



UNIVERSITÀ DI PARMA

UNIVERSITÀ DEGLI STUDI DI PARMA

Dottorato di ricerca in
INGEGNERIA INDUSTRIALE
Ciclo XXXVI°

HIGH-PRESSURE PROCESSING FOR FRUIT JUICES: A NON-THERMAL TREATMENT
ALTERNATIVE TO IMPROVE THE QUALITY OF RED BERRY JUICES WITH A FOCUS ON
THE ROLE OF OXIDATIVE ENZYMES

Coordinator: Prof. Gianni Royer-Carfagni

Tutor: Prof. Giuseppe Vignali

Co-tutors: Dr. Luca Cattani, Dr. Luca Sandei

Doctoral candidate: Andres Felipe Moreno Barreto

Anni accademici 2021 -2024

Table of contents

Acknowledgements	5
Abbreviations	7
Introduction	9
1. Red berries characterization	11
1.1. <i>Strawberries</i>	11
1.1.1. Nutritional composition	12
1.1.2. Relevant polyphenols for colour	14
1.2. <i>Sour Cherries</i>	16
1.2.1. Nutritional composition	16
1.2.2. Relevant polyphenols for colour	18
1.3. <i>Grapes</i>	19
1.3.1. Nutritional composition	20
1.3.2. Relevant polyphenols for colour	21
2. The role of enzymes in the stability of food products	24
2.1. <i>Colour change mechanisms associated with enzymes</i>	24
2.1.1. Polyphenol oxidase (PPO)	25
2.1.2. Peroxidase (POD)	26
2.1.3. β -glucosidase.....	26
2.2. <i>Texture-changing mechanisms associated with endogenous enzymes</i>	27
2.3. <i>Flavour change mechanisms associated with enzymes</i>	28
2.4. <i>Nutritional value changes associated with enzymes</i>	29
2.5. <i>Protein extraction and quantification</i>	30
3. Microorganisms involved in food deterioration and foodborne pathogens	34
3.1. <i>Salmonella</i>	35
3.2. <i>Enterohaemorrhagic Escherichia coli (EHEC)</i>	36
3.3. <i>Listeria monocytogenes</i>	37
4. High-Pressure Processing (HPP)	39
4.1. <i>Characteristics of the treatment</i>	39
4.2. <i>Effect on the medium (water)</i>	40
4.3. <i>Effect on microbial inactivation</i>	41
4.4. <i>Effect on enzymes</i>	45
4.5. <i>Effect on other nutrients and physicochemical parameters</i>	46
4.6. <i>HPP machinery</i>	46
4.7. <i>Packaging and HPP</i>	49
4.8. <i>High-Pressure Processing industry in numbers</i>	50
4.8.1. <i>Regulations about food preservation and food products</i>	50

5. Scope of the research	52
6. Methodological approach	54
7. Materials and methods	56
7.1. Materials	56
7.1.1. Fruits	56
7.1.2. Surrogate microorganisms	56
7.1.3. HPP machinery	57
7.2. Methods.....	57
7.2.1. Experimental Design - treatment plan	57
7.2.2. Nectar formulation	58
7.2.3. Microbiology sample preparation	59
7.2.4. Microbiology evaluation	59
7.2.5. Enzymatic extraction.....	60
7.2.6. Enzymatic measurements.....	60
7.2.7. Physicochemical parameters pH and Total soluble solids (TSS).....	62
7.2.8. Colour measurement	62
7.2.9. Total polyphenol content quantification	63
7.2.10. Antioxidant capacity quantification	64
7.2.11. Oxygen barrier test.....	64
7.3. Statistical analysis.....	65
8. Results and discussion	67
8.1. Enzymatic activity initial evaluation.....	67
8.2. HPP treatment in microbial inactivation	70
8.2.1. <i>Salmonella</i> surrogate (<i>E. coli</i> ATCC 11229)	72
8.2.2. EHEC surrogate (<i>E. coli</i> ATCC 8327).....	78
8.2.3. <i>Listeria monocytogenes</i> surrogate - Listeria cocktail.....	84
8.3. HPP treatment in enzymatic inactivation.....	88
8.3.1. Polyphenol oxidase (PPO)	89
8.3.2. Peroxidase (POD)	93
8.3.3. β -glucosidase.....	96
8.4. HPP treatment in total polyphenols content.....	97
8.5. HPP treatment in antioxidant capacity	104
8.6. Optimization of HPP treatment conditions	109
8.7. Shelf-life evaluation.....	111
8.7.1. Physicochemical parameters	112
8.7.2. Enzymatic evaluation.....	116
8.7.2.1. Strawberry nectar	116

8.7.2.2.	Sour cherry nectar	117
8.7.2.3.	Red grape juice.....	120
8.7.3.	Colour evaluation.....	122
8.7.4.	Total polyphenol content	126
8.7.5.	Antioxidant capacity	130
9.	Conclusions and recommendations	133
10.	Bibliography	135

Acknowledgements

To honour all the people involved in this journey, I will need to do a multilingual speech, so here I go:

Quisiera agradecer inicialmente a mi familia: papá, mamá y Esteban, mi querido hermano, que han estado siempre ahí, apoyándome y animándome para que llegue a donde quiera llegar, ahora desde la distancia pero con el mismo cariño y amor de toda la vida. Esto es por y para ustedes, gracias por siempre estar a mi lado.

Gracias también a mis amigos Paula, Dianita, Carito, Mario y Robin, que han estado acompañándome en este proceso desde la distancia, que han visto los sacrificios que ha implicado y que han estado ahí para darme su apoyo siempre que lo he necesitado.

Thanks also to Karen. Over the last few years, you have become not only one of my best friends but also a role model for me. Thanks for being there all this time, supporting me, giving me motivation, and, more than anything, thanks for this beautiful friendship that we have cultivated.

Grazie anche ai miei colleghi della SSICA: Luca, Elettra, Massimo, Rosanna, Valentina e tutti gli altri che mi hanno aiutato tantissimo in questi tre anni. Senza di voi non sarei riuscito a finire questo lavoro. Grazie per tutti i consigli, tutte le ore che abbiamo lavorato insieme e tutte le cose che sono riuscito ad imparare da voi. Sono contento di avere trovato un posto così per avere questa esperienza e di trovare persone così brave nei loro mestieri.

Grazie anche a te, Luca Cattani. Sei arrivato ad aiutarmi tantissimo nell'ultimo anno di questo percorso. Senza il tuo supporto, non sarei riuscito a finire. Grazie per trovare sempre una soluzione a ogni singolo problema e per avere sempre un'attitudine positiva.

Thanks to the HiStabJuice gang, particularly to Sebastian and Ibrahim, for our collaborations, the times that we shared together, and especially for the laughs. This time has been very challenging for us, but I am very happy for the chance to meet every one of you. Thanks also to the HiStabJuice project itself, for the opportunity.

Grazie a tutte le persone che ho conosciuto a Parma: Miriam, Fabio e i ragazzi dell'ufficio dottorandi al Tecnopolo. Voi avete reso i miei giorni qui più piacevoli.

Finalmente, gracias a mí, por la resiliencia, por todas las veces que estuve a punto de desistir de este proyecto y con fuerza de voluntad logré superar mis dudas y miedos.

This thesis has received funding for the Europe Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Innovative Training Networks (ITN). Grant agreement No. 956257.

Abbreviations

BHI: Brain Heart Infusion Broth

BSA: Bovine serum albumin

CDC: Center for Disease Control and Prevention

CFSAN: Center for Food Safety and Applied Nutrition

CFU: Colony-forming unit

DM: Dry matter

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

EC: Enzyme Commission number

ECDC: European Centre for Disease Prevention and Control

EDTA: Ethylenediamine tetraacetic acid

EFSA: European Food Safety Authority

EGTA: ethylene glycol tetraacetic acid

EHEC: Enterohaemorrhagic *Escherichia coli*

EVOH: Ethylene vinyl alcohol

FDA: U.S. Food and Drug Administration

FSIS: Food Safety and Inspection Service

FW: Fresh Weight

GAE: Gallic acid equivalent

HACCP: Hazard analysis and critical control points

HPL: Hydroperoxide lyase

HPP: High-Pressure Processing

LOX: Lipoxygenase

NT: Non-treated

OTR: Oxygen Transmission Rate

PA: Polyamide

PE: Polyethylene

PET: Polyethylene terephthalate

PG: Polygalacturonase

PME: Pectin methylesterase

PMSF: Phenylmethylsulfonyl fluoride

POD: Peroxidase

PP: Polypropylene
PPO: Polyphenol oxidase
PVP: Polyvinylpyrrolidone
PVPP: Polyvinylpolypyrrolidone
RA: Residual activity
RH: Relative Humidity
SRM: Surface response methodology
STEC: Shiga toxin-producing *Escherichia coli*
TA: Titratable acidity
TAC: Total antioxidant capacity
TEAC: Trolox equivalent antioxidant capacity
TMC: Total Microbial Count
TPC: Total Polyphenol Content
TSS: Total Soluble Solids
T: treated
USDA: United States Department of Agriculture

Introduction

Fruits and vegetables are an essential part of human diets and are consumed around the world in their fresh form. However, due to their high perishability and, in many cases, their limitations to grow throughout the year due to climate conditions, other alternatives to fresh products have been developed.

Red berries are not an exception; strawberries are one of the most consumed fresh fruits worldwide due to their characteristic flavour and appearance, and are also highly consumed in juices, jams, and other preparations. Sour cherries, unlike sweet cherries, are mainly consumed in prepared products such as liquors, jams, and juices because of their characteristic acidic taste that requires some processing to increase their palatability. Moreover, red grapes are a very important crop, not only as the fruit consumed fresh but also as the most important raw material of the winery industry. These three fruits, among other red berries, are rich in nutritional compounds and bioactive molecules, such as polyphenols, which are responsible for the promotion of health benefits mainly associated with their antioxidant capacity. These organoleptic and bioactive properties that make these fruits interesting for consumers can be altered by fruit spoilage; here, microbial growth can deteriorate the cell wall structure of the fruit, leading to visible structural damage. However, microorganisms are not the only responsible for fruit deterioration, and endogenous enzymes are also responsible for changes in the appearance when degrading pectin and colour by their action on anthocyanins, changing the colour from characteristic red to brown or even a faded colour. For these reasons, processing is required to preserve not only the microbial stability of the product, but also its properties.

Juice, as previously mentioned, is one of the multiple product alternatives currently in the market for fruits. Traditionally, thermal processing has been used to preserve fruit juices from a microbiological perspective, in addition to the enzymatic inactivation achieved with this technique. However, an increasing number of consumers are interested in minimally processed products, which is supported by the idea that certain processing steps are detrimental to the nutritional quality of the product. In the case of fruit juices, with the application of heat, thermally sensitive molecules, such as vitamins, aroma compounds, or polyphenols, can be degraded, leading to an overall reduction in quality despite food safety.

Therefore, alternative methods have been investigated in order to guarantee microbial stability while reducing food quality alterations as much as possible. It is here where High-Pressure Processing has become an interesting alternative in the juice industry. The principle is to use pressure instead of temperature to preserve food products, leading to a product with possibly less alteration in terms of organoleptic properties, considering that pressure will not alter, for instance, aroma compounds, maintaining the characteristics of the product similar to that of the fresh one. Nevertheless, despite the demonstrated effectiveness of this technology in terms of food preservation, enzymatic inactivation is limited under current industrially achievable conditions.

Furthermore, the effect of this technology on the preservation and maintenance of food quality is not only limited to the processing itself but is also related to the shelf stability of the product, which, in the long run, will be the product that consumers will find in the shelves and that is not only affected by the process but also the characteristics of the packaging material selected for each specific case. It is in the shelves where, based on the appearance of the product, potential consumers will judge the quality, which will ultimately influence purchase decision making.

The aim of this study was to evaluate the potential of High-Pressure Processing as a non-thermal pasteurisation technology, specifically in the preservation of juices and nectars from red berries, assessing its effectiveness in terms of microbial and enzymatic inactivation, as well as bioactive compound preservation, leading to the optimisation of process parameters to achieve the best possible conditions for these products. Furthermore, different packaging alternatives were compared in a shelf-life study to assess the behaviour of the product after treatment under optimised conditions.

1. Red berries characterization

1.1. Strawberries

Wild and cultivated strawberries (*Fragaria virginiana* and *Fragaria x ananassa*) belong to the genus *Fragaria* and Rosaceae family and are widely consumed worldwide as functional foods due to their chemical composition, which is rich in bioactive compounds such as sugars, organic acids, and polyphenols, among others (Aaby et al., 2007; Ganhão et al., 2019; González-Domínguez et al., 2020; Mandave et al., 2014). Strawberries are produced worldwide, with China being the main producer in 2020, followed by the United States, Egypt, and Mexico (FAOSTAT, 2020c). The structure of the berry is shown in Figure 1, where the outer layer is formed by epidermal cells and the internal parts constitute hypodermal and cortical cells (Jewell et al., 1973).

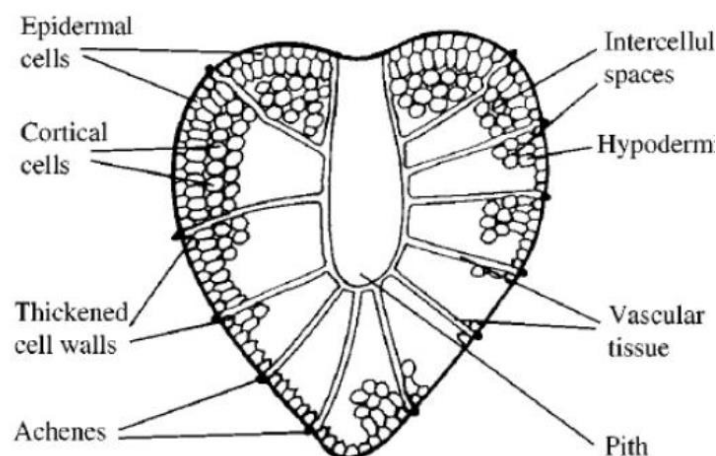


Figure 1. Strawberry structure (Jewell et al., 1973)

The cultivation time for strawberries is approximately 30 days to obtain fully ripened berries; however, the cultivation period is highly dependent on factors such as temperature, light, and soil composition, among others (Cordenunsi et al., 2002). Along with environmental factors, variety also plays a key role in the characteristics of the product, affecting its chemical composition and appearance attributes (Cordenunsi et al., 2002).

1.1.1. Nutritional composition

Strawberry composition has been extensively investigated considering its importance in terms of nutrition. According to the U.S. Department of Agriculture, strawberries are mainly composed of water, with accounts for about 90% of their weight composition, and only 10 % of total soluble solids (TSS), being sugars the major component of these solids, with approximately 8 g per 100 g of fresh product (Giampieri et al., 2012; USDA, 2018).

S. Y. Wang et al. in 1997 evaluated the chemical composition of 24 strawberry varieties, finding soluble solids ranging from 6.7% to 10.2 % (varieties “Settler” mid-season and “MDUS5036” late season, respectively) with a total average of 7.9%. Similar results were found by Ganhão et al. (2019), who evaluated the nutritional composition of three different strawberry cultivars (Primoris, Endurance, and Portola) and found moisture content between 86.3% and 89.9% and soluble solids between 4.89% and 8.51%, indicating the influence of the variety and possible cultivation conditions.

Soluble solids and moisture content are not only different between varieties but also during fruit development. Ornelas-Paz et al. (2013) evaluated the changes in the chemical composition of *Fragaria* × *ananassa* Duch, Cv. Albion, at six stages of ripening; soluble solids constantly increased from the first to the sixth stage (6.6% to 9.0%, respectively), while moisture content did not show a significant change. Comparable results were found by Pineli et al. (2011), who characterised two strawberry varieties along three ripening stages and found a significant increase in soluble solids for the Osogrande and Camino real varieties between the green and pink stages. Ripe Osogrande fruits presented similar values as pink stage and Camino real presented a decrease.

With respect to the specific sugar composition, fructose, glucose, and sucrose glucose levels have been reported by different researchers. S. Y. Wang et al. in 1997 reported the percentage of those sugars for 24 fruit varieties; the variability within fruit for these compounds is high, making it impossible to establish any trend with the presented data; however, a total sugar quantification shows “Settler” as the variety with the lowest total sugar (4.46%) and “MUDS5036” with the highest total sugar content (6.98%). Similarly, Cao et al. (2018) evaluated the chemical composition of four Chinese strawberry varieties (Hongyan, Tianxiang, Tongzi I, and Zhangji) and found that fructose was the most abundant

sugar among all varieties (from 4.65% to 2.88%), followed by glucose (4.25% to 2.36%) and sucrose (0.75% to 0.015%).

Variations with respect to the ripening stage can also be accounted for. Ornelas-Paz et al. (2013) also reported variation in these three sugars during the ripening stages, showing an increase in the concentration of all sugars during the second stage, followed by a reduction until the fourth stage. After this, only fructose and glucose showed a new increase, whereas sucrose remained at low levels.

Acidity is another crucial factor contributing to the flavour of strawberries. Despite not being as abundant as sugars, organic acids play an important role in flavour perception. Titratable acidity (TA) is measured to account for the total hydrogen ions in solution that can react with a strong base (Siggaard-Andersen, 1966). This parameter has been used by many authors to characterise fruits and vegetables. The study conducted by S. Y. Wang et al. in 1997 shows a variation from 0.44% in the varieties “Lester” and “Allstar” to 0.85% attributed to the variety “NJUS8219-2”. Comparable results were reported by Cao et al. (2018), finding TA values varying from 0.63% to 0.79% among the four varieties. This variation is also observed during the ripening process, with a reduction in TA content as ripening progresses (Ornelas-Paz et al., 2013; Pineli et al., 2011).

A relevant parameter useful for describing the variation between sugars and acids is the TSS/TA ratio. This factor allows for a comparison of different varieties and ripening stages. S. Y. Wang et al. in 1997 reported a TSS/RA ratio varying from 9.06 in “NJUS8219-12” to 18.93 in “Lester2. Similarly, it has been reported that there is a variation of this ratio between 8.28 and 13.80 for “Tongzi I” and “Hongyan”, respectively (Cao et al., 2018), accounting for variability between varieties. With respect to ripening stages, a trend of increasing TSS/TA ratio with ripening stages can be observed, mainly because of changes in the concentration of sugars, considering that TA is highly stable during the ripening process (Ornelas-Paz et al., 2013; Pineli et al., 2011).

Citric, malic, and tartaric acids are the most abundant organic acids present in strawberries. S. Y. Wang et al. (1997) reported a variation between 24 strawberry varieties for citric acid (between 0.31% in “Allstar” and 0.73% in “NJUS8219-2” varieties) and malic acid (between 0.02% in the varieties

“Lester”, “Lategrow” and “Jewel”, and 0.09% in the variety “Settler”). Similarly, González-Domínguez et al. (2020) investigated the chemical composition of five strawberry varieties (Aromas, Camarosa, Diamante, Medina, and Ventana), finding citric acid to be the most abundant organic acid, followed by malic and tartaric acid, maintaining values in similar ranges to those in the previous study. Changes during fruit development are also expected. The concentration of citric acid has been reported to decrease significantly as cultivation time increases, whereas the concentration of malic acid remains mostly constant during the same period (Ornelas-Paz et al., 2013).

Strawberries are also a source of minerals, such as calcium, magnesium, phosphorus, potassium, and vitamins, including vitamin C, at approximately 60 mg per 100 g (Giampieri et al., 2012; González-Domínguez et al., 2020). Ascorbic acid concentration has been reported by S. Y. Wang et al. in 1997, showing values from 70.4 mg/100 g FW for the “Northeastler” variety to 112.6 mg/100 g FW for the “Earlyglow” variety. Similar results have been reported for Chinese strawberry varieties, varying from 21.38 to 42.89 mg/100 g FW (Cao et al., 2018). An increase in ascorbic acid content during ripening has also been reported (Ornelas-Paz et al., 2013).

1.1.2. Relevant polyphenols for colour

The phenolic compounds in strawberries include flavonoids, derivatives of hydroxycinnamic and ellagic acids, and anthocyanins; the latter group is responsible for the attractive red colour present in the fruit (Aaby et al., 2007). A complete list of the phenolic compounds present in strawberries was collected by Giampieri et al. (2012). Similar to other chemical compounds, high variability has been found because of differences in cultivation conditions, variety, and ripening stages. A study conducted by Aaby et al. (2012) found that among the polyphenols present in strawberries, anthocyanins corresponded to glucosides and malonyl glucosides of both pelargonidin and cyanidin, in addition to rutinosides and acetyl glucosides of pelargonidin. In this study, 26 different cultivars were evaluated, and the most abundant anthocyanin was pelargonidin-3-glucoside, accounting for 60-95% of the total anthocyanin content, followed by pelargonidin-3-malonylglucoside (0-35%). Their molecular

structures are shown in Figure 2. Other anthocyanins, such as cyanidin-3-glucoside and pelargonidin-3-rutinoside, were also present in smaller amounts.

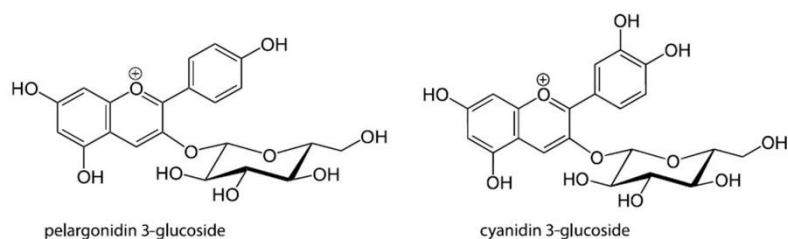


Figure 2. Most abundant anthocyanins in strawberry. Adapted from (Fornasaro et al., 2016)

These results agree with other studies that also evaluated anthocyanin content: Cao et al. (2018), evaluated the anthocyanin content of 4 different strawberry cultivars, finding pelargonidin-3-glucoside to be the most abundant anthocyanin among all the cultivars tested, followed by cyanidin-3-glucoside and pelargonidin-3-rutinoside. Furthermore, the study presented by González-Domínguez et al. in 2020, who evaluated the chemical composition of 5 different cultivars, also found pelargonidin-3-glucoside as the main anthocyanin present, followed by cyanidin-3-glucoside and pelargonidin-3-rutinoside. These results are in agreement with the findings of Dzhahfezova et al. (2020), who evaluated 18 cultivars harvested in two different years (2014 and 2015).

The relationship between the increase in the concentration of anthocyanins and plant development is expected, considering that, with ripening, the fruit acquires its characteristic red colour as a consequence of the increase in anthocyanins. Ornelas-Paz et al. (2013) conducted a study of one strawberry cultivar at six ripening stages. It shows an increase in the concentration of pelargonidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3-rutinoside, and pelargonidin-3-O-malonylglucoside while the ripening stages increased. This is also associated with an increase in Total Polyphenol Content (TPC) reported by other authors (Ornelas-Paz et al., 2013; Pineli et al., 2011).

1.2. Sour Cherries

Sour cherries (*Prunus cerasus* L.), which belong to the Rosaceae family, are characterised by their colour ranging from light red to dark red, sour taste, juicy flesh, and pleasant aroma. It is a fruit created by the natural hybridation between ground cherry (*Prunus fruticosa* Pall.) and sweet cherry (*Prunus avium* L.) (Alrgei et al., 2016; Sokół-Łętowska et al., 2020). The fruit was initially cultivated in the areas around the Caspian and Black Seas, and breeding processes were first successful in Russia and other countries in Europe (Germany, Romania, and Hungary), and further in the U.S. (Alrgei et al., 2016; Blando & Oomah, 2019). According to FAOSTAT (2020a), the top producers of sour cherries in 2020 were European countries, with Russia, Turkey, Ukraine, Serbia, and Poland being the largest producers. Sour cherry is widely used as fresh, but mainly as processed fruit; approximately 85% of sour cherry is processed in products such as juices (approximately 30 to 40%), canned fruit, brandy, and liqueurs, among others (Cairone et al., 2023; Sokół-Łętowska et al., 2020; Yılmaz et al., 2019). Its popularity has increased in recent years owing to its chemical composition, which is mostly related to its high content of polyphenols (anthocyanins, flavanols, and hydroxycinnamic acids) (Cairone et al., 2023; Yılmaz et al., 2019).

1.2.1. Nutritional composition

Several studies have reported the chemical composition of sour cherries. Bánáti et al. (2010) evaluated the chemical composition of 33 varieties cultivated under organic farming and integrated conditions in 2008 and 2009. They reported a range of TSS from 15.6% for the variety Érdi bôtermô bio (2008) to 21.1% for the variety VN-1 (2009). Equivalent results have been reported by other researchers: a range from 14.1 to 18.5% for TSS was found in a study evaluating 41 Oblaczynska sour cherry genotypes (Rakonjac et al., 2010). Moreover, in a study of 33 sour cherry varieties, TSS ranged from 13.5% to 25.15% (Wojdyło et al., 2014). These and other reports confirm the dependence of this parameter (and, in general, chemical composition) on variety, cultivation practices, and environmental conditions (Blando & Oomah, 2019; Sokół-Łętowska et al., 2020). Studies have also evaluated this parameter for

sour cherry products: Damar & Ekşi (2012), evaluated juices from 11 different Turkish sour cherry varieties, reporting TSS ranging from 16.0 to 26.3 %. With respect to ripening, Pedisić et al. (2007) reported an increase in the soluble dry matter with respect to fruit development for the Marasca variety, ecotype Recta, cultivated in two different regions of Croatia.

Regarding sugar composition, the main sugars present in sour cherries were fructose, glucose, and sucrose. Alrgei et al. (2016), investigated the sugar composition of 33 Oblaczynska clones, finding fructose (0.023–4.246 g/100g FW) and glucose (0.063–4.759 g/100g FW) to be the most abundant in all clones, followed by sucrose (0.009–0.432 g/100g FW). Other results were presented when evaluating 21 cultivars from different worldwide locations: in this case, glucose was the most abundant sugar present (2.18–5.68 g/100 g FW), followed by fructose (2.74–4.88 g/100 g FW). Interestingly, this study evaluated sorbitol (1.93–3.12 g/100 g FW), being most abundant than sucrose (0.0–1.41 g/100 g FW), and even some traces of galactose and xylose (Sokół-Łętowska et al., 2020). Other studies have also reported glucose to be the most abundant sugar in Hungarian sour cherry cultivars (Blando & Oomah, 2019).

Acidity is one of the most important factors that affect the taste of sour cherries. The study conducted by Bánáti et al. (2010) also evaluated TA for 33 cultivars, finding a minimum of 0.578% and a maximum of 2.134%, which are considerably higher than those of other berries, such as strawberries. Similar results were obtained in studies by Rakonjac et al. in 2010, which reported a range from 1.05 to 1.49%, and Wojdyło et al. in 2014, with a range from 0.94 to 2.79% TA. This parameter, in contrast to TSS, is not significantly affected by ripening (Pedisić et al., 2007). Comparable results were obtained when evaluating sour cherry juice, considering that organic acids, such as sugars, are water-soluble compounds that remain in the product after extraction. Damar & Ekşi (2012) reported a variation in TA in sour cherry juice from different cultivars, with values between 1.64 and 2.63%.

Among the most abundant organic acids, malic acid has been reported as the predominant one. In the study conducted by Sokół-Łętowska et al. in 2020, malic (1.027–1.976 g/100 g FW), malonic (0.213–0.406 g/100 g FW), oxalic (4.75–23.44 mg/100 g FW), shikimic (1.05–4.08 mg/100 g FW), and fumaric acids (0.15 – 0.35 mg/100 g FW) were reported to be present in 21 cultivars of sour cherry. Ascorbic

acid concentration has also been evaluated in numerous studies. Wojdyło et al. (2014) found a range for ascorbic acid from 5.45 to 22.18 mg/100g FW, which is considerably lower compared to strawberry fruit.

1.2.2. Relevant polyphenols for colour

The most abundant anthocyanins present in sour cherry fruit are derivatives of cyanidin, with cyanidin-3-*O*-glucosyl-rutinoside being the main constituent in this group according to the literature (Alrgei et al., 2016). In the evaluation of five Hungarian cultivars, Homoki et al. (2016) found cyanidin-3-rutinoside to be the most abundant anthocyanin, followed by cyanidin-3-glucoside and cyanidin-3-glucosylrutinoside, respectively. Similarly, cyanidin-3-*O*-glucosylrutinoside, cyanidin-3-*O*-sophoroside, and cyanidin-3-*O*-rutinoside have been identified as the main anthocyanins present in 33 sour cherry cultivars worldwide (Wojdyło et al., 2014). As expected, fruit development and anthocyanin concentration are directly correlated; an increase in total anthocyanins with an increase in harvesting time has been reported in a study comparing the same variety cultivated in two regions of Croatia (Pedisić et al., 2007).

The content and stability of anthocyanins in derivative products of sour cherry fruit are very important, considering that their consumption is strictly linked to the antioxidant capacity of polyphenols, and anthocyanins can play a role as an indicator of the retention of this attribute. Damar and Ekşi (2012) reported the concentration of anthocyanins in fruit juices coming from 11 different cultivars. In their report, cyanidin-3-glucosylrutinoside remained the most common anthocyanin, followed by cyanidin-3-rutinoside, and cyanidin-3-sophoside. With respect to stability, liqueurs prepared with sour cherry fruit (with and without the addition of sugar) were subjected to stability evaluation under two temperature conditions (15 and 30°C) for 24 weeks. The results showed cyanidin-3-*O*-glucosylrutinoside was the most abundant anthocyanin present in the product, followed by cyanidin-3-*O*-rutinoside. Comparing the results before and after storage, liqueurs with added sugar presented a

higher rate of anthocyanin degradation, which was not as significant as the effect of temperature; the most stable liqueurs were stored at lower conditions (Sokół-Łętowska et al., 2018).

1.3. Grapes

Grape vine (*Vitis* spp.) is a non-climacteric berry produced in different locations worldwide. The essential parts of the fruit include pulp, seeds, and skin (Granato et al., 2016). The skin consists of six to ten layers of thick-walled cells covering the flesh, the outermost layer of which is also covered with a coating that makes the berry waterproof. Following the skin, pulp is found, which is the majoritarian part of the berry in which the juice is contained. Moreover, in the centre of the berry, it is possible to find the seeds rich in tannins (Dharmadhikari, 1994). Grapes can be black, yellow, green, orange, blue, violet, or pink colour, depending on the particular characteristics of the variety. This is associated with the presence or absence of anthocyanins, mainly allocated in the skin (Dharmadhikari, 1994; Granato et al., 2016).

By 2020, the major grape provider worldwide was China, followed by well-known European countries in terms of wine production (Italy, Spain, and France) and other producers such as the U.S., Turkey, India, Chile, and Argentina (FAOSTAT, 2020b). Grapes are not only consumed as fresh fruits but also as juice or wine (Granato et al., 2016).

Grape juice is a clear or cloudy juice extracted from grape berries; its colour can be red, white, or rose, depending on the variety used (Cosme et al., 2018). Three main technologies are currently used to produce grape juice: initial steps for all technologies start with reception and cleaning of the fruit to follow the crushing step; after the difference can be identified, for the hot pressing method, crushed grapes are subjected to thermal treatment at 60-95°C for 30 to 60 min, with the addition of pectolytic enzymes (maceration), after which the grapes are pressed, and the mass follows a filtration step to separate the pomace from the juice. Further steps include pasteurisation of the juice, cooling, and packing. The cold press method differs from the previous method in that it does not increase the temperature during the maceration step. Finally, for the hot break process, a step after crushing is added:

here the temperature is increased between 57 and 85°C for a short period of time (5-10 minutes) to inactivate oxidative enzymes, after which the mass is cooled to maceration at 60°C (Cosme et al., 2018; Granato et al., 2016; G. G. Silva et al., 2019). The current interest in grape juice is due to its phenolic composition, which is related not only to colour-responsible compounds but also to non-coloured polyphenols responsible for antioxidant activity (Cosme et al., 2018; Kersh et al., 2023).

1.3.1. Nutritional composition

Similar to strawberries and sour cherries, sugars are the major constituent of the total composition of grapes, followed by organic acids (Dharmadhikari, 1994; Kersh et al., 2023). Their concentration is often referred to as the TSS and TA parameters. Different studies have provided information for these parameters: Lovino et al. (2006) evaluated the physicochemical parameters of 10 varieties of red grapes produced in the south of Italy, showing a range for TSS from 21.0 to 27.3% (varieties Uva di Troia and Frappato, respectively) and 4.5 to 9.7 g/L of total acidity (varieties Gaglioppo and Frappato, respectively), this last parameter is also used to describe acidity in food products, and its quantification includes all organic acids, being generally slightly higher than TA (Boulton, 1980). A similar variation was presented by Orak (2007), that characterised 16 red grape cultivars obtaining a variation from 13.89 to 24.46 % of TSS (cultivars Bogazkere and Gewürztraminer, respectively) and 3.3 to 9.53 g/L for total acidity (cultivars Md. Jean Mattihas and Papazkarası, respectively). Grape juice has also been characterised using these parameters; an extensive review by Granato et al. (2016) collected the chemical characteristics of different grape juices produced worldwide, identifying species, origin, physicochemical properties, chemical markers, and antioxidant activity. Other authors presented similar values for grape juice: Burin et al. (2010) reported TSS and TA for homemade, commercial, and organic grape juice, showing values of 10.0 to 19.0% and 5.60 to 9.84 g/L, respectively; Miele et al. (2015) compared 27 organic grape juices from the Brazilian region of Serra Gaucha, finding TSS content of 12.7 to 17.5% and TA of 48 to 114 meq g/L.

Regarding the specific sugar composition, glucose and fructose are the predominant sugars, present in similar amounts (ratio 1:1), and vary depending on cultivation practices, environmental conditions, and origin (Granato et al., 2016; Kersh et al., 2023). The organic acid composition is mainly distributed between tartaric and malic acids, accounting for approximately 90% of the total acid composition (Dharmadhikari, 1994; Granato et al., 2016; Kersh et al., 2023). A study for southern Italian red grapes investigated the concentration of tartaric and malic acid, finding ranges from 3.33 to 9.48 g/L for tartaric and 0.73 to 3.98 g/L for malic (Lovino et al., 2006). L. R. Silva and Queiroz (2016) showed tartaric and succinic were the most abundant, followed by malic, quimic, and fumaric acids, when evaluating the chemical composition of selected Portuguese red grapes.

Similar results have been reported for grape juice, where the concentrations of different organic acids were investigated. Toaldo et al. (2015) reported differences in the organic acid composition regarding agricultural practices (organic vs. conventional), showing however that malic and tartaric acids are the most abundant.

Studies have also reported that phosphorus, potassium, sodium, and calcium are the most abundant minerals (Granato et al., 2016; Miele et al., 2015; Toaldo et al., 2015).

1.3.2. Relevant polyphenols for colour

As previously stated, grapes are well-known for their polyphenol content; among them, anthocyanins are responsible for the characteristic colour of the fruit. Kersh et al. (2023) gathered in a review the major anthocyanins found in grapes or grape juice, according to their origin and variety. These compounds are not equally distributed in the berry; Castillo-Muñoz et al. (2009) reported malvidin-3-glucoside and peonidin-3-glucoside as the most abundant anthocyanins in Garnacha Tintorera, a grape cultivar produced in middle-southern Spain (mainly present in the skin and the flesh, respectively), 20 different anthocyanin compounds were identified and they varied depending on the part of the fruit where they were allocated. Other results confirm not only the high concentration of anthocyanins allocated in the skin, but also their high variability with respect to variety and origin. Zhu et al. (2012)

studied the phenolic composition of grape skins from varieties covering different regions (European, Asian, Euro-Asian, North American, Euro-American, and Muscadine grapes) and obtained interesting results. European varieties presented the same results as the study by Castillo-Muñoz et al. in 2009, which was expected considering that these grapes belong to this region but differ from others. There was no pattern in between regions, finding malvidin-3,5-diglucoside as the major anthocyanin in Asian varieties, delphinidin-3-glucoside as the most abundant in the Euro-Asian hybrids, the same as for the North American varieties, peonidin 3-glucoside for the Euro-American hybrids, and cyanidin 3,5-diglucoside for Muscadine grapes. This shows the high variability between fruits produced in different regions, which not only affects the content of principal macronutrients but also the composition of bioactive compounds.

Other European studies showed comparable results: Southern Italian varieties presented a similar profile compared to the Spanish and overall European varieties already presented (finding that the major constituent malvidin-3-glucoside, peonidin-3-glucoside, and delphinidin-3-glucoside varying depending on the variety). Additionally, this study showed how the same variety cultivated in different growing areas in the same region can affect the total content of specific anthocyanins (Lovino et al., 2006). Portuguese varieties are not an exception, presenting similar anthocyanin profiles compared to their Spanish and Italian peers (malvidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside were found to be majoritarian anthocyanins) (L. R. Silva & Queiroz, 2016).

The phenolic composition of the grape juice was also investigated. Considering that the skin contains a high amount of polyphenols, it is interesting to evaluate the effect of processing. When evaluating grape juice made with *V. labrusca* L. varieties Bordo, Isabel, and Niagara Branca produced in southern Brazil, the main anthocyanins found were malvidin-3,5-glucoside and cyanidin-3,5-glucoside, which presented similar anthocyanin profiles to conventional and organic farming practices (Toaldo et al., 2015). Additional work was gathered by Cosme et al. in 2018 showing the possible variability regarding three different aspects: processing conditions, when comparing maceration processes at 50 and 60 °C, finding a higher anthocyanin concentration at the highest temperature evaluated, varieties, and agricultural

practices, once again demonstrating the intrinsic variability of polyphenol content depending on the characteristics of the varieties and environmental conditions.

2. The role of enzymes in the stability of food products

Enzymes play a key role as catalysts in biological systems, being promoters of biological reactions that under no other natural conditions will occur. In plants, enzymes are essential for physiological development and metabolism, with different expression depending on the harvesting stage but remaining active post-harvest (Terefe et al., 2014). Their activity is crucial in promoting plant development and in cases where ripening is desired in postharvest storage; however, their presence might promote some undesirable effects, affecting important quality attributes such as colour, texture, flavour, and even possible nutritional value (Indrawati et al., 2002; Terefe et al., 2014).

No general statement can be made about the role of specific enzymes in the deterioration of fruit and vegetable quality, because factors such as nutritional composition, the type of enzymes present in the plant, and even the endogenous microbiota influence undesirable changes in plant-based products (Terefe et al., 2014). Nevertheless, it is possible to cluster enzymes according to their mechanisms of action and their effects on specific nutritional components that act as substrates.

2.1. Colour change mechanisms associated with enzymes

Colour plays an undeniable role in consumer acceptance, particularly for fruit and vegetable products. This is the first perceived quality indicator that drives the purchase process. Enzymes can highly affect the colour stability of fruits and vegetables and their products and are without doubt related to the colour responsible molecules associated with each product. Green products can be the target of some specific enzymes: LOX, which is mainly associated with flavour degradation, can also promote the loss of colour by degrading chlorophyll and carotenes (L. R. Ludikhuyze et al., 1998). Chlorophyllase has also been observed to degrade chlorophyll in the stems of pear cactus (Guevara et al., 2003), coleslaw cabbage (Heaton et al., 1996), and broccoli (Funamoto et al., 2002). Green decolourisation in processed garlic and pink colour loss in onion has been attributed to alliinase enzymes (Bai et al., 2005; Lee et al., 2007). Fruit products containing high amounts of anthocyanins present different enzymatic degradation pathways, which are also associated with different enzymes.

2.1.1. Polyphenol oxidase (PPO)

Polyphenol oxidase (EC 1.10.3.1) is a category of copper-containing enzymes responsible for the degradation of phenols in the presence of oxygen (Terefe et al., 2014; Yada & Jackman, 2012). PPO can be found in animals where is responsible for skin pigmentation, including freckle development (Sabarre Jr & Yagonia-Lobarbio, 2021). In the plant kingdom, PPO can be found as membrane-bound or soluble enzymes in plastids separated from their substrates (phenols), which are located in the vacuoles, meaning that both enzymes and substrates can be in contact only after processes such as aging or direct injury of the plant tissue (L. Ludikhuyze et al., 2003; Sabarre Jr & Yagonia-Lobarbio, 2021; Terefe et al., 2014). PPO is responsible for three types of reactions (Figure 3): cresolase activity, which consists of the hydroxylation of monophenols to form o-diphenols; catecholase activity, which is related to the oxidation of o-diphenols to produce o-quinones; and laccase activity, which transforms p-diphenols into p-quinones. After these reactions occur, o-quinones (yellow in colour and highly unstable) can undergo a non-enzymatic polymerisation stage, forming black, brown, and red pigments commonly known as melanins (Sabarre Jr & Yagonia-Lobarbio, 2021; Tomás-Barberán & Espín, 2001).

