Universidade de Aveiro
2014

Departamento de Química

Sílvia Alexandra Monteiro Moreira

Conservação de sopa a pressão elevada e comparação com refrigeração.

Hyperbaric preservation of soup and comparison with refrigeration.

Universidade de Aveiro
2014

Departamento de Química

Sílvia Alexandra Monteiro Moreira

Nº Mec.: 49447

Conservação de sopa a pressão elevada e comparação com refrigeração.

Hyperbaric storage of soup and comparison with refrigeration.

Dissertation submitted to the University of Aveiro to fulfil the requirements for the degree of Master of Biotechnology in the field of Food Biotechnology, done under the scientific supervision of Dr. Jorge Manuel Alexandre Saraiva, Assistant Researcher of the Department of Chemistry of the University of Aveiro.

Aos meus pais, com amor.

Júri

Presidente

Professor Doutor José António Teixeira Lopes da Silva
Professor Auxiliar do Departamento de Química da Universidade de Aveiro

Vogais

Professor Doutor Jorge Manuel Alexandre Saraiva (orientador)
Investigador auxiliar do Departamento de Química da Universidade de Aveiro

Professora Doutora Maria Ângela Sousa Dias Alves Cunha
Professora auxiliar do Departamento de Biologia da Universidade de Aveiro

Agradecimentos

Em primeiro lugar, agradeço de forma geral a todos quantos contribuiram e estiveram ao meu lado ao longo da realização desta tese.

Ao professor Jorge Saraiva, pelo desafio colocado para a realização deste trabalho, por ter acreditado em mim, pela porta aberta a qualquer hora e por todo o apoio que sempre demonstrou.

A todo o pessoal do laboratório que pertence ao "grupo HP", Liliana, Mauro, Rui e mais tarde, Rita, pelo apoio, amizade, convívio, risadas e partilha de conhecimentos. E em especial à Diana, por estar sempre presente, em qualquer altura.

Aos meus amigos de sempre, Patrícia, Sara e André, que apesar de longe, estavam lá sempre que era preciso desabafar e pedir conselhos.

Aos meus amigos e colegas de tese, ao Pedro, que sempre me acompanhou nestas aventuras, e também ao Ricardo, que se tornou um amigo com o qual se pode sempre contar.

Aos meus amigos de "cá", Micaela, Jonny e Emanuel. Obrigada pelo apoio, pela companhia ao longo da escrita, pelas aventuras e pelas risadas, mesmo nos dias em que rir era mais difícil.

E por fim, mas não menos importante, à minha família, mãe, pai, mano e meus queridos avós. Obrigado por nunca terem deixado de acreditar que eu era capaz.

«O único lugar onde sucesso vem antes do trabalho é no dicionário»

Albert Einstein

Palavras-chave Armazenamento sob alta pressão, refeições prontas-a-comer, temperatura ambiente, conservação de alimentos, cargas microbianas, refrigeração.

Resumo

O principal objectivo deste trabalho foi estudar os efeitos da aplicação da alta pressão hidrostática e se esta é eficaz no armazenamento de alimentos prontos a consumir, de modo a evitar a sua deterioração a nível microbiológico e fisico-quimico, permitindo assim o aumento do seu prazo de validade, sem necessidade de refrigeração.

O armazenamento sob alta pressão foi inicialmente estudado em sopa de cenoura comercial e comparado com o armazenamento em refrigeração. A sopa foi armazenada em diferentes condições de tempo (4 e 8 h), temperatura (4, 25 e 30 ºC) e pressão (0.1, 100 e 150 MPa). Foi ainda realizado um estudo à escala industrial, onde se armazenaram dois produtos comerciais reais, Caldo verde e Bacalhau com natas, durante 12 h, sob diferentes pressões (50, 100 e 150 MPa) à temperatura ambiente (~21 ºC). Em ambos os estudos compararam-se, com os respetivos controlos à pressão atmosférica, as cargas microbianas (mesófilos totais, *Enterobacteriaceae* e bolores e leveduras) e alguns parâmetros fisico-químicos como o pH, acidez titulável, conteúdo em açúcares redutores e cor.

O armazenamento sob alta pressão levou a resultados semelhantes ou melhores que a refrigeração, uma vez que foi visível uma inibição do crescimento microbiano para 100 MPa e ainda uma inativação microbiana para 150 MPa. A pressão mais elevada (150 MPa) e o período de estudo mais longo (8 h na escala laboratorial e 12 h na escala industrial) resultaram numa inactivação microbiana mais acentuada. Os mesófilos totais foram, dos microoganismos estudados, os menos susceptiveis à pressão. No que concerne aos parâmetros fisico-químicos analisados, o armazenamento sob alta pressão resultou, de forma geral, em valores semelhantes à refrigeração.

Portanto, o armazenamento de alimentos prontos a consumir sob alta pressão, sem necessidade de controlo de temperatura, parece ser uma possível alternativa à refrigeração no que toca à conservação destes alimentos.

Keywords Hyperbaric storage, ready-to-eat food products, room temperature, food preservation, microbial loads, refrigeration.

Abstract

The aim of this work was to investigate if hyperbaric storage, using high hydrostatic pressure, is effective in delaying ready-to-eat food products microbial and physicochemical spoilage allowing extending its shelf-life, with no need for refrigeration.

Inicially, the hyperbaric storage of a ready-to-eat carrot soup was studied and compared to refrigeration. Soup was stored at diferente time (4 and 8 h), temperature (4, 25 and 30 ºC), and pressure (0.1, 100 and 150 MPa) conditions. In addition, were also performed tests in an industrial equipment with two real commercial products, *Caldo verde* and *Bacalhau com natas* that were stored for 12 h at different pressures (50, 100 and 150 MPa) at room temperature (~21 ºC). For both cases, microbial loads (total aerobic mesophiles, *Enterobacteriaceae* and yeast and moulds) and physicochemical parameters such as pH, titratable acidity, reducing sugars and colour, were compared with the controls at atmospheric pressure at the same temperature.

Hyperbaric storage resulted in similar to better results when compared to refrigeration, since 100 MPa resulted in microbial growth inhibition, while for storage under 150 MPa microbial inactivation occured. Higher pressure (150 MPa) and a longer storage period (8 h for laboratorial scale, and 12 h for the tests in the industrial equipment) resulted in more pronounced microbial growth inactivation. Total aerobic mesophiles were found to be the less susceptible microorganisms to hyperbaric storage, compared to *Enterobacteriaceae* and yeast and moulds. Concerning the physycochemical parameters, storage under pressure, generally, was able to maintain the values similar to refrigeration.

Therefore, hyperbaric storage with no need for temperature control seems to be a possible new preservation methodology, and an alternative to refrigeration.

Contents

Figures Index I
Tables Index III
List of abbreviations IV
I. INTRODUCTION 1
1.1. High pressure processing 2
1.1.1. HPP history 2
1.1.2. HPP process description and equipment 3
1.1.2.1. Isostatic Rule 4
1.1.2.2. Le Chatelier's Principle 4
1.1.3. HPP and microbial inactivation 4
1.1.3.1. Bacteria and bacterial spores 5
1.1.3.2. Yeasts and moulds 5
1.1.4. HPP impact on food constituents 5
1.2. Hyperbaric storage 6
1.2.1. Hyperbaric storage at sub-zero temperatures 8
1.2.2. Hyperbaric storage at low temperatures 8
1.2.3. Hyperbaric storage at room temperatures 9
1.2.4. Hyperbaric treatment of fruits and vegetables 13
1.3. Soup 15
1.3.1. Carrot soup and *Caldo verde* 16
1.3.2. Soup spoilage/poisoning by microbial contamination 17
1.3.3. Soup processing and preservation 20
1.3.3.1. Soup processing 20
1.3.3.1.1. Pulsed Electric Fields (PEF) 20
1.3.3.1.2. Irradiation 21
1.3.3.1.3. High Pressure Processing (HPP) 21
1.3.3.2. Soup preservation 22
1.3.3.2.1. Modified Atmosphere Packaging (MAP) 22
II. OBJECTIVES AND SCHEDULE 23
III. MATERIALS AND METHODS 25

3.1. Chemicals 26
3.2. Preparation of samples 26
3.2.1. Carrot soup 26
3.2.1.1. First attempt to increase the microbial loads of the "new soup" 26
3.2.1.2. Second attempt to increase the microbial loads of the "new soup" 27
3.2.1.3. Inoculation of "new soup" with spoiled soup 27
3.2.2. *Caldo verde* and *Bacalhau com natas* 28
3.3. Storage experiments 28
3.3.1. Laboratorial scale 28
3.3.2. Experiments in an industrial equipment 29
3.4. Microbial analyses 30
3.4.1. Sample preparation and dilution 30
3.4.2. Count of total aerobic mesophiles microorganisms 30
3.4.3. Count of *Enterobacteriaceae* 30
3.4.4. Count of yeasts and moulds 31
3.4.5. Microbial counts 31
3.5. Physicochemical analyses 32
3.5.1. Determination of pH and titratable acidity 32
3.5.2. Reducing sugars 32
3.5.3. Colour 33
3.6. Statistical analyses 33
IV. RESULTS AND DISCUSSION 35
4.1. Laboratorial scale 36
4.1.1. Microbial analyses – general overview 36
4.1.1.1 Effect of hyperbaric storage on total aerobic mesophiles 38
4.1.1.2. Effect of hyperbaric storage on Enterobacteriaceae 38
4.1.1.3. Effect of hyperbaric storage on yeasts and moulds 39
4.1.1.4. Global effect 39
4.1.2. Physicochemical analysis 40
4.1.2.1. pH and titratable acidity 40
4.1.2.2. Reducing sugars 41
4.1.2.3. Colour 42

4.2. Tests in an industrial equipment 44
4.2.1. Microbial analyses in *Caldo verde* - overview 44
4.2.1.1. Effect of hyperbaric storage on microorganisms 45
4.2.1.2. Effect of post-hyperbaric storage at 0.1 MPa and 4 ºC 47
4.2.2. Microbial analyses in *Bacalhau com natas* - overview 47
4.2.2.1. Effect of hyperbaric storage on microorganisms 48
4.2.2.2. Effect of post-hyperbaric storage at 0.1 MPa and 4 ºC 48
4.2.3. Physicochemical analysis in *Caldo verde* 50
4.2.3.1. pH and titratable acidity 50
4.2.3.2. Colour 51
4.2.4. Physicochemical analysis in *Bacalhau com natas* 52
4.2.4.1. pH and titratable acidity 52
4.2.4.2. Colour 53
V. CONCLUSION AND FUTURE WORK 55
VI. REFERENCES 59
APPENDICES i
Appendix A. Preparation of soup samples ii
Appendix B. Standards preparation and calibration curve construction for the determination of reducing sugars content in soup iii
Appendix C. Microbial counts in the studied samples in initial and after HS for various combinations of time, pressure and temperature. iv
Appendix D. Curves showing linear increase/decrease for physicochemical parameters throughout storage time vi
Appendix E. Colour differences (ΔE^*) in the samples studied viii
Appendix F. pH and titratable acidity results of *Caldo verde* and *Bacalhau com natas* samples x

Figures Index

Figure 1. Relative representation of the pressures used to process food. Adapted from Considine et al. (2008). 4

Figure 2. Microbial counts in watermelon juice during 60 h of storage at naturally variable room temperature (18-21 ºC); and for 8 h at 30 ºC. Adapted from (Fidalgo et al., 2014). 10

Figure 3. Microbial counts in melon juice initially and after 8h of storage at 100 and 150 MPa at 25, 30 and 37°C. Discontinuous bars with indicate values higher than 6 and lower than 1 Log_{10} CFU/mL. Adapted from Queiros et al. (2014). 12

Figure 4. Carrot soup (a) and *Caldo verde* (b), a Portuguese traditional soup. 16

Figure 5. Schedule to organize work. 24

Figure 6. Carrot soup used in laboratorial storage experiments. 26

Figure 7. *Caldo verde com chouriço* and *Bacalhau com natas* used for the storage experiments in industrial equipment. 28

Figure 8. Hydrostatic presses used from hyperbaric experiments: (a) laboratorial scale and (b) industrial equipment. 30

Figure 9. Carrot soup. Microbial counts (Log_{10} CFU/g soup) in initial (blue bar) and after HS of carrot soup for various combinations of time, pressure and temperature: 4 and 8 hours, 100 and 150 MPa and 25 and 30 ºC; soup was also stored at control conditions for each temperature (25 and 30 ºC, at 0.1 MPa) and at refrigeration conditions (4 ºC, 0.1 MPa). Different letters indicate significant differences ($p<0.05$) between conditions. Values shown as 6 (#) and 1 (*) (white bars with discontinuous borders) log units, mean values higher than 6 and lower than 1 log units, respectively. 37

Figure 10. *Caldo verde*. Microbial counts (Log_{10} CFU/g soup) in initial (blue bar) and after hyperbaric storage of *Caldo verde* soup at 21 ºC for 12 h and after 3 days (Post-HS) at different pressures: 50, 100 and 150 MPa; soup was also stored at control conditions (21ºC at 0.1 MPa) and at refrigeration conditions (4 ºC, 0.1 MPa). Different letters indicate significant differences ($p < 0.05$) between conditions. Values shown as 6 (#) and 1 (*) (white bars with discontinuous borders) log units, mean values higher than 6 and lower than 1 log units, respectively. 46

Figure 11. *Bacalhau com natas.* Microbial counts (Log_{10} CFU/g soup) in initial (blue bar) and after hyperbaric storage of *Bacalhau com natas* at 21 ºC for 12 h and after 3 days (Post-HS) at different pressures: 50, 100 and 150 MPa; soup was also stored at control conditions (21ºC at 0.1 MPa) and at refrigeration conditions (4 ºC, 0.1 MPa). Different letters indicate significant differences ($p < 0.05$) between conditions. Values shown as 6 and 1 (white bars with discontinuous borders) log units, mean values higher than 6 and lower than 1 log units, respectively. 49

Figure 12. *Caldo verde* pH for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions..... 50

Figure 13. *Caldo verde* titratable acidity for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions. 51

Figure 14. Difference in colour (ΔE) in *Caldo verde* for all the studied stored conditions. Different letters indicate significant differences ($p<0.05$) between conditions. 51

Figure 15. *Bacalhau com natas* pH for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions..... 52

Figure 16. *Bacalhau com natas* titratable acidity for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions. 53

Figure 17. Difference in colour (ΔE) in *Bacalhau com natas* for all the studied stored conditions. Different letters indicate significant differences ($p<0.05$) between conditions. 53

Tables Index

Table 1. Studies of hyperbaric storage at sub-zero, low and room temperatures. Adapted from (Fernandes et al., 2014)..... 7

Table 2. Studies of hyperbaric treatment of fruits and vegetables. Adapted from (Fernandes et al., 2014). 14

Table 3. Components per 100 grams of carrot soup and *Caldo verde*. Adapted from (INSA, 2010b, INSA, 2010a). 17

Table 4. Common microorganisms found in soup. Data from Food Administration (FA) are for dried soup (FA, 1995). 19

Table 5. pH and titratable acidity (TA) values for soup stored for 4 and 8 hours at different temperature (ºC) and pressure (MPa) conditions. Different letters indicate significant differences ($p < 0.05$) between conditions (a-d) and storage time to the same storage condition (A-B). Values at 4 and 8 h showed at bold are statistically different from the initial value for each parameter and storage condition. 41

Table 6. Reducing sugars values for soup stored for 4 and 8 hours at different temperature (ºC) and pressure (MPa) conditions. Different letters indicate significant differences ($p < 0.05$) between conditions (a-d) and storage time to the same storage condition (A-B). Values at 4 and 8 h showed at bold are statistically different from the initial value for each parameter and storage condition. 42

Table 7. Colour parameters for soup stored for 4 and 8 hours at different temperature (ºC) and pressure (MPa) conditions. Different letters indicate significant differences ($p < 0.05$) between conditions (a-c) and storage time to the same storage condition (A-B). Values at 4 and 8 h showed at bold are statistically different from the initial value for each parameter and storage condition. 43

List of abbreviations

AA	Ascorbic acid
ACC	Aerobic colony count
ANOVA	Analysis of Variance
BMI	Body mass index
CFU	Colony forming units
DHAA	Dehydroascorbic acid
DNA	Deoxyribonucleic acid
DNS	3,5-dinitrossalicylic acid
ENT	*Enterobacteriaceae*
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
HHP	High hydrostatic pressure
HPH	High pressure homogenization
HPP	High pressure processing
HS	Hyperbaric storage
MAP	Modified atmosphere packaging
PATS	Pressure assisted thermal sterilization
PCA	Plate count agar
PEF	Pulsed electric fields
RBCA	Rose bengal chloramphenicol agar
RTE	Ready-to-eat
TA	Titratable acidity
TAM	Total aerobic mesophiles
UHP	Ultra high pressure
VRBDA	Violet red bile dextrose agar
WHO	World Health Organization
YM	Yeasts and moulds

.....

I. INTRODUCTION

Nowadays, there is an increasing demand for safe, natural and fresh-like food products, with all the sensory, nutritional and health-related properties preserved. Consumers also look for convenience, variety, long shelf-life, appropriate cost and environmental reliability **(Sanchez-Moreno et al., 2009)**. The solution found for the producers and manufacturers is ready-to-eat (RTE) food, but a refrigerated retail chain, which cannot be broken, is usually necessary **(Murcia et al., 2009)**. Soup is one of these RTE meals and is traditionally preserved by thermal treatments (pasteurization or sterilization) which are capable to delay spoilage and avoid potential human disease. But they are also responsible for loss of nutritional and organoleptic properties (protein denaturation, non-enzymatic browning and vitamin loss) of the treated food **(Ludikhuyze and Hendrickx, 2001, Lado and Yousef, 2002)**.

There are in development non-conventional food technologies, not only for preservation, but also for keeping food quality properties. They are called non-conventional technologies since they do not use heat to preserve food **(Williams, 1994)**, and they may not be easily accepted by the consumers since they are new technologies, which may lead to new products, with different properties (aroma, flavour, odour) **(Deliza et al., 2003)**. These new methods include treatments such as light pulses, ultrasound, PEF, cold plasma, ionizing or ultraviolet radiation and high pressure processing (HPP) **(Butz and Tauscher, 2002, Knorr et al., 2011).** Nevertheless, the consumers tend to be more aware of the food non-sensorial properties (nutritional quality, microbiological safety and environmental footprint).

1.1. High pressure processing

HPP technology is also known as ultra high pressure (UHP) or high hydrostatic pressure (HHP) being a promising technology for gentle food preservation since it does not use heat, while ensure safety by inactivation of microorganisms and enzymes **(Considine et al., 2008).**

1.1.1. HPP history

HPP technology has been known for more than a century as a preservation technique and it was **Hite (1899)** who first reported that milk and fruit can be preserved by HPP at 680 MPa. Also **Bridgman (1914)** has shown that it is possible to coagulate the egg

albumin under pressure, allowing explore new product textures. Since 1980, due to developments in equipment and processes, the interest of HPP food pasteurization restarted and the first commercial products appeared in Japan in 1990, and in USA and Europe since 1996 **(Barba et al., 2012)**.

Although it was initially developed to process packed foods, is nowadays used for preserving a wide range of products (solids or liquids) such as juices, fruits and vegetables in semi-continuous processes **(Lado and Yousef, 2002)**. Other potential applications of HPP in addition to food pasteurization are biotechnological developments such as inactivation of enzymes **(Rastogi et al., 2007)** and modification of proteins **(Tabilo-Munizaga and Barbosa-Cánovas, 2004)**.

1.1.2. HPP process description and equipment

HPP treatment is currently used at industrial scale to inactivate vegetative microorganisms in order to extend food shelf-life (process named as cold pasteurization). For treatment, the food product is placed in a carrier and automatically loaded into the vessel. The pressure media, usually water, is pumped into the vessel until the desired pressure is reached, not being necessary to expend more energy to maintain pressure during treatment time **(Knorr et al., 2011)**.

The non-availability of accessible equipment has been an obstacle for HPP use in industry. However, recent technological progresses predict that the equipment cost will come down in near future **(Rastogi et al., 2007)**. The volume of the vessel may vary from less than 100 mL (laboratory scales can operating up to 1000 MPa) to more than 500 L (for industrial scales, which can achieve up to 600 MPa due to its size and material resistance) **(Berg et al., 2001)**.

Pressure and temperature are two related factors and it is estimated that temperature increases 3-9 °C for each 100 MPa increase, depending on food matrix **(Knorr et al., 2011)**. HPP usually uses pressures in a range of 100-1000 MPa (see **Figure 1)** and temperatures of 0-100 °C, being the exposure time of a few seconds to above 20 min **(Butz and Tauscher, 2002).**

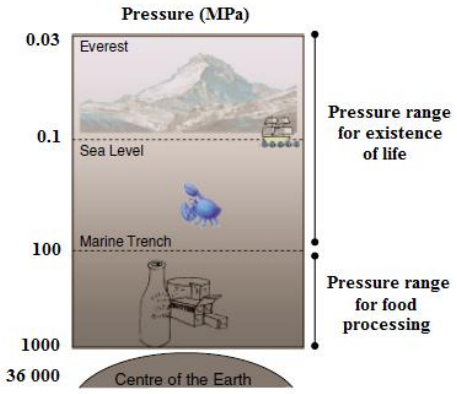

Figure 1. Relative representation of the pressures used to process food. Adapted from **Considine et al. (2008).**

1.1.2.1. Isostatic Rule

The isostatic rule states that pressure is instantaneously and uniformly transmitted through food, regardless of its size, shape, if it is in direct contact with compression media or in a flexible package **(Smelt, 1998)**.

1.1.2.2. Le Chatelier's Principle

This principle states that when an equilibrium system is perturbed, it tends to response in order to minimize the perturbation. So, any phenomenon accompanied with a volume decrease is favoured by pressure, and vice-versa **(Smelt, 1998)**.

