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Beyond Juice Heating: A Comparative Study of Thermal and Non-Thermal Processing Impact on Safety, Quality and Nutrition Parameters in Fruit Juices and Nectars

Coordinatore:
Prof. Gianni Royer Carfagni

Tutore:
Prof. Sara Rainieri
Co-Tutore:
Prof. Luca Cattani

Dottorando: Darío J. Pavón Vargas

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"My brain is only a receiver, in the Universe there is a core from which we obtain knowledge, strength and inspiration. I have not penetrated into the secrets of this core, but I know that it exists."

Nikola Tesla

Abstract

This study examines the effectiveness of Thermal treatment (TT), High-Pressure Processing (HPP), Pulsed Electric Fields (PEF), and Ohmic heating (OH) treatments on the microbial stability and physicochemical properties of strawberry and sour cherry nectars and juices and raspberry juice. Given the significance of juice matrix factors such as pH and sugar, this research highlights how these matrix elements impact processing outcomes, particularly with thermal methods. The findings indicate that each technology affects juice quality in distinct ways, with the impact varying based on the juice matrix. The effectiveness of each technology is thus both technology- and matrix-dependent. While thermal treatments ensure microbial safety, they can reduce anthocyanin content, affecting color and antioxidant properties. Conversely, thermal methods are advantageous for achieving textural consistency and enzymatic stability, particularly when combined with optimization of pH and sugar levels to minimize nutrient loss. In contrast, HPP preserves quality parameters in the juices while also assuring microbial inactivation, but increases viscosity due to pressure-induced changes, whereas PEF can achieve effective microbial reduction with minimal color and nutrient loss, though it demands precise parameter optimization. OH, while effective in reducing microbial content, the process also causes heat damage to the nutrients in the juices. Conclusions demonstrate the potential for optimization of the processes based on each matrix's unique attributes. This research is important for the juice industry, as the study supports a shift towards non-thermal alternatives, promoting both safety and high-quality juices and nectars, to meet consumer demands for nutritious and minimally processed products.

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List of Publications and Conferences

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Hassan Zia, Helen Murray, Mikko Hofsommer, Andrés Moreno-Barreto, **Darío Pavón-Vargas**, Alema Puzovic, Astrid Gędas, Sebastian Rincon, Manfred Gössinger, Ana Slatnar. **Comparing the impact of conventional and non-conventional processing technologies on water-soluble vitamins and color in strawberry nectar – a pilot scale study**, Food Chemistry, Volume 463, Part 1, 2025, 141078. https://doi.org/10.1016/j.foodchem.2024.141078

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Ngoc Quynh Anh Truong, **Darío Pavón-Vargas**, Krystina Simkova, Manfred Gössinger, Luca Cattani. **Comparative Modelling of *E. coli* Inactivation in Sour Cherry and Raspberry Juices Under Pulsed Electric Field Treatments**. Microbiol. Res. 2024, 15, Firstpage–Lastpage. https://doi.org/10.3390/xxxxx

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Comparative Analysis of Anthocyanin Content and Stability in Strawberry Nectar Under Various Preservation Techniques. 11th International Workshop on Anthocyanins and Betalains, 17 – 19 September 2024, Leeds, United Kingdom. (Poster)

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List of Abbreviations

AA	Ascorbic Acid
BI	Browning Index
BS	Buffer Solution
DPPH	2,2-Diphenyl-1-picrylhydrazyl
HPP	High-Pressure Processing
HTLT	High Temperature Long Time
MTLT	Mild Temperature Long Time
MS	Model Solution
OH	Ohmic Heating
PCA	Principal Component Analysis
PEF	Pulsed Electric Fields
POD	Peroxidase
PPO	Polyphenol Oxidase
RSA	Radical Scavenging Activity
SN	Strawberry Nectar
SP	Strawberry Purée
TAC	Total Anthocyanin Content
TPC	Total Phenolic Compounds
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
TSS	Total Soluble Solids
TT	Thermal Treatment
UHT	Ultra-High Temperature

Nomenclature

T	Temperature	°C
t	Time	min
pH	Acidity level	—
cp	Specific Heat Capacity	J/kg·K
E	Electric Field Strength	kV/cm
E_C	Critical electric field strength	kV/cm
L_S	Shoulder length	kV/cm
D	Decimal reduction time	min
D_P	Pressure-based D-value	min
δ	Scale parameter in Weibull model	min
p	Shape parameter in Weibull model	—
τ	Shear stress	Pa
K	Consistency coefficient in rheology	$Pa \cdot s^n$
k	First-order rate constant	min^{-1}
$\dot{\gamma}$	Shear rate	s^{-1}
n	Flow behavior index in rheology	—
ΔE	Total color difference	—
C*	Chroma (color intensity)	—
h	Hue angle (color tone)	°
k_{max}	Rate constant for microbial reduction	$(kV/cm)^{-1}$
μ	Viscosity	Pa·s

1 Introduction

In recent years, the fruit juice and nectar market has seen a rapid growth, driven by consumer demand for health and fresh-like options. These beverages are known for their nutrient-rich profiles, contain essential vitamins, minerals, antioxidants, and other bioactive compounds. Juices and nectars are vulnerable to spoilage due to microbial contamination, enzymatic reactions, and oxidative processes. This susceptibility has encouraged the development and the application of different preservation techniques to ensure product safety, quality, and shelf life. Traditional thermal processing methods have been the industry standard for decades, however novel preservation technologies offer viable alternatives to conventional thermal treatments, effectively reducing microbial load while better preserving the nutritional characteristics of the products.

This study investigates thermal and non-thermal processing techniques, focusing on their impact on quality attributes such as color, viscosity, and antioxidant activity in strawberry, sour cherry, and raspberry juices and nectars.

Despite extensive research on fruit and nectar juice processing, comparative studies that simultaneously evaluate both conventional and non-conventional stabilization methods are limited, and few studies have focused into the effects of juice matrix composition on treatment outcomes. Most studies isolate one method or one product, or compare only two at a time, creating a gap in understanding how different techniques interact when applied to various juice matrices. Moreover, the little insight into how matrix factors, such as pH, sugar content, and nutrient composition, might influence both safety and quality retention outcomes during thermal treatments underscores the need for more matrix-focused research.

To bridge these gaps, this study addressed several objectives, starting with a comparative analysis of conventional and non-conventional stabilization methods on juices and nectars. Secondly, the study of the microbial safety and preservation of the nutritional qualities using ohmic heating, evaluating its potential as method compared to traditional thermal processes. Thirdly, the research on how matrix components, specifically pH level and sugar concentration, affect microbial inactivation and other psychochemical properties, providing valuable insight into how these factors might be optimized for safety and quality retention.

This research holds significant implications for the fruit juice industry, as it could guide processors in selecting more precise and effective preservation methods. By understanding the comparative advantages of the different technologies and the influence of matrix components, juice manufacturers can better meet consumer demands for high-quality, minimally processed products while ensuring microbial safety. The findings aim in refining processing parameters, particularly as non-conventional technologies become more accessible and better understood.

This study underscores the importance of selecting processing methods that compliments of each fruit juice matrix. For instance, the acidic nature of strawberry nectar may call for milder thermal treatments to avoid degradation of some components, while higher pressures in High-Pressure Processing may benefit sour cherry juice by enhancing phenolic content. Such matrix-dependent responses highlight the need for a custom-made approach to juice processing, where both product microbial safety and consumer appeal are maintained without excessive nutritional losses.

This study is part of a larger research initiative titled "Establishing a Strong and Lasting International Training Network for Innovation in Food and Juice Industries: A 4D-Research Approach for Fruit Juice Processing". This collaborative project, involving multiple partners, enhances our ability to systematically explore diverse processing techniques and matrices.

2 *Literature Review: Thermal and Non-thermal Processing of Fruit Juices and Nectars*

Fruit juices are commonly consumed worldwide as part of a balanced diet and daily intake, valued for their rich nutritional profile, including vitamins, minerals, and antioxidants [1]. Juices and nectars are differentiated by their fruit content, with juices typically containing 100% fruit content and nectars being a blend of fruit puree, water, and sugar or sweeteners and/or some additives [2]. The global market for fruit juices and nectars has a significant growth over the past decades, with a global rate growth of 3.8% projected for 2024 [3,4]. This increase in consumption is mainly driven by the consumer demand for natural, healthy, and convenient beverage options [5]. The rise in the interest in beverages that retain the nutritional qualities of fresh fruits, have accelerate the development of various processing techniques aimed to preserve the sensory and nutritional properties of these products [6]. Conventional thermal processing remains the most widely use technology for shelf-life extension and preservation of fruit juices and nectars [7]. However, non-thermal processing technologies have emerged as viable alternatives to thermal processing, offering the potential to maintain the freshness and nutritional quality of fruit juices while extending their shelf-life [6,8]. It must be noticed that, to produce safe, high-quality fruit juice that meets consumer expectations, processing must address elimination of pathogenic microorganisms, destruction of spoilage microorganisms responsible for juice deterioration, inactivation of enzymes that degrade juice quality in terms of color and texture, and alongside these goals, minimizing the loss of beneficial nutrients like phenolics, carotenoids, and vitamins [9]. As consumers seek juices that maintain a fresh-like appearance, color, and sensory characteristics, industries should balance shelf-life extension with the retention of bioactive compounds [10].

This literature review will provide an overview of the evolving fruit juice and nectar market, focusing on current novel processing technologies and their impact on microbial reduction and product quality, and the challenges the industry faces in maintaining the balance between microbial safety, shelf life, and nutritional value.

2.1 Introduction to Thermal and Non-thermal Processing

Fruit juices and nectars are highly perishable products prone to spoilage due to microbial contamination and enzymatic reactions [11]. To ensure product safety and extend shelf life, various preservation techniques have been employed, ranging from traditional thermal technologies to emerging non-thermal technologies [10,12,13]. Different technologies have shown to have meaningful differences in maintaining the quality, flavor, and nutritional value of juices and nectars, more over while maintain the quality of the product these technologies should effectively control microbial load and endogenous enzymes activities [14,15].

Thermal processing, such as pasteurization and sterilization, have been the primary method for preserving fruit juices and nectars, ensuring microbiological safety and extending shelf life [9,16]. Various emerging non-thermal technologies have been proposed as alternatives to conventional thermal treatments, aiming to overcome the limitations associated with temperature [11].

Among the innovative technologies in fruit processing, several novel thermal methods have emerged, including microwave heating [17], radio frequency heating [18], ohmic heating [19], and infrared radiation heating [20]. On the non-thermal side, pulsed electric fields (PEF) [21], high-pressure processing (HPP) [22], cold plasma [23], ultrasound [24], and pulsed light [25] are key examples of the state-of-the-art technologies. While each of these technologies offers significant advantages, such as reduced energy consumption or improved nutrient retention [6,26], none have been widely adopted across the juice industry due to specific limitations. For instance, while HPP has been successfully implemented by several companies for juices and smoothies, the high cost of equipment and maintenance remains a barrier for broader industry adoption [26]. Similarly, PEF shows great promise, but scalability and complexity of the system limit its widespread use in commercial juice processing [27].

Nevertheless, among the various non-thermal technologies, HPP and PEF are the most industrially applied and studied, due to HPP effectiveness in ensuring quality and safety [28], and PEF effectiveness to inactivate enzymes and microbes without applying excessive heat, thus preserving the quality of the product [29,30]. Both PEF and HHP are often considered viable alternatives to traditional thermal treatments, offering the advantage of better preserving thermolabile compounds such as vitamins and phenolic compounds [31,32].

2.2 Physicochemical Properties of Strawberry, Sour Cherry, and Raspberry Juices and Changes due to Processing

Red fruits such as strawberries, sour cherries, and raspberries are rich in polyphenols and particularly anthocyanins, a group of natural pigments responsible for their vibrant colors [33,34]. Anthocyanins are known for their health benefits, including antioxidant, anti-inflammatory, anti-cancer, and cardiovascular health-promoting properties, making these fruits valuable in functional food applications [35–38]. Each fruit has its own unique nutritional profile, characteristics and benefits, for example:

Strawberries **(***Fragaria* × *ananassa***)** contain high number of antioxidants, and high levels of other nutrients [39], particularly vitamin C, folate, and manganese, and are among the top fruit sources of total phenolics and antioxidant capacity [40]. They are also high in phenolic compounds, particularly ellagic acid and flavonoids, which provide antioxidant and anti-inflammatory benefits [39]. Their anthocyanins, primarily pelargonidin, give strawberries their vibrant red color and contribute to cardiovascular protection [40,41].

Sour cherries **(***Prunus cerasus***)** are a more acidic fruit, known for their high anthocyanin content, especially cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside, which gives them their deep red color and tart taste [42]. Sour cherry anthocyanins are linked to significant anti-inflammatory and antioxidant properties [43]. Sour cherry juice has high antioxidant capacity and may inhibit enzymes like monoamine oxidase A, and α-glucosidase, potentially helping to prevent diseases [44].

Raspberries **(***Rubus idaeus***)** are known for their nutritional profile, containing high levels of fiber, vitamin C, and various phenolic compounds like quercetin and ellagic acid [45]. Raspberries are a rich source of ellagitannins, flavonoids, and anthocyanins, which give them their characteristic red coloration. The major anthocyanins in raspberries are cyanidin-based ones, and they contribute to the fruit's typical coloration and antioxidant properties [46]. These bioactive compounds support heart health, reduce oxidative stress, and may help in cancer prevention [45,47].