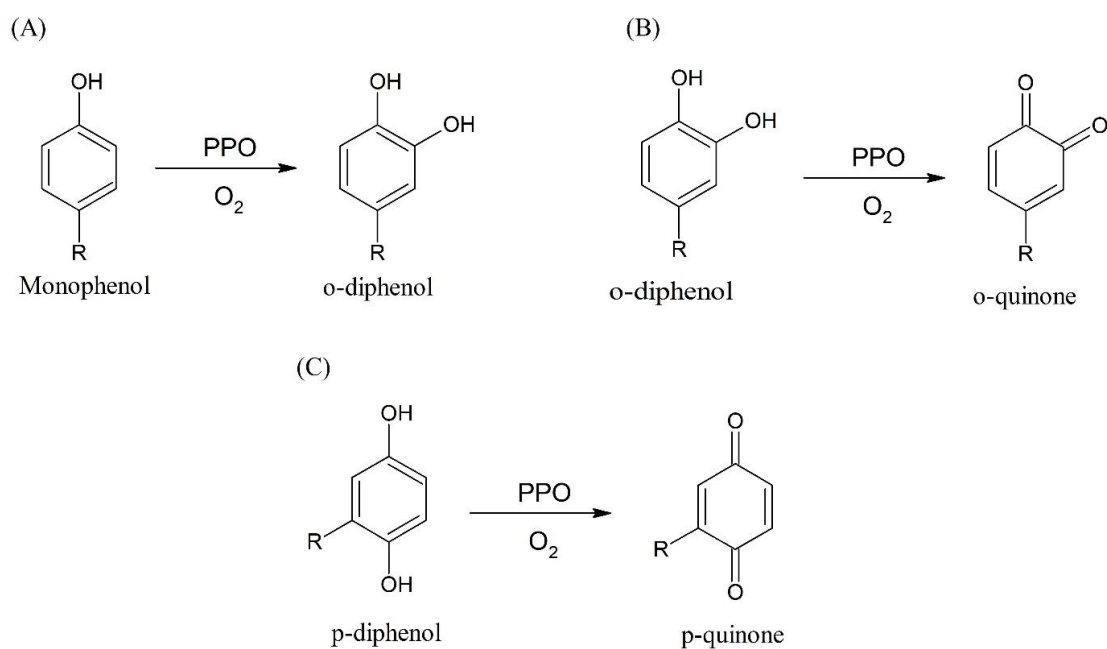


Figure 3. Reactions catalysed by PPO: (A) Cresolase activity, (B) Catecholase activity and (C) Laccase activity (Sabarre Jr & Yagonia-Lobarbio, 2021).

In terms of possible substrates, anthocyanins cannot be directly degraded by PPO due to steric conditions. To promote this reaction, it is necessary to remove the sugar moiety, job that is made by β -glucosidase enzymes, creating anthocyanidins susceptible to degradation by PPO enzymes. Alternatively, anthocyanins can react with products of the PPO oxidation pathway to produce brown compounds (Jiang, 2000).

2.1.2. Peroxidase (POD)

Peroxidase (EC 1.11.1.7) is widely distributed not only in the plant but also in the animal kingdom, being responsible for the control of peroxide levels in oxygenation reactions, reducing the formation of radicals potentially harmful for living forms. Plant POD, usually referred to as a single enzyme, corresponds to a wide variety of soluble and bound isoforms (Tomás-Barberán & Espín, 2001; van Dijk & Tijssens, 2000). POD is responsible for the catalysis of single-electron oxidation of different compounds, including phenols, aromatic amines, thioanisoles, halide ions, thiocyanate ions, and fatty acids in the presence of hydrogen peroxide (Shah et al., 2014; Tomás-Barberán & Espín, 2001). It catalyses the oxidation of phenolic compounds to form brown compounds including anthocyanins (López-Serrano & Ros Barceló, 2002; Zhang et al., 2005). Because its action is limited by the presence of hydrogen peroxide, its relevance is frequently questioned because of the low concentration of hydrogen peroxide present in the plant structure. However, it has been hypothesised that polyphenol oxidase (PPO) could act as a promoter, considering that the oxidation reaction of this enzyme generates hydrogen peroxide as a co-product after the oxidation of phenolic compounds (Tomás-Barberán & Espín, 2001).

2.1.3. β -glucosidase

β -glucosidases are responsible for catalysing the hydrolysis of aryl and alkyl β -glucosides to produce β -D-glucose. In plants, they are involved in important metabolic functions, such as chemical plant defence against pathogens and the activation of conjugates of plant growth regulators by the hydrolysis

of β -glycosidic bonds (Orruño et al., 2001; Terefe et al., 2014). These enzymes are highly specific; for example, anthocyanase (anthocyanin- β -glucosidase) is responsible for the hydrolysis of the sugar moieties of anthocyanins that produce anthocyanidins, which are highly unstable and susceptible to oxidation by PPO and POD enzymes (Chakraborty et al., 2014; Zhang et al., 2005). Orruño et al. (2001) characterised β -glucoside extracts from strawberries (*Fragaria* \times *ananassa*, cv. Elsanta), finding the optimal conditions for their activity with respect to pH (4.0) and temperature (55–60 °C).

2.2. Texture-changing mechanisms associated with endogenous enzymes.

Texture is one of the most important factors contributing to the quality of fruits and vegetables, and its deterioration is associated mainly with changes occurring in the cell wall, which is highly variable between different fruits and vegetables, making them more or less susceptible to quality loss (Terefe et al., 2014). Among the different structures present in the cell wall, pectin is the most significantly affected by enzymatic and non-enzymatic degradation (van Dijk & Tijskens, 2000). Three enzymes are known for their impact on pectin degradation:

Pectin Methylesterase (PME)

Pectin Methylesterase is found in not only all higher plants but also in phytopathogenic fungi and bacteria. Their role (considering the expression of different isoforms) has been associated with different physiological processes, such as stem elongation and tuber yield, root development, and fruit softening (Giovane et al., 2004).

The role of PME (EC 3.1.1.11) is to catalyse the de-esterification of pectin, producing pectin with a lower degree of esterification and methanol (Manmohit Kalia, 2015; Terefe et al., 2014). PME de-esterification can result in an apparent increase in firmness by producing unesterified carboxyl group blocks that can potentially form gels, promoted by their interaction with calcium ions (Giovane et al., 2004). This mechanism has inspired low-temperature blanching treatments aimed at reducing the softness after thermal processing (Ng et al., 1998; Stolle-Smits et al., 2000; van Dijk & Tijskens, 2000) or further degradation of the pectolytic structure owing to the action of other enzymes.

In the case of fruit juices, the main effect of PME action is not associated with softness, but with cloud loss (an attribute highly appreciated by consumers and used as an indicator of freshness). PME is then responsible for phase separation, which directly affects the appearance of the product (Baker & Cameron, 1999).

Polygalacturonase (PG)

Polygalacturonase (EC 3.2.1.15) is a wall-bound enzyme, present in many fruits and vegetables, and similarly to PME, it is also presented in fungi and bacteria (Terefe et al., 2014). PG is responsible for pectin depolymerisation by catalysing the cleavage of the alpha-(1-4) glycosidic bonds between two galacturonic acid residues. The action of this enzyme in combination with the resulting products after PME action results in texture degradation of fruits and vegetables (Terefe et al., 2014).

Peroxidase (POD)

In addition to colour, POD is also related to textural changes in fruit and vegetable products. At the structural level, POD catalyses the formation of phenolic cross-linking polymers allocated in the cell wall, providing further stability to the plant structure during thermal processing (van Dijk & Tijskens, 2000). The oxidation of phenolic compounds, such as ferulic acid (present in the cell wall), due to the action of POD enzymes can result in the formation of ferulic acid cross-links between polysaccharides, further promoting cell-to-cell adhesion, which could explain the slight softening perceived after cooking certain vegetables, such as Chinese water chestnuts, beet roots, or sugar beets (Ng et al., 1998; Van Buggenhout et al., 2009; Waldron et al., 1997).

2.3. Flavour change mechanisms associated with enzymes

Flavour is one of the most important quality parameters, as it is an indicator of overall product quality and plays an important role in consumer acceptability and the product launch decision-making process. It can also be affected by enzymatic action. However, flavour is a complex system involving taste and aroma, which makes it difficult to associate enzymatic action with the production of off-flavours in

food products (Terefe et al., 2014). Enzymes, such as β -glucosidases, are involved in the release of a wide variety of volatile compounds in fruits and vegetables using their glycosidic precursors as substrates (Orruño et al., 2001).

Lipoxygenase (LOX)

Lipoxygenase (EC 1.3.11.12) acts as a catalyst for oxidation of polyunsaturated fatty acids and esters (L. R. Ludikhuyze et al., 1998; Rodrigo et al., 2006). These hydroperoxides forms correspond to the substrates of other enzymes that will further produce volatile flavour compounds, usually responsible for the sensory notes “green” and “fresh” (Rodrigo et al., 2006, 2007).

Hydroperoxide lyase (HPL)

The degradation of the hydroperoxide forms of fatty acids generated by the action of LOX is conducted by HPL. The resulting compounds after the reaction are volatile aldehydes, ketones, and alcohols responsible for the green and fresh notes. The combined action of LOX and HPL should be controlled, considering that it is essential to produce the previously mentioned notes, which bring to the product a freshness perception, but that at higher concentrations can be considered as an off-flavour (Németh et al., 2004; Santiago-Gómez et al., 2007).

2.4. Nutritional value changes associated with enzymes

As a consequence of changes in colour and texture, enzymes are also responsible for changes in the nutritional value of fruits and vegetables. The action of LOX, which promotes the oxidation of unsaturated fatty acids, can cause the degradation of essential fatty acids, such as linoleic and linolenic acids, and indirectly, the degradation of proteins and vitamins by hydroperoxides and free radical intermediates. Enzymes responsible for colour degradation indirectly degrade the anthocyanins present in vegetable products, making them unable to provide their antioxidant capacity (Terefe et al., 2014).

2.5. Protein extraction and quantification

Protein extraction

As mentioned previously, all enzymes present in plant tissues are either soluble inside the plant cell or bound to one or several different structures. Therefore, it is necessary to break the cellular structure to release the enzymes, being at this step a major concern for stability and possible inactivation (Bonner & Bonner, 2007; Sabarre Jr & Yagonia-Lobarbio, 2021). Several factors influence enzyme stability during the extraction process:

- pH: biochemical processes, including enzymatic activity, are highly sensitive to pH changes. The selection of this parameter is crucial in the extraction process. Generally, enzymes have very specific pH ranges in which they are active, and exposure to pH conditions outside this range may cause denaturation and irreversible inactivation (Burgess & Deutscher, 2009; Eisenthal & Danson, 2002).
- Buffer type: a buffer is a solution of an acid and its conjugated base, the objective of which is to maintain a certain pH during enzymatic extraction (Bonner & Bonner, 2007). To select an adequate extraction buffer, factors such as the optimum pH of the target enzyme, the effects of the buffer on the enzymes, their sensitivity to temperature, and interactions with substrates and metals, among others, should be considered (Burgess & Deutscher, 2009).
- Temperature: the control of this variable is important considering that an increase in temperature might cause enzyme denaturation or activation of proteases (Bonner & Bonner, 2007; Rosenberg, 2013). Generally, during enzymatic extraction, the temperature is maintained in the range of (0 – 4 °C).
- Mass to solvent ratio: the adequate quantity of extraction buffer with respect to the amount of plant material is highly relevant. This ratio is a compromise between using the least possible buffer and obtaining the maximum extraction yield. A low buffer volume might cause a change in the pH, with the implicit consequences previously stated. However, the addition of extra buffer volume may have some implications at the cost level or even extraction yields. However, the ratio and its relationship with the extraction yield are highly dependent on the plant material

and the specific enzyme (Rocha et al., 1998; J. Wang et al., 2007; Wuyts et al., 2006). Sabarre Jr & Yagonia-Lobarbio (2021) gathered different ratios for PPO extraction from different plant materials, finding a common ground of at least 1:2.

- Extraction time: the period in which the extraction buffer is in contact with the plant material is also a crucial variable. This allows destruction of the cell structure, leading to the release of intracellular material. Nevertheless, during this period, enzymes may come into contact with compounds that might inactivate them (Hatti-Kaul & Mattiasson, 2003). In this context, the extraction time is a variable that should be optimised and is highly dependent on the specific plant material. Sabarre Jr & Yagonia-Lobarbio in 2021 gathered a variety of extraction times for PPO enzymes from different fruits and vegetables, varying between 1 min and 24 h.

The extraction buffer can be enriched with various additives to improve the extraction process. Some categories of possible additives can be included according to specific requirements:

- Protease inhibitors: during cell disruption, enzymes capable of breaking down proteins into individual amino acids (commonly known as proteases or proteinases) are released. These enzymes can also degrade other enzymes, and therefore, their activity should be controlled. Extractions performed at low temperatures are known to be effective against the action of proteolytic enzymes (Bonner & Bonner, 2007; Whitaker, 2018).

Different chemical agents can act as protease inhibitors; some examples include phenylmethylsulfonyl fluoride (PMSF), EDTA, and EGTA, among others. The use of these agents is recommended for purification steps when the concentration of enzymes can be effectively affected by proteases (Sabarre Jr & Yagonia-Lobarbio, 2021).

- Protective agents: as previously stated, phenols present in plant materials are possible substrates for enzymes involved in colour degradation. Therefore, it is imperative to remove these compounds to obtain enzymatically active extracts. Polymers such as polyvinylpyrrolidone (PVP) and polyvinylpolypyrrolidone (PVPP), can form stable hydrogen bonds with phenolic compounds (Cosme et al., 2019; Eisenthal & Danson, 2002; Ferreira et al., 2018). They can be

easily removed during clarification steps by filtration or centrifugation, and they do not interfere with enzymatic assays (Bonner & Bonner, 2007; Sabarre Jr & Yagonia-Lobarbio, 2021).

- Detergents: as previously mentioned, some enzymes are present in bound form on different membranes in the cell structure; in this context, the use of detergents is recommended to detach proteins from their original structures. Detergents are water-soluble lipid molecules because of the presence of a hydrophilic head attached to a hydrophobic tail (Chae et al., 2013). Detergents are capable of replacing the membranes binding the proteins to their hydrophobic tails (Sabarre Jr & Yagonia-Lobarbio, 2021).

Properties of detergents such as critical micelle concentration, micelle molecular weight, and hydrophile-lipophile balance, influencing enzymatic extraction and enzyme stability, are decisive factors to consider when selecting the appropriate agent to use (Rosenberg, 2013). Triton X-100 is a non-ionic detergent widely used in protein extraction, having as particular properties just a slight non-denaturation condition and the prevention of irreversible binding, among others (Bonner & Bonner, 2007). Other detergents can also be considered as possible membrane solubilisation agents (Chae et al., 2013; Das et al., 2021; Yoon & Robyt, 2005).

Protein quantification

After the cascade of purification steps, enzymatic activity should be quantified, and all factors affecting enzyme stability are even more important at this stage (particularly temperature and pH), considering that the concentration of enzymes is much higher than that in the plant material (Sabarre Jr & Yagonia-Lobarbio, 2021).

Substrate specificity

The enzymatic activity measurements are highly dependent on the selected substrate used for the assay. Primary substrates are found in the same plant material. However, after extraction, more suitable and stable substrates should be considered. Enzymes extracted from different plant sources can exhibit different kinetic parameters with respect to the same substrate (Jukanti, 2017), making it a relevant task

to identify the appropriate substrate with respect to a specific fruit or vegetable, the selected variety, and the target enzyme.

3. Microorganisms involved in food deterioration and foodborne pathogens

Food spoilage is a metabolic process responsible for changes in the sensory characteristics of food products, making them undesirable or unacceptable to consumers. These changes include textural modifications (generally associated with physical damage), chemical changes (oxidation or change in colour), or the generation of off-flavours and off-odours, all of which result from the action of microbial growth and consequently are products of microbial metabolism. Apart from fermented foods, where beneficial microorganisms are used to transform the inoculated matrix, spoiled food is always non-consumable, even if it might be affected by non-pathogenic microorganisms or microorganisms that do not release toxic compounds as secondary metabolites (Gram et al., 2002). Every food product possesses its own microbiota related to the processing stage (plant growth, harvesting, processing, and storage), and its effect on product quality is strictly linked to the concentration of contaminating microorganisms (Gram et al., 2002). However, general classifications can be made according to product categories: fruits and vegetables are nutrient-rich substrates for microorganisms, with predominantly acidic pH and high water activity. Soil, water, and air are the main sources of microorganisms in vegetable products. Their growth is highly dependent on pH, carbohydrate concentration, moisture content, and temperature of the contaminated matrix. Common spoilage microorganisms on these substrates include moulds (*Penicillium*, *Phytophthora*, *Alternaria*, *Botrytis*, and *Aspergillus*) and bacteria (*Pseudomonas*, *Erwinia*, *Bacillus*, and *Clostridium*) (Rawat, 2015).

On the other hand, there are microorganisms that can be found in food products, and their presence can be detrimental to human health. Commonly called foodborne pathogens, they are responsible for identified diseases associated with food consumption and they are also the main target for preservation technologies, considering that between spoilage microorganisms and foodborne pathogens, the latter group represents the highest health risk for consumers (Tauxe, 2002).

3.1. *Salmonella*

Salmonellae, belonging to the family *Enterobacteriaceae*, are considered to be important pathogens for human and animal health. *Salmonellae* are anaerobic gram-negative bacteria, and the genus is divided into two species, *S. bongori* and *S. enterica*. Subsequently, *S. enterica* is subdivided into the subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *andincica*, based on their biochemical and genomic characteristics (Brenner et al., 2000). Among other characteristics, the majority of *Salmonellae* are hydrogen sulphite producers, oxidase-negative, catalase-positive, and lactose fermenters. They are also able to grow using citrate as a carbon source, hydrolyse urea, and decarboxylate lysine (Andino & Hanning, 2015). Several serovars have been identified, most of which belong to the subspecies *enterica* (approximately 1500). The main niche for *Salmonella* has been identified as the intestinal tract of warm-blooded and cold-blooded animals as well as the environment (mainly soil) (Brenner et al., 2000).

The spread of *Salmonella* from animals begins with the excretion of faeces, which can further pollute water; its presence in water is an indication of faecal contamination (Andino & Hanning, 2015). *Salmonella* is highly adaptable to adverse survival conditions, being able to survive at pH ranges from 4.05 to 9.5 with optimal values between 6.5 and 7.5 and temperatures between +7 and +48°C, with optimal growth at +37°C (Fatica & Schneider, 2011).

This adaptability is crucial for the infective process in humans. Salmonellosis is characterised by specific symptoms such as diarrhoea, vomiting, craps, and fever in the general population. However, high-risk groups have been identified as young children, the elderly, and immunocompromised individuals. As with all infectious diseases, the outcome is highly dependent not only on the immune response of the organisms, but also on the inoculation dose, ranging from less than 100 CFU in the most extreme cases to a more regular concentration range of 10^6 to 10^8 CFU (Andino & Hanning, 2015; Fatica & Schneider, 2011).

Reported cases of salmonellosis have mainly been associated with the consumption of contaminated meat products or animal-origin products, such as milk or eggs; nevertheless, cases have been growing for non-animal-related products. The U.S. Center for Disease Control and Prevention (CDC) reported

the outbreaks produced in the United States in the period of 2016-2021, finding a total of 118 outbreaks with a maximum of 29 in 2019. From this number, 45 outbreaks were reported coming from animal products (meat, eggs, and dairy), and closely 32 of them came from fruits and vegetables (CDC, 2023). Similar results have been found in Europe, where confirmed cases of salmonellosis have decreased from 2016 to 2020, with origins in animal and animal-based products, followed by fruits and vegetables (EFSA & ECDC, 2021).

Different sources of contamination can be identified in plant-based products, with manure, faecal matter, animal contact, and water being the most common sources. During cultivation, irrigation with contaminated water can lead to the contamination of crops directly (via contact with water), but also indirectly (due to the penetration of water into the soil, root vegetables can also be indirectly affected). At the post-harvest stage, water microbial quality is also important for controlling *Salmonella* spread, in addition to the process temperatures that can lead to bacterial penetration when temperature increases, consequently providing a more suitable environment than the plant surface for microbial growth (Fatica & Schneider, 2011).

3.2. Enterohaemorrhagic *Escherichia coli* (EHEC)

Escherichia coli is a gram-negative, rod-shaped, facultative anaerobic, and coliform bacteria of the genus *Escherichia*. It is commonly found in the gastrointestinal tract as part of the microflora of humans and warm-blooded animals. However, some serovars have acquired virulence with different types of pathogenicity, among those enterohaemorrhagic *E. coli* (EHEC) (Bell, 2002; Berhanu & Pal, 2020). The EHEC action mechanism does not involve the colonisation of enterocytes, but it can produce verotoxins that can cause several damage to these cells. According to the produced toxins, the action mechanism differs; for instance, Shiga-Toxin producers (STEC) are capable of blocking protein synthesis with the inactivation of ribosomes (Berhanu & Pal, 2020; Kim et al., 2014). EHEC is highly infectious in humans at very low infective doses (less than 100 CFU), and it can be found naturally in

cows and other animals. Similarly to *Salmonella*, non-animal food products can be exposed to EHEC due to contamination with faeces, animal contact, or most likely faecal-contaminated water (Bell, 2002).

Foodborne illnesses caused by EHEC can lead to diarrhoea, several abdominal cramps, and even haemorrhagic colitis. This infection can lead to haemorrhagic diarrhoea and kidney failure. The most vulnerable populations consist of children under 5 years of age, the elderly, and immunocompromised people (Berhanu & Pal, 2020). Outbreaks in the U.S. for STEC were reported to be 38 between 2017 and 2021, the majority being confirmed from vegetable crops and beef respectively. In contrast, in Europe, meat and meat products have a higher incidence rate than fruits and vegetables (CDC, 2023; EFSA & ECDC, 2021).

Among fruits and vegetables, the most common products associated with EHEC are green leafy vegetables and sprouts. As for *Salmonella*, similar contamination sources during cultivation can include soil, manure, irrigation water, and human handling, among others. Postharvest sources, in addition to the quality of process water, include harvesting equipment, transport containers, processing equipment, and cross-contamination, among others. The survival of EHEC in these substrates is related to factors such as pH, temperature, moisture, and the availability of nutrients, among others (Luna-Guevara et al., 2019).

3.3. *Listeria monocytogenes*

Listeria monocytogenes is a gram-positive, non-spore-forming, facultative anaerobic bacterium that is motile from +22 to +28°C and non-motile above +30°C. Its ability to survive in a wide range of conditions has made this pathogen widely widespread in different food products, including dairy, meat, fish, raw vegetables, fruits, and ready-to-eat products. It is able to grow from -0.4 to +45°C with an optimal condition of +37°C, to survive in low water activity environments (water activity under 0.9), and to live at pH levels ranging from 4.6 to 9.5, as well as to tolerate salt concentrations up to 20% (Osek et al., 2022; Schlech, 2019; Wiktorczyk-Kapischke et al., 2023).

L. monocytogenes can cause different syndromes, including sepsis, meningitis, rhombencephalitis, and gastroenteritis, with children, the elderly and immunocompromised people being the most affected patients. The most common symptoms of a gastrointestinal episode are fever, headache, diarrhoea, nausea, vomiting, and abdominal pain, among others, with an incubation period of 24 h before the appearance of symptoms (Ooi & Lorber, 2005; Schlech, 2019). Reported outbreaks in the U.S. increased between 2017 and 2020, reaching a total of 18. The main food products associated with these outbreaks were dairy, fruit, and vegetables. Similar trends were followed in the period of 2016 to 2020 in the European Union, also attributing the majority of cases to the consumption of dairy and animal products (CDC, 2023; EFSA & ECDC, 2021)

4. High-Pressure Processing (HPP)

4.1. Characteristics of the treatment

High-pressure processing is based on the increase in pressure of the system to modify the properties of the food matrix, which subsequently generates a change in the characteristics of the food product. Unlike thermal or other technologies, the effect of high pressure is almost instantaneous and uniform, as the pressure transmission is not dependent on mass dimensions or time, making this process suitable for different flexible packaging options. In addition, the HPP effect is independent of machine configuration, making the process easily scalable (Mújica-Paz et al., 2011). The technology is governed by four main principles: isostatic, le Chatelier, microscopic order, and the transition state.

- The isostatic principle states that, under specific conditions of pressure or force, a fluid can be considered hydrostatic, in which case a force applied to a liquid surface is transported through it without friction, making it possible to apply pressure to food products regardless of their shape and size (Evrendilek, 2023). The distance between molecules changes with an increase in pressure, altering intermolecular forces such as van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions (Martinez-Monteagudo & Aranda Saldaña, 2014).
- Le Chatelier's principle explains the influence of pressure increase on chemical, biological, and physical phenomena. The principle, which originates from the second law of thermodynamics and is valid for reversible processes, states that a system in equilibrium subjected to a particular stress will shift to a new equilibrium state with some changes due to the applied stress. Applied to HPP, it explains the change in volume as an effect of increasing pressure, as well as the promotion of chemical reactions, phase transitions, and changes in molecular configuration (Martinez-Monteagudo & Aranda Saldaña, 2014; Martínez-Monteagudo & Balasubramaniam, 2016).
- The microscopic order principle states that the degree of ordering of substance molecules increases with increasing pressure at a constant temperature. By limiting translational,

rotational, and vibrational motions, the pressure increases the molecular order. In contrast, temperature has an inverse effect on molecular ordering (Balny & Masson, 1993).

- Transition State Theory: the principle aims to explain the effect of pressure on physical, biochemical, and chemical reactions. If the molar volume of an intermediate state differs from its reagent components, the reaction rate can be negatively or positively affected by an increase in the pressure of the system (Martinez-Monteagudo & Aranda Saldaña, 2014).

4.2. Effect on the medium (water)

Water is a very important component of high-pressure technology because it is the medium that ultimately transmits pressure in the system and is also one of the most abundant constituents in food products, which subsequently alters their characteristics under the treatment conditions. The behaviour of water compared to other liquids is particular; in the solid state, water can form thirteen different ice structures, nine of which are thermodynamically stable (Vega et al., 2005).

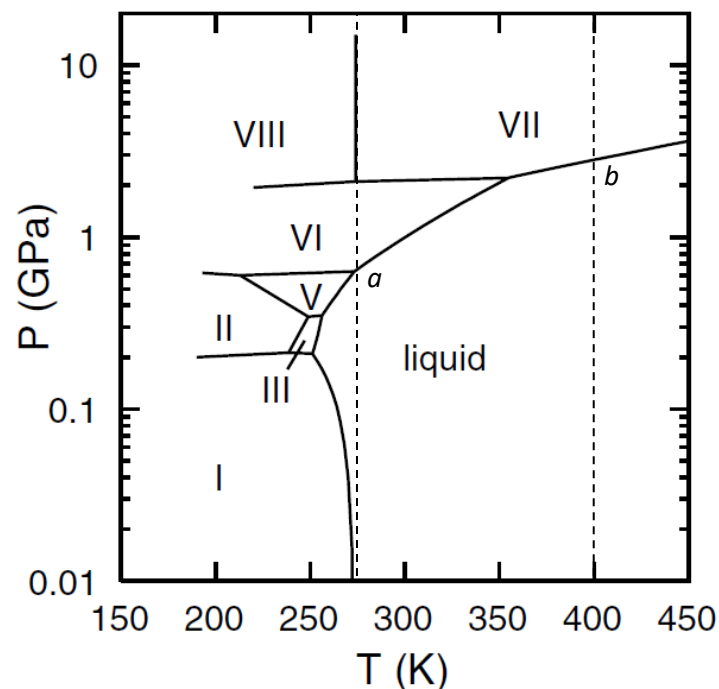


Figure 4. Experimental water phase diagram. Adapted from (Sanz et al., 2004)

The behaviour under pressure in isothermal conditions (typically promoted in the HPP process) can be elucidated with the interpretation of Figure 4. With the increase in pressure at low temperatures, where the water is in liquid state under atmospheric conditions (e.g., an isothermal process at 275 K represented as the vertical dotted line in the figure at 275 K), water remains in the same phase, reaching approximately 5500 MPa of pressure and maintaining its properties (point *a* in the figure). With the increase in temperature, water is capable of remaining in the liquid phase even at higher pressure conditions thanks to the increase in this property, which is responsible for the increase in energy in the system. For example, for an isothermal process at 400K (represented as a dotted line a 400K), water remains in the liquid state still 1.2 GPa (point *b* in the figure). This makes water an excellent medium for processing in terms of the pressure it can hold, and it is also considered an inert medium generally used in the food industry.

4.3. Effect on microbial inactivation

Microorganisms are affected by high-pressure processing due to alterations in their cellular structures. Damage to the cell membrane increases its permeability and consequently alters its metabolism, in addition to changes in the three-dimensional structure of molecules such as proteins and more complex cellular organelles (Agregán et al., 2021). However, not only does a single factor influence the effect of the treatment on the inactivation rates, the characteristics of the treatment, the specificity of the strain, and the medium conditions are all together responsible for microbial inactivation (Rendueles et al., 2011).

Generally, an increase in pressure, time, or a combination of both leads to greater microbial inactivation. A review made by Podolak et al. (2020) collected data related to the microbial inactivation of different pathogenic and spoilage microorganisms in fruit juices. Here is shown that with an increase in pressure or time, there is a reduction in the decimal reduction time, a parameter that quantifies the time required to reduce the microorganism population in a decimal unit. Comparable results have been reported in a review conducted specifically for *Salmonella* by Agregán et al. (2021). Another crucial factor to

consider is temperature, as it can exert a synergetic effect on microbial inactivation. However, the use of high-pressure processing as a preservation method is intended to maintain the nutritional and organoleptic properties that are normally affected by thermal processing. Therefore, low temperature conditions are preferred (Podolak et al., 2020). In this case, it would be desirable to increase the pressure or time to achieve the same microbial reduction compared to a milder combination of parameters at higher temperatures.

Another important combination of process parameters who play a significant role in the inactivation of microorganisms are the compression and decompression rates. It has been proposed that high speeds of compression and decompression can produce more stress on cells and, subsequently, more cell injuries. However, despite the supporting evidence presented by some researchers, contradictory results have been reported. For instance, in a study by Noma et al. (2002), fast decompression rates (1 ms to complete decompression) resulted in higher inactivation of *Salmonella enterica* serovar Typhimurium, *Pseudomonas aeruginosa*, and *E. coli* K-12 strains. Chapleau et al. (2006) reported significant higher inactivation using a combination of slow (1 MPa.s) and fast decompression rates (250 MPa.s) for *Salmonella typhimurium* and *Listeria monocytogenes*. Other studies have shown that changes in the pressurisation and depressurisation ramps are not relevant in terms of microbial inactivation. Rademacher et al. (2002) conducted an experiment combining two different sets of conditions (System A: 100 MPa/min and 500 MPa/min; System B: 500 MPa/min and 100 MPa/min, compression and decompression rates, respectively) for different holding times to evaluate the effect on the inactivation of *Listeria innocua*. No significant differences were observed between the two systems with respect to microbial inactivation. However, all these studies have been carried out in model solutions to avoid the effect of the food matrix on the inactivation of microorganisms. Syed et al. (2013) tested the effect of different compression and decompression rate combinations in three different matrixes (Tris buffer, skim milk, and orange juice) for *Escherichia coli* O157:H7. Comparing compression rates, all matrices presented a higher inactivation for the fast condition (11.4 MPa/s), which was highly significant only for orange juice. Regarding decompression rates, better results were obtained when slow depressurisation was maintained (2.6 MPa/s).

The specificity of microbial strains is also a determining factor that influences the effectiveness of HPP technology. The structure of the cell wall plays an important role, considering that it is the protective boundary of the cell that is affected by pressurisation during processing. Gram-negative and gram-positive bacteria have significant differences in their cell wall composition: gram-positive bacteria present a peptidoglycan structure constituting about the 90% of the total cell wall; in comparison, gram-negative bacteria only possess 5 to 10% of this polypeptide. Not only the percentage composition but also, in the case of gram-negative bacteria, the peptidoglycan structure is trapped between the outer membrane and the cytoplasmic membrane (Doyle et al., 2019). During the initial phase of a high-hydrostatic pressure process, the double-layer phospholipid structure of the membranes is compressed, and, during the depressurization stage, this structure changes, promoting the creation of pores that will cause leaking of the cytoplasmic content (Rendueles et al., 2011). The peptidoglycan outside layer present in gram-positive bacteria provides certain protection to the cell membrane structures, conferring higher resistance compared to gram-negative bacteria (Wuytack et al., 2002).

Differences in resistance to pressure processing may also be associated with genotype and phenotype variations. Several studies have demonstrated that strains possessed a significant variability in their pressure resistance. Tamber (2018) investigated the effect of HPP on the inactivation of 99 strains of *Salmonella enterica* from 24 different serovars collected over 26 years, finding no specific association between the serovars, origin, or years of collection with respect to microbial inactivation at 600 MPa for 3 min; however, the composition and properties of the cell membrane were indicated as possible indicators of pressure resistance behaviour. Other studies have suggested that there is a certain genetic similarity between strains resistant to pressure conditions. Duru et al. (2020) compared the genomic content of different pressure-resistant strains of *Listeria monocytogenes*, finding that 10 prophage genes were common in the most pressure-resistant strains. At the phenotypical level, high-pressure processing can induce the expression of proteins in charge of repair processed over those responsible for cell growth, as well as, in general, a reduction of the protein groups synthesised, prioritising the repairing process of the cells over other vital functions (Gouvea et al., 2023).

Pressure resistance of microorganisms is also highly dependent on the medium characteristics, being overly complex, and depending on the associated food matrix, the presence of proteins, fibres, sugars, and minerals can provide certain protection, considering that HPP cannot affect covalent bonds while maintaining their primary structures (Podolak et al., 2020). In the case of fruit juices, sugars are present as one of the main constituents of the food matrix; their presence directly affects water activity and further influences the resistance of microbial strains to pressure. Ogawa et al. (1990) evaluated the effect of the concentration of mandarine juice on the microbial inactivation of *Saccharomyces cerevisiae* and *Aspergillus awamori* under high pressures between 100 and 400 MPa at room temperature for 10 min. The results showed that the increase in soluble solids associated with the corresponding increase in juice concentration reduced the inactivation efficiency of HPP, showing higher microbial counts with increasing degrees Brix. However, water activity cannot be directly associated with microbial resistance behaviour; studies conducted such as those by Molina-Gutierrez et al. (2002), where comparable results with respect to pressure resistance were evaluated for *Lactococcus lactis* with variations in water activity, and in addition, Koseki & Yamamoto (2007) observed that the inactivation of *Listeria monocytogenes* was significantly different in solutions of sucrose, sodium chloride, and sodium phosphate buffer with similar water activity.

It is important to consider that sugar in juice is not the only component that contributes to the survival of microorganisms. The study by Simpson and Gilmour (1997) where the effect on microbial inactivation of *Listeria monocytogenes* under high pressure (300, 350, 357, 400, and 450 MPa for up to 30 min at ambient temperature) was investigated, used a model solution with different levels of protein (1 to 8% w/w BSA) and lipids (0 to 30% v/v olive oil). The results showed that the increase in the concentration of BSA was inversely proportional to the inactivation rate, being strain-dependent; additionally, the inactivation was greater in the samples where olive oil was present at all processing conditions. Without a doubt, pH is one of the main factors influencing microbial inactivation. Several studies on this topic have been conducted. Gouvea et al. (2023) collected several studies in reference to the effect of pH on the inactivation of *E. coli*, *Salmonella spp.* and *Listeria monocytogenes*. The

reduction in pH increased the inactivation rate in all cases. As explained in the review, this may be caused by the inability of damaged cells treated with HPP to recover and repair under acidic conditions.

4.4. Effect on enzymes

High-pressure processing is capable of inactivating many enzymes present in food products, being them responsible for changes in the quality and nutritional composition of food (Mújica-Paz et al., 2011). It has been studied that HPP is not capable of affecting covalent bonds, making the primary structure of proteins highly resistant to an increase in pressure (Heremans, 1993; Mozhaev et al., 1994), as cited in (L. Ludikhuyze et al., 2003). Similarly, the secondary structure of proteins is hardly affected by the increase in pressure; this is due to the low-pressure sensitivity of hydrogen bonds, which are only slightly altered under high pressure conditions (Balny & Masson, 1993).

In contrast, tertiary and quaternary structures are significantly affected by high-pressure processing mainly because of the disruption of ionic bonds and hydrophobic and electrostatic interactions. The increase in free water during the process consequently promotes an increase in the hydration of the protein due to the electrostriction of water around the newly exposed charged groups (L. Ludikhuyze et al., 2003; Podolak et al., 2020; Rendueles et al., 2011). Changes in the quaternary structure can be reversible. In such cases, the unfolded shape can be refolded after decompression, leading to a plausible reactivation (Chakraborty et al., 2014). However, only low-pressure conditions might produce reversible infolding (less than 200 MPa), pressures under which certain enzymes can even be activated, whereas high-pressure conditions produce consistent irreversible changes (Balny & Masson, 1993; Mújica-Paz et al., 2011). The reactivation of enzymes depends on a threshold of recompressibility with respect to pressure; this threshold is highly dependent on the nature of the enzyme and the characteristics of the food matrix. Although, within the threshold, enzymes can recover their structure but not be able to catalyse reactions entirely (J. Silva, 1993).

4.5. Effect on other nutrients and physicochemical parameters

High-pressure processing can also affect the organoleptic and nutritional characteristics of food products. Generally, low-molecular-weight compounds such as amino acids and vitamins, among others, are hardly affected by the increase in pressure (Rendueles et al., 2011). However, physicochemical parameters are more likely to change; changes in the appearance of HPP-treated grapes have been reported to be brighter than those of control samples. Additionally, it has been observed that colour migration from the skin to the flesh is induced by HPP treatment (Morata et al., 2015). It is important to note that much of the current research focuses on the effects of the use of this technology but not on storage evaluation, although this is usually the most relevant in terms of property changes (Aaby et al., 2018).

4.6. HPP machinery

Industrial high-pressure processing is commonly carried out in batch operation. Two configuration options are available for HPP machinery (Figure 5): a vertical arrangement where the packed food product is loaded into the equipment with the help of a carrier basket through the top opening of the pressure chamber. After treatment, the product is unloaded from the same opening (Balasubramaniam et al., 2016; Yamamoto, 2017). This configuration has some associated disadvantages that limit its use in large-scale applications. The first is the required infrastructure to maintain vertical operation, namely ceiling height, and additional equipment to charge and discharge the product, among others. Additionally, with this configuration, there is an associated risk of bypassing untreated products from the pressure treatment, considering that charging and discharging are always performed from the same cavity (Balasubramaniam et al., 2016).

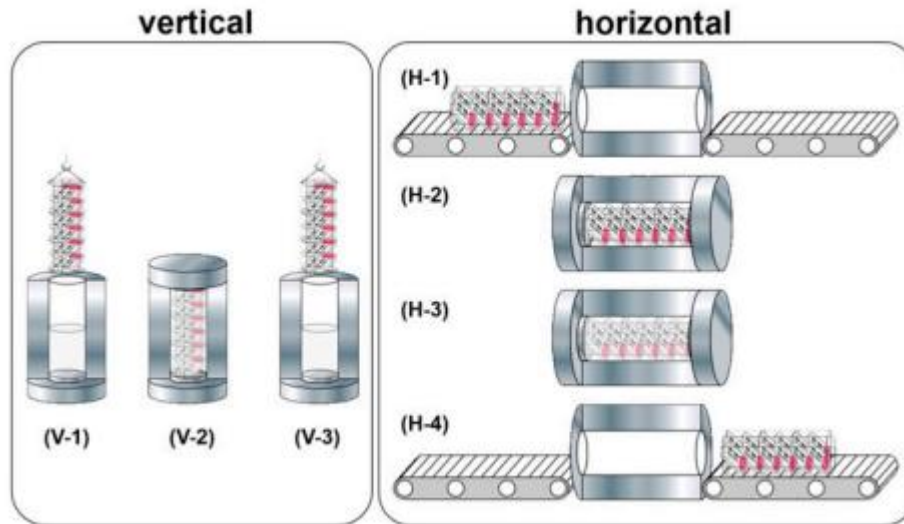


Figure 5. HPP equipment, vertical and horizontal configurations (Yamamoto, 2017)

Alternatively, there is a horizontal configuration, where the product is loaded into the pressure chamber from one side and, after treatment, unloaded from the opposite part, making this operation less risky in terms of a possible bypass for the treatment. In addition, the required infrastructure is simpler and involves fewer operations at high elevations. A typical High-Pressure horizontal unit is presented in Figure 6. A high-pressure system is formed by the following components (Balasubramaniam et al., 2016):

- Pressure vessel: this is the principal component of the entire unit. Here, the process takes place when the pressure pump introduces the transmission medium (generally water), and therefore, the system experiences a pressure increase. Different alternatives for the construction of this vessel can be found, all in order to guarantee the resistance of the material, which will be exposed to extreme mechanical stress. Monoblock, which is made of a monolithic material, is generally a low-alloy steel with high tensile strength. This is a less expensive option because it has the restrictions of working only under 400 MPa, relatively small diameters, and possible deformation and cracking under high-pressure conditions. Alternatively, multiwall vessels are constructed based on concentric cylinders (at least two), by assembling the external layers over the inner layers using heating and cooling treatment to promote expansion or shrinking (Elamin et al., 2015). Finally, wire-wound vessels are formed by layering high-strength wire on a wall

core. This last option, owing to its fabrication process, allows for higher operational performance compared to other alternatives (600 MPa being a typical operational condition), larger diameters, and the possibility of releasing pressure by leaking without compromising the integrity of the vessel.

