Universidade de Aveiro Departamento de Química
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Efeitos do processamento por alta pressão nas propriedades físico-químicas e na estabilidade microbiológica de uma salada de fruta.

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, ramo Alimentar, realizada sob a orientação científica do Doutor Jorge Manuel Alexandre Saraiva, Investigador Auxiliar do Departamento de Química da Universidade de Aveiro e da Professora Doutora Ivonne Delgadillo, Professora Associada com Agregação do Departamento de Química da Universidade de Aveiro

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palavras-chave

Processamento por alta pressão; salada de fruta; melão; pêra; maçã; capacidade antioxidante total; polifenol oxidase; estabilidade microbiológica; acastanhamento; perfil volátil.

resumo

Hoje em dia os consumidores estão mais conscientes dos efeitos da dieta na sua saúde, procurando alimentos naturais ou pouco processados. Assim, a investigação tem-se concentrado em processos que tornem os alimentos seguros sem afetar as suas propriedades nutricionais. Neste âmbito, o objetivo deste trabalho foi avaliar os efeitos do processamento por alta pressão (550 MPa/ 3 min/ 15º C) numa salada de fruta (composta por sumo de melão, pedaços de maçã Golden e pedaços de pera Rocha) ao longo de 35 dias de armazenamento refrigerado. Foi analisada a estabilidade microbiológica, propriedades físico-químicas, grau de acastanhamento, atividade enzimática, atividade antioxidante e perfil volátil.

Observou-se que a nível microbiológico, as amostras processadas demonstraram uma maior estabilidade do que as amostras controlo, sendo que os resultados da análise da acidez titulável corroboram estas observações. Relativamente ao acastanhamento, verificou-se um aumento significativo ($p<0.05$) nas amostras processadas. Foi feito um ensaio com adição de ácido ascórbico (100 mg/kg) visando a diminuição do acastanhamento das amostras, mas a concentração usada não foi suficientemente eficaz. No que toca à atividade antioxidante, em geral não foram observadas diferenças significativas entre amostras processadas e amostras controlo ao longo dos 35 dias de armazenamento. Observou-se um aumento da atividade da polifenol oxidase imediatamente após o processamento sendo em geral semelhante ou maior ao longo do armazenamento a 4 ºC, comparativamente às amostras controlo. No que diz respeito ao perfil volátil, verificou-se que os compostos associados ao aroma a melão constituem a maior percentagem relativa, como era esperado, tendo em conta que este é o componente maioritário do produto. No entanto, as amostras processadas revelaram uma diminuição na quantidade relativa destes compostos.

De uma forma geral, o processamento por alta pressão revelou ser eficaz em manter a estabilidade e qualidade geral do produto, apesar de se verificarem alguns efeitos no perfil volátil do produto. A composição em compostos bioativos deve ser analisada futuramente.

keywords

High pressure processing; fruit salad; melon; pear; apple; total antioxidant capacity; polyphenol oxidase; microbiological stability; browning; volatile profile.

abstract

Nowadays consumers are more aware of effects of their diet on their health, demanding natural or minimally processed food products. Thus, research has focused on processes that assure safe products without jeopardizing their nutritional properties. In this context, this work aimed to evaluate the effects of high pressure processing (550 MPa/ 3 min/ 15ºC) on a fruit salad (composed by melon juice and pieces of Golden apple and Rocha pear) throughout 35 days of refrigerated storage. It was analysed its microbiological stability, physicochemical properties, browning degree, enzymatic activity, antioxidant activity and volatile profile.

It was observed that processed samples were more microbiologically stable than raw samples, and the titratable acidity results corroborate this conclusion. Regarding browning degree, it was verified a significant increase ($p<0.05$) in processed samples. It was executed an assay with addition of ascorbic acid (100mg/kg) in order to decrease the browning of the samples, but the concentration used was inefficient. Concerning the antioxidant activity, in general there were no significant differences between raw and processed samples through the 35 days of storage. It was verified an increase in the activity of polyphenol oxidase immediately after processing, being generally similar or higher during storage at 4ºC, compared with raw samples. Regarding the volatile profile of the product, it was verified, as expected, that the compounds associated with melon represented the biggest relative percentage, given that melon juice is the major component of the product. However, processed samples revealed a decrease in the relative quantity of these compounds.

Broadly speaking, high pressure processing showed to be efficient in maintaining the stability and overall quality of the product, even though there were some negative effects on the volatile profile of the product. The composition in bioactive compounds must be analysed in the future.

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ABBREVIATIONS

3Z, 2E- EI	3Z,2E-enal isomerase
AA	Ascorbic acid
AAT	Alcohol acyl CoA transferase
ADH	Alcohol dehydrogenase
AER	Alkenal oxidoredutctase
AOC	Allene oxidase cyclase
AOS	Allene oxidase synthase
CAS Nº	Chemical abstract service number
CFU	Colony forming unit
CIFAC	Characteristic impact flavour and aroma compounds
FA	Fatty acids
HPL	Hydroperoxidase lyase
HPP	High pressure processing
HS-SPME	Headspace solid phase micro extration
HTST	High Temperature Short Time
JMT	Jasmonate methyltransferase
LOX	Lipoxygenase
OPR	12-oxo-phytodienoic acid reductase
PME	Pectin methylesterase
POD	Peroxidase
PPO	Polyphenol oxidase
RI	Retention index
RT	Retention time
TA	Titratable acidity
TAC	Total antioxidant capacity
TAM	Total aerobic mesophiles
TP	Total phenolics
TSS	Total soluble solids
YM	Yeasts and moulds

CONTEXTUALIZATION

This thesis is composed of 8 sections.

In the first chapter is presented a literature review which displays three separate sections. The first section addresses the importance of fruit consumption and the composition and properties of the fruits used in a fruit salad that is being studied under the scope of this master's thesis. Additionally, the preservation methods available for such kind of product are also discussed. The second section concerns High Pressure Processing, the technology used in this master's thesis work. A summary of its history is presented, along with its functional principles, equipment and current applications. The third section addresses known effects of this technology on microbiological, physicochemical and quality parameters important in fruit products. For each parameter, there is a brief introduction and then the main studies are presented, illustrating the effects observed by other authors.

In the second chapter, the objectives of the work being developed under the scope of this master's thesis are described.

The third chapter consists of a detailed description of materials and methods used to carry out this master thesis' work.

The obtained results and their discussion are presented in the fourth chapter, while conclusions and future work are in the fifth and sixth chapter, respectively.

The seventh chapter contains bibliographic references. Appendices with additional information are presented at the end of this thesis.

1. LITERATURE REVIEW

1.1. Introduction

Fruits are an integral part of a healthy diet since they are a source of vitamins, minerals, antioxidants, phytochemicals, sugars, dietary fibre, among others. The most popular form of consumption is in the form of juices and purées due to its convenience and practicality. However, these products spoil easily and therefore require the use of preservation technologies. Traditional preservation technologies for these products include canning, concentration, freezing, evaporation, and spray drying, still the most common is thermal pasteurization. However, the use of thermal techniques has negative effects on the nutritional and organoleptic properties of these foods. This often implies the use of additives in order to mask the effect of processing on colour and flavour, increasing the chemical load of the product [1]. The increased awareness regarding diet and health on the part of consumers has led to a greater exploration of alternative food processing technologies. These must ensure the products' microbial safety whilst preserving both the sensory and nutritional characteristics, allowing to obtain products more similar to fresh foods [2, 3]. These emerging technologies include high pressure processing (HPP), pulsed electric field, pulsed light, ultrasonication, ultraviolet irradiation, and alternative thermal-processing technologies such as microwave, radio frequency and ohmic heating [2].

HPP, also known as high hydrostatic pressure processing, is a non-thermal alternative for the extension of the shelf life of fruit-based products that is gaining popularity in this industry and was considered as one of the most important innovations in food processing during the past 50 years [4]. HPP treatments are effective in inactivating most pathogenic and spoilage vegetative microorganisms and may reduce significantly the enzymatic activity in acid fruit juices and fresh fruits, without greatly affecting vitamins, pigments, aroma, flavour and nutritional value [5]. HPP is nowadays industrially applied in a wide range of products such as fruit juices, sea-foods, meat, fruit-vegetable products, ready-to-eat foods, salads and sauces and even pet foods [5], being also promising when it comes to the impact on the environment and energy costs [6].

1.2. Fruit

1.2.1. Importance of consumption

According to the World Health Organization, low fruit and vegetable consumption is responsible for approximately 1.7 million (2.8%) of deaths being among the top 10 selected risk factors for global mortality. Insufficient intake of fruit and vegetables is estimated to cause around 14% of gastrointestinal cancer deaths, 11% of ischaemic heart disease

deaths and 9% of stroke deaths around the globe. Therefore, the World Health Organization recommends that at least 400 g/day of fruit and vegetables shall be consumed per person. In order to do so, consumers must eat a wide variety of fruits and vegetables including all forms: fresh, frozen, canned, dried, and 100% juices [7, 8].

The intake of fruits and vegetables lowers the risk of diseases related to oxidative stress and risk of developing chronic diseases (*e.g.* diabetes) since these products contain not only bioactive compounds (such as carotenoids and polyphenols) but also vitamins, minerals, and fibres [7, 9]. Hence, consumers should be obtaining phytochemicals from their diet, that must include a wide variety of fruits, instead of taking dietary supplements, which do not contain the balanced combination of phytochemicals found in whole foods [7].

1.2.2. Orange juice: Characteristics and benefits

Citrus juices are popular in many countries, in particular orange juice due to its high vitamin C content and delicate flavour [10]. Moreover, orange juice is a dietary source of flavonoids (hesperedin and naringenin). Actually, it contains, in average, 470-761 mg/L of hesperidin. The whole fruit may contain up to 5 times more than a glass of orange juice because of the fruits' solid parts, in particular, the white portion and the membranes separating the segments [11].

The main effect of flavonoids is the scavenging of free radicals that are involved in oxidative damage (related to ageing processes and chronic disease risk), which gives them anti-inflammatory, antiallergic, antiviral, hypocholesterolemic, and anticarcinogenic properties [7].

1.2.3. Melon: Characteristics and benefits

There is a wide range of melon cultivars, being galia, charentais, cantaloupe, honeydew and piel de sapo the most common [12]. Melon (*Cucumis melo* L.) is a highly perishable fruit given its low acidity (pH > 4.6), its high water activity, and its matrix, which provides a good environment for bacterial growth, especially during cutting prior to consumption or if the surface of the melon suffers damages [12].

Melons are composed by 90% of water as well as high amounts of protein (0.6%) and high content in sugars, which depends on the cultivar. These low caloric fruits are also rich in vitamin A (*ca.* 167 RAE μg/100 g) and in potassium (*ca.* 227 mg/ 100 g). Melons also have significant quantities of carotenes (*ca.* 1000mg/ 100g) and of vitamin C (*ca.* 30 mg/ 100 g), which are the main contributors for its antioxidant activity [13, 14].

1.2.4. Apple: Characteristics and benefits

Apples (*Malus sylvestris*) are the second most consumed fruits in the USA and are considered as the fourth most important fruits worldwide. There is a wide variety of apples, such as Golden, Pink Lady, and Granny Smith, and different varieties present different sizes, shapes, sugar contents, levels of acidity and texture [1].

Regarding the bioavailability of phenolics, apples have one of the highest level of free phenolics when compared with most fruits, which means that a larger quantity of these compounds are absorbed into the bloodstream [15]. The compounds most frequently found in apples consist of procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and quercetin conjugates [16].

Apple consumption is associated with reducing the risk of cancer (especially lung cancer) [17] and prevention of coronary heart disease (mainly due to its content in catechins) [18], cataracts, diabetes, Alzheimer disease, and even asthma [19].

Due to its high storability, apple is the symbol of convenient fruit available in retail throughout Europe in all seasons [20].

1.2.5. Rocha Pear: Characteristics and benefits

Pear (*Pyrus communis* L.) is very popular due to its desirable taste and high digestibility. Its production represents a significant economic activity to Portugal (around 190 000 tonnes *per* year), where the Portuguese exclusive cultivar Rocha accounts for 95% of the national production [21].

A study carried out by Salta *et al*. (2010) [21] evaluated the phenolic profile and antioxidant activity of Rocha pear. When compared to other varieties, Rocha pear presented the highest content of total phenolics (TP), being chlorogenic, syringic, ferulic and coumaric acids, arbutin and (-)-epicatechin the major components (Table 1).

Table 1 - Identification of phenolic compounds in five pear cultivars. Adapted from [21].

	Content in pear cultivar (mg/ 100g fruit)				
Phenolic compound	**G. Leclerc**	**Comice**	**Abate**	**Passe Crassane**	**Rocha**
Arbutin	2.6	5.6	3.6	3.1	**22.5**
Gallic acid	6.3	5.5	7.6	6.9	**4.4**
(+)-Catechin	6.9	1.2	2.4	10.3	**2.9**
Chlorogenic acid	4.3	4.3	7.9	5.3	**62.4**
Caffeic acid	8.5	3.5	12.9	9.4	**11.1**
Syringic acid	8.5	5.4	[a]	7.4	**24.7**
(-)- Epicatechin	nd	nd	nd	nd	**3.8**

Content in pear cultivar (mg/ 100g fruit)					
Phenolic compound	**G. Leclerc**	**Comice**	**Abate**	**Passe Crassane**	**Rocha**
Coumaric Acid	3.6	nd	4.8	4.8	**9.2**
Ferulic Acid	4.8	nd	9.6	3.3	**23.3**

nd – Below limit of detection

a – Excluded by the method used

1.3. High Pressure Processing

1.3.1. History and current use

The history of HPP applications dates to the late nineteenth century. Hite, in 1899, subjected milk to 680 MPa, being the first to prove the effectiveness of pressure in inactivating spoilage bacteria [22]. However, it wasn't until the 90s that high pressure (400–600 MPa) applications in the food industry started to be explored, namely in Japan, with the commercial introduction of pressure-treated jams and jellies [1]. Nowadays, the product spectrum ranges from fruit-vegetable based products (*e.g.*, apple juice, aloe vera gel), egg-dairy products (*e.g.*, Cheddar cheese), sea-foods and meat products (*e.g.*, atlantic mackarel, beef) to alcoholic beverages [5].

HPP market has now reached 9.8 billion USD with a projection of 12 billion USD for 2018, and more than 350 active HPP equipment worldwide [5]. The equipment service sector also grew to 330 Million USD (Figure 1). Nevertheless, regardless of the popularity of this technology, the investment needed to buy and set up a HPP equipment is still high, and can range anywhere between 500 000 to 2.5 million USD [5]. To overcome this obstacle, HPP-tolls (hiring the equipment for a short period) are now used by several companies. The equipment capacity reaches 10,000 L/h, now that Hiperbaric® has launched an innovative design that allows liquids to be processed in bulk before bottling [23] or 3000 kg/h using a machine of 525 L of capacity for most products in general [5].

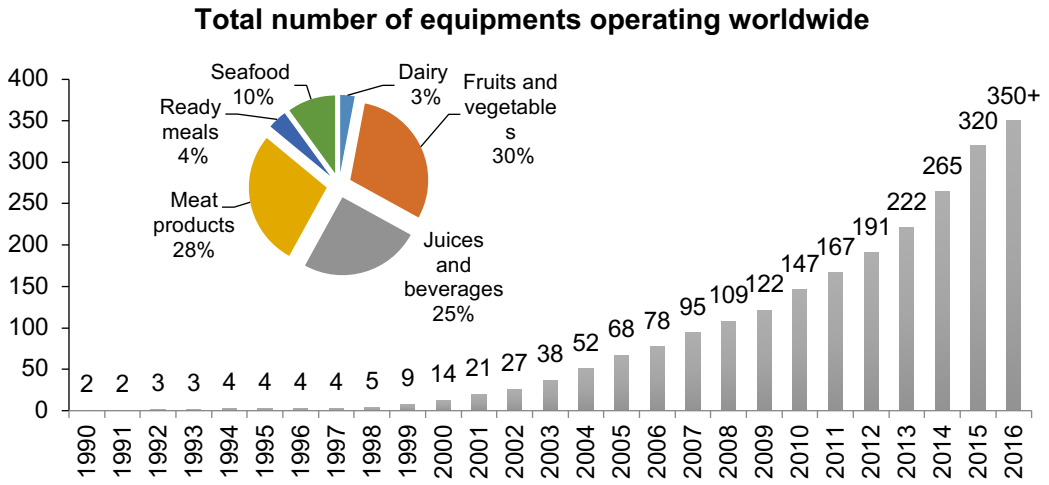


Figure 1 - Graphic representation of the number of operating HPP equipment worldwide and distribution of the type of products treated. Courtesy of Hiperbaric®.

It has been demonstrated that HPP technology can lead to reductions in environmental impacts when compared to conventional thermal processes [24]. This conclusion is based on the fact that there is no production of effluents, and is also based on the capacity of HPP to preserve foods without severe heating/cooling, consequently minimizing water and heat consumption, and on the use of electricity as a source of energy, with an important contribution of renewable resources instead of fossil fuels [5].

It is to be noted that a major milestone was achieved in 2009 when Food and Drug Administration approved Pressure-Assisted Thermal Sterilization. This variant of HPP consists on the pre-heating of the food product between 75 and 90 ºC, followed by the pressurization between 500 and 600 MPa at a pre-established temperature (usually from 90 to 120 ºC) for short periods of time (3 to 10 min) [1].

HPP is not just suitable for food pasteurization, it can be used for other kinds of applications. For example, the combination of high pressure and low temperatures has allowed the development of pressure supported freezing or thawing. Other applications are the shucking of bivalves and meat extraction from crustaceans, high pressure extraction of bioactive compounds or even pressure-ohmic-thermal sterilization [1, 25].

1.3.2. Governing principles

HPP uses a pressure-transmitting medium, usually water, to instantaneously transmit isostatic pressure (up to 600 MPa) to food, at cold, room or mild temperatures (about 60 °C), independent of size, shape or composition [1].

This process is governed by three principles:

- **Le Chatelier's principle:** This principle states that pressure application results in a change in equilibrium so that any phenomenon accompanied by a decrease in volume will be enhanced by increasing pressure. This means that pressure shifts the system to the state of lowest volume [1];
- **Principle of microscopic ordering:** At constant temperature, an increase in pressure will increase the degrees of ordering of molecules of a given substance [1];
- **Isostatic principle:** Presumes that pressure is uniformly applied and acts equally in all directions. This means that when pressure is applied to food, itself and its effects are distributed in a *quasi*-instantaneous and homogeneous manner, regardless of the product's shape or size. Therefore, this principle helps to understand why nonporous foods with high-moisture content are not macroscopically damaged by HPP. Since air and water have different compressibilities, the structure of the foods containing air pockets may suffer changes when under pressure [1].

1.4. Microbiological, physicochemical and quality parameters: Before and after HPP

1.4.1. Microbiological safety of fruit products

Acid-tolerant bacteria and fungi (yeasts and moulds) can cause spoilage of fruit products. If the contaminating microorganisms are pathogens, it can also cause human illness.

Yeasts and moulds are the most common contaminants in fruit products. Spoilage by fungi in fresh fruit products is characterized by the formation of haze, production of carbon dioxide and off-odours, off-flavours and changes in colour [26].

In a study carried out by Tournas *et al.* (2006) [26], the most common yeasts found in commercially available fruit salads were *Pichia* sp., *Rhodotorula* sp., *Candida pulcherrima*, *C. lambica, C. sake* and *Debaryomyces polymorphus*. The presence of filamentous fungi was evaluated in the same products, and there were mainly present the genera *Penicillium* and *Cladosporium*. Regarding the fruit juices analysed in the same study, only 22% of the samples were contaminated, being the main yeast species isolated *R. rubra, C. lambica, C. sake and Kloeckera api*. When it comes to moulds, *Penicillium* and *Fusarium* sp. were isolated in small quantities.