Strawberries, sour cherries, and raspberries are commonly processed into fruit juices that are appealing to consumers due to their red coloration and health benefits, these red juices have recently gained attention as consumers often associate their vibrant red color with a high antioxidant content [48]. However, to extend the shelf life of these juices and ensure their microbial safety, processing techniques must be applied, which can often lead to the degradation of important nutrients, such as anthocyanins [49]. Processing red fruits can result in significant changes in their nutritional and physicochemical properties. Heat treatments, such as thermal pasteurization, can degrade anthocyanins and vitamin C, resulting in color degradation and reduced antioxidant activity [50,51]. Phenolic compounds may also be reduced due to enzymes like polyphenol oxidase (PPO) and peroxidase (POD), that can further cause browning [52]. These changes are often undesirable and affect both the nutritional quality and the sensory properties of the juices.

2.2.1 Thermal Processing

Thermal processing has been the main method of preserving fruit juices and nectars for decades, offering a reliable method for inactivating microorganisms and enzymes [53,54]. Juices and nectars are typically acidic, with a pH below 4.6, which allows for pasteurization, a process that uses temperatures below 100°C to effectively eliminate spoilage microorganisms. While certain bacterial spores may survive in these acidic conditions, their growth and multiplication are inhibited [55].

Heat treatment involves exposing the product to high temperatures which effectively reduce the microbial load and prevent spoilage. Common thermal methods applied to juices include pasteurization, sterilization (canning), and ultra-high temperature (UHT) processing, which requires only a few seconds at 135°C for acidic foods [56]. The main mechanism of these processes is the denaturation of microbial proteins and enzymes by means of heat, but thermal processing has several disadvantages [57]. Thermally processed juices tend to lose some of their original flavor, color, and nutritional qualities such as vitamins, especially at higher temperatures and longer times [15].

To overcome these effects, hurdle technologies, which combine multiple preservation methods to improve food safety while reducing the need for high temperatures, have become popular in juice processing [58]. Additionally, preservatives are often used to inhibit microbial growth [59]. However, the use of preservatives is becoming increasingly unpopular with consumers, who are seeking more natural fruit juices without additives and are focusing on clean labeling [60]. Moreover, researchers have developed various pasteurization methods, each with a different heat intensity. High Temperature Long Time (HTLT) pasteurization, the most common for juices, uses temperatures between 60–80°C, and times around 5 to 20 min, and is ideal for low-acid juices with a pH above 4.5. This method can enhance certain bioactive compounds and reduce enzyme activity. High Temperature Short Time (HTST) pasteurization is designed to maintain high product quality by minimizing heat exposure, using temperatures above 80°C for less than 30 seconds to reduce enzymes such as PME, PPO, and POD. Mild Temperature Long Time (MTLT) pasteurization operates at temperatures below 80°C for more than 30 seconds and focuses on minimizing processing effects while extending shelf life [9,16].

Furthermore, UHT processing allows for short exposure times at very high temperatures, which reduces nutrient loss [61]. Thermal sterilization, such as the UHT process combined with aseptic filling, allows juice products to remain shelf stable at room temperature for extended periods due to the complete inactivation of enzymes, spores, and microbial load [61]. This is something that new technologies have yet to achieve. Nevertheless, despite optimization, thermal processing remains a compromise between microbial safety and juice quality preservation [53].

While novel technologies aim to preserve juice more effectively by maintaining its freshness and nutritional profile, they remain less accurate in addressing other common needs of the juice industry, such as concentration for easier transport and export. Due to this limitation, traditional thermal methods continue to play an essential role in these stages of production, ensuring stability for distribution on a global scale [62,63]. For example, more than 98% and approximately 75% of the orange juice consumed in the United States and Europe respectively, comes from Brazil [64]. Combined with the growing demand for tropical and non-seasonal flavors in the European juice market, make it necessary to use of thermal technologies for concentration [65,66]. This use of thermal concentration not only facilitates logistics but also allows shelf stability during transportation, ensuring that the juice maintains its quality until it reaches the consumer. On the other hand, some alternative non-thermal concentration technologies have been developed, such as osmotic and freeze concentration, but they are not as energy efficient as thermal concentration [67–69]. Thus, thermal processing remains a necessary component in the juice industry alongside emerging methods.

2.2.1.1 Effect of Thermal Processing on Functional Properties of Juices

T Thermal processing leads to several undesirable changes in fruit juices, including the degradation of heat-sensitive nutrients such as vitamins (especially vitamin C), phenolic compounds, and antioxidants [70]. Additionally, thermal processing can negatively affect the organoleptic properties of juice, leading to changes in color, flavor, and texture [16]. For example, the browning of fruit juices due to non-enzymatic browning reactions is a common challenge in shelf-stable fruit juices, resulting in lower consumer acceptance [71].

Thermal processing can lead to the degradation of anthocyanins, which are sensitive to high temperatures [72]. As a result, there is often a noticeable reduction in the color of juices, measured by an increase in total color difference, particularly in red fruits [51,71,73], as anthocyanins are responsible for these hues [74]. Polyphenols, while more stable than anthocyanins, are also affected by prolonged exposure to heat, leading to a decrease in their antioxidant properties [75]. These losses in polyphenols and anthocyanins can reduce the overall health benefits of the juice [35].

The antioxidant capacity of fruit juices and nectars, which is closely associated with their polyphenol content, can also be reduced by thermal treatments [76,77]. Thermal processing can affect the aroma and sensory qualities of juices, often leading to a decrease in total aroma compounds and the development of off-flavors [78]. These off-flavors can affect consumer acceptance of the juices [79]. Finally, vitamin C, or ascorbic acid, is especially thermolabile [80]. High temperatures and prolonged heating can cause significant losses of vitamin C, affecting not only the health benefits but also the antioxidant capacity [81]. However, the kinetics of vitamin C and anthocyanins degradation are generally slower than those required for effective microbial inactivation. Therefore, even if losses may occur during thermal treatment, a considerable portion can be retained

[80,82]. Table 2.1 summarizes some of the findings on the effect of thermal treatment on functional properties and nutrients, particularly for sour cherry, raspberry and strawberry fruit juices.

Table 2.1. Effect of Thermal treatment on functional properties of sour cherry, raspberry and strawberry juices

Fruit Juice	Properties	Treatment	Effect	References
Red raspberry	Anthocyanins	95°C for 5 min	More than 50% loss of anthocyanins.	[83]
Sour cherry	Anthocyanins	90°C for 4 min	Significant loss but depends on variety (25 analyzed).	[84]
Strawberry	Total phenolics, antioxidant capacity and β-carotene	95°C for 1 min	Reduction of total phenolics and antioxidant capacity but increase in β-carotene.	[85]
Strawberry	Anthocyanins, vitamin C and color	85 °C for 2 min	No significant differences, in anthocyanins and vitamin C with the control, ΔE 1.8±0.6.	[86]
Strawberry Nectar (60%)	Total phenolics, anthocyanins, vitamin C and antioxidant capacity	85°C for 5 min	A 14.1% loss of vitamin C, 14% reduction in total phenolics, 39% decrease in anthocyanins, and a 34% reduction in antioxidant capacity (TEAC).	[87]
Strawberry purée	Total phenolics, anthocyanins, vitamin C and color	90°C for 15 min, in 100 mL glass jars	Degradation of polyphenols (14%), anthocyanins (43%), vitamin C (61%), and color (ΔE > 3).	[88]
Strawberry purée	Anthocyanins, vitamin C and antioxidant capacity	70°C for 2 min	Degradation of vitamin C reached 21%, with significant reductions also observed in anthocyanin levels and antioxidant activity.	[89]

In summary, while thermal treatment is necessary for microbial safety, shelf-life extension, and transportation, it can compromise the nutritional and sensory qualities of fruit juices and nectars. This has increased the interest in optimizing thermal processes or exploring alternative non-thermal preservation methods to better preserve the functional components, color, and freshness [6].

2.2.2 Non-Thermal Processing

To address the limitations of traditional thermal treatments, non-thermal technologies have been studied in recent years [8]. Several non-thermal technologies have been investigated for their potential applications in the juice industry, and while numerous technologies have been proposed and studied, this chapter focuses specifically the HPP and PEF methods, as these were the technologies applied in this study.

2.2.2.1 High-Pressure Processing (HPP)

HPP is a non-thermal technology that uses extremely high isostatic pressure to inactivate microorganisms in food, up to 600 MPa and 6 min, the upper limit for commercial applications [8,90]. HPP has been recognized by the U.S. Food and Drug Administration, since 2001, as an approved method to achieve a 5-log reduction of the most common pathogens of fruit juices [91]. It is therefore equivalent to a thermal pasteurization.

The most common HPP treatment for juices in the industry is 300–600 MPa for 1–5 minutes at chilled or room temperatures, effectively inactivating pathogens [90]. The HPP process in foods commonly uses water as the pressure-transmitting fluid. When water is subjected to high pressure compression, its temperature increases by only 3 °C per 100 MPa

[92]. The water used for HPP treatment is often chilled to 4–8°C [90], to avoid reaching high temperatures during processing, due to the pressure increase.

HPP technology is based on two principles. The first is *Le Chatelier's* principle, which states that when pressure is changed in a system, the system tends to adjust by counteracting the change. The second is the isostatic principle, which guaranties that pressure is evenly distributed throughout the entire product, regardless of the size or geometry [93,94]. This contrasts with thermal processing, where processing times are influenced by the size and shape of the product [28]. Important factors that determine the effect of HPP on food products include processing time, temperature, pressure, packaging material, and the specific characteristics of the sample [95].

HPP systems are available in both batch and semi-continuous configurations; the main component of the batch design is the high-strength steel alloy cylindrical pressure vessel. Other key components include a yoke to hold the closure under pressure, a high-pressure pump and intensifier to achieve target pressures, and product handling vessels (carrier baskets). Batch processing is typically used for fruit juices. The HPP batch process is like retort systems used in thermal processing [94,96]. A schematic representation of a HPP system, and all its components is shown in Figure 2.1.

The main advantage of HPP is that it does not rely on heat, allowing the retention of heat sensitive compounds such as vitamins, antioxidants, and polyphenols [94]. This makes HPP an attractive option for producing high-quality juices that retain their fresh flavor and nutritional profile. Some drawbacks of this technology are that it can cause changes in the color and texture of juices [97,98], and it does not effectively inactivate spores [99]. Moreover, the high capital investment required for HPP equipment is a challenge to widespread adoption of the technology [96].

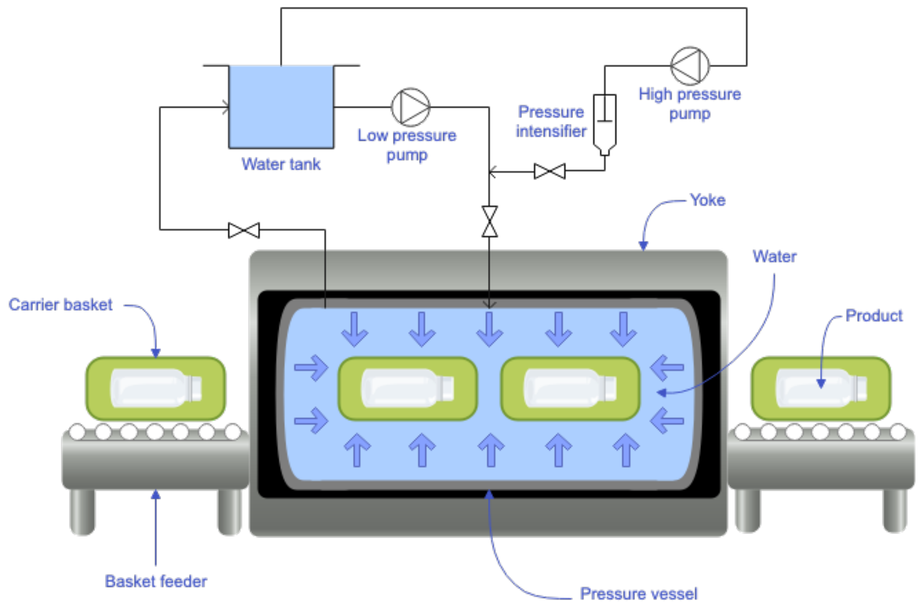

Figure 2.1. Scheme of a High-Pressure Processing (HPP) system and components. Adapted from Vignali et al. [26] and Elamin et al. [100]

2.2.2.1.1 *Effect of High-Pressure Processing (HPP) on the Functional Properties of Juices*

HPP shows great potential for industrial food applications, preserving important quality attributes such as color, flavor, and nutritional value [94]. Its limited effect on covalent bonds at moderate temperatures is a distinct advantage [101]. There are numerous studies showing that HPP at moderate temperatures has minimal or no significant effect on the vitamin and nutrients content of fruit and vegetable products [102–104].

Although, some studies show a decrease in oxidase enzymes such as PPO, POD, and PME after HPP, residual enzyme levels often remain, affecting juice quality and stability during storage, as these enzymes can degrade important juice components and affect their quality [105–107]. The use of temperature as a pretreatment and the combination of technologies can help to further mitigate this effect [108].

HPP treatment generally results in slight or negligible decreases in vitamin C content, with reductions that are typically less than those caused by thermal processing [102]. Lower pressures, up to 400 MPa, tend to have minimal impact on ascorbic acid levels, while pressures around 600 MPa can cause significant reductions [93]. For instance, in a study by Wibowo [109] on orange juice, a notable decrease in vitamin C content was observed in samples treated at 600 MPa. Conversely, e, vitamin C retention after thermal pasteurization tended to be more stable during storage, with vitamin C losses generally depending on the specific matrix, on the presence of endogenous enzymes or metal ions, and on the pressure applied [8].

Small increases in anthocyanin content and antioxidant activity, have been reported in various fruit juices treated with HPP [7,110,111], possibly due to the cell wall disruption caused by the high pressure. In terms of color degradation, HPP-treated samples typically show little differences compared to untreated samples [51,110].