- Closures: responsible for hermetic sealing of both extremities of the pressure vessel. This may be a major source of maintenance costs considering its wear. Plugs and wedges compose the closure system. Plugs prevent water leakage during operation, in addition to allowing the tubes to fill and empty the pressure vessel; wedges on the other hand, keep the plugs in position, maintain the sealing of the system during operation (HPP Advisors, 2019).
- Yoke: providing retention of the axial forces acting on the closures.
- Pumping system: responsible for transporting the transmitting pressure fluid. The increase in pressure can be direct or indirect. In a typical HPP commercial operation, the product is loaded into the pressure vessel, and after closure, the vessel is filled with water with the help of a low-pressure pump. When filled, the vessel is pressurised via external compression using external electrohydraulic intensifiers.
- Process control system: in charge of controlling and monitoring the system. Typically collects information about the pressure, temperature, and holding time. It is also responsible for corrective actions in case of equipment failure. It comprises a typical control unit that includes computer systems, sensors, and alarms.

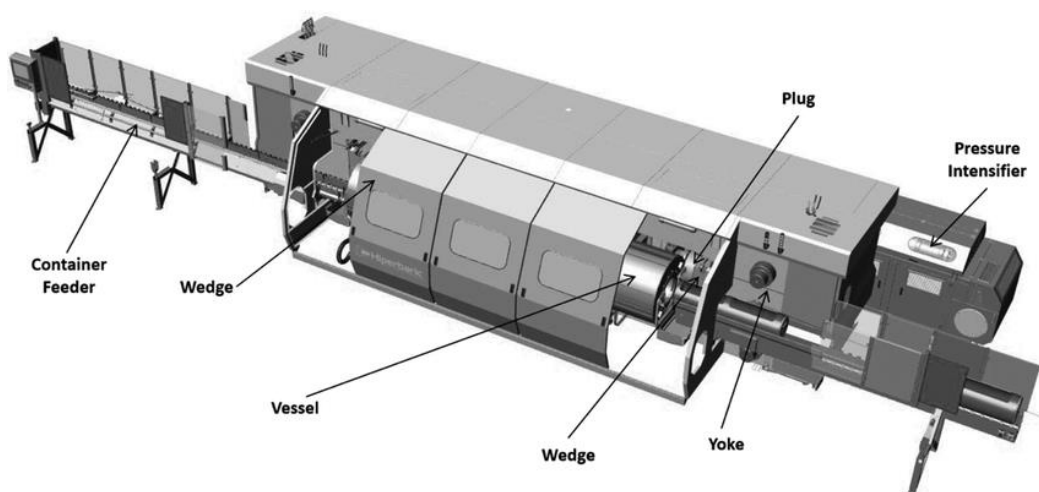


Figure 6. High-Pressure horizontal unit. (Hiperbaric, 2018)

Different companies in the market produce HPP machinery for industrial application. Balasubramaniam et al. (2016) and Elamin et al. (2015), created a list of current companies providing HPP equipment, stating the country of origin, capacity and maximum operational conditions. In general, manufacturers offer machines with capacities varying from 20 to 1400 L and a maximum operational condition of 600 MPa.

4.7. Packaging and HPP

Packaging is an integral and essential part of food products, and the selection of an adequate type of packaging is crucial for microbiologically preserving the product and ensuring storage stability. Specific characteristics are required to select a packaging material suitable for HPP: elasticity, making it resistant to high-pressure treatment and allowing pressure transmission, flexibility, to be able to recover its original shape after treatment. The sealing properties of the material should also be considered in order to avoid possible leakage of the product during and after processing (Elamin et al., 2015; Hiperbaric, n.d.-b).

Considering these characteristics, plastic bottles are typically used in HPP food applications, particularly in the juice industry. Bottles are made of materials such as PET, PP, PE, PA, or EVOH. These materials are not only suitable for bottles; other possibilities include trays and tubs, where ready-to-eat meals are usually packed. This type of application requires special attention to the sealing material used as well as the area for sealing. In addition, special attention should be paid to the corners of the rigid structure to ensure correct thickness, preventing a possible break in the structure (Hiperbaric, n.d.-b).

Alternative packaging applications can be used for HPP. Bags and pouches made of mono- and multilayer materials are widely used for soups, wet salads, salsas, baby food, fruit juices, and purees, among others. Regarding multilayer materials, there is special interest in this area considering their barrier properties (Elamin et al., 2015; Hiperbaric, n.d.-b). However, there is a risk in this type of

product associated with delamination, leakage, and mass transfer, among others (Ahmed et al., 2022; Caner et al., 2004; Fraldi et al., 2014; Galotto et al., 2008; López-Rubio et al., 2005; Mensitieri et al., 2013).

4.8. High-Pressure Processing industry in numbers

High-pressure processing is extensively used in different markets, from food products, including juices and beverages, meat and seafood products, dairy products, and ready-to-eat meals, among others, to pet food and pharmaceutical applications (Hiperbaric, n.d.-a). The focus of this technology is mainly on food safety, offering the benefit of providing non-thermally treated products that are stable under refrigerated storage, with nutritional and organoleptic characteristics similar to those of freshly produced products.

Industrial machinery production worldwide showed an exponential increase from 1990 to 2015, reaching more than 300 machines operating at that time. These facilities were mainly located in North America and Mexico, accounting for two-thirds of the total, followed by Europe (18%) and Asia (8%) (H.-W. Huang et al., 2017).

The HPP global food market also experienced an exponential increase; Peerun (2015) presented a forecast where by 2025, sales in the HPP food market will reach 55 billion, incrementally in all categories. By 2015, the market share was predominantly meat products (25%), juices and beverages (20%), vegetable products (20%), and toll processing (23 %).

4.8.1. Regulations about food preservation and food products

Food safety is a major concern when launching products in the market. With this in mind, establishing the processing conditions for different preservation technologies is an important task in which manufacturers, governmental agencies, and research institutions should participate. HPP is not an

exception; as a non-traditional pasteurisation method, specific regulations have been implemented to ensure its effectiveness and, consequently, consumer safety (H.-W. Huang et al., 2017).

Different policies have been put in place to regulate products under HPP treatment; in North America, HPP has been recognised as effective in pathogen inactivation and shelf-life extension by the FDA, USDA, and FSIS. Specific guides have been prepared for industries to assess the effectiveness of the process according to specific products (Hiperbaric, 2021).

European legislation, which initially classified HPP-processed foods under the “Novel Food” category, has removed this label, not considering HPP as a novel process anymore. Considering this, foods processed with HPP technology must comply with the general EU food safety and packaging regulations: EU Regulation 2073/2005, that establishes microbiological limits for food products, and EU Regulation 10/2011, which refers to plastic materials in contact with food (considering, as stated before, that the main packaging used in HPP processing is plastic) (EFSA Panel on Biological Hazards (BIOHAZ Panel) et al., 2022; Hiperbaric, 2021).

Asia has not established specific policies for HPP; however, Asian producers should consider an HACCP plan for HPP treatments to ensure adequate microorganism inactivation and additional compliance with specific regulations according to the commercialisation location. In contrast, in Oceania, as HPP is no longer considered a novel process, as in Europe, products with this treatment should comply with the requirements of the Food Standards Code. Other regions of the world might not have sufficient production using this technology, but they might be aligned with European and North American regulations (Hiperbaric, 2021).

5. Scope of the research

The intention of this research was to evaluate the effect of high-pressure processing technology on the characteristics of selected fruit nectars and juices (strawberry and sour cherry nectars and red grape juice), with special emphasis on the impact of the technology on the inactivation of foodborne pathogens (through the use of surrogate strains) associated with food safety of the product, and endogenous enzymes related to oxidation processes, which are responsible for the degradation of the food matrix and affect organoleptic parameters such as colour and appearance. Additionally, considering the antioxidant properties particularly attributed to red berry products, an evaluation of the polyphenol content, as well as their antioxidant capacity, will also be considered.

Ultimately, this project will lead to the optimisation of HPP unit parameters that will allow the treatment of fruit juices with higher quality. Moreover, considering that not only processing is a relevant factor in reference to product stability, an evaluation of the shelf life of the products will be performed to contrast the initial effect of the treatment on the previously selected properties and their evolution over time.

Research Question:

Is the HPP technology a feasible industrial alternative to inactivate enzymes but maintain the nutritional properties of fruit juices?

Research objectives:

Objective 1: To evaluate the efficiency of HPP in the inactivation of potential pathogenic microorganism and endogenous enzymes, while preserving the nutritional and organoleptic characteristics of fruit juices.

Objective 2: To optimise the HPP conditions to obtain the maximum enzymatic inactivation while maintaining the nutritional and organoleptic characteristics of the product, considering the microbial preservation requirements and the operational limitations of the system.

Objective 3: To validate the shelf stability of fruit juices produced under optimised HPP conditions, considering the effect of packaging material change.

Hypothesis:

Hypothesis 1: High-Pressure treatment has a significant impact on the inactivation potential pathogenic microorganisms and endogenous enzymes present in fruit juices.

Hypothesis 2: There is an optimal set of conditions in the HPP unit that maximise microbial reduction and enzymatic inactivation while maintain the nutritional properties of fruit juices.

Hypothesis 3: HPP is capable to treat fruit juices that under optimised conditions have a better performance in terms of stability compared to non-treated products, finding differences with respect to packaging barrier properties among treated samples.

6. Methodological approach

Definition of relevant parameters for the study

Considering the scope of the research, the following parameters were selected to carry out the study:

- Surrogate microorganisms for pathogen strains: to guarantee product safety as the baseline to evaluate other possible effects on the product with HPP treatment.
- Endogenous enzymes: PPO, POD, and β -glucosidase were selected considering their relevance in terms of colour stability.
- Physicochemical parameters: Total Soluble Solids (TSS) and pH were selected as parameters to track possible organoleptic changes. Characteristics of nectars and juice, in addition to their importance in the stability of other bioactive molecules and enzymes.
- Colour: one of the targets in terms of stability, a measurement of colour, was included to follow its evolution after treatments.
- Bioactive molecules analysis: total polyphenol content and total antioxidant capacity were selected as the target parameters to follow, considering the relevant amount of polyphenols present in the products.

According to the defined parameters that will be followed during the different stages of the study, suitable methods were selected based on their accuracy and detection limits.

Process optimisation strategy

Once the parameters and their corresponding methods were selected, the following optimisation strategy was used:

- Surface response methodology: considering the characteristics of HPP technology, three different variables can be used in the optimisation process: temperature, holding time, and pressure. Due to limitations in the operation of the equipment, temperature was not considered as a variable in this study. This because the required quantity of process water to be used for a machine with 300L capacity, requires a heating system that was not available in the facilities,

in addition to the fact that changes in the temperature of water will alter the normal operation of the production plant. Based on the surface response methodology (SRM), time and pressure variables were optimised for each specific parameter. A face-centred central composite design was used to create a surface response for each parameter to describe and further optimise the system.

With this design, nine time-pressure combinations were evaluated. A power analysis was performed to evaluate the accuracy of the design, resulting in a total of three repetitions per point.

The optimisation targets were defined as follows:

- Microbial inactivation: minimum 5-log reduction for each microorganism tested.
- Enzymatic inactivation: maximum inactivation of the target enzymes
- Bioactive molecules: maximum retention.

At this stage, the evolution of parameters over time after treatment, as well as changes in colour or physicochemical parameters, were not considered.

Shelf-life evaluation

To evaluate the efficacy of the optimised treatment, a shelf-life study was performed to follow the evolution of all defined parameters over time under controlled storage conditions. In this stage, a new variable was included: packaging material that might have an impact on the oxidation of the product due to the differences in oxygen migration according to their own characteristics.

7. Materials and methods

7.1. Materials

7.1.1. Fruits

Strawberry and sour cherry purees were obtained from different suppliers and red grapes were acquired in the local supermarket with attention to keep the same variety for the purchase; the details information about the varieties is presented in Table 1. In the case of strawberry puree, the supplier SICOLY was used for the process screening trials, whereas the supplier SVZ was used for the shelf-life study. The variety is not indicated in the SVZ product because it corresponded to a mix of different varieties.

Table 1. Fruit raw material characteristics.

Fruit	Variety	Supplier/Batch	Origin
Strawberry <i>Fragaria ananassa</i>	Senga sengana	Sicoly	Poland
	N.D	SVZ	The Netherlands
Sour Cherry <i>Prunus cerasus</i>	Oblaczynska	Mazzoni	Serbia
Red grapes <i>Vitis vinifera</i>	Red globe	UNES	Italy

7.1.2. Surrogate microorganisms

Three pathogenic microorganisms were selected as inactivation targets: enterohaemorrhagic *Escherichia coli* (EHEC), *Salmonella* spp. (using a cocktail of different strains as a reference), and *Listeria monocytogenes*. Non-pathogenic surrogates with respect to thermal inactivation were defined to be further used in the HPP evaluation trials. In the case of *Listeria monocytogenes*, 5 strains were selected as a suitable surrogate cocktail. A detailed list of all the surrogate microorganisms is presented in Table 2. The strain *E. coli* ATCC 11229 was selected as a suitable surrogate for *Salmonella* spp. considering its thermal resistance.

Table 2. Surrogate strains

Pathogenic Microorganism	Surrogate Strains
Enterohaemorrhagic <i>Escherichia coli</i> (EHEC)	<i>Escherichia coli</i> ATCC 8739
<i>Salmonella</i> spp.	<i>Escherichia coli</i> ATCC 11229
<i>Listeria monocytogenes</i>	<i>L. innocua</i> ATCC 33090, <i>L. innocua</i> 14S, <i>L. innocua</i> SSICA 95, <i>L. innocua</i> SSICA 99, <i>L. innocua</i> SSICA 101

7.1.3. HPP machinery

High-Pressure treatments were carried out at HPP Italia S.R.L. (Traversetolo, Parma, Italy). The equipment corresponded to a QFP 350L-600 equipment (Avure Technologies Inc., Kent, WA, USA).

7.2. Methods

7.2.1. Experimental Design - treatment plan

Process Screening Trials

Pouches containing 40 mL of strawberry and sour cherry nectars, and red grape juice were subjected to HPP treatment. Different conditions were required to inactivate microorganisms and enzymes. Initially a single design was envisioned for the process; however, after initial evaluation of microbially contaminated samples under 450 MPa, no survivals were found, meaning that two different sets of conditions were chosen to reach these targets (Table 3). Control samples were taken for all treatments to evaluate the effectiveness of the treatment with respect to the non-treated samples. All conditions were tested in triplicate to ensure statistical significance. The temperature of each run was maintained between 4 and 5 °C as informed by the technical team.

Table 3. HPP treatment conditions for microbial and enzymatic inactivation

	Pressure (MPa)	Time (min)
Microbiological inactivation	200	2, 4, 6
	250	
	300	

Enzymatic inactivation, TPC and antioxidant capacity	300	4, 8, 12
	450	
	600	

Shelf-life evaluation

Based on the results obtained in the screening trials, 600 MPa and 4 min were defined as the conditions to evaluate the shelf life of strawberry and sour cherry nectars and red grape juice in two different types of packaging: 200 mL transparent PET bottles and 80 mL multilayer flexible pouches. The study followed changes in physicochemical parameters (pH and TSS), colour, enzymatic activity, total polyphenol content, and antioxidant capacity. Samples were analysed over a 28 days period with a 7 days frequency for all the parameters. The oxygen barrier was tested on the initial and final dates of the study to evaluate the possible changes in this property.

7.2.2. Nectar formulation

Strawberry and sour cherry puree were transformed into nectars with 40% of fruit puree and 12 degrees Brix with the addition of citric acid as acidity modulator (having as target pH a range from 3.2 to 3.5), sucrose and water to obtain a formulation with the desired characteristics. Nectar characteristics were defined according to the suggestions of several partners in the project with expertise and knowledge regarding this type of products in the market. Standard formulation for strawberry and sour cherry nectars is presented in Table 4.

Table 4. Strawberry and sour cherry nectar formulation

Ingredient	Strawberry nectar (%)	Sour cherry nectar (%)
Puree	40	40
Water	50,72	54,71
Sucrose	9,17	5,13
Citric acid	0,11	0,15

On the other hand, grape juice was obtained with a juice extractor and further used in the experiments. No other ingredients were added in this case. Considering that the red grape juice was pressed and used immediately, different batches were necessary to complete the trials.

7.2.3. Microbiology sample preparation

Frozen surrogate strains for Salmonella, EHEC and Listeria were cultivated in Brain Heart Infusion (BHI; Oxoid, Basingstoke, UK) broth for 24 hours at 37°C, to reach an initial concentration of approximately 10⁸ CFU/mL. Each broth containing the cultivated strain (10 mL) was centrifuged at 4000 rpm for 15 min to obtain a pellet with concentrated microorganisms that was further resuspended in 1 mL of a peptone salt solution (8.5 g/l NaCl, 1 g/l tryptone).

Concentrated microorganism suspensions were inoculated into freshly prepared strawberry and sour cherry nectars, and grape juice. The initial cell concentration was measured and proved to be equivalent to approximately 10⁶ CFU/mL.

7.2.4. Microbiology evaluation

Endogenous microbial content

Freshly prepared strawberry and sour cherry nectars, and red grape juice were evaluated to determine their initial microbial load prior to surrogate inoculation. Total microbial counting was carried out on Tryptic Soy Agar (TSA; Oxoid, Basingstoke, UK). Plates were incubated at 37°C for 24 h to further counting. Yeasts and Moulds counting was carried out on Malt Extract Agar (MEA; Oxoid, Cambridge, UK) supplemented with 0.01% chlortetracycline (Sigma-Aldrich, St. Louis, MO, USA). Plates were incubated at 25°C for up to five days. A microscopic evaluation was performed to investigate the characteristics of the microflora found in the samples. Developed fungal colonies were further identified according to techniques proposed by Samson et al. (2019).

Surrogate-contaminated samples

Samples inoculated without treatment were analysed 24 h after the initial contamination to evaluate the effect of intrinsic parameters (pH, water activity, and sugar concentration in the matrix) on the initial microbial load of the products. Samples treated under the specified HPP conditions were placed into cold storage at 4°C for 24 h, after which counting was performed. For the evaluation of *E. coli*, plate counting was performed using Tryptone Bile X-Glucagar (TBX; VWR, Leuven, Belgium) agar, a chromophore-selective medium. Plates were incubated at 37°C for 24h for further counting. For the evaluation of *Listeria*, a surface method was used using Agar Listeria Ottaviani & Agosti (ALOA; Biolife, Milan, Italy), a similar chromophore medium selective for *Listeria*; the plates were incubated at 30°C for 72 h for further counting.

7.2.5. Enzymatic extraction

30 grams of strawberry and sour cherry nectars, and red grape juice were incubated with 20 mL of citrate buffer 0.2 M (pH 6.5) containing 0.05% w/v sodium ascorbate, 1% v/v TritonX100, 4% w/v PVPP, and 50 mM sodium chloride. The mixture was maintained in an agitated ice bath for 90 min.

After incubation, the suspension was centrifuged at 14000 rpm for 45 min at 4 °C, the supernatant was collected with the help of a sieve in order to retail possible parts of the pellet in the supernatant and submitting this to salting out with ammonium sulphate (80% saturation in the final solution). The salted supernatant was incubated for 30 min in an agitated ice bath.

7.2.6. Enzymatic measurements

Salted solutions were subjected to an initial centrifugation step for 15 min at 14000 rpm and 4 °C. The pellet was collected and resuspended in 4 mL of citrate buffer 0.1M (pH 6.5). After this, the samples were then subjected to a final centrifugation step for 10 min at 14000 rpm and 4°C to remove possible impurities. The supernatant obtained after this process was collected after a final filtration step with a

0.45 μm hydrophilic filter and subjected to enzymatic measurements. Spectrophotometric measurements were performed using a Spectrophotometer JASCO version V-630. The analysis of enzymatic reaction kinetics was performed using the Spectra manager version 2.05.03, incorporated in the spectrophotometer software. Enzymatic activity corresponded to the slope of the curve of absorbance vs. time for the linear part of the curve and was reported as abs/min/g of analysed nectar/juice. The residual activity (RA) was calculated according to the Equation 1.

Equation 1. Residual activity

$$RA = \frac{\text{enzymatic activity of treated product}}{\text{Enzymatic activity of fresh product}} \times 100$$

Polyphenol oxidase (PPO)

Enzymatic assay was performed using 20 μL of catechol 1M as substrate in 880 μL of citrate buffer 0.1 M (pH 6.5). 100 μL of the enzymatic extract were added to perform the measurement. A solution with the same composition, changing the enzymatic extract to citrate buffer, was used as a blank. The measurements were performed at 420 nm for 10 min.

Peroxidase (POD)

Enzymatic assay was performed using 2 μL of *o*-dianisidine 0.1M and 10 μL of hydrogen peroxide 3% v/v as substrates in 888 μL of citrate buffer 0.1 M (pH 6.5). 100 μL of the enzymatic extract were added to perform the measurement. A solution with the same composition, changing the enzymatic extract to citrate buffer, was used as a blank. The measurements were performed at 450 nm for 10 min.

β -glucosidase

Enzymatic assay was performed using 20 μL of 4-nitrophenyl- β -D-glucopyranoside 0.1 M as substrate in 880 μL of citrate buffer 0.1 M (pH 6.5). 100 μL of enzymatic extract were added to perform the measurement. A solution with the same composition, changing the enzymatic extract to citrate buffer, was used as a blank. The measurements were performed at 405 nm for 10 min at 45 $^{\circ}\text{C}$; for this reason, the buffer was preheated to maintain as much as possible the temperature reaction.

7.2.7. Physicochemical parameters pH and Total soluble solids (TSS)

Total soluble solids were measured after the preparation of strawberry and sour cherry nectars and red grape juice extraction. This in order to guarantee in the case of the nectars that the established targets for TSS were achieved. To measure the degrees Brix, a refractometer ATAGO model RX-5000 α was used. Samples were measured in triplicate.

Similarly, the pH was also established in the case of nectars as a target parameter. A pH meter METTLER TOLEDO model SevenDirect SD20 was used to perform the measurements. Measurements were performed in triplicate.

For the shelf-life study, both parameters were measured at each point of the storage time.

7.2.8. Colour measurement

Colour was analysed using a colorimeter HunterLab model LabScan XE supported by the software EasyMatchQC Version 4.9, SensorManager Version 4.45 ColorCalculator Version 4.13. The CIELAB coordinates L*, a*, and b* were reported. L* indicates brightness and has a value between black and white, while a* and b* indicate the direction of the colour, red for positive values of a*, and green for negative values. Similarly, yellow indicates positive values of b*, and blue indicates negative values.

The total colour difference was calculated using the Equation 2:

Equation 2. Total colour difference calculation

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

Where:

- $\Delta L^* = L_{sample}^* - L_{control}^*$
- $\Delta a^* = a_{sample}^* - a_{control}^*$
- $\Delta b^* = b_{sample}^* - b_{control}^*$

The total colour difference between the untreated and treated samples was interpreted using the following scale:

- No perceptible difference. $\Delta E = 0 - 0.5$
- Slightly perceptible difference: $\Delta E = 0.5 - 1.5$
- Evident difference: $\Delta E = 1.5 - 3.0$
- Clearly visible difference: $\Delta E = 3.0 - 6.0$
- Big colour difference: $\Delta E = 6.0 - 12.0$

7.2.9. Total polyphenol content quantification

Folin-Ciocalteu assay

In a 100 mL volumetric flask, 1 mL of the sample was added to 75 mL of distilled water. After 5 mL of Folin-Ciocalteu reagent 2N was added, the solution was agitated and incubated for 5 min at ambient temperature. After incubation, 10 mL of a saturated solution of sodium carbonate was added to the volumetric flask, and the volume was made up with distilled water. After agitation, the solution was incubated for one hour at ambient temperature to further be filtrated. The filtered samples were used for spectrophotometric measurements at 765 nm. The blank was prepared following the same method but with 1 mL of absolute ethanol instead of the sample.

Gallic acid calibration curve

Gallic acid standard was dissolved in absolute ethanol at concentrations ranging from 1000 ppm to 50 ppm. Samples obtained at different concentrations were subjected to the previously described method to generate a calibration curve for gallic acid.

Total polyphenol quantification

To determine the appropriate dilution factor of the samples, initial tests were performed at different dilutions to define the most adequate dilution factor for each substrate to be in the range of the calibration curve previously made. Strawberry and sour cherry nectar required a D5 dilution while red grape juice a D2. Results are presented as mg GAE/g.

7.2.10. Antioxidant capacity quantification

DPPH (2,2-Diphenyl-1-picrylhydrazyl) preparation

A solution of DPPH 0.1 mM in absolute ethanol was prepared and subjected to spectrophotometric measurement at 517 nm. The absorbance of the solution should be in the range of 1 to 1.1 and further adjustment could be required in order to achieve this concentration.

Sample preparation

Fruit juices were centrifuged at 9000 rpm for 10 min to further undergo filtration with a 0.45 µm hydrophilic filter. Samples were further diluted with absolute ethanol according to an initial test performed when the dilution factor D5 was established for strawberry and sour cherry nectar, whereas D2 was established for red grape juice.

Trolox calibration curve

Trolox was dissolved in absolute ethanol at concentrations ranging from 500 to 50 µmol/L. Samples obtained at different concentrations were subjected to the method described further to generate a calibration curve for Trolox.

Total antioxidant capacity assay

DPPH solution (2 mL) and absolute ethanol (2 mL) were incubated with the extracted sample (0.2 mL) under dark conditions for 30 min after agitation. Red grape juice required filtration with a 0.45 µm hydrophilic filter prior to measurement. After incubation, the samples were subjected to spectrophotometric measurements at 517 nm. Results are reported as TEAC µmol/g (Trolox equivalent antioxidant capacity).

7.2.11. Oxygen barrier test

The evaluation of the Oxygen Transmission Rate (OTR) was performed according to the standard DIN 53380-3, with the established parameters of temperature and relative humidity of 23°C and 50%

respectively (Deutsches Institut für Normung, 1998), using the Extra Solution TotalPerm with colorimetric determination. The results were reported in cm³/24h.

7.3. Statistical analysis

OriginPro 2023b (Learning Edition) was used for all statistical analysis. The comprehensive analysis included 2-way ANOVA. Tukey's post-hoc test was used to evaluate significant differences among groups.

Microbial inactivation modelling

Another way to characterise the obtained results is to use the kinetic parameters. Generally, thermal inactivation models are used to describe other technologies associated with first-order kinetics to establish an inactivation rate constant “*k*” (min⁻¹) (Buzrul, 2022). However, not all inactivation processes exhibit linear trends. This is the reason why more flexible mathematical models have been developed to adjust to one model of different behavioural inactivation kinetics. The Weibull model fills this characteristic (Equation 3). Its use is supported by different authors who have compared the correlation of this model in contrast with the classical first-order kinetics (Serment-Moreno, 2021; Usaga et al., 2021)

Equation 3. Weibull model

$$\log_{10} S(t) = -b * t^n$$

Where:

- *S(t)*: Microbial survival ratio defined as (*N/N₀*), where *N* is the microbial count at time *t* and *N₀* is the initial microbial count.
- *B*: Weibull model rate parameter, min⁻ⁿ
- *N*: Weibull model exponent or shape parameter, dimensionless

Surface response – Design of Experiments (DoE)

A mathematical model was fitted to represent the surface response to evaluate the results obtained during the process-screening trials. The characteristics of the model are given by Equation 4.

Equation 4. Fitted model for response variables dependant on time and pressure variables

$$RV = a t + b P + c t P + d P^2 + e t^2$$

Where RV is the response variable (microbial logarithmic reduction, enzymatic residual activity, etc.), and the letters a , b , c , d , and e correspond to the coefficients for time, pressure, time-pressure interaction, quadratic term for pressure, and quadratic term for time, respectively.

The fitting process started with the inclusion of all coefficients from Equation 4, followed by a refinement of the model according to the statistical significance for each term. The experimental design and subsequently fitting process was performed using OriginPro 2023b (Learning Edition), more specifically the DoE (Design of Experiments) tool which also performs tests for data normality and residuals analysis to validate the adequacy of the model.

8. Results and discussion

8.1. Enzymatic activity initial evaluation

Strawberry and sour cherry puree and red grape juice were tested in order to identify their response to enzymatic activity measurements. The results are shown in Figure 7. Strawberry puree samples included purees from Sicoly and SVZ and, as a reference, a sample of the variety 1171 (internal coding), which presented high enzymatic activity. As it is possible to observe in the figure, strawberry purees used in this study did not exhibit enzymatic activity with respect to PPO and β -glucosidase enzymes. In contrast, POD enzymes were detected in strawberry puree from the SVZ supplier, which was the only positive response for this substrate. All three enzymes were quantified in the sour cherry puree, leading to further quantification in subsequent stages of the project. Similar results were obtained for red grape juice; in this case, only active PPO (with a higher activity than that of sour cherry puree) and POD enzymes were observed.

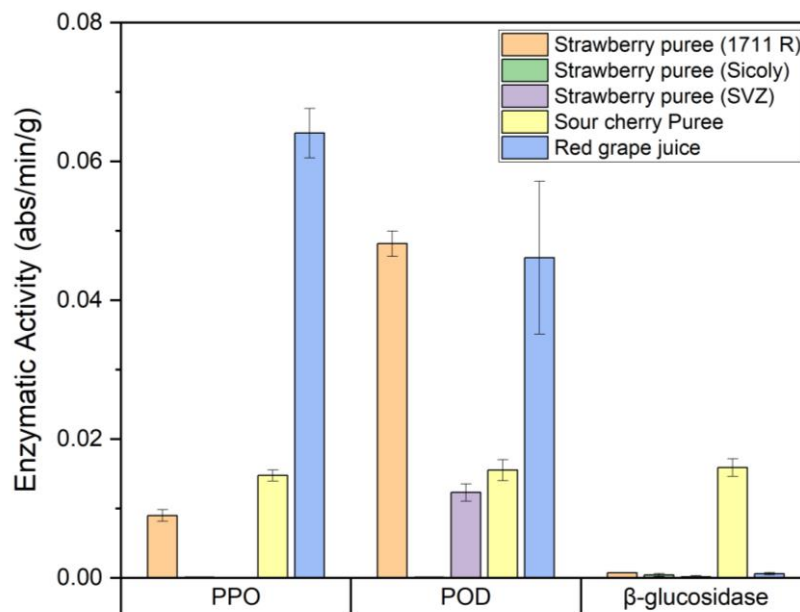


Figure 7. Initial evaluation of enzymatic activity. Substrates comparison

Several authors have reported the enzymatic activity of strawberries. In a study conducted by Holzwarth et al. (2012) where the objective was to evaluate the effect of different freezing and thawing methods on the colour and polyphenols retentions in strawberry, they also measured PPO activity. In this study, three different cultivars were evaluated, among them Senga Sengana (the same cultivar as that of the

Sicolý puree used in this study). They reported an enzymatic activity for PPO of 36.6 U/100g Dry Matter (DM), indicating the presence of this enzyme in the substrate. The differences between the two studies could be attributed to possible changes in enzymatic activity due to freezing or the harvesting year. Other authors have shown results similar to those presented in this study with respect to the variation among cultivars and varieties in their enzymatic content. In another study, Holzwarth et al. (2013) evaluated the effect of different inhibitors on the activity of PPO enzymes in strawberry puree. The values for PPO activity prior to inhibitors inoculation for the cultivars “Malwina”, “Clery” and “Elsanta” were reported as 26.5, 85.1 and 19.6 U/100g DM, showing a great variability with respect to cultivar. Cao et al. (2018) reported differences in the absolute activity of four Chinese cultivars. The variety Hongyan was the only variety with a significantly higher PPO activity compared to its POD activity and also to the PPO activity of the other varieties tested. For the Tianziang, Tongzi I, and Zhang I varieties, significantly higher activity of POD enzymes was observed when comparing their PPO enzymatic activity. In a more recent study, Teribia et al. (2021) evaluated PPO and POD enzymatic activity for 12 different cultivars prior to thermal processing, and the results showed high variation among cultivars, being more evident for POD enzymes, and also showing a general trend of higher activity for POD than PPO enzymes. Other studies have reported the activity for PPO, POD, and β -glucosidase enzymes, which mostly refer to residual activity after processing (Aguiló-Aguayo et al., 2008; Gössinger et al., 2009; Teribia, Buvé, Bonerz, Aschoff, Hendrickx, et al., 2021), including HPP (Aaby et al., 2018; Chakraborty et al., 2015b; Garcia-Palazon et al., 2004; Terefe et al., 2013). Based on the results obtained, it was not possible to evaluate the enzymatic activity of strawberries during the screening process, considering that only the puree from Sicolý was available. In contrast, when the shelf-life study was performed with the SVZ puree, POD enzymes were quantified during the storage period.

No previous study has quantified the enzymatic activity of sour cherries. Studies made by Pasquariello et al. (2015), evaluated the enzymatic activity of different enzymes (among them PPO and POD) in sweet cherry fruit (*Prunus avium* L.) for three different cultivars (“Ferrovia”, “Lapins”, and “Della Recca”) when fruits were covered by a chitosan coat. Their results showed enzymatic activity of

approximately $30 \text{ mmol kg}^{-1}\text{s}^{-1}$ for PPO enzymes and 10^6 U kg^{-1} for POD enzymes. There were no significant differences between varieties for either enzyme. Furthermore, no other studies have reported enzymatic activity values for sweet or sour cherries. In the present study, considering the high activity showed by all the enzymes included in this project, PPO, POD, and β -glucoside activities were evaluated for the process screening trials, as well as for the shelf-life study.

With respect to red grape juice, various authors have studied the enzymatic activity of diverse grape varieties. Troiani et al. (2003) evaluated the enzymatic activity of PPO and POD in the peel and pulp of three different cultivars (Rubi, Benitaka, and Borbon). Their results showed that enzymes are allocated differently in the fruit depending on the variety; the varieties Rubi and Benitaka presented higher PPO activity in the peel than in the pulp. In contrast, the Borbon variety showed higher POD activity in the pulp. With respect to PPO enzymes, the results were the opposite, with more enzymatic activity in the pulp for Rubi and Benitaka and lower PPO activity for Borbon. Other studies have also evaluated the enzymatic activity of PPO and POD in the Rubi and Benitaka varieties (Freitas et al., 2008). Their results showed higher enzymatic activity for POD with respect to PPO, which is comparable to those presented in the study conducted by Troiani et al. (2003). In this study, the enzymatic activity of PPO was higher than that of POD (with a higher standard deviation in the initial analysis). However, the type and concentration of enzymes are strictly linked to the variety, making it difficult to compare the data obtained by previous studies with those presented in this study, considering that the varieties differ. Studies such as those made by Orak (2007) show the variation of PPO activity in 16 different cultivars, finding values from 0.040 (cultivar Adakarasi) to $1.185 \text{ U min}^{-1} \text{ mL}^{-1}$ (cultivar Kokulu Siyah), accounting for the intrinsic variation of the enzymatic activity with respect to cultivar. Other authors have also studied the enzymatic activity of other grape varieties with respect to residual activity after processing, including HPP treatment (Del Pozo-Insfran et al., 2007; Li & Padilla-Zakour, 2021; Rapeanu et al., 2006; C. A. Weemaes et al., 1998).

8.2. HPP treatment in microbial inactivation

Samples of strawberry and sour cherry nectars, and red grape juice were treated with HPP. The relevant physicochemical parameters characterising these products (pH and degrees Brix) are presented in Table 5. As previously stated, strawberry and sour cherry nectars were formulated to achieve Total Soluble Solids (TSS) content of 12% and pH in the range 3.2-3.5. These results are in agreement with the defined values, which were slightly lower for sour cherry nectar. In contrast, red grape juice was extracted from the fruit, and no further alteration was made to it. As can be observed, there is a significant difference between the parameters corresponding to nectar and the juices, being in both cases higher for the juice. These parameters are relevant, as previously mentioned, for microbial growth, and directly impact the microbial survival rate.

Table 5. Physicochemical parameters of nectars and juices pre-treatment for microbiological evaluation

Substrate	°Brix	pH
Strawberry nectar	12.18 ± 0.12	3.28 ± 0.03
Sour Cherry nectar	11.52 ± 0.74	3.29 ± 0.00
Red Grape juice	16.99 ± 1.66	4.00 ± 0.11

An initial characterisation of the endogenous microflora was performed. The results are presented in Table 6. The microbial load present in the different products was quite different; for strawberry and sour cherry nectars, the identified microorganisms belonged to the genus *Bacillus*. On the other hand, red grape juice, which presented the highest microbial load among the studied samples, also contained microorganisms belonging to the genus *Lactobacillus* and a mould identified as *Aspergillus carbonarius*, which can be considered an associated species of grape fruits. These results were expected considering that red grape juice presents a less restrictive environment for microorganism growth.

Table 6. Total plate count - nectars and juices pre-treatment

Substrate	Total Plate Count (log CFU/mL)
Strawberry nectar	1.25
Sour Cherry nectar	3.62
Red Grape juice	4.01

These results are similar to those reported in previous studies were initial microbial load on strawberry puree and juice for the variety Senga Sengana (same variety to that used in this study), being 3.2 and 2.0 log CFU/mL, respectively, considering these values as low starting levels. Here, HPP at three different conditions was used to evaluate the effect of processing in strawberry puree and juice (300, 400, and 500 MPa for 3 min), finding no survivals after treatment (Aaby et al., 2018).

Other authors have reported much higher initial load for the same variety: Marszałek et al. (2015) reported initial values for total microbial count (TMC) of 4.86 log CFU/g Fresh Weight (FW) fresh strawberries, in addition to 4.6 and 3.82 log CFU/g FW for yeasts and moulds, respectively. In their study, and with respect to microbiological inactivation, HPP effectiveness was assessed by treating samples at 300 and 500 MPa for 1, 5, and 15 min at two different temperatures (0 and 50°C). The TMC was not significantly reduced for samples at 0°C, and a slightly significant reduction (approximately 1 logarithmic unit) was observed for samples at 50°C. Yeast and moulds were inactivated under all conditions at 50°C, while remaining accountable in samples subjected to 300 MPa for 1 and 5 min. Similarly, Cao et al. (2014) reported values of total aerobic bacteria for cloudy and clear strawberry juice (variety Tongzi I), being 4.23 and 3.56 log CFU/mL, respectively, in a study where juices were submitted to HHP at 0, 2, 4, and 6 minutes of holding time and 600 MPa. Only cloudy juice at holding times of 0 and 2 min presented survivals; for all other conditions, viable cells were not detected. In the case of coliforms, moulds, and yeast, all treatment conditions were effective in terms of inactivation.

Sour cherry nectar evaluated in this study presented a lower initial microbial load compared to previous findings. For instance, Tenuta et al. (2023) reported the concentration of total mesophilic bacteria and yeast and moulds, being 5.40 and 1.32 log CFU/mL, respectively. However, the cultivar used in this study was Amarena del Rio, cultivated in Modena (Italy) in 2021, to investigate the potential of HPP in the stabilisation of this fruit. With respect to the microbiological effectiveness, complete inactivation of bacteria, yeast, and moulds was reported after 3 min and 600 MPa after treatment and maintaining these results throughout the study. These differences can be attributed to the difference with respect to the variety used in this study. Another relevant study on sweet cherry (*Prunus avium* L.) reported initial total aerobic microbial and yeast counts as 3.5 and 4.7 log CFU/mL, respectively (similar values to

those found in this study, except for yeast). After HPP treatment at two different conditions (400 MPa for 5 min and 550 MPa for 2 min), the samples presented undetectable levels of bacteria and yeasts, maintaining these conditions during refrigerated storage for 28 days (Queirós et al., 2015).

Red grapes, being the substrate with more initial endogenous microflora, presented lower results compared to previous studies. Morata et al. (2015) reported 4 log CFU/mL for yeast and 2 log CFU/mL for bacterial population. In this study, grapes (*Vitis vinifera* L.) from the variety Tempranillo were treated with HPP at 200, 400, and 550 MPa for 10 min, finding yeast only after the treatment at 200 MPa (3 log CFU/mL); with respect to bacteria, a significant reduction was achieved with all treatments, but not a complete inactivation. Li & Padilla-Zakour (2021) also reported initial microbial load for Concord grape puree, being 6.33 and 6.31 log CFU/g FW, for total aerobic bacteria and yeast/moulds, respectively. This puree underwent HPP at 600 MPa for 3 min, showing a significant reduction of the load, which remained stable during refrigerated storage for 5 months. Other studies have reported the inactivation of native microorganisms in different fruit products, including pineapple, aronia berry, peach, and smoothies (Bleoanca et al., 2021; Chakraborty et al., 2015a; Hurtado et al., 2017; Picouet et al., 2016; Yuan et al., 2018).