1.1.3. HPP and microbial inactivation

The major objective of a food processing/preservation technique is to prevent the presence of pathogenic microorganisms and ensure food safety; then is necessary to inactivate spoilage microorganisms, in order to extend the product shelf-life **(Norton and Sun, 2008)**. Microorganisms are inactivated when are exposed to factors that alter cellular structure (DNA breakage, cell membrane rupture or damage, fluidity loss and detachment of membrane proteins) and/or physiological functions (key enzymes inactivation, membrane selectivity disabling, globular protein denaturation, ribosomal disintegration and inactivation of key enzymes essentials to maintain intracellular pH, such as ATPase) **(Lado and Yousef, 2002, Matser et al., 2004, Ulmer et al., 2002)**.

Bacteria and fungi can be inactivated at pressures lower than 800 MPa, being the growth and reproduction inhibited at pressures above 300 MPa **(Norton and Sun, 2008)**.

The growth stage and cell complexity are important factors to pressure resistance, since the prokaryotic cells and those in stationary phase tend to be more resistant than eukaryotes and those in exponential phase, because in stationary phase cells have smaller size, are more spherical and may accumulate components such as proteins or carbohydrates **(McClements et al., 2001, Patterson, 1999)**.

1.1.3.1. Bacteria and bacterial spores

Bacteria are responsible for food poisoning and gram-positive are considered more resistant to heat and pressure than gram-negative bacteria **(Smelt, 1998)**. This is due to the rigidity of teicoic acids in the thick peptidoglycan layer of the gram-positive cell wall **(Lado and Yousef, 2002)**. Cocci are also considered more resistant than rod-shaped bacteria due to its spherical form **(Smelt, 1998)**.

Spores are the most pressure resistant life forms known, and only pressures above 800 MPa can inactivate these microorganisms at high, ambient or low temperatures **(Ludikhuyze and Hendrickx, 2001)**. Bacterial spores can be induced to germinate at 50-300 MPa and the germinated spores can be inactivated by HPP **(Norton and Sun, 2008)**.

1.1.3.2. Yeasts and moulds

Fungi can be divided into two groups based on their vegetative structures: unicellular fungi (yeasts) and those producing hyphae (moulds). Generally, yeasts and moulds are sensitive to pressure and can be inactivated using relatively low pressures (100 MPa cause damage in cell wall of yeasts and at pressures of 200-300 MPa occur total inactivation of yeasts and moulds by damage of nuclear membrane, mitochondria and cytoplasm) **(Smelt, 1998)**.

1.1.4. HPP impact on food constituents

Besides microorganism's inactivation, HPP also acts in food constituents by protein denaturation/modification, enzymes activation/inactivation and alterations of carbohydrates and lipids **(Heremans, 1995)**. As HPP only acts in noncovalent bonds (ionic, hydrophobic and hydrogen bonds) and does not affect covalent bonds, it only causes alterations in larger molecules, such as proteins and lipids by alteration of secondary, tertiary and quaternary structures, and membranes by changes in components arrangement and architecture, leaving low molecular weight compounds intact, such as

peptides, vitamins and flavour and pigmentation compounds **(Linton and Patterson, 2000, Rastogi et al., 2007)**.

Generally, HPP treatment leads to good colour retention, contrarily to traditional thermal treatments, which cause browning **(Matser et al., 2004)**.

1.2. Hyperbaric storage

Beside all the benefits of new food preservation technologies, the storage and transportation of these products is usually carried out in refrigerated conditions, in order to achieve longer shelf-lives, retarding the food spoilage. Moreover, some raw foods are stored frozen, which has high energetic costs and causes undesirable modifications in texture and other quality parameters.

The first fact that evidenced the possibility of food storage under pressure and its viability came in consequence from the recovery of well preserved sandwiches, bouillon and apples found inside the submarine Alvin, sunken for 10 months, in deep sea, at refrigeration temperatures **(Jannasch et al., 1971)**. This discovery led to the development of another kind of combinations, with the aim to extend the products shelf-life, and the concept of storage under pressure at subzero, low and room temperature emerged (**Table 1** summarizes all the studies in these areas). "Hyperbaric storage" (HS) can be defined as the process of storing food or other biomaterials under pressure, in order to increase their stability and shelf-life since production until their distribution and consumption, by microbial growth inhibition **(Fernandes et al., 2014)**.

The use of HS at variable (not controlled) room temperature helps to potentially reduce industries energy costs, since there is no need for temperature control and energy is only needed to compression/decompression phases to reach the required pressure level, and no additional energy is necessary to maintain food products while under pressure throughout storage period.

Table 1. Studies of hyperbaric storage at sub-zero, low and room temperatures. Adapted from **(Fernandes et al., 2014)**.

Product	Conditions	Results	Reference
Sub-zero temperature			
Cod fish fillets	22.8 MPa for 36 days at -3 °C	Stable and consumable for at least 36 days. Classified with only 7 days of shelf life at 0.1 MPa.	**(Charm et al., 1977)**
Beef	200 MPa at -20 °C	Microbial load reduction and inactivation of yeasts and some bacteria.	**(Deuchi, 1990)**
Strawberries and tomatoes	50 to 200 MPa at -5 to -20 °C	Stable for a few more days/weeks. Fresh flavor and colour preserved.	**(Deuchi, 1992)**
Chicken and carp	170 MPa for 50 days at -8 and -15 °C	Stable for 50 days. Enzymatic activity reduced.	**(Ooide, 1994)**
Low temperature (Refrigeration)			
Bouillon, sandwiches and apples	15 MPa for 10 months at 3-4 °C	All the products were stable and consumable for the 10 months.	**(Jannasch et al., 1971)**
Rice, wheat and soy beans	3.5 MPa for 1 year at 1 °C	Stable for 1 year. Biochemical changes less pronounced in samples under pressure.	**(Mitsuda, 1972)**
Cod fish fillets	24.12 MPa for 21 days at 1 °C	Stable for 21 days. Classified with only 8.2 days old at 0.1 MPa.	**(Charm et al., 1977)**
Pollock	24.12 MPa for 12 days at 1 °C	Stable for 12 days. Classified with only 6.7 days old at 0.1MPa.	**(Charm et al., 1977)**
Room temperature			
Mushroom	3.5 MPa for 4 days at 20 °C	Reduction of moisture loss and browning. Larval forms growth inhibition.	**(Robitaille and Badenhop, 1981)**
Tilapia fillets	203 MPa for 12 hours at 25 ºC	Improved freshness and inhibition of deterioration only under pressure	**(Ko and Hsu, 2001)**
Strawberry juice	25, 100 and 220 MPa for 15 days at 20 °C	Stable for 15 days under pressure and more 15 days at refrigeration. Microbial load below the detection limits.	**(Segovia-Bravo et al., 2012)**
Watermelon juice	100 MPa at 18–21 ºC for 60 h	Inactivation/inhibition of microbial growth up to 60 h. Extended shelf life at 0.1 MPa after hyperbaric storage	**(Fidalgo et al., 2014)**
Melon juice	25, 50, 75, 100 and 150 MPa at 20, 30 and 37 ºC for 8 h	Stable at all temperatures under pressures above 50 MPa. Microbial growth inhibition at 50/75 MPa and reduction plus inhibition at 100/150 MPa	**(Queiros et al., 2014)**

1.2.1. Hyperbaric storage at sub-zero temperatures

The combined effect of high pressure and low temperatures results in a decreasing of the water freezing point, which makes possible to have liquid water at -22 °C and 209 MPa **(Norton and Sun, 2008)**, not being food products subject to freezing/thawing processes, allowing to maintain their structural and nutritional properties **(Kalichevsky et al., 1995)**.

Charm et al. (1977) were the first to study the potential of HS at sub-zero temperatures of codfish fillets (22.8 MPa, -3 °C, 36 days). Samples maintained its initial microbial loads, and a professional panel has characterized the pressurized fish as if it was only 7 days old. Also beef, strawberries and tomatoes maintained their fresh taste and colour during storage under 50-200 MPa, at -20 to -5 °C, with decrease of microbial counts of most microorganisms and inhibition of the enzymatic activity when compared to control **(Deuchi, 1990, Deuchi, 1992)**. Pressure also prevented raw pork from dripping, a characteristic of the thawing process **(Deuchi, 1991)**. A similar inhibitory effect on enzyme activity was obtained by **Ooide (1994)** who used pressure of 170 MPa, at -15 to -8 °C, for 50 days, on chicken and carp muscle, showing that meat texture can be preserved without significant protein denaturation.

For some cases, HS at sub-zero temperatures can be similar or even more efficient than refrigeration, but cannot inhibit the enzymatic activity as freezing **(Charm et al., 1977)**. Therefore, certain combinations of sub-zero temperatures and pressure can be used to extend food products shelf-life by enzyme and microbial inhibition.

1.2.2. Hyperbaric storage at low temperatures

As mentioned before, the first evidence of the viability of HS at low temperatures was the recovery of well preserved sandwiches, bouillon and apples from the sunken submarine Alvin, after 10 months in a depth of 1540 m (15 MPa), at 3-4 °C **(Jannasch et al., 1971)**. These food products remained stable for a few more weeks under refrigerated conditions and the authors attribute this stability to pressure action. **Charm et al. (1977)** studied the effect of 24 MPa, at 1 °C, for 12 and 21 days on pollock and codfish, respectively. The results showed that when stored under pressure, the fish maintained their fresh quality, classified by a panel with about half days old (12 days pollock was classified with 6.7 days old, and 21 days codfish with 8.2 days old), without microbial growth,

whereas those stored at 0.1 MPa were classified as unacceptable after the study period **(Charm et al., 1977)**. Another experiment was carried out with rice, wheat and soybeans that were stored at a depth of 30 m, at 1 °C, for 1 year, and were reported fewer modifications in seed moisture, fatty acids, vitamin B and reducing sugars, while germinative capacity, palatability and cooking quality were better for food stored under pressure than for those kept at 0.1 MPa, 1 ºC **(Mitsuda, 1972)**.

1.2.3. Hyperbaric storage at room temperatures

Both the aforementioned methods need temperature control throughout the entire process to maintain either sub-zero or refrigeration temperatures. As an alternative, it is in development the HS using room temperatures, a process optimization, which turns this method almost energy-independent, since, as stated before, there is no temperature control and energy is only needed for the compression/decompression phases until reaching the required pressure level, allowing maintaining food products under pressure for longer time periods **(Segovia-Bravo et al., 2012)**.

The first study regarding this methodology was the HS application on mushrooms at 3.6 MPa, at 20 °C, for 4 days, with an atmosphere composed by O_2, N_2 and CO **(Robitaille and Badenhop, 1981)**. The authors found out that mushrooms stored under pressure had a lower moisture loss, the respiration rate was not affected, was possible to observe a lower browning degree, and there was not larval forms growth, unless after one week later, on pressurized samples. Similar results were obtained for tilapia fillets, where not only an inhibitory effect was observed in the growth of total plate counts (for fillets stored under 101 MPa for 12 h at 25 ºC), as well was observed a reduction of about 2.0 log CFU/g (for fillets stored under 203 MPa for the same time period and temperature) **(Ko and Hsu, 2001)**. It was also evaluated the freshness quality index, which indicate a higher freshness for pressurized (203 MPa) fillets than for the controls (0.1 MPa). **Ko and Hsu (2001)** also studied the post-hyperbaric storage (post-HS) (12 h at 4 ºC) and verified that some enzymes and microbes reactivation occur, which means that when lower pressure is applied for longer times (preservation) it has an inhibitory effect, contrarily to the inactivation effect observed for higher pressures and shorter times used for processing.

The most recent studies are focused on fruit juices preservation. The first one was developed by **Segovia-Bravo et al. (2012)** who studied the effect of 25, 100 and 220 MPa,

at 20 °C, for 15 days on strawberry juice. The results showed that control juice (0.1 MPa, 20 °C) had high microbial counts (total aerobic mesophiles and yeasts and moulds), presenting an unpleasant smell after 15 days, while in pasteurized and pressurized juice the microbial counts were below the detection limits (<1.00 Log_{10} CFU/mL). Similar results were obtained for watermelon juice stored under 100 MPa, at naturally uncontrolled variable room temperature (18-21 °C) and above (30 °C), for 60 and 8 h, respectively (see **Figure 2**) **(Fidalgo et al., 2014)**.

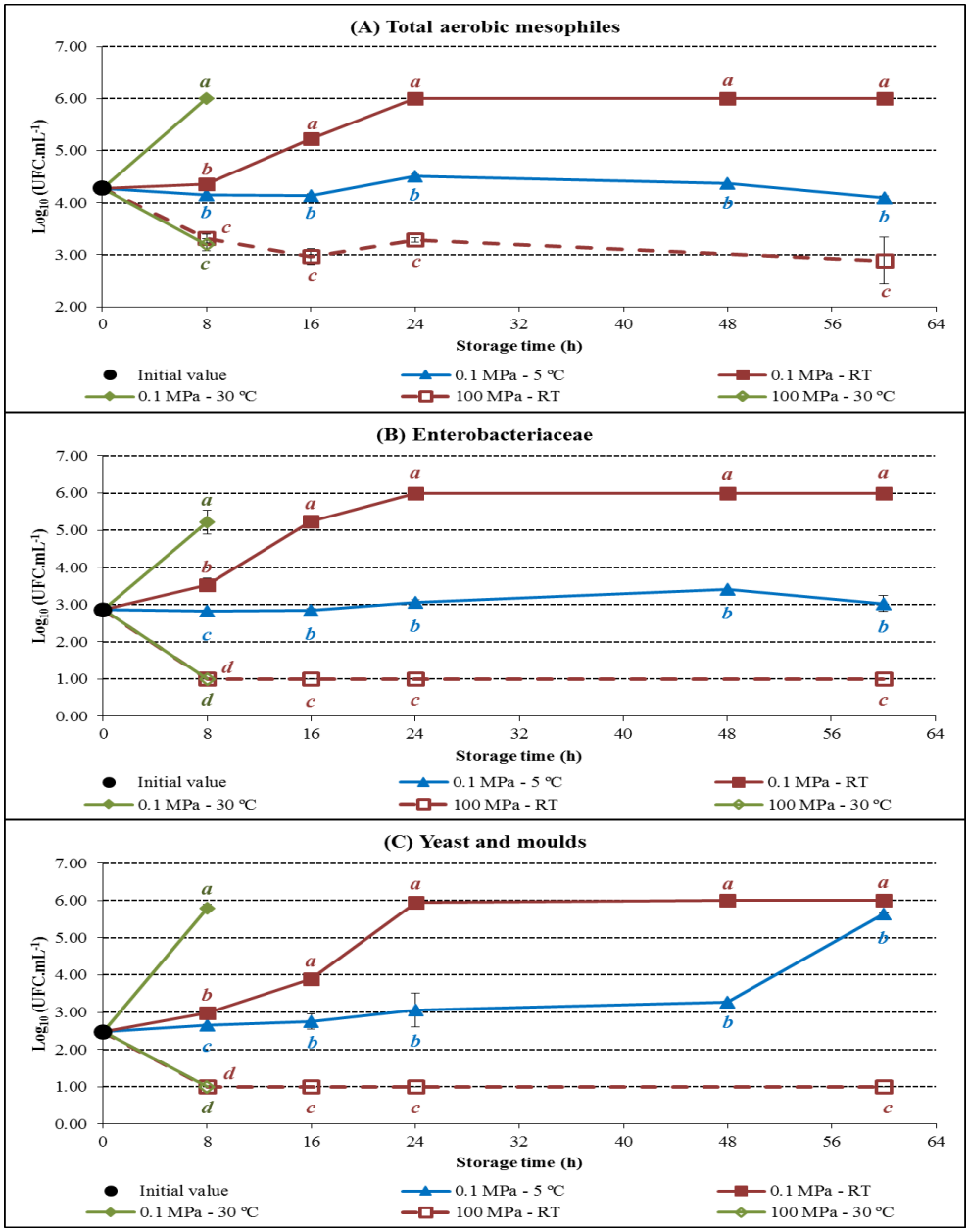

Figure 2. Microbial counts in watermelon juice during 60 h of storage at naturally variable room temperature (18-21 ºC); and for 8 h at 30 ºC. Adapted from **(Fidalgo et al., 2014)**.

Watermelon juice presented a high microbial load increase in the first 24 h at 18-21 ºC, 0.1 MPa, which led to an unpleasant odour. Nevertheless, the juice stored under refrigeration (0.1 MPa, 5 ºC) presented microbial counts similar to the initial value for all the studied microorganisms, confirming the refrigeration inhibitory effect on the microbial growth. Furthermore, the juice stored under pressure showed a microbial growth reduction in the first 8 h of storage, remaining this value unchanged throughout the storage period **(Fidalgo et al., 2014)**.

The last known study in this area was developed by **Queiros et al. (2014)**, who observed that pressurized melon juice showed a total aerobic mesophiles, *Enterobacteriaceae*, and yeasts and moulds growth reduction (the low microbial load resulted in an odour of fresh raw melon juice) while control juice at 0.1 MPa presented unpleasant odour and strong off-flavours, which was related to the higher microbial load. When stored under pressure at variable room temperatures (25, 30 and 37 ºC) for 8 h, the lower pressures (50 and 75 MPa) resulted in microbial counts similar to refrigeration, showing an inhibitory effect on microbial growth; in the case of the higher pressures studied (100 and 150 MPa) the results showed a reduction of the microbial loads, when compared to the initial value, resulting in better results when compared to samples stored under refrigerated conditions (see **Figure 3**) **(Queiros et al., 2014)**.

Segovia-Bravo et al. (2012) and **Fidalgo et al. (2014)** also studied the post-HS effect in strawberry and watermelon juices, respectively. In the case of strawberry juice, during the 15 days of post-HS there was not observed any microbial growth in pressurized or pasteurized samples, while in the juice stored in refrigeration the value kept increasing **(Segovia-Bravo et al., 2012)**. Similar results were observed by **Fidalgo et al. (2014)** since the microbial loads of the pressurized samples remained stable for 7 more days under refrigeration.

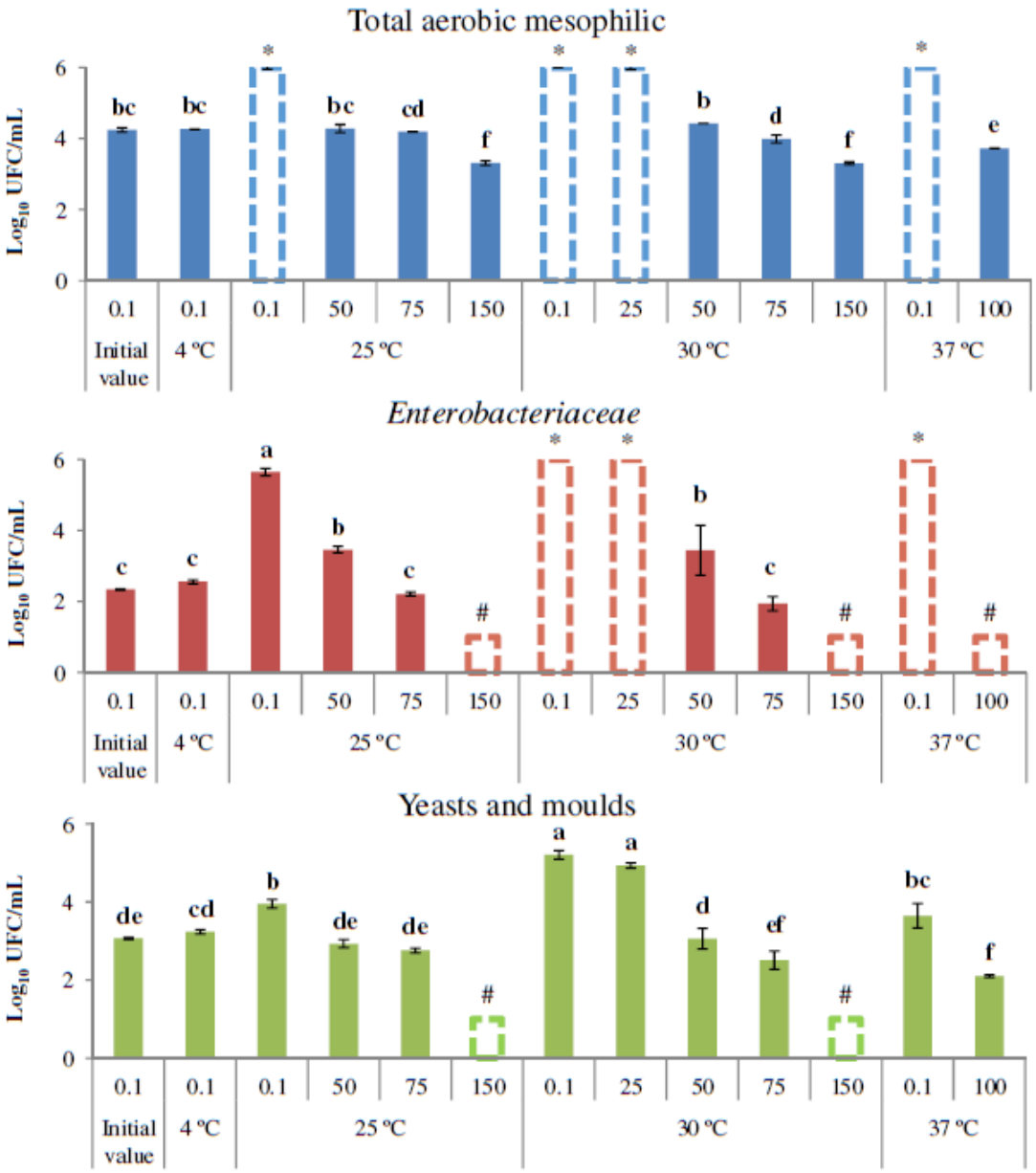

Figure 3. Microbial counts in melon juice initially and after 8h of storage at 100 and 150 MPa at 25, 30 and 37°C. Discontinuous bars with indicate values higher than 6 and lower than 1 Log_{10} CFU/mL. Adapted from **Queiros et al. (2014).**

Other parameters such as viscosity, cloudiness, juice colour and stability were studied after storage under pressure. Pressurized strawberry juice remained stable for the 15 days of storage, with higher viscosity decay attenuation for higher pressures **(Segovia-Bravo et al., 2012)**; for watermelon and melon juices, pressure also decreased cloudiness changes, being the juices similar to the initial one, without phase separation **(Queiros et al., 2014, Fidalgo et al., 2014)**. Concerning the colour changes, it was observed higher

colour decay for pasteurized strawberry juice, while the juice stored under pressure did not present significant colour degradation **(Segovia-Bravo et al., 2012)**. However, for watermelon juice, pressure caused higher colour changes which might be related to the different pH values between strawberry and watermelon juices **(Fidalgo et al., 2014)**. Relatively to the post-HS stability, strawberry juice remained stable for more 15 days, without viscosity or colour changes **(Segovia-Bravo et al., 2012)**. Similar results were obtained for watermelon juice for 7 days **(Fidalgo et al., 2014)**.