Studies have also reported that HPP treatment increases the permeability of plant cells, thereby improving the extraction of carotenoids and liposoluble vitamins [112].

Table 2.2 provides a more detailed view of the effect of HPP treatment on the functional properties and bioactive compounds in sour cherry, strawberry and raspberry juices is shown.

Table 2.2. Effect of High-Pressure Processing (HPP) on functional properties of sour cherry, raspberry and strawberry juices

Fruit Juice	Process Parameter	Results	Year	References
Black/Red Raspberry	400–600 MPa for 10 minutes.	PME partially inactivated at 600 MPa for 10 minutes (22%).	2022	[31]
Sour cherry	600 MPa for 3 min at 4 °C	HPP treatment did not affect the color, total phytochemical content, or antioxidant activity (measured by ABTS and DPPH assays).	2022	[113]
Strawberry	550 MPa, at 20 °C for 2 min	HPP-treated juice had higher antioxidant activity (+7.9%) and more zeaxanthin (+33.5%), polyphenols significantly higher in HPP (1100.04 mg GAE/L vs. 1002.66 mg GAE/L).	2016	[85]
Strawberry purée	300 or 500 MPa for 1, 5 or 15 min at 0 or 50 °C	No significant decrease in PPO (97.7%) or peroxidase (POD) (99.5%). Highest reductions in polyphenols (4%), anthocyanins (14%), and vitamin C (30%) occurred in HPP-treated samples at 500 MPa for 15 minutes at 50°C.	2015	[114]
Strawberry	400–600 MPa, 20 °C, 1.5 or 3 min	PPO activity did not decrease in HPP-treated samples, and vitamin C and anthocyanins declined faster during storage due to enzyme activity.	2018	[86]
Strawberry-based beverage	200–800 MPa for 1–15 min	Significant reduction of antioxidant capacity and flavonoid content by HTST pasteurization compared to HPP.	2012	[115]

2.2.2.2 Pulsed Electric Fields (PEF)

PEF technology uses short bursts of high-voltage electrical pulses to disrupt cell membranes, effectively inactivating microorganisms in fruit juices. The process takes place at room temperature or mild temperatures, preventing thermal damage to heat-sensitive components [8]. PEF is especially effective against bacteria, yeasts, and molds, while spores, like with HPP, remain more resistant. In PEF processing, short pulses of 1–100 μs and high electric fields (5–50 kV/cm) are typically applied to liquid food products as they pass through a narrow gap between two electrodes [92,116]. However, electric fields higher than 15 kV/cm electric fields may be required for effective pasteurization [30,117].

PEF offers several advantages, including preservation of fresh juice characteristics, such as flavor, color, and nutritional value [21]. The energy efficiency of PEF is another strength, as it requires relatively low energy inputs compared to thermal processes and HPP [26]. Furthermore, the process is fast, making it suitable for continuous production lines [118]. Nevertheless, the effectiveness of PEF is influenced by the electrical conductivity of the juice and may not be suitable for all the matrices [21].

During processing, the juice comes into direct physical and electrical contact with metal electrodes inside the treatment chamber. The system can operate in either batch or continuous mode. Higher energy short pulses are applied to the electrode. The most common pulse shapes used in PEF treatments are exponential or square-wave pulses, which can be monopolar or bipolar in nature [119]. A typical PEF system consists of several key components, including a high-voltage pulse generator, a treatment chamber, in which the electrodes can be configured in parallel plate, co-axial, or collinear designs, and in continuous operation a pump and a cooling system. In the parallel plate configuration, two flat electrodes face each other, creating a uniform electric field and commonly used in batch mode due to its simple setup. In the collinear configuration, electrodes are aligned along the flow path, with the electric field applied parallel to the flow for continuous treatment as food moves between the electrodes. The Co-axial configuration has a cylindrical setup, with an inner electrode and an outer electrode, allowing food to flow between them [27,119,120]. An overview of a continuous PEF system and cross section views of different treatment chamber configurations in presented in Figure 2.1. The treatment chamber shown in the figure is a collinear configuration, where the treatment areas lie between the high-voltage electrode (center) and the two ground electrodes (sides). This setup is insulated to ensure safe and controlled application of the electric field within the treatment zone.

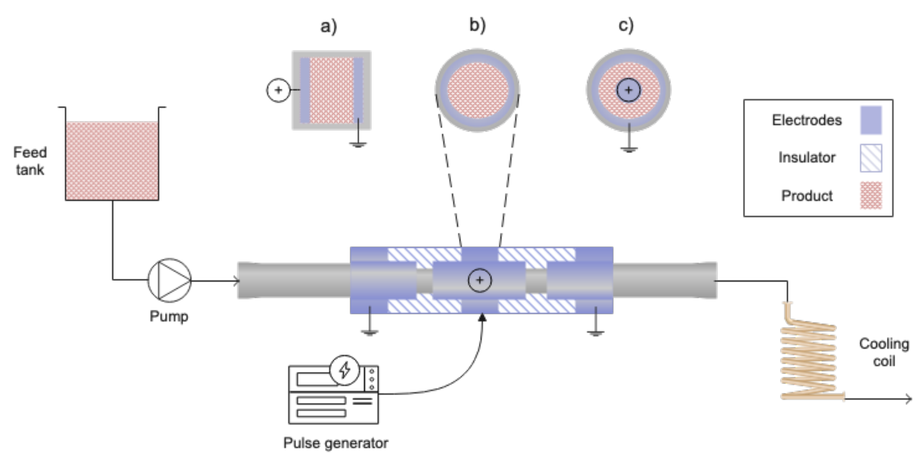

Figure 2.2. Scheme overview of a continuous Pulsed Electric Field (PEF) system and cross section views of different treatment chamber configurations: a) Parallel plate, b) Collinear, and c) Co-axial. Adapted from Vorobiev et al. [121] and Carpentieri et al. [122]

Applications of PEF include microbial inactivation in liquid foods such as juices, milk, and plant-based beverages [21,117,118,123]. When electrical pulses are applied, they can induce pore formation in the cell membranes, a process known as electroporation, the impact on biological cells will depends on the intensity of the treatment and the size and composition of the cells [8]. Depending on the intensity of the pulses, this pore formation can be either reversible or irreversible. Reversible electroporation allows for temporary permeabilization of the membrane, which can enhance mass transfer processes without damaging the cells, whereas irreversible electroporation can lead to cell death, which is particularly useful for microbial inactivation in juice processing, helping to preserve the product while minimizing thermal damage [21,119].

2.2.2.2.1 *Effect of Pulsed Electric Fields (PEF) on the Functional Properties of Juices*

Several studies have investigated the effects of PEF on the retention and extraction of bioactive compounds in fruit- and vegetable-based products [8]. In general, no significant decrease in bioactive compounds in liquid foods due to PEF processing has been observed [124]. When used as an alternative to thermal pasteurization in fruit juices, PEF treatment has been shown to be more effective in preserving bioactive compounds, including vitamin C [125] and polyphenols [126]. Some authors have also reported an increase in polyphenols and anthocyanins after PEF treatment [31]. For instance, a treatment at 35 kV/cm at 200 Hz, resulting in higher significant polyphenol levels in a fruit-based drink [127]. PEF also performs better than thermal pasteurization in retaining flavor and aroma [128].

PEF usually surpasses thermal methods in preserving other quality attributes and nutritional content, when equivalent treatments are compared [129–131]. In general, PEF maintain or improves the nutritional and sensorial quality of juices, for example, in the study of Rahaman et al.

[132] on apricot juice, PEF-treated samples showed notable increases in cloudiness, phenolic compounds, flavonoids, and antioxidant activity. In a study by Xiang et al. [133], carrot juice treated with PEF at 25 kV/cm retained higher levels of ascorbic acid, α-carotene, β-carotene, and lutein. The PEF-treated juices showed minimal differences from the control juices and when comparing PEF with thermal pasteurization, the inactivation of aerobic bacteria and mold was also comparable. A more detailed view of the effect of PEF on the functional properties of sour cherry, raspberry and strawberry juices is presented in Table 2.3.

Table 2.3. Effect of Pulsed Electric Fields (PEF) on functional properties of sour cherry, raspberry and strawberry juices

Fruit Juice	Process Parameter	Results	Year	References
Black/Red Raspberry	Frequency 100–500 Hz, pulse number 100, electric field strength from 11.3 to 23.3 kV/cm, and specific energy from 19.7 to 168.4 kJ/L	PEF treatment at 200 Hz/168.4 kJ/L resulted in 19% inactivation of PME, a 79% increase in total phenolic content (TPC), and a 77% increase in vitamin C.	2022	[31]
Sour cherry	Treatment time 350.9 μs at 6.78 kV/cm, and 98 Hz	The initial total antioxidant capacity increased significantly, with organic acids like citric acid showing no significant degradation and no formation of furfural or HMF.	2020	[134]
Sour cherry	Square wave bipolar pulses (3 μs), 20 μs delay, 50 mL/min, six chambers (0.23 cm gap), 24 kV/cm at 125, 250, and 400 Hz.	PEF treatment preserved 94% of sensory properties, 70% of physical properties, and significantly affected 57% of the 73 detected aroma compounds.	2016	[135]
Strawberry	Electric field strength 30 kV/cm, 100 Hz during 1.5, 3, and 4.5 min.	The combination of 1.5 minutes of PEF and 2.5 minutes of HPU was most like untreated samples, with the highest	2023	[136]

		DPPH antioxidant values observed in the PEF treatment of 2.7 minutes.		
Strawberry	Electric field strength 35 kV/cm, 27 μs with monopolar square pulses and a 2 μs pulse width.	PEF improved the retention of total phenolic content (4–5%), radical scavenging activity (18–19%), and total anthocyanin content (15–17%) compared to thermal pasteurization.	2021	[130]

2.3 Microbial Safety in Fruit Juices and Nectars: A Comparative View of Processing Techniques

Microbial safety is the most critical factor in juice production, because contamination with harmful pathogens can lead to serious foodborne illnesses. Juices, especially those that are not properly pasteurized or treated, can contain microorganisms such as *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes*, all of which have been associated with foodborne illnesses [137,138]. To ensure safety, regulatory agencies, such as the FDA, require that juice producers achieve a 5-log reduction in the most resistant pathogens of concern, denoting a 99.999% decrease in their microbial load [139]. This is the standard for reducing the risk of foodborne illness and ensuring that the juice is safe for consumption [9]. The challenge then, is to meet this requirement while maintaining the nutritional and sensory qualities of the product.

2.3.1 Thermal Inactivation of Microorganism in Fruit Juices

Thermal pasteurization is one of the most traditional and widely used methods for ensuring the safety of fruit juices, particularly those with a pH lower than 4.5, such as strawberry, cherry, and raspberry juices. The acidic environment in these juices already inhibits the growth of many spoilage and pathogenic microorganisms, but pasteurization is still essential to inactivate heat-resistant microbes [70].

Pasteurization involves heating the juice to a temperature between 60°C and 100°C for a short time, which effectively reduces the microbial load by denaturing proteins and enzymes critical for microbial survival [9]. The effectiveness of thermal inactivation depends on both the temperature and the duration of treatment, which must be controlled to avoid excessive degradation of nutrients such as vitamin C and anthocyanins [61].

When selecting a target microorganism to calculate the pasteurization treatment, juice processors should focus on the most heat-resistant pathogen that could be present in the juice. Due to their association with numerous outbreaks in unpasteurized juices, *E. coli* O157 and *Salmonella* are common choices, while *L. monocytogenes* is also considered due to its widespread presence [140].

The Pasteurization Norm, or *P-value*, represents the total time required at a given temperature to achieve a target reduction in microorganisms. Thermal inactivation of microorganisms is assumed to follow first-order degradation kinetics; therefore, *P-value* can be calculated as [141]:

$$P - value = D \cdot \log\left(\frac{N}{N_0}\right) \tag{2.1}$$

where, D is the *D-value* (Decimal Reduction Time) or the time required at a given temperature to achieve a 1-log reduction (or 90% reduction) in the population of a target microorganism, and $log(N/N_0)$ is the log reduction, or the reduction in microbial population achieved by a treatment. Here, N_0 represents the initial number of microorganisms, and N is the number remaining after treatment (e.g. a 2-log reduction corresponds to 99%).

Here, higher pasteurization temperatures reduce the total heating time needed (*P-value*) and vice versa. Weaker vegetative microorganisms will be inactivated below the set pasteurization temperature [141].To produce high-quality, safe fruit juices, minimal processing conditions should be used to minimize the thermal damage to quality attributes [16].

2.3.2 Microbial Inactivation with High-Pressure Processing (HPP)

Fruit juices and beverages are typically processed with HPP at pressures of 400 to 600 MPa for several minutes to reduce pathogenic and spoilage microorganisms and extend the shelf life of the products [142]. HPP works by disrupting the cell membranes and cellular functions of microorganisms without the need for high temperatures [94]. This makes it ideal for juices like strawberry, cherry, and raspberry, helping to preserve their bright colors, fresh flavors, and health-promoting compounds. HPP is generally effective against vegetative microorganisms but is less effective at inactivating bacterial spores, which are highly resistant to pressure alone. While HPP can damage spores at very high pressures (above 600 MPa) combined with moderate temperatures (40–80°C), complete spore inactivation cannot be achieved [99,143].

HPP treatment is performed at cold or ambient temperatures, and the pressure is uniformly distributed to minimize nutrient damage and ensure consistent microbial inactivation [95]. The sensitivity of microbial cells to HPP in juices is influenced by factors such as pressure, duration of the pressurization, temperature, rates of compression and decompression, the types of microorganisms present, and the intrinsic properties of the juices, such as pH and sugar content [28,99].