8.2.1. *Salmonella* surrogate (*E. coli* ATCC 11229)

Considering the inherent characteristics of each of the substrates evaluated in this study, a difference in the behaviour for the *Salmonella* surrogate (*E. coli* ATCC 11229) was expected. Model parameters obtained after fitting and optimising the surface response for the process (Equation 4) are presented in Table 7. All substrates were modelled considering the Pressure-Time interaction parameter (*c*). Only sour cherry nectar presented a better fit with the addition of quadratic parameters, in this case, the pressure–pressure coefficient (*d*). Coded and uncoded coefficients represent the change in the mean response (logarithmic reduction, enzymatic activity, etc.) associated with the increase in the specific variable to which they are referred, while others remain constant. Their magnitude assesses the weight of each variable in terms of its contribution to the response modification (Minitab, n.d.).

Table 7. Summary of surface response fitted model parameters for *E. coli* ATCC 11229 - *Salmonella* surrogate.

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Strawberry nectar	0.73099	0.93189	0.33403	-	-	0.857
Sour Cherry nectar	1.01926	0.89669	0.88147	-0.75365	-	0.895
Red Grape juice	0.75306	0.39278	0.63954	-	-	0.692

Some variations were also found in terms of these coefficients, for example, while for strawberry nectar pressure had a greater impact on the response (meaning that the coefficient *b* presented a higher value compared to the coefficient *a*), for red grape juice, time was the dominant variable, and the interaction term was greater than pressure. For sour cherry nectar, both factors have a similar impact as single variables; however, with the presence of the quadratic coefficient for pressure, this variable acquires more weight in the model. As can be seen in the table, the behaviour of the response cannot be attributed to only one variable, considering that the interaction coefficient has a similar weight (in order of magnitude) as those of the independent variables.

The Juice HACCP guidance for industry published by the Center for Food Safety and Applied Nutrition (CFSA) establishes a minimum requirement of 5-log pathogen reduction as the minimum level that should be achieved for microbial inactivation of specific pathogens; this will be the criteria for evaluating the efficiency of the HPP technology (CFSAN, 2004). The surface response corresponding to the logarithmic reduction for *E. coli* ATCC 11229 in the different substrates tested in this study are presented in Figure 8, Figure 9, and Figure 10. At the lowest time and pressure conditions (2 min and 200 MPa), strawberry nectar presented a logarithmic reduction higher than 2.5 units, further showing a profile that increased in a directly proportional manner with the increase in both variables. A 5-log reduction can be achieved with different combinations of parameters; the limit of inactivation was approximately 5.5-log reduction with the most extreme conditions tested for microorganisms in this study (Figure 8).

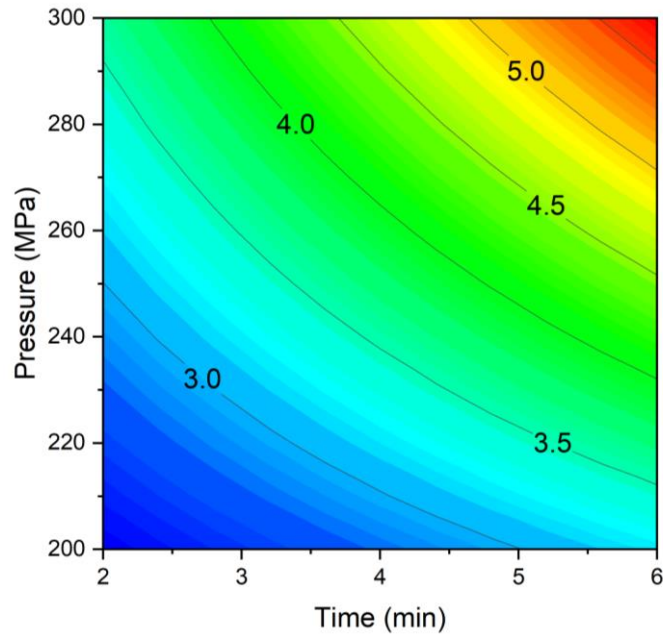


Figure 8. Contour Plot. Logarithmic reduction for *E. coli* ATCC 11229 - *Salmonella* surrogate in Strawberry nectar

In a study conducted by Y. Huang et al. (2013), different results were observed. A strawberry puree was inoculated with a *Salmonella* cocktail (*Salmonella enterica* serotypes Newport, Montevideo, and Stanley) at two different inoculum concentrations (low and high) and subjected to HPP under conditions ranging from 200 to 500 MPa at intervals of 50 MPa for 2 min at 21°C. For the low-inoculum samples, after treatment at 300 MPa, there was a log-reduction of approximately 3 units. Comparatively, for those with high inoculum, a 5 log-reduction was achieved under the same treatment conditions. These last results are similar to those presented in this study, even though the process temperature and the type of product were different (puree vs. nectar) and the variety was unknown. As expected, the physicochemical characteristics of the puree were different: the pH was slightly higher (3.6) and degrees Brix lower (8.8); however, the impact was not significant, reaching similar inactivation targets.

E. coli ATCC 11229 presented general higher resistance to low time-pressure combinations in sour cherry nectar, compared to strawberry nectar (Figure 9). At short times and at all pressures or at low pressures regardless of the treatment time, the inactivation was approximately 2.0 log reduction; just about the middle point of the experimental space (approximately 250 MPa and 4 min), the increase in

the inactivation rate accelerated, starting at that point in about 3.5-log reduction and reaching approximately 5.5-log reduction at the highest conditions evaluated.

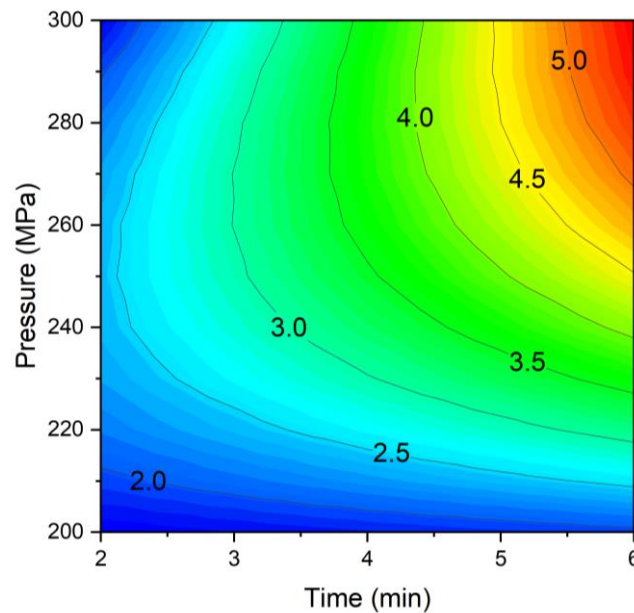


Figure 9. Contour Plot. Logarithmic reduction for *E. coli* ATCC 11229 -*Salmonella* surrogate in Sour Cherry nectar

Sour cherry juice was previously tested as substrate for *Salmonella enterica* subsp. *enteritidis* in a study conducted by Bayındırlı et al. (2006), where the juice was inoculated with this bacterium and underwent HPP at 250 MPa and 30°C for 5, 10, and 20 min, in addition to treatments at 350 MPa for 5 min at 30 and 40 °C. The results showed a 5.5-log reduction after treatment at 250 MPa for 5 min. and further reductions of 6.66 and 7.3 for 10 and 20 min, respectively. With respect to the effect of temperature at constant time and pressure, *S. enteritidis* was not detected under any condition. pH was the only parameter considered in the physicochemical characterisation (3.3), and no indications of the variety used were presented. With respect to this study, the increase in the effectiveness of HPP might be due to the difference in temperature (4 and 30°C) that might promote inactivation.

Finally, red grape juice presented a similar pattern to that of sour cherry nectar, but with lower inactivation values (Figure 10). At short times, even under the highest tested pressure conditions, *E. coli* ATCC 11229 concentration was only reduced to less than 2-log units. The same analysis can be made for low-pressure conditions at all treatment times, where the limit is 2.5-log units, similar to the

value achieved in the middle of the design space. The maximum inactivation achieved in this study corresponds to approximately 4-log units not being able to achieve the required 5-log reduction required for juices under these conditions.

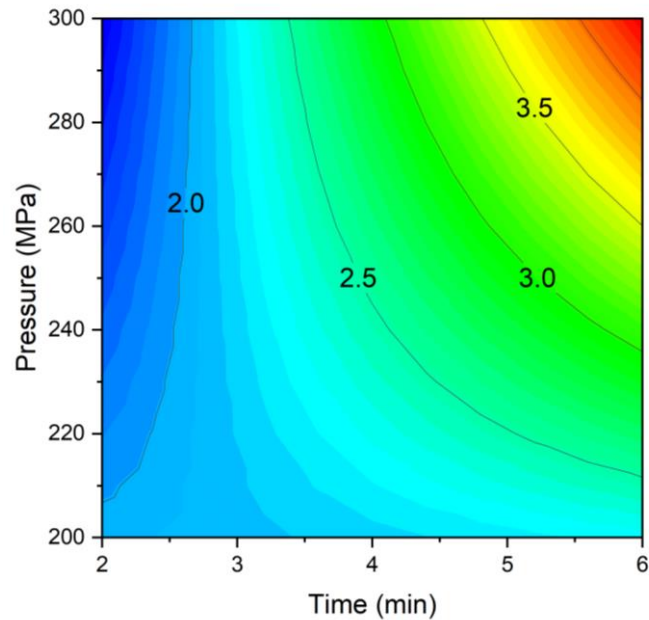


Figure 10. Contour Plot. Logarithmic reduction for *E. coli* ATCC 11229 -*Salmonella* surrogate in Red Grape juice

Considering the lack of adjustment of the experimental data for the surface response ($R^2 = 0.629$), it is relevant to evaluate the experimental data. Figure 11 shows the experimental data for *E. coli* ATCC 11229 in red grape juice. For 200 and 250 MPa, the experimental values were similar to those presented in the model, reaching a maximum inactivation of approximately 3-log units for all time points. The only difference with respect to the model was the point at 300 MPa and 6 min, where the inactivation achieved was approximately 5-log units while the model presented lower predictions.

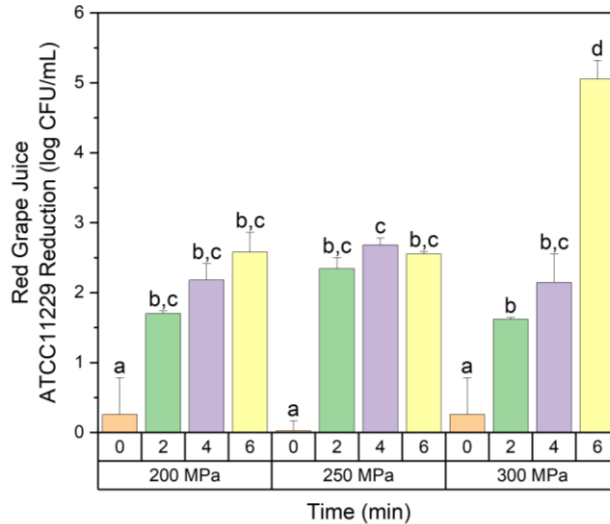


Figure 11. Experimental data. Logarithmic reduction for *E. coli* ATCC 11229–*Salmonella* surrogate in Red Grape juice. Different letters indicate statistically significant differences.

Previous studies for Concord grape juice used the surface response methodology to characterise the inactivation of a *S. enterica* cocktail (Petrus et al., 2020). Here samples of the juice were treated at different time and pressure combinations, following a methodology similar to that used in this study. The parameters ranged from 300 to 550 MPa and from 25 to 225 s. In their findings, a maximum of bacterial inactivation was found to be around the conditions 475 MPa and 150 seconds, reaching an inactivation of 8-log units. The pH and degrees Brix of this juice were measured, being 3.39 and 16.5, respectively. These parameters are similar to those of the juice produced in this study; however, a comparison of both datasets is not possible, considering that both experimental designs were conceptualised in different ranges of the variables of interest. Results obtained in the evaluation of pressure resistance of *Salmonella* strains in other matrices, such as orange, apples, and even culture medium or model solutions, are also available. Nevertheless, the comparison of these data with this study is not relevant, considering the substrate-specific behaviour of the strains (Bull et al., 2005; González-Angulo et al., 2021; Ogihara et al., 2009; Usaga et al., 2021).

Kinetic modelling

The results of the data modelled using the Weibull equation are presented in Table 8. As can be seen, the behaviour was linear only for red grape juice treated at 300 MPa, whereas the shape of the curve proved to be more curved (concave). The curvature was convex only for sour cherry nectar ($n = 1.307$).

Table 8. Kinetic parameters for *E. coli* ATCC 11229 - Weibull model.

Substrate	Pressure (MPa)	b	n	R ²
Strawberry nectar	200	1.58829	0.42246	0.999
	250	2.98663	0.17160	0.999
	300	1.99917	0.57874	0.996
Sour cherry nectar	200	0.98169	0.46033	0.999
	250	2.60146	0.21382	0.999
	300	0.5552	1.30671	0.999
Red grape juice	200	1.31561	0.37169	0.981
	250	2.43042	0.02969	0.984
	300	0.80631	1.00238	0.932

With respect to the rate parameter, strawberry nectar presented the highest parameters compared with the other substrates. There was no trend in terms of the increase in pressure for this parameter. The correlation coefficients remained high for all conditions.

8.2.2. EHEC surrogate (*E. coli* ATCC 8327)

As expected, the surface responses modelled for *E. coli* ATCC 8327 differed from those obtained for *E. coli* ATCC 11229. The coefficients corresponding to these models are presented in Table 9. Similar to the models created for the *Salmonella* surrogate, in this case, all three substrates included in their models the interaction coefficient (*c*) with a similar order of magnitude (i.e. the response cannot be attributed only to a single variable). Nevertheless, the strawberry and sour cherry nectar models required the inclusion of the quadratic coefficient for pressure (*d*), and this supports the fact that pressure is the most relevant variable in the model for those substrates. In contrast, for red grape juice, it has been difficult to establish a leading variable, considering the closeness of the coefficients and the correlation coefficient.

Table 9. Summary of surface response fitted model parameters for EC8327 - EHEC surrogate.

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Strawberry nectar	1.16192	1.25366	0.74953	0.78655	-	0.915
Sour Cherry nectar	1.09961	1.58712	0.55193	-1.54732	-	0.962
Red Grapes juice	0.61661	0.48180	0.42565	-	-	0.716

With respect to the inactivation of the EHEC surrogate, the surface responses for strawberry nectar, sour cherry nectar, and red grape juice are presented in Figure 12, Figure 13, and Figure 14. For strawberry nectar, *E. coli* ATCC 8327 presented a high resistance at short times at all pressures evaluated in this study; similarly, an increase in time at low-pressure conditions seemed ineffective in the inactivation of this microorganism (Figure 12). It seems effective only at high time-pressure combinations (more than 4 min and 250 MPa). The 5-log reduction target was achieved only at the very top corner of the design space, accounting for the most extreme conditions tested in this study.

Previous attempts to evaluate the pressure resistance of EHEC have been made by various authors. The study carried out by Y. Huang et al. (2013), included five strains of *Escherichia coli* serotype O157:H7 (strains H1730, Cider, 250, 251, and J58) that were inoculated in strawberry puree at two concentration levels. After treatment for 2 min at pressures between 200 and 500 MPa, samples with low inoculum presented a maximum log reduction of 1.5 at 300 MPa, which is similar to the results obtained in this study. Meanwhile, for those with a high inoculum, at 350 MPa there was only a reduction in 3.1 log units. Such results are more similar in terms of microbial load to those evaluated in this study; however, as previously stated, there are substantial differences in relevant variables such as pH, degrees Brix, and pulp content that influence the difference between the two experiments. Other studies have evaluated other *E. coli* strains in strawberry purees. Hsu et al. (2014) inoculated a cocktail containing five *E. coli* O157:H7 strains (ATCC 43888, 43889, 43890, 45756, and 11082) into fresh strawberry puree treated for 5, 15, and 30 min at pressures between 150 and 650 MPa, with an initial temperature of 10°C. The results showed that at 150 MPa after 30 min there was a reduction spectrum from 8.3 to 2.0 logarithmic units, referred to differences between strains. Starting from the treatment at 250 MPa for 5 min, all samples reached a 6.8-log reduction as the maximum achievable value, being below the detection limit (1.5 Log CFU/mL). Again, these results present differences compared to the data presented in this study, considering variables such as pH, TSS, or pulp content (100% in the case of purees and 40% in the present study), which might provide protection against the treatment.

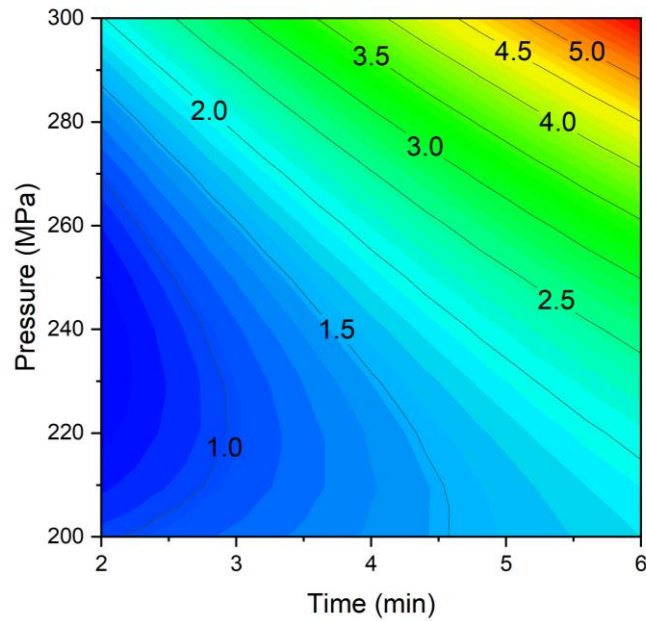


Figure 12. Contour Plot. Logarithmic reduction for EC8327 -EHEC surrogate in Strawberry nectar

Some authors have evaluated EHEC surrogates in strawberry juice. Yildiz et al. (2019), assessed the pressure resistance of an acid-adapted *E. coli* EHEC surrogate (ATCC 11775) in strawberry, treating with HPP inoculated samples at 200, 250, 300, 350, and 400 MPa for times up to 2 minutes. They found similar inactivation behaviour between 200 and 250 MPa, reaching a maximum inactivation of approximately 3-log units. Treatments under higher conditions proved to achieve at least 5-log reduction in times decreasing while pressure increased (about 1 min for 300 MPa). The juice was characterized, having pH and TSS values of 3.40 and 8.05, respectively. Compared to the data presented in this study, such results are similar showing a very homogeneous line at 2 min for pressure conditions between 200 and 250 MPa, apart from the fact that a 5-log reduction was achieved only after a longer treatment under the isobaric condition of 300 MPa.

With respect to sour cherry nectar, the prevalence of pressure over time was more notorious, especially at lower pressure values, where an increase in time proved to have a minor effect on inactivation (Figure 13). With an increase in pressure, time provides a more noticeable effect, leading to a 5-log target reduction that was achieved in the middle of the design space. This allowed for higher inactivation values to be achieved (maximum around 6.5-log reduction).

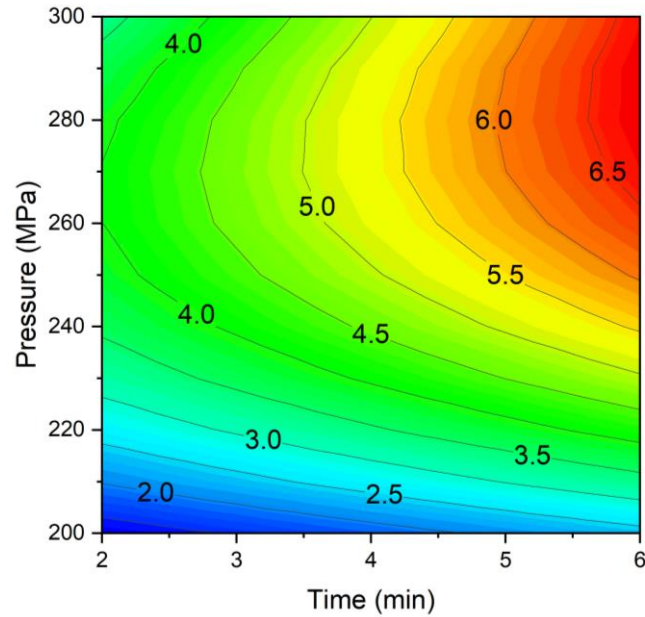


Figure 13. Contour Plot. Logarithmic reduction for EC8327 -EHEC surrogate in Sour Cherry nectar

Bayındırlı et al. (2006) evaluated the inactivation potential of *E. coli* O157:H7 in sour cherry juice, inoculating samples and treating them at 250 MPa for 5, 10, and 20 min at 30 °C, or at 350 MPa and 5 min at 30 and 40 °C. Their results showed that with an increase in time, *E. coli* O157:H7 was inactivated by 5.28 log units after 5 min of treatment, reaching a maximum inactivation value of 6.85 log after 20 min. With respect to the increase in temperature, samples treated at 30 °C at 350 MPa for 5 min showed a reduction of 7.67 log, whereas at 40 °C, no survivors were detected. These results are hardly comparable to those obtained in the present study, particularly because of the difference in temperature between the experimental designs.

Finally, regarding the inactivation of the *E. coli* EHEC surrogate in red grape juice, this microorganism presented a much higher resistance than strawberry and sour cherry nectar, reaching an inactivation lower than 3-log (Figure 14). The strain also showed high resistance at short treatment periods under all pressure conditions; similarly, under low-pressure conditions, time did not significantly influence microbial reduction.

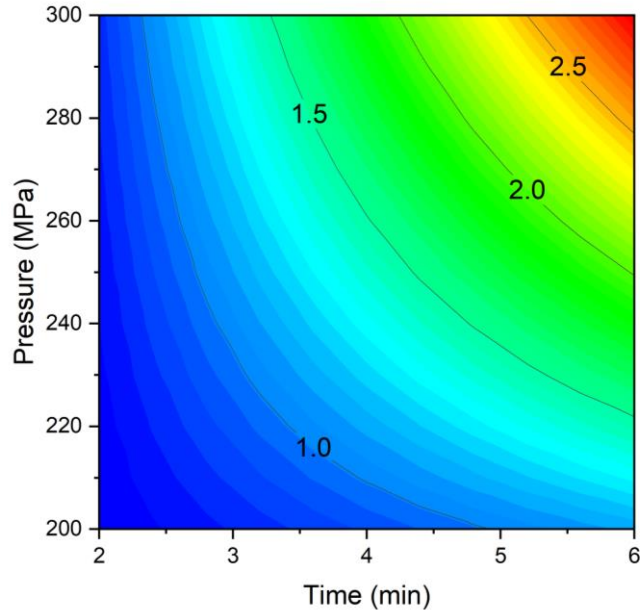


Figure 14. Contour Plot. Logarithmic reduction for *E. coli* ATCC 8327 -EHEC surrogate in Red Grape juice

Similar to the *E. coli* ATCC 11229 strain, the surface model adapted to the experimental data for *E. coli* ATCC 8327 showed poor correlation ($R^2 = 0.716$). The experimental values for this surrogate in red grape juice are presented in Figure 15. It showed a high resistance to pressure inactivation, except for the point taken at 6 min under all pressure conditions. These results agree with those represented by the surface response, despite its lack of fit.

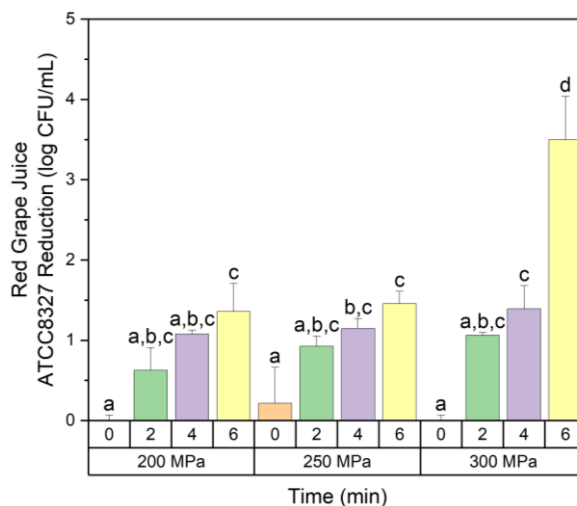


Figure 15. Experimental data. Logarithmic reduction for *E. coli* ATCC 8327 -EHEC surrogate in Red Grape juice. Different letters indicate statistically significant differences.

As for other substrates, previous authors have also evaluated grape juice as a substrate to inoculate EHEC strains. Petrus et al. (2020), who also evaluated the resistance of *S. enterica*, investigated the pressure resistance of an *E. coli* O157:H7 cocktail (strains C7927, ATCC 43890, ATCC 43894, ATCC43889, and ATCC 35150) in Concord grape juice. The results were modelled as a surface response, considering pressure conditions from 300 to 550 MPa and times from 25 to 225 s. In their study, an optimal inactivation value was achieved at approximately 500 MPa and 150 s (similar to that of the *S. enterica* cocktail tested in the same study). As stated before, even though the characteristics of the juice in that study were similar to those evaluated here, both the experimental design and fruit variety were different.

Kinetic modelling

Table 10 shows the kinetic parameters obtained for the *E. coli* EHEC surrogate for different substrates. With respect to the curvature, only strawberry nectar at 200 MPa presented a linear kinetic curve ($n=1.091$), while for the other conditions and substrates, kinetic models corresponded to a concave curve, except for strawberry nectar at 300 MPa, whose curve was the only convex in the group.

Table 10. Kinetic parameters for *E. coli* ATCC 8327 - Weibull model.

Substrate	Pressure (MPa)	b	n	R ²
Strawberry nectar	200	0.29618	1.09107	0.999
	250	1.10879	0.32362	0.996
	300	0.71893	1.20653	0.999
Sour Cherry nectar	200	0.83057	0.54031	0.975
	250	2.94104	0.39140	0.996
	300	2.09761	0.64089	0.996
Red Grape juice	200	0.42086	0.67747	0.999
	250	0.67547	0.41338	0.931
	300	0.58743	0.84914	0.967

Regarding the rate parameters, low values were found for 200 MPa in all substrates; the values for the 250 and 300 MPa treatments were the highest. Sour cherry nectar had the highest rate values in this study, in agreement with the corresponding surface response, where it was possible to achieve inactivation higher than 6-log units.

8.2.3. *Listeria monocytogenes* surrogate - Listeria cocktail

The inactivation of the *L. monocytogenes* surrogate presented a high variation with respect to the evaluated substrates, similar to the behaviour showed by other strains. The adjusted model parameters for the response surface of each substrate are presented in Table 11. In this case, strawberry and sour cherry nectar presented a similarity in terms of the order of magnitude of the coefficients, with the same structure in the equation for both, including the quadratic coefficient for pressure, making this variable the most relevant in terms of response modification. This resulted in high similarity in the contour plots compared to that of red grape juice. For this last substrate, its equation does not include the interaction term (*c*) but includes both quadratic coefficients for time (*e*) and pressure (*d*).

Table 11. Summary of surface response fitted model parameters for *Listeria cocktail - Listeria monocytogenes* surrogate.

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Strawberry nectar	0.67503	0.75155	-0.39355	0.82124	-	0.860
Sour Cherry nectar	0.6628	0.75681	-0.48011	0.64693	-	0.904
Red Grapes juice	0.52189	1.45158	-	0.66791	0.32313	0.932

The contour plots for all three substrates are shown in Figure 16, Figure 17, and Figure 18. In the case of strawberry nectar, and compared to Salmonella and EHEC surrogates, 5-log inactivation was achieved under milder conditions (**Figure 16**); time-pressure combinations at short times and high pressures or long times at low pressures allowed the inactivation target to be reached. Additionally, the iso-log lines in the upper part of the contour plot were flatter, meaning that at 290 MPa and at all times tested, it was possible to achieve logarithmic reductions from 5 to 5.5 logarithmic units.

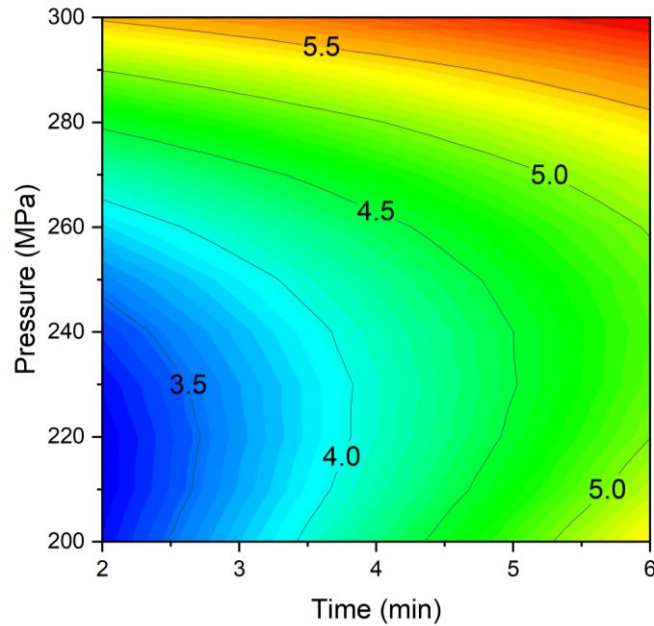


Figure 16. Contour Plot. Logarithmic reduction for *Listeria cocktail* -*Listeria monocytogenes* surrogate in Strawberry nectar

No previous attempts have been made to describe the inactivation of *L. monocytogenes* or its surrogates under high pressure conditions. Some authors have considered its stability when inoculated into strawberry juice or dry slices. Yildiz et al. (2019) evaluated the survival of *L. innocua* (ATCC 51742) in strawberry juice incubated at 25 °C for 48 hours. In the juice (with pH 3.40 and 8.05v degrees Brix) *L. innocua* showed a microbial reduction of about 2-log after 48 hours. A similar study using a *L. monocytogenes* cocktail (ILSI 4, ILSI 18, ILSI34, and ILSI 39) in strawberry dry slices showed a reduction in microbial load of 3-log units over a period of 330 days at 23 °C. However, none of these studies has evaluated the efficiency of HPP in the inactivation of these microorganisms in strawberry-based substrates.

As previously stated, sour cherry nectar presented a contour plot similar to that of strawberry nectar (Figure 17). However, in this substrate, the *L. monocytogenes* surrogate cocktail showed a higher resistance, achieving from 4.5 to 5-log units under the most extreme conditions tested in the study.

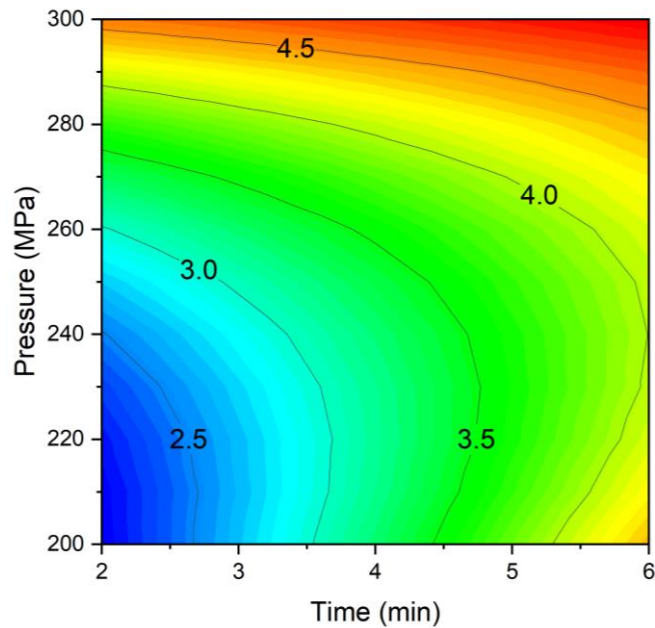


Figure 17. Contour Plot. Logarithmic reduction for *Listeria cocktail -Listeria monocytogenes surrogate* in Sour Cherry nectar

Only one study evaluated the inactivation of *L. monocytogenes* (strain CA) in sour cherry juice (pH 3.30), inoculated and treated at 250 MPa for 5 min at 30°C, finding 3.9 logarithmic reductions (Alpas & Bozoglu, 2003). This result agrees with those obtained in this study, despite the lack of information about the TSS concentration or the variety used there.

As for the other microorganisms evaluated in this study, red grape juice provided a more suitable environment for survival. A reduction of only 4-log units could be achieved at high time-pressure combinations (Figure 18). Under low-pressure conditions (lower than 240 MPa), the maximum reduction was equal to 2-log units. Although the increase in pressure at low times caused an increase in the logarithmic reduction, the inactivation proved low.

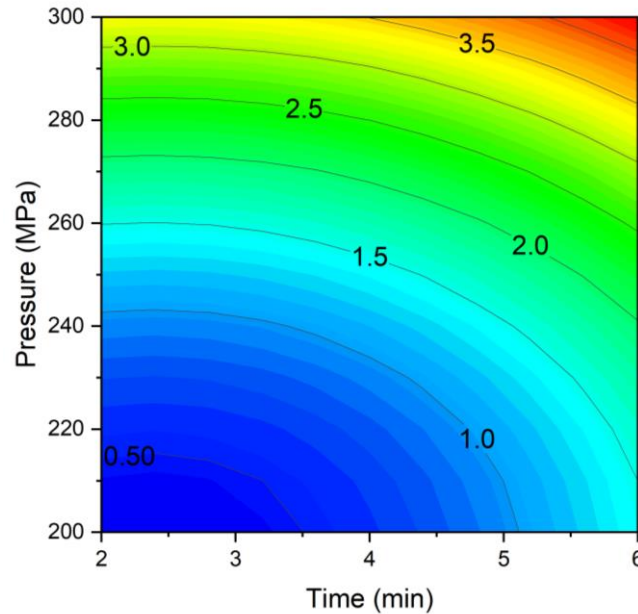


Figure 18. Contour Plot. Logarithmic reduction for *Listeria cocktail -Listeria monocytogenes surrogate in Red Grape juice*

Petrus et al. (2020) evaluated the inactivation of a *L. monocytogenes* cocktail (Lineage I serotype 4b FSL J1-108, Lineage I serotype 4d FSL J1- 107, Lineage II serotype 1/2a FSL R9-0506, FSL R9-5411, and FSL R9-5506) in Concord grape juice at pressures ranging from 330 to 550 MPa and times ranging from 25 to 225 s. The cocktail did not show growth under any conditions in this study. It is important to note that *L. monocytogenes* is a gram-negative bacterium; therefore, it should be more sensitive to HPP than *Salmonella* or EHEC, according to previous findings (Wuytack et al., 2002). However, substrate conditions seemed to play a more significant role than membrane structure.

Kinetic modelling:

As for the previous analysed microorganisms, kinetic modelling has been performed for these bacteria. The kinetic parameters are presented in Table 12. In this case, some systems could not be modelled: for strawberry nectar at 300 MPa, at all the considered times the obtained values corresponded to the detection limit of the analysis (10 CFU/mL), thus meaning a drastic reduction from the inoculation concentration to that limit, making impossible to fit a model that includes this variation. Additionally, for red grape juice treated at 200 MPa, no inactivation was detected during the first few minutes of the study, but after 6 min, a high inactivation was observed. Again, this behaviour cannot be modelled using

the Weibull equation, considering that a curve cannot be adjusted to such extreme changes in the microbial load.

Table 12. Kinetic parameters for *Listeria cocktail* - Weibull model.

Substrate	Pressure (MPa)	b	n	R ²
Strawberry nectar	200	2.93307	0.25797	0.993
	250	2.01965	0.55316	0.998
	300	N.D.	N.D.	N.D.
Sour Cherry nectar	200	1.52996	0.55764	0.979
	250	1.69942	0.5221	0.997
	300	4.52960	0.04060	0.996
Red Grape juice	200	N.D.	N.D.	N.D.
	250	1.49611	0.08838	0.975
	300	2.68108	0.24963	0.965

With respect to the modelled conditions, all curves presented a concave trend. In reference to the rate constant, for sour cherry nectar and red grape juice, the velocity of inactivation tended to increase with increasing pressure, whereas for strawberry nectar, the opposite trend was observed.

8.3. HPP treatment in enzymatic inactivation

The characterisation of strawberry and sour cherry nectars and red grape juice samples prior to HPP treatment is presented in Table 13. Target parameters were established for strawberry and sour cherry nectar as 12% for TSS and pH in the range of 3.2 to 3.5. The results obtained for the products agreed with the established targets. For red grape juice, as it did not undergo any further modifications after pressing, the parameters differed from those of the nectars, being considerably higher, which ultimately could affect the stability of enzymes and other bioactive molecules.

Table 13. Physicochemical parameters of nectars and juices pre-treatment for enzymatic evaluation

Substrate	°Brix	pH
Strawberry nectar	12.14 ± 0.02	3.27 ± 0.02
Sour Cherry nectar	12.48 ± 0.01	3.31 ± 0.03
Red Grape juice	17.94 ± 0.01	3.59 ± 0.01

As discussed in Section 8.1, strawberry nectar in this study did not exhibit enzymatic activity. In contrast, sour cherry nectar gave positive results for PPO, POD, and β -glucoside, whereas red grape juice presented positive results only for PPO and POD enzymes. Considering this, the following sections discuss the effect of HPP on enzyme inactivation.

8.3.1. Polyphenol oxidase (PPO)

The parameters obtained for enzymatic inactivation of PPO in sour cherry nectar and red grape juice are presented in Table 14. Here, the model of sour cherry nectar presents quadratic coefficients for both pressure (d) and time (e); interestingly, there is no interaction term. In contrast, red grape juice includes the interaction term (c) in addition to the quadratic coefficient for pressure. Considering the magnitude of the coefficients, in the case of sour cherry nectar, time presented the highest values when comparing single and quadratic terms; however, all coefficients presented a similar order of magnitude. For red grape juice, pressure (particularly its quadratic coefficient) was the most influential variable in the model. With respect to the correlation coefficient, red grape juice presents a low value.

Table 14. Summary of surface response fitted model parameters for PPO

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	a	b	c	d	e	
Sour Cherry nectar	-0.24821	-0.14748	-	-0.13353	0.20208	0.928
Red Grape juice	0.00958	-0.02368	0.09926	-0.4238	-	0.773

The contour plots obtained for PPO enzymatic inactivation in sour cherry nectar and red grape juices are presented in Figure 19 and Figure 20. For sour cherry nectar, inactivation occurred at different levels according to the specific conditions of the system (Figure 19); for short times, up to 6 min, there was a slight reduction in enzymatic activity when the pressure was under 450 MPa, and beyond this point, the maximum residual activity only reached approximately 55%. For long treatment times, at pressures under 450 MPa, it is possible to achieve 40% residual activity, and the zone between 8 to 12 min and 300 to 450 MPa is a very stable zone with no major changes in enzymatic activity. Finally, the region of high-pressure conditions and long treatment times is the one where the highest inactivation occurred,

reaching values of approximately 10% RA for the most extreme conditions. No previous studies have described the inactivation of PPO in sour cherry substrate, or even in other cherry varieties, making this study one of the first to characterise the inactivation in this substrate.

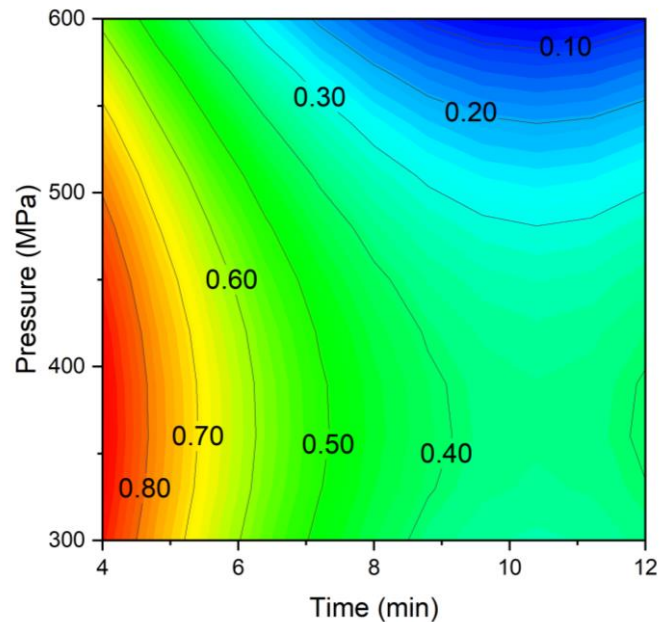


Figure 19. Contour Plot. Enzymatic residual activity for PPO in Sour Cherry nectar

PPO in red grape juice presented a particular behaviour with respect to pressure inactivation (Figure 20). Only under very specific conditions, being combinations of low times and high pressures and long times and low pressures, a slight inactivation was found (around 90% RA). Furthermore, the activation of PPO enzymes was observed, reaching a maximum of approximately 140% RA, located in the region around 450 MPa at all treatment times evaluated. When created, this model showed that time and pressure were non-significant variables; however, the interaction and quadratic terms for pressure were significant. This means that single variables had to be included in the model in order to evaluate the interaction. This can also be seen in the difference in the order of magnitude between the single variables and the interaction and quadratic terms.