1.2.4. Hyperbaric treatment of fruits and vegetables

Nowadays there is a crescent demand for fresh fruit and vegetables due to their high concentration of compounds with benefits to human health. However, fruits and vegetables are grown on regional and seasonal basis and its highly perishable nature makes necessary to search for better and efficient preservation methods in order to improve production, postharvest handling, processing and retailing **(Sinha et al., 2010)**.

The "hyperbaric treatment" applied to fruits and vegetables conservation consists on the use of low pressures (between 0.1 and 1.0 MPa), in order to preserve cell wall and membranes structure and extend these products shelf-life **(Goyette et al., 2012)** (see **Table 2** for summarized information of the studies concerning this area).

The first studies were developed by **Baba and Ikeda (2003)** who tried to preserve mume fruit under 0.1-0.5 MPa, at 4 °C, for 5 days. After HS, the fruit presented benefits against chilling, and decreased ethylene and CO_2 production. Five years later, when the same authors made the same test for 10 days, they obtained the same results for mume fruit but not for sweet basil and rock salad leaves, which exhibited browning injuries at 0.5 MPa **(Baba et al., 2008)**. A similar effect was observed for table grapes and sweet cherries when stored under 0.15 MPa, at 20 °C, for 4-24 h **(Romanazzi et al., 2008)**, who reported that the lesion diameter and the percentage of infected berries was decreased when stored under pressure.

Table 2. Studies of hyperbaric treatment of fruits and vegetables. Adapted from **(Fernandes et al., 2014).**

Hyperbaric Treatment			
Mume fruit	0.5MPa for 5 days at 5°C	Stable for at least 5 days. Acceptable color quality, decrease ethylene and CO_2 production.	**(Baba and Ikeda, 2003)**
Mume fruit, sweet basil	0.5 MPa for 10 days at 4 °C	Stable for 10 days. Inhibition of discoloration and chilling injuries for mume fruit. Sweet basil exhibited browning injuries.	**(Baba et al., 2008)**
Peach	0.414 MPa for 4 weeks at 4.4 °C	Decrease in total volatiles production.	**(Yang et al., 2009)**
Tomato	0.1, 0.3, 0.5, 0.7 and 0.9 MPa for 5, 10 and 15 days at 13 ºC	Decrease of respiration rate, weight loss and ripening process.	**(Goyette et al., 2012)**
Sweet cherries	0.15 MPa for 4 hours at 20 °C	Decrease of mould contamination (brow and total rots, grey and blue moulds).	**(Romanazzi et al., 2008)**
Table grapes	0.15 MPa for 1 day at 20 °C	Reduction of infected berry and percentage of lesion diameter.	**(Romanazzi et al., 2008)**
Tomato	0.1, 0.3, 0.5, 0.7 and 0.9 MPa for 4 days at 20 ºC	Lycopene synthesis inhibition during hyperbaric storage. No influence in total phenolics and ascorbic acid content.	**(Liplap et al., 2013a)**
Tomato	0.1, 0.3, 0.5, 0.7 and 0.9 MPa for 4 days at 20 ºC	Effective reduction of weight loss. Firmness conservation and retardment in ripening colour development.	**(Liplap et al., 2013b)**
Lettuce	0.1, 0.2, 0.4, 0.6 and 0.85 MPa for 3, 5 and 7 days at 20 °C	Product marketable for 5 days. Sensory and visual quality similar to refrigeration.	**(Liplap et al., 2014)**

To explore the viability of HS on tomato, **Goyette et al. (2012)** studied its characteristics under pressure of 0.1-0.9 MPa, at 13 °C, for 5, 10 and 15 days. The main conclusion was a decrease of respiration rate as pressure increased (with a maximum reduction at 0.9 MPa). In another study developed by **Liplap et al. (2013a)**, lycopene, phenolic compounds, ascorbic acid content, and antioxidant capacity of tomato stored under 0.1-0.9 MPa, at 20 °C, for 4 days, was evaluated. The results showed that lycopene synthesis is affected by pressure, time and temperature, while total phenolics, ascorbic acid and antioxidant capacity are only affected by time and remain stable under pressure. Similar conclusions were obtained for mume fruit, lettuce, and tomato stored under

pressures of 0.1-0.9 MPa, at 20 °C, for 4 days **(Liplap et al., 2013b, Liplap et al., 2014, Baba and Ikeda, 2003)**.

Concerning colour changes, some studies have confirmed that hyperbaric storage can retard red colour development in tomato and retain colour quality of lettuce and mume fruit **(Liplap et al., 2014, Liplap et al., 2013b, Baba and Ikeda, 2003, Baba et al., 2008)**. Also titratable acidity (TA) is an important parameter, since a lower TA is related to a faster ripening process. **Goyette et al. (2012)** reported that pressurized tomatoes had a lower TA value than the control at 0.1 MPa. Also, **Liplap et al. (2013b)** showed that refrigeration is more efficient in retarding the tomato ripening process. Another important quality parameter is aroma, and **Yang et al. (2009)** studied the effect of 0.414 MPa, at 4.4 °C, for 4 weeks, on peach volatiles. It was found that pressure reduced the total volatiles concentration, since inhibits ester biosynthesis, by enzymatic inactivation. This may be an undesirable effect of pressure, since odour is an important parameter to consumer's choice.

1.3. Soup

In today's society it is evident a crescent demand for RTE and ready-to-use meals, which are defined as an assembly of precooked foodstuffs, packed and sold together, being needed a constant refrigeration chain, with the propose to provide to the consumers a convenient meal **(Murcia et al., 2009)**. Due to a stressful life-style the population tend to adopt unhealthy food-habits which affect health and wellness. The better and easier way to improve health is through alimentation, since there are various foods with proven efficacy against a number of diseases that promote a healthier life-style. However, consumers demand tend to focus on food products that are tasty and convenient, which must have reduced levels of sugar, fat and salt **(Mitchell et al., 2011)**.

Accordingly to the World Health Organization (WHO) there are eight risk factors that are responsible for about 60% of cardiovascular diseases: alcohol and tobacco use, high blood pressure, high body mass index (BMI), high cholesterol, high blood glucose, low fruits and vegetable intake, and physical inactivity. These risks are usually associated with developed countries, but 84% of the total global diseases occur in developing countries and it is estimated that a decrease of exposure to these risks could increase global life expectancy by about 5 years **(WHO, 2009)**. Prevention is a more effective strategy than treatment of chronic diseases, and vegetable products consumption is strongly

recommended by several organizations such as European Food Safety Authority (EFSA), Food and Agriculture Organization (FAO) and WHO, which propose a daily fruits and vegetables intake of 400-600 g/d (approximately 5 pieces a day) **(Martínez-Tomás et al., 2012, Allende et al., 2006)**.

Soup can be defined as the most universal dish, having endless variety with different textures, tastes or forms, easily prepared and it is categorized among the most basic dishes **(Rumble, 2009)**.

The modern word "soup" derives from the old French words "sope" and "soupe", and, as a food, consists of a combination of meat, fish, poultry, vegetables or fruits stew with various other ingredients, in water, with the purpose of extract all its nutritional value **(Smith, 1950, Scully and Scully, 2002)**.

1.3.1. Carrot soup and *Caldo verde*

Vegetable products have higher storage stability when compared to fresh vegetables since they can be converted into a consumable form, such as soup. Carrot soup and *Caldo verde* were the two RTE soups studied in this work (**Figure 4**).

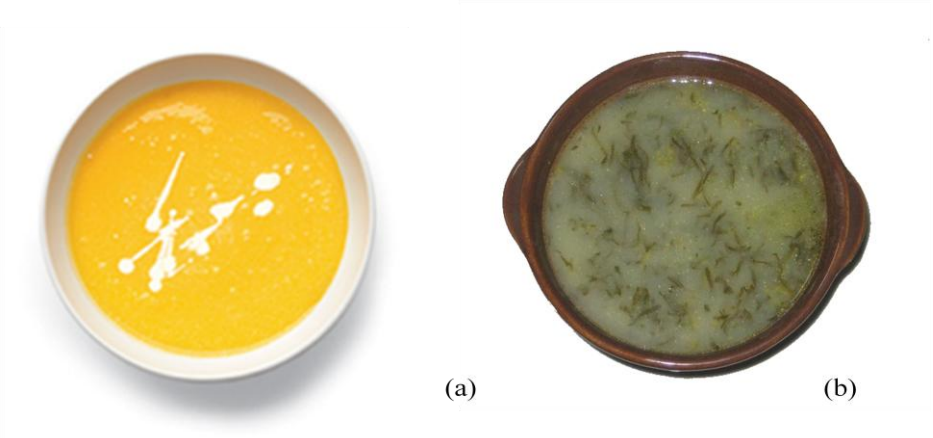

Figure 4. Carrot soup (a) and *Caldo verde* (b), a Portuguese traditional soup.

The carrot (*Daucus carota* var. Sativa) is one of the most important root crops grown around the world. This vegetable is consumed fresh, canned, dehydrated or in form of salad, juice or soups, and can masquerade as a fat substitute by serving as a thickener in soups and sauces **(Sarkar and Sharma, 2010)**.

As can be seen in **Table 3**, carrot soup is nutritionally very rich, with major amounts of water, carbohydrates (including dietary fibre) (55 %), carotenoids, retinol and

sodium and potassium, with low values of protein and calories **(INSA, 2010b)**. **Hedren et al. (2002)** defended that little nutritional value is lost in cooking and that in fact, nutrients in lightly cooked carrots are more usable (around 39 % of β-carotene is absorbed) by human body, than those in raw carrots (only 3 %), because cooking process breaks the cell walls and allows the β-carotene release. These results led to the hypothesis that carrot soup may have health benefits when included in human diet.

Caldo verde is a Portuguese traditional soup, constituted by potato puree and cabbage cut into thin stripes. It is a green thick soup usually served with a slice of chorizo and a slice of cornbread. In **Table 3** is possible to see that *Caldo verde* is very rich in water, carbohydrates (52 %), fat (37 %) and protein (12 %). It is also very rich in carotenoids, sodium and potassium **(INSA, 2010a)**.

Table 3. Components per 100 grams of carrot soup and *Caldo verde*. Adapted from **(INSA, 2010b, INSA, 2010a).**

Components per 100 g	Carrot soup	*Caldo verde*
Water (g)	92.7	90.1
Protein (g)	0.6	1.2
Fat (monosaturated fatty acids) (g)	1.1	1.1
Fat (polysaturated fatty acids) (g)	0.1	0.2
Carbohydrates (g)	4.8	5.7
Dietary fiber (g)	0.8	0.7
Retinol (ug)	128	28
Carotenoids (mg)	768	170
Sodium (mg)	242	267
Potassium (mg)	144	141
Calcium (mg)	12	31

1.3.2. Soup spoilage/poisoning by microbial contamination

The presence and detection of foodborne pathogens and spoilage microorganisms in RTE food products is very important since the majority of food poisoning cases has bacterial causes (60-90%), and can be in the origin of intoxication and infections **(Belitz et al., 2009)**. Spoilage can be achieved by enzyme activity, however, in soup, a heat

processed product, enzymatic activity can be neglected, so the major spoilage agents are microorganisms. Signs of soup spoilage may include change in colour and texture, and an unpleasant odour and sour taste. Spoilage microorganisms (such as total aerobic mesophiles, and yeast/moulds) develop relatively fast in products like soup, mostly due to the high pH (>4.5) and water activity (>0.95) that pose no considerable barriers to microbial growth, jeopardizing food safety and its shelf-life **(Shibeshi and Farid, 2011)**.

The spoilage microorganisms are inevitably introduced in food products during the processing, by slicing, packaging, and other manipulations, but this fact can be minimized by good hygiene of the equipment and the handlers **(HPA, 2009)**. The Aerobic Colony Counts (ACC), also known as the Total Aerobic Mesophiles (TAM), are an indicator of quality, not safety, and cannot directly contribute towards a safety assessment of RTE food product. In **Table 4** is possible to see that the maximum allowed level of TAM in soup is 10^6 CFU/g, meaning that below that value the flora is a mix of microorganisms and above this level there is, usually, a predominant organism, being the acceptability and the quality associated with the type of organism. The spoilage is noticeable by the production of taints, discolouration and slime **(HPA, 2009)**. *Enterobacteriaceae* is a hygiene indicator, since this group is associated with poor hygiene status of a food product, being these microorganisms generally killed by heat processes. They are a group of Gram-negative non-spore-forming bacteria, generally responsible for spoilage **(Lehner and Stephan, 2004)**. Therefore, their presence in heat treated foods indicates inadequate cooking or post-processing contamination **(HPA, 2009)**. Yeast and moulds cause spoilage by acid and gas production and its maximum level allowed is 10^6 CFU/g **(HPA, 2009)**. Many fungal species are able to produce mycotoxins that may jeopardize the safety of a wide range of foods, since this group of microorganisms is capable of grow under a wide range of pH (2 to 9), temperatures (5 to 35 ºC) and water activity (above 0.85) **(Tournas et al., 2006)**.

To have a more complete list of the entire microorganisms that can poison soup (see **Table 4**), it is necessary to take into account all the processing phases. Soup is usually made up from vegetables, which are in very close contact to soil until harvesting; carrots, potatoes and cabbage for being root vegetables, tend to have more pesticide residues than non-root vegetables. The most important soil microorganisms for food industry include yeast and moulds, and some strains from *Bacillus* and *Clostridium*. It is also common to find *E. coli* O157:H7 in unwashed vegetables **(Belitz et al., 2009)** due to manure

utilization to fertilize crops. After the harvest phase, contamination can come from water during washing and cooling steps. During processing, food can be contaminated from human handling and equipment, and the most concerning strains belong to *E. coli* O157:H7, *Staphylococcus* and *Shigella* **(Jay et al., 2005)**.

Table 4. Common microorganisms found in soup. Data from Food Administration (FA) are for dried soup **(FA, 1995)**.

	Minimal level (CFU/g)	Maxim level (CFU/g)	Possible cause	Reference
Quality/ hygiene indicator				
Aerobic colony count	$< 10^4$	$> 10^6$	-	(FA, 1995)
Enterobacteriaceae	$< 10^2$	$> 10^4$	Poor hygiene due to undercooking, food handlers or food contact surfaces	(HPA, 2009)
Yeast and moulds	-	$> 10^6$	-	(HPA, 2009)
Hazard				
C. perfringens	< 10	$> 10^2$	Improper storage or holding temperatures	(FA, 1995)
E. coli O157:H7	Detected	Not Detected	Inadequate processing; cross contamination	(HPA, 2009)
B. cereus	$< 10^3$	$> 10^5$	Poor processing, poor quality raw materials, poor temperature control	(HPA, 2009)

In soup is common to find *Bacillus cereus*, a group of bacteria that live scattered around the environment and its spores can survive to the cooking process **(HPA, 2009).** There is also a high risk of *Clostridium perfringens* contamination in vegetable and meat soups due to close soil contact of raw materials and its presence in digestive tract of human and animals **(De Jong et al., 2005, De Jong et al., 2004)**. These two microorganisms cause illness by releasing a toxin in the food product or in the digestive tract after consumption **(HPA, 2009)**. Also *E. coli*, an *Enterobacteriaceae*, is usually find in RTE foods (soup is frequently contaminated) **(Ryu et al., 2012)**, since this bacteria group is originated from intestinal tract of animals and humans, being related to equipment and surfaces inappropriate cleaning **(HPA, 2009)**.

1.3.3. Soup processing and preservation

Vegetable processing has three main objectives: (i) improving the shelf-life and vegetables quality; (ii) enhancing their palatability and digestibility; and (iii) inactivation of nutritional inhibitors that increase nutrients availability **(Butt and Sultan, 2010)**.

Preserving foods by heating has the main objective to kill vegetative bacteria, bacterial spores and inactivate native enzymes, by adjustment of adequate time and temperature. Low acid foods (pH > 4.6) are usually processed by thermal processes, and although this method ensures food safety and extends shelf-life, such drastic treatment affects food quality, fresh-like characteristics loss, and some nutrients degradation (vitamins are sensitive to different processing phases like trimming, blanching and canning) **(Sinha et al., 2010, Hui et al., 2008, Shibeshi and Farid, 2011)**.

1.3.3.1. Soup processing

Soup is a healthy and an easy-to-prepare dish, generally processed by heat (pasteurization) and has the inconvenient of fresh-quality characteristics loss and need constant refrigeration (high perishable nature) since production until its consumption. For so, nonthermal processing methods are in development, like pulsed electric fields, irradiation and high hydrostatic pressure.

1.3.3.1.1. Pulsed Electric Fields (PEF)

Another alternative to heat treatments is the use of PEF technology that uses short electric pulses (ms or μs) at high electric field strengths (kV/cm) and moderate temperatures **(Sun, 2005)**. It is common to use PEF as a preservation method for fruit juices and other vegetable products like soup. **Sanchez-Moreno et al. (2005)** studied the bioavailability of vitamin C from PEF-treated vegetable soup (*gazpacho*) in comparison with a fresh made soup. The authors concluded that there were no differences between the fresh and treated soup concerning to vitamin C concentration, and that PEF treatment allows maintaining soup for longer times. Similar results were obtained by **Elez-Martínez and Martín-Belloso (2007)** who also studied the effect of PEF in *gazpacho* antioxidant activity and concluded that this treatment does not affect antioxidant activity and causes minor loss of vitamin C, contrarily to the products treated with heat, which have lower antioxidant capacity.

1.3.3.1.2. Irradiation

Irawati et al. (2007) studied the effectiveness of medium irradiation doses at room temperature as a method to ensure microbial safety and to extend some RTE foods (such as soup) shelf-life. The authors observed that lower doses (1 kGy) treated samples quickly spoiled, similar to the non-irradiated control. However, the soups treated with higher doses (> 5 kGy) presented lower microbial loads (total aerobic mesophiles and yeast/moulds) when compared to the initial value, without affecting the physicochemical parameters **(Irawati et al., 2007)**. Nevertheless, it is noteworthy that this methodology is combined with refrigeration conditions (5 ºC), being necessary to control the temperature along the entire storage period (3 months).

1.3.3.1.3. High Pressure Processing (HPP)

Sánchez-Moreno et al. (2004) treated *gazpacho* soup using pressure of 400 MPa, at 40 °C for 60 s, in order to study the effect of HPP on vitamin C content and antioxidant activity. The results showed that consumption of HPP treated soup increased plasma concentration of vitamin C and decreased uric acid concentration in both genders, demonstrating that soup intake can improve human health.

The first study of HPP on a blended soup with additives was developed by **Plaza et al. (2006)** who compare two treatments at different pressures (150 and 350 MPa, for 15 min, at 60 ºC) with a soup without any treatment, on the carotenoids content and antioxidant capacity. HPP influence in total carotenoids extractability seems to be similar despite the pressure used, and does not cause carotenoids destruction. Although there was no difference in antioxidant activity right after treatment, and after 40 days storage at 4 °C, HPP treated soups showed higher activity than the soup without treatment, being the soup treated with lower pressure (150 MPa) the one that obtained better results; this shows that results are dependent of process parameters, such as temperature, time and pressure.

To inactivate bacterial spores, industry has to conjugate pressure with temperature using PATS (pressure assisted thermal sterilization). **Shibeshi and Farid (2011)** studied the effect of PATS on pumpkin soup with the purpose of comparing the inactivation efficacy of bacterial spores against thermal processing. The results showed that pressure and temperature combination (88 MPa, 115 °C, 20 min) increased the spores' inactivation rate when compared with thermal treatment (0.1 MPa, 121 °C, 20 min). PATS treatment is

considered a good method for soup preservation since it was not observed microbial growth during the posterior storage period at 4 °C.

1.3.3.2. Soup preservation

1.3.3.2.1. Modified Atmosphere Packaging (MAP)

MAP is used to increase product shelf-life by alteration of surrounding atmosphere in order to reduce respiration rate, to promote microbial growth inhibition, while retaining fresh-quality characteristics **(Murcia et al., 2003)**. **Murcia et al. (2009)** studied the effect of MAP on antioxidant activity of eight soups and purees and concluded that after MAP, vegetable soup and puree presented antioxidant activity, showing that this conservation method is good for preserving fresh food scavenging capacity.

Apart from the microbial inactivation effect of the methods described above for soup preservation, refrigeration throughout the storage period is necessary, with its inherent costs. Therefore, a preservation methodology, such as the HS, with no need for temperature control throughout the storage period, as a potential alternative to refrigeration is desirable.