Cell damage magnitude depends not only on the organisms' degree of tolerance, but also on the extent and duration of pressure treatment, and other environmental parameters (such as pH, water activity and temperature) [27]. Regarding fruit products, its inherent low pH reduces the optimal environment for the growth of microorganisms, resulting in a smaller

microbial load and in a more efficient inactivation during HPP, since bacterial vegetative cells are more pressure sensitive at low pH [28]. Broadly speaking, prokaryotes are more resistant to high pressure than eukaryotes. Yeasts and moulds are in general more pressure sensitive. In what concerns prokaryotes, gram positive microorganisms such as *Bacillus, Listeria, Staphylococcus* and *Clostridium*, are generally more pressure resistant than gram-negative microorganisms, given that they have a thicker peptidoglycan layer [29]. Furthermore, cells in the stationary phase show higher resistance towards pressure than those in exponential phase [1].

Studies regarding the effects of HPP on microorganisms in fruit products, selected based on the food matrix studied, are presented on Table 2.

Table 2 - Examples of reported effects of HPP on microbial populations in fruit products.

Fruit Product	Treatment	Effects immediately after processing	Reference
Acidified apple purée	400 and 600 MPa/ 5 min/ 20 °C	Initial load of 3.31 log CFU/mL of total aerobic mesophilic bacteria (TAM) and 3.22 log CFU/mL of moulds and yeasts (M&Y) suffered reductions to counts below the detection limit.	[30]
Mango Nectar	600 MPa/ 1 min/ 20 ºC	TAM reduction of 5.2 log CFU/mL and M&Y reduction of 3.1 log CFU/mL.	[31]
Pomegranate juice	350 MPa/ 2.5 min; 450-550 MPa/ 0.5, 1.5 and 2.5 min	Initial populations of TAM and M&Y of 2.98 and 3.79 log CFU/mL decreased to 1.0 log CFU/mL.	[32]
	400 MPa/ room temperature/ 5 min	Initial load of 6.05 log CFU/mL TAM and 3.69 log CFU/mL M&Y reduced to 1.52 log CFU/mL and < 1 log CFU/mL, respectively.	[33]
Red fruit based smoothies	350 MPa/ 5 min/ 10 ºC	Initial load of TAM suffered a reduction of 1.7 log CFU/mL, and M&Y suffered a reduction of 1.8 log CFU/mL.	[34]
Sweet cherry juice	400 MPa/ 5 min 550 MPa/ 2 min 10 ºC	Initial load of 3.50 log CFU/mL TAM and 4.70 log CFU/mL. M&Y was reduced to < 1 log CFU/mL.	[35]

The use of pressure levels between 300 and 350 MPa is not as efficient as the use of levels of and above 400 MPa in reducing the microbial load.

1.4.2. Vitamin C

Humans are incapable of synthesizing vitamin C, so they depend on their diet to get it. Besides the amount ingested through fruits and vegetables, this nutrient is widely consumed in the form of a dietary supplement.

Vitamin C has a protective effect against free radicals both in extracellular and intracellular spaces of biological systems. It shows numerous beneficial effects, *i.e.* eliminates several different reactive oxygen species, reduces the risk of diseases like atherosclerosis and cardiovascular diseases, is involved in the maintenance of healthy skin, gums, and blood vessels, among others [36]. Vitamin C shows antioxidant activity, however, at higher concentrations, it turns into a pro-oxidative drug that catalyses hydrogen peroxide production in tissues [37]. Since it is one of the most sensitive nutrients to processing conditions, any decline of vitamin C is interpreted as a decrease in product quality [36].

Broadly speaking, vitamin C is not very affected by HPP since most studies report a retention above 80% after processing [38]. It is generally accepted that HPP has limited effects on the content of vitamin C since it does not interfere with covalent bonds, having less impact on its content than thermal processing, as shown in many studies regarding the effects of HPP on vitamin C content in fruit products. Selected studies, based on the food matrix studied, regarding the effect of HPP in vitamin C content are presented in Table 3.

Table 3 – Examples of reported effects of HPP on vitamin C content in fruit products. Adapted from [36].

Fruit Product	Treatment	Results	Reference
Acidified apple purée	400 and 600 MPa/ 5 min/ 20 °C	Vitamin C content after HPP was 97% (400 MPa) and 94.5% (600 MPa).	[30]
Apple Juice	500 MPa/ 3 min/ 25 ºC	No significant difference.	[39]
Blueberry juice	200-600 MPa/ 5-15 min	Retention of 92%.	[40]
Fruit smoothies	T1: 450 MPa/ 20 ºC/ 5 min T2: 600 MPa/ 20 ºC/ 10 min	Increased content in HPP samples in 21.6% in T1 samples and 11.5% in T2 samples. The content in fresh smoothie was 81.1 mg/ 100mL.	[41]
Litchi	100, 200 and 300 MPa/ 5, 10, 15 min/ 27 ° C	Retention of 83.5%.	[42]
Orange juice	500-800 MPa/ 1 min/ 25 – 50 ºC	At 800 MPa and 25 ºC for 1 min, <20% loss after storage for 3 months at 4ºC or 2 months at 15 ºC.	[43]
	400 MPa/ 1 min/ 40 ºC	<8% less than untreated sample.	[44]
	250-450 MPa/ 0-60 min/ 25-50 ºC	350–450 MPa, 25 ºC, 30 min combinations, no significant differences ($p>0.05$) were detected, compared with that of the fresh juice. At 450 MPa at 45 ºC, content decreased.	[45]
	T1: 100 MPa/ 60 °C/ 5 min T2: 350 MPa/ 30 °C/ 2.5 min T3: 400 MPa/ 40 °C/ 1 min	T1 and T3 juices showed a decrease in vitamin C just after HPP, compared with fresh juices while T2 juices had the same level of vitamin C.	[46]

Fruit Product	Treatment	Results	Reference
Strawberry and blackberry purées	400/500/600 MPa/ 15 min/ 10–30 °C	No significant difference.	[47]

For example, pressures between 350 and 600 MPa almost did not affect the content of vitamin C of strawberry and blackberry purées [47] and orange juice [46]. In the case of strawberry and blackberry purées, studied by Patras *et al*. (2009), pressure treatments did not significantly affect vitamin C content. However, after thermal processing ($P_{70} \geq 2$ min), vitamin C degradation was *ca.* 21% [47]. Similarly, in the work of Keenan *et al.* (2012) [41], fruit smoothies were submitted to HPP or thermally pasteurized (70 ºC for 10 min) and it was observed that after HPP, the content of vitamin C was similar to fresh smoothies with a slight increase, probably due to higher extractability of vitamin C after HPP (Table 4). However, in the thermally pasteurized samples, vitamin C content was found to be 44% less than the pressurized samples.

Still, there are some exceptions, particularly at more severe HPP conditions. For instance, in the work developed by Kouniaki *et al.* (2004) [48] it was reported that the higher the level of pressure used, the higher the percentage of vitamin C losses. Also, Landl *et al.* (2010) [30] reported higher losses of vitamin C at 600 MPa comparatively to 400 MPa after processing on Granny Smith apple purée. The authors also compared the effects of HPP *versus* thermal pasteurization (75 ºC for 10 min). After pressurization treatments, the total vitamin C retention was *ca.* 94 % at 400 MPa and 79 % at 600 MPa, while thermal pasteurization had no major influence on the total vitamin C content, as *ca.* 95% was retained, which is explained by the authors as result of the stabilization of vitamin C by the low pH conditions of orange juice.

High levels of vitamin C can be maintained during 1-3 months at refrigerated storage after HPP [36]. For instance, in the study carried out by Plaza *et al*. (2006) [44], it was observed that vitamin C content showed a decrease lower than 8%, after refrigerated storage for 40 days at 4 ºC. The work of Kaushisk *et al.* (2013) [42] comes up as another example, since during post-processing refrigerated storage of litchi samples, processed samples still presented higher retention than unprocessed samples (untreated sample retained 48% of vitamin C after 17 days, whereas pressurized samples at 300 MPa for 10 and 15 min still retained 65 and 69% of vitamin C, respectively, after 42 days of storage). These results show that the use of lower temperatures during storage results in a better maintenance of the post-processing vitamin C content. Nonetheless, there are some contradictory results

in the literature. Valdramidis *et al*. (2009) [49] reported that the vitamin C content of HPP processed apple juice (400 MPa/ 15 min/ 10 ºC) stored at 4, 8 or 12 ºC decreased drastically, *ca.* 82%, during storage up to 36 days. There may be several reasons for different degradation rates, such as the type of cultivar [50], packaging and storage conditions, the presence of oxygen and enzymatic activity [49]. Vitamin C can be affected *in situ* by chemical and enzymatic reactions that may be enhanced by increasing pressure levels. For example, peroxidase (POD) of certain fruits is pressure-resistant and its residual activity can be a cause of vitamin C degradation after processing [36]. Moreover, many studies have reported that vitamin C content decreases when higher temperatures and longer processing times are used. Also, the food matrix has a prominent role not only regarding the effects of pressure on nutrients, but also regarding the effects of storage, which may justify the variability of results in the literature [36]. Taking into consideration the studies here mentioned, it is possible to infer that HPP shows a high preservation potential for vitamin C, since it increases its accessibility and retention, while delivering a safe product, even after long periods of storage [36].

1.4.3. Phenolics content

Phenolics are defined as compounds possessing one or more aromatic rings with one or more hydroxyl groups in the structures. These compounds are responsible for major organoleptic characteristics of fruits products, particularly colour and flavour. According to their structural differences, phenolic compounds are typically divided into four main categories: phenolic acids, flavonoids, stilbenes and lignans [51].

Given the antioxidant activity of phenolic compounds, that prevents phenomena such as low-density lipoprotein oxidation, red blood cell damage, DNA oxidative damage, among others, these compounds have shown protective effects against degenerative diseases, including cancers, cardiovascular and neurodegenerative diseases [52].

Actually, dietary phytochemicals with antioxidant activity capable of preventing low-density lipoprotein oxidation have been an important therapeutic approach since oxidized low-density lipoproteins play a key role in the initiation and progression of atherosclerosis [7].

The phenolic profile varies between products, and different phenolic compounds show different pressure sensitivity. HPP parameters, food matrices, storage condition, material and method of packing, additives/supplements, dissolved oxygen, enzymatic residual activity, and interactions between phenolic compounds and other ingredients may influence phenolics content in pressurized products. The variation of its content in fruit and vegetable products during HPP processing and storage may be attributed to enzymatic (due to

polyphenoloxidase (PPO) and POD activity) and/or non-enzymatic oxidation, condensation and polymerization [52].

Selected studies, based on the food matrix studied, regarding the effect of HPP in TP content are presented in Table 4.

Table 4 – Reported effects of HPP on total phenolics content in fruit products.

Fruit Product	HPP treatment	Results	Reference
Cashew apple juice	250 or 400 MPa/ 3, 5 and 7 min	In 3 and 5 min HPP samples, the contents were higher (17–28%).	[53]
Cloudy apple juice	500 MPa/ 25 ºC/ 3 min	HPP samples showed an increase of 28.7%.	[39]
Cloudy pomegranate juice	400 MPa/ room temperature/ 5 min	HPP treated samples showed an increased value in *ca.* 4%.	[33]
Fruit Smoothie	450 MPa/ 20 ºC/ 5 min	HPP samples had 11% more TP content than fresh smoothies.	[41]
Papaya beverage	550 MPa/ 20 ºC/ 5 min	No significant increase in TP content.	[54]
Strawberry and blackberry purées	500 or 600 MPa/ 20 ºC/ 15 min	At 500 MPa, strawberry and blackberry purées showed an increase of 8.3% and 1.8%, respectively, whilst at 600 MPa the increases were of 9.8% and 5.0%.	[47]
Sweet cherry juice	400 MPa/ 5 min 550 MPa/ 2 min 10ºC	After 28 days of refrigerated storage, TP content loss was higher in raw samples (26%) than in HPP treated samples (1%).	[35]

During HPP, more phenolic compounds become extractable due to the mechanical damage to tissues and cell membranes induced by high pressure, which may explain the higher values detected in the studies presented in Table 4 [52]. However, during storage, HPP samples show a decline in TP content, usually less pronounced than in untreated samples, which may happen due to the activity of enzymes such as PPO and POD that are usually not inactivated by HPP [35].

Broadly speaking, HPP [at moderate pressures (400-550 MPa), room temperature, for short holding times (2-5 min)] with subsequent refrigerated storage sustains more effectively the levels of phenolic compounds and antioxidant activity in fruit products, when compared to thermal pasteurization. This may be due to the inhibitory effect of HPP over non-enzymatic

oxidation and polymerization of phenolic compounds, and due to the prevention of oxidation of phenolic compounds through the use of lower temperatures during processing and storage [52]. For instance, in the work of Queirós *et al.* (2015), it was observed that HPP-treated sweet cherry juice samples showed higher stability during storage than those subjected to thermal pasteurization, which had a reduction of 20% in TP content during storage, whilst HPP samples had 11% and 1%, after 400 MPa/5 min and 550 MPa/2 min, respectively. In agreement with these results, Chen *et al*. (2015) verified that, although the TP contents in both HPP and High Temperatures Short Time (HTST) (110 ºC for 8.6 seconds) treated samples decreased, HPP samples showed higher TP content and antioxidant capacity, compared with the HTST treated samples, during 40 days of storage [54].

There are, however, some exceptions: Patras *et al.* (2009) [47] reported the increase of TP content using not only moderate (500 MPa), but also severe pressure (600 MPa), for a longer holding time (10 min), whilst Queiroz *et al.* (2010) [53] observed that when using a holding time of 7 min, there was a decrease of TP content, which may be a consequence of remaining enzymatic activity, as mentioned above.

So, resembling the conclusion regarding vitamin C, HPP is a highly efficient technology, having the capacity to retain TP content in fruit products and increase its availability.

1.4.4. Enzymes

As an alternative to thermal treatment, HPP can be used to inactivate enzymes in fruits and vegetables by itself or by adding enzyme inhibitors (e.g. acidulates, reducing agents, etc.) or by a combination of high pressure with anti-browning agents. It can be used to inactivate enzymes such as lipase, lipoxygenase (LOX), PME, PPO and POD, whilst maintaining the product's sensorial and nutritional features [28].

Inactivation of enzymes caused by high levels of pressure also depends on factors such as the type and origin of the enzyme, pH and temperature [2].

Broadly speaking, there is no consensus concerning the response of oxidative and hydrolytic enzymes to HPP treatments, as their activity can be reduced or enhanced.

1.4.4.1. Polyphenol oxidase

Polyphenol oxidase (PPO) (EC 1.14.18.1) is a copper-containing enzyme, which acts on phenols in the presence of oxygen [2]. It is present in fruit and vegetables and is responsible for enzymatic browning after bruising or cutting and for browning discoloration during processing and storage. Colour in real fruit products can change mainly due to the mixing

of polyphenol compounds with PPO enzyme since it catalyses two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones that either react with high molecular weight polymers or form macromolecular complex with amino acids and proteins. The non-enzymatic polymerization of these intermediate compounds and condensation of *o*-quinones leads to the formation of heterogeneous black, brown, or red pigments commonly called melanins [2, 28]. In addition, PPO is also believed to be involved in the oxidative degradation of ascorbic acid [2].

Several techniques have been used to control or inhibit PPO's activity in fruit and vegetables, such as thermal processing, refrigeration, lowering of the pH (it shows optimum activity at pH between 5 and 7), and enzyme inhibitors, depending on the type of product [2].

Browning due to PPO activity can be used as an indicator of the quality of HPP processing in fruit and vegetable products that darken after bruising, cutting or processing [28].

Concerning the HPP effect on this enzyme's activity, the results reported in the literature are not consensual, as it can be verified in Table 5, which compiles some selected studies.

Table 5 - Reported effects of HPP on fruit polyphenol oxidase.

Fruit Product	HPP treatment	Results	Reference
Cloudy apple juice	250-450 MPa/ 25-50 ºC/ 0-60 min	*ca.* 50% activity increase at 450 MPa/25 ºC/15 min; 90% inactivation at 450 MPa/ 50 ºC/ 60 min	[45]
	400 MPa/ 20 ºC/ 5 min 450 MPa/ 50 ºC/ 60 min	65% activity increase 9% residual activity	[55]
Peach juice	400-600 MPa/ 25 ºC/ 5-25 min	79% inactivation on maximum conditions.	[56]
Fruit Smoothies	350-600 MPa/ 10 ºC/ 3-5 min	PPO activity was not affected.	[57]
	450 MPa/ 20 ºC/ 5 min	PPO activity was reduced to two-thirds.	[41]
Strawberries	300, 450 or 600 MPa/ 20, 40 or 60 ºC/ 2, 6 or 10 min	PPO activity of the processed samples ranged from 71.8% at 600 MPa, 60 °C, 10 min to 118% at 300 MPa, 60 °C and 10 min comparing to the untreated sample.	[58]

Broadly speaking, PPO is very difficult to inactivate using HPP. In fact, looking at the results regarding cloudy apple juice [45, 55], it is also possible to infer that when using higher

temperature allied with pressure above 450 MPa, there is higher efficiency in inactivating PPO. However, the sensorial aspects of the product are highly affected when using high temperatures, which needs to be taken into consideration.

Despite the difficulty involved in comparing different products and different pressurizing conditions, the literature shows that PPO remains active in fruit treated by HPP, even if in small percentages [59].

1.4.4.2. Peroxidase

POD (EC 1.11.1.7) has as main physiological function to control the level of peroxides generated in oxygenation reactions to avoid excessive formation of radicals that are harmful, being found in almost all living organisms [2].

POD is believed to be involved in the degradation of horticultural products' colour and flavour. It catalyses the oxidation of phenolic compounds in the presence of hydrogen peroxide which suggests that PPO promotes POD activity, since hydrogen peroxide is generated during the oxidation of phenolic compounds catalysed by PPO [2]. That being said, even though the main responsible for enzymatic browning in fruits and vegetables is indeed PPO, there might be a synergistic interaction between PPO and POD that can not be excluded [60].

Seyderhelm *et al*. [61] found that the inactivation/inhibition of enzymes due to HPP followed the order: LOX, lactoperoxidase, pectinesterase, lipase, phosphatase, catalase, PPO, POD. They referred that there is a protective effect of food components in enzymatic inactivation, and that despite the fact that POD does not cause severe deteriorative effects in fresh fruit or vegetables, it is used as a quality indicator factor, since it is relatively resistant to heat and pressure [28, 61]. This way, it is assumed that if POD is inactivated, the other quality-degrading enzymes are also inactivated [2].

The effect of HPP on food POD differs according to the source of the enzyme and the composition of the surrounding food matrix [2]. Terefe *et al.* (2010) [62] studied various processing parameters for strawberry purée. Ranging pressures from 100-600 MPa, at temperatures between 24 ºC to 90 ºC, for 5 to 15 min, it was observed a strong synergistic relationship between pressure and temperature in inactivating POD at pressures above 400 MPa, and a slight antagonistic effect at 100-400 MPa. This antagonistic effect was explained by the authors as result of POD stabilization against thermal inactivation, since HPP might inhibit the loss of water molecules inherent to exposure to high temperature, given that it has a favourable effect on hydration of both charged and non-polar groups. It was observed a considerable inactivation of POD at 690 MPa at 90 ºC, regardless of the

holding time used. Similarly, Terefe *et al*. (2017) [63] concluded there was no significant inactivation of blueberry POD when processed at 30ºC, even when at the highest experimental pressure used, 690 MPa, the inactivation was ca. 23%. It was verified the existence of pressure-stable and labile POD fractions, and stated that HPP inhibits POD inactivation, only being possible to achieve significant inactivation when using temperatures above 80 ºC. Other authors have studied the effect of HPP on POD when using room temperature or similar, and reached the same conclusions as the authors previously mentioned. For instance, Prestamo *et al*. (2001) [64] studied the effect of HPP on apple POD, using 600, 800 or 1000 MPa, for 15 or 30 min, at 20 ºC. The authors concluded that this enzyme is highly resistant to pressure, showing limited inactivation even when submitted to pressures as high as 1000 MPa, regardless of the holding time used. Accordingly, Cao *et al.* (2011) [65] observed that when submitting strawberry purées to 400 or 500 MPa, at room temperature, POD activity reduced with increasing holding time, being the maximum reduction for 25 min of 56.5% or 74.6%, respectively. Liu *et al.* (2013) [66] reported a maximum inactivation of 42% for POD in watermelon juice after a treatment of 600 MPa/ 60 min/ 25 ºC.