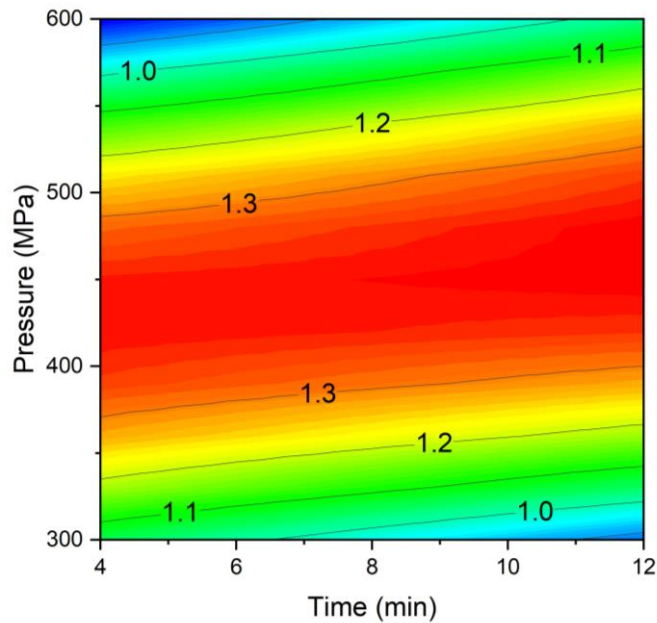


Figure 20. Contour Plot. Enzymatic residual activity for PPO in Red Grape juice

Considering the lack of fit for the surface response model ($R^2 = 0.773$), the experimental data was plotted to evaluate the differences between the actual points and the model (Figure 21). A similar trend to that presented in the model can be seen; for low-pressure conditions (200 MPa), there were no significant differences between the non-treated samples and all times evaluated, similar results were obtained for 600 MPa. A significant difference was observed for the condition at 450 MPa, where the treated samples differ significantly with respect to the control, all being higher than this.

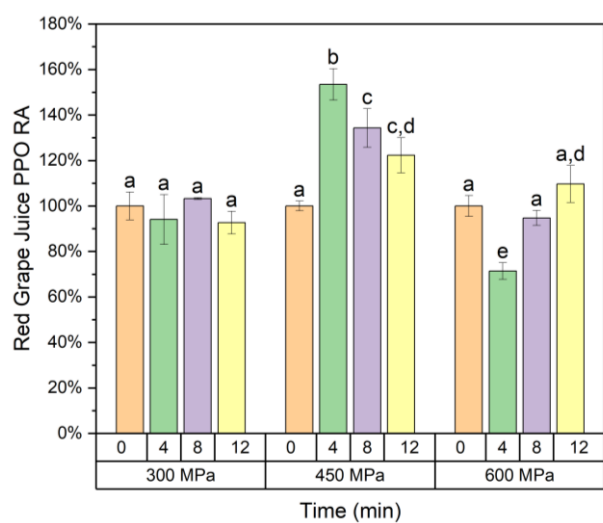


Figure 21. Experimental data. Enzymatic residual activity for PPO in Red Grape juice. Different letters indicate statistically significant differences.

Previous studies evaluated the stability of PPO enzymes in grapes. Weemaes et al. (1998) reported inactivation of PPO enzymes while treating white Italian grapes at 700 to 900 MPa for 3 min, without showing an increase in activity under milder conditions. In other study, Rapeanu et al. (2006) evaluated the stability of PPO enzymes in white grapes (Victoria variety from the south of Africa) under thermal and HPP conditions. Specifically, extracts at two different pH conditions were tested (4.0 and the defined optimal in their study 5.0), to be treated at pressures between 400 and 800 MPa for a total holding period of 2 min at four different temperatures (10, 25, 40, and 60 °C). Their findings showed a slight reduction with the increase in pressure and a more pronounced change when the temperature was increased in the system. With respect to pH, the optimal condition retained much of the enzymatic activity in comparison to the condition at pH 4.0; however, the reduction trend with respect to pressure and temperature was similar. Similarly, no increase in activity was reported in their study. A recent study evaluated the stability of PPO and POD enzymes over time in Concord grapes (*Vitis Labrusca* L.) treated at 600 MPa for 3 min. Their results showed an initial inactivation of approximately 20% for PPO after treatment (Li & Padilla-Zakour, 2021). The differences between the present study and previous findings can be attributed to differences in the variety, which plays a significant role in the characteristics of isoenzymes present in different substrates. With respect to the treatment, tertiary and quaternary structures can be affected by HPP, leading to a possible exposure of active sites of the enzymes, ultimately leading to an increase in enzymatic activity reflected in the activation observed (L. Ludikhuyze et al., 2003).

However, other studies have also reported an increase in activity with HPP. Del Pozo-Insfran et al. (2007) evaluated the stability of Muscadine grape juice (cv. Noble) under high-pressure conditions. In their study, grape juice was extracted and subjected to HPP at 400 and 500 MPa for 15 min. Their results showed an increase in the PPO activity of approximately 300% and 200% for treatments at 400 and 550 MPa, respectively. These results are in agreement with those of the current study, despite the fact that the increase in activity was less pronounced.

8.3.2. Peroxidase (POD)

Enzymatic inactivation of POD was also modelled using an optimised quadratic model. The coefficients obtained for the models are presented in Table 15. For sour cherry nectar, the quadratic term for pressure was required, whereas for red grape juice, the quadratic term for time was included in the model. The correlation coefficients in both models are considerably low, which is why the experimental data are analysed next to the predictive model.

Table 15. Summary of surface response fitted model parameters for POD

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Sour Cherry nectar	-0.24845	-0.12154	-	-0.23719	-	0.569
Red Grape juice	-0.15469	0.31081	-	-	0.34598	0.754

Figure 22 shows the predictive model for the response of POD enzymes in sour cherry nectar. It shows high resistance of the enzyme at short treatment times for all pressures, reaching a minimum of 60 to 50% RA. With an increase in treatment time, a reduction in RA was also observed, reaching values of 30% under low-pressure conditions and 10% under high-pressure conditions, both at the longest treatment period. However, resistance was observed at 450 MPa.

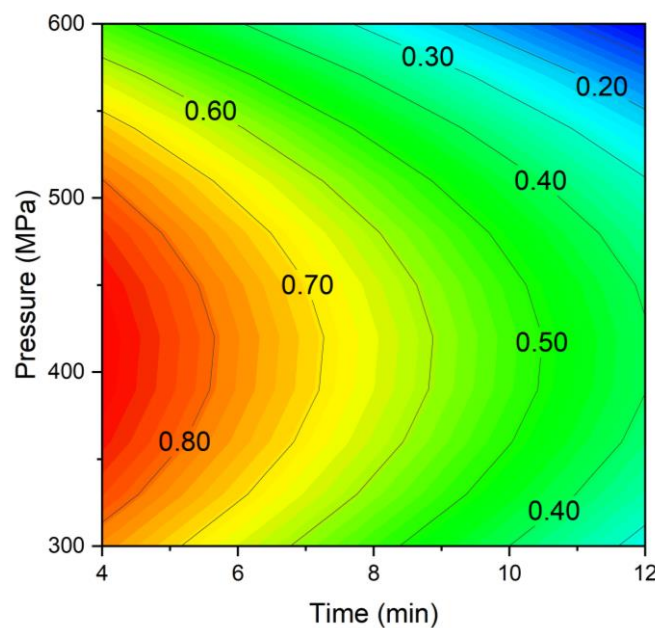


Figure 22. Contour Plot. Enzymatic residual activity for POD in Sour Cherry nectar

Contrasting these findings with the experimental data (Figure 23), it is possible to see that at 450 MPa, there is no significant difference between the control sample and that treated for 4 min, despite the fact that this sample presented a high standard deviation. It can also be observed that there is no significant difference between the treatment times at 600 MPa, which is contradictory when evaluating the surface response. Values allocated in the middle of the design space were all significantly different from those of the control samples (except for the case of 4 min at 450 MPa). No previous studies have been conducted on endogenous POD enzymes in sour cherries.

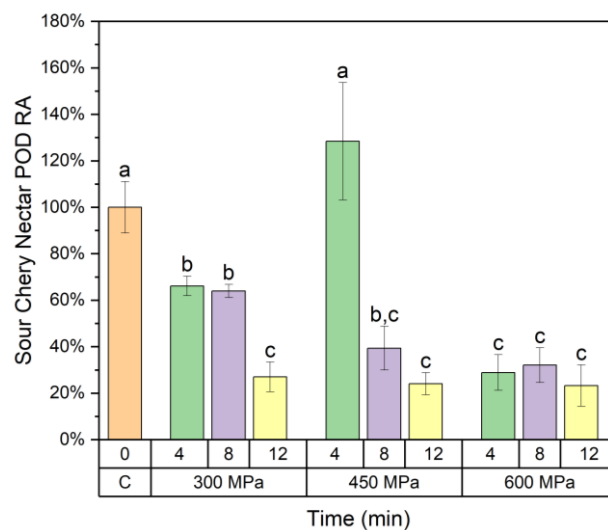


Figure 23. Experimental data. Enzymatic residual activity for POD in Sour Cherry nectar. Different letters indicate statistically significant differences.

Different results were obtained for POD enzymes in red grape juice. When modelling the residual activity (Figure 24), inactivation of POD was more pronounced when the treatment time was increased at low pressures, reaching values of approximately 30% RA after 7 min of treatment at 300 MPa. With increasing pressure, the reduction is less pronounced, and depending on the time, there could be certain enzymatic activation, particularly at short treatment times when it is possible to observe RA of approximately 130%.

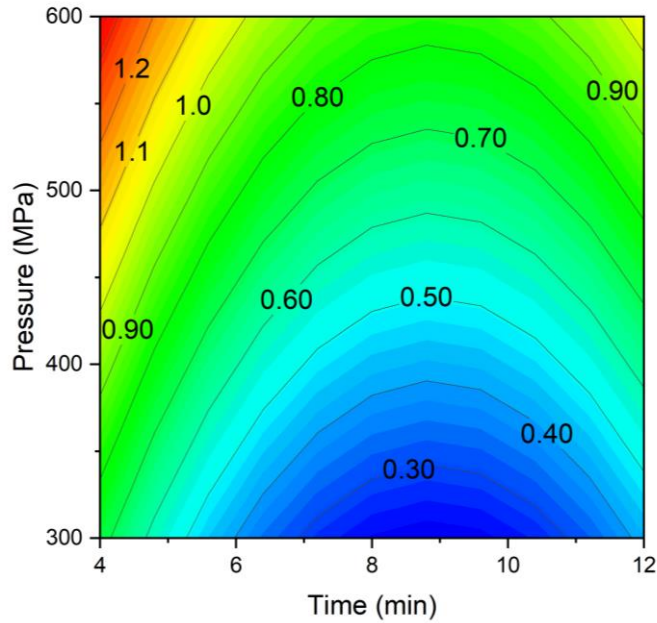


Figure 24. Contour Plot. Enzymatic residual activity for POD in Red Grape juice

Compared with the experimental data, there are some differences between the experimental data and the model (Figure 25). Starting with the similarities, there was a statistically significant reduction in enzymatic activity at 300 MPa, which is in agreement with what is predicted by the model. Additionally, at 600 MPa, there was only a significant difference between the control and the sample treated for 4 min, presenting an increase in the activity also predicted by the model. In the case of 450 MPa, there was a significant difference only for the sample treated for 8 min, presenting a strange behaviour with respect to the other treatment times in its cluster at 450 MPa. In the model, this could be represented by the curvature shown in the centre of the design space, which is slightly recovered at the lowest and highest treatment times.

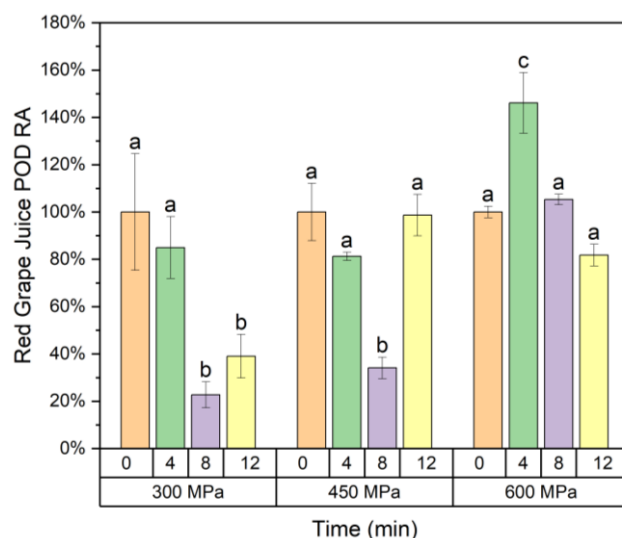


Figure 25. Experimental data. Enzymatic residual activity for POD in Red Grape juice. Different letters indicate statistically significant differences.

The study conducted by Li & Padilla-Zakour (2021) also evaluated the stability of POD enzymes in concord grapes treated with HPP at 600 MPa for 3 min. In their experiments, 80.7% RA was found immediately after treatment; results that differed from those found in this study were under similar conditions, and activation of 130% RA was observed. This could be attributed to differences in the variety; physicochemical parameters, such as pH and TSS, were similar between studies (3.35 and 18%, respectively).

8.3.3. β -glucosidase

Sour cherry nectar presented a well-correlated model for β -glucosidase enzymes (see Table 16). In the model, the linear coefficients for time and pressure, in addition to the quadratic coefficient for pressure, were included after the model optimisation stage. The presence of only these coefficients accounts for a more significant effect of pressure compared with time.

Table 16. Summary of surface response fitted model parameters for β -glucosidase

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Sour Cherry nectar	-0.07078	-0.30621	-	-0.18127	-	0.905

The surface response obtained from the modelled equation is presented in Figure 26. Under low-pressure conditions (less than 450 MPa), the effect of time was more pronounced. Here, the increase in treatment time accounted for a reduction in RA, with maximum values of approximately 60 to 70 %.

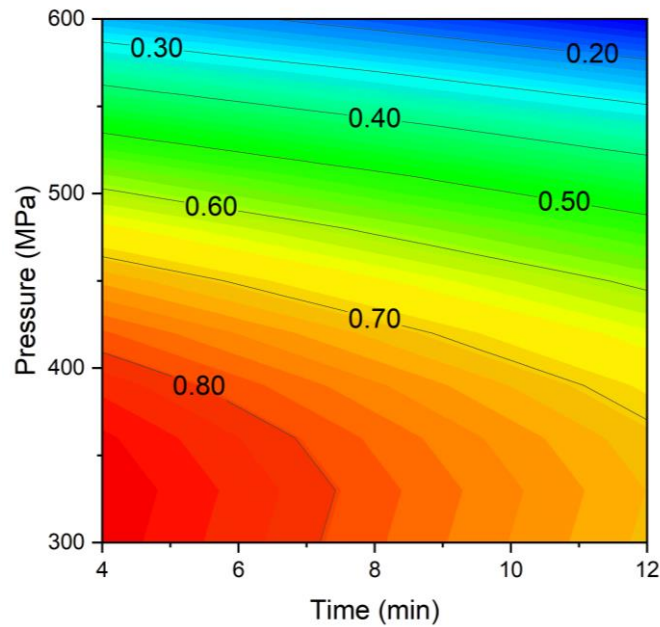


Figure 26. Contour Plot. Enzymatic residual activity for β -glucosidase in Sour Cherry nectar

In the high-pressure zone (above 450 MPa), the effect of time is less significant, indicating that the increase in pressure determines the RA remaining in the samples. The maximum inactivation in the system was achieved at 600 MPa and 12 min with RA values of RA around 20%; however, treatment at shorter times presented slightly different values (RA of approximately 30%). No previous studies have evaluated the presence or inactivation of this enzyme in sour cherry purees or their derived products.

8.4. HPP treatment in total polyphenols content

The total polyphenol content was modelled using an optimised quadratic model. Table 17 presents the coded coefficients for each substrate. Interestingly, compared with the models obtained for enzymatic inactivation, all models adjusted in this case included quadratic terms for time, a parameter usually excluded in previous models. The inclusion of many parameters can provide a better fit with respect to

the experimental data. However, all models that describe the behaviour of total polyphenols present low correlation coefficients, making it necessary to evaluate the experimental data while analysing the models. The interaction term (c) was excluded for nectar and presented a low value compared to other parameters for red grape juice, showing that there was no significant interaction between variables.

Table 17. Summary of surface response fitted model parameters for Total Polyphenol Content

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	a	b	c	d	e	
Strawberry nectar	-0.00586	-0.04615	-	-0.06113	0.04942	0.702
Sour Cherry nectar	-0.02103	-0.05032	-	-	-0.05483	0.475
Red Grapes juice	0.04518	0.06485	-0.0002	0.06872	-0.08105	0.460

Figure 27 shows the contour plot of the model adjusted for strawberry nectar. It can be seen that there is a slight reduction in the TPC when the pressure is increased compared with the increase in time. Low-pressure conditions, particularly at the extremes of the design space, exhibited the highest values for this parameter. In contrast, the pressures of approximately 600 MPa presented a reduction.

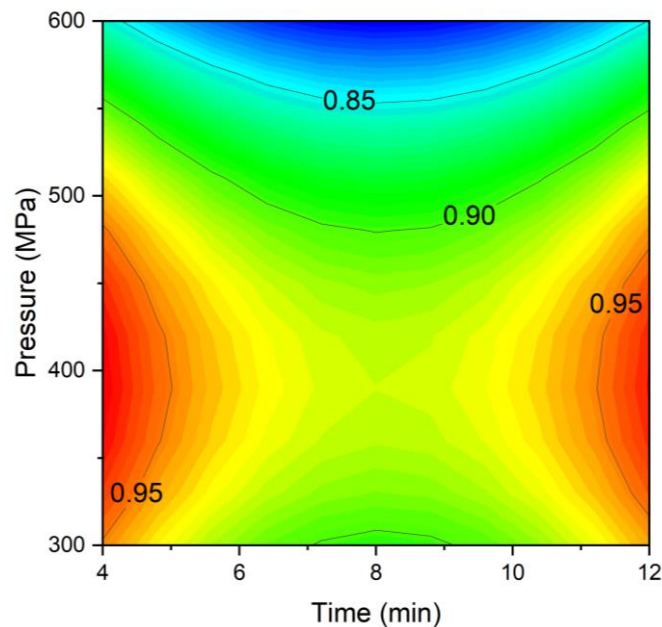


Figure 27. Contour Plot. Total Polyphenol Content in Strawberry nectar

The experimental results are similar to those presented in the model, despite the low correlation coefficient (Figure 28). There were no significant differences between the samples treated at 300 and

450 MPa for all treatment times with respect to the control samples. When evaluating the 600 MPa results, there was a significant difference with respect to the control at all treatment times; however, for 4 and 12 min, this difference was not significant when comparing these treatments with all treatment times at 300 MPa.

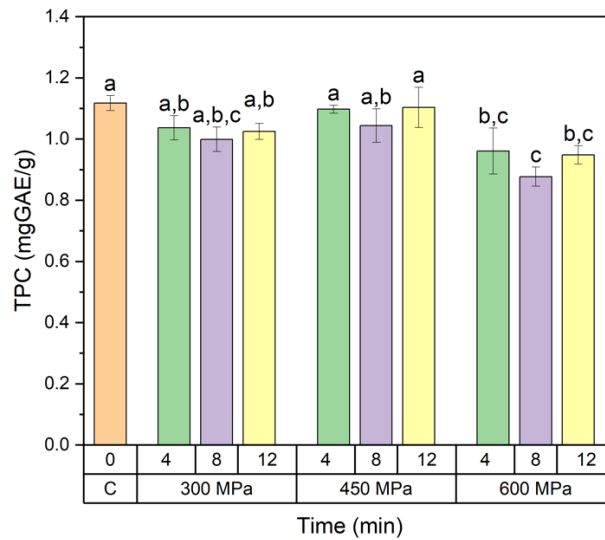


Figure 28. Experimental data. Total Polyphenol Content in Strawberry nectar. Different letters indicate statistically significant differences.

The variation between varieties has been presented by different authors (Cao et al., 2018; Dzhhanfezova et al., 2020; Ganhão et al., 2019) even at different ripening stages (Mandave et al., 2014; Ornelas-Paz et al., 2013; Pineli et al., 2011). Different results were obtained when evaluating the effect of HPP on the TPC. Cao et al. (2011), evaluated the change in TPC in strawberry juice (cultivar Tongzi I), after HPP treatment at 400, 500, and 600 MPa for 5 to 25 min at ambient temperature (approximately 25°C). Their results showed a significant reduction at 400 MPa for all treatment times. For treatments at 500 and 600 MPa, TPC also increased when the treatment time increased. Similar results were found in the study conducted by Terefe et al. (2013), who evaluated the impact of HPP and thermal processing on different parameters, including TPC, for three different cultivars (Camarosa, Festival, and Ruby Gem). HPP was conducted at 600 MPa for 5 min at 20°C. The results showed that, immediately after treatment Camarosa and Ruby Gem varieties showed a significant reduction of approximately 20% with respect to non-treated samples, while for cultivar Festival, the difference was not significant. Another study performed by Cao et al. (2014) evaluated the effect of HPP at 600 MPa for 4 min at ambient temperature

on different variables, including TPC in strawberry clear and cloudy juice (cultivar Tongzi I). The results showed no significant differences after treatment. More recent studies have evaluated strawberry pulp treated using thermal and non-thermal techniques, including HPP (600 MPa for 8 min). When quantifying TPC, there were no significant differences between treated and non-treated samples (Xing et al., 2023).

Marszałek et al. (2015) conducted a study where strawberry pulp was submitted to HPP coupled with thermal processing, and among the parameters evaluated, the TPC was included. The treatment was performed at two different temperatures (0 and 50 °C), two pressure conditions (300 and 500 MPa), and three treatment times (1, 5, and 15 min). Their results showed a clear dependence of temperature on the stability of polyphenols; significant differences with respect to the control samples were only found at 500 MPa for 5 min when the lowest temperature was analysed. At 50 °C, significant differences were found between the treatments at 300 and 500 MPa for 15 min.

Sour cherry nectar presented a different behaviour than strawberry nectar (Figure 29). The polyphenols present in sour cherries were stable at low pressure without a significant effect with respect to time. When the pressure increased, a reduction in TPC was observed, reaching maximum values at 600 MPa, particularly for short and long treatment times. The middle region presented a certain stability, being less than that of the control sample but without significant variation in the region.

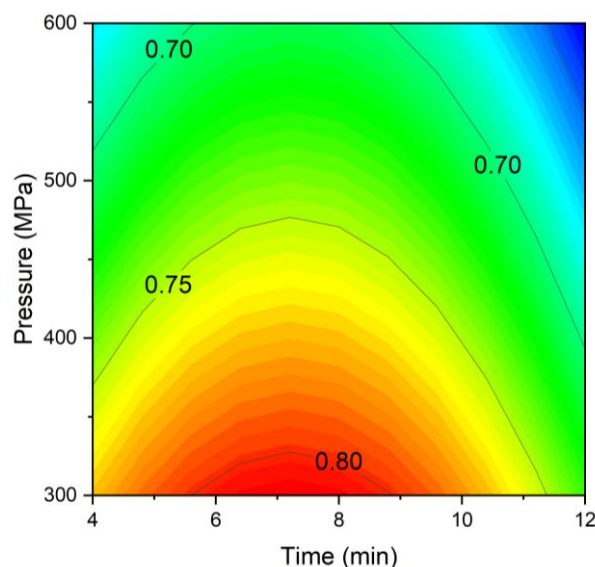


Figure 29. Contour Plot. Total Polyphenol Content in Sour Cherry nectar

The experimental data show a significant reduction for all time-pressure combinations with respect to the control sample (Figure 30). The stable region described previously was also evident when comparing the experimental results, showing no significant differences between all treatment times at 450 MPa. A significant change from that region, leading to lower values, was only observed at 8 and 12 min at 600 MPa. The variability of TPC in sour cherry fruit, juices, and derivate products with respect to cultivars has been presented by different authors, making it important not only to compare the obtained results with respect to the processing conditions but also to take into account the intrinsic variations in the raw material (Alrgei et al., 2016; Bánáti et al., 2010; Damar & Ekşi, 2012; Khoo et al., 2011; Sokół-Łętowska et al., 2018).

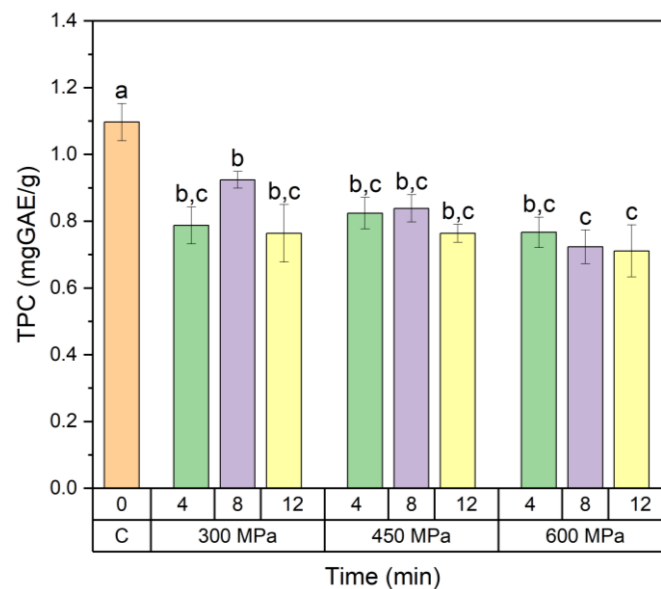


Figure 30. Experimental data. Total Polyphenol Content in Sour Cherry nectar. Different letters indicate statistically significant differences.

Few studies have evaluated the behaviour of TPC in cherry substrates with respect to HPP. Queirós et al. (2015) compared the effects of thermal and high-pressure treatments on sweet cherry (*Prunus avium* L.) from Cova da Beira in Portugal. Two HPP conditions were evaluated: 400 MPa for 5 min holding time and 550 MPa for 2 min, both treatments at 10 °C. Their results showed a significant difference only at the highest-pressure condition with respect to the control sample. A recent study evaluated the effects of HPP on different parameters, including TPC, in sour cherry (cultivar Amarena del Rio). The treatment was performed at 600 MPa for 3 min at 4 °C. Their results showed no significant change

immediately after treatment compared with the untreated sample (Tenuta et al., 2023). The difference between the present study and previous findings might be attributed to cultivar specificity and possible variations in temperature.

The contour plot obtained after modelling the TPC for red grape juice is shown in Figure 31. Its behaviour differs completely from that of the nectars previously evaluated. At short treatment times and low or mild pressure conditions, the TPC showed the lowest values in the study, while with increasing time and pressure, there was an increase in TPC, showing a certain stability in the region that comprises 6 to 12 min and 300 to 500 MPa. Highest TPC was observed at 600 MPa from 6 min treatments and longer times.

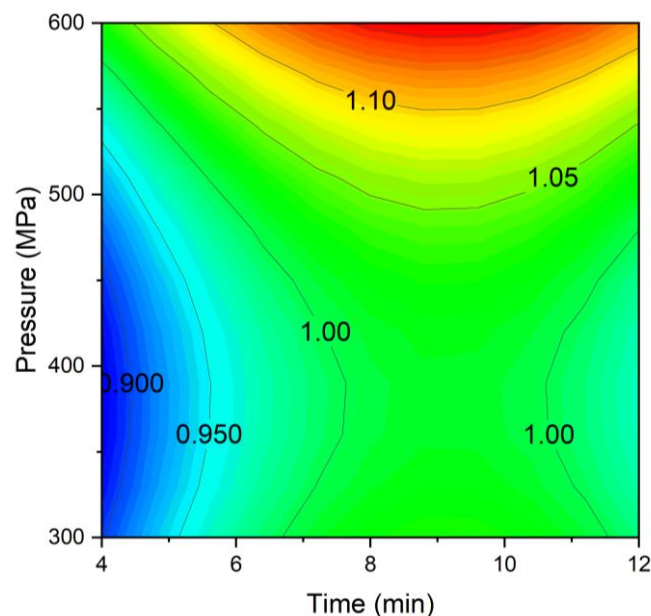


Figure 31. Contour Plot. Total Polyphenol Content in Red Grape juice

The evaluation of the experimental data for red grape juice, as previously done for enzymes, requires the evaluation of different control samples for each pressure condition because samples were prepared at different times for each set of treatments and extracted directly from red grapes. This type of analysis represents a realistic approach that considers possible intrinsic variations in the plant material. The experimental results for the TPC in red grape juice are presented in Figure 32. Comparing the control samples, it is possible to observe that TPC at 300 MPa is significantly different from those at 450 and 600 MPa. This could explain the low values presented in the surface response at 300 MPa. When

evaluating the results after treatment, it was observed that there were no significant differences between the control samples under all pressure conditions and their respective samples at all treatment times. This analysis demonstrates the importance of evaluating the experimental results in addition to the model when the correlation coefficient is low.

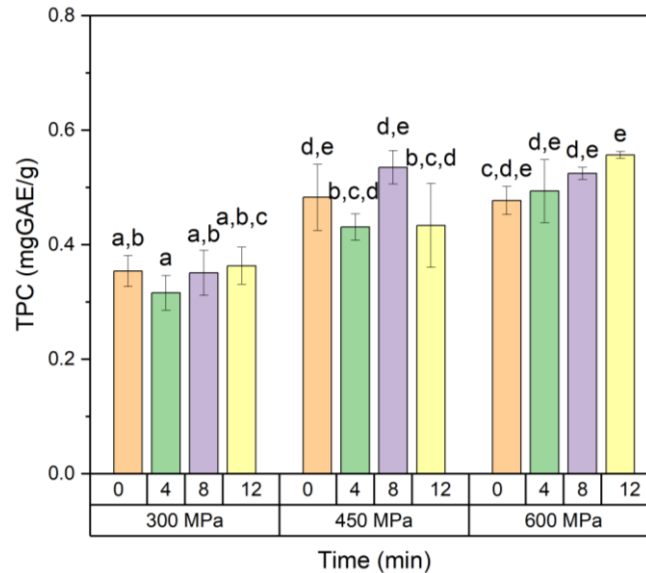


Figure 32. Experimental data. Total Polyphenol Content in Red Grape juice nectar. Different letters indicate statistically significant differences.

Several authors have investigated the differences in TPC between grape juices from different varieties and agricultural practices (Burin et al., 2010; Toaldo et al., 2015). Regarding high-pressure processing, Del Pozo-Insfran et al. (2007) evaluated the stability of anthocyanins in Muscadine Grapes (*Vitis rotundifolia*) after HPP treatment at 400 and 550 MPa for 15 min. Their results showed a significant reduction in total anthocyanins after treatment; however, these results are not comparable to those obtained in the present study, considering that anthocyanins are only a fraction of total polyphenols. Moreover, a recent study evaluated the effect of HPP on TPC, among other parameters, for Concord grape puree (*Vitis Labrusca* L.). The treatment was performed at 600 MPa for 3 min at 5 °C (Li & Padilla-Zakour, 2021). In their study, there was a significant difference between the HPP-treated and non-treated samples. All these studies showed changes with respect to the control samples; however, none of them worked with the same variety despite the fact that similar conditions were used.

8.5. HPP treatment in antioxidant capacity

Total antioxidant capacity was also modelled using the surface response methodology. The coded coefficients obtained for the nectars and juice are presented in Table 18. Only for the strawberry nectar was the quadratic coefficient necessary to describe the behaviour of antioxidant capacity, in this case the quadratic coefficient for pressure. In this system, it is important to note that the order of magnitude of the time coefficient is much lower than that of the pressure, meaning that the influence of pressure is more significant than that of time. A similar behaviour was observed for red grape juice. The interaction term was significant only for sour cherry nectar. In general, the correlation coefficients did not present a high value, requiring the analysis of the experimental data next to the modelled systems.

Table 18. Summary of surface response fitted model parameters for Antioxidant Capacity

Substrate	Fitted model coded coefficients (Equation 4)					R ²
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Strawberry nectar	0.00141	0.09925	-	-0.09214	-	0.528
Sour Cherry nectar	0.02193	0.08158	0.02208	-	-	0.784
Red Grapes juice	-0.00764	-0.0126	-	-	-	0.521

The contour plot obtained when modelling the experimental data for strawberry nectar is shown in Figure 33. As predicted, when analysing the coefficients, pressure shows a more significant impact than time. The increase in pressure showed an increase in antioxidant capacity, while time increments seemed to have no impact on this parameter.

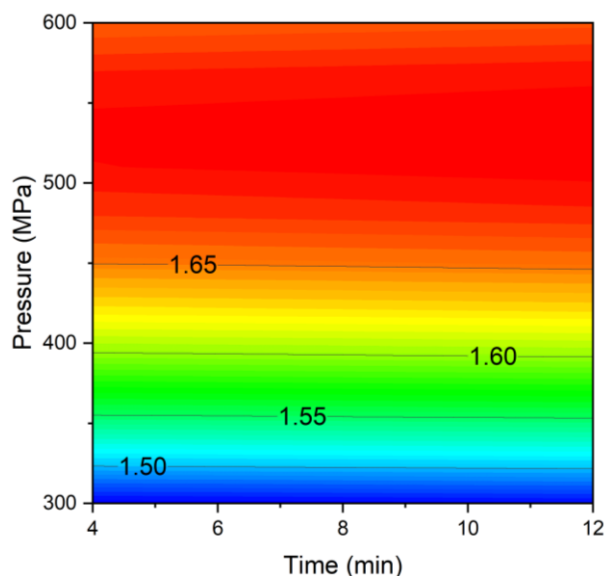


Figure 33. *Contour Plot. Antioxidant Capacity in Strawberry nectar*

When analysing the experimental values, it can be seen that only a few treatments were significantly different from the control samples (450 MPa for 8 min, 600 MPa for 4 and 12 min). With these results, it can be concluded that only under high-pressure conditions there is a significant difference, with values higher than the control; otherwise, HPP technology does not significantly affect the antioxidant capacity of strawberry nectar.

The variation between varieties with respect to total antioxidant capacity has been investigated by various authors (Cao et al., 2018; Dzhanfezova et al., 2020; Ganhão et al., 2019), some of them even evaluated differences with respect to ripening stages (Mandave et al., 2014; Pineli et al., 2011). With respect to the effect of HPP on antioxidant capacity, Cao et al. (2011) also evaluated the changes in antioxidant capacity of HPP-treated strawberry cloud and clear juice. Their results showed no significant differences between the control and the samples treated at 600 MPa for 4 min at ambient temperature, which is in agreement with the results of this study.

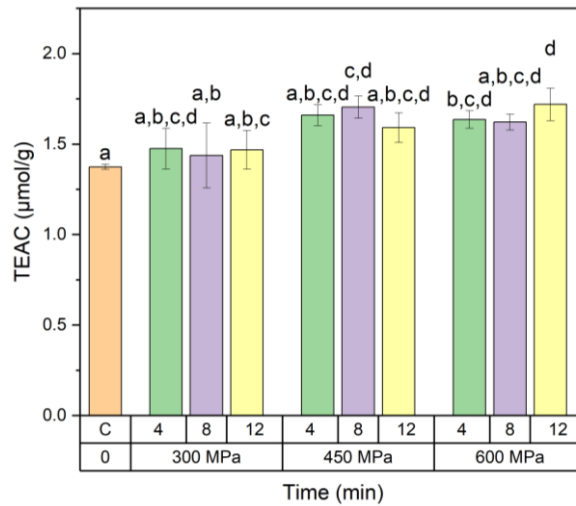


Figure 34. Experimental data. Antioxidant Capacity in Strawberry nectar. Different letters indicate statistically significant differences.

For sour cherry nectar, time was a more important variable compared with strawberry nectar (Figure 35). At low-pressure conditions (under 400 MPa), the effect of time seems low; when pressure increases, a major effect of time is perceived, leading to the highest values for all the design space in the top right corner (high pressure and long treatment time). When comparing the surface created using the model and the experimental data (Figure 36), it is possible to observe that there is a significant difference only for treatment at 600 MPa for 8 and 12 min.

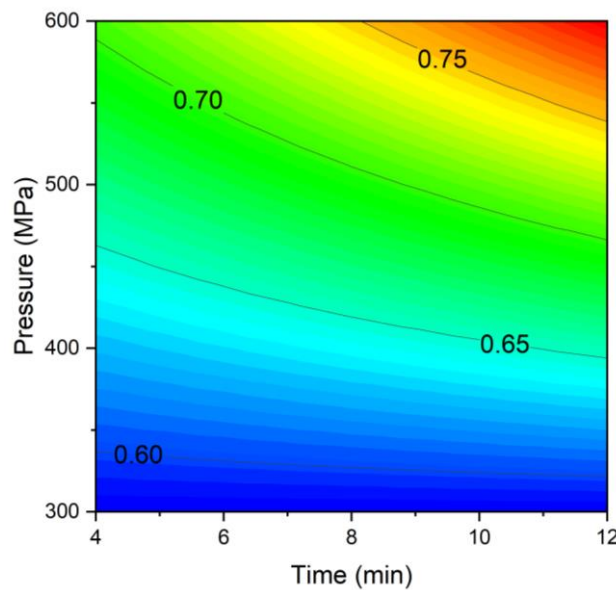


Figure 35. Contour Plot. Antioxidant Capacity in Sour Cherry nectar

The variability of total antioxidant capacity in sour cherry fruit, juices, and derived products with respect to cultivars has been presented by different authors, showing a high dependency of the substrate material with respect to the initial antioxidant capacity before any sort of treatment (Alrgei et al., 2016; Bánáti et al., 2010; Damar & Ekşi, 2012; Khoo et al., 2011; Sokół-Łętowska et al., 2018, 2020). Queirós et al. (2015) also investigated the effects of HPP treatment on the antioxidant capacity of sweet cherries. Their findings suggested that after treatment at 400 MPa for 4 min and 500 MPa for 2 min, there were no significant differences with respect to the control sample. Moreover, Tenuta et al. (2023) evaluated the effect of HPP processing on the antioxidant capacity of sour cherry (cultivar Amarena del Rio). Their results showed no significant differences compared to the control samples when the samples were subjected to treatment at 600 MPa for 3 min at 4 °C.

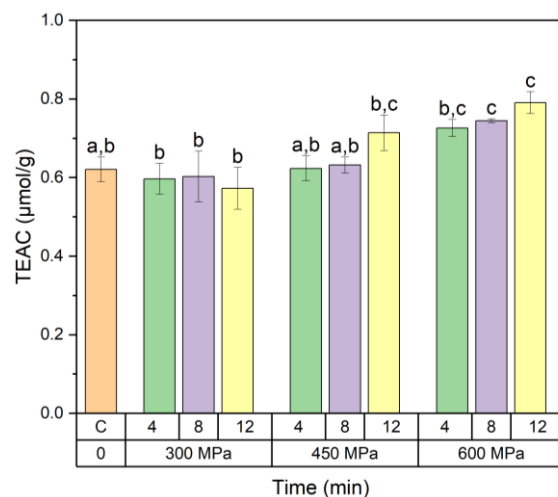


Figure 36. Experimental data. Antioxidant Capacity in Sour Cherry nectar. Different letters indicate statistically significant differences.

In the case of red grape juice, time and pressure presented a similar order of magnitude for their coefficients, leading to an equally significant effect of both variables on antioxidant capacity. A contour plot generated using the surface response methodology is shown in Figure 37. Here is possible to observe a diagonal trend with the highest values for antioxidant capacity in the corner with milder conditions and the lowest values at the opposite extreme. This is confirmed by the experimental data presented in Figure 38, where a significant reduction with respect to the control sample was only observed for the treatments at 600 MPa at all treatment times, in addition to the treatment at 400 MPa

for 12 min. For all other conditions, there were no significant differences compared to the control samples.

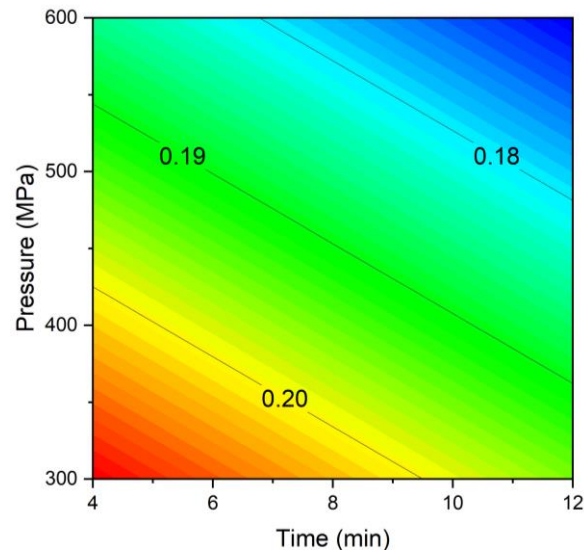


Figure 37. Contour Plot. Antioxidant Capacity in Red Grape juice

The diversity in antioxidant capacity among cultivars has been investigated by various researchers (Burin et al., 2010; Toaldo et al., 2015). When discussing the effects of HPP treatment, there have been some previous evaluations of different grape varieties. Talcott et al. (2003) evaluated different factors affected by HPP treatment at 600 MPa for 15 min, among those antioxidant capacity, when processing Muscadine grape juice (cultivar Noble). Their results showed a significantly higher antioxidant activity than that of the control sample. Other studies have shown different results; Del Pozo-Insfran et al. (2007) reported a significant reduction in antioxidant activity when red grape juice for the same cultivar was subjected to HPP at 400 and 550 MPa for 15 min. In a more recent study, Li & Padilla-Zakour (2021) evaluated the effect of HPP (600 MPa for 3 min at 5 °C) on antioxidant capacity, among other parameters, and found no significant differences with respect to treatment. The results in this matter were not aligned, showing differences even when evaluating the same cultivar.

