sweetness, astringency, pastiness, sandiness and flavour intensity (intensity scale 1-5 (low-high)). Flavour persistence was evaluated as well. Firmness in the mouth, acidity and astringency showed a decreasing tendency over time, in both ripen and unripen-harvested samples (Figure 34).

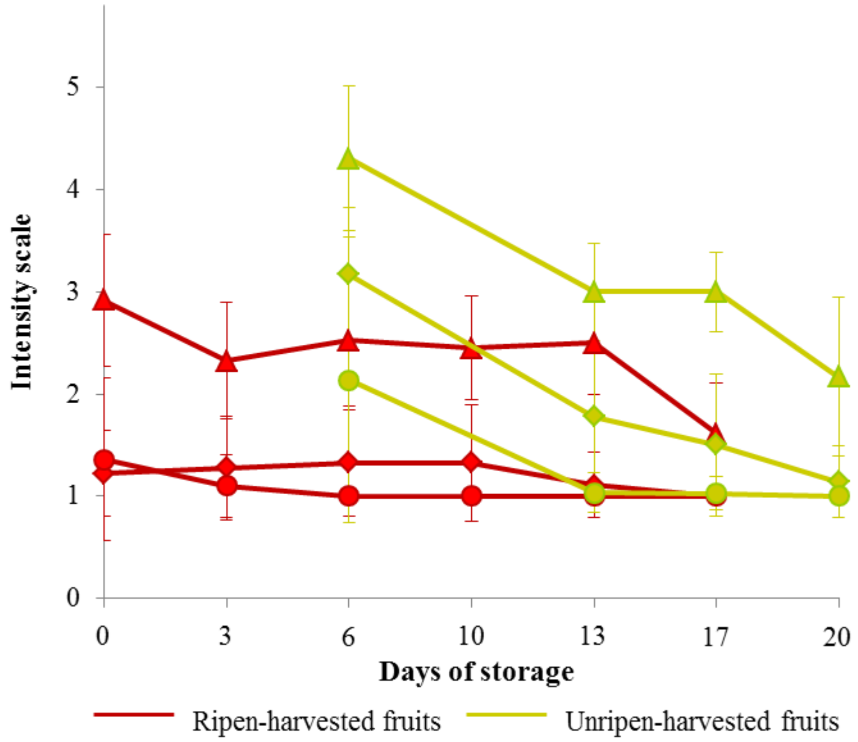


Figure 34 – Flavour of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC. ▲ Firmness, ♦ acidity, and ● astringency.

It is observed a decreasing tendency over storage time, however variations are not statistically significant (p<0.05) which means that harvesting fruits at different maturity degrees does not have a major influence as regards the firmness (in the mouth), acidity and astringency.

Sweetness, stoniness, pastiness and flavour intensity of *A. unedo* fruits during storage are shown in Figure 35.

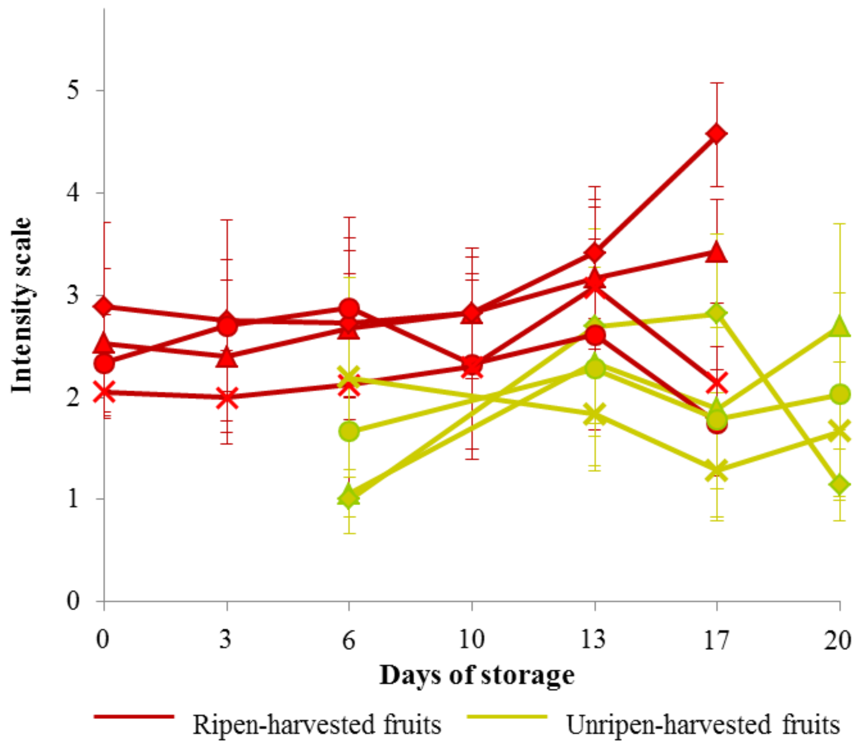


Figure 35 - Flavour of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC. ▲ Sweetness, ♦ pastiness, ● stoniness and × flavour intensity.

During storage sweetness showed an increasing trend in both ripen and unripen-harvested fruits, though the results are not statistically different ($p<0.05$). Regarding pastiness, the ripen fruits showed to become more pasty with storage. However, unripen-harvested fruits showed to have a higher variability in this parameter. Pastiness increases until day 13, decreases at day 17 and increases at the last day of storage. These values (concerning unripen-harvested fruits) are significantly different ($p>0.05$) from the ones of the ripen-harvested fruits. Stoniness showed a decreasing tendency through storage (not statistically significant ($p<0.05$)) over time in both ripen and unripen-harvested samples. Flavour intensity presented opposite trends ($p<0.05$) during storage on ripen and unripen-harvested samples: (1) on ripen harvested samples flavour intensity increases until day 13, moment when it decreases until the end of the storage, at day 17; (2) on unripen-harvested fruits, flavour intensity decreases until day 17, moment when it rises until the end of the storage, at day 20.

These results showed that despite the different maturation degrees at harvest, at the end of storage both samplings presented similar characteristics ($p<0.05$).

The persistence of *A. unedo* fruits flavor reflects the duration (in seconds) of the fruit's flavour in the mouth. The persistence was classified accordingly to the following established parameters: if flavour doesn't persist in the mouth (0 s) it corresponds to 1 in the intensity scale, if flavour persists for about 3-5 s it corresponds to 2, 7-11 s to 3, 12-15 s to 4 and >16 s to 5. The results of the persistence of *A. unedo* fruits in the mouth is presented in Figure 36.

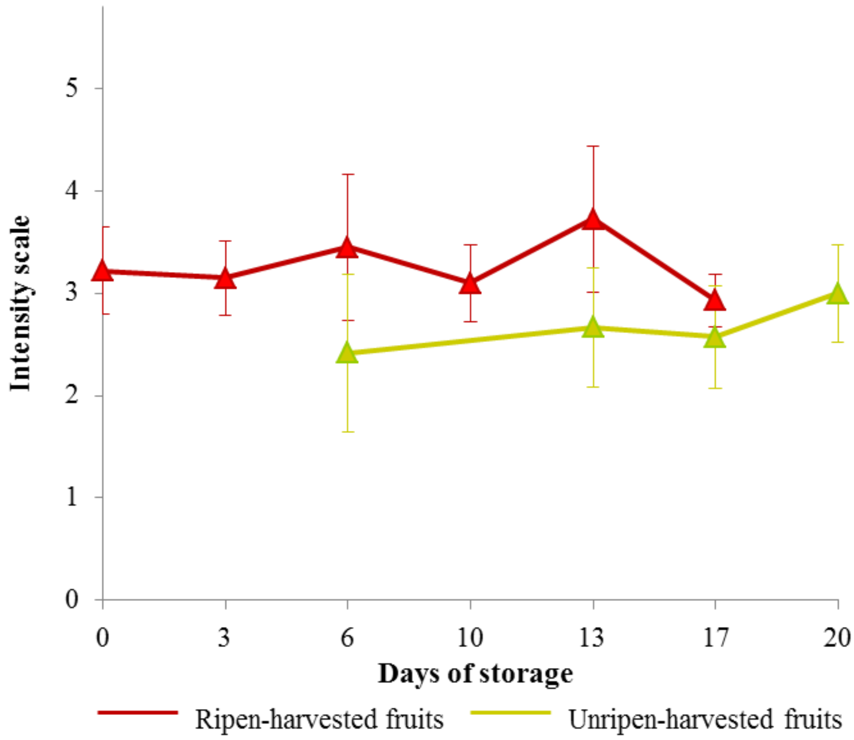


Figure 36 - Persistence of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

Regarding *A. unedo* fruits persistence it is possible to conclude that this parameter fluctuates during storage (the results are not statistically significant ($p<0.05$)), not showing a remarkable trend. This parameter, regardless of the maturity degree at harvest, shows similar values at the end of storage for both fruit samples.

Overall liking

The overall liking, regarding the sensory analysis performed (free choice profiling test at Figure 16), is a general classification of the fruits and is shown in Figure 37.