II. OBJECTIVES AND SCHEDULE

The aim of this work was to verify the effect of HS on RTE food products in order to assure its microbiological quality and comparing with refrigeration. The parameters studied were:

- Endogenous microflora: total aerobic mesophiles, *Enterobacteriaceae* and yeasts and moulds.
- Physicochemical changes: pH-values, titratable acidity, reducing sugars content, and colour.
- Laboratorial scale tests: carrot soup stored under refrigeration (4 ºC) and at room temperature (25 ºC) and up to 30 °C at atmospheric pressure (0.1 MPa) was studied for comparison with soup stored under pressure (hyperbaric storage). Different combinations of pressure level (100 and 150 MPa) and temperature (25 and 30 ºC) were studied for different storage times (4 and 8 h) and treatments compared with those obtained at atmospheric pressure (under the same temperature) and with refrigeration.
- Tests in an industrial equipment: *Caldo verde* and *Bacalhau com natas* stored under refrigeration and at room temperature (~21 °C) at atmospheric pressure were studied for comparison with samples stored under pressure (hyperbaric storage). Different combinations of pressure level (50, 100 and 150 MPa) were studied for 12 h at ~21 ºC and the treatments were compared with those maintained at atmospheric pressure (under the same temperature) and with refrigeration.

In order to organize the work and to predict the time when results would be obtained, a schedule was developed (see **Figure 5**).

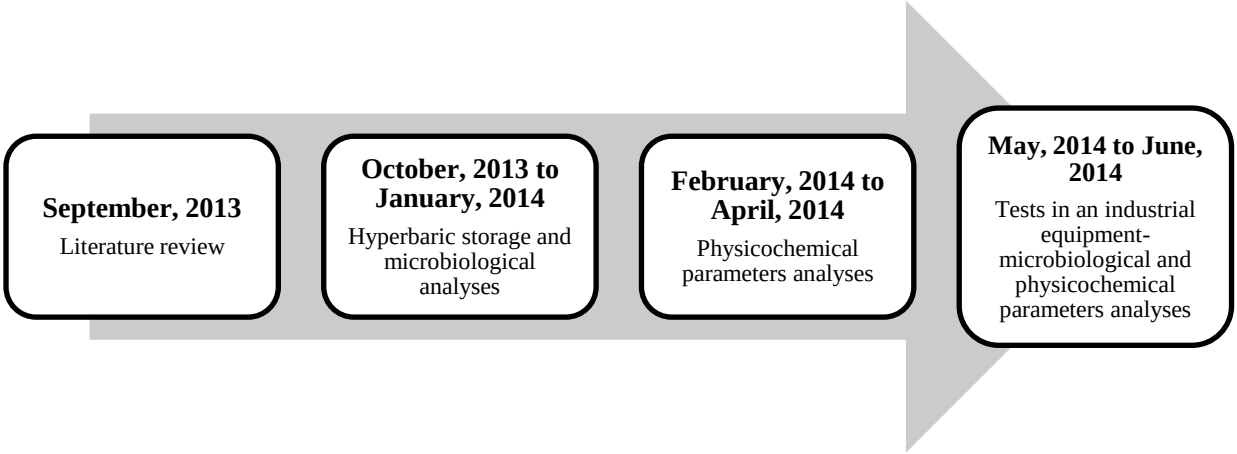

Figure 5. Schedule to organize work.

III. MATERIALS AND METHODS

3.1. Chemicals

The chemical 3,5-dinitrosalicylic acid was acquired from Acros (New Jersey, USA). Plate count agar (PCA), violet red bile dextrose agar (VRBDA) and rose bengal chloramphenicol agar (RBCA) were purchased from Merk (Darmstadt, Germany).

3.2. Preparation of samples

3.2.1. Carrot soup

Carrot soup (prepared with water, potato, carrot (25 %), onion, turnip, leek, garlic, salt and olive oil) was purchased from a local supermarket (see **Figure 6**). In sterile conditions, the product was separated in different aliquots (40 g) and immediately frozen and stored at -80 ºC until use. Since this soup was not enough to carry out all the experiments, another set of soup in couvets had to be bought. However, the new soup acquired showed microbial loads below the detection limit. This way, it was planned an experiment to increase the microbial loads to values similar to the initial set of soup samples.

Figure 6. Carrot soup used in laboratorial storage experiments.

3.2.1.1. First attempt to increase the microbial loads of the "new soup"

Initially, two aliquots of the "new soup" were thawed and mixed (in a total of ~80 g) and the mixture was left at room temperature (~ 20 ºC) in an open recipient for ~6 h, being mixed every each hour. To test which was the soup microbial load, 2.0 g of soup were diluted and homogenised with 18.0 mL of Ringer's solution (1:9). Dilutions from 10^{-1} up to 10^{-3} were performed with the same diluent and were made duplicates of sample and triplicates of each dilution for total aerobic mesophiles, which were plated on the

appropriated media. However, the total aerobic mesophiles counts were still below the detection limit (< 1.00 Log_{10} CFU/g soup) (see **Appendix A**), making necessary a new contamination.

3.2.1.2. Second attempt to increase the microbial loads of the "new soup"

Another soup aliquot (~80 g) was thawed, but this time the mixture was left at room temperature in an open container overnight (~12 h), then was placed in an incubator (30 ± 1 ºC) for ~6 h, and in cold (4 ºC) for another ~6 h. To ascertain if soup had already the microbial load required, the same protocol used in the first attempt was performed (soup homogenization with Ringer's solution (dilutions from 10^{-3} up to 10^{-6} were performed) and plated in appropriated media for total aerobic mesophiles). As can be seen in **Appendix A**, microbial load reached values above 6.00 Log_{10} CFU/g.

3.2.1.3. Inoculation of "new soup" with spoiled soup

Since the soup used in the first experiments had a total aerobic mesophiles initial load of 4.15 Log_{10} CFU/g, and the spoiled "new soup" had an initial load of 6.13 Log_{10} CFU/g, it was calculated the amount of the spoiled "new soup" necessary to add to the remaining "new soup" to have in the latter samples an initial load around 4.00 Log_{10} CFU/g, i.e, one hundred times less.

To perform this inoculation, all the remaining "new soup" (550 g) was thawed and mixed vigorously with 5.50 g of spoiled "new soup" in a large container (in sterile conditions). Then, also in sterile conditions, all the soup was separated in cups (~40 g each), and immediately frozen at -80 ºC.

3.2.2. *Caldo verde* and *Bacalhau com natas*

Caldo verde (prepared with water, potato, cabbage, onion, garlic, salt, olive oil and chorizo) (**Figure 7**) was provided by a local company, packaged in plastic bags of 200 g each, and initially tested for the presence of total aerobic mesophiles, after storage for 2 days at refrigeration and then frozen at -20 ºC. The results (**Appendix A**) showed that this soup batch had also microbial loads below the quantification limit (such as carrot soup). Similarly to the carrot soup, all the bags were thawed and left at room temperature (20-25 ºC) for 24 h. To ascertain if soup had already quantifiable microbial loads, the same protocol used in the laboratorial tests was performed (soup homogenization with Ringer's solution (1:9) and plated in appropriated media for total aerobic mesophiles). As can be seen in **Appendix A**, the microbial load was ca. 2.50 Log_{10} CFU/g. The same food company also provided *Bacalhau com natas* (prepared with potato, codfish, water, milk, onion and sour cream), that was also packaged in plastic bags of 200 g each.

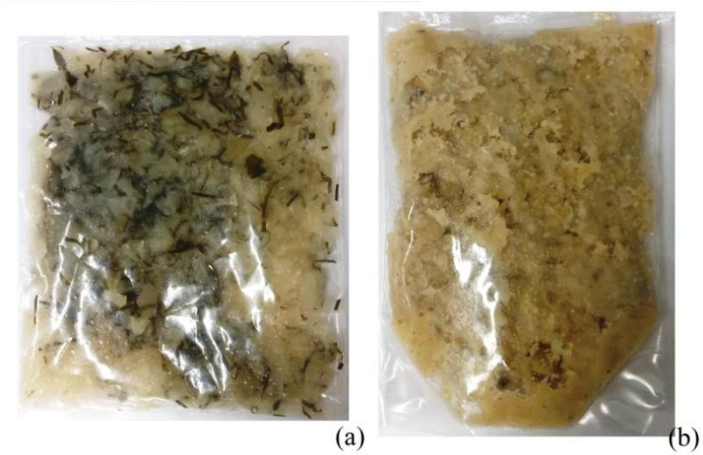

Figure 7. *Caldo verde com chouriço* and *Bacalhau com natas* used for the storage experiments in industrial equipment**.**

3.3. Storage experiments

3.3.1. Laboratorial scale

Before each experiment the soup was thawed at 4 ºC. HS experiments were carried out on a hydrostatic press (High pressure system U33, Institute of High Pressure Physics, Warsaw, Poland). This equipment has a pressure vessel of 35 mm inner diameter and 100 mm height surrounded by an external jacket, connected to a thermostatic bath (Huber Compatible Control CC1, New Jersey, USA) to control the temperature. A mixture of

propylene glycol and water (40:60) was used as a pressurising fluid and to control the temperature in the external jacket. The carrot soup samples were aseptically placed in low permeability polyamide–polyethylene bags (PA/PE-90, Albipack – Packaging Solutions, Águeda, Portugal), using a laminar flow cabinet (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain) to avoid contaminations. The bags were heat sealed manually with care to avoid as much as possible to leave air inside the bags. Each bag containing the soup was afterwards inserted in a second bag that was heat sealed under vacuum. The packaging film was previously sterilized by irradiation with UV light for 15 min (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain).

Different preservation combinations were performed for two different periods (4 and 8 h) under two pressure levels (100 and 150 MPa) at 25 and 30 ºC. Two control samples were maintained in all cases at atmospheric pressure (0.1 MPa): (i) one control was stored during the same time and under the same temperature conditions; (ii) and the second control was maintained during the same time and under refrigeration (4 ºC). Both controls were immersed in the same fluid used to generate the pressure and in the dark to avoid differences between samples.

3.3.2. Experiments in an industrial equipment

Before each experiment the soup was thawed overnight at 4 ºC. HS experiments were carried out on a hydrostatic press (Model 55, Hyperbaric, Burgos, Spain) (**Figure 8**). This equipment has a pressure vessel of 55 litters and a 200 mm diameter.

Different storage combinations were performed for 12 h under three pressure levels (50, 100 and 150 MPa) at room temperature (~21 ºC). Two control samples were maintained at atmospheric pressure (0.1 MPa): (i) one control was stored during the same time and under the same temperature conditions; (ii) and the second control was maintained during the same time and under refrigeration (4 ºC).

Post-hyperbaric storage (Post-HS) was also tested in the samples stored under 100 MPa for 12 h, during 3 more days under refrigeration (4 ºC) to ascertain if HS had any effect on subsequent storage under refrigeration at 0.1 MPa.

Figure 8. Hydrostatic presses used from hyperbaric experiments: (a) laboratorial scale and (b) industrial equipment.

3.4. Microbial analyses

3.4.1. Sample preparation and dilution

In the same day of the HS, 2.0 g aliquots were obtained aseptically and homogenised with 18.0 mL of Ringer's solution for 2 min in a Stomacher 80 Biomaster. For the tests in an industrial equipment, since it was used a larger amount of soup, 10 g of soup were homogenised with 90 mL of Ringer's solution for 4 min in a Stomacher 80 Biomaster. From the 10^{-1} dilution, other decimal dilutions were prepared with the same diluent (up to dilution of 10^{-4} allowing a maximum microbiological quantification of 6.00 Log_{10} CFU/g). Duplicates of each sample and triplicates of each dilution were plated on the appropriate media. All the samples were analysed for counts of total aerobic mesophiles, *Enterobacteriaceae* and yeast and moulds.

3.4.2. Count of total aerobic mesophiles microorganisms

Total aerobic mesophiles (TAM) counts were determinate in Plate Count Agar (PCA) after aerobic incubation at 30 ± 1 ºC for 72 ± 3 h (ISO 4833-1:2013). The method used was the pour-plated, using 1.0 mL of diluted solution sample.

3.4.3. Count of *Enterobacteriaceae*

Enterobacteriaceae (ENT) counts were quantified in Violet Red Bile Dextrose Agar (VRBDA), being incubated aerobically at 37 ± 1 ºC for 24 h (ISO 21528-1:2004). The method used was the pour-plated, using 1.0 mL of diluted sample.

3.4.4. Count of yeasts and moulds

Yeasts and moulds (YM) were counted on Rose Bengal Choramphenicol Agar (RBCA) after incubation at 25 ± 1 ºC for 5 days (ISO 21527-1:2008). RBCA was prepared every experiment eve according to the necessary quantity, taking into account the number of dilutions to be performed in the next day. The spread-plate method used 200 µL per sample and five replicates for each dilution.

3.4.5. Microbial counts

The petri dishes considered contained 15-300 colony forming units (CFU) and the results were expressed as logarithmic of CFU per gram of soup (Log_{10} CFU/g). In RBCA media, were considered 1-150 colonies. The microbial counts were calculated following **Equation 1 (ISO, 4833:2003).**

$$N = \frac{\sum \text{characteristic colonies}}{V\,[(n1 + 0.1 \times n2) \times d]}$$

Equation 1

being:

N – Colony forming units per gram of soup (CFU/g)

V – Sample volume (mL) – 1.0 mL for TAM and ENT, and 200 µL for YM

n1 – Number of plates countable in the first dilution

n2 – Number of plates countable in the second dilution

d – First countable dilution

Results presented in this thesis were obtained from duplicate of sample and triplicate of analysis for TAM and ENT and quintuplicate of analyses for YM. Values below the quantification limit were considered < 1.00 Log_{10} CFU/g and above it were considered > 6.00 Log_{10} CFU/g.

3.5. Physicochemical analyses

3.5.1. Determination of pH and titratable acidity

Samples (2.00 g) were mixed with 8.0 mL of distilled water with an Ultraturrax T25 homogeniser (Janke & Kunkel IKA-Labortechnik). For the tests in industrial equipment, since it was used a larger amount of sample, 4.00 g were homogenised with 16.0 mL of distilled water.

The pH value of the samples was measured at 20 °C with a properly calibrated glass electrode (Crison, Barcelona, Spain) which was calibrated with pH 4.0 and 7.0 buffer. The solution was titrated with 0.02 M sodium hydroxide solution to pH 8.1, using an automatic titrator (Crison Titromatic 1S, Crison). The pH and titratable acidity (TA) resulted from duplicate of sample and triplicate of analysis and TA was expressed in milligram of malic acid per 100 grams of sample (mg malic acid/100 g).

3.5.2. Reducing sugars

One gram of soup was diluted in 20 mL of distilled water at room temperature and homogenised for 15 seconds with an Ultraturrax T25 homogeniser. The mixture was centrifuged for 15 minutes at 2000 rpm at 20 ºC. Into an aluminium coated tube were added 1.0 mL of the supernatant soup and 1.0 mL of DNS reagent. The mixture was heated to 100 ºC for 5 minutes and then placed at ice for rapid refrigeration and diluted until a final volume of 10 mL with distilled water. Absorbance at 540 nm was measured using a Multiskan Go microplate spectrophotomeeter (Thermo Scientific) with Brand plate of 96 wells. Initially was measured the absorbance of the empty plate and then, to each well, 300 µL of mixture were added, being the blank prepared by adding 1.0 mL of distilled water instead of centrifuged soup. Then, the plate was shaken for 10 seconds and the absorbance at 540 nm was measured. The reducing sugars value resulted from the difference between the absorbance of the plate with samples and the empty plate. Six quantifications (duplicate of sample and triplicate of analysis) were performed and the value was determined using the calibration curve represented in **Equation 2** (see **Appendix B**) and expressed in milligram of glucose equivalents per gram of sample (mg glucose/g).

$$\text{Abs}(540\text{ nm}) = 5.54 \text{ x C(glucose)} - 9.30 \text{ x } 10^{-3} \quad R^2 = 0.998$$

Equation 2

3.5.3. Colour

The colour parameters were determined taking into account the parameters *a** (red/green colour) and *b** (yellow/blue colour), and *L** (luminosity). The analyses were performed using the CIE*Lab* space at 25 ºC after homogenization of the sample for 30 seconds using an Ultraturrax T25 homogeniser. A Konica Minolta CM 2300d (Minolta Konica, Japan) spectrophotometer was used. The CIE*Lab* parameters were determined using the original SpectraMagic™ NX Software, Konica Minolta, USA, according to regulations of the International Commission on Illumination. The *L**, *a** and *b** values resulted from duplicate of sample and six random measures of each sample.

In addition, the difference in colour (ΔE) was calculated using **Equation 3 (Krapfenbauer et al., 2006)**.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Equation 3

3.6. Statistical analyses

Differences between samples stored at different conditions were assessed at a 5% level of significance using One-way Analysis of Variance (ANOVA) followed by Tukey's HSD Test. The results were expressed as mean ± standard deviation.

IV. RESULTS AND DISCUSSION

4.1. Laboratorial scale

4.1.1. Microbial analyses – general overview

As stated in section 3.2.1. "Preparation of soup samples", in Chapter III, "Materials and methods", the "new soup" didn't have quantifiable microbial counts and was necessary to increase them. After inoculation, and taking into account the initial values obtained from the first soup used in the first experiments, the initial average counts of TAM, ENT and YM were 4.72 ± 0.36, 2.38 ± 0.17 and 2.26 ± 0.27 Log_{10} CFU/g soup, respectively (see **Figure 9** and **Table C1** in **Appendix C**). These results were obtained by the mean of all the initial values registered before each HS experiment.

One control sample was maintained in refrigerated conditions (4 ºC, 0.1 MPa) for the same time period of HS experiments (4 or 8 h). As expected, refrigeration acted on growth inhibition (see **Figure 9**), since the results, for all the studied microorganisms, remained stable ($p>0.05$) after 4 and 8 h of storage, comparatively to the initial. These results are according to the traditional soup storage under refrigeration, being possible to maintain the microbial loads below the limit of 6.00 Log_{10} CFU/g (recommended limit for dried soup) for 3-4 days **(Kilinc, 2010)**. However, the growth rate can vary according to soup composition and package type.

Generally, storage for 4 and 8 h at atmospheric pressure (0.1 MPa), at 25 or 30 ºC, the microbial loads increased significantly ($p<0.05$) relatively to the initial value, reaching a maximum after storage at 30 ºC (> 6.00 Log_{10} CFU/g for TAM and ~3.3 Log_{10} CFU/g for ENT and YM). These results were expected since soup is a low acid food (pH > 4.6), with high water activity and, so with no main hurdler to microbial proliferation **(Shibeshi and Farid, 2011)**.

When soup was stored under both pressures (100 and 150 MPa), the microbial counts obtained were lower ($p<0.05$) than those verified for storage at 0.1 MPa, for the same temperature and time period. HS for 4 h, revealed a preservation effect similar ($p>0.05$) to refrigeration, since microbial growth inhibition occurred for all the studied microorganisms to levels equivalent to refrigeration. In addition, after 8 h of storage, the microbial counts indicated microbial inactivation, since the loads were equal or lower than the initial and refrigerated values. These results clearly revealed an equal to better performance of HS at 25 and 30 ºC, when compared to refrigeration.

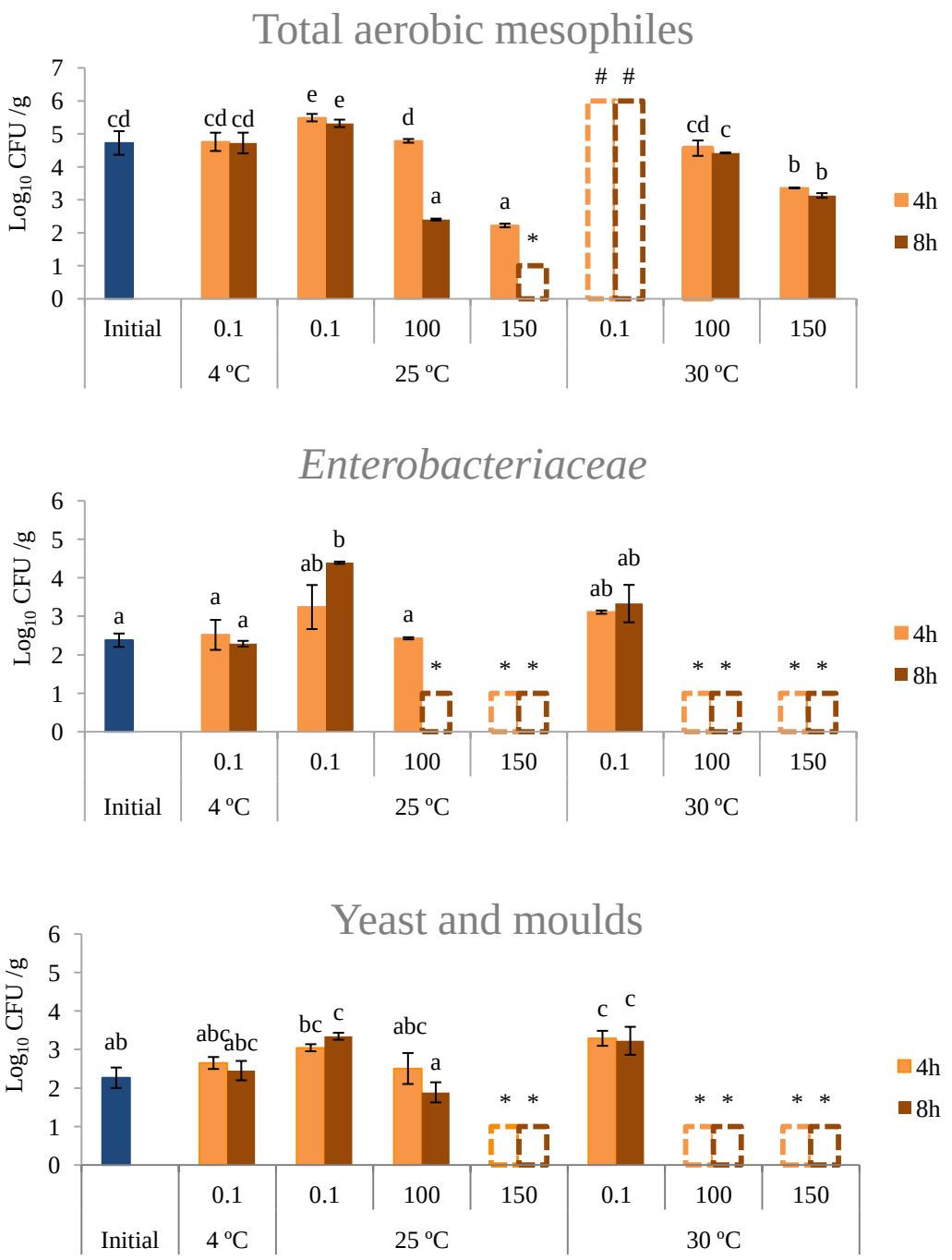

Figure 9. Carrot soup. Microbial counts (Log_{10} CFU/g soup) in initial (blue bar) and after HS of carrot soup for various combinations of time, pressure and temperature: 4 and 8 hours, 100 and 150 MPa and 25 and 30 ºC; soup was also stored at control conditions for each temperature (25 and 30 ºC, at 0.1 MPa) and at refrigeration conditions (4 ºC, 0.1 MPa). Different letters indicate significant differences ($p<0.05$) between conditions. Values shown as 6 (#) and 1 (*) (white bars with discontinuous borders) log units, mean values higher than 6 and lower than 1 log units, respectively.