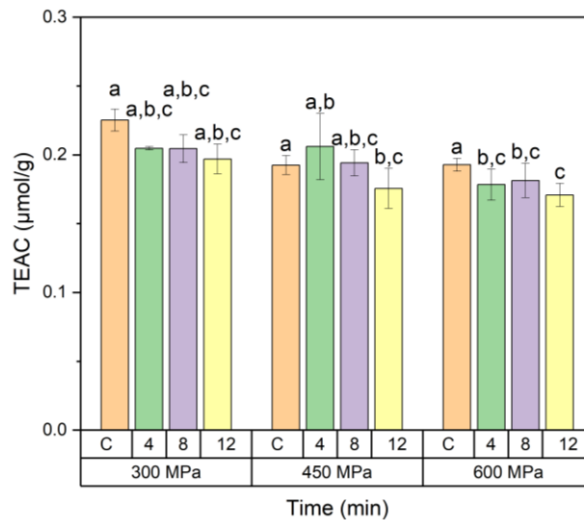


Figure 38. Experimental data. Antioxidant Capacity in Red Grape juice. Different letters indicate statistically significant differences.

8.6. Optimization of HPP treatment conditions

Considering that one of the objectives of this study was to optimise the HPP conditions in order to obtain red fruit juices with better colour stability, a multi-objective optimisation should be performed based on the different analyses performed. In general, the system did not present zones considered as maximum or minimum to be optimised; only PPO in red grape juice presented a region of maximisation that should be avoided. Evaluating each of the parameters tested, these are the optimised conditions for each parameter:

- Microbial inactivation: a 5-log reduction was possible for *E. coli* ATCC 11229 (*Salmonella* surrogate), *E. coli* ATCC 8327 (*EHEC* surrogate), and *Listeria* cocktail (*L. monocytogenes* surrogate) in strawberry and sour cherry nectars under conditions of approximately 300 MPa for 6 min. For red grape juice, all surrogate microorganisms tended to resist the conditions tested in the design space. However, further experiments showed that after treatment at 450 MPa for 4 min, all surrogate strains were successfully inactivated (data not shown).
- Enzymatic inactivation: each of the enzymes evaluated had a different behaviour with respect to its specific substrate. For PPO, the highest inactivation was achieved only at 600 MPa for longer treatment times in the case of sour cherry nectar, whereas for red grape juice, little

inactivation was observed at longer treatment times at low pressure or at high-pressure conditions for a short time.

POD enzymes, in the case of sour cherry nectar, exhibited a behaviour similar to that of PPO in the same substrate. On the contrary, for red grape juice, only long treatment times at low pressure were effective in enzymatic inactivation; high-pressure conditions at short times showed activation of enzymes. Finally, for β -glucosidase enzymes, all treatments at 600 MPa resulted in high inactivation.

- Total polyphenol content retention: once again, each product exhibited a different behaviour with respect to this parameter. While for strawberry nectar, a significant reduction was observed only at 600 MPa, similar results were obtained under all treatment conditions for sour cherry nectar. Moreover, red grape juice showed no significant differences under any treatment conditions, presenting the most stable profile among the evaluated products.
- Antioxidant capacity retention: with respect to antioxidant capacity, strawberry and sour cherry nectar showed a significant increase under high-pressure conditions and longer treatment times, whereas for red grape juice, a significant decrease was observed under the same treatment conditions.

Industrial processing factors should be considered as a restriction of the system because it is not feasible to perform processes with long treatment times on a normal basis because the characteristics of the machine subjected to long periods of stress are more susceptible to wear, increasing not only the operational costs but also the risk of the process. In addition, HPP is an energy – intensive process in which the total production cost increases when the holding time increases. At the industrial level, 5 min treatments are commonly used for different products, resulting in optimal conditions in terms of microbial inactivation and production costs.

Considering all previous factors, the most relevant restrictions are those involving microbial safety and industrial process limitations. In the case of microbial inactivation, as found in this study, conditions above 450 MPa allow the production of microbially safe products (achieving 5-log reduction); however, the limitation in terms of treatment time allows only a maximum holding time of 5 min. All other

possible restrictions, such as the maximisation of enzymatic inactivation and the retention of antioxidant capacity and polyphenol content, are becoming a result of the selected process.

The conditions selected as the optimised treatment correspond to 600 MPa and 4 min, which present at the same time advantages and disadvantages depending on the specific product:

Advantages:

- Microbial inactivation was achieved for all three products.
- The conditions were in accordance with the machine limitations.
- PPO inactivation for sour cherry nectar (approximately 50%) and red grape juice (approximately 25%).
- POD inactivation for sour cherry nectar (approximately 70%).
- β -glucosidase inactivation for sour cherry nectar (approximately 75%).
- Retention of total polyphenol count for red grape juice.
- Increase in antioxidant capacity for strawberry and sour cherry nectar.

Disadvantages:

- POD activation for red grape juice (approximately 40%)
- Reduction in total polyphenol content for strawberry and sour cherry nectars (approximately 10 and 30%, respectively).
- Decrease in antioxidant capacity for red grape juice (approximately 5%)

With the selected conditions, the next step was to evaluate storage behaviour. The results obtained directly after treatment were compared with those obtained previously.

8.7. Shelf-life evaluation

The shelf stability of HPP-treated and non-treated strawberry and sour cherry nectars, and red grape juice was evaluated over a period of 28 days, considering two different packaging alternatives: PET

bottles and multilayer pouches. The results obtained for the OTR for both materials, pre- and post-treatment are presented in Table 19.

Table 19. Oxygen transmission rates for PET bottles and multilayer pouches

Packaging Material	OTR (cm ³ /24h)
PET Bottles pre-treatment	0.054
PET Bottles post-treatment	0.056
Multilayer pouches pre-treatment	0.039
Multilayer pouches post-treatment	0.016

These results show a higher barrier respect to oxygen permeability for multilayer pouches compared to PE bottles. This was expected, considering the fact that one of the layers included correspond to aluminium foil, which presents a high oxygen barrier. However, the reported values after treatment for multilayer pouches were not expected, considering that if the integrity of the packaging was compromised, an increase in the oxygen transmission rate will be higher than those of the same type of packaging without treatment; nevertheless, when comparing both packaging alternatives pouches present a higher oxygen barrier than bottles.

8.7.1. Physicochemical parameters

Physicochemical parameters were evaluated during the shelf-life study to identify possible variations in the organoleptic characteristics of the product, mainly related to aroma, acidity, and sweetness. No formal sensory evaluation was performed in this study, which is why these parameters acted as indirect measurements for these product characteristics. When the pH was evaluated, this parameter was very stable throughout the study for all three products. When performing 2-way ANOVA analysis, strawberry nectar did not show significant differences in storage time. For sour cherry nectar and red grape juice, some significant differences were observed; however, the actual variation in pH was very low.

The total soluble solids showed significant variations in the study. Figure 39 shows the variation in degrees Brix for non-treated (NT) and treated (T) samples during the storage period of 28 days.

Treatment was found to be non-significant, and it is evident in the figure that both NT and T samples follow the same trend. In both cases, there was a significant but slight increase in the TSS content. However, despite the differences between storage days, the overall range of degrees Brix ranged from 12 to 12.6, corresponding to a low variation.

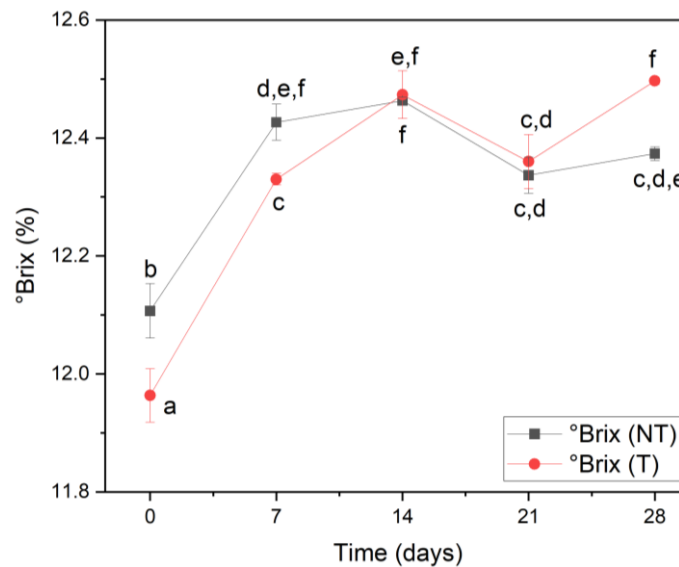


Figure 39. TSS evolution during shelf life for strawberry nectar. Different letters indicate statistically significant differences.

The case of sour cherry nectar was different (Figure 40): the treated samples (T) did not show significant variation during the storage period, while there was a significant change in non-treated (NT) samples. This was more evident after 21 days of cold storage and was significantly different after 28 days. The reduction in TSS in NT samples could be associated with possible fermentation. Without being processed with HPP technology, samples could contain bacteria, yeast, and moulds that could use the available sources of sugar to grow. This hypothesis is supported by two specific factors that were detected while processing the samples for analysis: first, after day 14, NT samples presented a fermented smell that was intensified in the following days of the study; second, NT samples stored in pouches were visibly inflated, which is a product of fermentation processes, leading to the damage of the packaging of some samples on days 21 and 28 of the study.

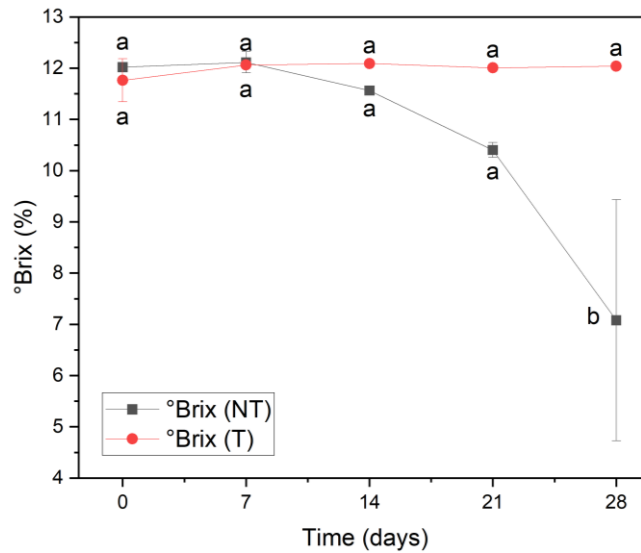


Figure 40. TSS evolution during shelf life for sour cherry nectar. Different letters indicate statistically significant differences.

The results for red grape juices showed an intermediate behaviour compared to that of strawberry and sour cherry nectars (Figure 41). A decrease in TSS was observed in NT samples after day 21, similar to sour cherry nectar, with the difference that this change was maintained until the end of the study. These samples presented a slightly fermented smell, compared to NT sour cherry nectar, and pouches containing the NT product did not suffer any alteration. With respect to the treated samples, a slight significant increase was observed only on day 14 with respect to the control samples; however, this increase was not significant with respect to the other storage days.

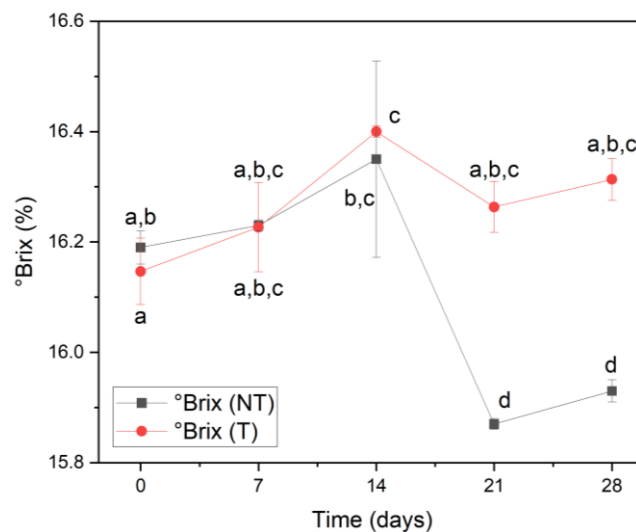


Figure 41. TSS evolution during shelf life for red grape juice. Different letters indicate statistically significant differences.

Previous studies have evaluated the evolution of physicochemical parameters with respect to storage time for samples treated with HPP technology. Aaby et al. (2018) evaluated the evolution of pH and TSS for strawberry puree and juice (cultivar Senga Sengana) in a storage period of 49 days. The treatments were conducted for 3 min at 400, 500, and 600 MPa at 20 °C. Their results showed a slight but significant decrease in pH after day 14 with respect to the control sample for both puree and juice. TSS content remained almost constant throughout the study for puree and juice, with a slight increase after treatment at 400 and 500 MPa. Moreover, the study performed by Yildiz et al. (2021), which compared different processing technologies to treat strawberry juice, including HPP (300 MPa for 1 min at 18.3 °C), showed no significant changes in pH or TSS after a storage period of 42 days.

No previous studies have evaluated the effect of HPP on sour cherry products with respect to the storage time. However, the study performed by Queirós et al. (2015) evaluated the evolution of pH and TSS in sweet cherry juice after treatment at 400 MPa for 5 min and 550 MPa for 2 min, for a storage period of 28 days. Their findings showed that there was a significant decrease in pH and TSS after day 14 under both conditions. The untreated samples did not present a drastic reduction in TSS, as observed in this study. This might be a consequence of the availability of free sugars in the juice compared to nectars, which are potential substrates for endogenous microorganisms, as well as the initial microbial load present in each product and its diversity.

Similarly, only one study has evaluated the effect of HPP on grapes during their shelf life. Li & Padilla-Zakour (2021) evaluated the storage stability of Concord grape puree treated at 600 MPa for 3 min for 4 months in refrigerated storage. Their results showed no significant differences with respect to pH or TSS during the study.

8.7.2. Enzymatic evaluation

8.7.2.1. Strawberry nectar

As previously mentioned, the shelf-life study was conducted using the puree of the supplier SVZ, which, after the initial evaluation, showed POD activity. Therefore, this is the only enzyme evaluated for strawberry nectar in this study. After treatment, the initial inactivation of POD enzymes was quantified, which resulted in 50.2% RA. However, after 7 days of study, no enzymatic activity was observed. Samples were evaluated for quantification after long periods of time (about 1 h), and no increase in the absorbance was detected; even overnight samples were left at ambient temperature, but no positive results were found. The evolution of enzymatic residual activity for NT samples of strawberry nectar in bottles and pouches is presented in Figure 42. Pouches presented a more stable behaviour compared to bottles, showing only significant differences with respect to the control sample after day 21, with a slight increase (about 140% RA) followed by a significant decrease (about 80% RA). Bottles on the other side showed a significant decrease after day 7, a trend that was maintained until day 28, when a sudden increase in activity was detected (approximately 120% RA). This increase could be related to changes in the food matrix during storage, that subsequently will release the enzymes increasing the enzymatic activity.

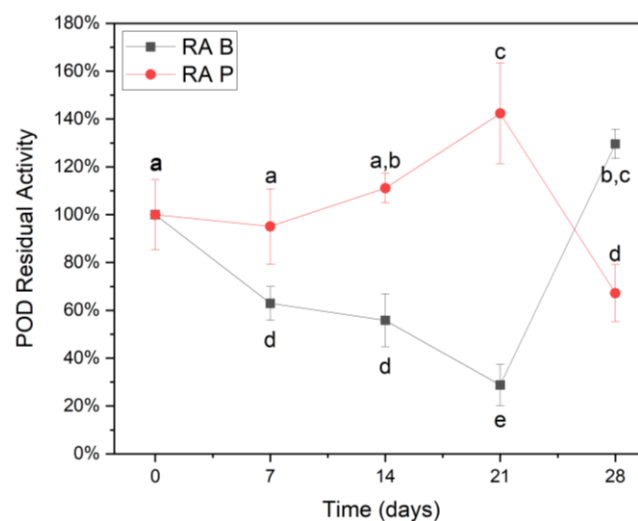


Figure 42. POD Residual activity evolution during shelf life for strawberry nectar packed in pouches (P) and bottles (B). Different letters indicate statistically significant differences (non-treated samples).

Several authors have investigated the potential of HPP to reduce the enzymatic activity of strawberry substrates. Garcia-Palazon et al. (2004) studied the effect of HPP in the stability of endogenous enzymes (PPO, POD and β -glucosidase) when treated at 400, 600, and 800 MPa for 5, 10, and 15 min) at temperatures between 18 and 22 °C in strawberry (cultivar not specified). Their results for POD enzymes showed slight activation after 5 min for samples treated at 400 MPa, followed by a slight decrease (95% RA after 15 min). For treatments at 600 and 800 MPa, a decreasing trend was observed, reaching the most extreme conditions with similar inactivation values (approximately 65% RA). Similar results were obtained by Cao et al. (2011), who evaluated the resistance of PPO, POD, and β -glucosidase enzymes, among other parameters, for strawberry pulps (cultivar Tongzi I) treated at 400, 500, and 600 MPa for 5 to 25 min. Their findings, with respect to POD inactivation, showed a decreasing trend at 400 and 500 MPa, starting with a RA of approximately 75 and 55%, respectively. At 600 MPa, there was an initial inactivation of 65% (35% RA) after 5 min of treatment, followed by an increase with 10 min of treatment (approximately 70% RA), and a decreasing trend similar to that of the other treatments. Terefe et al. (2013) also evaluated the enzymatic activity of POD enzymes, but in this case for three different strawberry cultivars (Camarosa, Ruby Gem, and Festival) after HPP treatment at 600 MPa for minutes at 20 °C. Their results showed RA of approximately 60 % for the cultivars Camarosa and Festival, while Ruby Gem presented RA of approximately 70%. Other studies have also evaluated the effect of pressure-temperature-time combinations (Terefe et al., 2010). All these previous results are in agreement with the findings presented in this study, considering that the varieties differ between articles, as well as the type of product tested; 600 MPa for short treatment times is capable of partially inactivating POD enzymes in strawberry substrate.

8.7.2.2. Sour cherry nectar

Inactivation of PPO enzymes for samples with and without treatment and its evolution was evaluated in two packaging configurations (Figure 43). For NT samples, the behaviour was very stable; for pouches, a slight but significant reduction in RA was observed only at day 14 with respect to the value of day 0, after which a recovery was seen, leading to non-significant differences with respect to the

initial value. In the case of bottles, the behaviour showed a significant increase on day 14, followed by a significant decrease on day 21. Finally, the last day of the storage period showed no significant differences compared to day 0.

The treated samples exhibited an initial residual activity of 25%, which was significantly lower than the expected value (55% RA when evaluating the surface response). The trends for both pouches and bottles were erratic, but finally led to non-significant differences when comparing day 0 and day 28 in the case of pouches and a significant increase (approximately 50% RA) for bottles comparing the same storage times.

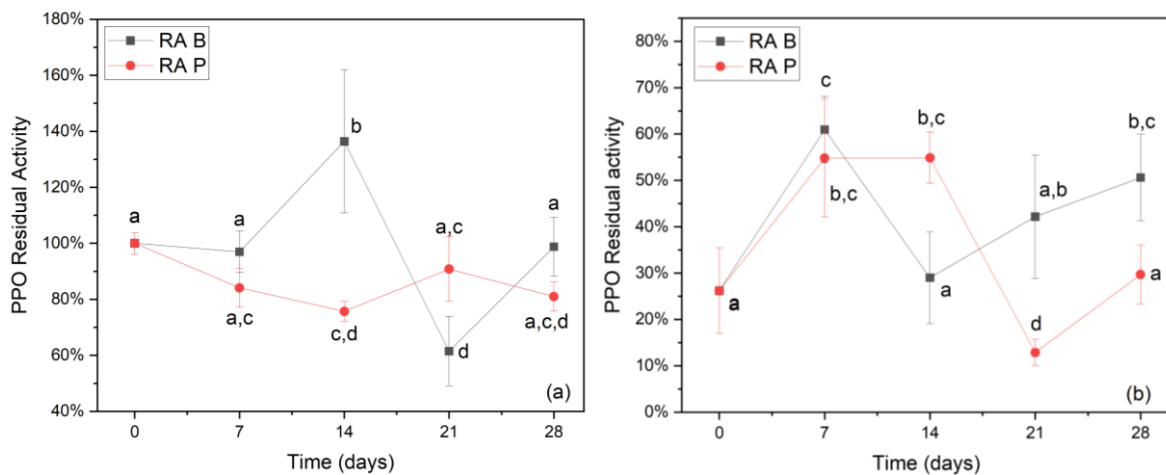


Figure 43. PPO Residual activity evolution during shelf life for sour cherry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

The POD results for the treated and non-treated samples are shown in Figure 44. The NT samples presented a decreasing trend throughout the study, reaching approximately 60% and 30% for pouches and bottles, respectively. In contrast, the treated samples presented an initial RA of approximately 70%, which was considerably higher than the values found when evaluating the surface response (approximately 30% RA). The storage evolution showed differences between packaging materials: pouches presented a more stable behaviour, aside from a reduction in RA on days 14 and 21, followed by an increase in RA, reaching statistically equal values compared to day 0. In contrast, bottles showed a significant reduction in RA, reaching approximately 50% RA at the end of the storage period.

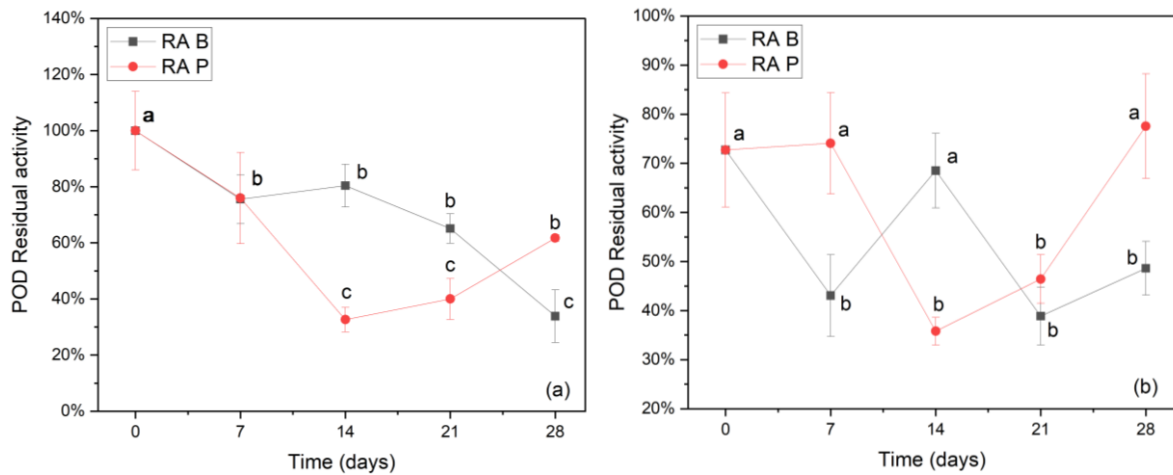


Figure 44. POD Residual activity evolution during shelf life for sour cherry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

The evolution of RA for β -glucosidase is shown in Figure 45. The non-treated samples presented a general decreasing trend in RA, with a final value after the study of approximately 50% RA. The initial enzymatic inactivation of the treated samples corresponded to previous findings in this study (approximately 25% RA). With respect to storage time and packaging characteristics, pouches did not present significant differences with respect to the initial value during the study, aside from a significant reduction on day 14. For bottles, a slight but significant increase was observed on day 28 (approximately 40% RA), breaking the stable trend prior to this point.

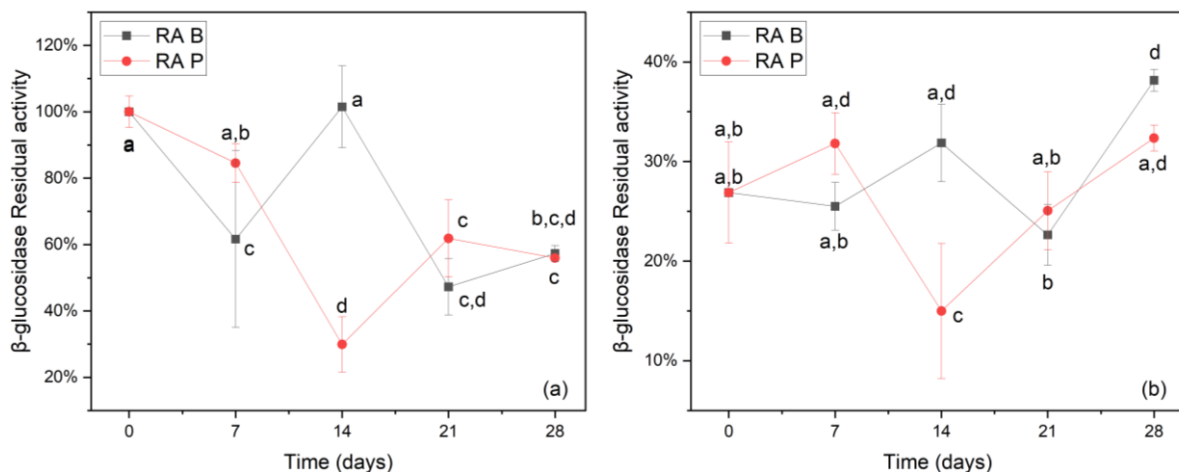


Figure 45. β -glucosidase Residual activity evolution during shelf life for sour cherry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

No previous studies have evaluated the storage evolution of enzymatic activity for endogenous enzymes in sour cherries after HPP treatment. However, Pasquariello et al. (2015) showed results for PPO and POD, among other enzymes, in non-treated sweet cherries (cultivars Ferrovia, Lapins, and Della Recca) under refrigerated storage. Their results showed an increase in both enzymes during storage, which differs from the results presented in this study. However, the substrates were different.

8.7.2.3. Red grape juice

The PPO and POD enzymes were also evaluated in red grape juice. The results of the evolution of enzymatic activity under refrigerated storage for PPO enzymes are presented in Figure 46. For NT samples, a general trend of increasing RA was observed, reaching values between 130 and 140% RA at the end of the study. Different trends were observed for the treated samples. The initial inactivation after treatment was not effective, as predicted by the analysis presented in this study. However, when storage time was considered as a variable, bottles presented a stable behaviour, leading to non-significant differences between RA at days 0 and 28. In contrast, pouches showed a decrease in RA during the study period, leading to a RA of approximately 70% after 28 days of refrigerated storage.

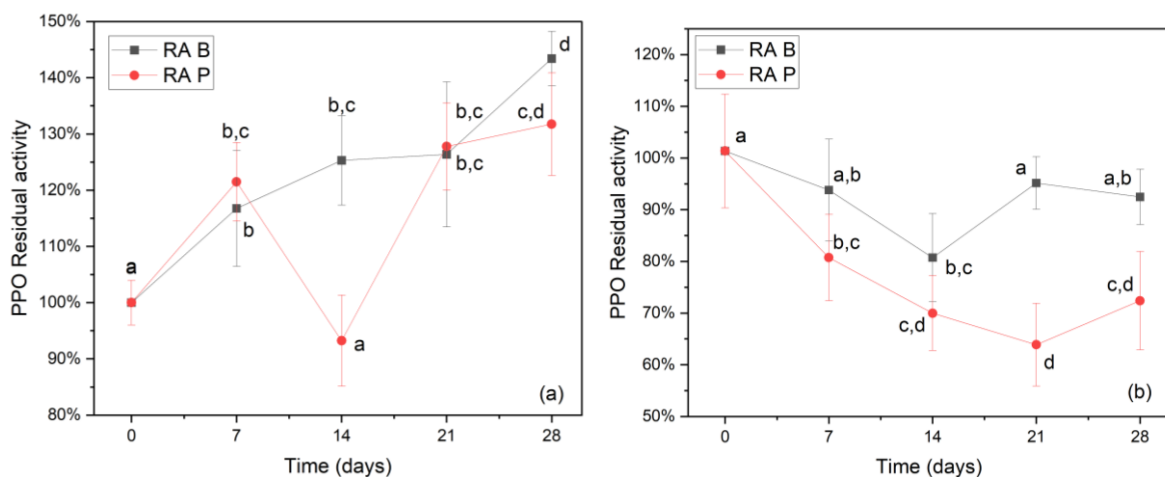


Figure 46. PPO Residual activity evolution during shelf life for red grape juice packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

POD enzymes showed a different behaviour than PPO enzymes (Figure 47). The NT samples showed a reduction in RA after the storage period, with a minimum of 80% RA for both packaging materials.

A more pronounced reduction was observed in the treated samples. An initial inactivation was observed immediately after treatment (approximately 75% RA), which differed with respect to the values obtained in the surface response where an activation was observed (approximately 140% RA). With respect to the RA evolution over time, both packaging materials presented similar behaviour: a decrease in RA was observed throughout the study, reaching minimum values of approximately 45% after 28 days.

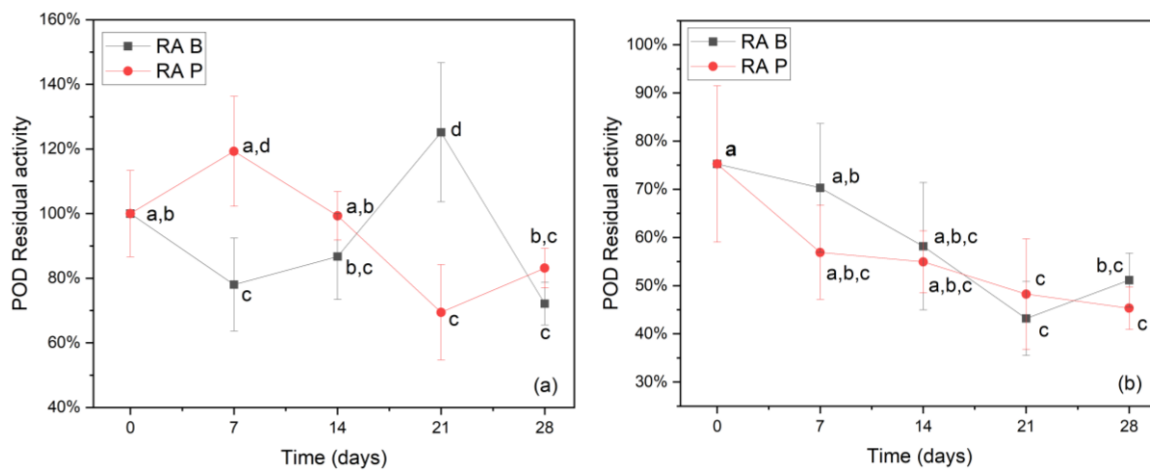


Figure 47. POD Residual activity evolution during shelf life for red grape juice packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

Only Li & Padilla-Zakour (2021) evaluated the behaviour of PPO and POD enzymes after HPP treatment and kept them in refrigerated storage for grapes. In their study, Concord grape puree was subjected to HPP (600 MPa for 3 min) and stored for 5 months at 4 °C. Their results showed inactivation of approximately 20% for PPO enzymes, showing a stable behaviour over the 5 months, corresponding to the results obtained in this study despite the differences in raw materials. For POD enzymes, an initial reduction of 20% in enzymatic activity was observed, which was maintained during the storage period. In this case, the results differ from those of the present study, but differences between treatment conditions and, more importantly, raw materials could be the cause of this variation.

8.7.3. Colour evaluation

Colour is one if not the most interesting parameter to follow in this shelf-life study, considering that the implemented optimisation strategies were taken in place in order to promote the colour stability of the fruit juices and to maintain bioactive molecules relevant for this type of product minimally unaltered or even stable. The comparison of bottles and pouches for strawberry nectar over a total period of 28 days of refrigerated storage is presented in Figure 48. For this product, the effects of time and packaging material are significant for colour changes. For NT samples, evident differences were observed from day 7, which were clearly visible for samples stored in pouches. Bottles maintained their changes in the range of 1.5 to 3.0 (evident difference), while for pouches, there was an increasing trend, reaching values of approximately 3.5 at the end of the study. For the treated samples, the difference in behaviour between pouches and bottles was most evident. Pouches presented clearly visible changes after day 7, similar to the non-treated samples, with an incremental trend reaching to about 6.0 at day 28. On the other side, bottles showed an incremental but not so pronounced trend with no significant differences in between day 7 and 14 and also non-significant differences between days 21 and 28, keeping values always under 3.0 (evident difference).

The most relevant changes were observed in the L* and a* parameters, which decreased throughout the study, accounting for the brightness and red colour, which are usually related to residual enzymatic activity. However, considering the obtained results for treated samples in this study, where no enzymatic activity was detected after day 7, the changes in total colour difference might be related to non-enzymatic browning, such as ascorbic acid degradation, despite these changes being more associated with post-storage effects in thermally treated samples (Buvé et al., 2018). Moreover, in NT samples where enzymatic activity was observed, the changes were less pronounced than those in the treated samples, providing further insight into the non-enzymatic colour degradation phenomena. To explain the differences between non-treated and treated samples, it can also be hypothesised that after HPP, anthocyanins are more susceptible to ascorbic acid present in the food matrix, being easily attacked by it and consequently leading to larger changes in colour when compared to the non-treated product.

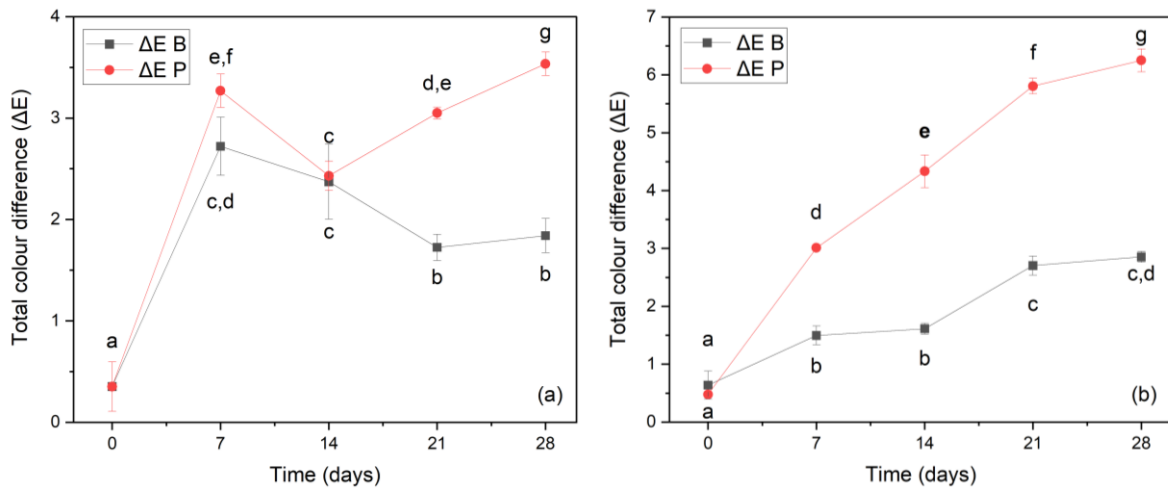


Figure 48. Colour evolution during shelf life for strawberry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

This hypothesis was also investigated by Cao et al. (2012), who evaluated the differences during storage of clear and cloudy strawberry juice at 4 and 25 °C, after HPP treatment at 600 MPa for 4 min, and for a total storage period of 6 months. In their study, among other parameters, the colour, anthocyanin content, and ascorbic acid content were measured. For colour, significant differences were observed when comparing both cloudy and clear at the same temperature condition and the same type of juice at different temperatures. Comparing cloudy vs. clear juice, an increase in total colour difference was observed for both; however, this trend was more pronounced for cloudy juice, where ΔE reached a maximum of 7.8 after 6 months, compared to 5.36 for the same storage time. For the temperature comparison, trends were also similar (increase in total colour difference) with a more pronounced increase for juices stored at 25 °C, with a significant difference in the L^* and a^* parameters when comparing both storage temperatures (both decreasing throughout the study). In addition, their findings showed that colour measurements, except for b^* , were correlated with the anthocyanin content. Moreover, a high correlation between a^* values and ascorbic acid content was also observed, supporting the idea that changes in strawberry colour could be associated with possible interactions between anthocyanins and ascorbic acid, as well as the enzymatic degradation of anthocyanins.

Similar results were reported by Aaby et al. (2018), who compared HPP at 400, 500, and 600 MPa for 3 min at 20 °C for strawberry puree and evaluated different parameters after refrigerated storage for 49

days. In their results, L^* was very stable throughout the study; however, an increment in ΔE was observed for all tested conditions and paired with a decrease in ascorbic acid and total anthocyanins.

With respect to the packaging material, Buvé et al. (2018) evaluated the colour changes during storage in pasteurised strawberry juice (thermal treatment at 95 °C for 120 s with pre-heating and post-cooling phases both at 65 °C) packed in two different types of bottles: a standard PET monolayer bottle and a multilayer bottle with an extra oxygen barrier. Samples were stored at 20, 28, 35 and 42 °C and kept under those conditions for a maximum of 32 weeks. Their results showed that for strawberry juice stored in monolayer material, the decrease in parameter a^* was accelerated compared to that in multilayer bottles. In both cases, an increase in the temperature led to a faster decrease in a^* . Variations in ΔE were associated only with changes in redness, as L^* and b^* were nearly invariant. These results disagree with those found in this study, considering that pouches contain multiple layers specifically designed to enhance the oxygen barrier.

Sour cherry nectar colour behaviour with respect to storage time is shown in Figure 49. For the NT samples, there were significant differences with respect to the packaging material. An exponential increase in ΔE was observed for products packed in pouches, leading to approximately 7 after 28 days, in contrast to samples in bottles that after presenting an increase after day 7, a reduction and a subsequent more stable behaviour was observed; the maximum ΔE value was observed at day 7 being approximately 4.5. For the treated samples, at all storage times, regardless of the packaging material, the samples remained under a ΔE of 3. Pouches and bottles presented similar but less pronounced trends compared to the NT samples.

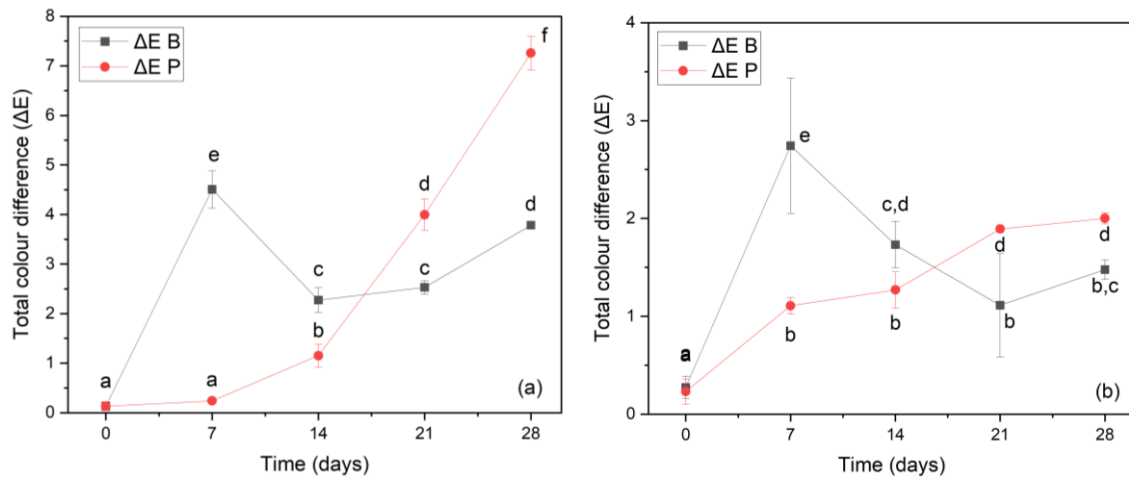


Figure 49. Colour evolution during shelf life for sour cherry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

Tenuta et al. (2023) evaluate the colour changes of sour cherry juice over a period of 5 months refrigerated storage, after HPP at 600 MPa for 3 min. In their findings, colour was maintained at levels similar to those of non-treated products after 3 months of storage, after which a decrease in the a^* parameter was observed. In the present study, a^* changed constantly in all sour cherry nectar samples; however, changes in L^* and b^* were also observed, particularly for NT samples. These results show that even with partial inactivation of enzymes, it is possible to control the colour degradation of sour cherry nectar.