HPP pressure and the duration of pressure-holding affects the inactivation of target microorganisms. In general, higher pressures and longer times lead to greater microbial cell destruction. However, in some cases, tailing occurs, where a portion of the microbial population becomes more resistant over time, causing a slower inactivation rate as treatment continues [99,144]. In addition to the treatment pressure time, there is the time required to reach the desired pressure, known as the come-up time (CUT). A long CUT affects significantly the overall process time and can contribute to microbial inactivation [99].

Studies on HPP treatment in juices and nectars have shown that the inactivation of microorganisms, like *E. coli* O157 and *L. monocytogenes*, follows a first-order kinetics model [145,146]. According to these kinetics, z-values for HPP treatment on *E. coli* and *L. monocytogenes* have been observed to range between 128 and 570 MPa [99]. Nevertheless, the decrease in microbial population due to pressure tends to be exponential. The first-order approach to describing microbial inactivation in thermal treatments assumes that all the cells in a population have the same sensitivity to the lethal agent. This model often differs from real data in HPP treatments, which show survival curves with irregular shapes like shoulders and tails. The Weibull model offers a better fit for such non-linear data by including subpopulations with different inactivation rates [147,148]. This approach has been successfully used to modeling the microbial inactivation kinetics of HPP-treated juices [149]. The Weibull model for HPP microbial reduction follow the equation [148]:

$$\left(\frac{\mathrm{N}}{\mathrm{N_0}}\right) = e^{-\left(\frac{t}{\delta}\right)^{p}} \tag{2.2}$$

Where, t is the treatment time, δ is the time needed to achieve significant inactivation, and p is the shape parameter, indicating how the rate of inactivation changes over time. When $p > 1$, the inactivation rate accelerates over time, while $p < 1$ indicates a deacceleration.

HPP treatments in food processing typically range from 100 to 1000 MPa [150], with specific pressure and duration combinations designed to achieve the desired microbial reductions. Treatment pressures and times can vary significantly depending on the juice matrix. For example, black/red raspberry juices treated at 400–600 MPa for 10 minutes resulted in log reductions of molds (1.85 to 3.72 log) and yeast cells (3.19 log), with only a partial reduction on PME enzyme activity (22%) at 600 MPa for 10 minutes [31]. However, in apple juice, a pressure of 400 MPa for 1 min at 25°C totally inactivate *Escherichia coli* (29055) population (10^8 CFU/mL) [145].

2.3.3 Microbial Inactivation with Pulsed Electric Fields (PEF)

The effect of PEF on reducing bacterial load has been studied for over 30 years [151]. This process aims at microbial inactivation while maintaining food quality and offers a potential alternative to traditional heat treatments [119]. The mechanism involved in microbial inactivation is based on the application of short bursts of high voltage electric fields (typically 15–40 kV/cm) to the juice, which creates pores in the microbial cell membranes. PEF treatment minimizes spoilage and pathogenic microorganisms at low temperatures (< 55°C) and in short treatment times (up to 1200 μs) [152]. When the electric field strength exceeds a critical threshold, the pores are irreversibly damaged, resulting in cell death [21,153]. PEF have been proven to be effective against a broad spectrum of vegetative microorganisms [118], but its effect on spores is limited, making it best suited for acidic juices where spores are inhibited by low pH [117].

The electroporation process occurs in three stages: (1), membrane expansion and swelling, (2) pore formation, and (3) an increase in pore size and number, the sensitivity of microorganisms to PEF varies based on factors such as cell size, shape, type, and growth stage [152]. Studies indicate that PEF exerts antimicrobial effects not only by disrupting cell membranes but also by generating reactive molecules like free oxygen, hydroxyl, and hydroperoxyl radicals [154]. In addition, the inactivation efficiency of PEF is influenced by parameters such as pH, water activity (a_w), and electrical conductivity of the fruit juice [21,119]. Moreover, Gram-positive bacteria, such as *Listeria monocytogenes* and *Staphylococcus aureus*, are surrounded by multiple thick layers of peptidoglycan, making them more resistant to PEF compared to Gram-negative bacteria such as *Escherichia coli* and *Salmonella enterica*. This structural difference results in higher effectiveness of PEF in inactivating gram-negative bacteria than Gram-positive ones [151,155].

PEF pasteurization parameters for juices vary widely depending on factors such as equipment type, pulse energy level, and juice composition. Generally, electric field strengths above 15 kV/cm are considered effective for achieving a 5-log reduction in microbial populations [117]. However, studies indicate that much higher electric field strengths and more intense treatments are often required for effective inactivation. For example, sour cherry juice treated with electric field strengths from 17 to 30 kV/cm and 131 μs treatment time resulted in a maximum *E. coli* O157 inactivation of 4.90±0.23 log CFU/mL and a *L. monocytogenes* reduction of 6.62±0.00 log CFU/mL when 30 kV/cm was applied [156]. Additionally, a PEF treatment at 35 kV/cm for 27 μs in strawberry juice reduced the *E. coli* population by 5.16±0.15 log [32]. It is also important to note that at higher energy levels, particularly above 80 kJ/L, ohmic heating effect becomes the primary factor contributing to microbial reduction during PEF treatments [157]. This heating effect, which is often not reported, shifts the process towards a mild thermal treatment rather than a true non-thermal treatment, and has a significant impact on both microbial inactivation effectiveness and juice quality.

2.4 Ohmic Heating: a Promising Thermal Alternative for Juice Processing

Ohmic heating (OH), also called Joule heating, uses electrical currents to heat the juice directly, relying on the liquid's electrical conductivity to generate heat uniformly throughout the product. This rapid, uniform heating allows for effective microbial inactivation while minimizing the thermal gradients that often cause quality deterioration in conventional heat treatments [158,159]. OH, involves the application of electric fields ranging from 20 to 100 V/cm, with frequencies between 50 Hz and 30 kHz, and maximum power levels up to 480 kW [160]. Ohmic heating methods are suitable for foods with conductivity in the range of 0.1 to 10 S/m and can be applied in both batch and continuous processes. Key factors such as electric field strength and electrode configuration, can influence the heating rate. Additionally, the heating rate also depends on the efficiency of the energy source, the design of the equipment, properties of the food medium, including conductivity, viscosity, and specific heat capacity [19,161].

2.4.1 Working Principle of Ohmic Heating (OH)

The fundamental principle of OH is to pass an alternating electric current (I) through two electrodes placed in the food, which generates heat. According to *Joule's Law* (2.3), the power generated is directly proportional to both the square of the applied electric field (E) and electrical conductivity of the food (σ), making these parameters critical to the efficiency of the OH process [158,162]. The power generated (W, in Joules) can be expressed as [160]:

$$W = \sigma \cdot E^2 \cdot t \tag{2.3}$$

where σ is the electrical conductivity of the food (S/m), E represents the electric field strength (V/m), and t is the time during which the electric field is applied (s).

For effective OH, the food must have a certain level of electrical conductivity to allow the current to flow. This conductivity varies with temperature, frequency, and the food's composition. Uneven conductivity within foods can lead to temperature differences, the current naturally flows through areas with higher conductivity, causing *hot spots*, while areas with lower conductivity remain cooler, resulting in *cold spots*.

Another critical factor is the dependence of conductivity on temperature, which can further affect uniform heating [92,160]. In contrast, juices typically have a more uniform matrix, allowing for more even heating under ohmic treatment. However, their electrical conductivity generally ranges from 0.1 to 1.6 S/m, close to the minimum required for efficient ohmic heating. This low conductivity is a drawback, because it can limit heating efficiency [159,160,162].

2.4.2 Ohmic Heating (OH) Equipment Setup

Ohmic heating is a century-old technology, but advances have addressed the issues faced by earlier systems. Modern systems now incorporate titanium electrodes, improved insulating materials, and optimized electrode positioning, all of which have improved the stability, efficiency, and applicability of the process [159,161,163].

The most important component in an ohmic device is a power supply, or generator, and the electrodes connected to it. The electrodes are in direct contact with the food to be heated and allow the electric current to flow through the food. The distance between the electrodes, called the electrode gap, can vary depending on the size of the system. Adjusting this gap changes the strength of the electrical field. Modern ohmic heaters are predominantly designed for integration into continuous processing lines rather than batch processes [158,160]. Two primary electrode configurations are typically used in continuous OH systems: transverse field and collinear field modes. In transverse mode, the electric field and current are perpendicular to the mass flow direction, while in collinear mode, they are parallel to it. The flexibility of electrode placement and gap adjustment provides valuable versatility, enabling precise control over electric field strength. Industries prefer continuous processing systems because they are efficient and easily scalable, making them well suited for handling large production volumes [158,164]. A continuous collinear OH system scheme with three stage is shown in Figure 2.3.

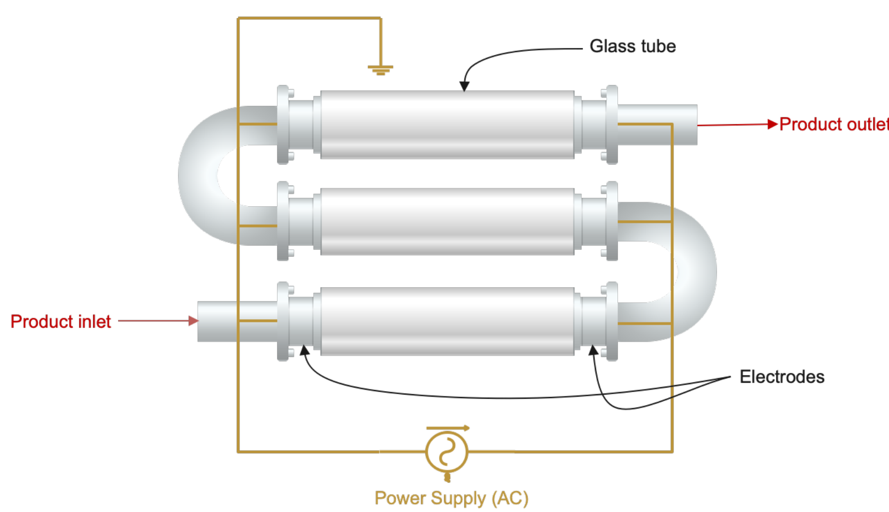

Figure 2.3. Continuous collinear ohmic heating system scheme. Adapted from Icier and Bozkurt [165]

The use of glass as the tube material prevents electrical conductivity from passing through the tube. Additionally, glass helps minimize the fouling effect by providing a smooth, non-reactive surface.

In food processing, both OH and PEF apply electric currents to achieve preservation effects, yet they differ significantly in the intensity of the electric field applied and the resulting effects on the product. The treatment outcome depends on the strength of the electric field and the energy input. This can lead to either a more thermal effect or a primary focus on cell electroporation. With field intensities of OH < 100 V/cm and PEF in the range of 1–100 kV/cm, OH operates at relatively low electric field strengths, that primarily generate heat, while conventional PEF employs higher electric fields that cause cell electroporation at non-lethal temperatures. Each approach has distinct mechanisms to achieve preservation, with OH focusing on heating and PEF focusing on cell disruption by electroporation [160].

2.4.3 Microbial inactivation with Ohmic Heating (OH)

Most research on microbial inactivation by OH has concentrated on the kinetics of inactivation, with only a few studies investigating the mechanisms involved [160]. The inactivation of vegetative cells in OH treatments is generally attributed to thermal effects [166], although some authors suggest that electroporation may also play a role when an electric field is applied [164,167]. While PEF using high electric intensities combined with mild temperatures (< 50°C) is known to induce electroporation of bacterial membranes, the electric fields used in OH are considerably lower and therefore no electroporation is expected [160].

The dual mechanism of microbial reduction in OH, whether it's purely thermal or includes electroporation, is a topic of debate. Nevertheless, several studies have shown no significant difference between microbial inactivation kinetics achieved by conventional thermal methods and those achieved by OH [166,168,169].

Nevertheless, the advantage remains in the reduced time to achieve the microbial inactivation. Astráin-Redín et al. [168] applied an ohmic treatment of 2.5 kV/cm and 50 Hz for the pasteurization of agar cylinders contaminated with *L. monocytogenes* and observed a reduction of the treatment time of 83.3–99.3% compared to conventional heating.

2.4.4 Application of Ohmic Heating (OH) in the Fruit Juice Industry

Electric pasteurization was first commercially used for milk in the early 20th century [170]. Today, OH is widely applied in the pasteurization and sterilization of diverse food products including juices, achieving high-quality results [19,163]. Nowadays, OH is also used for ultra-high temperature (UHT) sterilization, which is particularly suitable for foods with large particles (up to 2.5 cm) which are difficult to sterilize using traditional methods [158]. The application of OH allowed to reduce processing times and energy consumption by the application of rapid volumetric heating [160,161]. Giuliangeli et al, [171] apply an ohmic treatment of 0.21 V/cm at 60 Hz at 60, 70, and 80 °C on guava pulp, resulted in a 93% reduction in energy consumption and achieved a steady heating rate of 0.16 °C/s, which was 2.6 times faster than conventional heating.

Ohmic pasteurization in juices achieves rapid and uniform heating, which helps retain heat-sensitive nutrients such as vitamin C. The technology is especially beneficial for juices with high viscosity or those containing particulates, as it allows for a more uniform heating distribution without the risk of *hot spots*. Additionally, OH has shown advantages in energy efficiency, reducing overall processing costs [160,161,164].

2.4.5 Effect of Ohmic Heating (OH) on the Functional Properties of Juices

Ohmic technology offers several benefits over traditional methods, including better retention of nutritional components, effective destruction of bacteria and enzymes, and reduced fouling [163]. Numerous studies have explored various applications of ohmic heating to different food products [19], [82], [154], [156], [159], [168].