Still, in the work of Prestamo *et al.* (2001) [64] it was observed an increase in POD activity when processed at 600 MPa, which was considered a consequence of increased enzyme extraction, due to the already mentioned damage in membranes inherent to HPP.

POD usually is not susceptible to HPP treatments, requiring the exposure to high temperatures to be significantly inactivated, and/or long holding times. However, it is not recommended the use of such high temperatures when aiming for fresh-like fruit products, since the product would be cooked, affecting sensorial and nutritional properties typical of fresh products, and the use of lower temperatures did not deliver considerable inactivation, implying the use of long holding times, which is not industrially desirable.

1.4.4.3. Pectin methylesterase

PME (EC 3.1.1.11) catalyses the de-esterification of pectin to acidic pectin with a lower degree of esterification and methanol, being involved in fruit ripening and in cell wall extension. The activity of PME destabilizes pectinaceous materials in fruit juices and concentrates and modifies the texture of fruit and vegetable products, since the de-esterified pectin precipitates or forms gels such as calcium pectinate or pectate, which results in serious quality defects like cloud loss in fruit juices and gelation of juice concentrates [2]. In the food industry, PMEs can be used for extraction and increase of fruit juice yield, fruit juice clarification, enzymatic peeling of fruits, rheological property characterization of purées and

pastes (mainly for tomato products), among others [67]. There are numerous isoforms of PME in oranges, for example, with both the heat-sensitive and heat-stable forms being commercially relevant [68].

It has been shown that high pressure is inefficient in inactivating PME and, in some cases, it even enhances its activity, since the de-esterification of pectin represents a volume reduction, and any reaction accompanied by a volume reduction is favoured under pressure [2].

For example, Bayindirli *et al*. (2006) [45] observed that after submitting orange juice to various combinations of pressure/time/temperature, after 450 MPa at 50 ºC for 30 minutes, the residual PME activity was *ca.* 7%. The authors claimed that pressure-resistant isoenzymes might have been responsible for the final residual activity. They also suggested a synergistic interaction between temperature above 40 ºC and pressure above 400 MPa. Accordingly, Liu *et al*. (2013) [51] also verified PME post-processing residual activity in watermelon juice, being the lowest 23.2% after HPP at 600 MPa for 60 min, which represents extremely harsh conditions. This lack of efficiency was explained by the author through two correlated possibilities: there is only one isoform of PME in watermelon juice and that the proportion of this liable isoenzyme might be too significant to inactivate completely.

Contrarily, Baron *et al*. (2006) [69] stated that, regarding cloudy apple juice, increasing the pressure or holding time at moderate temperatures (15 – 40 ºC) resulted in increased PME activity, which also showed to be stable when submitted to pressures ranging from 100 to 600 MPa at 25 ºC. Only HPP at temperatures above 40 ºC and up to 65 ºC resulted in some inactivation. Actually, PME activation has a commercial interest in the development of new products. For instance, there is a production process for fruit gel snacks, which is based on the activation of PME under pressure, that is already patented by Mars® [2].

Hurtado *et al*. (2017) [70] observed no effect of HPP on PME activity, both immediately after processing and during refrigerated storage, when using pressures between 350 and 400 MPa for 5 min at 10 ºC. Using 600 MPa, even for less time (3 min), it was verified a PME inactivation of two thirds.

Looking at the results presented throughout this section, it is possible to confirm that HPP is not highly efficient in inactivating PME, and that only more severe pressures allied to high temperatures can cause a significant reduction in this enzyme's activity.

1.4.5. Browning degree

Sensory perception and consumer acceptance of foods is intensely determined by their colour, which is an important quality parameter of fruits and its derived products.

The colour is derived from the natural pigments, which may suffer changes along the maturation and ripening of the fruit. The primary pigments imparting colour quality are chlorophylls (green), carotenoids (yellow, orange, and red), anthocyanins (red, blue), flavonoids (yellow), and betalains (red). These pigments may be affected by pH changes, temperature and browning (enzymatic and non-enzymatic) [71].

Regarding the effect of HPP upon the browning degree of fruit products, in general, HPP does not inhibit browning, as mentioned before, but shows less severe browning degree when compared with thermal processing. For instance, Gomes *et al*. (1996) [72] observed that apples browned markedly after HPP at pressures in the range of 200-600 MPa for 10 min. Zhang *et al*. (2011) [73] compared the effect of to HPP (300, 600, and 900 MPa for 5, 20, 40, and 60 min at 60 °C) and thermal processing (5, 20, 40, and 60 min at 60ºC) on watermelon juice and reported that the browning degree of the samples subjected to HPP was lower when compared to thermally processed samples. Accordingly, Kaushik *et* al. (2018) [74] verified that the browning degree of mango pulp increased significantly ($p < 0.05$) during both HPP (600 MPa/ 52 ºC/ 10 min) and thermal processing (95 ºC/ 15 min) when compared with untreated samples, being browning more intense in thermally processed samples.

1.4.6. Volatile organic compounds

Given the important role of volatile organic compounds (VOCs) in organoleptic quality of foods and beverages, these compounds have been widely studied in fruit matrices [75].

The volatile profiles of fruit are complex and depend on the cultivar, ripeness, pre and post-harvest conditions, the fruit sample itself (either intact fruit, slices, or homogenized samples), and analytical methods utilized [76]. Volatile compounds produced in fresh fruits, comprise various classes of chemicals, including esters, alcohols, aldehydes, ketones, lactones, and terpenoids. Even though the number of chemical compounds identified as volatile compounds in fresh fruit is vast, only a fraction of these compounds is considered to have an impact of fruit flavour, based on their quantitative abundance and thresholds. Esters, for instance, are important volatile compounds in many fruits, imposing a distinct “fruity” odour [77]. These compounds are the most abundant volatile compounds emitted by apples, although some possess strong “pear-like” aromas (*eg.* hexyl acetate, butyl acetate,

pentyl acetate, butyl butanoate, 2-methylpropyl acetate) and C_9 acetate esters are among the major determinants of melon quality [76].

VOCs can be formed by different metabolic pathways but are mainly formed from fatty acids (FA) or amino acids [76, 77].

FAs are precursors for a large number of VOCs. Many of them are important compounds that are responsible for fresh fruit flavours, that usually have straight-chain carbons ranged from C1 to C20. Degradation of FAs occurs mainly by three different oxidative routes: α- and β-oxidation, oxidation by the LOX pathway, and autoxidation. The widest variety of flavour compounds formed from lipids arises via LOX activity. Many of the aliphatic esters, alcohols, acids, and carbonyls found in fruits are derived from the oxidative degradation of linoleic and linolenic acids (Figure 2), which can also be autoxidized. Hexanal and 2,4-decadienal are the primary oxidation products of linoleic acid, while autoxidation of linolenic acid produces 2,4-heptadienal as the major product. Further autoxidation of these aldehydes leads to the formation of other volatile products [76, 77].

Some VOCs can be produced by the action of enzymatic systems on amino acids such as such as alanine, valine, leucine, isoleucine, phenylalanine and aspartic acid. (Figure 3). Amino acids are precursors for some branched aliphatic compounds such as 2-methyl-1-butanol and 3-methyl-1-butanol that are formed during the amino acid catabolism. These compounds can be further synthesized to form esters. As they share the same precursor pyruvate, which is generated from glycolysis, the interaction between FAs and branched amino acids is another important factor in the volatile biosynthesis of fruits [76, 77].

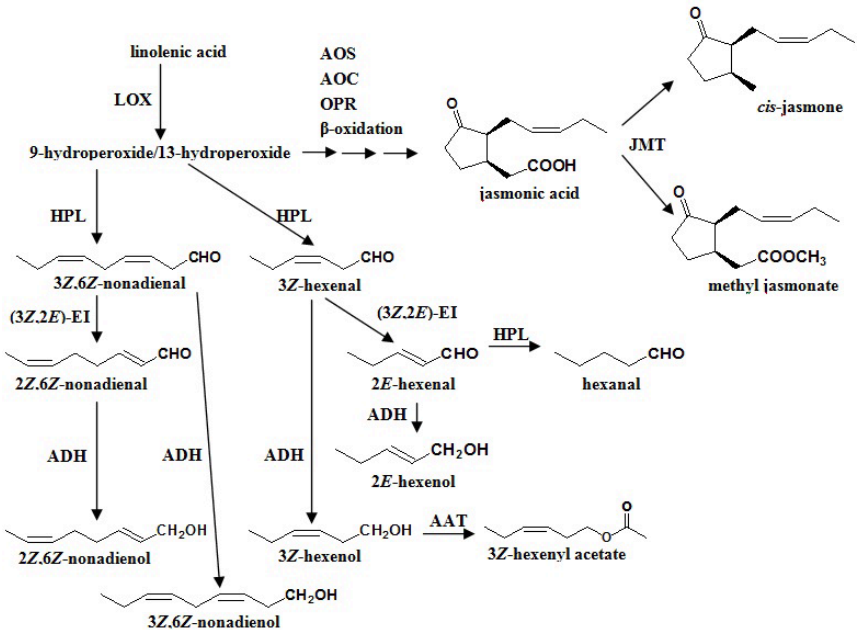


Figure 2 – Schematic representation of linolenic acid-derived flavour molecules. Taken from [76]. AAT, alcohol acyl CoA transferase; ADH, alcohol dehydrogenase; AER, alkenal oxidoreductase; AOC, allene oxide cyclase;

AOS, allene oxide synthase; HPL, hydroperoxide lyase; JMT, jasmonate methyltransferase; OPR, 12-oxo-phytodienoic acid reductase; 3Z,2E-EI, 3Z,2E-enal isomerase.

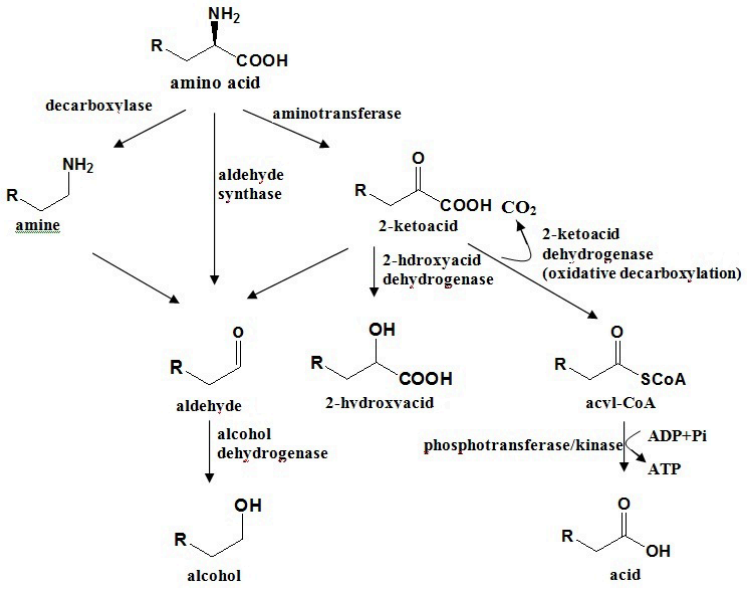


Figure 3 - Schematic representation of amino acid-derived flavour molecules. Taken from [76].

Different strategies for evaluation of volatile content on fruit matrices have been applied, with isolation prior to their identification. Solid phase micro extraction is a solventless extraction procedure, which does not induce modifications in the volatile compounds due to temperature or solvent effects. This type of extraction involves the adsorption/absorption of analytes onto a fused silica fiber coated with a suitable stationary phase and their subsequent desorption immediately before chromatographic analysis [78]. The use of solid phase micro extraction in the headspace mode (HS-SPME) has been successfully applied for the determination of the volatile profiles of several fruits, including those targeted in this work [75, 79–85]. This methodology presents advantages such as a good compound pre-concentration in vapour and liquid phases, a solventless sample preparation method, short extraction times, and the possibility of using different sorbent materials depending on the nature of target analytes. However, it also presents some disadvantages in the quantification process: the amount of analytes transferred from a sample to the SPME fiber is highly dependent on the sample matrix, adsorption capability, and several other extraction-related parameters [75]. These variables make quantification of volatile bay SPME a difficult task, even when using external standards. Therefore, most studies report qualitative profiles while others, making use of internal standards, use a semi-quantitative approach.

Along with this sample preparation technique, gas chromatography coupled to mass spectrometry (GC-MS) has been used successfully for separation/identification of the compounds [78].

Diverse VOCs commonly identified in fruit samples, namely in the fruits used to prepare the fruit salads studied in this work (apple, pear and melon), were selected from the literature and are presented in Table 6.

Table 6 – Aromatic compounds identified in the samples' headspace present in the literature.

Compound family	Compound name	Matrix	Reference
Acetate Esters	2-Methyl-1-butanol acetate	Melon	[83]
		Pear	[86]
	Ethyl acetate	Apple	[79, 85]
		Melon	[81, 87]
		Pear	[88, 89]
	Propyl acetate	Melon	[87]
		Pear	[89]
	Butyl acetate	Apple	[82]
		Melon	[83]
		Pear	[88, 89]
	Pentyl acetate	Apple	[82]
		Melon	[81]
		Pear	[88]
	Hexyl acetate	Apple	[79, 82]
		Melon	[83, 87]
		Pear	[80, 89]
	2-Hexen-1-ol acetate	Apple	[85]
	Heptyl acetate	Melon	[75]
		Pear	[88, 89]
	(Z)- 6-Nonenyl acetate	Melon	[90]
	Nonyl acetate	Melon	[90]
Non-acetate esters	Hexyl 2-methylbutyrate	Apple	[79, 91]
Aldehydes	Hexanal	Apple	[91]
		Melon	[75, 81, 84, 87]
		Pear	[89]
	(E)-2-Hexenal	Apple	[91]
		Melon	[81]
		Pear	[89]
	Heptanal	Melon	[75, 81, 84]
	(E)-2-Heptenal	Melon	[75]
	Octanal	Melon	[75, 84]
	(E)-2-Octenal	Melon	[75]
	(Z)-6-Nonenal	Melon	[75]
	Nonanal	Melon	[75, 81, 84, 87]
	(E)-2-Nonenal	Melon	[75]
	(Z)-6-Nonenal	Melon	[75]
	(E,Z)-2,6-Nonadienal	Melon	[75]
	(E,E)-2,4-Nonadienal	Melon	[75, 81]
	Decanal	Melon	[75, 81, 84, 87]
Alcohols	1-Hexanol	Apple	[82, 85]
		Melon	[75, 84]
		Pear	[80, 89]
	2-ethyl-1-hexanol	Melon	[87]

Compound family	Compound name	Matrix	Reference
	1-Octanol	Melon	[75]
		Pear	[91]
	1-Octen-3-ol	Melon	[92]
	1-Nonanol	Melon	[75]
		Pear	[89]
	(E)-2-Nonen-1-ol	Melon	[92]
		Pear	[88]
	(Z)-3-Nonen-1-ol	Melon	[75, 81]
		Pear	[88]
	2,6-Nonadien-1-ol	Melon	[75]
Terpenes	α-pinene	Apple	[93]
	Limonene	Apple	[85]
	α-farnesene	Apple	[79]
		Pear	[80, 88]

2. OBJECTIVES

The main goal was to evaluate the effects of HPP on a fruit salad, not only on a microbiological level, but also concerning the nutritional and physicochemical characteristics of the final product. The product's behaviour during post-processing refrigerated storage was also addressed.

Fruit salad was chosen since the available literature regarding the effects of HPP on a product with these characteristics is scarce. Moreover, this is a very popular product in Portugal, with commercial relevance. However, it shows a very limited shelf life due to the perishability of fresh cut fruit. Given the results of HPP in other fruit products, this work's goal is to show that HPP is a feasible alternative, that allows lessening the food waste due to the quick spoilage of fruit, without the need to use additives.

In this study, the fruit salad was firstly subjected to 550 MPa, for 3 minutes, at 15 ºC. Enzymatic activity, browning index, antioxidant activity, physicochemical and microbiological parameters, were analysed immediately after processing (day 0) and after 3, 7, 14, 21, 28 and 35 days of storage at 4 °C. The impact of HPP on the volatile profile of the product was also addressed.

The first assay was carried out with a fruit salad composed by orange juice, golden apple and Rocha pear pieces. However, after 3 weeks of storage, no microbial growth was detected both in control and processed samples. Also, when attempting to perform enzymatic analysis (PME, POD and PPO), it was verified that the samples' pH was extremely acidic, *ca.* 2.7 to 3.0, and was inhibiting the microbial growth and hindering the detection of enzymatic activity using the available methods, described in the next section. Therefore, a new assay was initiated, in which was used a non-acidic fruit juice, namely melon juice, along with golden apple and Rocha pear pieces. Given the browning of the samples, which is presented later in this thesis, a third assay was performed, with the addition of ascorbic acid as an antioxidant.

3. MATERIALS AND METHODS

3.1. Reagents and solutions

Folin-Ciocalteu reagent, gallic acid, sodium carbonate, 2,2'-azinobis(3-ethylbenzthiazolin-6-sulfonate) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), sodium 2,6-dichloroindophenolate hydrate (DCIP), polyvinylpolypyrrolidone, oxalic acid, 4-methylcatechol, Triton X-100 and 2-phenylethanol were obtained from Sigma-Aldrich (Seelze, Germany). Potassium persulphate and absolute ethanol were purchased from Carlo ERBA Reagents (Val de Reuil, France). Bromothymol blue was purchased from GURR. Sodium hydroxide and hydrogen peroxide were purchased from VWR (Leuven, Belgium). Acetic acid was acquired from ChemLab (Zedelgem, Belgium). Sodium acetate and L-ascorbic acid were purchased from Panreac (Barcelona, Spain). Pectin was purchased from Riedel-de Haën (Hanover, Germany). Sodium phosphate was purchased from Acros Organics (Geel, Belgium). Sodium dihydrogen phosphate anhydrous was purchased from Scharlau (Barcelona, Spain). Plate count agar (PCA) and rose-bengal chloramphenicol agar (RBCA) were acquired from Liofilchem (Teramo, Italy), while Ringer tablets were purchased from Merck (Darmstadt, Germany). Food grade ascorbic acid (AA) was kindly supplied by Nutre®.

3.2. Fruit salad preparation and processing

Golden delicious apples (*Malus spp*.), Rocha pear (*Pyrus communis* L.), oranges (*Citrus sinensis* L.) and melons (*Cucumis melo* L.), grown in Portuguese territory, were purchased at commercial maturity from a local supermarket and kept at 4 °C until use.

Regarding the fruit salads from the first assay, these were prepared as followed: the fruits were washed in running water and manually peeled and ginned. Apples and pears were cut in cilindrical pieces with 1 cm of diameter and 0.5 cm of thickness and the orange juice was prepared using a squeezer (Braun CJ 3000). Then, *ca.* 20 mL of orange juice were mixed with 2 pieces of apple and 2 pieces of pear in 30 mL flasks (Thermo Scientific™ Nalgene™ Wide-Mouth Lab Quality HDPE Bottles). The control group was immediately stored at 4ºC, and the HPP samples were immediately processed at 450 and 550 MPa, for 3 minutes, at 15 ºC (Hiperbaric 55; Hiperbaric, Spain) and stored at 4 ºC. This HPP equipment has a pressure vessel of 200 mm inner diameter and 2000 mm length and a maximum operation pressure of 600 MPa. It is connected to a refrigeration unit (RMA KH 40 LT, Ferroli, San Bonifacio, Italy) that allows to control the temperature of the input water used as a pressurizing fluid.