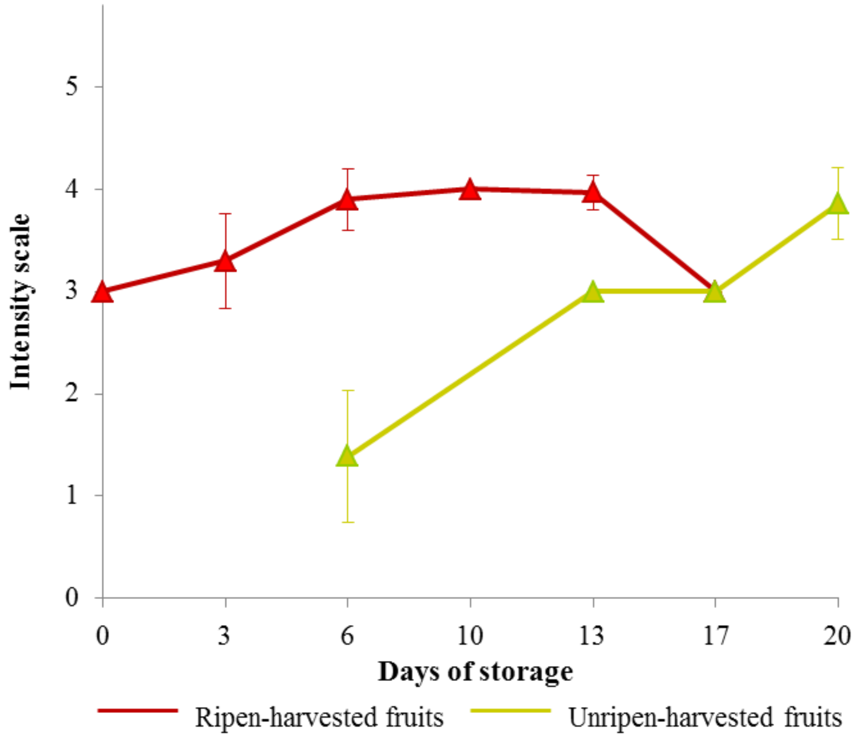


Figure 37 - Global classification (Figure 16) of ripen and unripen-harvested *A. unedo* fruits during storage at ≈5 ºC

It is shown that overall liking, regarding the ripen-harvested samples, remains constant ($p<0.05$) from day 3 to day 13. Thereafter, a decreasing tendency takes place. As regards the unripen-harvested samples, an increasing trend takes place; however, the results are statistically different ($p>0.05$) at 6th and at the 20th day of storage. According to the Figure 37, both ripen and unripen-harvested fruits, at the best eating-point (13th day of storage for ripen-harvested fruits and 20th day of storage for the unripen-harvested fruits), exhibit similar classification values ($p<0.05$). This result reflects that harvesting fruits at different maturity degree does not have a major impact on the overall liking of the fruits

In Figure 38 is shown the chemical and sensory profile of *A. unedo* fruits at the best time for consumption: at 13th day of storage for the ripen-harvested samples and at 20th day of storage for the unripen-harvested samples.

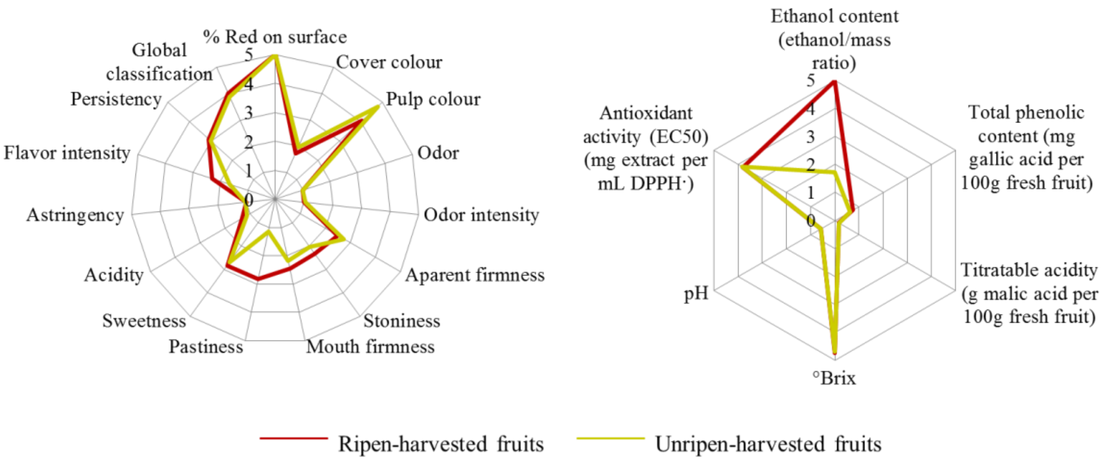


Figure 38 - Sensory (a) and chemical (b) profile of *A. unedo* fruits best ranked for consumption: at 13th day of storage for the ripen-harvested samples and at 20th day of storage for the unripen-harvested samples. Chemical parameters are adjusted to a 0-5 scale.

According to the Figure 38, unripen and ripen-harvested samples at their best eating point exhibited similar sensory and chemical characteristics. It may be important to address that at this point unripen-harvested fruits showed best pastiness and significantly lower ethanol content. The unripen-harvested fruits also presented lower mouth firmness, considered a negative point; however it's not significantly different from the ripen-harvested fruits.

In conclusion, considering that: (1) the ethanol content is 3 times lower in unripen than in ripen-harvested samples and that (2) unripen samples can be stored for a longer period of time, harvesting unripen *A. unedo* fruits for further consumption seems to be the best choice. Assessing the impact of cold storage on fresh fruits can be used as a decision support for producers of *A. unedo* fruits, in order to reach new consumers and new markets.

3.3 Chemical characterization of the lipophilic extractives from *A. unedo* fruits

As far as we know there is still a lack of information regarding the lipophilic composition of *A. unedo* fruits and the published data deal mainly with fatty acids profile. In order to add value to strawberry tree fruits and boost their consumption is important to understand the chemical composition, namely the lipophilic fraction. In this work the dichloromethane extracts of the ripe *A. unedo* fruits were prepared and analyzed by GC-MS, before and after alkaline hydrolysis, in order to study the free and esterified compounds. The fruits of the last harvest, December 11th, from the six different locations described before were used. During this chapter the fruits of different locations will be identified as B1-B6, accordingly to the harvesting location.

The composition of dichloromethane extract will be described below. The dichloromethane extraction yields (Table 5) of ripen strawberry tree fruits ranged between 0.722 and 1.66 %, being location 1 and 4 the ones which presented higher results.

Table 5 - Dichloromethane extraction yields (% w/w) for *A. unedo* harvested from six different locations

Location	Extraction yield (% w/w)
1	1.66
2	1.56
3	1.48
4	1.58
5	1.55
6	0.722

Until now there are no reports on literature about extractive yields of *A. unedo* fruits and of plants of the same genus as well. The lipophilic fraction of *A. unedo* fruits harvested from six different locations presented similar compositions regarding the qualitative analysis, either BH or AH. The chromatogram of the derivatized dichloromethane extract is illustrated in Figure 39.

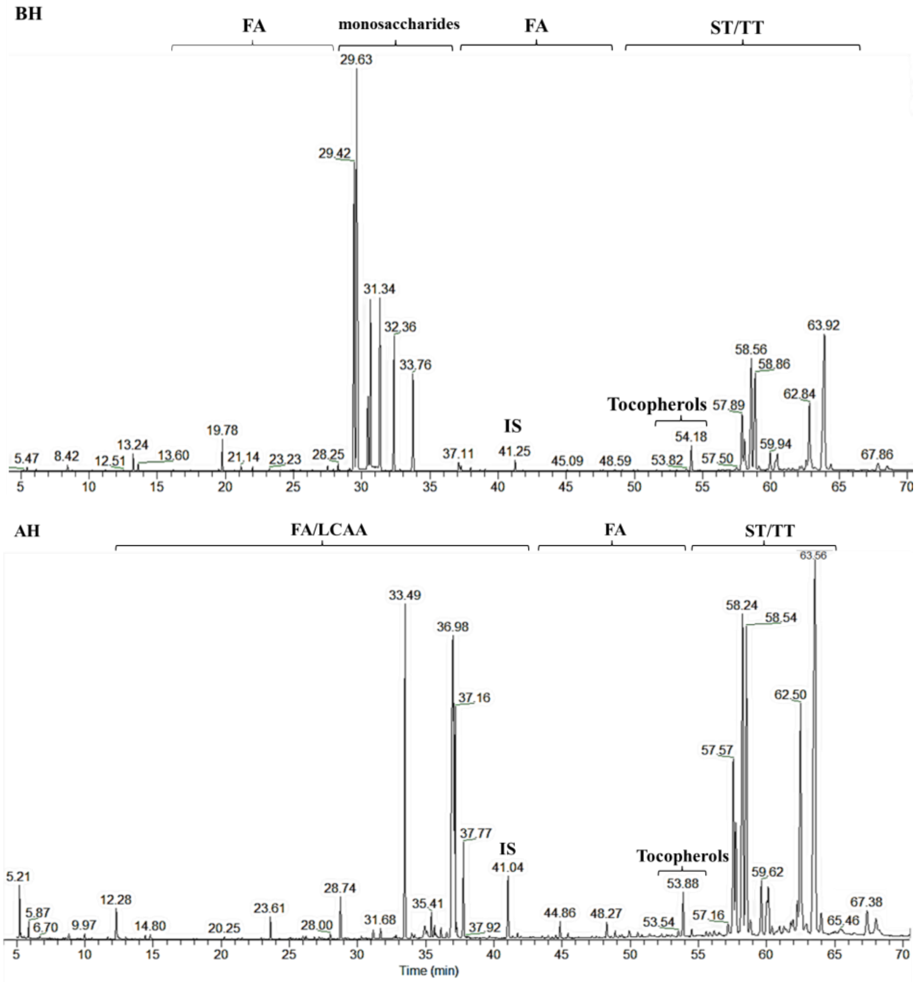


Figure 39 - GC-MS chromatogram of the TMS-derivatized dichloromethane extract of *A. unedo* fruits before (BH) and after (AH) alkaline hydrolysis. Abbreviations: IS, internal standard; FA, fatty acids; LCAA, long chain aliphatic alcohols; ST, sterols; TT, triterpenoids

Figure 40 shows the main families of compounds identified in the dichloromethane extract of *A. unedo* fruits BH and AH. Triterpenoids, as well as fatty acids are the major families found in all fruits, from B1 to B6. AH it was observed a large increase in fatty acids content which confirms the presence of esterified compounds. Also, long chain aliphatic alcohols are not detected BH but they are described AH, which also confirms the presence of esterified structures. The proportion of sterols remains roughly unchanged AH which means that the proportion of free and esterified sterols is very similar AH and BH in *A. unedo* extractives.