Research on OH specifically targeting the three selected fruits (sour cherry, raspberry, and strawberry) remains relatively limited. However, Table 2.4 presents a summary of studies that have investigated the effects of OH on various red fruits that are rich in anthocyanins. These studies provide insight into the potential impact of OH on color stability, anthocyanin retention, and other physicochemical properties in fruits with similar profile than those mentioned.

The degradation of bioactive compounds in OH is comparable to that observed with traditional thermal treatments. For instance, in the study by Barrón-García et al. [169] when mango pulp was treated with both methods, OH at 15–20 V/cm at 60 Hz, and thermal pasteurization at 72°C for 2 minutes, polyphenol oxidase (PPO) inactivation reached 95.7% within 15 seconds in both, demonstrating similar efficacy in reducing enzymatic activity. Moreover, Sarkis et al. [170] investigated the kinetics of anthocyanin degradation in blackberry pulp under OH (23–125 V) and conventional heating (70–90°C). They found similar anthocyanin degradation rates at low voltage (23 V) for both methods, while higher voltages led to greater degradation due to electrochemical reactions.

However, the authors also noted that higher voltage had a positive effect on the retention of antioxidant activity. On the other hand, OH has also demonstrated better preservation of anthocyanins, antioxidant activity, and vitamin C retention in orange juice compared to conventional thermal treatment (helical heat exchanger) [171]. In general, the degradation of bioactive compounds in OH increases with higher voltage [157], [163].

Table 2.4. Effect of Ohmic Heating (OH) on functional properties of red fruits

Fruit Juice	Process Parameters	Results	Year	References
Blackberry	23–25 V at 60 Hz.	Anthocyanin degradation was like thermal at low voltage, but OH caused higher degradation at higher voltage.	2019	[174]
Blueberry	0 to 240 V at 60 Hz	In OH at lower electric fields the degradation % was like conventional heating. However, at higher, OH caused higher anthocyanin degradation.	2013	[176]
Mulberry	15–30 V/cm	Phenolic content in OH concentrated samples was 3.0–4.5 times higher than with conventional thermal. OH consumed 4.6–5.3 times less energy.	2020	[177]
Mulberry	19 V/cm at 80, 85 and 90°C	Increase of total phenolic content of 6.6%, after 30 minutes at 90°C.	2019	[178]
Sour cherry	Concentration, 10-14 V/cm	Increased the performance of the evaporation process in terms of energy	2020	[179]
Sour cherry	10, 12 and 14 V/cm, 65 ◦C	Antioxidant capacity increased after all treatments, there was no significant difference between Conventional and OH treatments.	2019	[180]
Whey-raspberry beverage	25 V, 10, 100 and 1000 Hz, 65 ◦C	DPPH and FRAP assays showed that Ohmic heating (OH) treated samples had higher antioxidant capacity compared to conventional heating	2019	[172]

In summary, OH would allow shorter heat treatments compared to traditional ones, which could improve the quality of the juice but, depending on the matrices treated and the parameters could in some cases negatively affect certain bioactive compounds.

2.4.6 Advantages and Limits of Ohmic Heating (OH) on Foods

The main advantage of OH is its rapid processing, which reduces the time food spends at high temperatures, thus helping to preserve heat-sensitive nutrients like vitamins and phenolic compounds. Additionally, the even heat distribution minimizes the risk of localized overheating, preventing color degradation and browning. OH, also offers greater energy efficiency compared to traditional heating methods [157], [158]. In industry, continuous OH is applied to highly viscous foods and offers advantages such as technical simplicity, improved energy efficiency, and low maintenance costs [157].

However, the effectiveness of OH can vary depending on the conductivity and composition of the juice. Furthermore, equipment costs and the need for careful monitoring of electrical parameters may limit its application [154], [159]. Furthermore, a significant drawback of OH is the potential for electrochemical reactions and electrode erosion, especially when applied directly to foods. To address this, alternating current and electrode materials like titanium are used, which have shown promising results. Operating at higher frequencies in the kilohertz range further mitigates these effects. Another potential drawback is that non-uniform conductivity within the food matrix that can lead to inconsistent heating, creating hot and cold spots if not properly controlled [82], [156], [177].

2.5 Conclusions

This literature review on thermal and non-thermal processing of fruit juices and nectars concludes that while traditional thermal methods, such as pasteurization, are effective for ensuring microbial safety and extending shelf life, they often degrade sensitive nutrients, colors, and flavors. Non-thermal alternatives, particularly High-Pressure Processing (HPP) and Pulsed Electric Fields (PEF), offer promising solutions for preserving these qualities while achieving microbial safety. HPP and PEF are noted for retaining heat-sensitive nutrients and enhancing sensory attributes, though they face scalability and equipment cost challenges.

Emerging thermal technologies, like ohmic heating, provide more uniform heat distribution and energy efficiency, reducing the risk of nutrient degradation and improving process control. The choice between thermal and non-thermal methods often involves decision in between equipment costs, energy efficiency, and product quality outcomes, with no single method universally ideal for all juice types and processing conditions. The review highlights the importance of balancing microbial safety, nutrient preservation, and consumer appeal to meet market demands for natural, minimally processed juice products.

2.6 References

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3 A Study on Strawberry and Cherry Nectar: Matrix Effects, Thermal Treatments, and High-Pressure Processing

Fruit nectars are produced by combining fruit juice or puree with water, along with sugar or sweeteners. As per the European Directive 2001/112/EC, a limited number of additives, mainly antioxidant or acids, can be included. Additionally, the fruit content in nectar must be between 25% and 50%, and up to 20% w/w (20°Brix) of sugars can be added [1]. These beverages are appreciated for their sensorial quality and nutritional profile. However, quality in juices and nectars is greatly influenced by the matrix and the processing method. Preserving essential characteristics such as color, viscosity, antioxidant activity, and microbial safety is crucial for both consumer appeal and product shelf life [2,3]. The juice matrix, specifically the pH and sugar content, plays a significant role in maintaining these quality parameters, meanwhile the type of processing, whether thermal or non-thermal, can be also affected by the matrix. These variables can significantly affect juice color, viscosity, antioxidant levels, and microbial stability [4–6].

Also, pH is a key factor not only for the quality attributes of fruit juices but also for their microbial safety [7,8]. For instance, fruit juices and nectars with a lower pH, such as and strawberry nectar, as well as cherry nectar, are classified as high-acid foods. High-acid foods generally require milder pasteurization conditions to achieve microbial stability, as acidity inhibits the growth of many spoilage microorganisms and pathogens [9]. Additionally, pH can also influence quality parameters such as antioxidant activity and color stability. For instance, polyphenols and anthocyanins, which are abundant in cherries and strawberries, are sensitive to changes in pH, and their degradation can affect both the health benefits and visual appeal of the juice [10,11]. Anthocyanins, in particular, are responsible for the deep red coloration of cherries and strawberries and are linked to numerous health benefits due to their antioxidant properties [12,13].

Given the importance of both pH and sugar levels in determining the final quality and safety of processed juices, this chapter explores the matrix effects of these variables in strawberry and cherry nectars. Specifically, it investigates how varying pH and sugar content interact with temperature and thermal pasteurization intensity, and on the other side, compare the thermal pasteurization and high-pressure processing (HPP) processing influence in color, and viscosity. This study seeks to provide insights into how juice matrix composition can be optimized to maintain product quality during processing while ensuring microbial safety.

Thermal processing is widely utilized in the food industry due to its efficacy in reducing microbial loads, inactivating undesired enzymes and extending shelf life [2]. However, temperature changes during thermal treatments can also lead to notable changes in the physicochemical properties of juices and nectars [14]. Elevated temperatures can cause the degradation of heat-sensitive compounds such as antioxidants, including vitamin C and anthocyanins [15]. Furthermore, thermal treatments may affect juice color, which is a critical factor in consumer perception of fruit juices [16]. Non-thermal technologies like HPP, while effective in microbial reduction, tend to better preserve the color and nutritional content of juices and nectars compared to thermal treatments [17,18].

The following sections will discuss the experimental methods used for matrix analysis in strawberry and cherry nectars, followed by an analysis that will address how pH and sugar levels affect both the physicochemical and microbial properties of these nectars, and an evaluation of color and viscosity changes under different processing treatments and conditions, providing a comprehensive understanding on the matrix and thermal treatment effects in fruit nectar processing.

3.1 Matrix Effect (pH and Sugar Content) on Antioxidant Activity and Color in Thermal Treated Strawberry Nectar – a Box-Behnken Design Study

Pasteurization is the most common technique used to ensure microbial safety and extend the shelf life of fruit juices and nectars [19]. However, it often alters the product quality parameters [20]. Therefore, understanding how the composition, such as pH, sugar levels, and the processing temperature, affect the antioxidant activity and color of strawberry nectar is important for optimization of the nutritional and sensory properties of thermally treated products.

It is known that the matrix, which includes pH and Total Soluble Solids (TSS), can affect the antioxidant activity and color stability in juices treated with thermal pasteurization [21,22]. These parameters play a significant role in the overall quality and shelf life of processed juices [23]. In this study a Box-Behnken experimental design was employed to evaluate the interaction effects of pH (3.0 - 4.0), total soluble solids (10 - 14°Brix), and pasteurization on strawberry nectar

The Box-Behnken design (BBD) is a type of Design of Experiments (DOE) that is particularly well-suited for studies with three or more variables. BBD is efficient to explore quadratic response surfaces and requires fewer experimental points than a full factorial design while still providing valuable information about the interactions between variables [24]. With three factors (pH, sugar content, and temperature) being varied, the BBD can examine the effects of each factor and their interactions on the outcome variables. This approach will provide a comprehensive understanding of the matrix effects on the antioxidant activity, browning index and color, and can be used to optimize the processing conditions for the best balance between sensory attributes and nutritional quality in strawberry nectar.

3.1.1 Materials and Methods

3.1.1.1 Raw Material

Strawberry nectar was prepared from frozen, untreated strawberry (*Fragaria x ananassa*) puree of the 'Senga Sengana' variety, purchased from Sicoly (France). The puree was packaged in 1 kg sealed packages, and thawing was performed at refrigeration temperature overnight prior to nectar preparation.

3.1.1.2 Nectar Formulation

The nectar formulation was determined using the initial measurements of total soluble solids (TSS) and acidity in the strawberry puree, which were 7 °Brix and 7.7 g/kg (as citric acid), respectively. The fruit content was set at 40% w/w, with the final acidity adjusted to 5 g/kg by adding citric acid. Sucrose was added to reach a final sugar content of 12% w/w (12 °Brix), and filtered tap water was then added to complete the formulation. For a 1 kg batch of nectar, the required ingredients were: 400 g of puree, 1.64 g of citric acid, 90 g of sucrose, and 508.36 g of water.

3.1.1.3 Thermal Treatment

For the thermal treatments, 14 mL of nectar were sealed in polyethylene bags (50.8 mm × 75.2 mm, 50.8 μm). The treatments were conducted in a thermostatic water bath (MPM Instruments, Italy) at three different temperatures, with a set treatment time of 3 minutes for all conditions, to specifically evaluate the effect of temperature. Immediately after treatment samples were cooled down on an ice bath.

3.1.1.4 Colorimetry

The color of the samples was analyzed using a Minolta Colorimeter (CM 2600D, Minolta Co., Japan), equipped with a standard illuminant D_{65}. The nectar was poured into transparent glass petri dishes with a 60 mm diameter, and measurements were taken at 3 different points on the surface. The measured color coordinates were L* (lightness), a* (redness), and b* (yellowness). The coordinates were then utilized to calculate the total color difference (ΔE) after treatment by:

$$\Delta E = [(\Delta \mathrm{L}^*)^2 + (\Delta \mathrm{a}^*)^2 + (\Delta \mathrm{b}^*)^2]^{1/2} \tag{3.1}$$

where, ΔL*, Δa*, and Δb* represent the differences between the color coordinates of the treated samples and the initial (untreated) values.

Additionally, the Chroma (C*) and Hue angle (h) values were also considered to evaluate the color characteristics of the strawberry nectar samples. Chroma (C*), that represents the intensity of the color, was calculated as**Error! Reference source not found.**:

$$C^* = [(a^*)^2 + (b^*)]^{1/2} \tag{3.2}$$

where:

- a* is the redness/greenness component and
- b* is the yellowness/blueness component.

and, hue Angle (h), that describes the actual color and is expressed in degrees, was calculated as:

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{3.3}$$

These calculations allowed for a more detailed information of the color changes in the samples under different conditions, providing insights into the intensity and tone of the redness of the nectar.

This measurement was carried out for all samples in triplicate across all treatment conditions to quantify the color differences due to the thermal processes and matrix changes.

3.1.1.5 Antioxidant Activity by DPPH Radical Scavenging Method

The antioxidant activity of the nectar samples was measured using the DPPH (α-diphenyl-β-picrylhydrazyl) radical scavenging method following the method described by Brand-Williams et al. [25], measuring the absorbance at 517 nm following a 60 min reaction period in the dark at room temperature. The radical inhibition percentage (%) was calculated as Radical scavenging activity (RSA) [26], following the equation:

$$RSA(\%) = \left(\frac{A_c - A_s}{A_c}\right) \cdot 100 \tag{3.4}$$

where A_C is the absorbance of the control samples, with the addition of methanol instead of nectar and A_S is the absorbance of testing sample.