4.1.1.1 Effect of hyperbaric storage on total aerobic mesophiles

Concerning TAM counts, storage for both 4 and 8 h, 25 ºC, 0.1 MPa caused a microbial load increase of about 1.00 Log_{10} CFU/g. The counts obtained for storage at 0.1 MPa were higher ($p<0.05$) than those obtained for storage under both pressures (100 and 150 MPa) for the same time period. The storage for 4 h, at 100 MPa caused an inhibitory growth effect, since the microbial loads (4.79 ± 0.06 Log_{10} CFU/g soup) were similar ($p>0.05$) to refrigeration (4.76 ± 0.28 Log_{10} CFU/g). Moreover, when the soup was stored under 150 MPa, was observed an additional microbial inactivation effect, since was obtained a microbial load reduction of 2.50 Log_{10} CFU/g, resulting in values lower ($p<0.05$) than refrigeration. The HS at 150 MPa for 8 h had a similar effect since it caused a clear growth inactivation, with TAM counts being <1.00 Log_{10} CFU/g. The HS feasibility was also studied above room temperature, at 30 ºC. In the soup stored at 0.1 MPa for 4 and 8 h, was detected a microbial load increase (to values above 6.00 Log_{10} CFU/g) comparatively to the initial value. When the soup was stored under 100 MPa, it was observed a microbial growth inhibition for TAM (4.57 ± 0.23 and 4.42 ± 0.01 Log_{10} CFU/g for both 4 and 8 h of storage, respectively), being the values similar ($p>0.05$) to refrigeration. However, when using 150 MPa, TAM loads reduced ($p<0.05$) about 1.50 and 1.00 Log_{10} CFU/g for 4 and 8 h, respectively, confirming the inactivation effect of HS.

Interestingly, the effect of HS on TAM was more pronounced at 25 ºC compared to 30 ºC. This might be due to the fact that optimum growth temperature for TAM at atmospheric pressure is 30 ºC, being TAM less susceptible to growth inhibition and inactivation at this temperature.

4.1.1.2. Effect of hyperbaric storage on Enterobacteriaceae

Storage for 4 and 8 h at 0.1 MPa, for both 25 and 30 ºC, led to a microbial growth increase relatively to the initial value. However, when stored for 4 h at 100 MPa, 25 ºC, was observed a microbial growth inhibition, being the loads (2.43 ± 0.03 Log_{10} CFU/g) similar ($p>0.05$) to refrigeration (2.52 ± 0.39 Log_{10} CFU/g). ENT showed to be more susceptible to HS than TAM, since all the remained HS conditions led to ENT reduction to values below the detection limits (<1.00 Log_{10} CFU/g).

4.1.1.3. Effect of hyperbaric storage on yeasts and moulds

YM showed a similar behaviour to ENT, since when stored at 0.1 MPa, the microbial loads tended to increase ($p<0.05$) relatively to the initial about 1.00 Log_{10} CFU/g, and when stored under pressure, the microbial loads tended to reduce to values below the detection limits, unless for storage at 100 MPa, 25 ºC (2.51 ± 0.40 and 1.89 ± 0.26 Log_{10} CFU/g for both 4 and 8 h of storage, respectively), where the values remained similar ($p>0.05$) to refrigeration. It is noteworthy that HS effect was more accentuated at 30 ºC than to 25 ºC. This fact can be related to the YM optimum growth temperature (25 ºC), being YM less susceptible to growth inactivation at this temperature.

4.1.1.4. Global effect

Globally, the present results show the potential of refrigeration replacement by HS, since soup can be preserved under 100 or 150 MPa, at and above room temperature (so with no need for temperature control), being the microbial loads similar, or even lower than those for the soup stored under refrigeration. Generally, TAM were the microorganisms less susceptible to growth inhibition/inactivation, compared to ENT and YM.

The microbial inactivation effect appears to be temperature dependent (differing from the optimum growth temperature of each microorganism at atmospheric pressure), showing better results for the higher temperature (30 ºC) studied, being also more accentuated for the higher pressure (150 MPa) (however more studies are necessary to confirm this observation). This is in accordance with **Mota et al. (2013)** and **Queiros et al. (2014)**, who showed that for pressures up to 100 MPa was observed a general growth inhibition, with an additional growth inactivation when using higher pressures.

Similar results to those reported in this work were observed in raw juice storage (strawberry, watermelon and melon juices), where refrigeration kept the microbial loads unchanged (comparatively to initial) during storage, while HS allowed not only an inhibition effect for lower pressures (25-100 MPa) as also an inactivation effect for higher pressures (150-220 MPa) for storage at room temperature **(Segovia-Bravo et al., 2012, Fidalgo et al., 2014, Queiros et al., 2014)**. **Segovia-Bravo et al. (2012)** stored strawberry juice under 25-220 MPa at 20 ºC for 15 days and showed that pressure inhibited and/or inactivated microbial growth along storage time, depending on the pressure level used.

Similar results were obtained by **Fidalgo et al. (2014)** using uncontrolled naturally variable room temperature (18-21 ºC) at 100 MPa for 60 h. For melon juice, it was observed that higher pressures (100 and 150 MPa) have an inactivation effect on microbial growth, while lower pressures (50 and 75 MPa) allow obtaining results similar to refrigeration, showing a growth inhibition effect **(Queiros et al., 2014)**.

This fact turns possible to extend perishable food products shelf-life under moderate pressure with potential energy savings, since no energy is required to control temperature (contrarily to refrigeration) since it only needs energy to compression/decompression phases, allowing to reduce the energy associated to preservation of a RTE product. Therefore, the present work shows that soup can be preserved at 100 and 150 MPa, with no need for refrigeration temperatures, with similar or better microbial loads compared to refrigeration in some of the studied cases. Nevertheless, other studies are necessary to extend the knowledge in this new area, like the study of longer storage times, other pressures and other microbial populations.

4.1.2. Physicochemical analysis

4.1.2.1. pH and titratable acidity

According to values found in the literature, soup pH vary generally in a range of 4-6, depending on composition **(Plaza et al., 2006, Shibeshi and Farid, 2011, Pinilla et al., 2005, Gadekar et al., 2009)**. Carrot soup initial pH was 5.65 ± 0.07 and, as can be seen in **Table 5**, this value did not significantly ($p>0.05$) changed regardless of the pressure level. The major changes were verified for storage for 4 and 8 h at 30 ºC, 0.1 MPa, where it was observed a linear decrease ($r^2=0.988$; $y = -0.148x + 5.66$; **Appendix D**) throughout the storage time, reaching a pH minimum after 8 h of storage (4.46 ± 0.14). These results can be related to the higher microbial load of TAM and YM in this condition (**Figure 9**).

Soup initial TA was 63.65 ± 5.59 $mg_{malic\ acid}$/100 g (**Table 5**). The maximum value of TA was obtained after 8 h of storage at 30 ºC, 0.1 MPa (90.50 ± 2.62 $mg_{malic\ acid}$/100 g), and as pH decreased, TA tended to increase linearly throughout time ($r^2=0.9898$; $y = 3.355x + 64.44$; **Appendix D**). Soup stored under pressure did not significantly changed ($p>0.05$) relatively to the initial value and to the refrigerated value.

Table 5. pH and titratable acidity (TA) values for soup stored for 4 and 8 hours at different temperature (ºC) and pressure (MPa) conditions. Different letters indicate significant differences ($p < 0.05$) between conditions (a-d) and storage time to the same storage condition (A-B). Values at 4 and 8 h showed at bold are statistically different from the initial value for each parameter and storage condition.

Conditions		pH		Titratable acidity ($mg_{malic\ acid}$/ 100 g)	
		4 h	8 h	4 h	8 h
Initial	**0.1 MPa**	5.65±0.07 aA	5.65±0.07 abcA	63.65±5.59 abcA	63.65±5.59 aA
4 ºC	**0.1 MPa**	5.57±0.04 aA	5.63±0.17 abcA	66.20±4.14 abcA	70.04±4.57 acA
25 ºC	**0.1 MPa**	5.66±0.20 aA	5.72±0.01 bcA	**50.89±3.15** aA	53.46±1.43 bA
	100 MPa	5.54±0.05 a	*	63.59±3.00 abc	*
	150 MPa	5.66±0.01 aA	**5.87±0.02** cB	**55.35±1.85** abA	47.28±1.12 bB
30 ºC	**0.1 MPa**	**5.10±0.15** bA	**4.46±0.14** dB	**79.44±5.33** cA	90.50±2.62 dA
	100 MPa	5.61±0.03 aA	**5.44±0.01** aB	65.69±2.11 abcA	76.06±1.73 cB
	150 MPa	5.74±0.12 aA	5.58±0.01 abA	70.81±8.41 bcA	66.95±1.86 aA

* - Experiments were not carried out in these conditions.

4.1.2.2. Reducing sugars

The major sugars present in carrot are sucrose, glucose and fructose, being the last two reducing sugars the most abundant **(Cazor et al., 2006)**. Glucose can also result from potato α-amylase and glucoamylase residual activity (both enzymes are thermo- and baro-stable, with optimal temperature of 70 ºC and 60 ºC for both enzymes, respectively, and inactivated by pressures above 500 MPa **(van der Maarel et al., 2002, Weemaes et al., 1996)**.

In **Table 6** is possible to observed that the reducing sugars initial value was 6.40 ± 0.32 mg glucose/g and that value remained, generally, stable ($p>0.05$) after soup storage for 4 and 8 h, regardless of temperature or pressure level used. The only exceptions were verified for storage at 30 ºC, 0.1 and 100 MPa, where the values reached a maximum after 8 h (8.01 ± 0.36 and 9.43 ± 0.19 mg glucose/g, respectively).

These results are in agreement with the presence of a higher microbial load (TAM and YM) at 30 ºC that leads to the hydrolysis of the starch present in carrot and potato, producing glucose for fermentation and microbial growth.

Table 6. Reducing sugars values for soup stored for 4 and 8 hours at different temperature (ºC) and pressure (MPa) conditions. Different letters indicate significant differences ($p < 0.05$) between conditions (a-d) and storage time to the same storage condition (A-B). Values at 4 and 8 h showed at bold are statistically different from the initial value for each parameter and storage condition.

Conditions		Reducing sugars (mg glucose/g)	
		4 h	8 h
Initial	**0.1 MPa**	6.40±0.32 abA	6.40±0.32 aA
4 ºC	**0.1 MPa**	**6.89±0.24** abA	6.72±0.30 aA
25 ºC	**0.1 MPa**	6.42±0.35 abA	6.10±0.13 aA
	100 MPa	7.56±0.80 b	*
	150 MPa	7.13±0.34 abA	6.85±0.59 aA
30 ºC	**0.1 MPa**	**5.97±0.28** aA	**8.01±0.36** bB
	100 MPa	6.01±0.50 aA	**9.43±0.19** cB
	150 MPa	6.13±0.21 abA	6.62±0.29 aA

* - Experiments were not carried out in these conditions.

4.1.2.3. Colour

The initial soup presented a bright orange colour (L^* = 52.44 ± 1.07), tending to red (a^* = 4.35 ± 0.16) and yellow (b^* = 33.03 ± 2.12) (**Table 7**). The L^* value (luminosity) remained stable after refrigerated storage. However, after storage at 30 ºC, 0.1 MPa, L^* tended to linearly increase (r^2 = 0.997; y = 0.368x + 52.5; **Appendix D**) throughout the storage time, with a maximum of 55.39 ± 0.56 after 8 h. Furthermore, when soup was stored under pressure, the L^* value did not significantly ($p>0.05$) changed relatively to the initial or the refrigeration values, except when stored at 25 ºC, for 4 h, at 150 MPa, where reached a minimum of 46.17 ± 0.41. Thus, it was observed that lightness parameter was not significantly changed in soup samples stored under pressure, except in samples stored during 4 h at 150 MPa and 25 ºC, being the higher changes verified in samples stored at 0.1 MPa.

The redness (a^*) and yellowness (b^*) parameters behaviour was similar, since the soup stored under pressure did not significantly ($p>0.05$) changed comparatively to refrigeration, except the storage for 4 h, 25 ºC, 100 MPa, where a^* reached a maximum (6.09 ± 0.07) and the storage for 4 h, 30 ºC, 150 MPa, where reached a minimum (3.60 ± 0.15). It is noteworthy that although these results are significantly different ($p<0.05$) from

refrigeration, they are similar ($p>0.05$) to the initial value, showing that HS is able to maintain the soup original colour. The same pattern is observed with *b** parameter, since it reached a maximum of 37.91 ± 0.24 when soup was stored for 4 h, at 25 ºC and 100 MPa, and a minimum of 30.14 ± 0.53 after 4 h at 30 ºC, 150 MPa, while the remaining conditions did not caused significant ($p>0.05$) variation relatively to initial or refrigeration values.

A noticeable difference between food products colours is perceptible when they differ by $\Delta E^*>3.5$ **(Krapfenbauer et al., 2006)**. For the soup stored at 0.1 MPa, 25 and 30 ºC the ΔE^* obtained was higher than 3.5 (see **Table E1** in **Appendix E**), indicating that storage in these conditions caused visible colour changes. However it is noteworthy that after HS under 100 MPa, for both 4 and 8 h, the ΔE^* values remained below the limit established by **Krapfenbauer et al. (2006)**.

Table 7. Colour parameters for soup stored for 4 and 8 hours at different temperature (ºC) and pressure (MPa) conditions. Different letters indicate significant differences ($p < 0.05$) between conditions (a-c) and storage time to the same storage condition (A-B). Values at 4 and 8 h showed at bold are statistically different from the initial value for each parameter and storage condition.

Conditions		Colour					
		L*		***a****		***b****	
		4 h	**8 h**	**4 h**	**8 h**	**4 h**	**8 h**
Initial	**0.1 MPa**	52.44±1.07 aA	52.44±1.07 aA	4.35±0.16 abA	4.35±0.16 aA	33.03±2.12 abA	33.03±2.12 abA
4 ºC	**0.1 MPa**	53.60±1.34 aA	53.43±0.88 abA	**5.36±0.34** bcA	4.85±0.22 bcA	34.94±0.91 abA	34.13±1.15 abA
25 ºC	**0.1 MPa**	**49.46±0.28** bA	52.68±1.19 aB	4.37±0.11 abA	**5.52±0.51** cB	31.68±0.19 aA	**35.43±1.85** aB
	100 MPa	53.70±0.24 a	*	6.09±0.07 c	*	37.91±0.24 b	*
	150 MPa	**46.17±0.41** cA	52.25±0.09 aB	**4.91±0.13** abcA	**4.72±0.18** abA	33.31±1.34 abA	33.94±0.43 abA
30 ºC	**0.1 MPa**	54.05±1.68 aA	**55.39±0.56** cA	4.40±0.65 bcA	5.24±0.42 bcA	32.83±2.53 abA	35.52±1.26 aA
	100 MPa	52.49±1.42 aA	52.35±0.38 aA	4.04±0.78 abA	4.24±0.24 aA	32.41±1.71 aA	32.27±0.57 bA
	150 MPa	**51.56±0.28** abA	**54.23±0.01** bcB	**3.60±0.15** aA	4.80±0.21 abB	30.14±0.53 aA	34.04±0.23 abB

* - Experiments were not carried out in these conditions.

4.2. Tests in an industrial equipment

The HS experiments in an industrial equipment were performed in the new industrial high pressure equipment of University of Aveiro (seen in **Figure 8**) using larger amounts of real commercial RTE food products. These experiments were performed using *Caldo verde*, since the RTE food supplier company, no longer produce carrot soup. Since the new pressure equipment has a large usable volume (55 L), it was decided to take advantage of the experiments with *Caldo verde*, to study another food product. The food product chosen was *Bacalhau com natas*, a solid RTE meal that the same local company could produce.

Generally the results were in good agreement with the ones just presented for laboratorial scale, demonstrating that HS can be successfully applied in a larger scale and are discussed below.

4.2.1. Microbial analyses in *Caldo verde* - overview

Similar to what happen with the carrot soup in the laboratorial scale experiments, the *Caldo verde* had also initially no quantifiable total aerobic mesophiles counts. This was expected since it was a real commercial soup, for so the microbial loads should be as lower as possible. So, as it was described at section 3.2.2., *Caldo verde* was left at room temperature (20-25 ºC) for 24 h, to allow some microbial growth. After the first microbial analyses, the initial average counts of TAM and YM were 2.39 ± 0.04 and 2.01 ± 0.16 Log_{10} CFU/g, respectively, and below the detection limits (< 1.00 Log_{10} CFU/g) for ENT (see **Figure 10**, **Table C2** in **Appendix C**).

The *Caldo verde* samples were stored at different pressure conditions (50, 100 and 150 MPa) for 12 h at 21 ºC. Two controls were maintained at atmospheric pressure, one at room temperature (~21 ºC) and the other at 4 ºC. It was possible to observe that, generally, refrigeration for 12 h was capable to maintain the microbial loads (2.44 ± 0.08 and 1.85 ± 0.12 Log_{10} CFU/g for TAM and YM, while ENT remained below the detection limit) similar to the initial value ($p>0.05$), which is in agreement to the literature **(Kilinc, 2010)**. The second control was stored for 12 h at atmospheric pressure and room temperature (~21 ºC), and it was visible a clear microbial load increase ($p<0.05$) for all the studied microorganisms comparatively to the initial and the refrigerated values: TAM increased about 1.50 Log_{10} CFU/g, ENT about 2.50 Log_{10} CFU/g and YM increased about 0.50

Log_{10} CFU/g. These results were expected since *Caldo verde* has a perishable nature, with a neutral pH and high water activity.

4.2.1.1. Effect of hyperbaric storage on microorganisms

Globally, the microbial counts after the HS experiments were statistically ($p<0.05$) lower than the values obtained for storage at 0.1 MPa at room temperature after 12 h, indicating a growth inhibition effect of HS on TAM, ENT and YM. The only exception occur for HS at 50 MPa, where the pressure level allowed a slight microbial growth of TAM (2.83 ± 0.03 Log_{10} CFU/g) when compared to the initial and refrigerated value, but still lower than the value obtained for storage at 0.1 MPa (4.09 ± 0.07 Log_{10} CFU/g). However, for ENT and YM, 50 MPa allowed to maintain the microbial counts similar ($p>0.05$) to the initial and the refrigerated values.

Concerning TAM counts, HS at 100 MPa allowed not only to inhibit the microbial growth, as also enabled a slight microbial reduction to 2.19 ± 0.02 Log_{10} CFU/g, being this value statistically lower ($p<0.05$) relatively to refrigeration. Furthermore, when a higher pressure was used (150 MPa) it was observed a clear TAM counts reduction (about 1.50 Log_{10} CFU/g), being the value obtained below the detection limit (<1.00 Log_{10} CFU/g), showing the pressure inactivation effect on TAM.

Relatively to ENT, as expected, the initial load was below the detection limit, since the *Caldo verde* in study was a real commercial product, which indicate that the soup was made in a clean and hygienic environment. After storage at 0.1 MPa and room temperature (~21 ºC) the microbial load increased to 2.52 ± 0.17 Log_{10} CFU/g. Nevertheless, it is noteworthy that when stored under pressure, at all the pressure levels, the microbial loads remained below the detection limit, showing the inhibitory effect of pressure in this group of microorganisms.

YM showed a growth pattern similar to TAM, since after storage for 12 h under 50 MPa the microbial loads (1.90 ± 0.03 Log_{10} CFU/g) remained similar ($p>0.05$) to the initial and refrigerated values, and when the soup was stored under 100 MPa the same inhibitory effect was observed (1.85 ± 0.21 Log_{10} CFU/g). In addition, when *Caldo verde* was stored under 150 MPa for 12 h, a reduction of the microbial loads was observed to values below the detection limits (<1.00 Log_{10} CFU/g).