The fruit salads regarding the second assay were similarly prepared. The fruits were washed in running water and manually peeled and ginned. Apples and pears were cut in cylindrical pieces with 1 cm of diameter and 0.5 cm of thickness and the pieces of melon were crushed with a blender (Braun MR 6500/500, Kronberg, Germany), producing the juice. The melon juice was inoculated with previously prepared and spoiled melon juice, in order to achieve the initial load of 5.50 log TAM and 2.17 log YM. Then, the samples were prepared by mixing *ca.* 40 mL of melon juice with 4 pieces of apple and 4 pieces of pear in flasks of 60 mL (destined for preparation for physicochemical and enzymatic analysis), or by mixing *ca.* 20 mL of melon juice with 2 pieces of apple and 2 pieces of pear in 30 mL flasks (destined for microbiological testing). The control group was immediately stored at 4ºC, and the HPP samples were immediately processed at 550 MPa, for 3 minutes, at 15 ºC and stored at 4 ºC.

The samples for the assay with the addition of AA were prepared the same way as the ones from the second assay, but food grade AA was added in the concentration of 100 mg/kg [94] to the melon juice. Then, *ca.* 20 mL of melon juice with AA were mixed with 2 pieces of apple and 2 pieces of pear in 30 mL flasks. The control group was immediately stored at 4ºC, and the HPP samples were immediately processed at 550 MPa, for 3 minutes, at 15 ºC and stored at 4 ºC.

3.3. Samples preparation and homogenization

To clarify the samples for physicochemical and enzymatic analysis, the samples in 60mL bottles were manually ground and then homogenized (Miccra D-9 Homogenizer, Miccra GmbH, Heitersheim, Germany). Afterwards, the samples were centrifuged at 11600 rpm, at 4 ºC for 20 min (Heraeus Biofuge Stratos Centrifuge, Thermo Electron Corporation, D-37520 Osterode, Germany). The supernatant was filtered (MN 640 w) and stored at -80 ºC until further use.

3.4. Total soluble solids

Total soluble solids (TSS) content was determined by measuring the brix degree at 20 ºC based on the official AOAC Official Method 932.12 (AOAC International, 1932) and the results were expressed as ºBrix.

3.5. Titratable acidity and pH

The pH value of the samples was measured at 25 °C with a properly calibrated glass electrode (pH electrode 50 14, Crison Instruments, S.A., Spain). Titratable acidity (TA) was

determined by titrating 25 mL of diluted sample (1:10) to pH=8.9 with a standardized 0.01 M sodium hydroxide solution, using an automatic titrator (Titromatic 1S, Crison Instruments, S. A., Barcelona, Spain), based on AOAC Official Method 942.15. The results were expressed as g citric acid/g of fruit salad. The followings equations were used to calculate the results:

$$TA\ (M\ citric\ acid) = \left(\frac{[NaOH] \times V_{NaOH}(ml)}{V_{sample}(ml)}\right) \div \frac{dilution\ factor}{3} \quad \textbf{(1)}$$

$$TA\ (g\ citric\ acid/L) = TA\ (M\ citric\ acid)\ \times\ MW_{citric\ acid} \quad \textbf{(2)}$$

To convert to g citric acid/g fruit salad, it was used the relationship between weight and extract volume of each sample.

3.6. Microbiological stability

To carry out the analysis, each sample was aseptically homogenized with Ringer's solution in a proportion of 1:10, in a Stomacher homogenizer (Stomacher 80 Biomaster; Seward Laboratory Systems Inc., FL, USA) for 3 min at high speed. Then, further decimal dilutions were made and droplets (20 μL) of the dilutions were plated on the surface of proper media in triplicate, based on the colony count method described by Miles and Misra (1938) [95]. TAM were enumerated in PCA, after incubation at 30 °± 1°C for 72 ± 3 h (ISO 4833-2:2013) and YM were counted on RBCA after incubation at 25 °± 1°C for 5 days (ISO 21527-1:2008). Results were expressed as logarithmic of colony-forming units (CFU) per mL of blended fruit salad (log CFU/mL), and the detection and quantification limits considered were 2.70 log CFU/mL and 3.40 log CFU/mL.

3.7. Browning degree

The browning degree value was determined by measurement of the absorbance of the samples at 420 nm in a UV–VIS spectrophotometer (Microplate Spectrophotometer Multiskan Go, ThermoScientific, USA) [96]. Higher values of absorbance at 420nm correspond to higher browning.

3.8. Total phenolics content

The TP content was measured using the Folin-Ciocalteu colorimetric method [97]. Following the mentioned method, 125 μL of sample were added to 125 μL of the Folin-Ciocalteu

solution and 500 µL of distilled water. Then the solution was placed in the dark for 6 min. Afterwards, 1.25 mL of a 7% sodium carbonate solution was added, and the solution was homogenized again. After 60 min at room temperature, the solution absorbance was read at 720 nm using a UV–VIS spectrophotometer (Microplate Spectrophotometer Multiskan Go, ThermoScientific, USA). TP was calculated using a predetermined calibration curve (appendix B), with gallic acid as the standard, and expressed as mg gallic acid equivalents (GAE)/g of fruit salad.

3.9. Vitamin C content

The determination of vitamin C content was based on the method described by Guldas *et al*. (2003) [98], based on the reduction of DCIP by L-ascorbic acid. DCIP turns pink in when in an acidic environment and is colourless when completely reduced. This method suffered some modifications. Firstly, 234 µl of DCIP 36 mg/L and 26 µL of sample were mixed. Absorbance was read at 540 nm using a UV–VIS spectrophotometer (Microplate Spectrophotometer Multiskan Go, ThermoScientific, USA). Samples blanks were made by substituting DCIP for distilled water. Vitamin C content was calculated using a predetermined calibration curve (appendix C), in which AA was diluted in oxalic acid 0.4% in concentrations ranging from 0 to 100 mg/L and expressed as mg/g of fruit salad.
In order to obtain the values correspondent to the DCIP that reacted with vitamin C, the absorbance of the 0 mg vitamin C/L solution was subtracted from the absorbance measured.

3.10. Total antioxidant capacity

Total antioxidant capacity (TAC) of extracts was measured according to the method described by Re *et al.* (1999) [99]. This method allows to quantify both water and lipid-soluble antioxidants, via direct production of the $ABTS^{\bullet+}$ chromophore (blue/green) by reaction of ABTS and potassium persulfate. The $ABTS^{\bullet+}$ solution was prepared by addition, in a proportion of 1:1 (v/v), of 7 mM ABTS diammonium salt to 2.45 mM potassium persulfate solutions, and left to react in the dark for 16 h. In order to obtain an absorbance of 0.700 ± 0.020, at 734 nm, the $ABTS^{\bullet+}$ solution was duly diluted in distilled water. To 2 mL of diluted $ABTS^{\bullet+}$ solution, it was added 120 µL of the clarified sample and, after reacting for 6 min in the dark, absorbance at 734nm was measured, using a UV–VIS spectrophotometer (Microplate Spectrophotometer Multiskan Go, ThermoScientific, USA). It was used a predetermined calibration curve with Trolox as standard (0–100 mg/mL)

(appendix A), and the results were expressed as Trolox equivalent antioxidant activity (TEAC) in mg/g of fruit salad.

3.11. Enzymatic activity

PPO activity was assayed based on the method described by Juarez-Henriquez *et al.* (2015) [100], but with slight modifications. First, 43 μL of the sample were mixed with 130.0 μL of 50 mM sodium phosphate buffer (pH 6.5) and incubated at 25ºC. This mixture was considered the blank. Then, 87 μL of 4-methylcathecol 50 mM (substrate) were added and the absorbance was measured at 420 nm, at 25 ºC, every 10 seconds for 3 minutes, using a UV–VIS spectrophotometer (Microplate Spectrophotometer Multiskan Go, ThermoScientific, USA).

The quantification of POD activity was performed based on the method described by Siguemoto *et al.* (2017) [101]. 162 µL of 67 mM phosphate buffer (pH 6.0) and 54 µL of the sample were added to a microplate well. The mixture was then incubated at 25 °C for 1 min; then, 22 µL of 1.7 mM ABTS solution and 22 µL of 0.8 mM hydrogen peroxide solution were added. Absorbance at 405 nm was monitored every 20 s for 5 min (Microplate Spectrophotometer Multiskan Go, ThermoScientific, USA).

PME activity was measured based on the method described by Hagerman and Austin, (1986) [102]. Before proceeding to the analysis, all solutions were adjusted to a pH of 7.5 using 2.0 M sodium hydroxide. To 566 µL of sample were added 1.33 mL of citrus pectin solution (0.5%, w/v), 100 µL of bromothymol blue (0.01%, w/v). The absorbance was measured at 620 nm (Lambda 35 UV/Vis spectrometer, PerkinElmer Instruments Inc., MA, USA) during 1 min.

All the enzymatic activities were expressed as ΔAbs/min.

3.12. Volatile organic compounds analysis

Volatile analysis was performed by gas chromatography – mass spectroscopy GC-MS as described by Amaro *et al.* (2013) [103], with slight modifications, using a 7890A gas chromatograph coupled to an 5977 B mass selective detector, both from Agilent Technologies (USA). Control samples stored for 0, 3 and 7 days and HPP samples stored for 0, 3, 7, 14 and 21 days were analysed.

Fruit salads were homogenized using glass spheres and a vortex. A 2.5g amount of pulp was weighted in 20 ml headspace precision thread Vials (LA-PHA-PACK, GMBH, Germany) and mixed with 25 µL of 2-phenylethanol (internal standard) prepared at 0.5 mg/mL in water, followed by 500 µL of NaCl 20% (w/v) to facilitate the volatile release to the headspace. The

vials were sealed using magnetic screw caps with silicone transparent blue/PTFE white septa (LA-PHA-PACK) and placed in a heating plate at 40 °C for 40 min to equilibrate the headspace. HS-SPME procedure was carried out using a 50/30 µm (1 cm) preconditioned DVB/CAR/PDMS Stableflex 24 Ga fiber (Supelco, Bellefonte, PA, USA), which was in the injection port at 270 ◦C for 1 h, according to manufacture instructions. The SPME fiber was exposed to the headspace for 30 min absorbing volatiles at 40 ºC. After extraction, the volatiles were desorbed from the SPME fiber into the gas chromatograph injection port set at 250 ºC for 10 min, equipped with a SPME/direct (Supelco) liner, in the splitless mode with a constant pressure of 14.9 psi. Volatiles were separated on a 30 m × 0.25 mm i.d. × 0.25 µm thickness ultra-inert capillary column (HP-5MS, Agilent Technologies). The carrier gas was helium with a nominal initial flow rate of 1.9 mL min−1. The initial oven temperature was 35 ºC, followed by a ramp of 3 ºC min−1 up to 75 ºC, and then at 20 ºC min−1 to reach a final temperature of 250 ºC, which was held for 5 min, with a total chromatogaphic time of 30 minutes. Mass spectra were obtained by electron ionization (EI) at 70 eV, in a full scan mode, with a spectrum range of ion mass captured between 40 and 450 m/z and an average of 3.5 scans s−1 (sample rate of 2). The mass spectra were evaluated using Enhanced ChemStation software (Version F.01.03.2357, Agilent Technologies). The peaks were identified using a mass spectrometer (5977 B mass selective detector, Agilent Technologies) coupled to the gas chromatograph by comparison of experimental spectra with those of the National Institute for Standards and Technology (NIST MS version 2.2) data bank. Only compounds with match above 860 were considered. Out of these, the most important compounds were selected based on their presence and relevance in the literature, presented previously in the section Literature Review of this thesis. Of the selected VOC, only 5 showed a match below 900. Results were expressed in relative percentage of the total area counts in the full scan mode, excluding the area occupied by the internal standard and are presented in section 4.7. Results in mg/ kg of internal standard equivalents are presented in appendix G.

3.13. Statistical analysis

All analyses were performed in triplicate and expressed as a mean ± standard deviation. The results were statistically analysed using one-way Analysis of variance (ANOVA), followed by Turkey's honest significant differences test at 5% of significance.

4. RESULTS AND DISCUSSION

The results presented and discussed in this section correspond to the analysis performed on the samples with melon juice.

4.1. Total soluble solids, pH and titratable acidity

The initial TSS was 10.80 ± 0.20 and 11.07 ± 0.12 ºBrix, for raw and HPP samples respectively. The results show that TSS content did not suffer significant changes ($p > 0.05$), as expected, neither derived from the storage time, nor resultant of the subjection to high pressure, as showed in Appendix D. This was also verified by Queirós *et al.* (2015) [35] in sweet cherry juice and Chen *et al*. (2013) [33] in pomegranate juice, and in both works was concluded that HPP had no significant effect in TSS ($p > 0.05$). Wolbang *et al.* (2008) [50] studied the effect of HPP on nutritional value and quality attributes of *Cucumis melo* L. and came to the same conclusion that TSS was not significantly affected by HPP ($p > 0.05$).
The results regarding pH variation are summarily presented in the following figure.

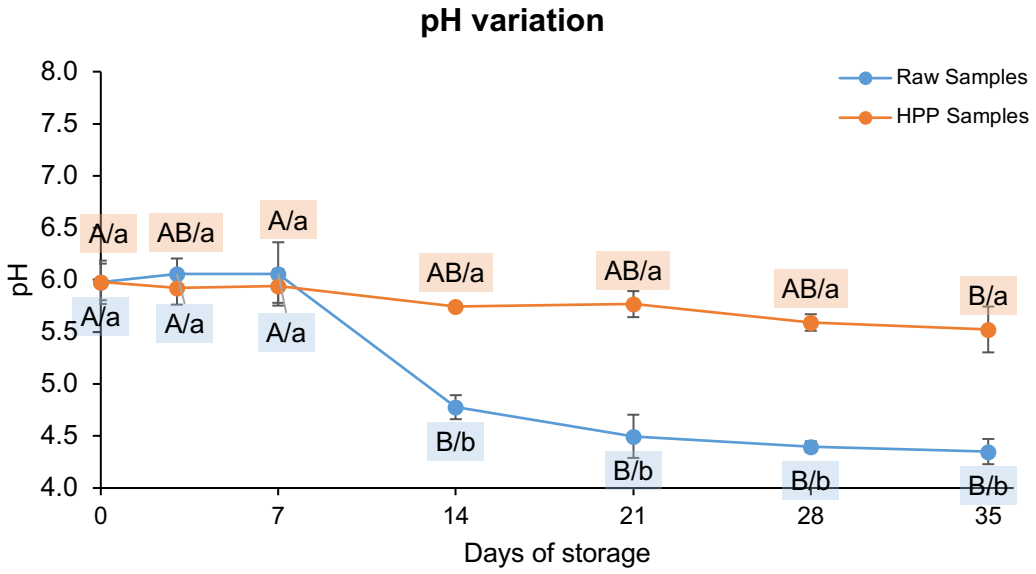


Figure 4 – pH variation through time in cold storage and respective one-way ANOVA results. Different letters represent significant differences ($p < 0.05$) at the same conditions (capital letters; effect of storage) or between samples at the same time of storage (noncapital letters; effect of HPP).

The pH of both raw and HPP samples was approximately 6, confirming the non-acidic profile of this product. These samples suffered an accentuated decrease ($p < 0.05$) in pH between the 7th and 14th day stored at 4 ºC, which is most likely associated with the increasing microbial activity during this period. Regarding HPP samples, these decreased slowly over time. These results are concordant with TA results, which are summarily presented in Figure

5. In fact, there is a large statistical correlation (|R|=0.938, p = 0.002) between pH and TA values, both in raw and HPP samples.

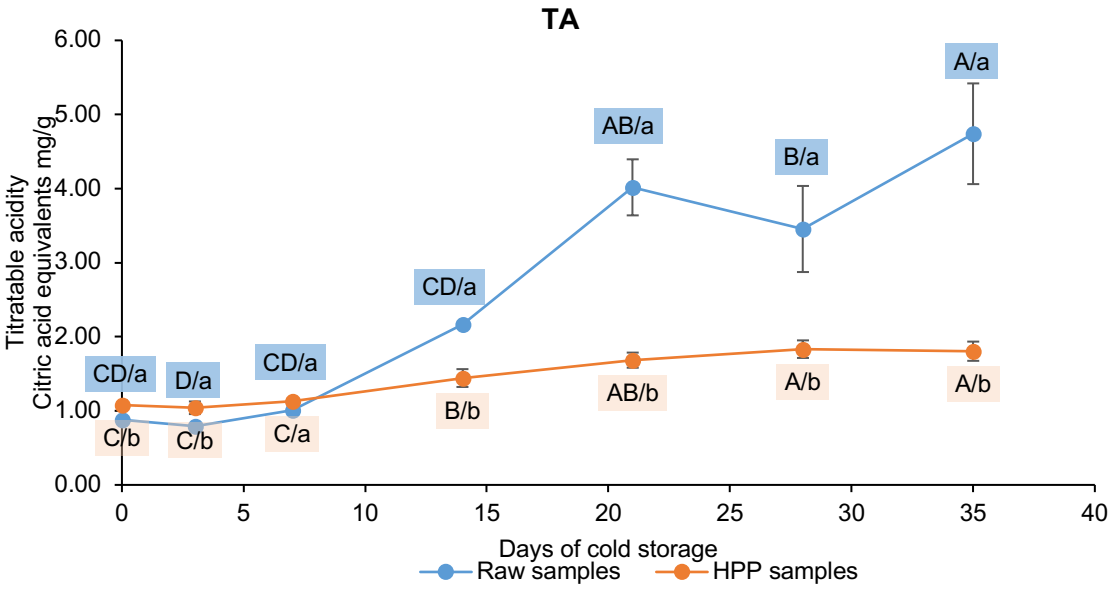


Figure 5 – Titratable acidity results and respective one-way ANOVA results. Different letters represent significant differences (p < 0.05) at the same conditions (capital letters; effect of storage) or between samples at the same time of storage (noncapital letters; effect of HPP).

When comparing TA in raw and HPP samples, initially even though there is a statistical difference (p < 0.05), the difference in TA is subtle and may result from a probable gradual organic acids leakage from the vegetable cell organelles to the juice matrix after HPP [105]. However, from the 14th day onwards it is possible to notice an accentuated difference (p < 0.05) between the two sets of samples, which reflects the difference in microbial load. Raw samples reach higher levels of acidity fairly quicker than HPP samples due to their higher microbial load. Regarding HPP samples, TA starts to rise slowly after the 14th day in refrigerated storage, which is concordant to when TAM colonies started being detected in microbiology counts. In fact, there is a large statistical correlation (|R|=0.991, p=0.000) between the TA increase and TAM colonies growth. Microbiology stability results are presented in the next section of this document.

The changes in TA are, therefore, attributed to microbial growth and not to HPP itself. In accordance, in the work of Wolbang *et al.* (2008) [50], HPP showed no impact on TA on fresh cut melon.

4.2. Microbiological stability

TAM and YM growth over time were assessed as spoilage parameters. The results are presented in Figures 6 and 7.

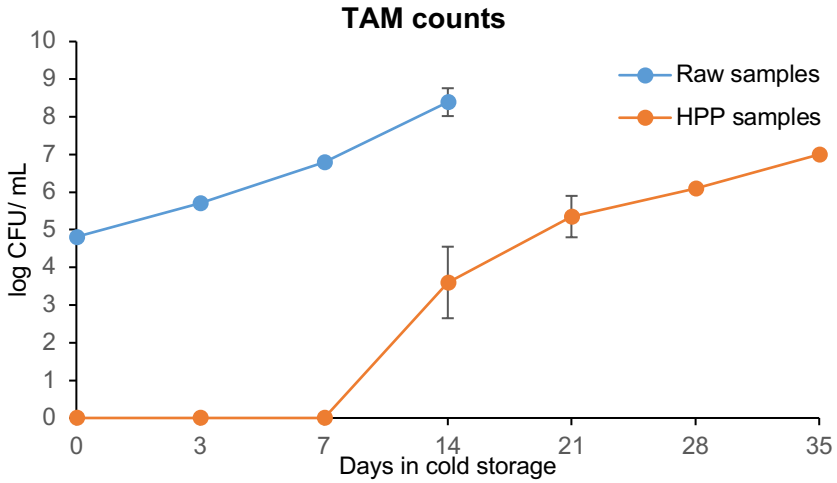


Figure 6 – Graphic comparison of total aerobic mesophiles counts overtime. The absence of error bars in the points of the 28th and 35th day is related with problems obtaining the results in duplicate or triplicate. The value for the storage days where the colonies are undetectable is registered as 0.00. Raw samples stopped being analysed after day 14 given the extremely high microbial load.