3.1.1.6 Browning Index (BI)

The browning index (BI) was determined using spectrophotometric measurements as follow: 10 mL of the nectar samples were centrifuged at 10 000g for 3 minutes in a centrifuge (5810 R, Eppendorf), the supernatant was filtered with a 0.45 µm syringe filter (Eppendorf) and diluted in water at a ratio of 1:4. Absorbance (ABS) was assessed at two wavelengths: 420 nm and 520 nm [11]. BI was calculated as the rate between 420 nm and 520 nm (ABS_{420nm}/ABS_{520nm}) [27].

3.1.1.7 Design of Experiments (DOE)

A Design of Experiments (DOE) approach was applied to evaluate the effects and interactions of the evaluated factors (pH, TSS, and Temperature) on strawberry nectar parameters. The sampling points and temperatures followed a surface response methodology, based on a Box-Benhken design (BBD). Three factors were varied, each with three levels: pH from 2.5 to 3.5, TSS from 8 to 16°Brix, and temperature from 70 to 90°C. This design is particularly useful when studying three-level-three-factors [28]. The design involved 12 experimental points, along with 4 central points (C) used for replication and to check the consistency of the model. The experimental points and levels are shown in a 3D model diagram (Figure 3.1). For pH adjustment of samples, anhydrous citric acid, 99% (Merck KGaA) was added to lower the pH, and a 0.1 N NaOH solution was used to increase the pH to the desired levels. The TSS content was calculated as previously explained.

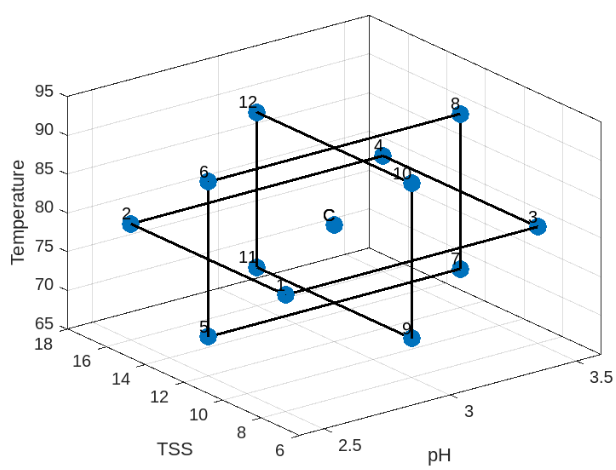


Figure 3.1. 3D Box-Behnken design model diagram

Figure 3.1 represent the 3D model of the BBD, the points indicate the different experimental conditions, including the central point (C) and the various combinations of the factors (pH, TSS, and temperature). Each number corresponds to a specific experimental run. The specific parameter levels of each run are outlined in Table 3.1.

Table 3.1. Experimental points, factors and levels of the Box-Benhken design

Name	pH	TSS (°Brix)	T (°C)	Point Type
1	2.5	8.0	80	1
2	2.5	16.0	80	1
3	3.5	8.0	80	1
4	3.5	16.0	80	1
5	2.5	12.0	70	1
6	2.5	12.0	90	1
7	3.5	12.0	70	1
8	3.5	12.0	90	1
9	3.0	8.0	70	1
10	3.0	8.0	90	1
11	3.0	16.0	70	1
12	3.0	16.0	90	1
C	3.0	12.0	80	0
C	3.0	12.0	80	0
C	3.0	12.0	80	0
C	3.0	12.0	80	0

3.1.1.8 Data Analysis

Data analysis for the BBD was conducted using OriginPro 2023b (version 10.0.5.157). The analysis evaluated the main effects and two-way interactions between the experimental factors. The significance of the model was assessed by examining the p-values < 0.05. Additionally, a *Lack of Fit* test was performed to assess whether the model adequately fits the data. A *Lack of Fit* p-value greater than 0.05 indicated that the model was suitable, and that the unexplained variation was not significant.

The results were analyzed using main effect and two-way interactions and the standardized effects. Main effects plot is used to illustrate the impact of each factor on a response variable by showing the mean response at each level of a factor [24]. ANOVA tables of main and two-way interactions are shown in appendix section A.1.

3.1.2 Results and Discussion

3.1.2.1 Analysis of the Matrix Effect on Color Parameters

Figure 3.2 shows the hue angle (h) values for the strawberry nectar samples are shown in polar coordinates, h represents the visual perception of color [29], the values vary between 35° and 45° degrees. Hue angles in this range typically indicates red coloration. In general, samples treated at lower temperatures (e.g., 70°C) tend to have higher hue angles, indicating they retain more of the original reddish hue, especially at lower pH levels (e.g., pH 2.5). In contrast, samples treated at higher temperatures (e.g., 90°C) show a decreased hue angle, probably shifting into a darker coloration, particularly at higher pH (e.g., pH 3.5).

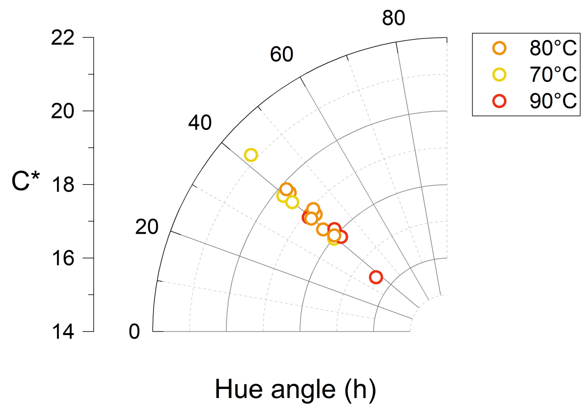


Figure 3.2. Chromaticity diagram for Chroma (C*) and Hue angle (h) values of strawberry nectar at different treatment temperatures for 70°C (○), 80°C (○), and 90°C (○)

This color shift could be related to both the pH effect on anthocyanins, which are sensitive to pH changes, and thermal degradation of these pigments at elevated temperatures, leading to a loss in color intensity and vibrancy [10,21].

As can be seen in the figure at 70°C and a lower pH of 2.5, both the hue angle and chroma (C*) are notably higher, measuring 42.03 and 21.17, respectively. In contrast, at 90°C and a higher pH of 3.5, the hue angle decreases to 37.41 and the chroma drops to 16.43, which are the maximum and minimum points observed across all samples. Higher pH tends to promote degradation of pigments such as anthocyanins, resulting in a color shift towards a less saturated tones, meanwhile, lower pH conditions can stabilize anthocyanins, which tend to retain more vivid colors under acidic conditions [10,30].

The ΔE of the strawberry nectar as affected by (A) pH, (B) TSS, and (C) temperature are show in Figure 3.3. The figure shows significant influence of pH, TSS, and temperature on the ΔE f the samples, which is confirmed by the p-values of the ANOVA table (Table A.1.1).

In (A), the ΔE increases as pH increases from 2.5 to 3.5, indicating that higher pH levels lead to more noticeable changes in color. This trend suggests that as the pH increases, there may be more degradation or alteration of anthocyanins, which are sensitive to pH changes [30]. In (B) the relationship between TSS and total color difference is quadratic, with ΔE peaking around 12 Brix.

This suggests that at moderate levels of soluble solids, color changes are more pronounced. Finally, in (C) the ΔE shows a clear growing trend with temperature, possibly due to degradation of ascorbic acid and other thermolabile molecules [31], especially at temperatures above 80°C. In summary, both high pH and high temperature lead to an increasing ΔE in the nectar.

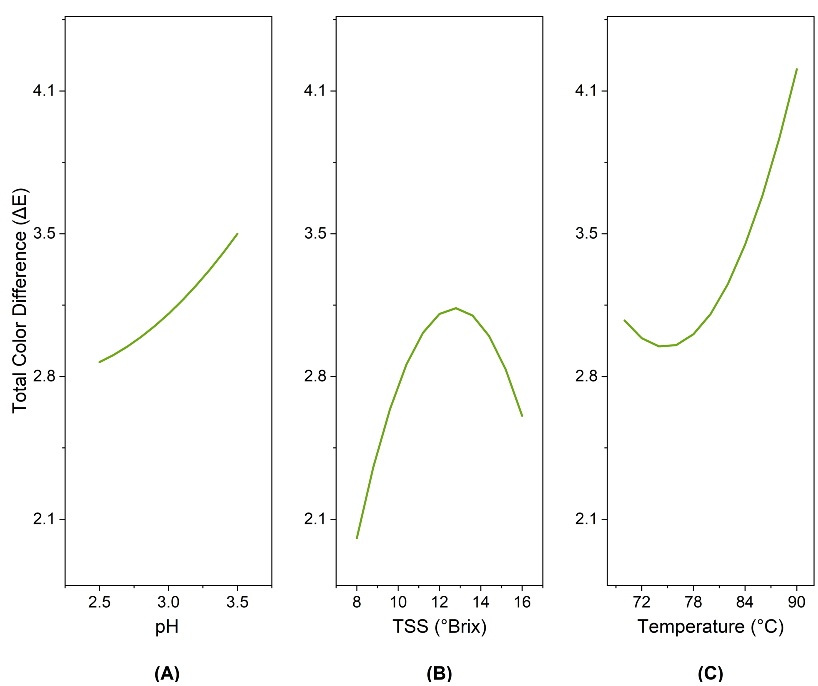


Figure 3.3. Main effects of (A) pH, (B) Total soluble solids (TSS), and (C) Temperature on Total color difference (ΔE) of strawberry nectar.

The standardized effects plot (Figure A.1.1) demonstrates that pH and TSS are the most significant factor affecting the ΔE, followed by temperature. Regarding the two-way interactions, the ANOVA table shows no significative effect between factors. While the two-way interaction between TSS and temperature did not show a statistically significance, the main effects of TSS and temperature on ΔE are evident in the response surface plot in Figure 3.4.

The color gradient of the response surface plot (Figure 3.4) indicates that higher TSS levels (16 °Brix) and higher temperatures (90°C) lead to the greatest ΔE, represented by the darker purple area (ΔE ~ 4.3). At lower TSS values (8 °Brix) and moderate temperatures (75°C), the total color difference remains minimal. These conditions may be favorable for maintaining the visual quality of the product.

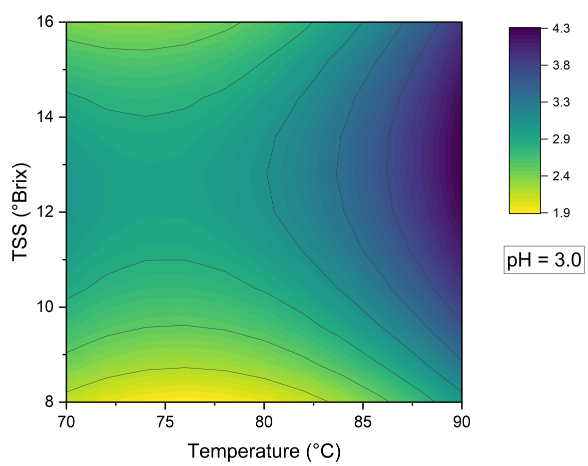


Figure 3.4. Response surface of total color difference (ΔE) as a function of TSS and Temperature at pH = 3.0 in strawberry nectar.

3.1.2.2 Analysis of the Matrix Effect on Browning Index (BI)

The browning index (BI) is a quantitative measure used to evaluate the degree of browning in juices. Browning can occur due to various chemical reactions, including enzymatic browning and non-enzymatic browning, which includes the Maillard reaction and caramelization. Browning is an important indicator of product quality as it can affect not only the visual appearance but also the flavor and aroma [32]. As samples were treated with temperatures above 70°C, minimal polyphenol-oxidase activity is predicted an no enzymatic browning of the samples is expected [33].

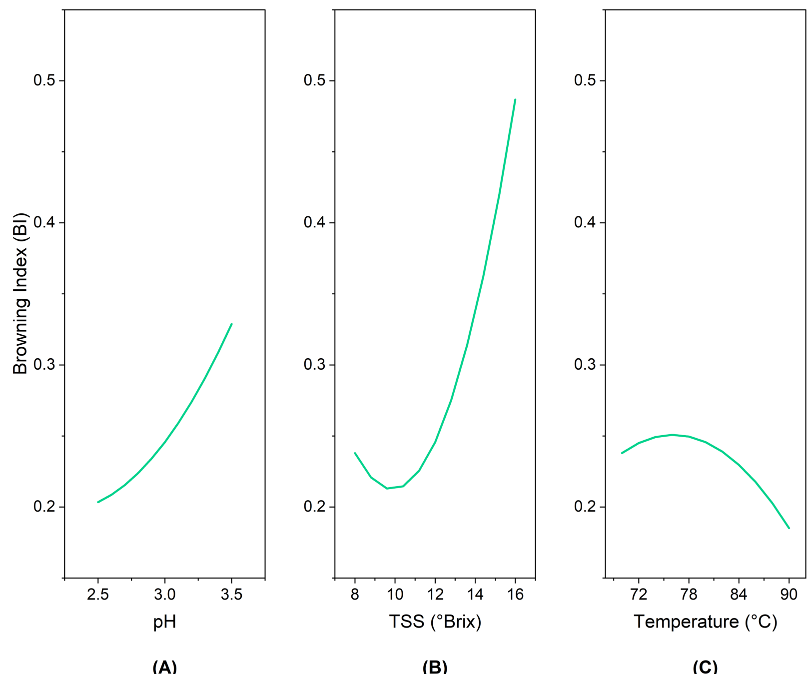


Figure 3.5. Main effects of (A) pH, (B) Total soluble solids (TSS), and (C) Temperature on browning index (BI) of strawberry nectar.