Finally, the colour stability of red grape juice was influenced by the treatment as well as the packaging material (Figure 50). For NT samples, bottles presented a more rapid increment in total colour difference, reaching a maximum value of 3.0 after day 28. Meanwhile, for pouches, the increase was less pronounced, reaching a maximum of 2.0 after day 28. Both pouches and bottles remained under the limit of clearly visible differences. For treated samples, pouches presented a more pronounced increase in the total colour difference compared to the bottles. Nevertheless, these changes were lower than those presented in the NT samples, leading to less perceptible changes. The parameters responsible for these changes were mainly L^* and a^* . This similarity in the increase in colour change could be associated with the activity of PPO and POD enzymes. As previously shown, only a small reduction in enzymatic activity was observed for POD enzymes after treatment, which could account for the deceleration of the change in the case of treated samples compared to non-treated samples.

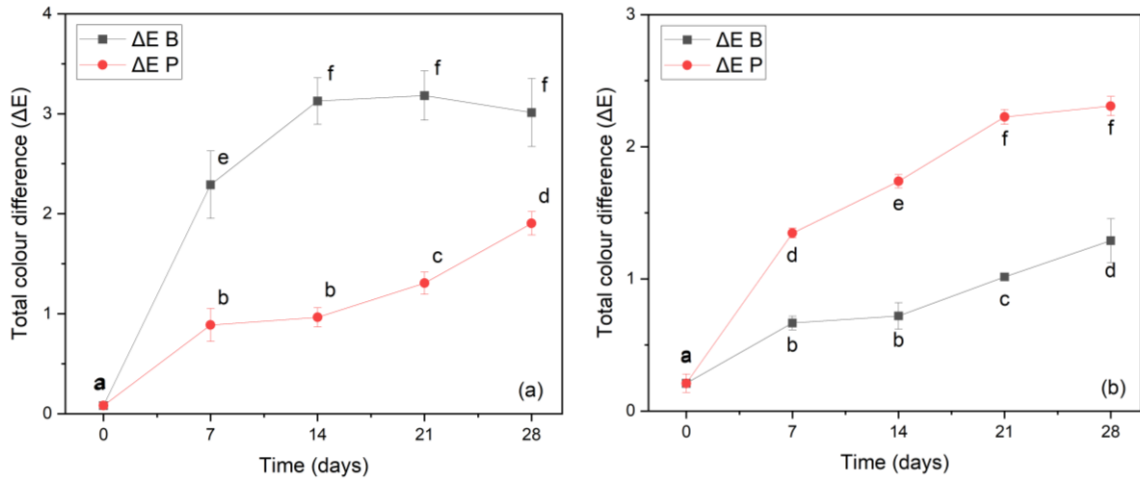


Figure 50. Colour evolution during shelf life for red grape juice packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

The study conducted by Li & Padilla-Zakour (2021) also evaluated the colour changes in concord grape puree after HPP treatment (600 MPa for 3 min at 5 °C) during refrigerated storage for 4 months. Their results showed no significant changes during the storage period for any of the colour parameters evaluated, showing an even higher colour stability than that presented in this study.

8.7.4. Total polyphenol content

The effect of refrigerated storage on the degradation of polyphenols was evaluated for strawberry and sour cherry nectars and red grape juice. The comparison between non-treated and treated strawberry nectar samples is presented in Figure 51. In general, the TPC was lower than that reported in the analysis of the surface response. NT samples presented very stable behaviour regardless of the packaging characteristics, leading to non-significant differences between samples at all points evaluated during the storage period. In the case of the treated samples, a reduction in the total polyphenol count was observed when comparing the values obtained in the surface response analysis, reaching approximately 25% reduction compared to 10% in previous findings. These differences (and those of the NT samples) can be directly attributed to the puree used in the preparation of the nectar for the shelf-life study (supplier SVZ), considering that it is different from that of the surface response analysis (supplier Sicoly). With respect to the storage effect, a similar behaviour was observed when comparing different

packaging materials; samples packed in bottles showed a higher stability until day 21, with a further significant reduction in polyphenols content. Moreover, for pouches, the decrease in polyphenols was also significant with respect to the initial treated samples only on day 28.

Many researchers have evaluated the stability of strawberry polyphenols under refrigerated and non-refrigerated storage conditions. Cao et al. (2012) evaluated the effect of refrigerated and non-refrigerated storage (4 and 25 °C, respectively) of clear and cloudy strawberry juice treated at 600 MPa for 4 min, for a total storage time of 6 months. In their study, a constant decrease in total polyphenols was observed for both temperature conditions, which was more pronounced for samples stored at 25 °C. Similar results were observed by Terefe et al. (2013), who evaluated the storage stability of strawberry puree of three different cultivars (Camarosa, Ruby Gem and Festival) after treatment at 600 MPa for 5 min at 20 °C. The samples were maintained at 4 °C for 3 months. For the cultivars Camarosa and Ruby Gem, there was a significant reduction in TPC after treatment, result that was different for the variety Festival where there was not significant difference. After 3 months, all cultivars showed a significant decrease in TPC. No further data was presented between post-treatment evaluation and three months of refrigerated storage. Moreover, in a most recent study of Yildiz et al. (2021), strawberry juice (cultivar non-specified) was submitted to HPP (300 MPa for 1 min), and samples were evaluated for different parameter (among those TPC) for a total refrigerated storage period of 42 days. Their results are in agreement with those presented in the previous findings reported in this document, showing a decreasing trend during the studied period. These results indicate that the strawberry nectar treated in this study presented a certain stability for a longer period when compared with other studies.

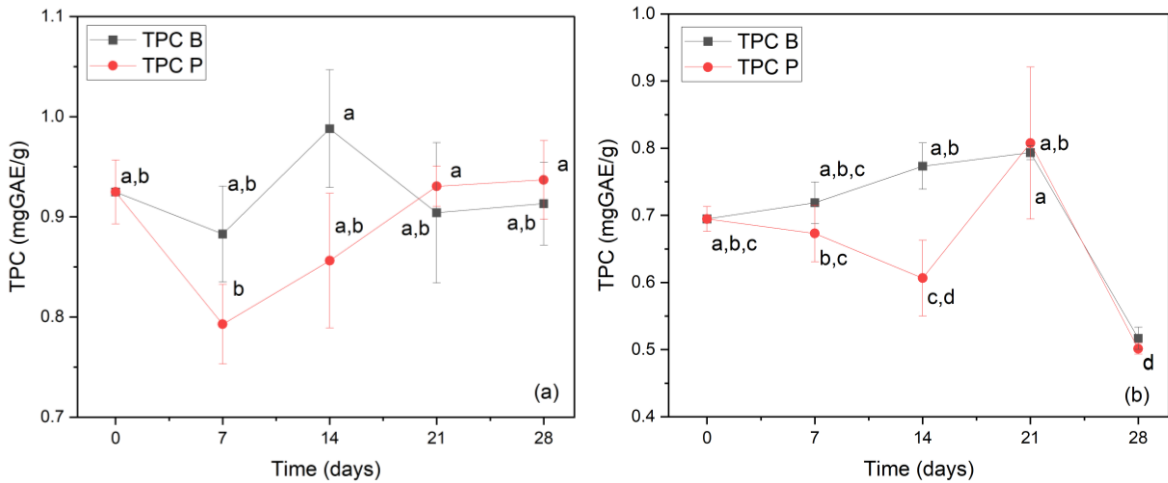


Figure 51. Total polyphenol content evolution during shelf life for strawberry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

The effect of storage on sour cherry nectar after treatment is shown in Figure 52. For the NT samples, packaging material showed a significant effect. The initial values were consistent with those reported in the surface response analysis of the NT samples. Pouches presented a very stable behaviour until day 21. After that, a significant increase in TPC was observed. On the other hand, bottles presented a slight reduction, which was only significant at day 28. With respect to the treated samples, the initial reduction in TPC was higher for the shelf-life study than for the surface response (approximately 40 and 30%, respectively). With respect to the behaviour during storage, packaging was not an influential variable, and samples remained stable throughout the studied period.

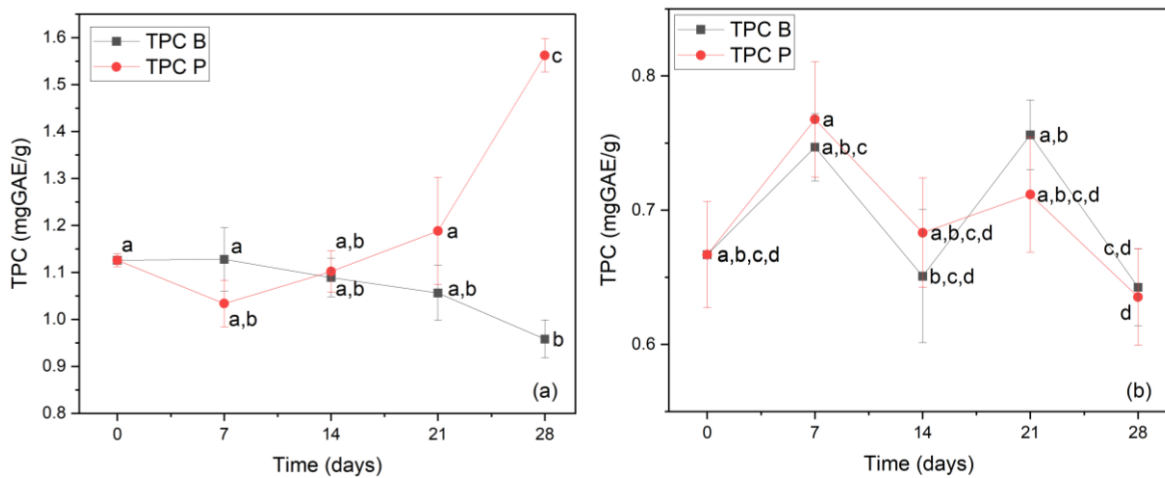


Figure 52. Total polyphenol content evolution during shelf life for sour cherry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

The storage stability of polyphenols in sweet cherry juice after two different HPP treatment combinations (400 MPa for 4 min and 550 MPa for 2 min) under refrigerated storage for 28 days was evaluated by Queirós et al. (2015). Their results showed a significant decrease after day 21 for the mildest conditions, reaching an 11% reduction. Under the strongest conditions, the samples remained stable. Similar results were presented by Tenuta et al. (2023); in their study, sour cherries (cultivar Amarena del Rio) were subjected to HPP (600 MPa for 3 min at 4 °C) for further storage at 4 °C for 5 months. Their results showed significant differences only after 94 days of storage, presenting similar results to those found in this study, being also more accurate considering that they correspond to the same substrate despite differences between varieties.

Finally, polyphenols in red grape juice exhibited the most stable behaviour among all the evaluated products (Figure 53). The initial values for both the treated and non-treated samples corresponded to those initially found in the surface response study. The NT samples did not present significant differences throughout the study, and these results were similar for the treated samples. Packaging was not a relevant variable for the TPC in this substrate.

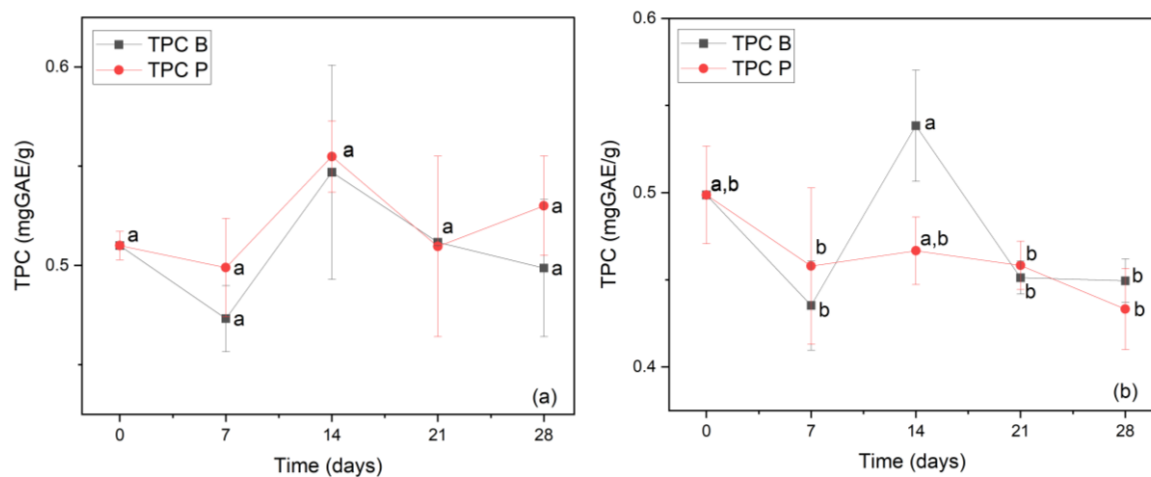


Figure 53. Total polyphenol content evolution during shelf life for red grape juice packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

Li & Padilla-Zakour (2021) evaluated the storage stability of grape juice (cultivar Concord) treated with HPP technology (600 MPa for 3 min) at 4 °C for months. Their results showed a significant decrease in TPC after the first month and further stability between months 1 and 4 of the study. The differences

between their study and this could be related to the characteristics of the substrate, as expressed before, an important factor with respect to the quantity and stability of polyphenols.

8.7.5. Antioxidant capacity

Antioxidant capacity was also evaluated during shelf life, as well as for the total polyphenols in each substrate. The results obtained when comparing non-treated and treated samples in different packaging for strawberry nectar are presented in Figure 54. The initial TAC values for the non-treated and treated samples differed from those found in the surface response analysis; however, a significant increase immediately after treatment was observed. This can be attributed to the differences in the strawberry puree used in the two stages. With respect to shelf-life evolution, NT samples presented differences with respect to packaging material; for bottles, an initial significant reduction was observed at day 7 with a slow recovery after that point, reaching values at day 28 without significant differences with respect to day 0. Pouches, on the other hand, also showed a significant reduction on day 7, without a recovery, leading to lower values with respect to the control. Treated samples showed a similar behaviour; both pouches and bottles presented a significant reduction on day 7 (most pronounced for pouches) and after that, in the case of bottles, a relatively constant trend, while for pouches, a less pronounced but significant decrease continued until the end of the study.

Cao et al. (2012) also evaluated the evolution of TAC under the same conditions as those previously presented for TPC. Their results showed linear decreasing trends for clear and cloudy strawberry juices, which were more pronounced for the samples stored at 25 °C. Compared with the data currently being presented, this puree seems to be more stable, as was also observed when comparing the TPC data.

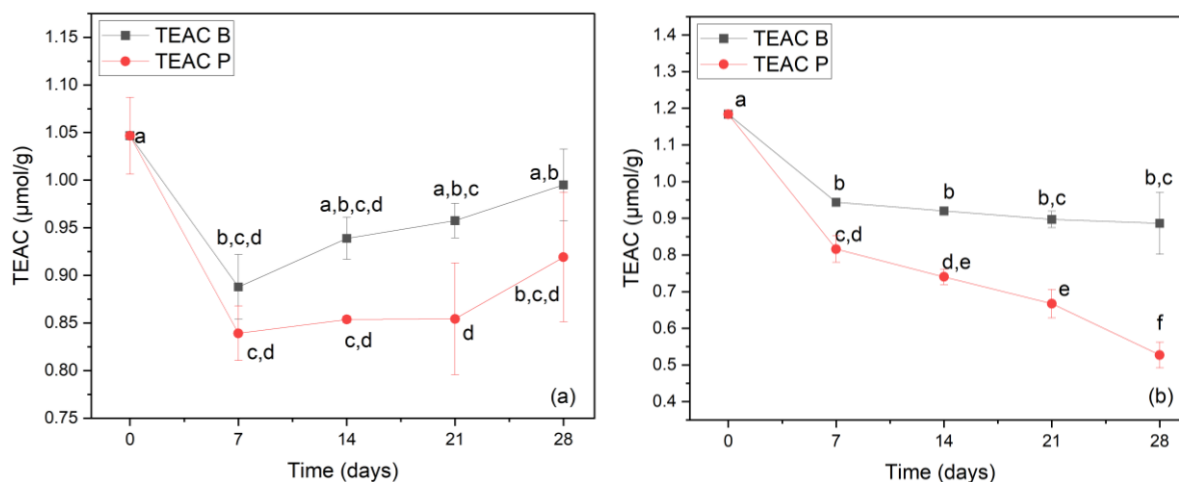


Figure 54. Antioxidant Capacity evolution during shelf life for strawberry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

In the case of sour cherry nectar, when comparing the data obtained for the SRM and the shelf-life study, higher values were found for the shelf life; nevertheless, no significant differences were found between the NT and T samples, similarly to the results previously obtained. As shown in Figure 55, NT samples did not present significant differences at all storage times, and similar stability was observed for the treated samples, despite a specific point for pouches on day 14 where a diminution was significant.

Previous studies are not in full agreement with these findings; Queirós et al. (2015) also evaluated the changes in TAC for sweet cherry juice under the conditions presented in the TPC section. Their observations concluded that there were no significant differences after treatment between the two HPP conditions; however, the mildest condition presented a significantly higher value than the control. With respect to storage evolution, the mildest conditions showed a significant increase in TAC on day 28, whereas the strongest conditions showed a significant increase from day 21. A more related study by Tenuta et al. (2023) in sour cherry, which was intensively discussed in this document, also evaluated the antioxidant capacity evolution, and their results showed a constant decreasing trend over the 150 days of the study. In this case, sour cherry nectar showed more stability than in previous findings.

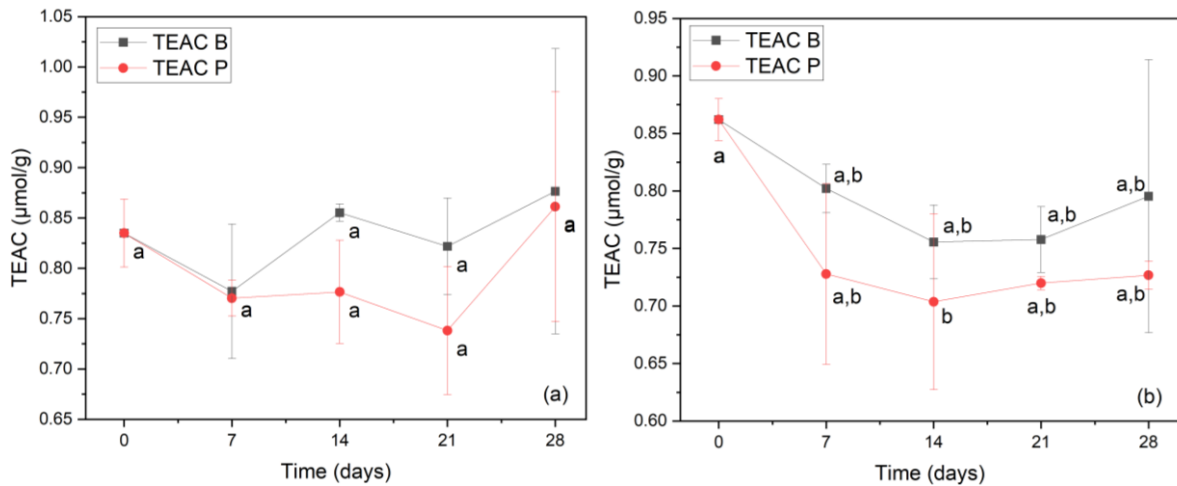


Figure 55. Antioxidant Capacity evolution during shelf life for sour cherry nectar packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

Finally, for red grape juice, a general decreasing trend was observed in all cases (Figure 56). First, the values obtained for the shelf-life study coincided with those of the SRM, as well as a slight reduction after the HPP treatment. NT samples showed a decrease in TAC evident in the case of pouches from day 14, contrary to bottles that only showed significant changes at day 28.

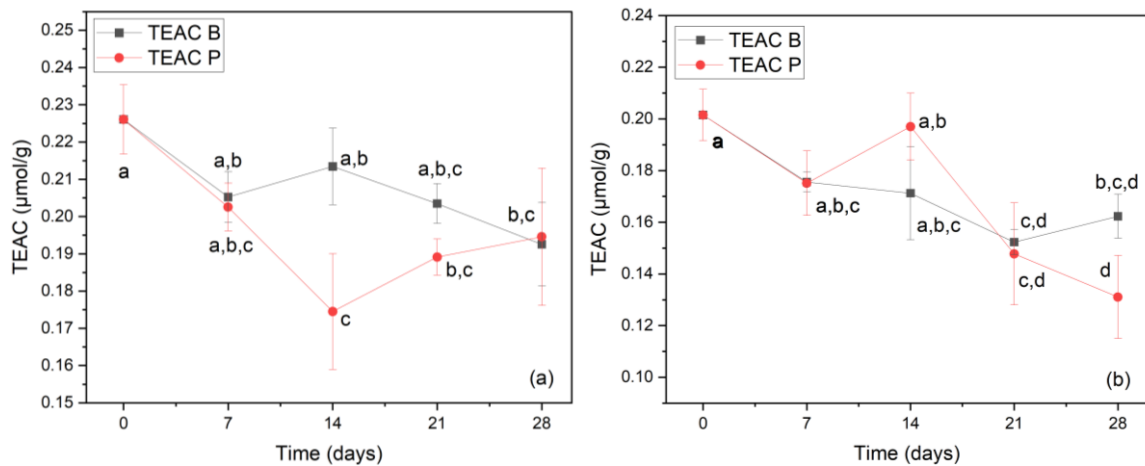


Figure 56. Antioxidant Capacity evolution during shelf life for red grape juice packed in pouches (P) and bottles (B). (a) non-treated samples, (b) treated samples. Different letters indicate statistically significant differences.

In the study by Li & Padilla-Zakour (2021), TAC evolution was also analysed for Concord grape puree. Their findings conclude that, there is not significant differences in TAC after 4 months refrigerated storage. These results might differ from those of this study because of differences in the raw materials.

9. Conclusions and recommendations

Addressing the main research question of this study, HPP is a feasible industrial alternative able to provide inactivation for potential pathogenic bacteria and, at the same time, reaching enzymatic inactivation of endogenous enzymes. This capacity is highly dependent on the treated product and, conditions such as pH, TSS, initial enzymatic and microbial content, and other intrinsic characteristics of the substrates can promote or limit the effect of this technology, making each product independent and hardly relatable to other products even within the same category. This variation is also extended to diversity in cultivars for the same fruits.

Considering this high variability between different products, optimisation conditions might differ from each treated product, as well as the particular target (microorganisms, enzymes, or polyphenols). In particular, microbial inactivation for the three surrogates tested in this study at a level of 5-log, was possible at 300 MPa for 6 min for strawberry and sour cherry nectars, while for red grape juice this was possible only at higher conditions (450 MPa for 4 min). Moreover, enzymatic inactivation was even more substrate specific, leading to different conditions for each enzyme type in each substrate. Finally, for TPC, strawberry and sour cherry nectar showed significant reduction at 600 MPa while for red grape juice this parameter was stable. For TAC strawberry and sour cherry nectar presented a significant increase while red grape juice showed a decrease under the same conditions. In this complex panorama, and to provide a homogenous treatment for all products having into consideration that they belong to the same category, the treatment of 600 MPa and 4 min was selected as the optimal point considering also restrictions of the equipment and having in mind that these parameters will not correspond to the optimal conditions for all products.

The product life cycle does not finish after the production step. Big changes occur in their characteristics during the storage period, and this study evaluated how the condition selected as optimal could impact the shelf stability of these products. Again, each product presented its own particularities but some general trends were observed. HPP was able to prevent microbial growth and aroma and taste of the products compared to untreated ones, being this evaluated based on the pH, TSS and aroma perception.

The reduction of enzymatic activity was observed in all products, maintaining a certain stability during the storage period for all products regardless the packaging material. These results did not reflect directly in the colour stability of the products. Particularly, strawberry nectar packaged in pouches (where no enzymatic activity was detected) presented a very unstable behaviour during the whole study, even with lower performance than the untreated product. On the other hand, sour cherry nectar and red grape juice (both with partial enzymatic inactivation) presented a more stable behaviour after treatment. This demonstrates that colour is a parameter involved in a complex phenomenon, not only depending on enzymatic inactivation, but also on other components of the food matrix, in this case possibly ascorbic acid and its interaction with anthocyanins. Finally, polyphenols presented a very stable behaviour for all packaging conditions at all storage times in all products, while antioxidant capacity presented a stable behaviour for sour cherry nectar and a decrease for strawberry nectar and red grape juice, all very similar to the untreated product.

These results provide scientific evidence of the effectiveness of the technology in controlling the microbial growth, while inactivating, even partially, endogenous enzymes and maintaining the nutritional properties of the products, under certain limitations detailed in this work. However, the limitations of this study should be considered in the future to obtain a more detailed panorama of the efficiency of the technology. In particular, the use of a food-model solution with controlled pH, TSS and viscosity at different levels/concentrations could be an interesting approach to evaluate the microbial inactivation effectiveness of the technology, considering these variables were crucial for the microbial survival in this study. Moreover, exploring the increase in temperature and the modification of the compression and decompression rates could be beneficial in terms not only of microbial but also on enzymatic inactivation.

10. Bibliography

- Aaby, K., Ekeberg, D., & Skrede, G. (2007). Characterization of Phenolic Compounds in Strawberry (*Fragaria × ananassa*) Fruits by Different HPLC Detectors and Contribution of Individual Compounds to Total Antioxidant Capacity. *Journal of Agricultural and Food Chemistry*, 55(11), 4395–4406. <https://doi.org/10.1021/jf0702592>
- Aaby, K., Grimsbo, I. H., Hovda, M. B., & Rode, T. M. (2018). Effect of high pressure and thermal processing on shelf life and quality of strawberry purée and juice. *Food Chemistry*, 260, 115–123. <https://doi.org/10.1016/j.foodchem.2018.03.100>
- Aaby, K., Mazur, S., Nes, A., & Skrede, G. (2012). Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry*, 132(1), 86–97.
- Agregán, R., Munekata, P. E. S., Zhang, W., Zhang, J., Pérez-Santaescolástica, C., & Lorenzo, J. M. (2021). High-pressure processing in inactivation of *Salmonella* spp. In food products. *Trends in Food Science & Technology*, 107, 31–37. <https://doi.org/10.1016/j.tifs.2020.11.025>
- Aguiló-Aguayo, I., Sobrino-López, Á., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric field processing on lipoxygenase and β -glucosidase activities in strawberry juice. *Innovative Food Science & Emerging Technologies*, 9(4), 455–462. <https://doi.org/10.1016/j.ifset.2007.12.007>
- Ahmed, J., Mulla, M. Z., Al-Zuwayed, S. A., Joseph, A., & Auras, R. (2022). Morphological, barrier, thermal, and rheological properties of high-pressure treated co-extruded polylactide films and the suitability for food packaging. *Food Packaging and Shelf Life*, 32, 100812. <https://doi.org/10.1016/j.fpsl.2022.100812>
- Alpas, H., & Bozoglu, F. (2003). Efficiency of high pressure treatment for destruction of *Listeria monocytogenes* in fruit juices. *FEMS Immunology & Medical Microbiology*, 35(3), 269–273. [https://doi.org/10.1016/S0928-8244\(02\)00446-7](https://doi.org/10.1016/S0928-8244(02)00446-7)
- Alrgei, H. O. S., Dabić, D. Č., Natić, M. M., Rakonjac, V. S., Milojković-Opsenica, D., Tešić, Ž. L., & Fotirić Akšić, M. M. (2016). Chemical profile of major taste- and health-related compounds of Oblačinska sour cherry. *Journal of the Science of Food and Agriculture*, 96(4), 1241–1251. <https://doi.org/10.1002/jsfa.7212>
- Andino, A., & Hanning, I. (2015). *Salmonella enterica*: Survival, Colonization, and Virulence Differences among Serovars. *The Scientific World Journal*, 2015, 1–16. <https://doi.org/10.1155/2015/520179>
- Bai, B., Chen, F., Wang, Z., Liao, X., Zhao, G., & Hu, X. (2005). Mechanism of the Greening Color Formation of “Laba” Garlic, a Traditional Homemade Chinese Food Product. *Journal of Agricultural and Food Chemistry*, 53(18), 7103–7107. <https://doi.org/10.1021/jf051211w>
- Baker, R. A., & Cameron, R. G. (1999). Clouds of citrus juices and juice drinks. *Food Technology (Chicago)*, 53(1), 64–69.
- Balasubramaniam, V. M., Barbosa-Cánovas, G. V., & Lelieveld, H. L. M. (Eds.). (2016). *High Pressure Processing of Food: Principles, Technology and Applications*. Springer New York. <https://doi.org/10.1007/978-1-4939-3234-4>
- Balny, C., & Masson, P. (1993). Effects of high pressure on proteins. *Food Reviews International*, 9(4), 611–628. <https://doi.org/10.1080/87559129309540980>

- Bánáti, D., Tóth-Markus, M., Adányi, N., Boross, F., Vámos-Falusi, Zs., Daood, H. G., Szabó, T., & Nyéki, J. (2010). Composition and sensory properties of sour cherry cultivars. *International Journal of Horticultural Science*, *16*(3). <https://doi.org/10.31421/IJHS/16/3/889>
- Bayındırlı, A., Alpas, H., Bozoğlu, F., & Hızal, M. (2006). Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. *Food Control*, *17*(1), 52–58. <https://doi.org/10.1016/j.foodcont.2004.09.002>
- Bell, C. (2002). Approach to the control of entero-haemorrhagic Escherichia coli (EHEC). *International Journal of Food Microbiology*, *78*(3), 197–216.
- Berhanu, G., & Pal, M. (2020). Emerging role of enterohemorrhagic Escherichia coli as a global foodborne pathogen. *Journal of Emerging Environmental Technologies and Health Protection*, *3*, 60–68.
- Blando, F., & Oomah, B. D. (2019). Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. *Trends in Food Science & Technology*, *86*, 517–529. <https://doi.org/10.1016/j.tifs.2019.02.052>
- Bleoanca, I., Patraşcu, L., & Borda, D. (2021). Quality and Stability Equivalence of High Pressure and/or Thermal Treatments in Peach–Strawberry Puree. A Multicriteria Study. *Foods*, *10*(11), 2580. <https://doi.org/10.3390/foods10112580>
- Bonner, P., & Bonner, P. L. (2007). *Protein purification*. Taylor & Francis.
- Boulton, R. (1980). The Relationships between Total Acidity, Titratable Acidity and pH in Wine. *American Journal of Enology and Viticulture*, *31*(1), 76–80. <https://doi.org/10.5344/ajev.1980.31.1.76>
- Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., & Swaminathan, B. (2000). Salmonella nomenclature. *Journal of Clinical Microbiology*, *38*(7), 2465–2467.
- Bull, M. K., Szabo, E. A., Cole, M. B., & Stewart, C. M. (2005). Toward Validation of Process Criteria for High-Pressure Processing of Orange Juice with Predictive Models. *Journal of Food Protection*, *68*(5), 949–954. <https://doi.org/10.4315/0362-028X-68.5.949>
- Burgess, R. R., & Deutscher, M. P. (2009). *Guide to protein purification*. Academic Press.
- Burin, V. M., Falcão, L. D., Gonzaga, L. V., Fett, R., Rosier, J. P., & Bordignon-Luiz, M. T. (2010). Colour, phenolic content and antioxidant activity of grape juice. *Food Science and Technology*, *30*, 1027–1032.
- Buvé, C., Kebede, B. T., De Batselier, C., Carrillo, C., Pham, H. T. T., Hendrickx, M., Grauwet, T., & Van Loey, A. (2018). Kinetics of colour changes in pasteurised strawberry juice during storage. *Journal of Food Engineering*, *216*, 42–51. <https://doi.org/10.1016/j.jfoodeng.2017.08.002>
- Buzrul, S. (2022). The Weibull Model for Microbial Inactivation. *Food Engineering Reviews*, *14*(1), 45–61. <https://doi.org/10.1007/s12393-021-09291-y>
- Cairone, F., Frascetti, C., Menghini, L., Zengin, G., Filippi, A., Casadei, M. A., & Cesa, S. (2023). Effects of Processing on Chemical Composition of Extracts from Sour Cherry Fruits, a Neglected Functional Food. *Antioxidants*, *12*(2), 445. <https://doi.org/10.3390/antiox12020445>
- Caner, C., Hernandez, R. J., & Harte, B. R. (2004). High-pressure processing effects on the mechanical, barrier and mass transfer properties of food packaging flexible structures: A critical review: HIGH PRESSURE AND EFFECTS ON PACKAGING. *Packaging Technology and Science*, *17*(1), 23–29. <https://doi.org/10.1002/pts.635>

- Cao, X., Bi, X., Huang, W., Wu, J., Hu, X., & Liao, X. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science & Emerging Technologies*, *16*, 181–190. <https://doi.org/10.1016/j.ifset.2012.05.008>
- Cao, X., Liu, F., Wu, J., Liao, X., & Hu, X. (2014). Effects of High Hydrostatic Pressure Combined with Blanching on Microorganisms and Quality Attributes of Cloudy and Clear Strawberry Juices. *International Journal of Food Properties*, *17*(9), 1900–1920. <https://doi.org/10.1080/10942912.2013.766887>
- Cao, X., Wang, Y., Liao, X., & Hu, X. (2018). *Characterization of physico-chemical and biochemical compositions of selected four strawberry cultivars*. <https://doi.org/10.5073/JABFQ.2018.091.021>
- Cao, X., Zhang, Y., Zhang, F., Wang, Y., Yi, J., & Liao, X. (2011). Effects of high hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps: Effects of high hydrostatic pressure on strawberry pulps. *Journal of the Science of Food and Agriculture*, *91*(5), 877–885. <https://doi.org/10.1002/jsfa.4260>
- Castillo-Muñoz, N., Fernández-González, M., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2009). Red-Color Related Phenolic Composition of Garnacha Tintorera (*Vitis vinifera* L.) Grapes and Red Wines. *Journal of Agricultural and Food Chemistry*, *57*(17), 7883–7891. <https://doi.org/10.1021/jf9002736>
- CDC. (2023, July 24). *Summary of Possible Multistate Enteric (Intestinal) Disease Outbreaks in 2021 / CDC*. <https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/annual-summaries/annual-summaries-2021.html>
- CFSAN. (2004). *Guidance for Industry: Juice Hazard Analysis Critical Control Point Hazards and Controls Guidance, First Edition*. FDA. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-juice-hazard-analysis-critical-control-point-hazards-and-controls-guidance-first>
- Chae, P. S., Wander, M. J., Cho, K. H., Laible, P. D., & Gellman, S. H. (2013). Carbohydrate-containing Triton X-100 analogues for membrane protein solubilization and stabilization. *Molecular BioSystems*, *9*(4), 626. <https://doi.org/10.1039/c3mb25584k>
- Chakraborty, S., Kaushik, N., Rao, P. S., & Mishra, H. N. (2014). High-Pressure Inactivation of Enzymes: A Review on Its Recent Applications on Fruit Purees and Juices: HPP of enzymes in fruit purees & juices.... *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 578–596. <https://doi.org/10.1111/1541-4337.12071>
- Chakraborty, S., Rao, P. S., & Mishra, H. N. (2015a). Empirical model based on Weibull distribution describing the destruction kinetics of natural microbiota in pineapple (*Ananas comosus* L.) puree during high-pressure processing. *International Journal of Food Microbiology*, *211*, 117–127. <https://doi.org/10.1016/j.ijfoodmicro.2015.06.017>
- Chakraborty, S., Rao, P. S., & Mishra, H. N. (2015b). Kinetic modeling of polyphenoloxidase and peroxidase inactivation in pineapple (*Ananas comosus* L.) puree during high-pressure and thermal treatments. *Innovative Food Science & Emerging Technologies*, *27*, 57–68. <https://doi.org/10.1016/j.ifset.2014.11.003>
- Chapleau, N., Ritz, M., Delépine, S., Jugiau, F., Federighi, M., & de Lamballerie, M. (2006). Influence of kinetic parameters of high pressure processing on bacterial inactivation in a buffer system. *International Journal of Food Microbiology*, *106*(3), 324–330. <https://doi.org/10.1016/j.ijfoodmicro.2005.09.009>

- Cordenunsi, B. R., Oliveira do Nascimento, J. R., Genovese, M. I., & Lajolo, F. M. (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *Journal of Agricultural and Food Chemistry*, *50*(9), 2581–2586.
- Cosme, F., Ferreira, S. S., Alves, A. S., Filipe-Ribeiro, L., & Nunes, F. M. (2019). Recycling of PVPP used in the wine industry: An opportunity for obtaining reusable PVPP and bioactive phenolic compounds. *BIO Web of Conferences*, *15*, 02020. <https://doi.org/10.1051/bioconf/20191502020>
- Cosme, F., Pinto, T., & Vilela, A. (2018). Phenolic Compounds and Antioxidant Activity in Grape Juices: A Chemical and Sensory View. *Beverages*, *4*(1), 22. <https://doi.org/10.3390/beverages4010022>
- Damar, İ., & Ekşi, A. (2012). Antioxidant capacity and anthocyanin profile of sour cherry (*Prunus cerasus* L.) juice. *Food Chemistry*, *135*(4), 2910–2914. <https://doi.org/10.1016/j.foodchem.2012.07.032>
- Das, A., Bysack, A., & Raghuraman, H. (2021). Effectiveness of dual-detergent strategy using Triton X-100 in membrane protein purification. *Biochemical and Biophysical Research Communications*, *578*, 122–128. <https://doi.org/10.1016/j.bbrc.2021.09.031>
- Del Pozo-Insfran, D., Del Follo-Martinez, A., Talcott, S. T., & Brenes, C. H. (2007). Stability of Copigmented Anthocyanins and Ascorbic Acid in Muscadine Grape Juice Processed by High Hydrostatic Pressure. *Journal of Food Science*, *72*(4). <https://doi.org/10.1111/j.1750-3841.2007.00316.x>
- Deutsches Institut für Normung. (1998). *Testing of plastics—Determination of gas transmission rate—Part 3: Oxygen-specific carrier gas method for testing of plastic films and plastics mouldings (DIN 53380-3)*. <https://www.din.de/en>
- Dharmadhikari, M. (1994). Composition of grapes. *Vineyard Vintage View Mo State Univ*, *9*(7/8), 3–8.
- Doyle, M. P., Diez-Gonzalez, F., & Hill, C. (Eds.). (2019). *Food microbiology: Fundamentals and frontiers* (5th edition). ASM Press.
- Duru, I. C., Andreevskaya, M., Laine, P., Rode, T. M., Ylinen, A., Løvdal, T., Bar, N., Crauwels, P., Riedel, C. U., Bucur, F. I., Nicolau, A. I., & Auvinen, P. (2020). Genomic characterization of the most barotolerant *Listeria monocytogenes* RO15 strain compared to reference strains used to evaluate food high pressure processing. *BMC Genomics*, *21*(1), 455. <https://doi.org/10.1186/s12864-020-06819-0>
- Dzhanfezova, T., Barba-Espín, G., Müller, R., Joernsgaard, B., Hegelund, J. N., Madsen, B., Larsen, D. H., Martínez Vega, M., & Toldam-Andersen, T. B. (2020). Anthocyanin profile, antioxidant activity and total phenolic content of a strawberry (*Fragaria × ananassa* Duch) genetic resource collection. *Food Bioscience*, *36*, 100620. <https://doi.org/10.1016/j.fbio.2020.100620>
- EFSA & ECDC. (2021). The European Union One Health 2020 Zoonoses Report. *EFSA Journal*, *19*(12), e06971. <https://doi.org/10.2903/j.efsa.2021.6971>
- EFSA Panel on Biological Hazards (BIOHAZ Panel), Koutsoumanis, K., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Castle, L., Crotta, M., ... Allende, A. (2022). The efficacy and safety of high-pressure processing of food. *EFSA Journal*, *20*(3). <https://doi.org/10.2903/j.efsa.2022.7128>