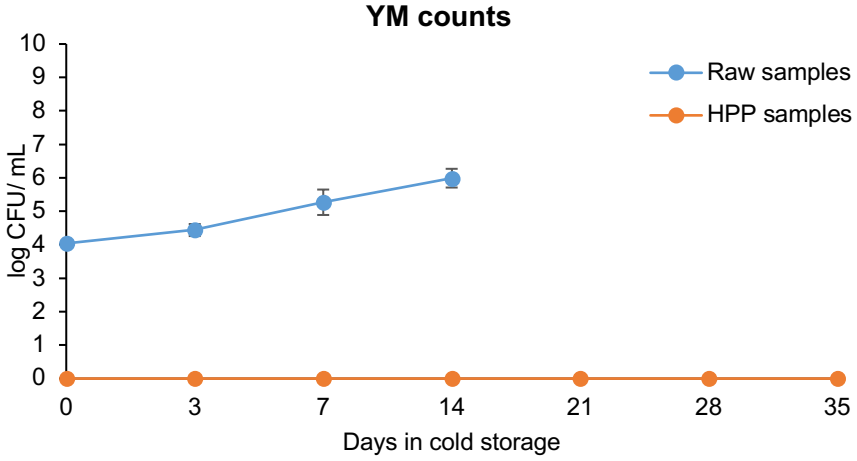


Figure 7 – Graphic comparison of yeasts and moulds counts overtime. Raw samples stopped being analysed after day 14 given the extremely high microbial load. The value for undetectable is registered as 0.00. Raw samples stopped being analysed after day 14 given the extremely high microbial load.

The initial load of raw samples resulted of the inoculation previously mentioned. This inoculation allowed to get a better understanding of the magnitude of the effect of HPP on the product's microbial load.

Hence, it is possible to verify that immediately after HPP, both YM and TAM counts suffered a reduction of *ca.* 4 and 5 log units, respectively, to below the limit of detection. Broadly speaking, inactivation of microorganisms can be achieved by using pressures of 350-600

MPa. HPP shows multi-targeted effects, for example, it induces unfolding of globular proteins, induces disintegration of ribosomes, affects metabolic pathways and leads to an inability to control intracellular pH, and to proliferate among other essential processes. These effects are reversible at low pressures (< 350 MPa), but irreversible at higher pressures, where, ultimately, the permeabilization of the cell membrane causes cell death [106]. As all important cell functions are successively compromised with the increase of pressure, it becomes impossible to withstand and survive at these hostile conditions, leading to loss of cell viability. Hence, a pressure treatment of 550 MPa caused cell death [27]. Similar results were obtained in other fruit products, for instance, Chen *et al*. (2013) [33] compared the effects of 300 MPa and 400 MPa in cloudy pomegranate juice, under holding times between 2.5 and 25 minutes, at room temperature. The results showed that the use of 400 MPa allowed shorter holding times and assured bigger decimal cycles reductions. For example, for a holding time of 5 minutes, TAM showed a reduction of 4.53 log CFU/mL and M&Y decreased from 3.69 log CFU/mL to below the limit of detection. Using 300 MPa and the same holding time, these values were of 3.23 log CFU/mL and 1.89 log CFU/mL, respectively. Varela-Santos *et al*. (2012) [32], in a study also starring pomegranate juice, reported that, in general, pressures from 350 MPa on are more effective in reducing the microbial loads to values below the limit of detection. This can be explained by the fact that the irreversible denaturation of proteins may occur above 300 MPa, which is one of the main reasons behind the inactivation of vegetative cells, as mentioned before [34].

When it comes to YM in HPP samples, the low value remained constant throughout the 35 days of storage at 4ºC. Relatively to raw samples, YM were already present in the inoculate, and showed a less accentuated growth than TAM, probably due to the high bacterial load, which might have inhibited YM. The non-acidic food matrix may also have had influence.

At day 14, the raw samples were already highly contaminated, with 8.39 log CFU/mL regarding TAM and 5.99 log CFU/mL regarding YM, and showed clear signs of spoilage with an uncharacteristic and unpleasant odour. Contextualizing the results, the French legislation [107] imposes as the maximum acceptable value of 5×10^6 CFU TAM/g for raw foods of vegetable nature ready to use, which means that at 14 days of storage at 4ºC, raw samples were clearly inappropriate for consumption. Given the high microbial load at this point, raw samples stopped being analysed.

Concerning TAM counts on HPP samples, these only started being detected after 14 days of cold storage, and remained within the acceptable limits until the 21st day of cold storage. The samples analysed after 28 days of storage showed unpleasant aromas, similar to raw samples at the 14th day of storage, which demonstrates that the microbial growth in HPP

samples was slower than in raw samples. These results are backed by the TA results, which were presented previously, and together give basis to infer that the product is microbiologically stable for 21 days after HPP at most. Therefore, it can be interpreted that HPP can indeed reduce considerably the microbial load in fruit products, which allows shelf life extension. Similar results can be found in the literature for other fruit products, for instance, Hurtado *et al.* (2017) [34, 59] reported that HPP processed red-fruit based smoothies microbiologically stable retained their "fresh-like" properties for at least 14 days at 4ºC. Accordingly, Queirós *et al.* (2015) [35] reported that sweet cherry juice subjected to HPP showed TAM and M&Y values below the limit of detection throughout 28 days of storage at 4ºC. Landl *et al.* (2010) [30] also concluded that HPP processed acidified apple purée reached 3 weeks of refrigerated storage without microbial growth. Chen *et al*. (2013) [33] verified that HPP processed pomegranate juice still met the Chinese hygienic standard for fruit juices (≤100 CFU/mL TAM and ≤20 CFU/mL M&Y) after 90 days of storage at 4ºC. These results, along with Patterson *et al.* (2012) [108] and many other works not hereby mentioned, allow to infer that HPP has been giving proofs of its efficiency, assuring the microbial safety of fruit-based products not only immediately after processing, but for a long period of time.

Due to the inoculation, it is not possible to infer exactly for how many days the raw sample would be microbiologically stable, but due to the product's low acidity and high water activity, it would not have been more than a few days.

4.3. Browning degree

In the first assay performed with melon juice, without the addition of any antioxidant compound, it was verified a noticeable difference in the visual perception between raw and HPP samples. This effect was also reported in other studies, for example in the work of Wolbang *et al.* (2008) [50] regarding melon juice and of Guerrero-Beltrán *et al*. (2005) [109] regarding mango purée. An exemplifying picture is shown in Figure 8 and results are presented in Figure 10.

Figure 8 - Picture of samples without ascorbic acid, taken at the 14th day of storage. The first three, from left to right, are raw samples, while the other three are HPP samples.

Given the significant browning of HPP samples, which is most likely explained by the increased activity of PPO in these samples, which is explained in more detail in section 4.6.1., it was decided that it was pertinent to perform another assay, this time adding an antioxidant, namely AA. Visually, it was still perceptible a difference in the colour, being HPP browner than raw samples, but this difference was less intense than in the previous assay. An exemplifying picture is presented below, and results are presented in Figure 9.

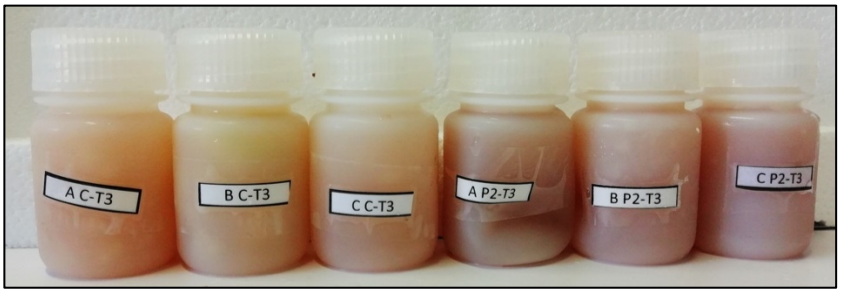
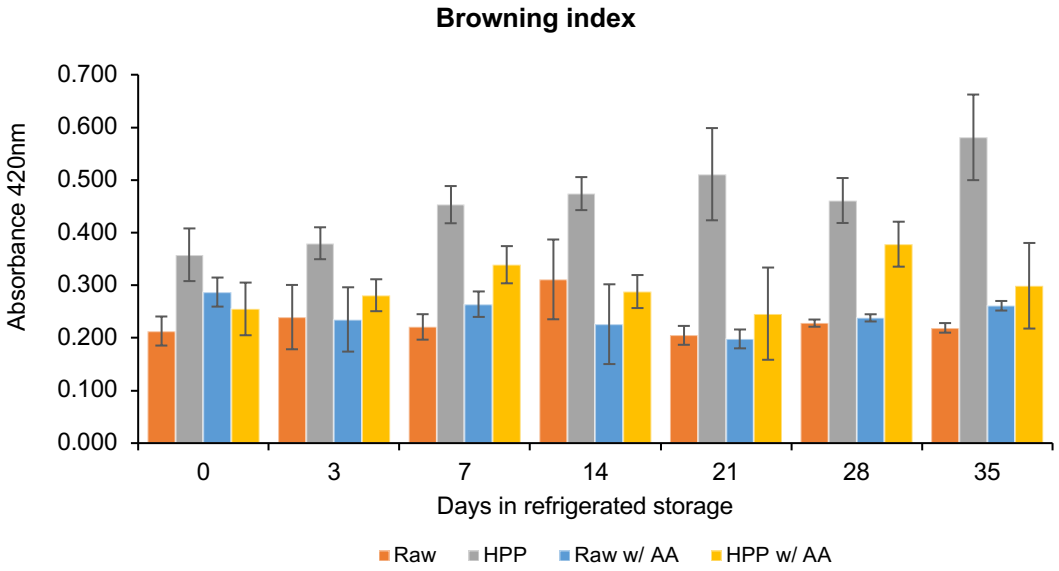


Figure 9 - Picture of samples with ascorbic acid, taken at the 14thday of storage. The first three, from left to right, are raw samples, while the other three are HPP samples.



Storage days		0	3	7	14	21	28	35
Without AA	Raw	A / b	A / b	A / b	A / b	A / b	A / b	A / b
	HPP	B / a	B / a	AB / a	AB / a	AB / a	AB / a	A / a
With AA	Raw	A / a	A / a	A / b	A / a	A / a	A / b	A / a
	HPP	AB / a	AB / a	AB / a	AB / a	B / a	A / a	AB / a

Figure 10 – Browning index results regarding the assays with and without addition of ascorbic acid and respective one-way ANOVA results. Different letters represent significant differences ($p < 0.05$) at the same conditions (capital letters; effect of storage) or between samples at the same time of storage (noncapital letters; effect of HPP). All data is presented in Appendix E.

In the assay without AA, there was always a significant difference ($p < 0.05$) between raw and HPP samples throughout the 35 days of storage. Raw samples did not show significant changes ($p > 0.05$) in their browning index during storage, while HPP samples show a significant difference ($p < 0.05$) when comparing day 0 (0.358 ± 0.050) and the 3rd (0.380 ± 0.030) and with the 35th (0.581 ± 0.081) day of storage.

In what concerns the assay with the addition of AA, the behaviour of raw samples was similar to those without AA, given that no significant changes ($p > 0.05$) were detected in their browning index during storage. Regarding HPP samples with AA, between those from day 0 to the 14th day and the 35th, there are no significant changes. However, on the 28th day there is a significant rise ($p < 0.05$) followed by a decrease at the 35th day. This sudden increase can be a consequence of the heterogeneity of the samples themselves.

Based on the graphic presented above, it is possible to notice differences when comparing the two assays, namely between the bars representing HPP samples. Even though the browning was not completely solved with the quantity of AA used, as showed in Figure 9, it

showed improvements and higher concentrations shall be tested in order to stabilize the products visual perception.

4.4. Total phenolics and vitamin C content

No phenolics nor vitamin C were detected when performing the methods described previously, most likely because their detection limits might be too high for the quantity present in these samples.

According to Oms-Oliu *et al.* (2008) [14], unprocessed 'Piel de sapo' melon (the major component of the product used in this work) contains small amounts of TP (15.4 – 20 mg gallic acid/100 g fw). In the same work, it was reported a moderate vitamin C concentration of unprocessed 'Piel de Sapo' melon (41.7–48.7 mg/100 g fw). During the preparatory steps of the samples, the natural protection of fruit, the peel, is removed, and therefore they become highly susceptible to oxidation. If there was a decrease in these compounds content due to slicing, cutting, blending, processing the sample or enzymatic activity, it is possible that the values were too small to be detected by the chosen methods. Due to the time available to perform all the analysis of this work, the availability of financial resources and of the equipment, it was not possible to test other methods such as HPLC within the timeframe. This quantification using other methodologies must be performed in the future.

4.5. Total antioxidant capacity

The results of TAC are graphically presented below, in Figure 11.

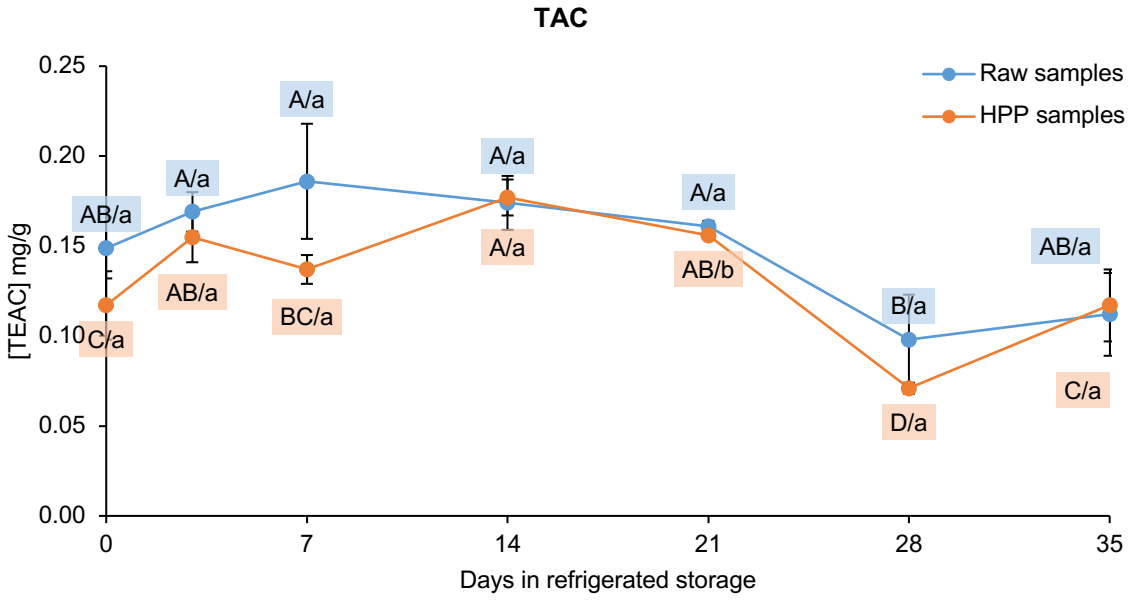


Figure 11 - Antioxidant activity expressed as mg TEAC/g. Different letters represent significant differences ($p < 0.05$) at the same conditions (capital letters; effect of storage) or between samples at the same time of storage (noncapital letters; effect of HPP). Data presented in Appendix F .

Even though it was not possible to quantify TP and vitamin C, it was possible to detect antioxidant activity in the samples. In fact, a high concentration of β-carotene is linked to melon's nutritional quality, and given that melon juice is the major component of the studied fruit salad, these compounds might be responsible for the observed TAC [110]. According to Rúa et. al (2018) [111], "Piel de sapo" melon juice has 0.107 ± 0.012 mg TEAC/ g, which is in the same range of values determined in this work.

Regarding the results themselves, these did not show a clear tendency. Despite the results' variability throughout storage time, there were no significant differences ($p > 0.05$) between raw and HPP samples except for the samples analysed after 21 days of refrigerated storage, in which statistical analysis reported significant differences ($p < 0.05$) even though TEAC concentration of raw and HPP samples appears very similar when analysing the graphic representation of the results.

Storage time did not have significant influence ($p > 0.05$) on TAC in raw samples up to the 28th day. The fact that only the samples from this day reveal significant changes may be a consequence of the samples' heterogeneity.

Concerning results in HPP samples, these also do not show a clear tendency but have a similar behaviour to those of raw samples and, as mentioned above, are not significantly

different than raw samples, in general. This way, it is possible to infer that HPP does not have a compelling effect on the antioxidant activity of the samples as reported in other studies. For instance, Fernández-García *et al.* (2000) [112] applied HPP (600 MPa/ 60 ºC/ 30 min) to apple juice and no significant alterations were found in TAC of apple juice immediately after processing and during refrigerated storage for a month at 4 ºC. Fernández-García *et al.* (2001) [10] also did not find significant changes ($p > 0.05$) in antioxidant capacity (using DPPH method) immediately after subjecting orange–lemon–carrot juice to HPP (500 to 800 MPa/ room temperature/ 5 min) or when it was subsequently stored at 4 ºC for 21 days, similarly to the work presented in this thesis.

4.6. Enzymatic activity

4.6.1. Polyphenol oxidase activity

The results of PPO activity are graphically presented below, in Figure 12.

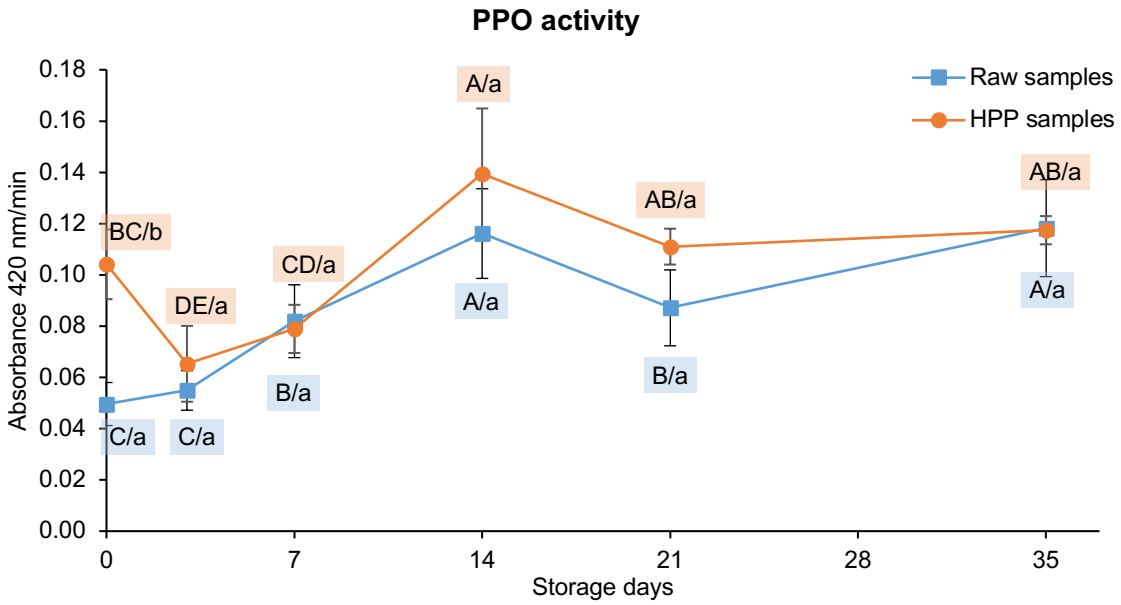


Figure 12 – Polyphenol oxidase activity variation through time in cold storage and respective one-way ANOVA results. Different letters represent significant differences ($p < 0.05$) at the same conditions (capital letters; effect of storage) or between samples at the same time of storage (noncapital letters; effect of HPP).