The browning index provides a numerical value of the intensity of these browning changes, in this study, based on the calculations, the BI will show an increase as the browning increases, so BI will provide an idea of the degree of browning [11]. The main effects plot (Figure 3.5) shows significant effects for pH and TSS, corroborated by the ANOVA table (Table A.1.2). As can be seen, in (A), as pH increases, browning increase, as shown by the higher values of BI. The plot in (B) shows that at higher values of TSS are associated with higher browning values, especially above

12 °Brix. It is known that higher sugar content accelerates browning, due to caramelization or Maillard reactions [34]. Temperature (C), on the other side, has a relatively weaker effect on browning, with lower degree of browning as the temperature increases.

The impact of pH on the BI is closely related to the stability of phenolic compounds. In more acidic environments, anthocyanins tend to be more stable, which helps reduce browning reactions [10,11]. The results in pH effect differs from the work of Pham et al. [35] who demonstrated that lowering the pH of a model juice system from 3.8 to 1.5 significantly accelerated the degradation of ascorbic acid, and therefore promotes increased in non-enzymatic browning reactions, however this effect during storage. Conversely, higher pH and higher TSS levels resulted in higher BI, suggesting lower stability of certain components under these conditions. This stability may be related with anthocyanins, as an increase in browning was associated with the degradation of anthocyanins and other phenolic compounds, which are key contributors to the color and stability of the matrix [11,27].

The interaction between pH and TSS are the most influential to the BI, as shown in the effect plot (Figure A.1.2), followed by the individual effect of TSS, then pH and lastly the temperature effect. A response surface plot, showing the relation between pH and TSS on the BI of the samples are shown in Figure 3.6.

The interaction between pH and TSS is now interpreted as a synergistic effect where higher level of pH and TSS lead to more pronounced browning. This means that, in these conditions, the product would experience significant color degradation, likely due to the combined effects of oxidative reactions and less stable pigments. In moderate conditions (12°Brix and pH 3), browning is minimum, meaning the color remains more stable under these conditions.

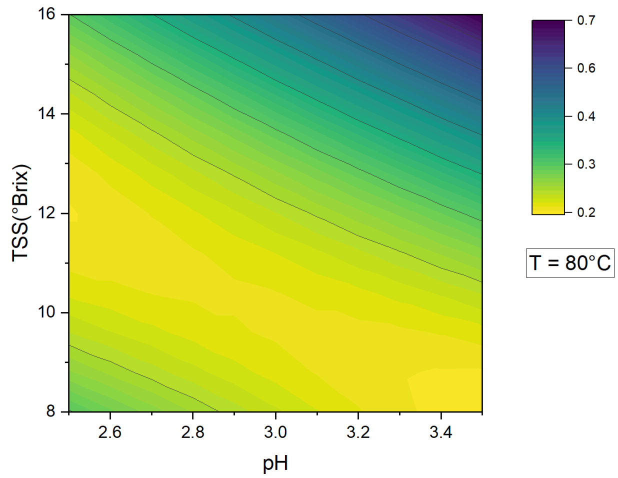


Figure 3.6. Response surface of browning index (BI) as a function of pH and TSS at Temperature (T) = 80°C in strawberry nectar.

3.1.2.3 Analysis of the Matrix Effect on Antioxidant Activity – DPPH Radical Scavenging Method

The main effects plot (Figure 3.7) for the antioxidant activity, showing the radical scavenging activity (RSA) of the samples, provides important insights into the influence of pH, TSS, and temperature on the antioxidant capacity of the nectar. Although the significant effects plot visually suggests an interaction between pH and TSS (Figure A.1.3), the ANOVA results (Table A.1.3) indicate that the interaction pH and TSS is not significant. This means that while there may be an apparent trend in the data, it does not reach the threshold for statistical significance. Thus, the interaction observed visually may not represent a true underlying effect, as confirmed by the ANOVA. In general, the main effects plot shows that lower pH, moderate TSS, and moderate temperature ranges (75 – 80°C) are ideal for enhancing the antioxidant activity of the samples.

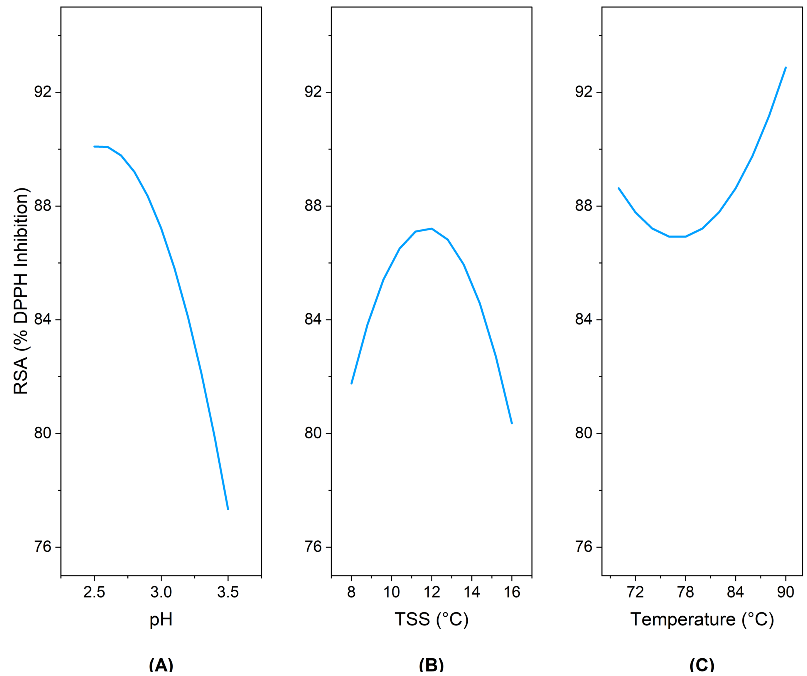


Figure 3.7. Main effects of (A) pH, (B) Total soluble solids (TSS), and (C) Temperature on radical scavenging activity (RSA) of strawberry nectar.

The mild conditions in TSS, particularly 12°Brix, which showed the lowest BI values, are further corroborated by the highest peaks in antioxidant activity (panel B). This suggests that under moderate TSS levels, the stability of antioxidant compounds is enhanced, therefore contributing to decrease in browning. The antioxidant capacity can be influenced by antioxidative Maillard reaction by products, formed during pasteurization [21].

In (A), the antioxidant activity decrease as pH increases from 2.5 to 3.5. This suggests that more basic conditions (higher pH) are more favorable for oxidative reactions. In panel (C), antioxidant activity exhibits a slight drop as the temperature rises from 70°C to around 80°C, but then rises again at 90°C. This indicates that heating may increase the antioxidant potential, likely by releasing phenolic compounds [21]. This effect is only observed at higher temperatures (> 80°C). Regarding pH, the results of this study are in line with those of Torskangerpoll et al. [10] which showed that under highly acidic conditions, anthocyanins are more stable, thereby enhancing antioxidant activity. The decrease of the contents of phenolic compounds, and total anthocyanins led to a reduction of the antioxidant capacity during the processing of strawberries [21].

Figure 3.8 illustrates the response surface for the pH and TSS interaction, providing a visual representation of their combined effects. It can be noticed the quadratic effect of TSS on the response. Additionally, the figure shows that at elevated temperatures, the Radical Scavenging Activity (RSA) is generally higher. Antioxidant activity may increase at higher temperatures due to the release or extraction of antioxidant compounds from the matrix. Elevated temperatures can facilitate the breakdown of cell structures, potentially allowing more phenolics and anthocyanins to become bioavailable, thus increasing the activity [21].

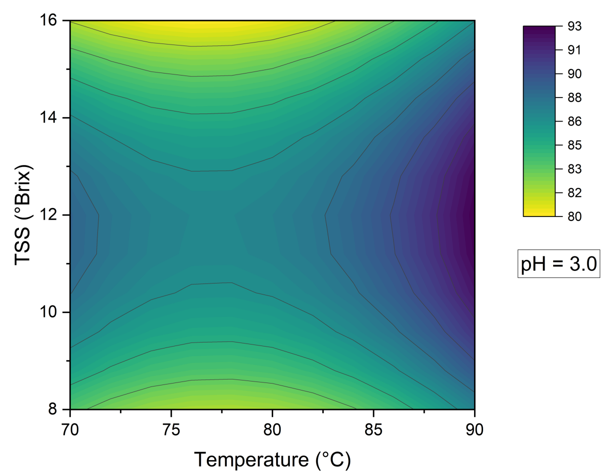


Figure 3.8. Response surface of Radical Scavenging Activity (RSA) as % of DPPH inhibition as a function of pH and TSS at Temperature (T) = 80°C in strawberry nectar.

An optimization of the parameters indicated that to achieve minimal color reduction, maximize antioxidant activity, and reduce the browning index, the conditions are 9.55°Brix, 70°C, and a pH of 2.5.

3.1.3 Conclusions

The matrix effect, including variations in pH and sugar content, in strawberry nectar samples, significantly impacts BI, color, and antioxidant activity. Overall, color was more affected by temperature, with higher temperatures leading to greater ΔE. Browning increased with higher TSS and lower pH, while mild conditions around 12 °Brix resulted in the least browning, although this was associated also with lower color stability but higher antioxidant activity. pH and TSS were the main factors affecting DPPH inhibition, with lower pH and TSS around 12 °Brix significantly increasing antioxidant activity, likely due to the stability of phenolic and other antioxidant compounds.

3.2 Effects of the Matrix (pH and Sugar Content) on the Stability of Color, Antioxidants, Enzymes, and Microbial Safety in Strawberry and Sour Cherry Nectar

The stability of antioxidants, enzymes, and microbial safety in fruit-based beverages, such as strawberry and sour cherry nectar, is highly influenced by the matrix composition [36], specifically the pH and sugar content. These parameters not only affect the physicochemical properties of the nectars but also play a significant role in determining the stability of phenolic and antioxidant compounds [37]. These compounds are important in maintaining the nutritional and sensory qualities of the product [38]. Phenolic compounds contribute to both the antioxidant capacity and the color of the nectars [27], at the same time, antioxidant compounds can help mitigate the browning reactions that happened in the matrix. Nevertheless, enzymes present in fruit nectars, such as polyphenol oxidase (PPO) and peroxidase (POD), can contribute to color changes by catalyzing reactions that can lead to degradation of phenolic compounds and browning of the product [33].

Although thermal treatments are the most common method for fruit juices and nectars and is effective in inactivating microorganism, thereby extending shelf life and ensuring product safety for consumers, they can also degrade phenolic compounds and antioxidants, such as vitamin C [2,9]. This degradation can lead to a reduction in antioxidant activity and color stability [14].

Balancing the application of thermal treatments to ensure adequate microbial and enzyme inactivation, while simultaneously minimizing the degradation of antioxidant and phenolic compounds, is essential for maintaining both the safety and quality of the final product. Achieving this balance is critical, as excessive heat can degrade valuable compounds like vitamin C and phenolics, leading to a reduction in antioxidant activity and a loss of color and nutritional value.

This section explores how variations in pH and sugar content affect the stability of these quality parameters in both strawberry and sour cherry nectars. Two equivalent thermal treatments were chosen: one mild pasteurization and one high-temperature short-time treatment, chosen based on *E. coli* ATCC8739 kinetics, as an adequate surrogate for strawberry and cherry nectar pasteurization [39]. In addition to microbial safety, color stability and browning were also evaluated to understand how these factors influence the overall quality of both strawberry and cherry nectars under varying matrix conditions.

3.2.1 Materials and Methods

3.2.1.1 Nectar preparation

Strawberry (*Fragaria* × *ananassa*) puree was obtained from SVZ International BV (The Netherlands) produced from Spanish varieties, while the cherry puree was prepared at SSICA facilities (Italy) from 'Oblačinska' variety of sour cherry (*Prunus cerasus*), purchased from from Miker Produkt (Serbia). Both purees had a 5 mm particle diameter. The nectar formulation and preparation followed the methodology detailed in 3.1.1.2. The pH adjustment was based on the acidity of the puree, for the sour cherry puree, 13.1 g/kg, and for strawberry, 7.0 g/kg.

3.2.1.2 Experimental Design

A full factorial design was employed with three factors: pH (three levels: lower level 2.2, upper level 3.2), Brix (two levels: 8 and 12), and treatment (three conditions: T0, control; T1, 70°C for 3 min; T2, 90°C for 30 s). The thermal treatments were calculated to achieve equal 5-log microbial reduction of *E. coli* ATCC8739 surrogate, with kinetic parameters derived from previous study by Gędas et al. [39]. For pH-adjusted samples, citric acid anhydrous (Fisher bioreagents) was added to lower the pH, while a 0.1 N NaOH (Sigma-Aldrich) solution was used to increase it. The sugar content was added based on the calculation as previously described.

3.2.1.3 Thermal Treatments and Microbial Inoculation

For thermal treatment of samples, two different setups were used based on the experimental requirements. One for thermal treatment of the main samples and other for the microbial analysis. For the main samples, 12 mL of nectar were sealed in polyethylene bags (50.8 mm × 75.2 mm, 50.8 µm), and the thermal processing was carried out in a water bath (Thermo Scientific, Precision™) at the designated temperatures (70°C or 90°C) for the specified times (3 minutes and 30s), followed by immediate cooling in an ice bath. The small size and thickness of the bags allowed for rapid temperature increase and control of heating time. The temperature was monitored using a thermocouple placed in the bath.

On the other hand, microbial reduction analysis was conducted using sterile 15 mL Eppendorf tubes, with a thermocouple inserted into one of the tubes to monitor the temperature at the center. All material and media were prepared and handled under aseptic conditions in a laminar flow hood. The difference in setup was necessary to maintain sterility and minimize contamination risk, ensuring reliable microbial reduction results.

In microbial samples, a total of 9 mL of nectar was inoculated with 1 mL of *E. coli* ATCC 8739 as inoculum (~10^8 CFU/mL), which was previously thawed and incubated in tryptic soy broth (TSB) for 24h at 37°C.