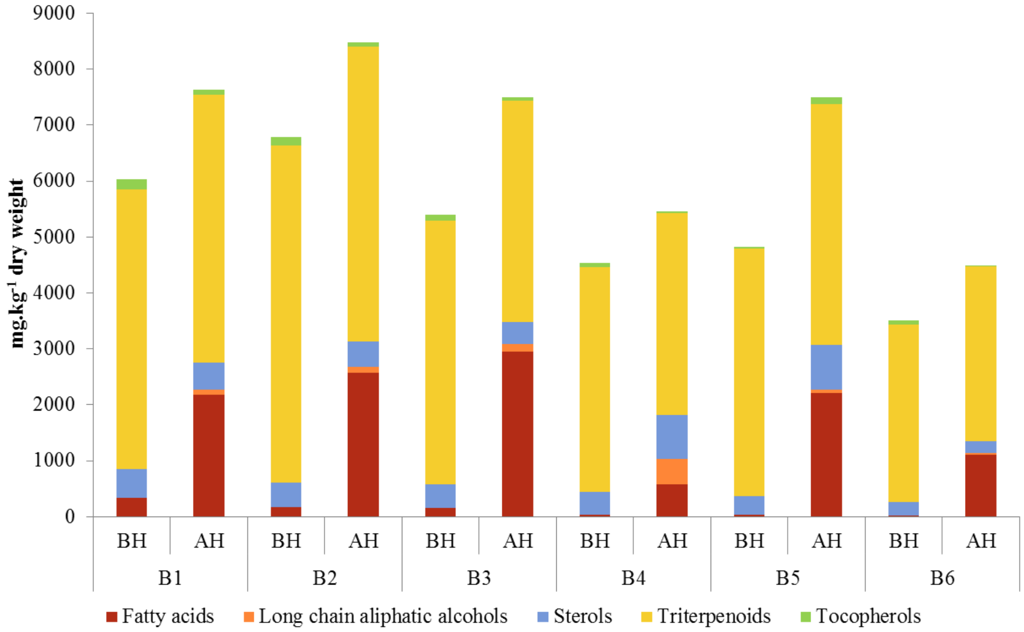


Figure 40 - Major lipophilic components identified in *A. unedo* fruits extracts collected from 6 different locations (B1-B6), before (BH) and after hydrolysis (AH).

Table 6 shows the different families and individual compounds identified in the dichloromethane extracts, BH and AH, as well as their quantification in B1-B6 fruits. The different families of lipophilic components present on *A. unedo* fruits will be described below.

Fatty acids

Fatty acids were identified as TMS derivatives based on their fragmentation pattern and elution order. [86–89] The most abundant peaks in the mass spectra are those at *m/z* 73 and 75 and the $[M\text{-}CH_3]^+$ ion (or $[M\text{-}15]^+$). The ions at *m/z* 73 $[(CH_3)_3Si]^+$ and 75 $[(CH_3)_2Si\text{-}OH]^+$ provide negligible or no structural information since they are ubiquitous in the mass spectra of all TMS derivatives. Other prominent peaks appear at *m/z* 117, 129, 132, 145. Ions at *m/z* 132 and 145 are McLafferty type rearrangement ions. [88,89] The ions referred are visible, for example, in the mass spectrum of the TMS derivative of hexadecanoic acid shown in Figure 41.

Fatty acids represented one of the major groups of lipophilic compounds present in *A. unedo* fruits corresponding to 10-39 % of the total amount of detected compounds in dichloromethane extract after hydrolysis.

Table 6 – Composition (mg compound per Kg dry weight) of dichloromethane extracts from *A. unedo* fruits collected from six locations [a]

			B1		B2		B3		B4		B5		B6	
Rt (min)	Compound		BH	AH	BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
	Fatty acids		**346**	**2186**	**168**	**2569**	**154**	**2954**	**35**	**587**	**33**	**2205**	**29**	**1104**
	Saturated		209	659	108	561	116	748	7	291	8	790	9	478
8.46	Hexanoic acid C6:0		nd	tr	nd	tr	nd	tr	nd	tr	nd	tr	nd	tr
11.68	Octanoic acid C8:0		nd	2	nd	1	nd	3	nd	2	nd	7	nd	1
14.87	Nonanoic acid C9:0		nd	4	nd	2	nd	2	nd	5	nd	9	nd	0
17.96	Decanoic acid C10:0		nd	2	nd	1	nd	2	nd	2	nd	3	nd	1
23.61	Dodecanoic acid C12:0		2	20	2	4	2	13	1	7	2	9	3	16
26.94	Tridecanoic acid C13:0		nd	tr	nd	tr	nd	tr	nd	tr	nd	tr	nd	tr
28.74	Tetradecanoic acid C14:0		nd	54	nd	18	nd	40	nd	15	nd	40	nd	50
31.78	Pentadecanoic acid C15:0		nd	tr	nd	tr	nd	tr	nd	tr	nd	tr	nd	tr
33.49	Hexadecanoic acid C16:0		194	386	92	312	105	414	nd	115	nd	317	nd	300
35.64	Heptadecanoic acid C17:0		nd	9	nd	tr	nd	tr	nd	87	nd	215	nd	6
37.77	Octadecanoic acid C18:0		9	156	11	210	6	254	4	40	5	140	5	95
41.73	Eicosanoic acid C20:0		nd	9	nd	6	nd	9	nd	10	nd	24	nd	7
45.45	Docosanoic acid C22:0		1	6	nd	4	tr	5	tr	7	tr	22	tr	1
47.08	Tricosanoic acid C23:0		nd	tr	nd	tr	nd	tr	nd	tr	nd	tr	nd	tr
48.91	Tetracosanoic acid C24:0		3	12	3	4	3	7	2	2	2	6	2	1
	Unsaturated		137	1527	60	2007	37	2206	27	296	24	1415	20	626
32.68 32.81	Palmitic acid C16:1$^{(\Delta 9)}$ Palmitic acid C16:1$^{(\Delta 9)}$	(*cis* + *trans*)	tr	7	tr	6	tr	10	tr	21	tr	1	tr	343
36.98	Linoleic acid C18:2$^{(\Delta 9,\ 12)}$ + α-Linolenic acid C18:3$^{(\Delta 9,\ 12,\ 15)}$		123	1108	56	1989	34	2186	26	272	23	1414	17	183
37.16 37.26	Oleic acid C18:1$^{(\Delta 9)}$ Oleic acid C18:1$^{(\Delta 9)}$	(*cis* + *trans*)	14	412	4	12	4	11	2	3	2	1	3	101
	Long Chain Aliphatic Alcohols		**0**	**86**	**0**	**118**	**0**	**130**	**0**	**453**	**0**	**72**	**0**	**30**
26.84	Tetradecanol		nd	2	nd	1	nd	2	nd	25	nd	5	nd	1
31.68	Hexadecanol		nd	10	nd	14	nd	29	nd	233	nd	33	nd	6
35.41	Octadec-9-en-1-ol		nd	65	nd	101	nd	94	nd	195	nd	33	nd	16
36.12	Octadecan-1-ol		nd	9	nd	1	nd	6	nd	1	nd	1	nd	6

Table 6 – continued.

	Sterols	**516**	**493**	**453**	**451**	**427**	**407**	**421**	**793**	**341**	**805**	**233**	**217**
55.09	Cholestan-3-one	nd	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
55.83	Campesterol	nd	24	nd	25	nd	1	nd	6	nd	137	nd	7
56.65	Stigmasterol	9	10	8	12	5	7	8	10	4	5	tr	4
57.75	Sitosterol	507	459	445	414	422	399	413	777	337	663	233	205
	Triterpenoids	**5207**	**4931**	**6247**	**5494**	**4908**	**4110**	**4178**	**3757**	**4589**	**4417**	**3235**	**3196**
57.16	Amyrin derivative	54	2	51	56	50	4	51	314	33	48	43	12
57.33	Lupenone	95	54	86	77	96	77	80	74	69	29	18	18
57.57	β-Amyrin	69	29	22	21	41	36	41	686	15	193	25	442
58.24	α-Amyrin	952	875	949	832	829	719	941	914	753	1197	1203	1646
58.54	Lupeol	1158	1165	1319	1161	855	781	805	66	960	1329	102	0
59.62	Lupenyl acetate	174	160	210	191	154	139	32	30	104	107	61	93
62.10	Olean-12-en-3β,23-diol	113	91	139	140	90	83	83	80	96	86	49	50
62.25	Uvaol	19	72	922	831	676	587	72	108	561	232	30	21
62.50	Oleanolic acid	779	715	62	36	42	57	443	542	38	321	324	265
63.56	Ursolic acid	1795	1769	2486	2149	2074	1627	1632	943	1959	876	1381	649
	Tocopherols	**176**	**82**	**153**	**80**	**104**	**61**	**71**	**32**	**21**	**115**	**76**	**21**
53.88	α-Tocopherol	40	27	73	52	31	26	14	31	16	63	42	7
54.18	γ-Tocopherol	136	55	80	28	74	35	58	1	5	52	34	14

[a] Abbreviations: nd, not detected; tr, traces.