HPP samples showed an increased activity immediately after processing (0.1041 ± 0.0084 Abs/min) when compared to raw samples (0.0496 ± 0.0136 Abs/min). When under pressure, even though covalent bonds are not affected, the main stabilizers of the three-dimensional conformation of the enzyme, such as disulphide bonds, hydrogen bonds and hydrophobic, electrostatic, and van der Waals interactions, are disturbed. These changes may result in an increase or decrease of biological activity and may alter the substrate

specificity. In fact, it has been observed that the application of pressure may activate some enzymes, especially monomeric enzymes such as PPO [2]. Moreover, HPP destabilizes the compartmentalization in the intact cells of the substrates and enzymes, leading to their interaction [113]. The non-acidic profile of the product may also present itself as an explanation for the resistance of PPO to HPP, given that low pH is known to destabilize PPO [97]. These results are in accordance with other reports where HPP was performed near room temperature [45, 55, 100]. In order to achieve higher PPO inactivation, higher temperatures should be used [55, 58, 114], but that would go against the purpose of maintaining the fresh-like attributes of the product and would also make the process less economically attractive and environmentally friendly.

On the 3rd day, it had decreased abruptly in HPP samples while raw samples showed a slight increase, resulting in similar enzymatic activity ($p > 0.05$) in both samples. From then on, PPO activity showed no significant differences ($p > 0.05$) between raw and HPP samples, except for the samples taken at the 21st and 28th day. This may be caused by the heterogeneity of the samples themselves.

Falguera *et al.* (2013) [115] studied PPO inactivation in apple juices made from six apple varieties. Looking at the results of this study, it is possible to conclude that, in general, apple PPO is extremely pressure-resistant if the process is carried out at approximately room temperature (25 °C), since the maximum inactivation after 16 min at 600 MPa in Golden Delicious PPO was one of the most resistant under these conditions (residual activity 93 %). Hurtado *et al.* (2015) [57] observed no effect of HPP on PPO activity in fruit smoothies, unlike Keenan *et al.* (2012) [41], where higher inactivation has been achieved at near-ambient temperatures. However, the authors did not present any explanation for this disparity in results. Rao *et al.* (2013) [56] reported 79% inactivation of PPO in peach juice at 600 MPa/25 min/25 °C, even though the residual activity increased *ca.* 7.3% after processing at 400 MPa for 5 min. This activation of PPO has been observed in other products, such as cloudy apple juice [45, 55] (results presented in Table 5 in section 1.4.4.1.). The most pertinent explanation for this phenomenon, and that has been verified, is that there are two PPO isoforms: one isoenzyme is sensitive to pressure and the other is stable [55, 56, 62, 66].

Considering the aforementioned results, it is possible to state that PPO did not suffer inactivation and was the major contributor to the extreme browning observed (section 4.3). The use of AA as an antioxidant was explored and must be optimized in order to minimize the effects of PPO activity on the sensorial properties of these fruit salads.

4.6.2. Pectin methylesterase and peroxidase activity

PME and POD activities were analysed following the methods described in section 3.11. However, no activity was detected in both enzymes. Regarding POD, this can be a consequence of the methodology used, that has not the appropriate limit of detection, or a consequence of the contact with high amounts of O_2 during peeling and slicing of the fruit, which caused oxidation of POD itself and reduced its activity to below limit of detection.

Concerning PME, it is believed that the problem resided with the methodology itself, since it requires to be carried out at a very specific pH (7.5), and there were problems bringing the samples' pH to that value given the small volume available. That being stated, the method must be further optimized and tested in future work, and other non-spectrophotometric methodologies with lower limits of detection must be taken into consideration.

4.7. Volatile organic compounds

The main VOCs identified in fruit salads composed by melon juice and pieces of apple and pear, without the addition of AA, are presented in Table 7, in relative percentage of the total area counts in the full scan mode, and in Table 11 in Appendix G in mg/ kg of internal standard. Also, two chromatograms, in which the peaks representing bigger areas with clear differences between the two groups of samples were highlighted, are presented in Figure 13 as an example. These chromatograms refer to samples of day 0. Figure 14 presents graphic representations of some results obtained.

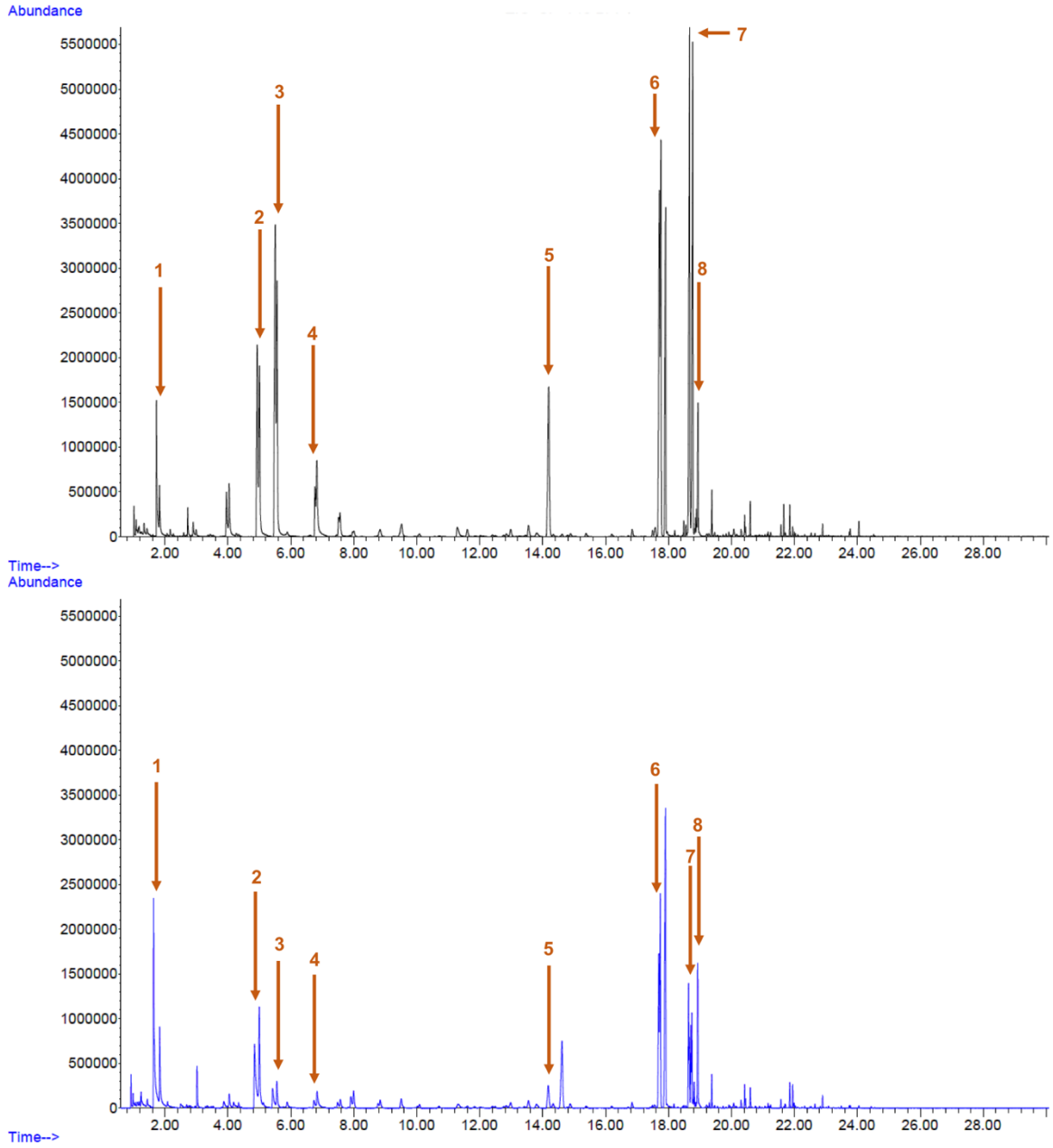


Figure 13 - Total ion chromatograms referring to samples from day 0. The black line refers to a raw sample, while the blue line refers to an HPP sample. 1- Ethyl acetate; 2- Hexanal; 3- Butyl acetate; 4- (E)-2-Hexenal; 5- Hexyl acetate; 6- (Z)-6-Nonenal; 7- (E,Z)-2,6-Nonadienal; 8- (E)-Z-Nonenal.

Table 7- Main volatile organic compounds extracted by HS-SPME measured by GC-MS. Results expressed in relative percentage of the total area counts in the full scan mode.

					Compound relative percentage (%)							
					Raw samples stored at 4ºC			HPP samples stored at 4ºC				
Compound family	**RT[a]**	**Compound name**	**CAS Nº**	**RI[b]**	**Day 0**	**3rd day**	**7th day**	**Day 0**	**3rd day**	**7th day**	**14th day**	**21st day**
Acetate esters	1.72-1.88	**Ethyl acetate**	141-78-6	612	3.47 ± 0.66 A/b	2.74 ± 1.48 A/b	3.29 ± 0.28 A/a	8.04 ± 1.67 A/a	7.35 ± 0.99 A/a	4.80 ± 1.56 A/a	5.17 ± 0.50 A	5.13 ± 3.9 A
	2.88/2.98	**Propyl acetate**	109-60-4	708	0.42 ± 0.06 A	0.54 ± 0.01 A/a	0.52 ± 0.11 A/b	nd	0.56 ± 0.40 A/a	0.79 ± 0.04 A/a	0.30 ± 0.11 A	nd
	5.4-5.6	**Butyl acetate**	123-86-4	812	15.7 ± 0.44 A/b	14.2 ± 2.2 A/b	8.35 ± 1.75 B/b	19.7 ± 0.6 AB/a	21.5 ± 0.1 A/a	21.5 ± 0.1 A/a	13.4 ± 3.3 BC	11.2 ± 2.4 C
	7.9-8.05	**2-Methyl-1-butanol acetate**	624-41-9	880	0.24 ± 0.01 A/b	0.13 ± 0.02 B/a	0.11 ± 0.06 B/a	1.76 ± 0.45 A/a	0.18 ± 0.05 B/a	0.15 ± 0.07 B/a	0.02 ± 0.01 B	nd
	9.4-9.6	**Pentyl acetate**	628-63-7	911	0.68 ± 0.05 B/a	1.20 ± 0.13 AB/a	1.77 ± 0.42 A/a	0.69 ± 0.22 A/a	0.46 ± 0.14 AB/b	0.49 ± 0.10 AB/b	0.26 ± 0.06 B	0.23 ± 0.07 B
	14.2	**Hexyl acetate**	142-92-7	1011	8.41 ± 2.32 A/a	6.85 ± 0.86 A/a	6.37 ± 1.12 A/a	5.02 ± 2.53 A/a	3.85 ± 0.78 AB/b	3.85 ± 0.78 AB/a	1.11 ± 0.11 B	0.64 ± 0.15 AB
	14.32-14.35	**2-Hexen-1-ol acetate**	2497-18-9	1016	0.17 ± 0.09 B/a	0.39 ± 0.07 A/a	0.31 ± 0.03 AB/a	0.27 ± 0.04 A/a	0.10 ± 0.05 B/b	0.10 ± 0.05 B/b	nd	nd
	17.96	**Heptyl acetate**	112-06-1	1113	0.15 ± 0.01 C/b	0.49 ± 0.03 B	0.83 ± 0.11 A	0.21 ± 0.01 a	nd	nd	nd	nd
	20.42	**6-Nonenyl acetate**	35854-86-5	1308	0.24 ± 0.03 A/b	0.23 ± 0.04 A/a	0.22 ± 0.02 A/a	0.62 ± 0.04 A/a	0.26 ± 0.03 B/a	0.26 ± 0.02 B/a	0.16 ± 0.02 C	0.10 ± 0.01 C
	20.44	**Nonyl acetate**	143-13-5	1308	0.10 ± 0.01 A	0.12 ± 0.02 A	0.14 ± 0.02 A	nd	nd	nd	nd	0.03 ± 0.00
		Σ Acetate esters			29.3 ± 1.5 A/b	26.52 ± 3.2 AB/b	21.71 ± 3.5 B/b	36.45 ± 2.4 A/a	34.3 ± 0.7 A/a	31.8 ± 1.4 A/a	20.0 ± 2.8 B	17.3 ± 5.4 B
Non-acetate esters	19.73	**Hexyl 2-methylbutyrate**	10032-15-2	1236	0.02 ± 0.01 B/a	0.03 ± 0.01 AB/a	0.05 ± 0.02 A/a	0.03 ± 0.02 B/a	0.06 ± 0.03 AB/a	0.13 ± 0.05 A/a	0.01 ± 0.00 B	0.03 ± 0.01 B
Aldehydes	4.6-5.08	**Hexanal**	66-25-1	800	9.46 ± 0.26 A/b	2.28 ± 0.47 B/b	2.79 ± 0.69 B/b	12.3 ±1.3 A/a	9.88 ± 1.68 A/a	8.40 ± 1.27 A/a	8.82 ± 2.42 A	10.5 ± 3.9 A
	6.7-6.95	**(E)-2-Hexenal**	6728-26-3	854	4.66 ± 0.21 A/a	2.18 ± 0.60 B/a	1.38 ± 0.16 B/a	2.18 ± 0.29 A/b	1.42 ± 0.08 B/a	1.34 ± 0.21 B/a	1.07 ± 0.26 B	1.61 ± 0.42 AB
	8.66-8.92	**Heptanal**	111-71-7	901	0.40 ± 0.02 C/b	1.40 ± 0.19 B/a	2.44 ± 0.29 A/a	0.78 ± 0.03 B/a	0.81 ± 0.13 B/b	1.09 ± 0.14 AB/b	1.26 ± 0.16 A	1.03 ± 0.13 AB
	11.25-11.45	**2-Heptenal**	18829-55-5	958	0.51 ± 0.05 B/a	0.64 ± 0.02 A/a	0.62 ± 0.03 A/a	0.60 ± 0.07 B/a	0.79 ± 0.11 B/a	0.71 ± 0.13 B/a	1.67 ± 0.09 A	1.65 ± 0.15 A
	13.55	**Octanal**	124-13-0	1003	0.39 ± 0.05 A/b	0.43 ± 0.10 A/a	0.44 ± 0.07 A/a	0.57 ± 0.04 A/a	0.48 ± 0.12 A/a	0.47 ± 0.10 A/a	0.36 ± 0.06 A	0.43 ± 0.13 A

Compound family	RT[a]	Compound name	CAS Nº	RI[b]	Compound relative percentage (%)							
					Raw samples stored at 4ºC			HPP samples stored at 4ºC				
					Day 0	3rd day	7th day	Day 0	3rd day	7th day	14th day	21st day
	16.2	**2-Octenal**	2548-87-0	1060	0.10 ± 0.01 B/b	0.12 ± 0.02 AB/b	0.13 ± 0.01 A/a	0.18 ± 0.02 B/a	0.23 ± 0.07 AB/a	0.27 ± 0.12 AB/a	0.39 ± 0.00 A	0.37 ± 0.03 A
	17.71	**(Z)-6-Nonenal**	2277-19-2	1101	10.7 ± 1.1 B/a	13.8 ± 0.6 A/a	9.74 ± 0.12 B/a	6.74 ± 0.17 A/b	4.93 ± 0.58 B/b	4.44 ± 0.41 BC/b	3.84 ± 0.72 BC	3.25 ± 0.05 C
	17.74	**Nonanal**	124-19-6	1104	7.04 ± 0.42 A/a	6.47 ± 0.40 AB/a	5.53 ± 0.38 B/a	7.53 ± 0.39 A/a	5.95 ± 1.23 AB/a	5.15 ± 1.01 B/a	3.94 ± 0.44 B	4.26 ± 0.71 B
	18.62-18.68	**(E,Z)-2,6-Nonadienal**	557-48-2	1155	11.8 ± 1.7 A/a	12.9 ± 1.1 A/a	10.9 ± 0.7 A/a	5.71 ± 0.46 B/b	7.25 ± 0.57 B/b	6.69 ± 0.74 B/b	13.9 ± 0.7 A	13.1 ± 0.9 A
	18.7-18.8	**(E)-2-Nonenal**	18829-56-6	1162	9.67 ± 0.40 A/a	11.0 ± 2.1 A/a	10.5 ± 0.4 A/a	5.58 ± 0.59 BC/b	7.19 ± 0.19 ABC/b	4.42 ± 6.99 B/a	14.4 ± 0.4 A	14.0 ± 1.4 AB
	19.37	**Decanal**	112-31-2	1206	0.58 ± 0.13 A/b	0.52 ± 0.06 A/b	0.62 ± 0.12 A/b	0.99 ± 0.12 A/a	1.01 ± 0.13 A/a	1.29 ± 0.36 A/a	0.66 ± 0.21 A	0.67 ± 0.07 A
	19.46	**(E,E)-2,4-Nonadienal**	5910-87-2	1213	0.09 ± 0.01 B/b	0.13 ± 0.02 A/a	0.14 ± 0.01 A/a	0.13 ± 0.03 C/a	0.16 ± 0.04 C/a	0.20 ± 0.09 BC/a	0.31 ± 0.02 AB	0.40 ± 0.01 A
		Σ Aldehydes			55.4 ± 2.9 A/a	51.8 ± 4.2 AB/a	45.2 ± 0.9 B/a	43.3 ± 2.8 AB/b	39.9 ± 3.9 AB/b	34.5 ± 6.0 B/b	50.6 ± 3.2 A	51.3 ± 6.8 A
Alcohols	7.47-7.65	**1-Hexanol**	111-27-3	868	1.57 ± 0.06 B/a	2.00 ± 0.06 B/a	3.70 ± 0.51 A/a	1.44 ± 0.13 A/a	1.37 ± 0.48 A/a	1.41 ± 0.40 A/b	1.63 ± 0.33 A	1.38 ± 0.15 A
	12.07	**1-Heptanol**	111-70-6	970	0.03 ± 0.01 A/b	0.31 ± 0.02 A/a	1.25 ± 0.24 B/a	0.11 ± 0.03 A/a	0.09 ± 0.01 A/b	0.07 ± 0.02 A/b	0.08 ± 0.01 A	0.08 ± 0.05 A
	12.49	**1-Octen-3-ol**	3391-86-4	980	0.04 ± 0.00 C/b	0.07 ± 0.01 B/b	0.12 ± 0.02 A/a	0.14 ± 0.01 B/a	0.15 ± 0.01 B/a	0.16 ± 0.02 B/a	0.35 ± 0.06 A	0.28 ± 0.04 A
	14.86	**2-ethyl-1-hexanol**	104-76-7	1030	0.14 ± 0.03 A/a	0.07 ± 0.01 B/b	0.13 ± 0.02 A/b	0.20 ± 0.07 B/a	0.54 ± 0.01 B/a	0.47 ± 0.11 B/a	0.18 ± 0.03 B	1.11 ± 0.25 A
	16.84	**1-Octanol**	111-87-5	1071	0.24 ± 0.04 B/b	0.30 ± 0.05 B/a	0.47 ± 0.04 A/a	0.42 ± 0.02 A/a	0.39 ± 0.05 A/b	0.33 ± 0.06 A/b	0.45 ± 0.02 A	0.41 ± 0.13 A
	18.6	**(Z)-3-Nonen-1-ol**	10340-23-5	1143	nd	nd	0.48 ± 0.02	0.05 ± 0.00 A	0.06 ± 0.01 A	nd	nd	nd
	18.85	**(E,Z)-2,6-Nonadien-1-ol**	7786-44-9	1169	0.24 ± 0.01 B/a	0.24 ± 0.03 B/a	0.66 ± 0.09 A/a	0.18 ± 0.02 A/b	0.14 ± 0.02 A/b	0.14 ± 0.03 A/b	0.14 ± 0.03 A	0.16 ± 0.05 A
	18.89	**(E)-2-Nonen-1-ol**	31502-14-4	1176	0.37 ± 0.01 B	0.35 ± 0.06 B	0.64 ± 0.09 A	nd	nd	nd	nd	nd
	18.92-18.96	**1-Nonanol**	28473-21-4	1173	2.17 ± 0.09 C/b	4.09 ± 0.20 B/a	8.67 ± 1.01 A/a	5.45 ± 0.31 A/a	3.39 ± 0.74 B/a	3.27 ± 0.53 B/b	3.16 ± 0.55 B	2.57 ± 0.06 B
		Σ Alcohols			4.8 ± 0.2 C/b	7.4 ± 0.3 B/a	16.1 ± 1.8 A/a	7.98 ± 0.47 A/a	6.12 ± 0.39 AB/b	5.81 ± 0.32 B/b	5.99 ± 0.84 B	5.99 ± 0.22 AB