- Eisenthal, R., & Danson, M. J. (2002). *Enzyme assays: A practical approach* (Vol. 257). Practical Approach.
- Elamin, W. M., Biological and Agricultural Engineering, Faculty of Engineering, University of Khartoum, Khartoum, Sudan, Endan, J. B., Department of Process and Food Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Yosuf, Y. A., Department of Process and Food Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Shamsudin, R., Department of Process and Food Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Ahmedov, A., & Department of Process and Food Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia. (2015). High Pressure Processing Technology and Equipment Evolution: A Review. *Journal of Engineering Science and Technology Review*, 8(5), 75–83. <https://doi.org/10.25103/jestr.085.11>
- Evrendilek, G. A. (2023). Non-Thermal Processing Operations. In *Principles of high pressure processing and its equipment* (pp. 301–318). Woodhead Publishing.
- FAOSTAT. (2020a). *Cherries, sour* / UNdata. <https://data.un.org/Data.aspx?d=FAO&f=itemCode%3A530>
- FAOSTAT. (2020b). *Grapes* / UNdata. <https://data.un.org/Data.aspx?d=FAO&f=itemCode%3A560>
- FAOSTAT. (2020c). *Strawberries* / UNdata. <https://data.un.org/Data.aspx?d=FAO&f=itemCode%3A544>
- Fatica, M. K., & Schneider, K. R. (2011). Salmonella and produce: Survival in the plant environment and implications in food safety. *Virulence*, 2(6), 573–579. <https://doi.org/10.4161/viru.2.6.17880>
- Ferreira, S. S., Alves, A. J., Filipe-Ribeiro, L., Cosme, F., & Nunes, F. M. (2018). Holistic and Sustainable Approach for Recycling and Valorization of Polyvinylpyrrolidone Used in Wine Fining. *ACS Sustainable Chemistry & Engineering*, 6(11), 14599–14606. <https://doi.org/10.1021/acssuschemeng.8b03208>
- Fornasaro, S., Ziberna, L., Gasperotti, M., Tramer, F., Vrhovšek, U., Mattivi, F., & Passamonti, S. (2016). Determination of cyanidin 3-glucoside in rat brain, liver and kidneys by UPLC/MS-MS and its application to a short-term pharmacokinetic study. *Scientific Reports*, 6(1), 22815. <https://doi.org/10.1038/srep22815>
- Fraldi, M., Cutolo, A., Esposito, L., Perrella, G., Pastore Carbone, M. G., Sansone, L., Scherillo, G., & Mensitieri, G. (2014). Delamination onset and design criteria of multilayer flexible packaging under high pressure treatments. *Innovative Food Science & Emerging Technologies*, 23, 39–53. <https://doi.org/10.1016/j.ifset.2014.02.016>
- Freitas, A. A. D., Francelin, M. F., Hirata, G. F., Clemente, E., & Schmidt, F. L. (2008). Atividades das enzimas peroxidase (POD) e polifenoxidase (PPO) nas uvas das cultivares benitaka e rubi e em seus sucos e geléias. *Ciência e Tecnologia de Alimentos*, 28(1), 172–177. <https://doi.org/10.1590/S0101-20612008000100025>
- Funamoto, Y., Yamauchi, N., Shigenaga, T., & Shigyo, M. (2002). Effects of heat treatment on chlorophyll degrading enzymes in stored broccoli (*Brassica oleracea* L.). *Postharvest Biology and Technology*, 24(2), 163–170. [https://doi.org/10.1016/S0925-5214\(01\)00135-1](https://doi.org/10.1016/S0925-5214(01)00135-1)
- Galotto, M. J., Ulloa, P. A., Hernández, D., Fernández-Martín, F., Gavara, R., & Guarda, A. (2008). Mechanical and thermal behaviour of flexible food packaging polymeric films materials under high pressure/temperature treatments. *Packaging Technology and Science*, 21(5), 297–308. <https://doi.org/10.1002/pts.807>

- Ganhão, R., Pinheiro, J., Tino, C., Faria, H., & Gil, M. M. (2019). Characterization of Nutritional, Physicochemical, and Phytochemical Composition and Antioxidant Capacity of Three Strawberry “*Fragaria × ananassa* Duch.” Cultivars (“Primoris”, “Endurance”, and “Portola”) from Western Region of Portugal. *Foods*, 8(12), 682. <https://doi.org/10.3390/foods8120682>
- Garcia-Palazon, A., Suthanthangjai, W., Kajda, P., & Zabetakis, I. (2004). The effects of high hydrostatic pressure on β -glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria × ananassa*). *Food Chemistry*, 88(1), 7–10. <https://doi.org/10.1016/j.foodchem.2004.01.019>
- Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., & Battino, M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, 28(1), 9–19.
- Giovane, A., Servillo, L., Balestrieri, C., Raiola, A., D’Avino, R., Tamburrini, M., Ciardiello, M. A., & Camardella, L. (2004). Pectin methylesterase inhibitor. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1696(2), 245–252. <https://doi.org/10.1016/j.bbapap.2003.08.011>
- González-Angulo, M., Serment-Moreno, V., Clemente-García, L., Tonello, C., Jaime, I., & Rovira, J. (2021). Assessing the pressure resistance of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* to high pressure processing (HPP) in citric acid model solutions for process validation. *Food Research International*, 140, 110091. <https://doi.org/10.1016/j.foodres.2020.110091>
- González-Domínguez, R., Sayago, A., Akhatou, I., & Fernández-Recamales, Á. (2020). Multi-Chemical Profiling of Strawberry as a Traceability Tool to Investigate the Effect of Cultivar and Cultivation Conditions. *Foods*, 9(1), 96. <https://doi.org/10.3390/foods9010096>
- Gössinger, M., Moritz, S., Hermes, M., Wendelin, S., Scherbichler, H., Halbwirth, H., Stich, K., & Berghofer, E. (2009). Effects of processing parameters on colour stability of strawberry nectar from puree. *Journal of Food Engineering*, 90(2), 171–178. <https://doi.org/10.1016/j.jfoodeng.2008.06.018>
- Gouvea, F. S., Koutchma, T., Ferreira, E. H. R., Walter, E. H. M., & Rosenthal, A. (2023). Resistance of *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* in high and low-acidity juices processed by high hydrostatic pressure. *International Journal of Food Microbiology*, 395, 110189. <https://doi.org/10.1016/j.ijfoodmicro.2023.110189>
- Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage—Interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78(1–2), 79–97.
- Granato, D., Magalhães Carrapeiro, M. D., Fogliano, V., & Van Ruth, S. M. (2016). Effects of geographical origin, varietal and farming system on the chemical composition and functional properties of purple grape juices: A review. *Trends in Food Science & Technology*, 52, 31–48. <https://doi.org/10.1016/j.tifs.2016.03.013>
- Guevara, J. C., Yahia, E. M., Brito De La Fuente, E., & Biserka, S. P. (2003). Effects of elevated concentrations of CO₂ in modified atmosphere packaging on the quality of prickly pear cactus stems (*Opuntia* spp.). *Postharvest Biology and Technology*, 29(2), 167–176. [https://doi.org/10.1016/S0925-5214\(03\)00021-8](https://doi.org/10.1016/S0925-5214(03)00021-8)
- Hatti-Kaul, R., & Mattiasson, B. (2003). *Isolation and purification of proteins*. CRC Press.
- Heaton, J. W., Yada, R. Y., & Marangoni, A. G. (1996). Discoloration of Coleslaw Is Caused by Chlorophyll Degradation. *Journal of Agricultural and Food Chemistry*, 44(2), 395–398. <https://doi.org/10.1021/jf9504475>

- Heremans, K. (1993). The behaviour of proteins under pressure. *High Pressure Chemistry, Biochemistry and Materials Science*, 443–469.
- Hiperbaric. (n.d.-a). High Pressure Processing (HPP) Applications. *Hiperbaric*. Retrieved November 16, 2023, from <https://www.hiperbaric.com/en/hpp-technology/hpp-applications/>
- Hiperbaric. (n.d.-b). HPP Packaging. *Hiperbaric*. Retrieved November 15, 2023, from <https://www.hiperbaric.com/en/hpp-technology/hpp-applications/hpp-packaging/>
- Hiperbaric. (2018, September 19). HPP, an alternative to Conventional Food Processing. *Hiperbaric*. <https://www.hiperbaric.com/en/alternatives-to-conventional-food-processing-hpp/>
- Hiperbaric. (2021, July 30). HPP Technology: Global Regulatory Compliance. *Hiperbaric*. <https://www.hiperbaric.com/en/hpp-technology-global-regulatory-compliance/>
- Holzwarth, M., Korhummel, S., Carle, R., & Kammerer, D. R. (2012). Evaluation of the effects of different freezing and thawing methods on color, polyphenol and ascorbic acid retention in strawberries (*Fragaria x ananassa* Duch.). *Food Research International*, 48(1), 241–248. <https://doi.org/10.1016/j.foodres.2012.04.004>
- Holzwarth, M., Wittig, J., Carle, R., & Kammerer, D. R. (2013). Influence of putative polyphenoloxidase (PPO) inhibitors on strawberry (*Fragaria x ananassa* Duch.) PPO, anthocyanin and color stability of stored purées. *LWT - Food Science and Technology*, 52(2), 116–122. <https://doi.org/10.1016/j.lwt.2012.10.025>
- Homoki, J. R., Nemes, A., Fazekas, E., Gyémánt, G., Balogh, P., Gál, F., Al-Asri, J., Mortier, J., Wolber, G., Babinszky, L., & Remenyik, J. (2016). Anthocyanin composition, antioxidant efficiency, and α -amylase inhibitor activity of different Hungarian sour cherry varieties (*Prunus cerasus* L.). *Food Chemistry*, 194, 222–229. <https://doi.org/10.1016/j.foodchem.2015.07.130>
- HPP Advisors. (2019, February 18). *Learning About HPP Equipment*. Aftermarket Parts for the HPP Food Industry. <https://hppadvisors.com/2019/02/18/learning-about-hpp/>
- Hsu, H., Sheen, S., Sites, J., Huang, L., & Wu, J. S.-B. (2014). Effect of high pressure treatment on the survival of Shiga toxin-producing *Escherichia coli* in strawberry puree. *Food Microbiology*, 40, 25–30. <https://doi.org/10.1016/j.fm.2013.11.019>
- Huang, H.-W., Wu, S.-J., Lu, J.-K., Shyu, Y.-T., & Wang, C.-Y. (2017). Current status and future trends of high-pressure processing in food industry. *Food Control*, 72, 1–8. <https://doi.org/10.1016/j.foodcont.2016.07.019>
- Huang, Y., Ye, M., & Chen, H. (2013). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. In strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. *International Journal of Food Microbiology*, 160(3), 337–343. <https://doi.org/10.1016/j.ijfoodmicro.2012.11.008>
- Hurtado, A., Guàrdia, M. D., Picouet, P., Jofré, A., Ros, J. M., & Bañón, S. (2017). Stabilization of red fruit-based smoothies by high-pressure processing. Part A. Effects on microbial growth, enzyme activity, antioxidant capacity and physical stability. *Journal of the Science of Food and Agriculture*, 97(3), 770–776. <https://doi.org/10.1002/jsfa.7796>
- Indrawati, Van Loey, A., Fachin, D., Nguyen, B., Verlent, I., & Hendrickx, M. (2002). Overview: Effect of High Pressure on Enzymes Related to Food Quality - Kinetics as a Basis for Process Engineering. *High Pressure Research*, 22(3–4), 613–618. <https://doi.org/10.1080/08957950212420>

- Jewell, G. G., Rantsios, A., & Scholey, J. (1973). Factors influencing the breakdown of fruit in strawberry jam. *Journal of Texture Studies*, 4(3), 363–370.
- Jiang, Y. (2000). Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. *Journal of the Science of Food and Agriculture*, 80(3), 305–310. [https://doi.org/10.1002/1097-0010\(200002\)80:3<305::AID-JSFA518>3.0.CO;2-H](https://doi.org/10.1002/1097-0010(200002)80:3<305::AID-JSFA518>3.0.CO;2-H)
- Jukanti, A. (2017). *Polyphenol oxidases (PPOs) in plants*. Springer.
- Kersh, D. M. E., Hammad, G., Donia, M. S., & Farag, M. A. (2023). A Comprehensive Review on Grape Juice Beverage in Context to Its Processing and Composition with Future Perspectives to Maximize Its Value. *Food and Bioprocess Technology*, 16(1), 1–23. <https://doi.org/10.1007/s11947-022-02858-5>
- Khoo, G. M., Clausen, M. R., Pedersen, B. H., & Larsen, E. (2011). Bioactivity and total phenolic content of 34 sour cherry cultivars. *Journal of Food Composition and Analysis*, 24(6), 772–776. <https://doi.org/10.1016/j.jfca.2011.03.004>
- Kim, H. J., Koo, M., Jeong, A.-R., Baek, S.-Y., Cho, J.-I., Lee, S.-H., & Hwang, I.-G. (2014). Occurrence of pathogenic *Escherichia coli* in commercially available fresh vegetable products in Korea. *Journal of the Korean Society for Applied Biological Chemistry*, 57(3), 367–370. <https://doi.org/10.1007/s13765-014-4073-5>
- Koseki, S., & Yamamoto, K. (2007). Water activity of bacterial suspension media unable to account for the baroprotective effect of solute concentration on the inactivation of *Listeria monocytogenes* by high hydrostatic pressure. *International Journal of Food Microbiology*, 115(1), 43–47. <https://doi.org/10.1016/j.ijfoodmicro.2006.10.005>
- Lee, E., Cho, J., Kim, J., & Lee, S. (2007). Green pigment in crushed garlic (*Allium sativum* L.) cloves: Purification and partial characterization. *Food Chemistry*, 101(4), 1677–1686. <https://doi.org/10.1016/j.foodchem.2006.04.028>
- Li, Y., & Padilla-Zakour, O. I. (2021). High pressure processing vs. Thermal pasteurization of whole Concord grape puree: Effect on nutritional value, quality parameters and refrigerated shelf life. *Foods*, 10(11), 2608.
- López-Rubio, A., Lagarón, J. M., Hernández-Muñoz, P., Almenar, E., Catalá, R., Gavara, R., & Pascall, M. A. (2005). Effect of high pressure treatments on the properties of EVOH-based food packaging materials. *Innovative Food Science & Emerging Technologies*, 6(1), 51–58. <https://doi.org/10.1016/j.ifset.2004.09.002>
- López-Serrano, M., & Ros Barceló, A. (2002). Comparative Study of the Products of the Peroxidase-Catalyzed and the Polyphenoloxidase-Catalyzed (+)-Catechin Oxidation. Their Possible Implications in Strawberry (*Fragaria × ananassa*) Browning Reactions. *Journal of Agricultural and Food Chemistry*, 50(5), 1218–1224. <https://doi.org/10.1021/jf010902z>
- Lovino, R., Baiano, A., Pati, S., Faccia, M., & Gambacorta, G. (2006). Phenolic composition of red grapes grown in southern Italy. *Italian Journal of Food Science*, 18(2).
- Ludikhuyze, L. R., Indrawati, Van Den Broeck, I., Weemaes, C. A., & Hendrickx, M. E. (1998). High Pressure and Thermal Denaturation Kinetics of Soybean Lipoxygenase: A Study based on Gel Electrophoresis. *LWT - Food Science and Technology*, 31(7–8), 680–686. <https://doi.org/10.1006/fstl.1998.0424>
- Ludikhuyze, L., Van Loey, A., Indrawati, Smout, C., & Hendrickx, M. (2003). Effects of Combined Pressure and Temperature on Enzymes Related to Quality of Fruits and Vegetables: From Kinetic

- Information to Process Engineering Aspects. *Critical Reviews in Food Science and Nutrition*, 43(5), 527–586. <https://doi.org/10.1080/10408690390246350>
- Luna-Guevara, J. J., Arenas-Hernandez, M. M. P., Martínez De La Peña, C., Silva, J. L., & Luna-Guevara, M. L. (2019). The Role of Pathogenic *E. coli* in Fresh Vegetables: Behavior, Contamination Factors, and Preventive Measures. *International Journal of Microbiology*, 2019, 1–10. <https://doi.org/10.1155/2019/2894328>
- Mandave, P. C., Pawar, P. K., Ranjekar, P. K., Mantri, N., & Kuvalekar, A. A. (2014). Comprehensive evaluation of in vitro antioxidant activity, total phenols and chemical profiles of two commercially important strawberry varieties. *Scientia Horticulturae*, 172, 124–134. <https://doi.org/10.1016/j.scienta.2014.03.002>
- Manmohit Kalia, P. K. (2015). Pectin Methylsterases: A Review. *Journal of Bioprocessing & Biotechniques*, 05(05). <https://doi.org/10.4172/2155-9821.1000227>
- Marszałek, K., Mitek, M., & Skąpska, S. (2015). The effect of thermal pasteurization and high pressure processing at cold and mild temperatures on the chemical composition, microbial and enzyme activity in strawberry purée. *Innovative Food Science & Emerging Technologies*, 27, 48–56. <https://doi.org/10.1016/j.ifset.2014.10.009>
- Martinez-Montegudo, S., & Aranda Saldaña, M. D. (2014). Chemical Reactions in Food Systems at High Hydrostatic Pressure. *Food Engineering Reviews*, 6. <https://doi.org/10.1007/s12393-014-9087-6>
- Martínez-Montegudo, S. I., & Balasubramaniam, V. M. (2016). Fundamentals and Applications of High-Pressure Processing Technology. In V. M. Balasubramaniam, G. V. Barbosa-Cánovas, & H. L. M. Lelieveld (Eds.), *High Pressure Processing of Food* (pp. 3–17). Springer New York. https://doi.org/10.1007/978-1-4939-3234-4_1
- Mensitieri, G., Scherillo, G., & Iannace, S. (2013). Flexible packaging structures for high pressure treatments. *Innovative Food Science & Emerging Technologies*, 17, 12–21. <https://doi.org/10.1016/j.ifset.2012.10.007>
- Miele, A., Rizzon, L. A., Queiroz, S. C. D. N. D., & Gianello, C. (2015). Physicochemical composition, minerals, and pesticide residues in organic grape juices. *Food Science and Technology (Campinas)*, 35(1), 120–126. <https://doi.org/10.1590/1678-457X.6540>
- Minitab. (n.d.). *Coded coefficients table for Analyze Variability* [Mtbtopic]. Retrieved December 2, 2023, from <https://support.minitab.com/en-us/minitab/21/help-and-how-to/statistical-modeling/doe/how-to/factorial/analyze-variability/interpret-the-results/all-statistics-and-graphs/coded-coefficients-table/#coef>
- Molina-Gutierrez, A., Rademacher, B., Gänzle, M. G., & Vogel, R. F. (2002). Effect of sucrose and sodium chloride on the survival and metabolic activity of *Lactococcus lactis* under high-pressure conditions. *Progress in Biotechnology*, 19, 295–302. [https://doi.org/10.1016/S0921-0423\(02\)80115-0](https://doi.org/10.1016/S0921-0423(02)80115-0)
- Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2015). Grape Processing by High Hydrostatic Pressure: Effect on Microbial Populations, Phenol Extraction and Wine Quality. *Food and Bioprocess Technology*, 8(2), 277–286. <https://doi.org/10.1007/s11947-014-1405-8>
- Mozhaev, V. V., Heremans, K., Frank, J., Masson, P., & Balny, C. (1994). Exploiting the effects of high hydrostatic pressure in biotechnological applications. *Trends in Biotechnology*, 12(12), 493–501.

- Mújica-Paz, H., Valdez-Fragoso, A., Samson, C. T., Welti-Chanes, J., & Torres, J. A. (2011). High-Pressure Processing Technologies for the Pasteurization and Sterilization of Foods. *Food and Bioprocess Technology*, 4(6), 969–985. <https://doi.org/10.1007/s11947-011-0543-5>
- Németh, Á. S., Márczy, J. S., Samu, Z., Hágger-Veress, Á., & Szajáni, B. (2004). Biocatalytic production of 2(E)-hexenal from hydrolysed linseed oil. *Enzyme and Microbial Technology*, 34(7), 667–672. <https://doi.org/10.1016/j.enzmictec.2004.03.003>
- Ng, A., Harvey, A. J., Parker, M. L., Smith, A. C., & Waldron, K. W. (1998). Effect of Oxidative Coupling on the Thermal Stability of Texture and Cell Wall Chemistry of Beet Root (*Beta vulgaris*). *Journal of Agricultural and Food Chemistry*, 46(8), 3365–3370. <https://doi.org/10.1021/jf9711101>
- Noma, S., Shimoda, M., & Hayakawa, I. (2002). Inactivation of Vegetative Bacteria by Rapid Decompression Treatment. *Journal of Food Science*, 67(9), 3408–3411. <https://doi.org/10.1111/j.1365-2621.2002.tb09598.x>
- Ogawa, H., Fukuhisa, K., Kubo, Y., & Fukumoto, H. (1990). Pressure Inactivation of Yeasts, Molds, and Pectinesterase in Satsuma Mandarin Juice: Effects of Juice Concentration, pH, and Organic Acids, and Comparison with Heat Sanitation. *Agricultural and Biological Chemistry*, 54(5), 1219–1225. <https://doi.org/10.1080/00021369.1990.10870118>
- Ogihara, H., Yatuzuka, M., Horie, N., Furukawa, S., & Yamasaki, M. (2009). Synergistic effect of high hydrostatic pressure treatment and food additives on the inactivation of Salmonella enteritidis. *Food Control*, 20(11), 963–966. <https://doi.org/10.1016/j.foodcont.2008.11.004>
- Ooi, S. T., & Lorber, B. (2005). Gastroenteritis due to *Listeria monocytogenes*. *Clinical Infectious Diseases*, 40(9), 1327–1332.
- Orak, H. H. (2007). Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. *Scientia Horticulturae*, 111(3), 235–241. <https://doi.org/10.1016/j.scienta.2006.10.019>
- Ornelas-Paz, J. D. J., Yahia, E. M., Ramírez-Bustamante, N., Pérez-Martínez, J. D., Escalante-Minakata, M. D. P., Ibarra-Junquera, V., Acosta-Muñiz, C., Guerrero-Prieto, V., & Ochoa-Reyes, E. (2013). Physical attributes and chemical composition of organic strawberry fruit (*Fragaria x ananassa* Duch, Cv. Albion) at six stages of ripening. *Food Chemistry*, 138(1), 372–381. <https://doi.org/10.1016/j.foodchem.2012.11.006>
- Orruño, E., Apenten, R. O., & Zabetakis, I. (2001). The role of β -glucosidase in the biosynthesis of 2, 5-dimethyl-4-hydroxy-3 (2H)-furanone in strawberry (*Fragaria* × *ananassa* cv. Elsanta). *Flavour and Fragrance Journal*, 16(2), 81–84.
- Osek, J., Lachtara, B., & Wiczorek, K. (2022). *Listeria monocytogenes* – How This Pathogen Survives in Food-Production Environments? *Frontiers in Microbiology*, 13, 866462. <https://doi.org/10.3389/fmicb.2022.866462>
- Pasquariello, M. S., Di Patre, D., Mastrobuoni, F., Zampella, L., Scortichini, M., & Petriccione, M. (2015). Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit. *Postharvest Biology and Technology*, 109, 45–56. <https://doi.org/10.1016/j.postharvbio.2015.06.007>
- Pedisić, S., Levaj, B., Dragović-Uzelac, V., & Kos, K. (2007). Physicochemical composition, phenolic content and antioxidant activity of sour cherry cv. Marasca during ripening. *Agriculturae Conspectus Scientificus*, 72(4), 295–300.

- Peerun, S. (2015). The Food High Pressure Processing (HPP) Technologies Market Forecast 2015–2025: Pascalization & Bridgmanization. *Visiongain Ltd.: London, UK*.
- Petrus, R., Churey, J., & Worobo, R. (2020). Searching for high pressure processing parameters for *Escherichia coli* O157: H7, *Salmonella enterica* and *Listeria monocytogenes* reduction in Concord grape juice. *British Food Journal*, *122*(1), 170–180.
- Picouet, P. A., Hurtado, A., Jofré, A., Bañon, S., Ros, J.-M., & Guàrdia, M. D. (2016). Effects of Thermal and High-pressure Treatments on the Microbiological, Nutritional and Sensory Quality of a Multi-fruit Smoothie. *Food and Bioprocess Technology*, *9*(7), 1219–1232. <https://doi.org/10.1007/s11947-016-1705-2>
- Pineli, L. D. L. D. O., Moretti, C. L., Dos Santos, M. S., Campos, A. B., Brasileiro, A. V., Córdova, A. C., & Chiarello, M. D. (2011). Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *Journal of Food Composition and Analysis*, *24*(1), 11–16. <https://doi.org/10.1016/j.jfca.2010.05.004>
- Podolak, R., Whitman, D., & Black, D. G. (2020). Factors Affecting Microbial Inactivation during High Pressure Processing in Juices and Beverages: A Review. *Journal of Food Protection*, *83*(9), 1561–1575. <https://doi.org/10.4315/JFP-20-096>
- Queirós, R. P., Rainho, D., Santos, M. D., Fidalgo, L. G., Delgadillo, I., & Saraiva, J. A. (2015). High pressure and thermal pasteurization effects on sweet cherry juice microbiological stability and physicochemical properties. *High Pressure Research*, *35*(1), 69–77.
- Rademacher, B., Werner, F., & Pehl, M. (2002). Effect of the pressurizing ramp on the inactivation of *Listeria innocua* considering thermofluidodynamical processes. *Innovative Food Science & Emerging Technologies*, *3*(1), 19–24. [https://doi.org/10.1016/S1466-8564\(02\)00002-4](https://doi.org/10.1016/S1466-8564(02)00002-4)
- Rakonjac, V., Akšić, M. F., Nikolić, D., Milatović, D., & Čolić, S. (2010). Morphological characterization of ‘Oblačinska’ sour cherry by multivariate analysis. *Scientia Horticulturae*, *125*(4), 679–684. <https://doi.org/10.1016/j.scienta.2010.05.029>
- Rapeanu, G., Loey, A. V., Smout, C., & Hendrickx, M. (2006). Biochemical characterization and process stability of polyphenoloxidase extracted from Victoria grape (*Vitis vinifera* ssp. *Sativa*). *Food Chemistry*, *94*(2), 253–261. <https://doi.org/10.1016/j.foodchem.2004.10.058>
- Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research*, *5*(4), 47–56.
- Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Science and Technology*, *44*(5), 1251–1260. <https://doi.org/10.1016/j.lwt.2010.11.001>
- Rocha, A. M. C. N., Cano, M. P., Galeazzi, M. A. M., & Morais, A. M. M. B. (1998). Characterisation of ‘Starking’ apple polyphenoloxidase. *Journal of the Science of Food and Agriculture*, *77*(4), 527–534. [https://doi.org/10.1002/\(SICI\)1097-0010\(199808\)77:4<527::AID-JSFA76>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0010(199808)77:4<527::AID-JSFA76>3.0.CO;2-E)
- Rodrigo, D., Jolie, R., Van Loey, A., & Hendrickx, M. (2006). Combined thermal and high pressure inactivation kinetics of tomato lipoxygenase. *European Food Research and Technology*, *222*(5–6), 636–642. <https://doi.org/10.1007/s00217-005-0128-5>
- Rodrigo, D., Van Loey, A., & Hendrickx, M. (2007). Combined thermal and high pressure colour degradation of tomato puree and strawberry juice. *Journal of Food Engineering*, *79*(2), 553–560. <https://doi.org/10.1016/j.jfoodeng.2006.02.015>

- Rosenberg, I. M. (2013). *Protein analysis and purification: Benchtop techniques*. Springer Science & Business Media.
- Sabarre Jr, D. C., & Yagonia-Lobarbio, C. F. (2021). Extraction and Characterization of Polyphenol Oxidase from Plant Materials: A Review. *Journal of Applied Biotechnology Reports*, 8(2). <https://doi.org/10.30491/jabr.2021.255549.1308>
- Samson, R. A., Houbraeken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2019). *Food and indoor fungi*. Westerdijk Fungal Biodiversity Institute.
- Santiago-Gómez, M. P., Vergely, C., Policar, C., Nicaud, J.-M., Belin, J.-M., Rochette, L., & Husson, F. (2007). Characterization of purified green bell pepper hydroperoxide lyase expressed by *Yarrowia lipolytica*: Radicals detection during catalysis. *Enzyme and Microbial Technology*, 41(1–2), 13–18. <https://doi.org/10.1016/j.enzmitec.2006.11.017>
- Sanz, E., Vega, C., Abascal, J. L. F., & MacDowell, L. G. (2004). Phase Diagram of Water from Computer Simulation. *Physical Review Letters*, 92(25), 255701. <https://doi.org/10.1103/PhysRevLett.92.255701>
- Schlech, W. F. (2019). Epidemiology and clinical manifestations of *Listeria monocytogenes* infection. *Microbiology Spectrum*, 7(3), 7.3. 3.
- Serment-Moreno, V. (2021). Microbial Modeling Needs for the Nonthermal Processing of Foods. *Food Engineering Reviews*, 13(3), 465–489. <https://doi.org/10.1007/s12393-020-09263-8>
- Shah, S., Borah, D., & Yadav, R. N. S. (2014). Extraction and characterization of peroxidase from *Camellia sinensis*. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 84(2), 343–348. <https://doi.org/10.1007/s40011-013-0211-9>
- Siggaard-Andersen, O. (1966). Titratable acid or base of body fluids. *Annals of the New York Academy of Sciences*, 133(1), 41–58. <https://doi.org/10.1111/j.1749-6632.1966.tb50707.x>
- Silva, G. G., Dutra, M. D. C. P., De Oliveira, J. B., Rybka, A. C. P., Pereira, G. E., & Dos Santos Lima, M. (2019). Processing methods with heat increases bioactive phenolic compounds and antioxidant activity in grape juices. *Journal of Food Biochemistry*, 43(3), e12732. <https://doi.org/10.1111/jfbc.12732>
- Silva, J. (1993). Pressure Stability of Proteins. *Annual Review of Physical Chemistry*, 44, 89–113. <https://doi.org/10.1146/annurev.physchem.44.1.89>
- Silva, L. R., & Queiroz, M. (2016). Bioactive compounds of red grapes from Dão region (Portugal): Evaluation of phenolic and organic profile. *Asian Pacific Journal of Tropical Biomedicine*, 6(4), 315–321.
- Simpson, R. K., & Gilmour, A. (1997). The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. *Journal of Applied Microbiology*, 83(2), 181–188. <https://doi.org/10.1046/j.1365-2672.1997.00215.x>
- Sokół-Łętowska, A., Kucharska, A. Z., Hodun, G., & Gołba, M. (2020). Chemical Composition of 21 Cultivars of Sour Cherry (*Prunus cerasus*) Fruit Cultivated in Poland. *Molecules*, 25(19), 4587. <https://doi.org/10.3390/molecules25194587>
- Sokół-Łętowska, A., Kucharska, A. Z., Szumny, A., Wińska, K., & Nawirska-Olszańska, A. (2018). Phenolic Composition Stability and Antioxidant Activity of Sour Cherry Liqueurs. *Molecules*, 23(9), 2156. <https://doi.org/10.3390/molecules23092156>

- Stolle-Smits, T., Beekhuizen, J. G., Recourt, K., Voragen, A. G. J., & Van Dijk, C. (2000). Preheating Effects on the Textural Strength of Canned Green Beans. 1. Cell Wall Chemistry. *Journal of Agricultural and Food Chemistry*, 48(11), 5269–5277. <https://doi.org/10.1021/jf990941z>
- Syed, Q. A., Buffa, M., Guamis, B., & Saldo, J. (2013). Lethality and injuring the effect of compression and decompression rates of high hydrostatic pressure on *Escherichia coli* O157:H7 in different matrices. *High Pressure Research*, 33(1), 64–72. <https://doi.org/10.1080/08957959.2013.767898>
- Talcott, S. T., Brenes, C. H., Pires, D. M., & Del Pozo-Insfran, D. (2003). Phytochemical Stability and Color Retention of Copigmented and Processed Muscadine Grape Juice. *Journal of Agricultural and Food Chemistry*, 51(4), 957–963. <https://doi.org/10.1021/jf0209746>
- Tamber, S. (2018). Population-Wide Survey of Salmonella enterica Response to High-Pressure Processing Reveals a Diversity of Responses and Tolerance Mechanisms. *Applied and Environmental Microbiology*, 84(2), e01673-17. <https://doi.org/10.1128/AEM.01673-17>
- Tauxe, R. V. (2002). Emerging foodborne pathogens. *International Journal of Food Microbiology*, 78(1–2), 31–41. [https://doi.org/10.1016/S0168-1605\(02\)00232-5](https://doi.org/10.1016/S0168-1605(02)00232-5)
- Tenuta, M. C., Artoni, E., Fava, P., Bignami, C., & Licciardello, F. (2023). Shelf Life Extension and Nutritional Quality Preservation of Sour Cherries through High Pressure Processing. *Foods*, 12(2), 342. <https://doi.org/10.3390/foods12020342>
- Terefe, N. S., Buckow, R., & Versteeg, C. (2014). Quality-Related Enzymes in Fruit and Vegetable Products: Effects of Novel Food Processing Technologies, Part 1: High-Pressure Processing. *Critical Reviews in Food Science and Nutrition*, 54(1), 24–63. <https://doi.org/10.1080/10408398.2011.566946>
- Terefe, N. S., Kleintschek, T., Gamage, T., Fanning, K. J., Netzel, G., Versteeg, C., & Netzel, M. (2013). Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different strawberry cultivars. *Innovative Food Science & Emerging Technologies*, 19, 57–65. <https://doi.org/10.1016/j.ifset.2013.05.003>
- Terefe, N. S., Yang, Y. H., Knoerzer, K., Buckow, R., & Versteeg, C. (2010). High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Science & Emerging Technologies*, 11(1), 52–60. <https://doi.org/10.1016/j.ifset.2009.08.009>
- Teribia, N., Buvé, C., Bonerz, D., Aschoff, J., Goos, P., Hendrickx, M., & Van Loey, A. (2021). The effect of thermal processing and storage on the color stability of strawberry puree originating from different cultivars. *LWT*, 145, 111270. <https://doi.org/10.1016/j.lwt.2021.111270>
- Teribia, N., Buvé, C., Bonerz, D., Aschoff, J., Hendrickx, M., & Loey, A. V. (2021). Impact of processing and storage conditions on color stability of strawberry puree: The role of PPO reactions revisited. *Journal of Food Engineering*, 294, 110402. <https://doi.org/10.1016/j.jfoodeng.2020.110402>
- Toaldo, I. M., Cruz, F. A., Alves, T. D. L., De Gois, J. S., Borges, D. L. G., Cunha, H. P., Da Silva, E. L., & Bordignon-Luiz, M. T. (2015). Bioactive potential of *Vitis labrusca* L. grape juices from the Southern Region of Brazil: Phenolic and elemental composition and effect on lipid peroxidation in healthy subjects. *Food Chemistry*, 173, 527–535. <https://doi.org/10.1016/j.foodchem.2014.09.171>
- Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, 81(9), 853–876. <https://doi.org/10.1002/jsfa.885>

- Troiani, E. D. P., Tropiani, C. T., & Clemente, E. (2003). Peroxidase (POD) and polyphenoloxidase (PPO) in grape (*Vitis vinifera* L.). *Ciência e Agrotecnologia*, 27(3), 635–642. <https://doi.org/10.1590/S1413-70542003000300019>
- Usaga, J., Acosta, Ó., Churey, J. J., Padilla-Zakour, O. I., & Worobo, R. W. (2021). Evaluation of high pressure processing (HPP) inactivation of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in acid and acidified juices and beverages. *International Journal of Food Microbiology*, 339, 109034. <https://doi.org/10.1016/j.ijfoodmicro.2020.109034>
- USDA. (2018). *Strawberry, raw—FoodData Central*. <https://fdc.nal.usda.gov/fdc-app.html#/food-details/167762/nutrients>
- Van Buggenhout, S., Sila, D. N., Duvetter, T., Van Loey, A., & Hendrickx, M. (2009). Pectins in processed fruits and vegetables: Part III—Texture engineering. *Comprehensive Reviews in Food Science and Food Safety*, 8(2), 105–117.
- van Dijk, C., & Tijssens, L. M. M. (2000). Mathematical modelling of enzymatic reactions as related to the. *Discovering the Future: Modelling Quality Matters*, 125.
- Vega, C., Abascal, J. L. F., Sanz, E., MacDowell, L. G., & McBride, C. (2005). Can simple models describe the phase diagram of water? *Journal of Physics: Condensed Matter*, 17(45), S3283–S3288. <https://doi.org/10.1088/0953-8984/17/45/013>
- Waldron, K. W., Smith, A. C., Parr, A. J., Ng, A., & Parker, M. L. (1997). New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends in Food Science & Technology*, 8(7), 213–221. [https://doi.org/10.1016/S0924-2244\(97\)01052-2](https://doi.org/10.1016/S0924-2244(97)01052-2)
- Wang, J., Jiang, W., Wang, B., Liu, S., Gong, Z., & Luo, Y. (2007). Partial properties of polyphenol oxidase in mango (*Mangifera indica* L. cv. “Tainong”) pulp. *Journal of Food Biochemistry*, 31(1), 45–55. <https://doi.org/10.1111/j.1745-4514.2007.00097.x>
- Wang, S. Y., Galletta, G. J., & Maas, J. L. (1997). Chemical Characterization of 24 Strawberry Cultivars and Selections. *Journal of Small Fruit & Viticulture*, 5(1), 21–36. https://doi.org/10.1300/J065v05n01_02
- Weemaes, C. A., Ludikhuyze, L. R., Van Den Broeck, I., Hendrickx, M. E., & Tobback, P. P. (1998). Activity, Electrophoretic Characteristics and Heat Inactivation of Polyphenoloxidases from Apples, Avocados, Grapes, Pears and Plums. *LWT - Food Science and Technology*, 31(1), 44–49. <https://doi.org/10.1006/fstl.1997.0302>
- Weemaes, C., Ludikhuyze, L., Broeck, I., & Hendrickx, M. (1998). High Pressure Inactivation of Polyphenoloxidases. *Journal of Food Science*, 63(5), 873–877. <https://doi.org/10.1111/j.1365-2621.1998.tb17917.x>
- Whitaker, J. R. (2018). *Principles of enzymology for the food sciences*. Routledge.
- Wiktorczyk-Kapischke, N., Skowron, K., Wałęcka-Zacharska, E., Grudlewska-Buda, K., Wnuk, K., Buszko, K., & Gospodarek-Komkowska, E. (2023). Assessment of the influence of selected stress factors on the growth and survival of *Listeria monocytogenes*. *BMC Microbiology*, 23(1), 27. <https://doi.org/10.1186/s12866-023-02766-4>
- Wojdyło, A., Nowicka, P., Laskowski, P., & Oszmiański, J. (2014). Evaluation of Sour Cherry (*Prunus cerasus* L.) Fruits for Their Polyphenol Content, Antioxidant Properties, and Nutritional Components. *Journal of Agricultural and Food Chemistry*, 62(51), 12332–12345. <https://doi.org/10.1021/jf504023z>

- Wuytack, E. Y., Diels, A. M. J., & Michiels, C. W. (2002). Bacterial inactivation by high-pressure homogenisation and high hydrostatic pressure. *International Journal of Food Microbiology*, 77(3), 205–212. [https://doi.org/10.1016/S0168-1605\(02\)00054-5](https://doi.org/10.1016/S0168-1605(02)00054-5)
- Wuyts, N., De Waele, D., & Swennen, R. (2006). Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande naine) roots. *Plant Physiology and Biochemistry*, 44(5–6), 308–314. <https://doi.org/10.1016/j.plaphy.2006.06.005>
- Xing, Y., Ma, Q., Wang, K., Dong, X., Wang, S., He, P., Wang, J., & Xu, H. (2023). Non-thermal treatments of strawberry pulp: The relationship between quality attributes and microstructure. *Ultrasonics Sonochemistry*, 98, 106508. <https://doi.org/10.1016/j.ultsonch.2023.106508>
- Yada, R. Y., & Jackman, R. L. (2012). *Protein structure-function relationships in foods*. Springer Science & Business Media.
- Yamamoto, K. (2017). Food processing by high hydrostatic pressure. *Bioscience, Biotechnology, and Biochemistry*, 81(4), 672–679. <https://doi.org/10.1080/09168451.2017.1281723>
- Yildiz, S., Pokhrel, P. R., Unluturk, S., & Barbosa-Cánovas, G. V. (2019). Identification of equivalent processing conditions for pasteurization of strawberry juice by high pressure, ultrasound, and pulsed electric fields processing. *Innovative Food Science & Emerging Technologies*, 57, 102195. <https://doi.org/10.1016/j.ifset.2019.102195>
- Yildiz, S., Pokhrel, P. R., Unluturk, S., & Barbosa-Cánovas, G. V. (2021). Shelf life extension of strawberry juice by equivalent ultrasound, high pressure, and pulsed electric fields processes. *Food Research International*, 140, 110040. <https://doi.org/10.1016/j.foodres.2020.110040>
- Yılmaz, F. M., Görgüç, A., Karaaslan, M., Vardin, H., Ersus Bilek, S., Uygun, Ö., & Bircan, C. (2019). Sour Cherry By-products: Compositions, Functional Properties and Recovery Potentials – A Review. *Critical Reviews in Food Science and Nutrition*, 59(22), 3549–3563. <https://doi.org/10.1080/10408398.2018.1496901>
- Yoon, S.-H., & Robyt, J. F. (2005). Activation and stabilization of 10 starch-degrading enzymes by Triton X-100, polyethylene glycols, and polyvinyl alcohols. *Enzyme and Microbial Technology*, 37(5), 556–562. <https://doi.org/10.1016/j.enzmictec.2005.04.002>
- Yuan, B., Danao, M.-G. C., Stratton, J. E., Weier, S. A., Weller, C. L., & Lu, M. (2018). High pressure processing (HPP) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innovative Food Science & Emerging Technologies*, 47, 249–255. <https://doi.org/10.1016/j.ifset.2018.03.009>
- Zhang, Z., Pang, X., Xuwu, D., Ji, Z., & Jiang, Y. (2005). Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chemistry*, 90(1–2), 47–52. <https://doi.org/10.1016/j.foodchem.2004.03.023>
- Zhu, L., Zhang, Y., & Lu, J. (2012). Phenolic Contents and Compositions in Skins of Red Wine Grape Cultivars among Various Genetic Backgrounds and Originations. *International Journal of Molecular Sciences*, 13(3), 3492–3510. <https://doi.org/10.3390/ijms13033492>