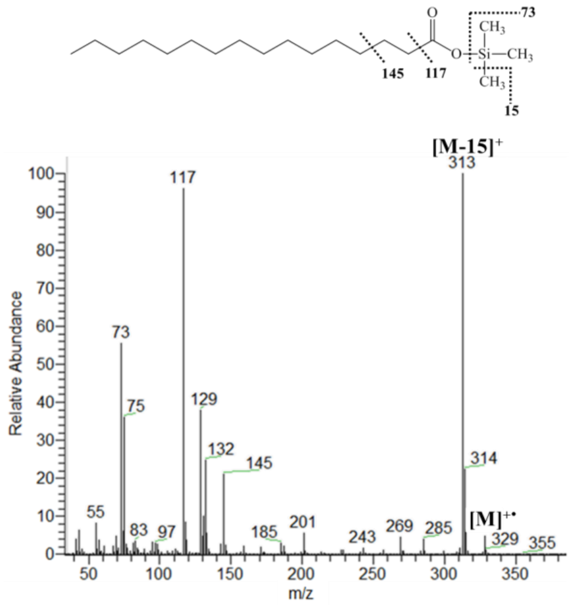


Figure 41 - Mass spectrum of the TMS derivative of hexadecanoic acid

Fatty acids represented one of the major groups of lipophilic compounds present in *A. unedo* fruits corresponding to 10-39 % of the total amount of detected compounds in dichloromethane extract after hydrolysis. BH *A. unedo* extract comprised between 1-6 % of fatty acids. Both saturated fatty acids and unsaturated fatty acids were identified in *A. unedo* fruits. After hydrolysis, saturated fatty acids accounted for from 5 to 10 % of the total compounds and unsaturated fatty acids for from 5 to 29 %. The content of unsaturated fatty acids was found considerably higher than that of saturated fatty acids in all samples, with location 5 being the exception. The fruits collected on location 5 presented about the same amount of saturated fatty acids and unsaturated fatty acids AH. Total fatty acids content increased until 70 times after hydrolysis which confirms the presence of esterified structures such as steryl esters, glycerides among others in the original extract.[89] The identified fatty acids ranged from hexanoic (C8:0) to tetracosanoic (C24:0), including six unsaturated structures (C16 and C18) (Table 6). Palmitic acid (C16:0) is the most abundant saturated fatty acid in *A. unedo* fruits with the highest content observed in location 3 (414 $mg.kg^{-1}$ of dw) and the lowest in location 4 (115 $mg.kg^{-1}$ of dw). Linoleic and α-linolenic acids were the major compounds of the unsaturated fatty acids group reaching up 2186 $mg.kg^{-1}$ of dw (in location 3). These were followed by *cis*- and *trans*- oleic acids (412 $mg.kg^{-1}$ of dw in location 1) and *cis*- and *trans*- palmitic acids (343 $mg.kg^{-1}$ of dw in location 6). Is important to highlight that palmitic acid, along with palmitoleic, oleic, linoleic and α-linolenic acids represent the major fraction BH and are still the most abundant fatty acids AH (Figure 42). Consequently these fatty acids are the main esterified with glycerol and also with sterols and alcohols.

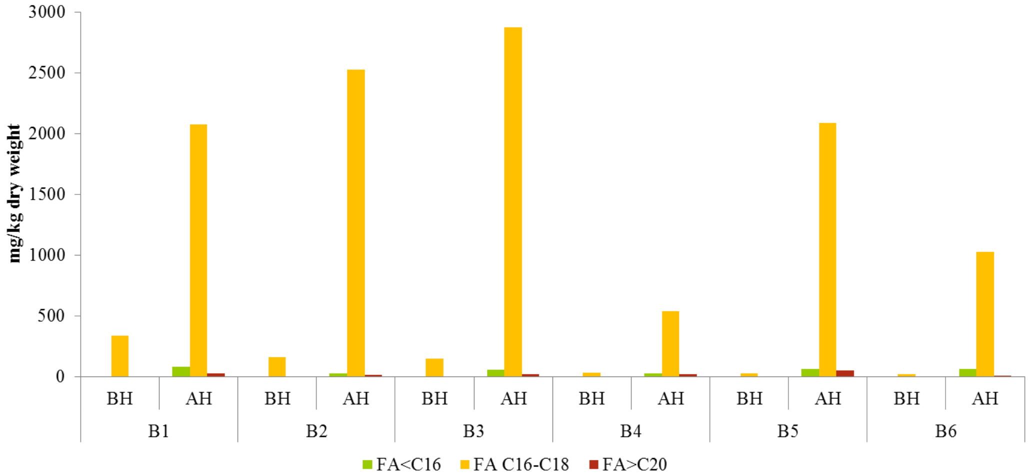


Figure 42 - Major fatty acids identified in the dichloromethane extract of *A. unedo* fruits, before and after alkaline hydrolysis. FA<C16: fatty acids lower than C16; FA C16-C18: fatty acids between C16-C18; FA>C20: fatty acids higher than C20.

The compounds identified in the present study have already been reported in *A. unedo* fruits however, only the content of palmitic, oleic, linoleic and α-linolenic acids was determined (AH).[32,38,45] Linoleic and α-linolenic acids were the major compounds with 92.1 ± 6.6 mg and 83.5 ± 16.3 mg.100 g^{-1} fresh weight. Palmitoleic and oleic acids accounted for 50.01 ± 1.0 and 39.9 ± 9.2 mg.100 g^{-1} fresh weight respectivelly.[32] Our results show that linoleic and α-linolenic acids content represent about 120 mg.100 g^{-1} fresh fruit, about 23 mg of oleic acid and about 24 mg of palmitic acid. Our results are also in accordance with the previously reported data regarding the fatty acids profile: 1) the unsaturated fatty acids are the most representative fraction, with linoleic and α-linolenic acids in higher amounts and 2) the saturated fatty acids were the minor fraction.

Polyunsaturated fatty acids like linoleic and α-linolenic acids are ω-3 and ω-6, essential nutrients that must be obtained from the diet as they are not synthetized by the human body. [25,47] These compounds have been associated with the prevention and reduced risk of cardiovascular disease, depression and Alzheimer's, management of obesity, reducing weight, suppressing appetite and modulation of body fat and plasma lipids. [25] Here we assume that the intake of *ca.* 20 g *A. unedo* fruits provides until 24 mg of essential fatty acids. Aforementioned there were reported the adequate intakes for linoleic and α-linolenic acids as 16 and 1.6 $g.day^{-1}$ respectively, for healthy adults.[49] Considering this information it is possible to conclude that an edible portion (*ca.* 20 g fresh weight) of fruits contribute to the intake of 0.13 % of essential fatty acid. The role of ω-3 and -6 FA's in human health and their presence in *A. unedo* fruits contributes to value these fruits and to establish a nutritional profile.

Long chain aliphatic alcohols

Long chain aliphatic acids were identified as TMS derivatives based on their fragmentation pattern and elution order.[86,87,89] The most abundant peaks in the mass spectra are *m/z* 73 and 75 and the ion $[M-15]^+$. Also, there are other prominent peaks that appear at *m/z* 89 and 103 $[(CH_3)_3SiOCH_2]^+$ and help to distinguish aliphatic alcohols from fatty acids. [88,89] The ions referred are visible in the mass spectrum of the TMS derivative of octadecan-1-ol shown in Figure 43.

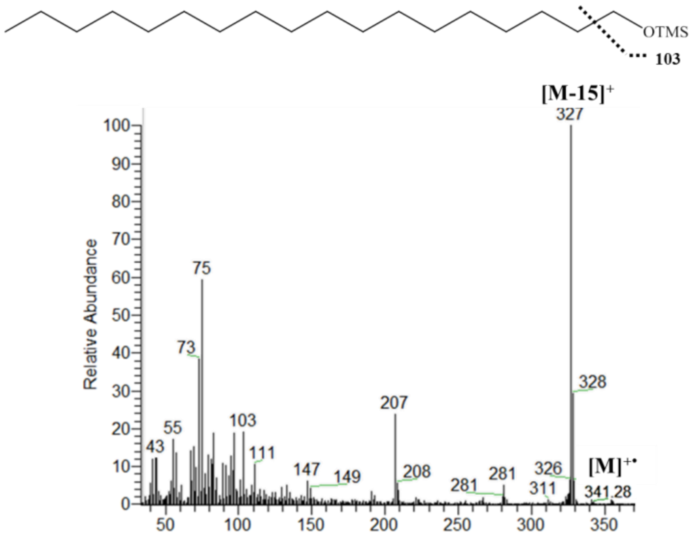


Figure 43 - Mass spectrum of the TMS derivative of octadecan-1-ol.