Compound family	RT[a]	Compound name	CAS Nº	RI[b]	Compound relative percentage (%)							
					Raw samples stored at 4ºC			HPP samples stored at 4ºC				
					Day 0	3rd day	7th day	Day 0	3rd day	7th day	14th day	21st day
Furans	12.98	2-pentyl-furan	3777-69-3	993	0.32 ± 0.03 C/b	0.67 ± 0.04 B/a	0.85 ± 0.02 A/b	0.63 ± 0.05 C/a	1.28 ± 0.45 BC/a	2.63 ± 0.21 A/a	2.39 ± 0.23 A	2.21 ± 0.58 AB
	13.45	cis-2-pentenylfuran	70424-13-4	1002	0.07 ± 0.01 C/b	0.18 ± 0.01 B/a	0.22 ± 0.02 A/b	0.17 ± 0.03 B/a	0.31 ± 0.13 B/a	0.79 ± 0.16 A/a	1.11 ± 0.11 A	1.10 ± 0.17 A
		Σ Furans			0.39 ± 0.04 C/b	0.85 ± 0.05 B/a	1.07 ± 0.04 A/b	0.80 ± 0.07 B/a	1.59 ± 0.58 B/a	3.43 ± 0.03 A/a	3.51 ± 0.26 A	3.32 ± 0.76 A
Terpenes	10.08	α-pinene	80-56-8	937	0.09 ± 0.02 B/b	0.12 ± 0.01 B/b	0.20 ± 0.03 A/a	0.25 ± 0.05 A/a	0.32 ± 0.06 A/a	0.25 ± 0.06 A/a	0.15 ± 0.07 A	0.27 ± 0.11 A
	14.6	Limonene	5989-54-8	1030	1.81 ± 2.65 A/a	2.87 ± 4.11 A/a	0.75 ± 0.52 A/a	2.25 ± 3.58 A/a	4.27 ± 0.33 A/a	2.18 ± 2.02 A/a	0.87 ± 0.57 A	2.06 ± 3.11 A
	21.94	α-farnesene	502-61-4	1508	0.13 ± 0.01 B/a	0.14 ± 0.04 B/b	0.24 ± 0.03 A/a	0.11 ± 0.09 B/a	0.31 ± 0.07 AB/a	0.38 ± 0.11 A/a	0.21 ± 0.03 AB	0.32 ± 0.14 AB
		Σ Terpenes			2.03 ± 2.65 A/a	3.12 ± 4.09 A/a	1.19 ± 0.50 A/a	2.61 ± 3.59 A/a	4.89 ± 0.19 A/a	2.81 ± 1.97 A/a	1.23 ± 0.65 A	2.65 ± 3.09 A

[a] -Retention time in minutes

[b]- Retention index reported in NIST MS version 2.2.

Different letters represent significant differences ($p < 0.05$) at the same conditions (capital letters; effect of storage) or between samples at the same time of storage (noncapital letters; effect of HPP).

nd- not detected

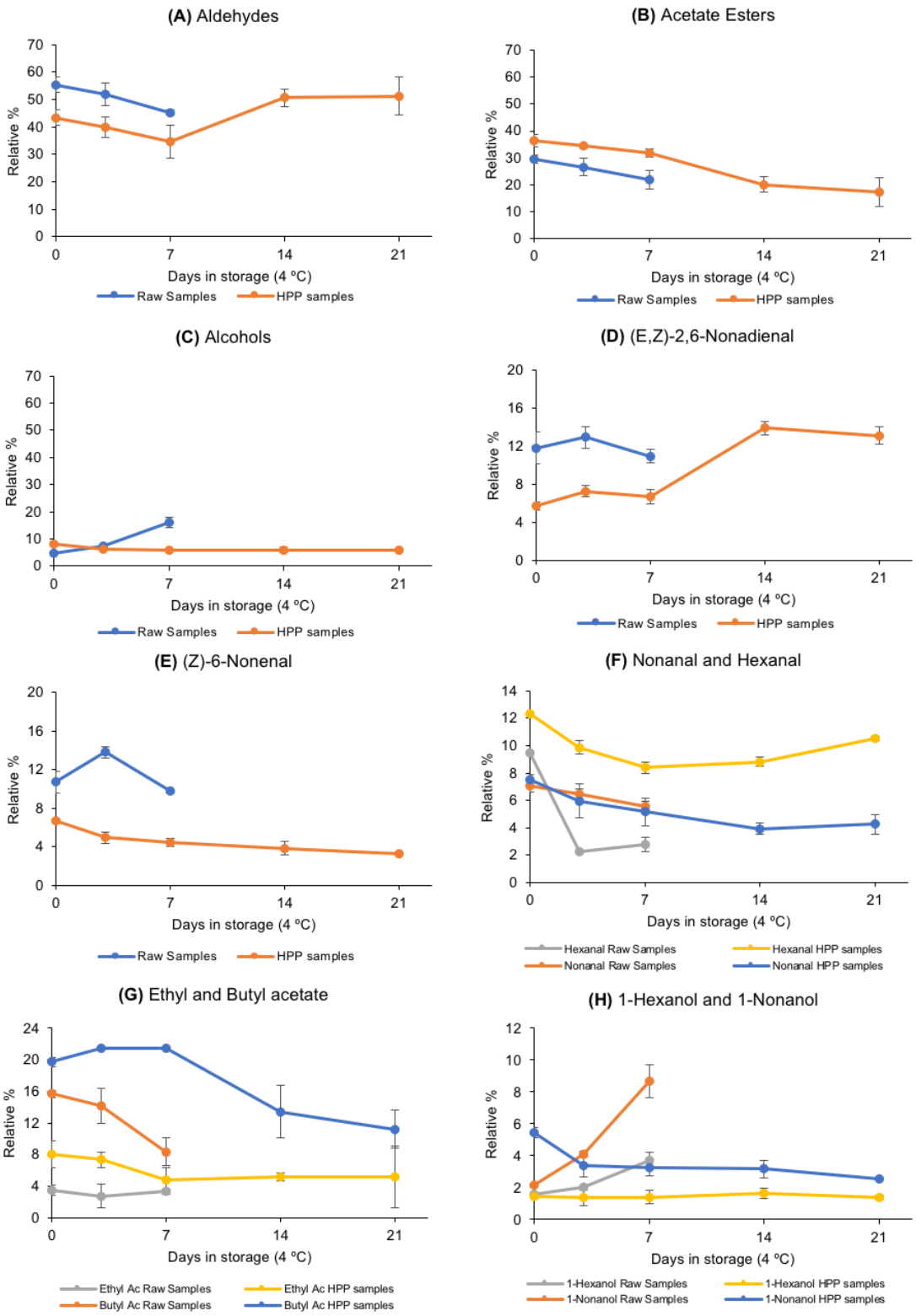


Figure 14 – Graphical representation of the results obtained for certain families of compounds and individual compounds mentioned in the discussion.

Given that apple and pear were not the major component of the fruit salad and were in solid pieces while the melon was in the form of juice, it was not expected that typical VOCs of apple and pear would appear as major contributors to the products aroma profile. This way, melon aroma is a major concern in this work, considering melon juice is the major component of the studied fruit salads

Aldehydes (Figure 14) represented the majority of the VOCs identified, followed by acetate esters, both in raw and HPP samples. Other classes were found but in significantly smaller proportions.

Regarding aldehydes (Figure 14A) in raw samples, these showed a gradual relative reduction during storage at 4 ºC, as observed in melons in the work of Amaro *et al.* (2012) [116]. The relative percentage was significantly ($p < 0.05$) higher in day 0 when compared with the 7th day. Concerning the impact of HPP, samples subjected to HPP presented a significantly ($p < 0.05$) lower relative percentage of aldehydes (43.3 $\pm$ 2.8 %) immediately after processing than raw samples also from day 0 (55.4 $\pm$ 2.9 %). The relative amounts in HPP samples showed a slow tendency to increase, given that at the 14th day of storage it had increased to 50.6 $\pm$ 3.2 %.

Acetate esters (Figure 14B) stand as the second most representative class of VOCs present in these fruit salads volatile profile. This class showed a tendency to decrease over storage time in raw samples, being significantly lower ($p < 0.05$) after 7 days of refrigerated storage. In samples subjected to HPP, the proportions significantly increased ($p < 0.05$) immediately after processing in comparison with raw samples from day 0. Nevertheless, HPP samples showed the same tendency to decrease with storage.

Oh *et al.* (2011) [92] also used HS-SPME in *Cucumis melo* L. and considered (Z)-6-nonenal, nonanal, (E,Z)-2,6-nonadienal and (E)-2-nonenal characteristic impact flavour and aroma compounds (CIFAC) of melon. These compounds were also found in the samples analysed in this work in significant relative percentages. (Z)-6-Nonenal (Figure 14E) did not show a clear tendency to increase or decrease with storage time in raw samples. However, there was a significant ($p < 0.05$) reduction (4% less) in HPP samples immediately after processing, and there was a clear decrease in the proportion of (Z)-6-nonenal over storage time. The proportion of nonanal (Figure 14F) did not show significant differences ($p > 0.05$) between raw and HPP samples, and in both groups of samples was observed a decreasing tendency in nonanal with storage time. Regarding (E,Z)-2,6-nonadienal (Figure 14D), there were no significant ($p > 0.05$) changes in raw samples over 7 days of storage at 4ºC. Nonetheless, it was verified a significant reduction ($p < 0.05$) after HPP, having a 6% difference from raw samples in day 0. The content of (E,Z)-2,6-nonadienal content in HPP

samples increased along the 21 days of storage. Finally, (E)-2-nonenal showed a behaviour similar to (E,Z)-2,6-nonadienal, having underwent a significant reduction after HPP and increased over storage time in processed samples.

The evolution of the total alcohols is pictured in Figure 14C. The alcohols correspondent to the previously highlighted aldehydes, namely 1-nonanol (Figure 14H), (E)-2-nonen-1-ol, (Z)-3-nonen-1-ol and (E,Z)-2,6-nonadien-1-ol, were detected in the studied fruit salads in much smaller proportions. Besides its association to melon aroma, 1-nonanol, (Z)-3-nonen-1-ol and (E)-2-nonen-1-ol are also associated with pear aroma [88, 89]. Different behaviours in raw and HPP samples were verified. 1-nonanol was detected in both groups of samples, however it increased over storage time in raw samples and decreased over time in HPP samples. (E)-2-nonen-1-ol was present in raw samples, and significantly increased but was not detected in samples subjected to HPP. The absence of this compound in samples subjected to HPP was also verified by Sumitani *et al.* (1994) in peaches [117]. The content in (Z)-3-nonen-1-ol increased in raw samples, given it was not detected in the first 3 days of storage, but at the 7th day represented 0.48 $\pm$ 0.02 %. In HPP samples, it was only detected in the first 3 days of refrigerated storage and in very small percentages (0.05 $\pm$ 0.00 % and 0.06 $\pm$ 0.01 %, respectively). The increase of these alcohols' content in raw samples may result from the reduction of the corresponding aldehydes [92]. However, as explained in section 1.4.6., aldehydes are enzymatically reduced to their corresponding alcohols. And as also explained in the literature review, high pressure affects enzymatic activity causing both activation and inhibition, depending on the enzyme itself, its origin, matrix, among others. The observed difference regarding the behaviour of melon CIFACs and its corresponding alcohols in HPP samples may result in changes in the activity of enzymes involved in the biosynthetic pathways that originate these VOCs. For example, the quantity of (E)-2-nonenal increased over storage time while no (E)-2-nonen-1-ol was detected. This indicates that the metabolic pathway was somehow affected.

In what concerns C6 aldehydes, hexanal (Figure 14F), which is characteristic of the three fruits composing the fruit salad, decreased abruptly from day 0 to day 3 in raw samples and remained similar at the 7th day of storage. When comparing hexanal in raw and HPP samples in day 0, processed samples showed significantly ($p < 0.05$) higher proportions, as it was verified by Navarro *et al.* (2012) in strawberry purées, and did not suffer significant changes ($p > 0.05$) during storage, remaining always significantly ($p < 0.05$) higher than raw samples. On the other hand, (E)-2-hexenal, that decreased over storage time in raw samples, suffered a reduction immediately after processing, unlike what was observed in

the work of Navarro *et al.* [118] and remained similar ($p > 0.05$) to raw samples from the 3rd day onwards.

Regarding acetate esters, ethyl acetate and butyl acetate (Figure 14G) showed a significant increase in samples subjected to HPP. The content of ethyl acetate showed a twofold increase (approximately) on day 0 and 3 than in raw samples, but did not suffer significant changes ($p > 0.05$) during storage in both groups of samples. Butyl acetate is usually found in great amounts in melon [116], and its content was also significantly ($p < 0.05$) greater in HPP samples and decreased over storage time in both groups of samples. Pentyl acetate and heptyl acetate both showed an increase during storage in raw samples but showed different behaviours in processed samples. Pentyl acetate registered a slow decrease through storage while heptyl acetate was only detected immediately after processing. The increasing relative percentage of these compounds in raw samples may be related with microbial activity, given the load these samples presented. If pentyl and heptyl acetate do result from microbial metabolism, the reduction of the microbial load in processed samples can also explain why their relative percentage did not increase. Moreover, according to Yi *et al.* (2018) [119], acetate esters' decrease during storage may be linked to esterase activity. In fact, and as mentioned previously, high pressure can either activate or inhibit enzymatic activity. This way, given the decrease in the quantity of these two acetate esters in HPP samples, the activation of esterase presents itself as a possible justification.

Regarding the presence of furan-related compounds, these have been detected before in various thermally treated food products, and their formation is related to Maillard reaction and oxidation of triple unsaturated FA [120]. It was hypothesized that these could be artefacts formed by chemical reactions in the course of isolation of volatiles. However, given that these increased with time, this hypothesis was discarded. It was also hypothesized that these compounds could be a result of HPP. This theory was also discarded given the low temperature (15 ºC) at which HPP was performed and given the presence of these compounds in raw samples. The only explanation left is that these compounds are naturally present in the samples. In fact, Yajima *et al.* (1985) [121] identified furan-related compounds for the first time as naturally occurring flavour components, when investigating watermelon. In this case, it remained uncertain whether these compounds were produced enzymatically or whether they resulted of the isolation of volatiles.

Compounds typically associated to apple's aroma, such as 2-hexen-1-ol acetate, hexyl 2-methylbutyrate and α-pinene were detected in both raw and HPP samples, but in small percentages, as shown in Table 7. 2-hexen-1-ol acetate showed a slight increase throughout storage time in raw samples, but in HPP samples decreased until it was no

longer detected. Hexyl 2-methylbutyrate showed a significant ($p < 0.05$) increase in raw samples in the 7 days of storage at 4ºC. No significant difference ($p > 0.05$) was verified between the content in raw and HPP samples, having showed the same increasing tendency in processed samples up until the 7th day of storage, after which it abruptly decreased. α-pinene also significantly increased ($p < 0.05$) in raw samples after 7 days at 4ºC. The content of this terpene was significantly higher ($p < 0.05$) in HPP samples immediately after processing when compared with the content in day 0 in raw samples, and also increased with storage time. α-farnesene, present in apples and pears, was also found in small relative quantities, in both groups of samples. Similarly to α-pinene, it increased with storage time in both groups of samples, but HPP did not have a significant ($p > 0.05$) impact on α-farnesene content immediately after processing. HPP did not show benefits regarding melon's CIFAC, decreasing its abundancy. Given that HPP effect on the VOC profile of fruit products is very scarcely reported in the literature, further studies on this matter should be performed.

5. CONCLUSION

Nowadays, consumers present a higher demand for fresh and natural products and show a preference for raw or minimally processed products. Hence, non-thermal food processing technologies are being developed and tested. HPP has been deeply studied over the past 20 years and comes out as a feasible, eco-friendly and efficient way to extend fruit products' shelf life while assuring its nutritional properties. However, some effects of HPP still need to be further investigated.

This work aimed to study the effects of HPP in numerous quality parameters of a non-acidic fruit salad. Standing as a case-study, a fruit salad composed of melon juice and pieces of Golden apple and Rocha pear was subjected to 550 MPa for 3 minutes, at 15ºC, and stored for 35 days at 4ºC. When compared to equal samples that did not suffer any processing and went directly to refrigerated storage, some interesting results were observed.

Regarding TSS, HPP did not implicate any significant differences ($p > 0.05$) between the two groups of samples. Regarding microbial growth, HPP reduced drastically the microbial load in samples up to 21 days, as it was to be expected. However, since the samples were inoculated, it was not possible to determine the exact shelf life of the product. The differences in microbial activity also implicated differences in TA and in pH.

Concerning enzymatic activity, results showed that HPP was not efficient inactivating PPO. In fact, the browning index in processed samples was significantly higher ($p < 0.05$), which corroborates the idea that pressure caused activation of the enzyme by rupturing the cellular compartments that separated the enzyme from its substract. In order to find a solution for the poor visual attractiveness of the product, an assay with the addition of AA as an antioxidant was performed. The browning of the samples was not completely solved, but showed some improvements which led to believe that higher concentrations of the additive may be the solution to improve the products' colour.

When it comes to TAC, no significant changes ($p > 0.05$) were detected. However, it was not possible to measure vitamin C nor TP content due to the inadequacy of the chosen methods, which most likely present a limit of detection too high.

Finally, the volatile profile of the samples was analysed. The major concern in this matter was the maintenance of the key compounds responsible for the melon aroma of the product, given that melon juice was the major component of the fruit salad. It was verified that HPP samples had less content in melon CIFACs than raw samples and different behaviours of these compounds and others related to them where observed. This may indicate that HPP caused changes in the enzymatic activity of the enzymes involved in the metabolic

pathways that originate these VOCs and it is a matter that requires further and deeper studies.

Summarily, HPP showed to be effective in preserving the fruit salad overall quality. However, further studies must be performed in this type of product in order to reach a final conclusion.

6. FUTURE WORK

Further experiments using more sensitive methods regarding the effect of HPP on TP content, vitamin C, PME and POD activities are needed in order to reach a better understanding of how fruit salads respond to this kind of processing. Future work should also comprise quantification of β-carotene and its behaviour post-HPP and during storage, as well as the evaluation of alterations in the texture of the solid components of the product. Controlling the browning of the product is also mandatory, in order to preserve its visual properties. Therefore, more concentrations of ascorbic acid must be tested.

Filling the gap in literature regarding HPP effect on metabolic pathways that lead to aroma compounds in fruit products should also be a goal in the future.

Moreover, since this thesis stands as a case-study, more combinations of fruit most be tested, with different acidities and nutritional matrices.