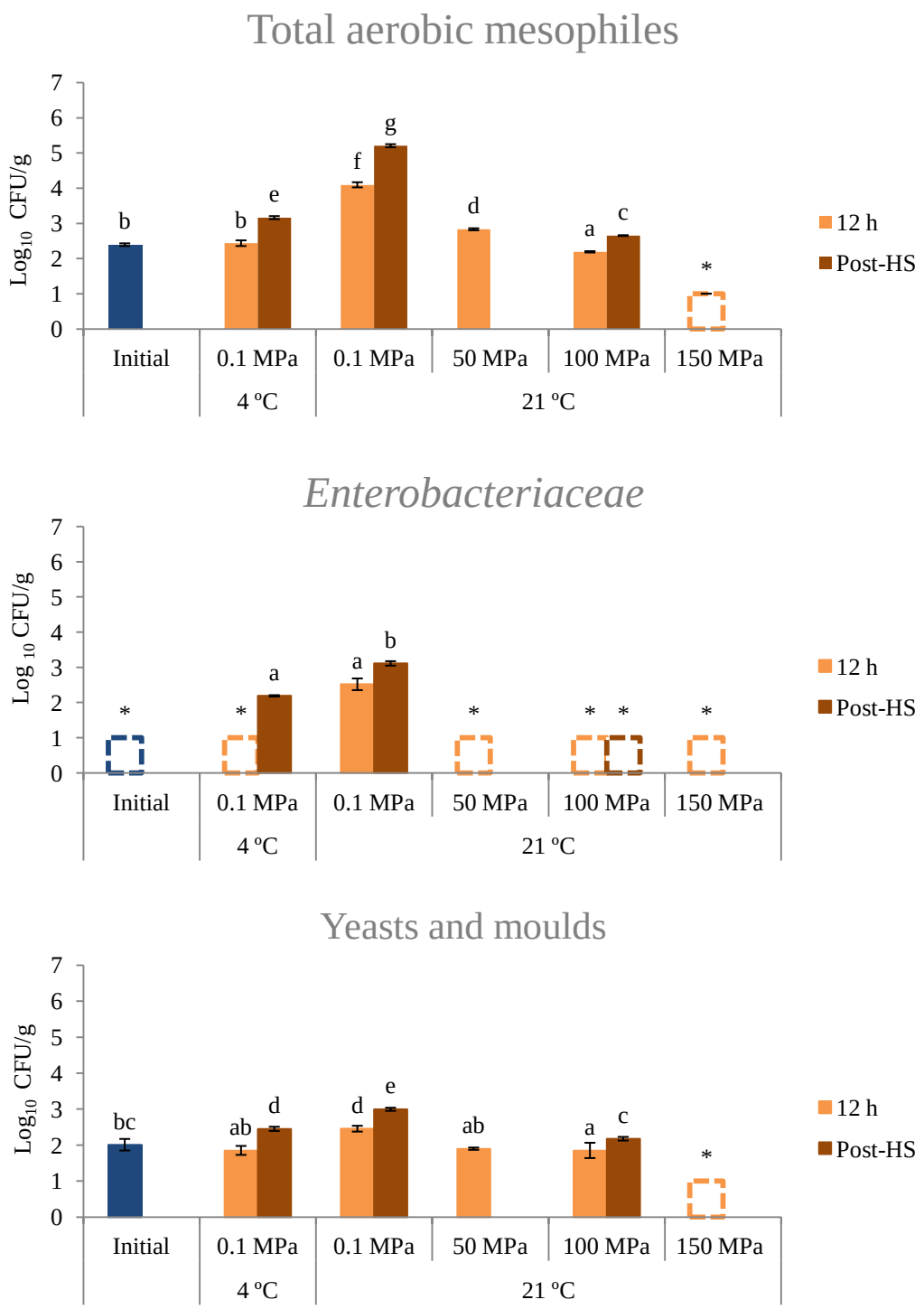

Figure 10. *Caldo verde*. Microbial counts (Log_{10} CFU/g soup) in initial (blue bar) and after hyperbaric storage of *Caldo verde* soup at 21 ºC for 12 h and after 3 days (Post-HS) at different pressures: 50, 100 and 150 MPa; soup was also stored at control conditions (21ºC at 0.1 MPa) and at refrigeration conditions (4 ºC, 0.1 MPa). Different letters indicate significant differences ($p < 0.05$) between conditions. Values shown as 6 (#) and 1 (*) (white bars with discontinuous borders) log units, mean values higher than 6 and lower than 1 log units, respectively.

The results after HS at 50 MPa were similar to refrigeration, while HS at 150 MPa at room temperature allows for a better microbial preservation of *Caldo verde*, compared to refrigeration causing even a decrease of the initial microbial load of the soup.

4.2.1.2. Effect of post-hyperbaric storage at 0.1 MPa and 4 ºC

Since the *Caldo verde* samples stored at 100 MPa showed an inhibitory effect on the microbial growth of all the studied microorganisms, it was studied the post-hyperbaric storage (post-HS) at 4 ºC and 0.1 MPa for 3 days. After 3 days of storage at 4 ºC the microbial loads (2.65 ± 0.01, <1.00, 2.17 ± 0.05 Log_{10} CFU/g for TAM, ENT and YM, respectively) were found to be higher than those obtained after the 12 h under pressure, but were, still, lower than those obtained for the control stored in refrigeration for the same time period (3.16 ± 0.04, 2.19 ± 0.02 and 2.45 ± 0.06 Log_{10} CFU/g for TAM, ENT and YM, respectively). These results reveal that when the soup is stored under pressure there is a microbial growth inhibition leading to lower microbial loads that cause a longer shelf-life under refrigerated conditions at 0.1 MPa. This effect was already observed by **Segovia-Bravo et al. (2012)** and **Fidalgo et al. (2014)** who studied strawberry and watermelon juices previously stored under pressure that were afterwards maintained at refrigeration storage for 15 and 7 days, respectively, at 0.1 MPa.

4.2.2. Microbial analyses in *Bacalhau com natas* - overview

As stated before, *Bacalhau com natas* was used as another case study of a commercial RTE food product. The initially microbial loads in this product were 4.38 ± 0.30 and 1.35 ± 0.40 Log_{10} CFU/g for TAM and YM and below the detection limits (<1.00 Log_{10} CFU/g) for ENT (**Figure 11**, **Table C3** in **Appendix C**). Once again, the microbial level of ENT reflects the high hygiene of the production process of the RTE meal.

Following the same methods used before, two controls were left at atmospheric pressure, one at room temperature (~21 ºC) and the other at 4 ºC to mimic the refrigerated condition. The control at 4 ºC revealed the same pattern showed before, since refrigeration allowed to maintain the microbial loads (4.36 ± 0.08 and 1.85 ± 0.12 Log_{10} CFU/g for TAM and YM, while ENT remained below the detection limits) similar ($p>0.05$) to the initial value throughout the storage period. Concerning the storage for 12 h at 0.1 MPa and ~21 ºC, the TAM counts reached values above 6.00 Log_{10} CFU/g, while ENT and YM

both increase their microbial loads by about 2.50 Log_{10} CFU/g. These results were expected since this RTE food product has a high perishable nature, with a pH close to neutral and high a_w.

4.2.2.1. Effect of hyperbaric storage on microorganisms

Similar to the results obtained before, all the samples stored at HS conditions obtained lower microbial counts than those stored at 0.1 MPa at the same temperature conditions. Generally, the microbial growth inhibition under pressure increased progressively with the pressure level. For example, concerning TAM counts, storage for 12 h at 50 MPa allowed some microbial growth (about 1.00 Log_{10} CFU/g), but this value was not high enough to be statistically different ($p>0.05$) from refrigeration. However, when *Bacalhau com natas* was stored for 12 h at 100 MPa, the expected inhibitory effect was observed, being the microbial loads similar ($p>0.05$) to the initial and refrigerated values. Furthermore, when HS was performed using 150 MPa, although not statistically different ($p>0.05$) was observed a reduction in the microbial counts of about 1.00 Log_{10} CFU/g.

Relatively to ENT loads, all the HS conditions studied led to microbial counts below the detection limits. The same effect was observed for YM, except for HS at 50 MPa, where was observed a microbial growth similar ($p>0.05$) to refrigeration.

These results indicate that HS at 50 and 100 MPa at room temperature performs similar to refrigeration for all the studied microorganisms for preservation of *Bacalhau com natas.* At 150 MPa results better than refrigeration were obtained.

4.2.2.2. Effect of post-hyperbaric storage at 0.1 MPa and 4 ºC

Also in *Bacalhau com natas* was studied a post-HS period of 3 days under refrigeration. After 3 days at 4 ºC and 0.1 MPa, the samples previously pressurized showed higher microbial loads when compared to those obtained right after the 12 h: TAM increased about 1.00 Log_{10} CFU/g, ENT about 2.00 Log_{10} CFU/g and YM increased about 1.50 Log_{10} CFU/g. However it is noteworthy that these values were similar to those obtained for the samples stored under refrigeration during all the studied period (4.88 ± 0.10, 3.53 ± 0.15 and 3.06 ± 0.07 Log_{10} CFU/g for TAM, ENT and YM, respectively).

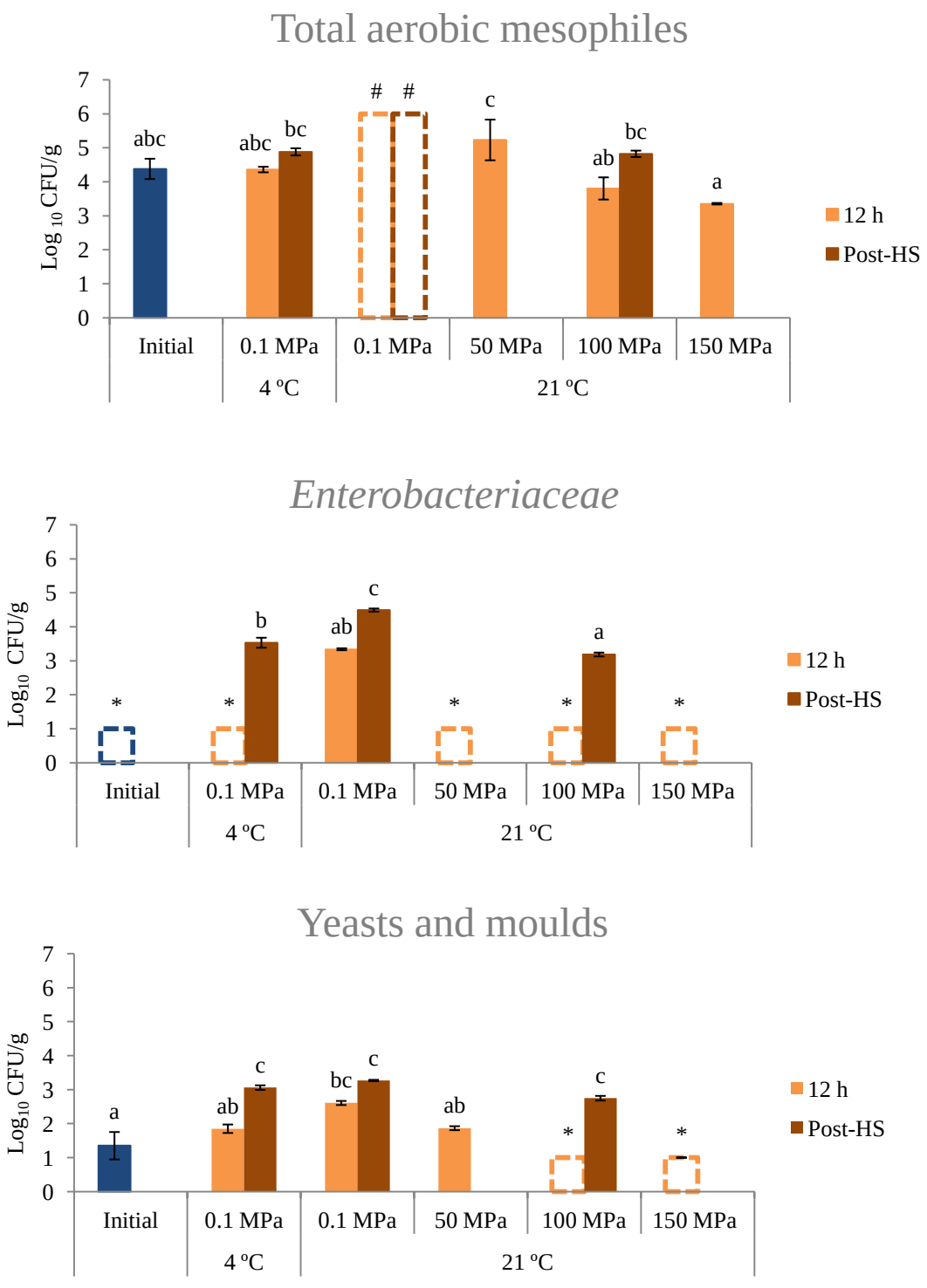

Figure 11. *Bacalhau com natas.* Microbial counts (Log_{10} CFU/g soup) in initial (blue bar) and after hyperbaric storage of *Bacalhau com natas* at 21 ºC for 12 h and after 3 days (Post-HS) at different pressures: 50, 100 and 150 MPa; soup was also stored at control conditions (21ºC at 0.1 MPa) and at refrigeration conditions (4 ºC, 0.1 MPa). Different letters indicate significant differences ($p < 0.05$) between conditions. Values shown as 6 and 1 (white bars with discontinuous borders) log units, mean values higher than 6 and lower than 1 log units, respectively.

These results clearly show that when *Bacalhau com natas* was stored under pressure for the 12 h, a microbial growth inhibition effect was visible. When the samples were placed in refrigeration for 3 more days, the microbial loads in these samples had a similar level to those stored at all the studied period under refrigeration condition.

4.2.3. Physicochemical analysis in *Caldo verde*

4.2.3.1. pH and titratable acidity

The initial *Caldo verde* pH was 6.22 ± 0.03 and that value remained stable ($p>0.05$) for all the studied storage conditions (**Figure 12**). This value is in agreement with the data found in the literature **(Gadekar et al., 2009, Plaza et al., 2006)**.

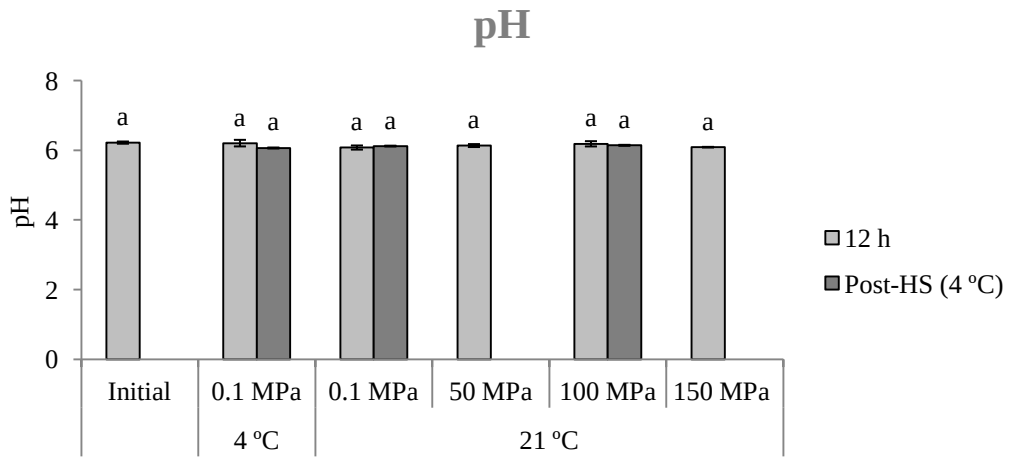

Figure 12. *Caldo verde* pH for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions.

Concerning the TA, the initial value was 50.47 ± 0.68 $mg_{malic\ acid}$/100 g (see **Table F1** in **Appendix F**). Although there were visible some variations (**Figure 13**), the TA value in all the HS conditions was similar ($p>0.05$) to the initial and refrigerated value. Only to the soup stored during the post-HS period (after storage at 0.1 MPa and 21 ºC for 12 h), was observable a higher value (54.89 ± 5.46 $mg_{malic\ acid}$/100 g).

HS was effective to attenuate the increase of TA, as well as to prevent the decrease of pH that occurred at 0.1 MPa. Therefore, HS caused fewer changes on physicochemical parameters of *Caldo verde* than at atmospheric pressure and same temperature (**Figure 13**).

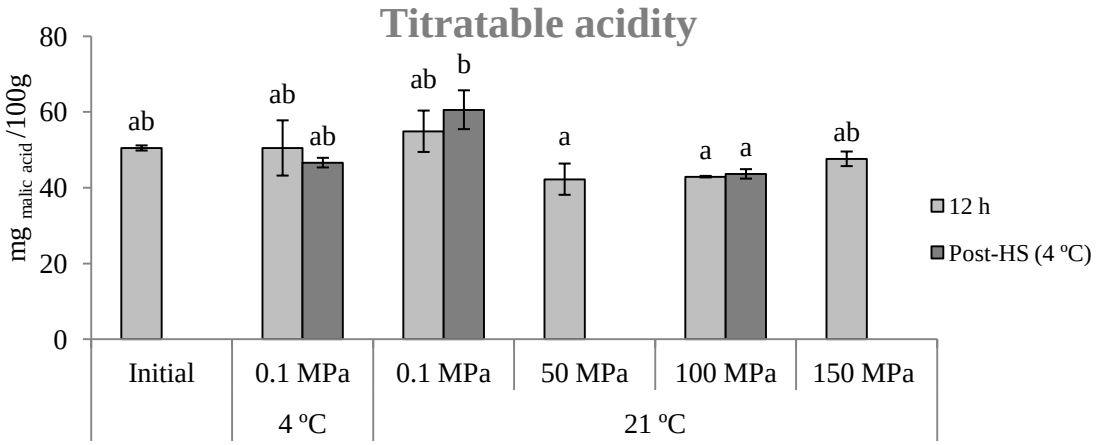

Figure 13. *Caldo verde* titratable acidity for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions.

4.2.3.2. Colour

As said before, a noticeable difference between two products colour is perceptible by a casual viewer when the *ΔE** is above 3.5 **(Krapfenbauer et al., 2006)**. The *ΔE** values of *Caldo verde* (**Figure 14** – see values in **Table E2** in **Appendix E**) reflect the heterogeneity of the sample, since it consists in a yellowish potato puree with green striped cabbage, making it difficult to measure the colour differences, resulting in higher standard deviations. However, when comparing to the initial value, it is possible to observe a higher ΔE value for the samples stored at 0.1 MPa, than for those stored under pressure.

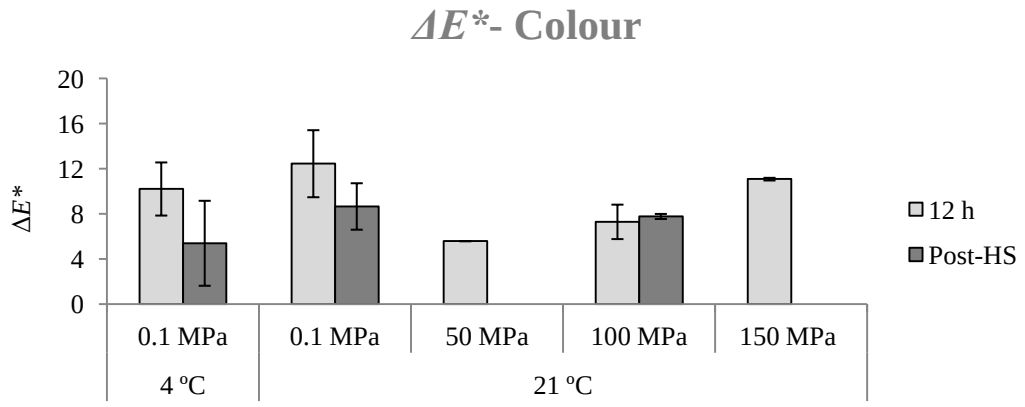

Figure 14. Difference in colour (ΔE) in *Caldo verde* for all the studied stored conditions. Different letters indicate significant differences ($p<0.05$) between conditions.

4.2.4. Physicochemical analysis in *Bacalhau com natas*

4.2.4.1. pH and titratable acidity

The initial pH of *Bacalhau com natas* was 6.70 ± 0.07 (**Figure 15**), which, although a little higher, is in agreement with data of commercial *Bacalhau com natas* (in a range of 6.0-6.5). The pH remained unchanged ($p>0.05$) for all the studied storage conditions, except for the storage at room temperature at 0.1 MPa, where was observed a lower value (5.82 ± 0.08 and 6.01 ± 0.04 for 12 h of storage and the post-HS period, respectively), that can be the result from the higher microbial loads verified at these conditions (see **Figure 11**).

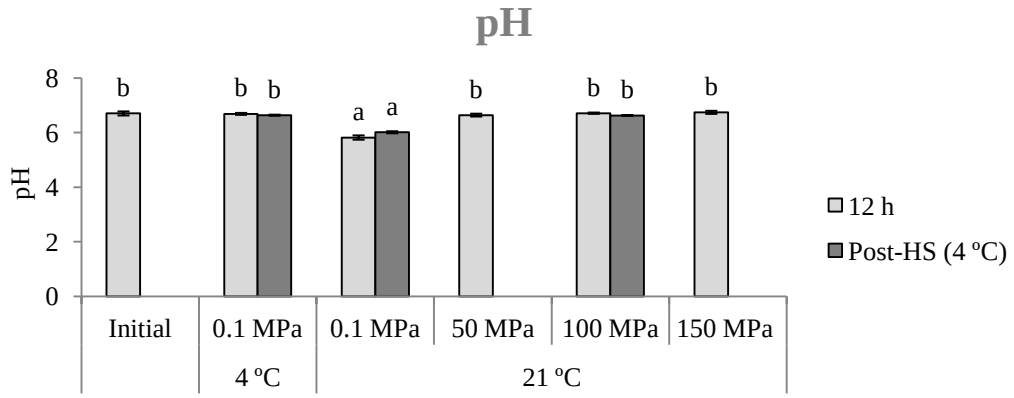

Figure 15. *Bacalhau com natas* pH for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions

Bacalhau com natas initial TA was 112.44 ± 1.44 $mg_{malic\ acid}$/100 g (**Figure 16**). The higher values were obtained in the samples stored at room temperature, 0.1 MPa (191.72 ± 17.82 and 236.71 ± 7.81 $mg_{malic\ acid}$/100 g, for 12 h of storage and the post-HS period, respectively), which are accordingly to the lower pH values observed for storage in the same conditions. Refrigeration and HS did not caused significant changes ($p>0.05$) in samples TA, being all the values similar to the initial. These results show, once again, that HS can preserve the physicochemical characteristics of a RTE food product, maintaining the values similar to the initial and to refrigeration.