Long-chain aliphatic alcohols were detected in quite low amounts, representing about 1-8 % of the total lipophilic extractives of ripen *A. unedo* fruits. There were not detected aliphatic alcohols BH. AH octadec-9-en-1-ol was the most abundant compound, representing 52 % of the total long chain aliphatic alcohols present, followed by hexadecan-1-ol, octadecan-1-ol and tetradecan-1-ol. To the best of our knowledge this is the first time these compounds were described in *A. unedo* fruits.

Sterols

In general, the mass spectra of sterols exhibit the presence of abundant TMS-containing groups because this strongly directs the fragmentation of the molecules. The ions at *m/z* 73 and 75 provide no structural information and correspond to the loss of $(CH_3)_3Si$ and $(CH_3)_2Si\text{-}OH$ respectively. Ions at *m/z* 129 and $[M\text{-}129]^+$ correspond to the loss of the TMS group together with a carbon fragment of ring A containing the C-1, C-2 and C-3. Another important structural ion is at *m/z* $[M\text{-}90]^+$ which corresponds to the 1,2-elimination of the trimethylsilanol group. Indirect information about the molecular weight can be obtained from *m/z* $[M\text{-}15]^+$. The ions referred to above and some fragmentations are visible in the mass spectrum of the TMS-derivative of β-sitosterol shown in Figure 44.

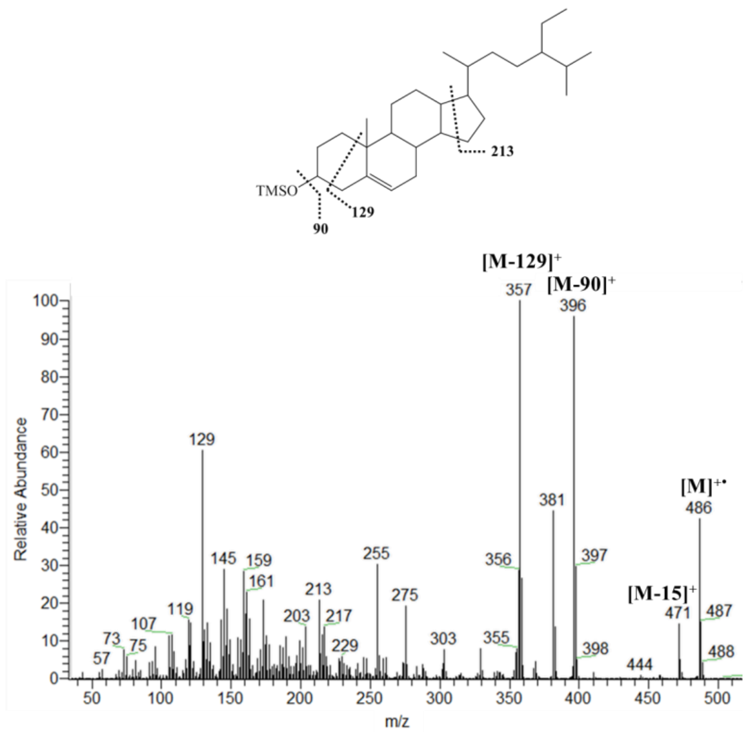


Figure 44 - Mass spectrum of the TMS derivative of β-sitosterol

The sterols found in *A. unedo* fruits extract were cholestan-3-one, campesterol, stigmasterol and β-sitosterol. These phytosterols represents up to 9 % of the total extract BH and up to 14 % AH. β-Sitosterol was the main component of this family in all samples representing between 82 and 100 % of total sterols content. Campesterol was only detected AH accounting for 1-137 $mg.kg^{-1}$ of dw. β-Sitosterol was detected BH accounting for 233-507 $mg.kg^{-1}$ of dw and AH the β-sitosterol content ranged between 205-777 $mg.kg^{-1}$ of dw.

Stigmasterol was detected BH accounting for 4-9 $mg.kg^{-1}$ of dw and AH accounting for 4-12 $mg.kg^{-1}$ of dw. Cholestan-3-one was found in trace amounts in the majority of the samples. All the sterols identified in this work were already reported in the literature for *A. unedo* fruits except campesterol.

The role of phytosterols in human health is mainly associated with non-pharmacological lipid-altering therapies, reducing exogenous cholesterol absorption by displacement in the intestinal lumen. As a result, both total cholesterol and HDL are reduced. Consumption of 1.8-2.0 g of phytosterols per day has been shown to lower cholesterol absorption.[64] Strawberry tree fruits can contribute to the intake of between 2 and 6 mg phytosterols per 20 g in the human diet. Although the consumption of 20 g of fresh fruit only contributes to the intake of 0.11-0.33 % of the phytosterols content, *A. unedo* fruits are a safe and practical option for the consumption of bioactive compounds.

Triterpenoids

Similarly to the other identified families, triterpenoids were identified based on their typical fragmentation pattern and elution order. There are some characteristic signals found at *m/z* 189, 203, 279, 307 and 320, typical of ions arising from fragments containing A and B rings and in some cases of the C ring. The most important signals are found at *m/z* 600 $[M]^{+\cdot}$, 585 $[M-15]^+$, 510 $[M-TMSOH]^+$, 495 $[M-TMSOH-CH_3]^+$, 482 $[M-TMSOOCH]^+$, 393 $[M-TMSOH-TMSOC]^+$ and 392$[M-TMSOH-TMSOC]^+$. In Figure 45 are described some of the ions referred to above in the mass spectrum of the TMS-derivative of ursolic acid.

Triterpenoids represented the major family of the dichloromethane extract of *A. unedo* fruits. This family accounted for up to 90 % of the total amount of detected compounds in strawberry tree fruits extract BH. Ursolic acid is the major compound representing 34 to 43 % of all identified compounds BH and 20 to 40 % of all triterpenoids BH. Ursolic acid is present in higher amounts (649-2149 $mg.kg^{-1}$ of dw AH) followed by oleanolic acid and lupeol. Other identified triterpenoids include olean-12-en-3β,23-diol, uvaol, β- and α-amyrin and amyrin and lupeol derivatives.

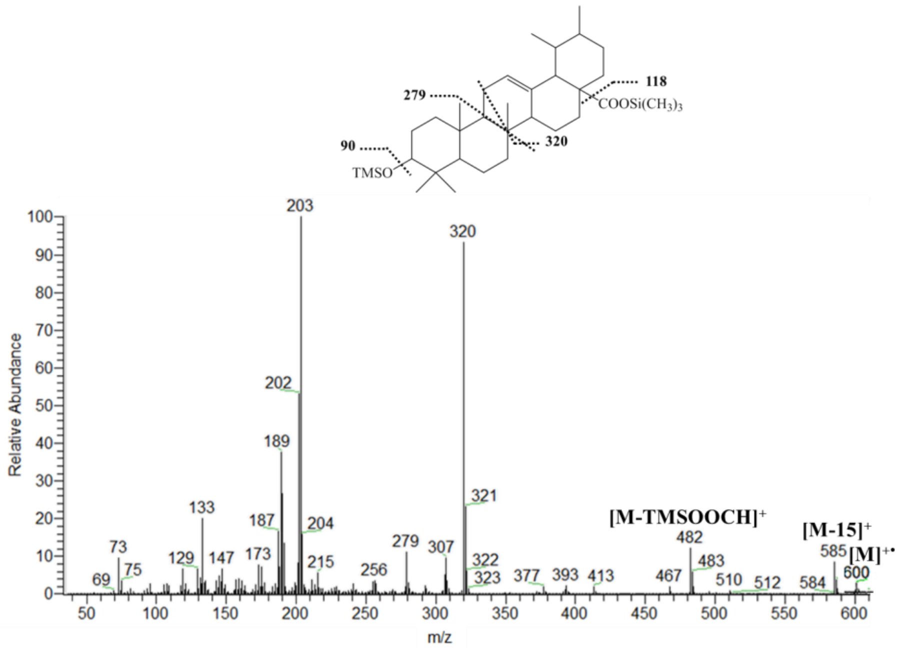


Figure 45 - Mass spectrum of the TMS derivative of ursolic acid

The total amount of triterpenoids BH and AH is practically the same. All the triterpenoids identified in this work were already reported in the literature for *A. unedo* fruits, which were identified in Portuguese berries but quantification was not assessed.[58] So, to the best of our knowledge this is the first time that amount of triterpenoids is reported in *A. unedo* fruits.

Reports on the role of triterpenoids in health suggest an improvement of glucose tolerance and a decrease in blood glucose and plasma lipids, and a hypocholesterolemic effect, as well as antiinflamatory and antitumour among many others.[27–29,90] Their antibacterial activity has also been reported, namely, ursolic and oleanolic acids showed moderate to good antibacterial activity, but limited to Gram-positive bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus*.[91] The daily consumption of an edible portion (*ca*. 20g) of *A. unedo* fruits contributes to the intake of 25-48 mg triterpenoids. Given the composition put forward above, the consumption of *A. unedo* fruits can represents an important contribution for the intake of phytochemicals with recognized nutritional and health benefits.

3.4 Development of new food products based on *A. unedo* fruits

The development of new food products goes through several stages as aforementioned (Figure 12). In this thesis it is explored a strategy surrounding the development of new food products based on *A. unedo* fruits. During chapter one (3.4.1) the results regarding the knowledge and consumption of *A. unedo* fruits and products in Portugal are presented and discussed as well as the SWOT analysis and the ideas generation: brainstorming. On chapter two (3.4.2) there are detailed the product's characteristics and the product development.