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8. APPENDICES

8.1. Appendix A – Standard curve for determination of total antioxidant capacity

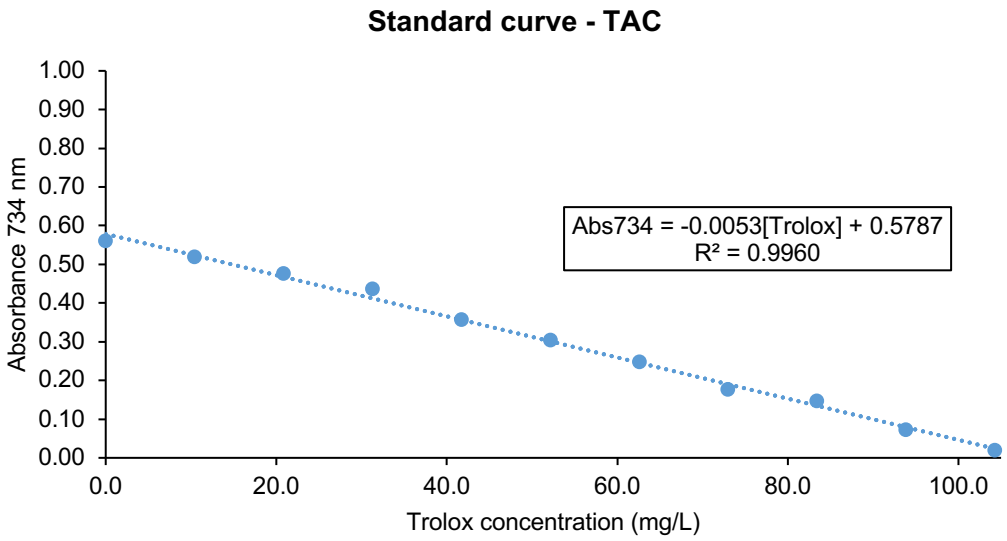

Figure 15 – Standard curve of absorbance at 734 nm *versus* Trolox concentration (mg/L).

8.2. Appendix B – Standard curve for determination of total phenolics content

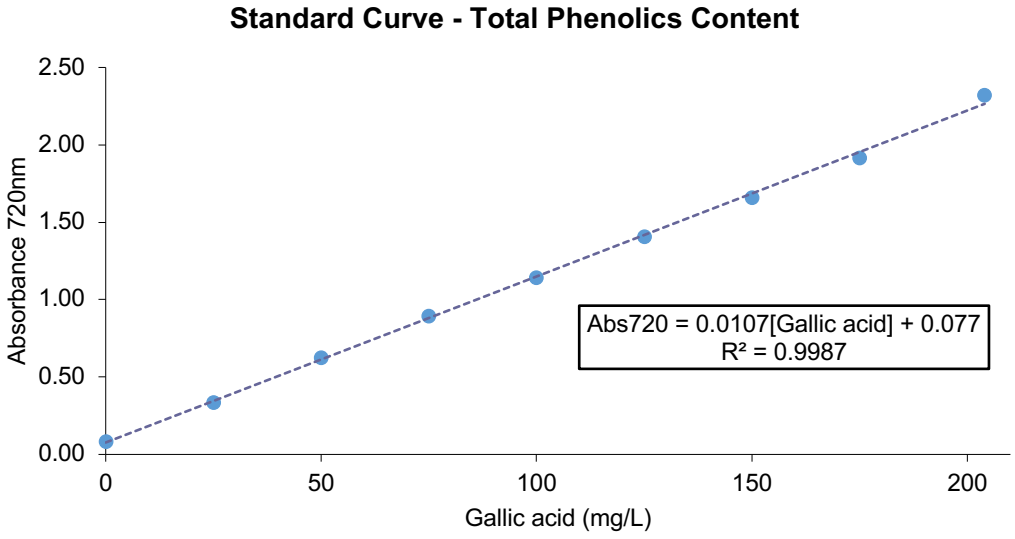

Figure 16 - Standard curve of absorbance at 720 nm *versus* gallic acid concentration (mg/L).

8.3. Appendix C – Vitamin C determination standard curve

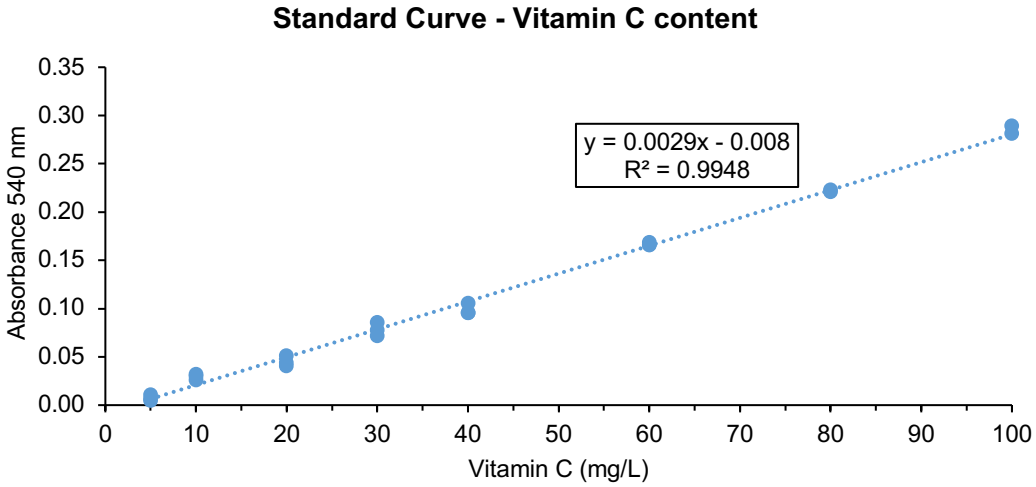

Figure 17 - Standard curve of absorbance at 540 nm *versus* vitamin C concentration (mg/L).

8.4. Appendix D – Total soluble solids data

Table 8 – Total soluble solids data.

Sampling day	Raw Samples	Standard deviation	ANOVA	HPP samples	Standard deviation	ANOVA
0	10.80	0.20	A/a	11.07	0.12	A/a
3	10.87	0.42	A/a	10.40	0.20	A/a
7	10.80	0.35	A/a	10.43	1.01	A/a
14	10.20	0.80	A/a	10.93	0.46	A/a
21	10.30	0.17	A/a	10.20	0.53	A/a
28	10.33	0.64	A/a	10.00	1.06	A/a
35	10.73	0.12	A/a	11.27	0.46	A/a

8.5. Appendix E - Browning index data

Table 9 - Browning index instrumental data in which Figure 10 was based on.

	Sampling day	Raw Samples	Standard error	ANOVA	HPP	Standard deviation	ANOVA
Without AA	0	0.213	0.028	A/a	0.358	0.050	A/b
	3	0.239	0.061	A/a	0.380	0.030	B/b
	7	0.221	0.024	A/a	0.453	0.035	AB/b
	14	0.311	0.076	A/a	0.474	0.031	AB/b
	21	0.205	0.018	A/a	0.511	0.088	AB/b
	28	0.228	0.007	A/a	0.461	0.043	AB/b
	35	0.219	0.009	A/a	0.581	0.081	B/b
With AA	0	0.287	0.043	A/a	0.255	0.042	AB/a
	3	0.235	0.071	A/a	0.281	0.093	AB/a
	7	0.264	0.017	A/a	0.339	0.023	AB/b
	14	0.226	0.040	A/a	0.288	0.025	AB/a
	21	0.198	0.048	A/a	0.246	0.047	B/a
	28	0.238	0.024	A/a	0.378	0.035	A/b
	35	0.261	0.032	A/a	0.299	0.039	AB/a

8.6. Appendix F – Total antioxidant capacity results

Table 10 – Total antioxidant capacity results. Data on which Figure 11 was based on.

Sampling day	Raw Samples	Standard deviation	ANOVA	HPP samples	Standard deviation	ANOVA
0	0.149	0.017	AB/a	0.117	0.019	C/a
3	0.169	0.011	A/a	0.155	0.014	AB/a
7	0.186	0.032	A/a	0.137	0.008	BC/a
14	0.174	0.015	A/a	0.177	0.010	A/a
21	0.161	0.003	A/a	0.156	0.001	AB/b
28	0.098	0.025	B/a	0.071	0.003	D/b
35	0.112	0.023	AB/a	0.117	0.020	C/a

8.7. Appendix G – Volatile organic compounds

Table 11 - Main volatile organic compounds extracted by HS-SPME measured by GC-MS. Results expressed in mg/kg of internal standard.

					Compound relative concentration (mg/ kg internal standard)							
					Raw samples stored at 4ºC			HPP samples stored at 4ºC				
Compound family	**RT[a]**	**Compound name**	**CAS**	**RI[b]**	**Day 0**	**3rd day**	**7th day**	**Day 0**	**3rd day**	**7th day**	**14th day**	**21st day**
Acetate Esters	1.72-1.88	**Ethyl acetate**	141-78-6	612	2.16 ± 0.26	1.43 ± 0.54	1.52 ± 0.35	2.96 ± 0.23	3.05 ± 0.46	2.04 ± 0.33	2.13 ± 0.13	2.45 ± 1.33
	2.88/2.98	**Propyl acetate**	109-60-4	708	0.28 ± 0.08	0.19 ± 0.05	0.24 ± 0.08	0.02 ± 0.03	0.35 ± 0.07	0.16 ± 0.06	0.11 ± 0.04	0.14 ± 0.13
	5.4-5.6	**Butyl acetate**	123-86-4	812	10.5 ± 2.6	6.33 ± 0.91	3.83 ± 1.08	2.60 ± 2.72	3.07 ± 4.10	0.66 ± 0.01	4.69 ± 2.91	0.25 ± 0.11
	7.9-8.05	**2-Methyl-1-butanol acetate**	624-41-9	880	0.17 ± 0.04	0.08 ± 0.02	0.07 ± 0.02	0.63 ± 0.03	0.07 ± 0.01	0.04 ± 0.02	0.01 ± 0.00	0.01 ± 0.00
	9.4-9.6	**Pentyl acetate**	628-63-7	911	0.45 ± 0.14	0.50 ± 0.16	0.81 ± 0.24	0.25 ± 0.03	0.20 ± 0.09	0.19 ± 0.01	0.11 ± 0.05	0.13 ± 0.01
	14.2	**Hexyl acetate**	142-92-7	1011	5.62 ± 2.40	3.09 ± 0.46	2.89 ± 0.49	1.07 ± 0.72	0.53 ± 0.58	0.94 ± 0.03	0.31 ± 0.33	0.25 ± 0.00
	14.32-14.35	**2-Hexen-1-ol acetate**	2497-18-9	1016	0.10 ± 0.03	0.19 ± 0.04	0.14 ± 0.02	0.10 ± 0.03	0.04 ± 0.02	0.02 ± 0.01	nd	nd
	17.96	**Heptyl acetate**	112-06-1	1113	0.10 ± 0.03	0.27 ± 0.03	0.38 ± 0.04	0.08 ± 0.02	nd	nd	nd	nd
	20.42	**6-Nonenyl acetate**	35854-86-5	1308	0.15 ± 0.02	0.12 ± 0.00	0.10 ± 0.02	0.23 ± 0.06	0.14 ± 0.03	0.05 ± 0.02	0.07 ± 0.01	0.04 ± 0.00
	20.44	**Nonyl acetate**	143-13-5	1308	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	nd	0.01 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
		Σ Acetate esters			19.6 ± 4.9	12.1 ± 2.0	10.04 ± 2.25	7.93 ± 3.62	7.50 ± 3.51	4.09 ± 0.32	7.44 ± 3.51	3.27 ± 1.39
Non - acetate esters	19.73	**Hexyl 2-methylbutyrate**	10032-15-2	1236	0.01 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Aldehydes	4.6-5.08	**Hexanal**	66-25-1	800	6.35 ± 1.57	2.96 ± 0.13	1.33 ± 0.25	4.33 ± 0.55	4.24 ± 0.66	3.64 ± 0.27	3.58 ± 0.56	3.73 ± 1.20
	6.7-6.95	**(E)-2-Hexenal**	6728-26-3	854	2.97 ± 0.87	1.36 ± 0.27	0.58 ± 0.20	0.79 ± 0.09	0.52 ± 0.05	0.52 ± 0.06	0.44 ± 0.04	0.55 ± 0.18
	8.66-8.92	**Heptanal**	111-71-7	901	0.26 ± 0.05	0.89 ± 0.15	1.11 ± 0.11	0.31 ± 0.03	0.36 ± 0.12	0.40 ± 0.08	0.52 ± 0.02	0.37 ± 0.05
	11.25-11.45	**2-Heptenal**	18829-55-5	958	0.33 ± 0.05	0.33 ± 0.09	0.27 ± 0.09	0.21 ± 0.01	0.36 ± 0.04	0.56 ± 0.09	0.69 ± 0.08	0.67 ± 0.13
	13.55	**Octanal**	124-13-0	1003	0.26 ± 0.04	0.23 ± 0.06	0.20 ± 0.06	0.23 ± 0.01	0.20 ± 0.03	0.17 ± 0.03	0.15 ± 0.01	0.17 ± 0.01
	16.2	**2-Octenal**	2548-87-0	1060	0.07 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.00	0.10 ± 0.03	0.12 ± 0.04	0.16 ± 0.03	0.15 ± 0.03

Compound family	RT[a]	Compound name	CAS	RI[b]	Compound relative concentration (mg/ kg internal standard)							
					Raw samples stored at 4ºC			HPP samples stored at 4ºC				
					Day 0	3rd day	7th day	Day 0	3rd day	7th day	14th day	21st day
	17.71	**(Z)-6-Nonenal**	2277-19-2	1101	6.96 ± 1.04	8.84 ± 1.79	4.48 ± 0.74	2.27 ± 0.19	2.20 ± 0.16	1.92 ± 0.24	1.59 ± 0.27	1.43 ± 0.14
	17.74	**Nonanal**	124-19-6	1104	4.64 ± 0.91	3.71 ± 0.85	2.55 ± 0.33	3.04 ± 0.18	2.55 ± 0.37	2.16 ± 0.35	1.63 ± 0.13	1.44 ± 0.02
	18.62-18.68	**(E,Z)-2,6-Nonadienal**	557-48-2	1155	7.62 ± 1.21	6.92 ± 1.96	5.06 ± 1.17	1.73 ± 0.15	3.28 ± 0.20	5.59 ± 1.24	5.81 ± 1.11	5.30 ± 1.04
	18.7-18.8	**(E)-2-Nonenal**	18829-56-6	1162	6.41 ± 1.42	6.00 ± 2.31	4.82 ± 0.99	2.17 ± 0.09	3.18 ± 0.25	4.30 ± 1.46	6.00 ± 1.13	5.63 ± 0.95
	19.37	**Decanal**	112-31-2	1206	0.37 ± 0.01	0.27 ± 0.02	0.28 ± 0.07	0.36 ± 0.04	0.34 ± 0.06	0.24 ± 0.01	0.27 ± 0.06	0.27 ± 0.05
	19.46	**(E,E)-2,4-Nonadienal**	5910-87-2	1213	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	0.09 ± 0.02	0.13 ± 0.03	0.17 ± 0.03
		Σ Aldehydes			36.3 ± 6.9	31.7 ± 5.7	20.8 ± 3.8	15.5 ± 0.7	17.4 ± 0.6	19.7 ± 3.24	21.0 ± 2.23	19.9 ± 2.38
Alcohols	7.47-7.65	**1-Hexanol**	111-27-3	868	0.95 ± 0.25	0.97 ± 0.06	1.55 ± 0.07	0.56 ± 0.07	0.61 ± 0.23	0.97 ± 0.42	0.61 ± 0.12	0.50 ± 0.07
	12.07	**1-Heptanol**	111-70-6	970	0.02 ± 0.01	0.17 ± 0.04	0.58 ± 0.15	0.04 ± 0.01	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
	12.49	**1-Octen-3-ol**	3391-86-4	980	0.03 ± 0.00	0.04 ± 0.02	0.06 ± 0.02	0.05 ± 0.00	0.08 ± 0.01	0.10 ± 0.03	0.14 ± 0.00	0.11 ± 0.02
	14.86	**2-ethyl-1-hexanol**	104-76-7	1030	0.09 ± 0.02	0.09 ± 0.06	0.06 ± 0.01	0.07 ± 0.02	0.24 ± 0.02	0.05 ± 0.00	0.07 ± 0.00	0.40 ± 0.11
	16.84	**1-Octanol**	111-87-5	1071	0.16 ± 0.01	0.17 ± 0.04	0.19 ± 0.03	0.14 ± 0.01	0.17 ± 0.02	0.14 ± 0.04	0.19 ± 0.03	0.16 ± 0.00
	18.6	**(Z)-3-Nonen-1-ol**	10340-23-5	1143	nd	nd	0.24 ± 0.07	0.02 ± 0.00	0.03 ± 0.01	nd	nd	nd
	18.85	**(E,Z)-2,6-Nonadien-1-ol**	7786-44-9	1169	0.16 ± 0.04	0.12 ± 0.02	0.33 ± 0.11	0.06 ± 0.01	0.07 ± 0.00	0.05 ± 0.00	0.06 ± 0.1	0.05 ± 0.1
	18.89	**(E)-2-Nonen-1-ol**	31502-14-4	1176	0.25 ± 0.06	0.23 ± 0.09	0.29 ± 0.05	nd	nd	0.04 ± 0.03	nd	nd
	18.92-18.96	**1-Nonanol**	28473-21-4	1173	1.43 ± 0.28	2.14 ± 0.56	3.71 ± 0.98	1.70 ± 0.32	1.49 ± 0.09	1.18 ± 0.25	1.30 ± 0.20	1.23 ± 0.05
		Σ Alcohols			3.08 ± 0.64	3.95 ± 0.74	7.03 ± 1.40	2.65 ± 0.11	2.72 ± 0.21	2.55 ± 0.15	2.41 ± 0.29	2.48 ± 0.15
Furans	12.98	**2-pentyl-furan**	3777-69-3	993	0.21 ± 0.07	0.34 ± 0.11	0.39 ± 0.06	0.18 ± 0.02	0.55 ± 0.19	1.12 ± 0.24	1.00 ± 0.23	1.13 ± 0.24
	13.45	**cis-2-pentenylfuran**	70424-13-4	1002	0.05 ± 0.01	0.09 ± 0.03	0.10 ± 0.02	0.05 ± 0.00	0.13 ± 0.06	0.39 ± 0.09	0.46 ± 0.04	0.55 ± 0.08
		Σ Furans			0.26 ± 0.08	0.43 ± 0.14	0.49 ± 0.08	0.24 ± 0.02	0.68 ± 0.25	1.51 ± 0.33	1.46 ± 0.27	1.68 ± 0.32

Compound family	RT[a]	Compound name	CAS	RI[b]	Compound relative concentration (mg/ kg internal standard)							
					Raw samples stored at 4ºC			HPP samples stored at 4ºC				
					Day 0	3rd day	7th day	Day 0	3rd day	7th day	14th day	21st day
Terpenes	10.08	**α-pinene**	80-56-8	937	0.06 ± 0.02	0.05 ± 0.01	0.10 ± 0.03	0.08 ± 0.01	0.15 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.10 ± 0.02
	14.6	**Limonene**	5989-54-8	1030	1.42 ± 2.19	1.84 ± 2.68	0.32 ± 0.22	0.67 ± 1.05	1.90 ± 0.31	0.38 ± 0.27	0.34 ± 0.20	0.12 ± 0.04
	21.94	**α-farnesene**	502-61-4	1508	0.09 ± 0.02	0.07 ± 0.02	0.08 ± 0.04	0.04 ± 0.03	0.15 ± 0.05	0.16 ± 0.09	0.09 ± 0.00	0.12 ± 0.14
		Σ Terpenes			1.56 ± 2.22	1.96 ± 2.67	0.49 ± 0.27	0.80 ± 1.03	2.19 ± 0.22	0.60 ± 0.35	0.48 ± 0.22	0.34 ± 0.07

[a] -Retention time in minutes

[b]- Retention index reported in NIST MS version 2.2.

nd- not detected