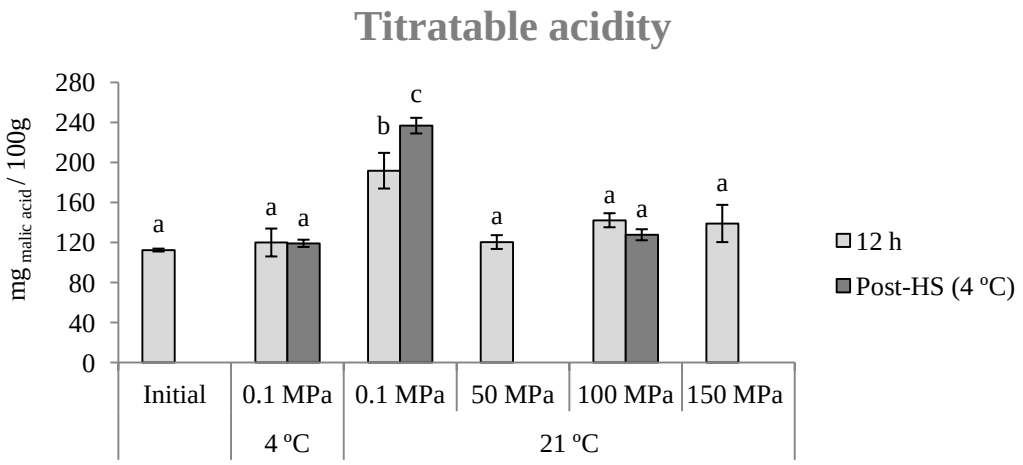

Figure 16. *Bacalhau com natas* titratable acidity for all the studied conditions. Different letters indicate significant differences ($p<0.05$) between conditions.

4.2.4.2. Colour

The *Bacalhau com natas* is reasonably homogeneous relatively to colour. The *ΔE** was calculated and all the studied samples were below the limit of perception to verify colour changes (**Figure 17**) (*ΔE**<3.5), defined by **Krapfenbauer et al. (2006)**. However, it is observable a tendency for the samples stored at 0.1 MPa (3.46 ± 1.11 and 3.30 ± 0.99, at 4 and 21 ºC, respectively) to have a higher *ΔE** when compared to the samples stored under pressure (2.58 ± 0.53, 2.23 ± 0.01 and 2.05 ± 0.33 for 50, 100 and 150 MPa, respectively).

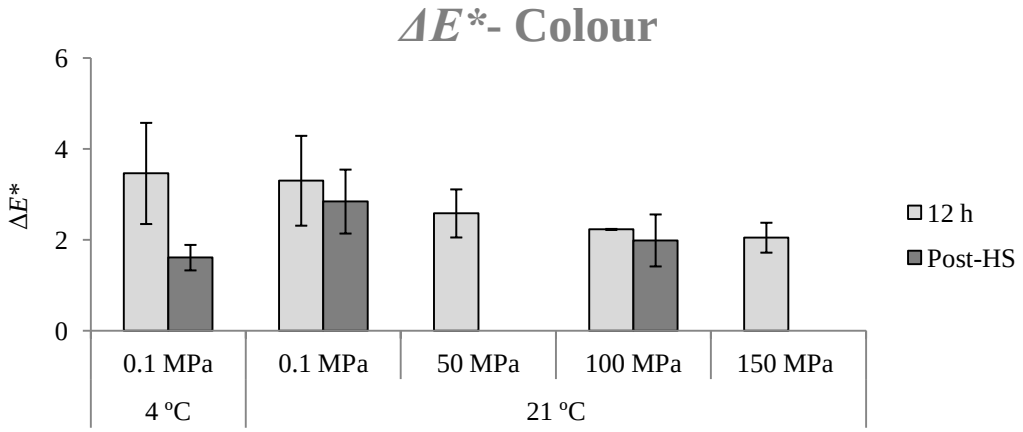

Figure 17. Difference in colour (ΔE) in *Bacalhau com natas* for all the studied stored conditions. Different letters indicate significant differences ($p<0.05$) between conditions.

V. CONCLUSION AND FUTURE WORK

Currently, there are no published studies concerning the HS of RTE food products. On this regard, the present work analyzed several microbiological and physicochemical parameters, in order to observe the effect of different pressure, storage periods and temperature conditions in three different RTE products.

The HS of a RTE carrot soup at and above room temperature (25 and 30 ºC) showed equal to better microbiological and physicochemical results compared to refrigeration, since HS not only caused microbial growth inhibition as even led to microbial reduction. Furthermore, storage at and above room temperature at 0.1 MPa led to higher microbial loads when compared to the initial, being above 6.00 Log_{10} CFU/g for TAM, when the soup was stored for 4 and 8 h at 30 ºC. Microbial growth inhibition prevailed for the lower pressure and the shorter storage period studied (100 MPa and 4 h), while microbial inactivation was more pronounced at 150 MPa (for both 4 and 8 h of storage). After HS at 150 MPa, TAM reduced about 2.50 Log_{10} CFU/g after 4 h, while for storage for 8 h the reduction was about 3.70 Log_{10} CFU/g (to values below the detection limit). Concerning ENT and YM, HS at 150 MPa led to values below the detection limit for both 4 and 8 h of storage. The most pressure resistant microorganisms were TAM, showing mainly growth inhibition. On the other hand, ENT and YM were easily inhibited/inactivated by pressure, resulting in values below the detection limits.

Physicochemical parameters revealed small changes, generally, having no significant differences between the soup stored under pressure or in refrigeration. HS was capable to maintain the pH, titratable acidity, reducing sugars content and colour values similar to refrigeration.

The experiments in an industrial equipment with larger amounts of real RTE food products allowed us to show that this new preservation methodology has similar effect when applied to larger amounts of samples. For *Caldo verde*, storage under pressure (50, 100 and 150 MPa) allowed its microbiological preservation at least up to 12 h, at room temperature (~21 ºC) as demonstrated by TAM, ENT and YM microbial loads. For *Bacalhau com natas*, for storage at 50 MPa a slightly higher value than the initial for TAM and YM was found, while for the higher pressures (100 and 150 MPa) inhibition/inactivation was observed.

In conclusion, HS is a new preservation methodology, showing great potential as possible alternative to refrigeration as a food preservation technology. Contrarily to the

latter, HS is basically energetically costless, since there is no need to control temperature and energy is only needed at the beginning to generate the pressure and at the end to decompress, being no need to spend energy to maintain the pressure level.

Nevertheless, considering all the results obtained in the present work, other studies are necessary in order to advance the scientific knowledge in this area, such as longer storage periods, different pressure/time/temperature combination, other microbial populations and other quality-related parameters. Although, the equipment limitations (laboratorial or industrial) does not allow, for now, to study longer storage periods, and this should also be done in the future.

As a novel conceptual food preservation methodology, HS may become a main trend in food preservation research in the future. It was already possible to publish an article concerning a hyperbaric storage literature review, being another article submitted concerning the laboratorial results obtained in this thesis and it was in development one last article with the results obtained for the experiments in an industrial equipment.

VI. REFERENCES

ALLENDE, A., TOMÁS-BARBERÁN, A., F. & GIL, M. I. 2006. Minimal processing for healthy traditional foods. *Trends in Food Science & Technology,* 17**,** 513-519.

BABA, T. & IKEDA, F. 2003. Use of high pressure treatment to prolong the postharvest life of mume fruit (Prunus mume). *Issues and Advances in Postharvest Horticulture.,* 628**,** 373-377.

BABA, T., ITO, S., IKEDA, F. & MANAGO, M. 2008. Effectiveness of high pressure treatment at low temperature to improve postharvest life of horticultural commodities. *Proceedings of the International Symposium on the Role of Postharvest Technology in the Globalisation of Horticulture.,* 768**,** 417-422.

BARBA, F. J., ESTEVE, M. J. & FRÍGOLA, A. 2012. High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: a review. *Comprehensive Reviews in Food Science and Food Safety,* 11**,** 307-322.

BELITZ, H. D., GROSCH, W. & SCHIEBERLE, P. 2009. Food contamination. *In:* SPRINGER (ed.) *Food Chemistry.* Heidelberg, Germany.

BERG, R., HOOGLAND, H., LELIEVELD, H. L. M. & SCHEPDAEL, L. 2001. High pressure equipment designs for food processing applications. *In:* HENDRICKX, M. G., KNORR, D., LUDIKHUYZE, L., LOEY, A. & HEINZ, V. (eds.) *Ultra High Pressure Treatments of Foods.* Springer US.

BRIDGMAN, P. W. 1914. The coagulation of albumen by pressure. *Journal of Biological Chemistry,* 19**,** 511-512.

BUTT, M. S. & SULTAN, M. T. 2010. Nutritional profile of vegetables and its significance to human health. *In:* WILEY (ed.) *Handbook of vegetables and vegetable processing.* Iowa, USA.

BUTZ, P. & TAUSCHER, B. 2002. Emerging technologies: chemical aspects. *Food Research International,* 35**,** 279-284.

CAZOR, A., DEBORDE, C., MOING, A., ROLIN, D. & THIS, H. 2006. Sucrose, glucose, and fructose extraction in aqueous carrot root extracts prepared at different temperatures by means of direct NMR measurements. *Journal of Agricultural and Food Chemistry,* 54**,** 4681-6.

CHARM, S. E., LONGMAID, H. E., 3RD & CARVER, J. 1977. A simple system for extending refrigerated, nonfrozen preservation of biological material using pressure. *Cryobiology,* 14**,** 625-36.

CONSIDINE, K. M., KELLY, A. L., FITZGERALD, G. F., HILL, C. & SLEATOR, R. D. 2008. High-pressure processing-effects on microbial food safety and food quality. *FEMS Microbiology Letters,* 281**,** 1-9.

DE JONG, A. E., BEUMER, R. R. & ZWIETERING, M. H. 2005. Modeling growth of *Clostridium perfringens* in pea soup during cooling. *Risk Analysis,* 25**,** 61-73.

DE JONG, A. E. I., ROMBOUTS, F. M. & BEUMER, R. R. 2004. Effect of cooling on *Clostridium perfringens* in pea soup. *Journal of Food Protection,* 67**,** 352-356.

DELIZA, R., ROSENTHAL, A. & SILVA, A. L. S. 2003. Consumer attitude towards information on non conventional technology. *Trends in Food Science & Technology,* 14**,** 43-49.

DEUCHI, T., HAYASHI, R. 1990. A new approach for food preservation: Use of non-freezing conditions at sub-zero temperature generated under moderate high-pressure. *In:* HAYASHI, R. (ed.) *Pressure Processed Food-Research and Development.* San-El Publishing Co., Kyoto, Japan.

DEUCHI, T., HAYASHI, R. 1991. Pressure application to thawing of frozen foods and to food preservation under subzero temperature. *In:* HAYASHI, R. (ed.) *High Pressure Science for Food.* San.El Publishing Co., Kyoto, Japan.

DEUCHI, T., HAYASHI, R. 1992. High pressure treatments at subzero temperature: application to preservation, rapid freezing and rapid thawing of foods. *In:* BALNY C, H. R., HEREMANS K, MASSON P (ed.) *High Pressure and Biotechnology.* Montrouge, France: Colloque INSERM/John Libbey Eurotext Ltd.

ELEZ-MARTÍNEZ, P. & MARTÍN-BELLOSO, O. 2007. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry,* 102**,** 201-209.

FA 1995. Microbiological reference criteria for food. *In:* SAFETY, F. (ed.). Food Administration Manual.

FERNANDES, P. R., MOREIRA, S., FIDALGO, L., SANTOS, M., QUEIRÓS, R., DELGADILLO, I. & SARAIVA, J. 2014. Food preservation under pressure (Hyperbaric Storage) as a possible improvement/alternative to refrigeration. *Food Engineering Reviews***,** 1-10.

FIDALGO, L., SANTOS, M., QUEIRÓS, R., INÁCIO, R., MOTA, M., LOPES, R., GONÇALVES, M., NETO, R. & SARAIVA, J. 2014. Hyperbaric storage at and above room temperature of a highly perishable food. *Food and Bioprocess Technology,* 7**,** 2028-2037.

GADEKAR, Y. P., ANJANEYULU, A. S. R., THOMAS, R., MENDIRATTA, S. K. & KONDAIAH, N. 2009. Quality changes in soup from deboned chicken frames at refrigerated (4 ± 1 °C) and frozen (−18 ± 1 °C) storage. *International Journal of Food Science & Technology,* 44**,** 1763-1769.

GOYETTE, B., VIGNEAULT, C., CHARLES, M. T. & RAGHAVAN, V. G. S. 2012. Effect of hyperbaric treatments on the quality attributes of tomato. *Canadian Journal of Plant Science,* 92**,** 541-551.

HEDREN, E., DIAZ, V. & SVANBERG, U. 2002. Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *European Journal of Clinical Nutrition,* 56**,** 425-30.

HEREMANS, K. 1995. High pressure effects on biomolecules. *In:* D. A. LEDWARD, D. E. J., R. G. EARNSHAW, & A. P.M. HASTING (EDS.) (ed.) *High pressure processing of foods.* Leicestershire, UK: Nottingham University Press.

HITE, B. H. 1899. The effects of pressure in the preservation of milk. *Bulletin of the West Virginia University Agricultural Experimental Station Morgantown,* 58**,** 15-35.

HPA 2009. Guidelines for assessing the microbiological safety of ready-to-eat foods. *In:* AGENCY, L. H. P. (ed.).

HUI, Y. H., NIP, W.-K., NOLLET, L. M. L., PALIYATH, G. & SIMPSON, B. K. 2008. *Food biochemistry and food processing,* Iowa, USA, Wiley.

INSA. 2010a. *Detalhe do alimento - sopa, caldo verde* [Online]. Instituto Nacional de Saúde Doutor Ricardo Jorge. Available: http://www.insa.pt/sites/INSA/Portugues/AreasCientificas/AlimentNutricao/AplicacoesOnline/TabelaAlimentos/PesquisaOnline/Paginas/DetalheAlimento.aspx?ID=IS782 [Accessed 17/06/2014.

INSA. 2010b. *Detalhe do alimento - sopa, creme de cenoura* [Online]. Instituto Nacional de Saúde Doutor Ricardo Jorge. Available: http://www.insa.pt/sites/INSA/Portugues/AreasCientificas/AlimentNutricao/AplicacoesOnline/TabelaAlimentos/PesquisaOnline/Paginas/DetalheAlimento.aspx?ID=IS788 [Accessed 16/06/2014.

IRAWATI, Z., NATALIA, L., NURCAHYA, C. M. & ANAS, F. 2007. The role of medium radiation dose on microbiological safety and shelf-life of some traditional soups. *Radiation Physics and Chemistry,* 76**,** 1847-1854.

ISO 4833:2003. Total Aerobic Plate Counts (Based on the BS EN ISO 4833:2003 Standard Protocol).

JANNASCH, H. W., EIMHJELLEN, K., WIRSEN, C. O. & FARMANFARMAIAN, A. 1971. Microbial degradation of organic matter in the deep sea. *Science,* 171**,** 672-5.

JAY, J. M., LOESSNER, M. J. & GOLDEN, D. A. 2005. Microorganisms in foods. *In:* ASPEN PUBLISHERS, I. (ed.) *Modern food microbiology*. Las Vegas, Nevada: Springer.

KALICHEVSKY, M. T., KNORR, D. & LILLFORD, P. J. 1995. Potential food applications of high-pressure effects on ice-water transitions. *Trends in Food Science & Technology,* 6**,** 253-259.

KILINC, B. 2010. Preparation of liquid anchovy (*Engraulis encrasicholus*) soup and microbiological and sensory changes during refrigerated storage. *Journal of Muscle Foods,* 21**,** 451-458.

KNORR, D., FROEHLING, A., JAEGER, H., REINEKE, K., SCHLUETER, O. & SCHOESSLER, K. 2011. Emerging technologies in food processing. *Annual Review of Food Science and Technology,* 2**,** 203-35.

KO, W.-C. & HSU, K.-C. 2001. Changes in K value and microorganisms of tilapia fillet during storage at high-pressure, normal temperature. *Journal of Food Protection,* 64**,** 94-98.

KRAPFENBAUER, G., KINNER, M., GOSSINGER, M., SCHONLECHNER, R. & BERGHOFER, E. 2006. Effect of thermal treatment on the quality of cloudy apple juice. *Journal of Agricultural and Food Chemistry* 54**,** 5453-60.

LADO, B. H. & YOUSEF, A. E. 2002. Alternative food-preservation technologies: efficacy and mechanisms. *Microbes and Infection,* 4**,** 433-40.

LEHNER, A. & STEPHAN, R. 2004. Microbiological, epidemiological, and food safety aspects of Enterobacter sakazakii. *Journal of Food Protection,* 67**,** 2850-7.

LINTON, M. & PATTERSON, M. F. 2000. High pressure processing of foods for microbiological safety and quality (a short review). *Acta Microbiologica et Immunologica Hungarica,* 47**,** 175-82.

LIPLAP, P., BOUTIN, J., LEBLANC, D. I., VIGNEAULT, C. & VIJAYA RAGHAVAN, G. S. 2014. Effect of hyperbaric pressure and temperature on respiration rates and quality attributes of Boston lettuce. *International Journal of Food Science & Technology,* 49**,** 137-145.

LIPLAP, P., CHARLEBOIS, D., CHARLES, M. T., TOIVONEN, P., VIGNEAULT, C. & RAGHAVAN, G. S. V. 2013a. Tomato shelf-life extension at room temperature by hyperbaric pressure treatment. *Postharvest Biology and Technology,* 86**,** 45-52.

LIPLAP, P., VIGNEAULT, C., TOIVONEN, P., CHARLES, M. T. & RAGHAVAN, G. S. V. 2013b. Effect of hyperbaric pressure and temperature on respiration rates and quality attributes of tomato. *Postharvest Biology and Technology,* 86**,** 240-248.

LUDIKHUYZE, L. & HENDRICKX, M. G. 2001. Effects of high pressure on chemical reactions related to food quality. *In:* HENDRICKX, M. G., KNORR, D., LUDIKHUYZE, L., LOEY, A. & HEINZ, V. (eds.) *Ultra High Pressure Treatments of Foods.* Springer US.

MARTÍNEZ-TOMÁS, R., PÉREZ-LLAMAS, F., SÁNCHEZ-CAMPILLO, M., GONZÁLEZ-SILVERA, D., CASCALES, A. I., GARCÍA-FERNÁNDEZ, M., LÓPEZ-JIMÉNEZ, J. Á., NAVARRO, S. Z., BURGOS, M. I., LÓPEZ-AZORÍN, F., WELLNER, A., PLAZA, F. A., BIALEK, L., ALMINGER, M. & LARQUÉ, E. 2012. Daily intake of fruit and vegetable soups processed in different ways increases human serum β-carotene and lycopene concentrations and reduces levels of several oxidative stress markers in healthy subjects. *Food Chemistry,* 134**,** 127-133.

MATSER, A. M., KREBBERS, B., VAN DEN BERG, R. W. & BARTELS, P. V. 2004. Advantages of high pressure sterilisation on quality of food products. *Trends in Food Science & Technology,* 15**,** 79-85.

MCCLEMENTS, J. M., PATTERSON, M. F. & LINTON, M. 2001. The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. *Journal of Food Protection,* 64**,** 514-522.

MITCHELL, M., BRUNTON, N. P. & WILKINSON, M. G. 2011. Impact of salt reduction on the instrumental and sensory flavor profile of vegetable soup. *Food Research International,* 44**,** 1036-1043.

MITSUDA, J., KAWAI, F., AND YAMAMOTO, A. 1972. Underwater and underground storage of cereal grains. *Food Technology,* 26.

MOTA, M. J., LOPES, R. P., DELGADILLO, I. & SARAIVA, J. A. 2013. Microorganisms under high pressure — Adaptation, growth and biotechnological potential. *Biotechnology Advances,* 31**,** 1426-1434.

MURCIA, M. A., JIMÉNEZ-MONREAL, A. M., GARCÍA-DIZ, L., CARMONA, M., MAGGI, L. & MARTÍNEZ-TOMÉ, M. 2009. Antioxidant activity of minimally processed (in modified atmospheres), dehydrated and ready-to-eat vegetables. *Food and Chemical Toxicology,* 47**,** 2103-2110.

MURCIA, M. A., MARTINEZ-TOME, M., NICOLAS, M. C. & VERA, A. M. 2003. Extending the shelf-life and proximate composition stability of ready to eat foods in vacuum or modified atmosphere packaging. *Food Microbiology,* 20**,** 671-679.

NORTON, T. & SUN, D. W. 2008. Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology,* 1**,** 2-34.

OOIDE, A., KAMEYAMA, Y., IWALA, N., UCHIO, R., KARINO, S. AND KANYAMA, N. 1994. Non-freezing preservation of fresh foods under subzero temperature. *In:* HAYASHI, R. & KUNUGI, S., SHIMADA, S. AND SUZUKI, A. (eds.) *High Pressure Bioscience.* Publishing Co., Kyoto, Japan.

PATTERSON, M. 1999. High-pressure treatment of foods. *In:* ROBINSON RK, B. C. & PATEL PD, E. (eds.) *The Encyclopedia of Food Microbiology.* Academic Press, New York.

PINILLA, M. J., PLAZA, L., SÓNCHEZ-MORENO, C., DE ANCOS, B. & CANO, M. P. 2005. Hydrophilic and lipophilic antioxidant capacities of commercial Mediterranean vegetable soups (Gazpachos). *Journal of Food Science,* 70**,** S60-S65.

PLAZA, L., SÁNCHEZ-MORENO, C., ANCOS, B. & CANO, M. P. 2006. Carotenoid content and antioxidant capacity of Mediterranean vegetable soup (gazpacho) treated by high-pressure/temperature during refrigerated storage. *European Food Research and Technology,* 223**,** 210-215.

QUEIROS, R. P., SANTOS, M. D., FIDALGO, L. G., MOTA, M. J., LOPES, R. P., INACIO, R. S., DELGADILLO, I. & SARAIVA, J. A. 2014. Hyperbaric storage of melon juice at and above room temperature and comparison with storage at atmospheric pressure and refrigeration. *Food Chemistry,* 147**,** 209-14.