3.4.1 Market opportunities

3.4.1.1 Searching for information about consumption of *A. unedo* fruits and products in Portugal

The research on consumption and initial impression of *A. unedo* fruits by the Portuguese consumers was made based on surveys, via internet and also via postal. The characteristics of those surveyed are presented in Figure 46.

A total of 689 individuals from almost all districts answered the survey. The results showed that nearly 75 % of those surveyed had an age between 17 and 50 years and that more than half of those surveyed were female (57 %). Most of the answers came from Viseu and Aveiro and the students represented the major fraction of the respondents. Regarding the research on consumption and initial impression of *A. unedo* fruits by the Portuguese consumers, an overview is presented in Figure 47.

The results showed that most respondents (83 %) knew the strawberry tree fruits, of which 76 % had already consumed it. Those interviewed which had already eaten this fruit were asked to rate *A. unedo* fruits taste, on a scale from 1 (bad) to 5 (excellent). The results showed that 44 % of people classifies them as good (3), 48 % as very good (4), 6 % classified their taste as excellent (5) and only 2 % rated *A. unedo* fruits as less good (1-2). To the 24 % of those surveyed that had never consumed strawberry tree fruits were asked why. Most respondent (86 %) stated that there are no food products based on strawberry tree fruits available on the market.

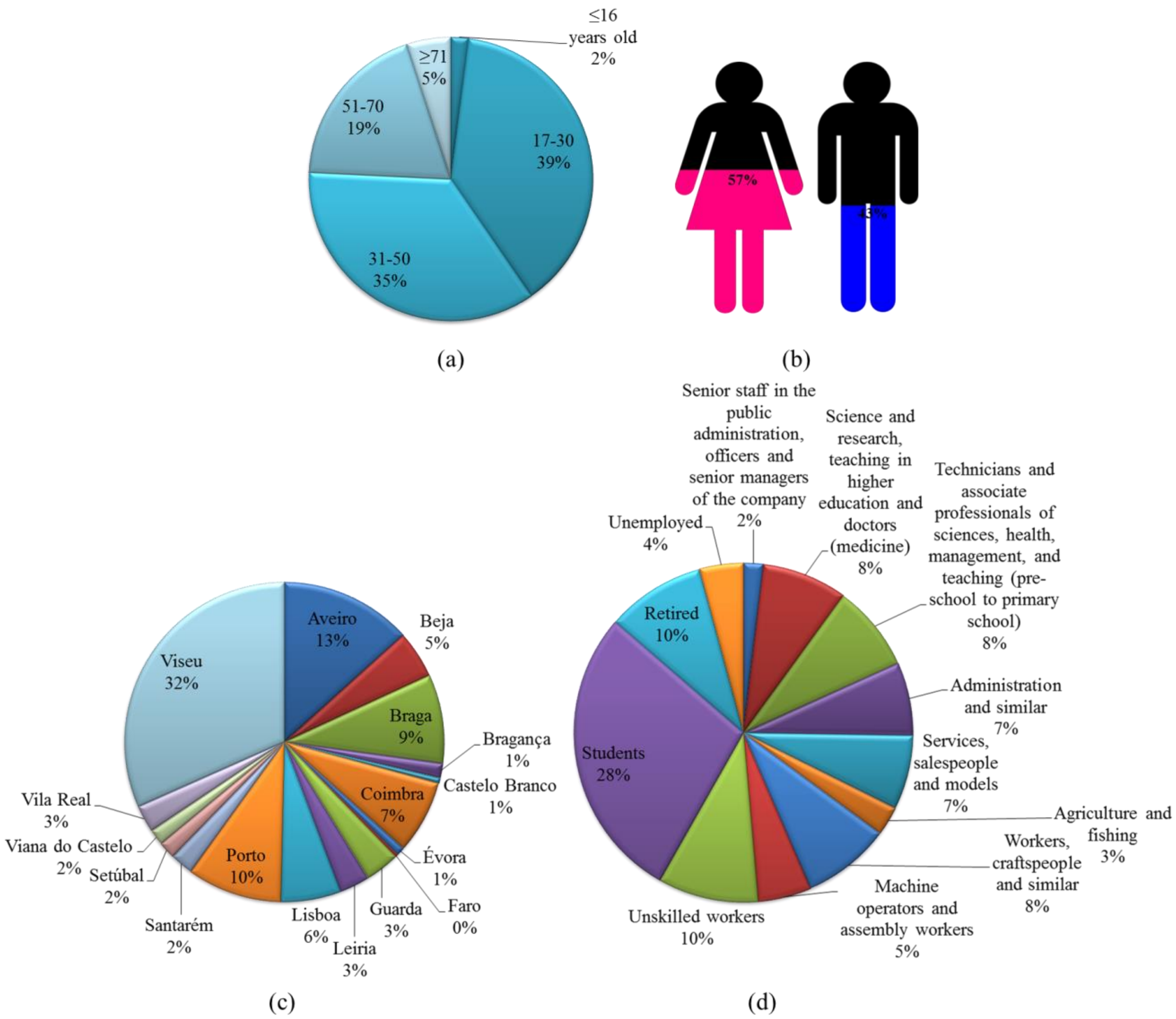


Figure 46 – Results regarding characteristics of those surveyed: age, gender, address and job.

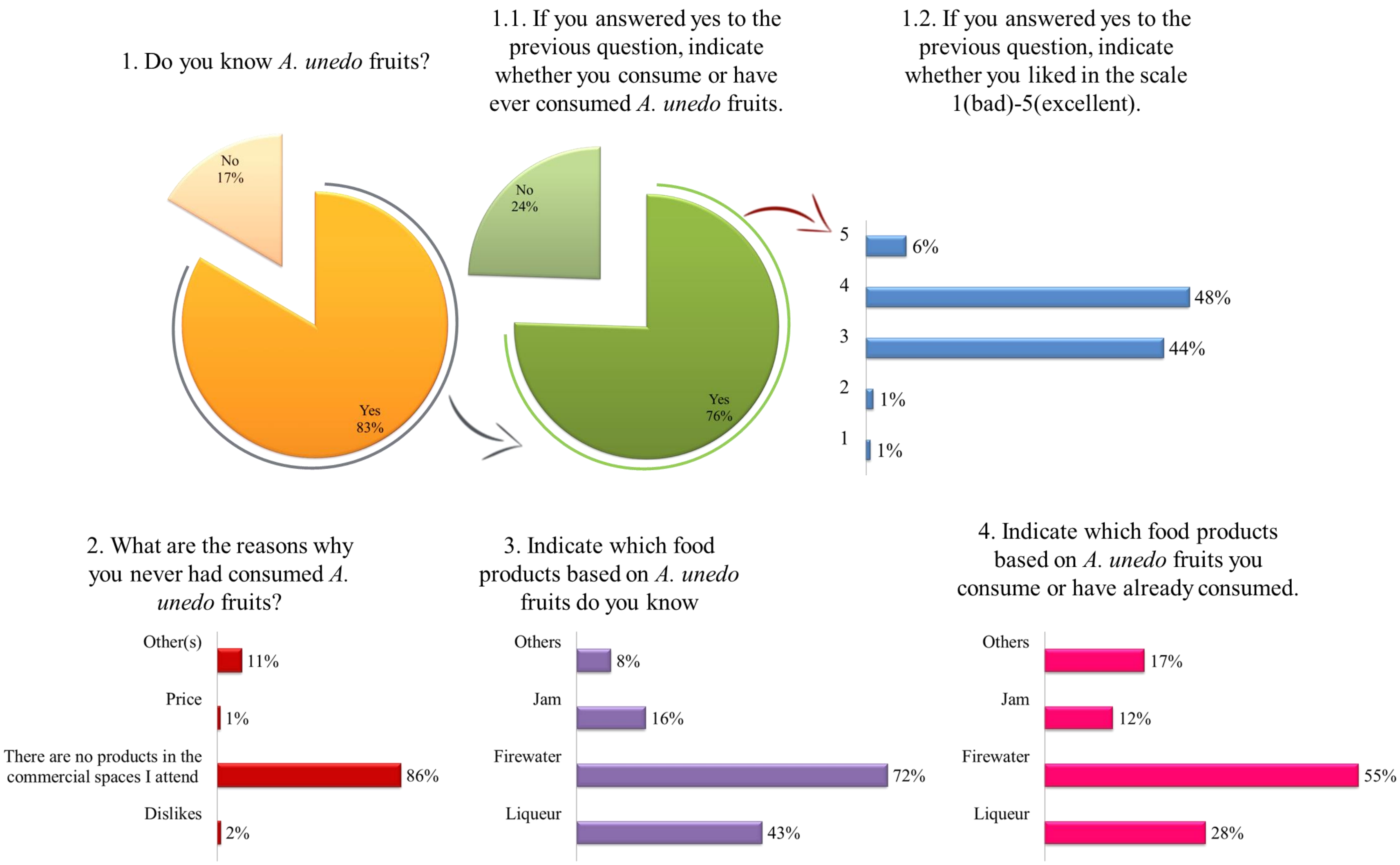


Figure 47 - Results of the survey regarding the questions

11 % stated that they have never been interested to look for these products and only a minor fraction of those interviewed (1 %) stated that the price was the determining reason. Lastly, it was asked what food products based on *A. unedo* fruits did people knew and which they consumed. All interviewed knew food products based on *A. unedo* fruits. Medronheira, the strawberry tree fruits spirit, is the most popular product (52 % of all answers), followed by liqueur (31 %) and jam (11 %). About 6% of all respondents refer to other products, mainly home-made, such as deserts and cocktails. Almost all surveyed (81 %) that knew food products based on *A. unedo* fruits had already consumed it. Medronheira, is the most consumed product (49 % of all answers), followed by liqueur (25%) and jam (11 %). About 16 % of all answers refer to other products, mainly home-made or restaurant-made, such as deserts, cocktails or a dipping sauce (chutney).

In conclusion, it is possible to recognize that the majority of those surveyed know the fruit and had also consumed it in some form. Also, they rate *A. unedo* fruits taste as good and very good. Consumer acceptance of strawberry tree fruits flavor is a key success factor. Based on the aforementioned collected data, the development of new food products with strawberry tree fruits can be a good market opportunity. As already mentioned, food products based in this fruit are scarce and only a few consumers had consumed cocktails and other home-made products. At this point, it was necessary to explore the product development process in order to focus on the different factors determining products manufacture and acceptance.

3.4.1.2 SWOT analysis

A SWOT analysis helps to take best advantage of the abilities of *A. unedo* fruits: consider strengths, exploit the opportunities, understand the weaknesses and manage the threats. In Figure 48 are identified and categorized the main factors faced in the development of new products based on strawberry tree fruits. The strengths are mainly related to the fruit’s appearance. Because they are not very popular the novelty and the exoticness are the main prominent factors. When looking at the weaknesses, stoniness is a non-appreciated sensory characteristic. Also, fruit’s seasonality and perishability are factors that decrease potential applicability. Novelty has a dual rating since it can be considered as weakness and strength as well.

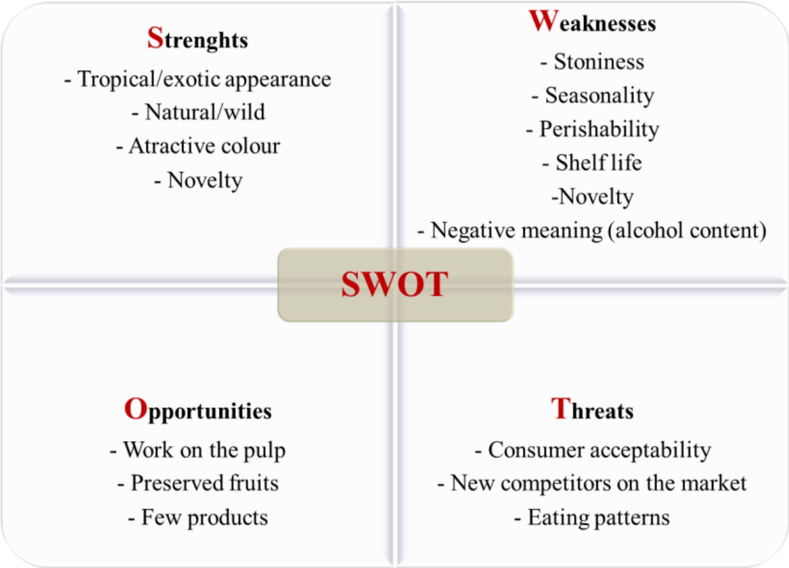


Figure 48 - SWOT analysis of *A. unedo* fruits in the development of new food formulations.

Consumers like to experience new flavors, new sensations and new products, but novelty is a potential factor for controversy and the fruit products can be rejected. *A. unedo* fruits can reach till 0.5 % alcohol when overripe, so this gives a negative connotation near population. Study solutions to overcome weaknesses can open up opportunities. So, from this point of view there were considered the weaknesses presented above and some resolutions came up: work only on pulp's fruit is important to avoid stoniness, and adopt storage strategies is a way of fight seasonality and provide the fruit through all year. Moreover, it is important to emphasize that there are only a few food products based on *A. unedo* fruits commercially available, which opens up market opportunities and allows creating a greater variety of new formulations. When looking at the threats, factors such as consumer acceptability, new market competitors and eating habits were mentioned. These are the faced obstacles and which makes the development of new food process more vulnerable. SWOT analysis helped to focus on *A. unedo* strengths to minimise weaknesses, and to take the greatest possible advantage of opportunities available.

3.4.1.3 Idea generation: brainstorming

As any other product, *A. unedo* fruits are defined by the complexity of its characteristics, which define their value and use. In order to explore initial impressions of strawberry tree fruits a brainstorming was made, allowing exploring fruit's characteristics and uncovering possible applications for these fruits (Figure 49).

(1) What does the appearance of *A. unedo* fruits reminds you?

- natural, wild, exotic, healthy and fun

(2) Accordingly to the actual market trends in which categories can the food products based on *A. unedo* fruits be inserted?

- healthy, wellness, sustainability and ethics

(3) Accordingly to the actual trends and the SWOT analysis performed, which new products based on *A. unedo* fruits can you suggest?

- cookies, cereal bars, ice cream, yoghurts, dehydrated snacks, chocolate bars, drinks, jellies, fruits purée and fruit topping (to ice cream and cakes for example)

Figure 49 - Overview of brainstorming regarding *A. unedo* fruits characteristics and future applications

This brainstorming allowed obtaining answers to the following questions: (1) What does the appearance of *A. unedo* fruits reminds you?, (2) Accordingly to the actual market trends in which categories can the food products based on *A. unedo* fruits be inserted? and (3) Accordingly to the actual trends and the SWOT analysis performed, which new products based on *A. unedo* fruits can you suggest? The general description of strawberry tree fruits gave the following characteristics: natural, wild, exotic, healthy and fun. Upon the previous description it was possible to connect *A. unedo* fruit's characteristics and the actual market trends: healthy, wellness, sustainability and ethics.

The market offers only a few products based on *A. unedo* fruits: liqueurs, spirit and jams, which allows for a broad scope of choices for future merchandise. [6,15] Based on *A. unedo* fruits characteristics a broad range of ideas came out: a new group of products with *A. unedo* fruit flavor and/or pieces such as cookies, cereal bars, ice cream, yoghurts, dehydrated snacks, chocolate bars, drinks, jellies, fruits purée and fruit topping (to ice cream and cakes for example). The discussion allowed eliminating unpromising ideas and the following ones were selected: cookies filled with fruits pulp, cereal bars with fruit pieces, filled chocolates and yoghurt with fruit.

3.4.2 Product concept development

At this stage details and technical aspects of the proposed ideas were given, considering attitudes and behaviors towards the food products. The product idea concept details the benefits that consumers want from the products (Table 7).

Table 7 – Proposed product idea concepts and descriptions

Product idea concept	Product idea description
Cookies	Artisan cookies filled with *A. unedo* fruits
Fruit & Yogurt	Smooth, creamy natural yoghurt with *A. unedo* fruits pulp and no synthetic colours or flavours
Cereal bars	Healthy crunchy cereal bar with dehydrated fruit and high fibre content
Filled Chocolates	Gourmet product; miniature desserts; an exceptional sensorial experience

These products were moved through the next stages and also there were conceived new formulations (Figure 50).

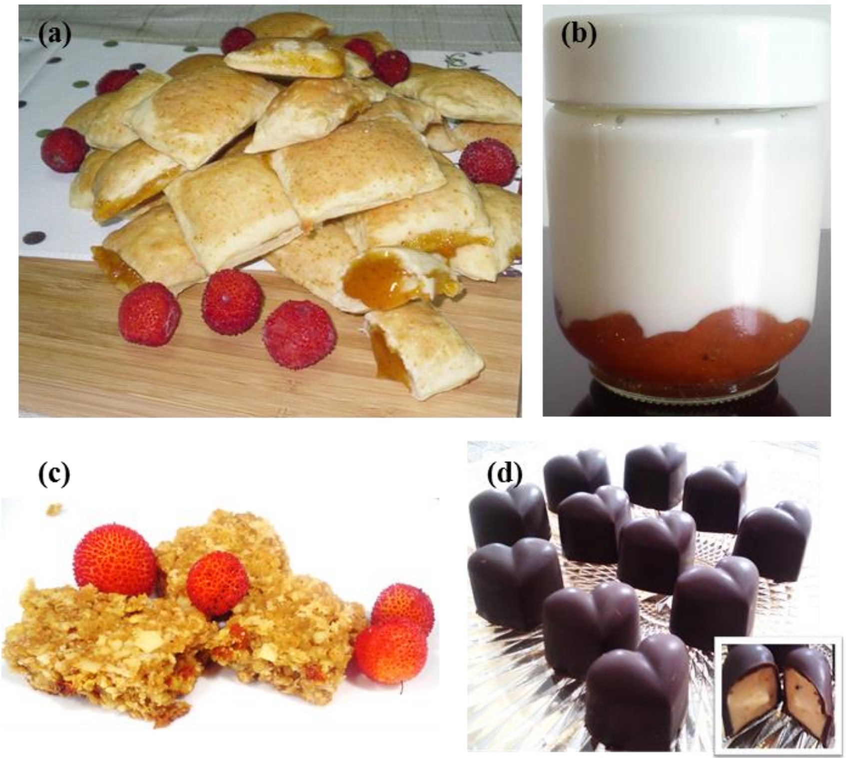


Figure 50 – Formulations prepared with *A. unedo* fruit: (a) cookies filled with *A. unedo* fruit, (b) yoghurt with *A. unedo* fruit at the bottom, (c) cereal bars with dehydrated fruit pieces and (d) chocolates.