RASTOGI, N. K., RAGHAVARAO, K. S., BALASUBRAMANIAM, V. M., NIRANJAN, K. & KNORR, D. 2007. Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition,* 47**,** 69-112.

ROBITAILLE, H. A. & BADENHOP, A. F. 1981. Mushroom response to postharvest hyperbaric storage. *Journal of Food Science,* 46**,** 249-253.

ROMANAZZI, G., NIGRO, F. & IPPOLITO, A. 2008. Effectiveness of a short hyperbaric treatment to control postharvest decay of sweet cherries and table grapes. *Postharvest Biology and Technology,* 49**,** 440-442.

RUMBLE, V. R. 2009. *Soup through the ages: a culinary history with period recipes,* North Carolina, USA, McFarland, Incorporated Publishers.

RYU, S.-H., LEE, J.-H., PARK, S.-H., SONG, M.-O., PARK, S.-H., JUNG, H.-W., PARK, G.-Y., CHOI, S.-M., KIM, M.-S., CHAE, Y.-Z., PARK, S.-G. & LEE, Y.-

K. 2012. Antimicrobial resistance profiles among *Escherichia coli* strains isolated from commercial and cooked foods. *International Journal of Food Microbiology,* 159**,** 263-266.

SÁNCHEZ-MORENO, C., CANO, M. P., DE ANCOS, B., PLAZA, L., OLMEDILLA, B., GRANADO, F. & MARTÍN, A. 2004. Consumption of high-pressurized vegetable soup increases plasma vitamin C and decreases oxidative stress and inflammatory biomarkers in healthy humans. *Journal of Nutrition,* 134**,** 3021-3025.

SANCHEZ-MORENO, C., DE ANCOS, B., PLAZA, L., ELEZ-MARTINEZ, P. & CANO, M. P. 2009. Nutritional approaches and health-related properties of plant foods processed by high pressure and pulsed electric fields. *Critical Reviews in Food Science and Nutrition,* 49**,** 552-76.

SANCHEZ-MORENO, C., PILAR CANO, M., DE ANCOS, B., PLAZA, L., OLMEDILLA, B., GRANADO, F., ELEZ-MARTINEZ, P., MARTIN-BELLOSO, O. & MARTIN, A. 2005. Intake of Mediterranean vegetable soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Sciences and Nutrition,* 56**,** 115-24.

SARKAR, B. C. & SHARMA, H. K. 2010. Carrots. *In:* WILEY (ed.) *Handbook of vegetables and vegetable processing.* Iowa, USA.

SCULLY, D. E. & SCULLY, T. 2002. *Early french cookery: sources, history, original recipes and modern adaptations,* University of Michigan Press.

SEGOVIA-BRAVO, K. A., GUIGNON, B., BERMEJO-PRADA, A., SANZ, P. D. & OTERO, L. 2012. Hyperbaric storage at room temperature for food preservation: A study in strawberry juice. *Innovative Food Science and Emerging Technologies,* 15**,** 14-22.

SHIBESHI, K. & FARID, M. M. 2011. Scale-up unit of a unique moderately high pressure unit to enhance microbial inactivation. *Journal of Food Engineering,* 105**,** 522-529.

SINHA, N. K., HUI, Y. H., EVRANUZ, E. Ö., SIDDIQ, M. & AHMED, J. 2010. *Handbook of vegetables and vegetable processing,* Iowa, USA.

SMELT, J. 1998. Recent advances in the microbiology of high pressure processing. *Trends in Food Science & Technology,* 9**,** 152-158.

SMITH, H. 1950. *The master dictionary of food & cookery and menu translator,* Practical Press.

SUN, D. W. 2005. *Emerging technologies for food processing,* Elsevier Science.

TABILO-MUNIZAGA, G. & BARBOSA-CÁNOVAS, G. V. 2004. Ultra high pressure technology and its use in Surimi manufacture: an overview. *Food Science and Technology International,* 10**,** 207-222.

TOURNAS, V. H., HEERES, J. & BURGESS, L. 2006. Moulds and yeasts in fruit salads and fruit juices. *Food Microbiology,* 23**,** 684-688.

ULMER, H. M., HERBERHOLD, H., FAHSEL, S., GANZLE, M. G., WINTER, R. & VOGEL, R. F. 2002. Effects of pressure-induced membrane phase transitions on inactivation of HorA, an ATP-dependent multidrug resistance transporter, in Lactobacillus plantarum. *Applied and Environmental Microbiology,* 68**,** 1088-95.

VAN DER MAAREL, M. J., VAN DER VEEN, B., UITDEHAAG, J. C., LEEMHUIS, H. & DIJKHUIZEN, L. 2002. Properties and applications of starch-converting enzymes of the alpha-amylase family. *Journal of Biotechnology,* 94**,** 137-55.

WEEMAES, C., DE CORDT, S., GOOSSENS, K., LUDIKHUYZE, L., HENDRICKX, M., HEREMANS, K. & TOBBACK, P. 1996. High pressure, thermal, and combined pressure-temperature stabilities of alpha-amylases from Bacillus species. *Biotechnology and Bioengineering,* 50**,** 49-56.

WHO 2009. Global Health Risks: mortality and burden of disease attributable to selected major risks.

WILLIAMS, A. 1994. New technologies in food preservation and processing: part II. *Nutrition & Food Science,* 94**,** 20-23.

YANG, D. S., BALANDRÁN-QUINTANA, R. R., RUIZ, C. F., TOLEDO, R. T. & KAYS, S. J. 2009. Effect of hyperbaric, controlled atmosphere, and UV treatments on peach volatiles. *Postharvest Biology and Technology,* 51**,** 334-341.

APPENDICES

Appendix A. Preparation of soup samples

Table A1. Total aerobic mesophiles counts in "new soup" after increasing the microbial loads (first and second attempt).

		10^{-1}			10^{-2}			10^{-3}			10^{-4}			10^{-5}			10^{-6}			Log_{10} (N)
1st attempt	**1**	4	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	< 1.0
	2	2	3	1	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	< 1.0
2nd attempt	**1**	-	-	-	-	-	-	inc	inc	inc	142	129	154	6	16	15	1	0	1	6.15
	2	-	-	-	-	-	-	inc	inc	inc	138	101	136	13	18	16	2	1	3	6.11

Table A2. Total aerobic mesophiles counts in *Caldo verde*

		10^{-1}			10^{-2}			10^{-3}			10^{-4}			10^{-5}			Log_{10} (N)
Initial	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	< 1.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	< 1.0
After 24h at RT	**1**	inc	17	22	11	12	23	7	7	15	0	0	0	0	0	0	2.47
	2	22	20	24	18	23	29	5	1	13	0	0	0	0	0	0	2.63

Appendix B. Standards preparation and calibration curve construction for the determination of reducing sugars content in soup

B1. DNS reagent and standards preparation

The 3,5-dinitrossalicylic acid (DNS) reagent was performed by dissolution of 5 g of DNS acid in 100 mL of heated 2 M NaOH solution. This solution was mix with 150 g of sodium potassium tartrate to a final volume of 500 mL with distilled water. A glucose solution at concentration of 1.00 g/L was prepared with distilled water and used as stock solution. Several standards ranging from 0.10 to 1.00 g/L were prepared by dilution of stock solution with distilled water, and used to build a calibration curve (See **Figure B1**).

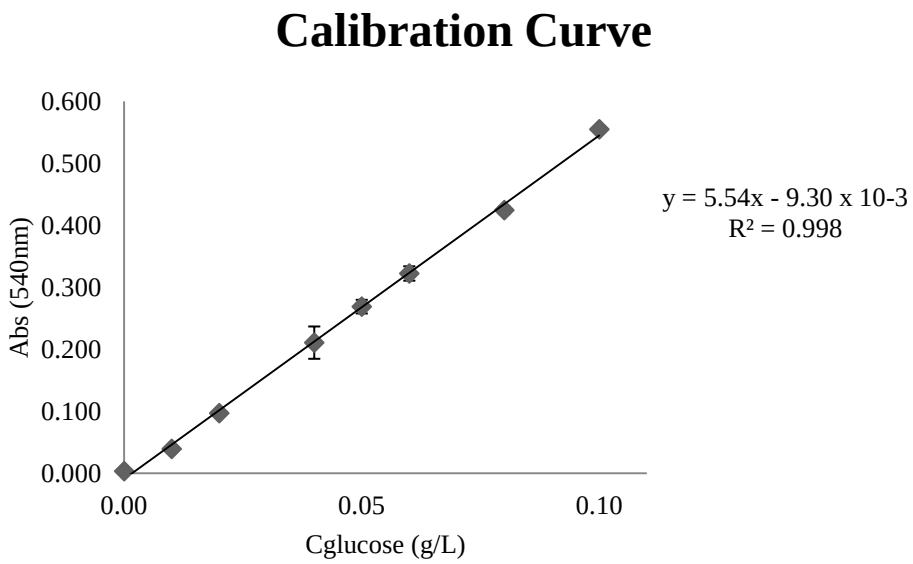

Figure B1. Standard curve used for determining reducing sugars content in soup.

Appendix C. Microbial counts in the studied samples in initial and after HS for various combinations of time, pressure and temperature.

Table C1. Microbial counts in carrot soup samples. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		TAM		*Enterobacteriaceae*		YM	
		4 h	**8 h**	**4 h**	**8 h**	**4 h**	**8 h**
Initial		4.72 ± 0.36 cd		2.38 ± 0.17 a		2.26 ± 0.27 ab	
4 ºC	**0.1 MPa**	4.76 ± 0.28 cd	4.72 ± 0.31 cd	2.52 ± 0.39 a	2.29 ± 0.07 a	2.65 ± 0.16 abc	2.45 ± 0.25 abc
25 ºC	**0.1 MPa**	5.49 ± 0.11 e	5.32 ± 0.11 e	3.24 ± 0.57 ab	4.40 ± 0.03 b	3.05 ± 0.09 bc	3.34 ± 0.09 c
	100 MPa	4.79 ± 0.06 d	2.40 ± 0.03 a	2.43 ± 0.03 s	< 1.00	2.51 ± 0.40 abc	1.89 ± 0.26 abc
	150 MPa	2.22 ± 0.06 a	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
30 ºC	**0.1 MPa**	> 6.00	> 6.00	3.11 ± 0.04 ab	3.33 ± 0.49 ab	3.29 ± 0.20 c	3.23 ± 0.36
	100 MPa	4.57 ± 0.23 cd	4.42 ± 0.01 c	< 1.00	< 1.00	< 1.00	< 1.00
	150 MPa	3.36 ± 0.01 b	3.13 ± 0.07 b	< 1.00	< 1.00	< 1.00	< 1.00

Table C2. Microbial counts in *Caldo verde* samples. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		TAM		*Enterobacteriaceae*		YM	
		12 h	**Post-HS**	**12 h**	**Post-HS**	**12 h**	**Post-HS**
Initial		2.39 ± 0.04 b		< 1.00		2.01 ± 0.16 bc	
4 ºC	**0.1 MPa**	2.44 ± 0.08 b	3.16 ± 0.04 e	< 1.00	2.19 ± 0.02 a	1.85 ± 0.12 ab	2.45 ± 0.06 d
25 ºC	**0.1 MPa**	4.09 ± 0.07 f	5.21 ± 0.04 g	2.52 ± 0.17 a	3.11 ± 0.06 b	2.45 ± 0.08 d	2.99 ± 0.04 e
	50 MPa	2.83 ± 0.03 d	*	< 1.00	*	1.90 ± 0.03 ab	*
	100 MPa	2.19 ± 0.02 a	2.65 ± 0.01 c	< 1.00	< 1.00	1.85 ± 0.21 a	2.17 ± 0.05 c
	150 MPa	< 1.00	*	< 1.00	*	< 1.00	*

* - Experiments were not carried out in these conditions.

Table C3. Microbial counts in *Bacalhau com natas* samples. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		TAM		*Enterobacteriaceae*		YM	
		12 h	**Post-HS**	**12 h**	**Post-HS**	**12 h**	**Post-HS**
Initial		4.38 ± 0.30 abc		< 1.00		1.35 ± 0.40 a	
4 ºC	**0.1 MPa**	4.36 ± 0.08 abc	4.88 ± 0.10 bc	< 1.00	3.53 ± 0.15 b	1.85 ± 0.12 ab	3.06 ± 0.07 c
25 ºC	**0.1 MPa**	> 6.00	> 6.00	3.34 ± 0.03 ab	4.49 ± 0.05 c	2.61 ± 0.06 bc	3.27 ± 0.02 c
	50 MPa	5.23 ± 0.60 c	*	< 1.00	*	1.87 ± 0.06 ab	*
	100 MPa	3.80 ± 0.33 ab	4.82 ± 0.09 bc	< 1.00	3.18 ± 0.06 a	< 1.00	2.75 ± 0.07 c
	150 MPa	3.35 ± 0.02 a	*	< 1.00	*	< 1.00	*

* - Experiments were not carried out in these conditions.

Appendix D. Curves showing linear increase/decrease for physicochemical parameters throughout storage time

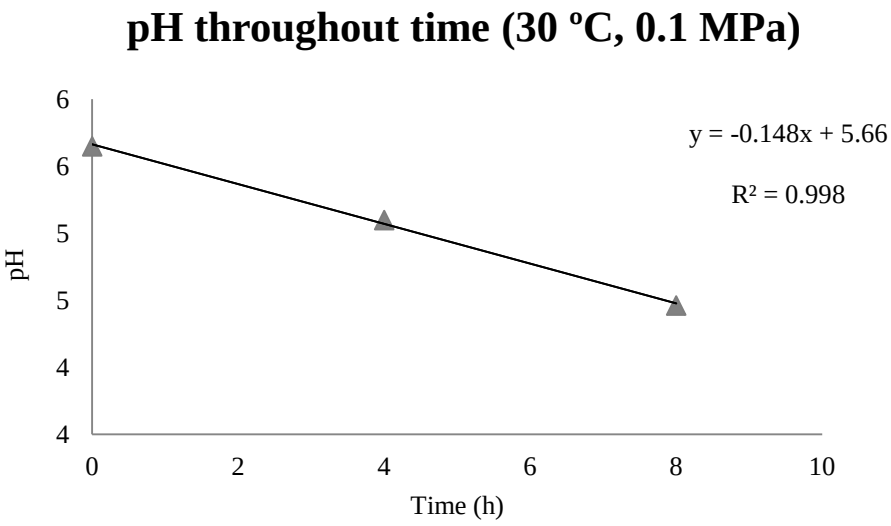

Figure D1. pH variation throughout storage time at 30 ºC, 0.1 MPa.

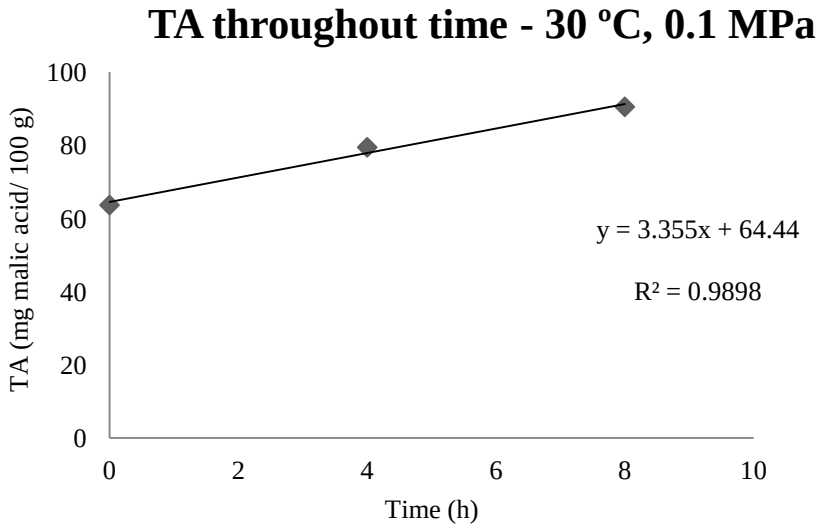

Figure D2. Titratable acidity variation throughout storage time at 30 ºC, 0.1 MPa.

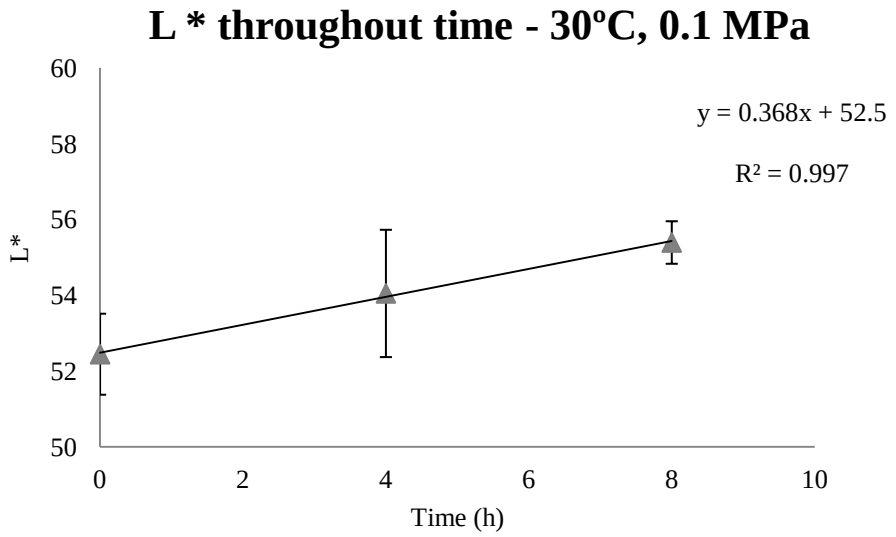

Figure D3. Luminosity variation throughout storage time at 30 ºC, 0.1 MPa.

Appendix E. Colour differences (*ΔE**) in the samples studied

Table E1. Colour differences (*ΔE**) in carrot soup samples.

Conditions		***ΔE**** **– Carrot soup**	
		4 h	**8 h**
4 ºC	**0.1 MPa**	3.21 ± 1.12	1.77 ± 0.67
25 ºC	**0.1 MPa**	5.65 ± 0.18	7.53 ± 2.20
	100 MPa	2.65 ± 0.01	*
	150 MPa	6.27 ± 0.47	5.71 ± 1.14
30 ºC	**0.1 MPa**	1.74 ± 0.50	3.99 ± 1.10
	100 MPa	1.53 ± 0.49	1.27 ± 0.80
	150 MPa	2.89 ± 0.72	2.19 ± 0.10

* - Experiments were not carried out in these conditions.

Table E2. Colour differences (*ΔE**) in *Caldo verde* samples. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		***ΔE**** **–** ***Caldo verde***	
		12 h	**Post-HS**
4 ºC	**0.1 MPa**	10.22 ± 2.35	5.41 ± 3.76
25 ºC	**0.1 MPa**	12.46 ± 2.97	8.67 ± 2.06
	50 MPa	5.60 ± 0.01	*
	100 MPa	7.30 ± 1.53	7.78 ± 0.22
	150 MPa	11.09 ± 0.12	*

* - Experiments were not carried out in these conditions.

Table E3. Colour differences (*ΔE**) in *Bacalhau com natas* samples. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		***ΔE**** **– *Bacalhau com natas***	
		12 h	**Post-HS**
4 ºC	**0.1 MPa**	3.46 ± 1.11	1.61 ± 0.28
25 ºC	**0.1 MPa**	3.30 ± 0.99	2.84 ± 0.70
	50 MPa	2.58 ± 0.53	*
	100 MPa	2.23 ± 0.01	1.99 ± 0.57
	150 MPa	2.05 ± 0.33	*

* - Experiments were not carried out in these conditions.

Appendix F. pH and titratable acidity results of *Caldo verde* and *Bacalhau com natas* samples

Table F1. pH and titratable acidity (TA) values for *Caldo verde* stored for 12 h at ~21 ºC and 3 days at 4ºC (Post-HS) at different pressure conditions. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		pH		Titratable acidity (mg malic acid/ 100 g)	
		12 h	Post-HS	12 h	Post-HS
Initial	**0.1 MPa**	6.22 ± 0.03 a		50.47 ± 0.68 ab	
4 ºC	**0.1 MPa**	6.20 ± 0.09 a	6.06 ± 0.01 a	50.48 ± 7.29 ab	46.61 ± 1.27 ab
25 ºC	**0.1 MPa**	6.08 ± 0.06 a	6.12 ± 0.01 a	54.89 ± 5.46 ab	60.57 ± 5.12 b
	50 MPa	6.13 ± 0.04 a	*	42.24 ± 4.13 a	*
	100 MPa	6.18 ± 0.08 a	6.14 ± 0.01 a	42.90 ± 0.20 a	43.63 ± 1.25 a
	150 MPa	6.09 ± 0.01 a	*	47.61 ± 1.93 ab	*

* - Experiments were not carried out in these conditions.

Table F2. pH and titratable acidity (TA) values for *Bacalhau com natas* stored for 12 h at ~21 ºC and 3 days at 4ºC (Post-HS) at different pressure conditions. Different letters indicate significant differences ($p < 0.05$) between conditions.

Conditions		pH		Titratable acidity (mg malic acid/ 100 g)	
		12 h	Post-HS	12 h	Post-HS
Initial	**0.1 MPa**	6.70 ± 0.07 b		112.44 ± 1.44 a	
4 ºC	**0.1 MPa**	6.69 ± 0.03 b	6.64 ± 0.02 b	119.98 ± 13.94 a	119.15 ± 3.62 a
25 ºC	**0.1 MPa**	5.82 ± 0.08 a	6.01 ± 0.04 a	191.72 ± 17.82 b	236.71 ± 7.81 c
	50 MPa	6.64 ± 0.05 b	*	120.43 ± 7.01 a	*
	100 MPa	6.71 ± 0.02 b	6.63 ± 0.01 b	142.20 ± 7.01 a	127.71 ± 5.51 a
	150 MPa	6.74 ± 0.06 b	*	138.97 ± 18.63 a	*

* - Experiments were not carried out in these conditions.