A general production scheme for these formulations is presented at Figure 18 .During this phase, there was used *A. unedo* fruit jam as the filling. First, the jam was made from frozen fruits. Regarding the cookies production: the ingredients used were mixed together; the dough was turned on to a floured board, kneaded for a few minutes and then stretched out thinly. Small square-like pieces were cut, the jam was placed above each one of them and the edges were sealed. The cookies were placed into a greased backing sheet and baked in a moderate oven. As in the cookies, there was used *A. unedo* fruits jam in the bottom of the yoghurts. The cereal bars were made by mixing the ingredients together with the dehydrated fruit pieces. The mixture is then stretched out thinly and the cereal bars were placed into a greased backing sheet and baked in the oven. The cereal bar got cold and then was cut into square-like little pieces. The filled chocolate prototype was the last to be made. It was used a mixture containing *A. unedo* fruits jam to fill the chocolates.

The formulations produced in this last phase are in line with the current market trends. During this thesis there were made home-feasible products, however a new range of products, regarding higher technological requirements may arise. It is possible to conclude that the strawberry tree fruits can have a wide range of applications in the food area.

CHAPTER IV – Concluding Remarks and Future Work

Concluding Remarks

In this thesis, the general quality parameters (ºBrix, pH, titratable acidity, total phenolic content and antioxidant activity) of *A. unedo* fruits during maturation were assessed, by using berries from Serra da Beira. To the best of our knowledge, the present work represents the first evaluation of the impact of maturation on the berries quality parameters under study. An increase in ºBrix and pH was observed in all samples (harvested in six locations) during maturation, whereas titratable acidity, total phenolic content and antioxidant activity exhibited no remarkable tendency. The Table 8 summarizes the range values obtained for the ripen berries from Serra da Beira and also the values available on the literature concerning other Portuguese fruits [13,35,38,42,45] , indicating that, with the exception of total phenolic content, the berries under study exhibited a similar profile that the others already studied. It is important, to point out, that a significant higher phenolic content was observed for the berries under study.

Table 8 – Summary of the chemical characteristics of ripen *A. unedo* fruits

Parameters	ºBrix	Titratable Acidity[a]	pH	Total Phenolic Content[b]	Antioxidant activity[c]
Serra da Beira	22.4 - 30.1	0.81 - 0.91	2.97 - 3.17	1160 - 2222	0.28-0.59
Literature (Portuguese)[ref]	≈22[13]	-	-	≈445-941[35]	0.25-0.79[38,42,45]

[a], titratable acidity expressed as g malic acid per 100 g fresh weight, [b], total phenolic content expressed as mg gallic acid per 100 g fresh weight, [c], antioxidant activity expressed as mg extract per mL DPPH·.

The effect of storage on the quality of the fresh *A. unedo* fruits was assessed through chemical and sensory analysis. The results showed that the ripen-harvested fruits (21 ºBrix) can be stored up to 17 days whereas the unripen-harvested fruits (19 ºBrix) can be stored up to 20 days. At the best eating-point, berries from both sampling moments presented similar sensory and chemical characteristics. However, the unripen-harvested fruits were less pasty in the mouth and had significantly lower levels of ethanol. These results suggest that the harvest of fruits with *ca*. 19 ºBrix, pH 2.7 and titratable acidity of 1 g malic acid per 100 g fresh fruit, contributes to increasing their shelf-life, while maintaining the firmness and reduced ethanol content. Assessing the impact of maturation and cold storage on fresh fruits can be used as a decision tool to support *A. unedo* fruits producers of in order to reach new consumers and new markets.

The lipophilic extractives profile was analyzed by GC-MS, which represent the first phytochemical study regarding this fraction from ripen *A. unedo* fruits. It was identified 41 lipophilic compounds which belong to five chemical families: triterpenoids, fatty acids, sterols, tocopherols and long chain aliphatic alcohols. Triterpenoids (3196 - 5494 $mg.kg^{-1}$ dw) and fatty acids (587 - 2954 $mg.kg^{-1}$ dw), including ω-3 and ω-6, represented the major groups, followed by sterols (217 - 805 $mg.kg^{-1}$ dw), long chain aliphatic alcohols (30 - 453 $mg.kg^{-1}$ dw) and tocopherols (21 - 115 $mg.kg^{-1}$ dw). Given the composition put forward above, the presence of compounds with reported health benefits, particularly ω-3 and ω-6 fatty acids, phytosterols and triterpenoids, can be a determining factor on the valuation of *A. unedo*.

The final phase of this thesis included several stages, namely, research on consumer habits of *A. unedo* fruits by the Portuguese population, the assessment of market opportunities for this type of product, and the design of some products that were in line with current market trends. Thus, were conceived and tested various formulations of cookies, yoghurt, cereal bars and chocolates. The last phase of this work confirms that this fruit has numerous applications in the food area, which are in line with current market trends.

Future work

The results presented here allowed taking an important step on the knowledge regarding the *A. unedo* fruits. In the future new tasks can be taken and developed in a number of ways:

- Study the phenolic composition of *A. unedo* fruits, in order to characterize the chemical profile regarding the bioactive compounds;
- Study the biological activity of *A. unedo* extracts in order to infer about its potential effects and applicability;
- Promote the marketing and launch of the developed products;
- Study and valuate the residues of *A. unedo* fruits exploitation in order to implement recovery strategies.

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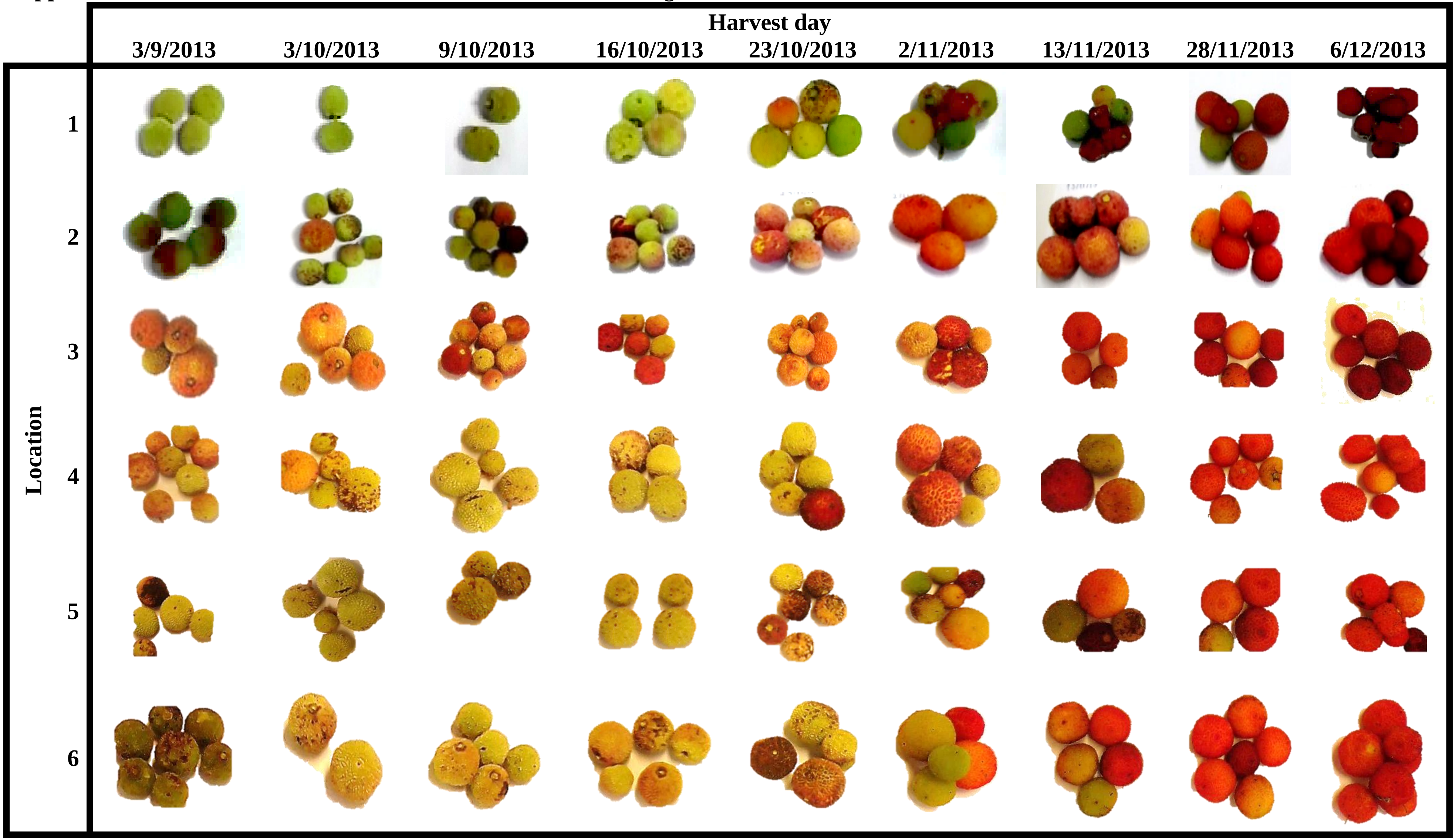
Appendix

Appendix 1. *A. unedo* fruits from different locations collected through time



Appendix 2.

Technical record

This inquiry was made by Daniela Fonseca, master's degree student, between April 10th and September 17th 2014. The target universe was composed of Portuguese individuals of all ages and inquiries were made via internet (social networking site) and also via postal. 689 answers were obtained of which 57 % are females and 43 % males. 38 % of population had between 17-30 years, 35 % between 31-50, 19 % between 51-70, 5 % plus 71 years old and only 2 % of inquired has less than 17 years old.