The thermal treatments were carried out in a water bath (Thermo Scientific, Precision™), the control sample was not treated. Treatment time was recorded when the center of the tube reached the target temperature, after treatment the samples were cooled down in an ice bath and stored at 4 °C until analysis.

3.2.1.4 Microbial analysis

The *E. coli* counts were determined by plating on tryptic soy agar (TSA). Serial dilutions of the treated nectar samples were prepared, and the plates were incubated at 37°C for 24h. Colony counts were performed on duplicate plates, and all experiments were conducted in duplicate.

3.2.1.5 Colorimetry

Color parameters were measured on triplicate, on polystyrene (PS) plates (35 mm x 10 mm). The CIEL*a*b* parameters were measured with a CR-400 chroma meter (Konica Minolta, Japan) with illuminant D_{65} and 10° observer angle. The measured color coordinates were L* (lightness), a* (redness), and b* (yellowness). Total color differences (ΔE) were calculated from **Error! Reference source not found.**. and control correspond to the untreated samples.

3.2.1.6 Browning index (BI) and Antioxidant activity

The antioxidant activity of the nectar samples was measured following the method described in section 3.1.1.5 absorbance readings were taken at 517 nm, using absolute ethanol (VWR Chemicals) as the solvent. Radical scavenging activity (RSA) was calculated using **Error! Reference source not found.**. Ethanol was used as the control for the initial measurement.

For the determination of BI, nectar samples were first centrifuged at 10 000g for 3 minutes, and the resulting supernatant diluted in water at a 1:4 ratio. Absorbance measurements were taken at two wavelengths: 420 nm and 510 nm, and BI calculated as the ratio of absorbance at 420 nm to 510 nm, following the methodology by Aamer et al. [27], for strawberry nectar. Higher BI values will indicate an increase in browning.

A UV/Vis spectrometer (Lambda 25, PerkinElmer) was used to record the absorbance values at the respective wavelengths for both tests.

3.2.1.7 Enzymatic Activity Measurements – Polyphenol-oxidase activity

Enzymatic measurements were performed according to the method described by García-Palazon et al. [40]. The enzyme extraction solution consisted of a 0.2 M sodium phosphate buffer (pH 6.5) containing 4% (w/v) PVPP (Polyvinylpolypyrrolidone) and 1% (v/v) Triton™ X-100, both from Sigma-Aldrich. A 5 g sample of fruit was mixed with 10 mL of the enzyme extraction solution and homogenized using a vortex (TopMix, Fisher Scientific) for 5 minutes. The homogenate was then centrifuged at 10 000 g for 30 minutes at 4°C in a centrifuge (ST 8R, Thermo Scientific). The resulting supernatant was collected and used for enzymatic activity analysis.

For the polyphenol-oxidase (PPO) assay, 75 μL of the enzyme extract was mixed with 3.0 mL of a 0.07 M catechol (o-diphenol) solution in 0.05 M sodium phosphate buffer (pH 6.5). The blank was prepared in the same manner, with distilled water replacing the fruit extract. Absorbance readings were done in a UV/Vis spectrometer (Lambda 25, PerkinElmer) and measurement were taken at 420 nm every 30 seconds for the first 3 minutes and then at 1-minute intervals for up to 10 minutes. The blank was measured alongside the samples to ensure accurate comparisons. The rate of reaction was calculated from the linear slope of activity curves. Enzyme activity was expressed in terms of relative activity, which compares the rate of absorbance change of treated samples to that of the untreated (initial) sample, without acid or sucrose addition. Relative activity was calculated as a percentage and the untreated sample initial activity was used as the baseline (100% activity).

3.2.1.8 Data Analysis

The experimental data were analyzed using a general full factorial design in OriginPro 2023b (version 10.0.5.157). A third-order interaction model was employed, and the results were evaluated with a 95% confidence interval. The model was assessed through the coefficient of determination (R^2) and adjusted R^2 values to ensure model accuracy and fit.

The analysis included main effects, two-way interactions, and three-way interactions. An ANOVA table was generated to evaluate the statistical significance of each factor and their interactions. Additionally, plots of the main effects, as well as interaction plots, were created and analyzed to support the discussion of the results (appendix section A.2).

3.2.2 Results and Discussion

3.2.2.1 Effect of the Matrix on Log Reduction of *E. coli* Surrogate in Strawberry Nectar

To estimate the microbial reduction of *E. coli* surrogate in strawberry nectar, two temperature-time combinations were calculated. These treatments were based on the kinetic parameters obtained from a previous study [39]: D-value at 65°C (D_{65}) of 1.16 minutes and a z-value of 20.1°C.

Using the Bigelow equation for thermal inactivation [41], the time required to achieve a 5-log reduction was calculated by:

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \tag{3.5}$$

where:

- (N/N_0) is the microbial reduction ($1/10^{-5}$, or 5-log reduction),
- t is the time required for the desired reduction,
- D is the D-value at the specific temperature.

For the selected treatment temperatures: At 70°C, a time of 3 minutes was calculated (T1: 70°C, 3 min). This temperature was chosen based on the previous section which identified 70°C as the optimal treatment temperature for strawberry nectar. At 90°C, an equivalent reduction was achieved in 30 seconds (T2: 90°C, 30 s). Additionally, T0 was set as control to assess changes in microbial reduction, and other parameters compared to the untreated sample.

Figure 3.9 illustrates the effect of pH, TSS, and the different treatments (T0, T1, and T2) on the reduction of *E. coli* ATCC8739 in strawberry nectar.

One key observation is that the desired 5-log reduction was not achieved across any of the conditions. This is probably due to the limitations of the treatment conditions, particularly in relation to the plastic Eppendorf tubes used during processing, affecting the overall inactivation rate. Even though, all samples were treated under the same batch conditions for each treatment combination.

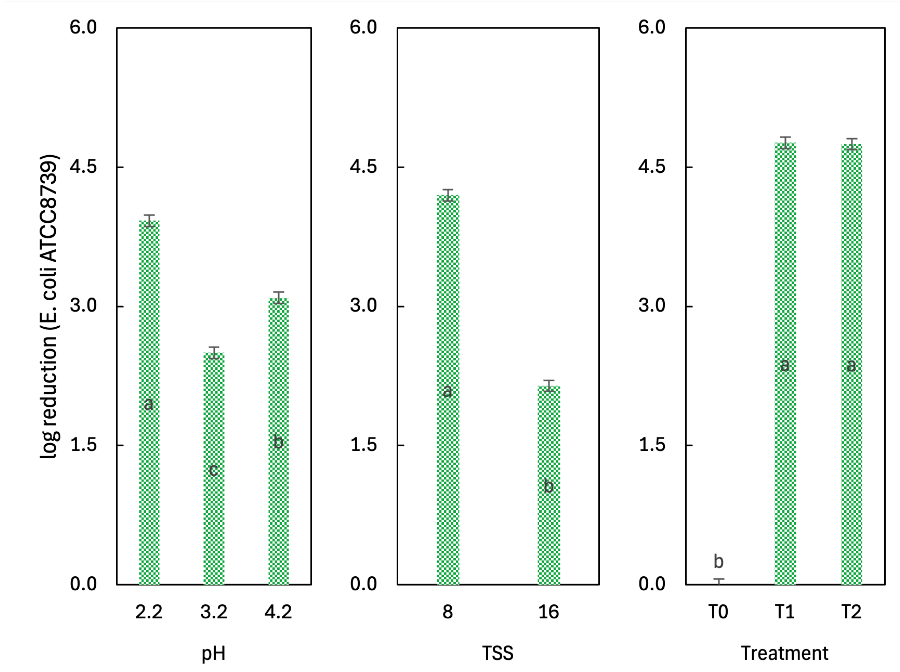


Figure 3.9. Effect of pH, total soluble solids (TSS), and treatment on *E. coli* surrogate log reduction in strawberry nectar and. (T0 = control, T1, T2)

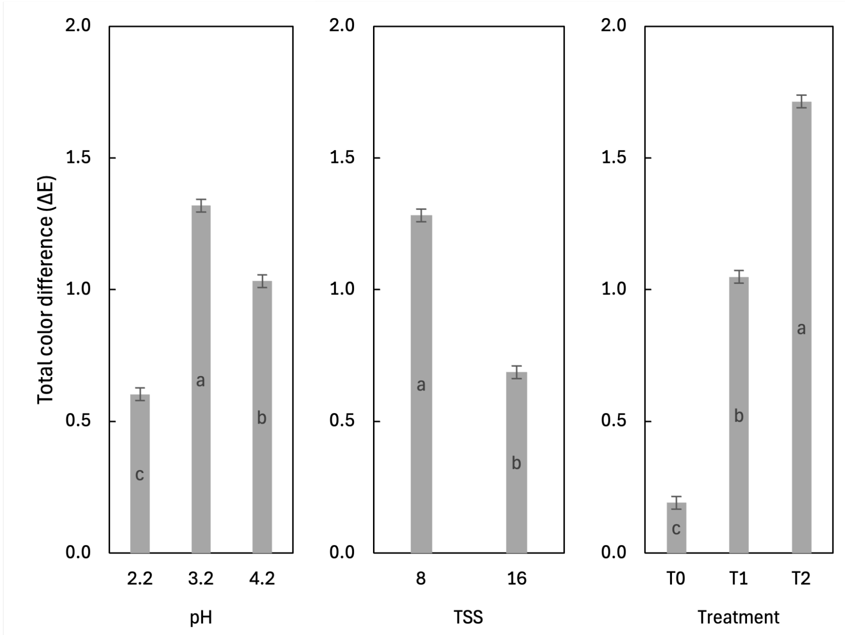
In panel (A) pH has a significant effect on microbial inactivation, showing a quadratic trend with the lowest reduction at pH 3.2. The pH level directly impacts the environment for microbial growth, therefore, changes in acidity will contribute significantly to microbial inactivation [7,8]. Regarding panel (B), TSS has a significant effect on microbial reduction, as demonstrated by a higher reduction at 8°Brix. This result contrasts with findings in the literature, which generally indicate that higher TSS levels reduce water activity thus increasing microbial inactivation [33]. This difference suggests a possible synergistic effect between TSS and pH or a protective effect of sugar against osmotic pressure, as proposed in the study by Podolak et al. [8].

The ANOVA table (Table A.2.1) highlights the significant interactions between pH and TSS. Microbial inactivation response to one factor will depends on the levels of the other, e.g. TSS effect on *E. coli* reduction will depend on pH level. From the two-way interactions, it can be noticed that at higher TSS the effect on microbial reduction is more pronounced for lower pH levels. Therefore, optimizing these parameters together would be critical to achieve the effective microbial reduction.

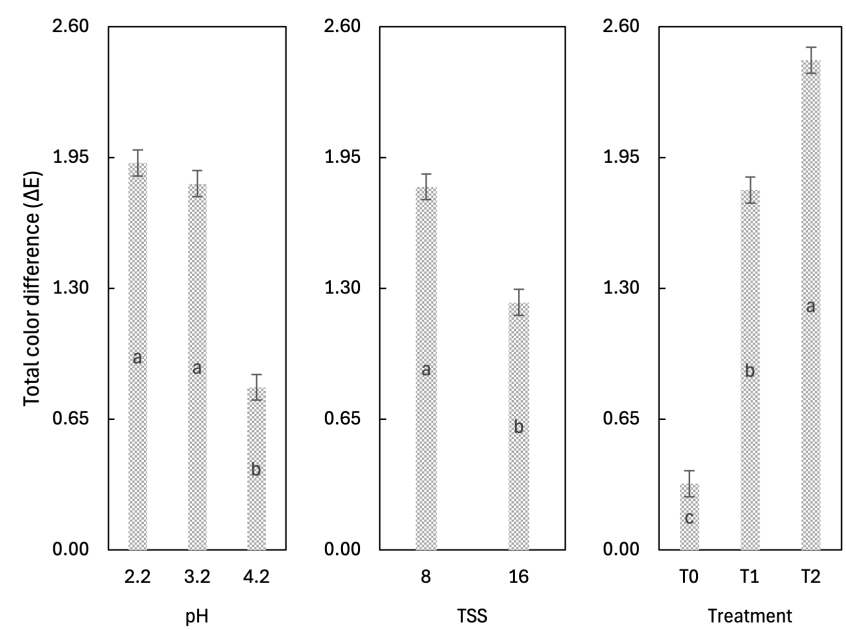
3.2.2.2 Effect of the Matrix on Color of Strawberry and Sour Cherry Nectar

Figure 3.10 shows the ΔE for sour cherry nectar (A) and strawberry nectar (B) under different pH, TSS, and treatment conditions. In general, pH plays an important role in color stability in both sour cherry and strawberry nectars. In sour cherry lower pH (2.2) results in smaller color changes, while higher pH (4.2) shows greater ΔE, in strawberry the effect is the contrary showing greater ΔE at lower pH values. However, the overall magnitude of color change is higher in strawberry nectar compared to sour cherry nectar, suggesting that the matrix of strawberry nectar is more sensitive to pH changes. As can be seen in Figure 3.10, panel (B), the strawberry nectar exhibited higher color differences at lower pH levels. This may be attributed to the degradation of vitamin C in highly acidic conditions, which accelerates the formation of Maillard-type byproducts [42]. The degradation of vitamin C under these conditions can lead to browning reactions [35,43], contributing to the greater color differences observed, even at lower pH values, where phenolic compounds are mean to be more stable.

Both nectars show a significant impact of TSS on ΔE. In both cases, higher TSS (16°Brix) results in lower ΔE, while lower TSS (8°Brix) results in more color change. This indicates that higher sugar content may provide some protective effect on color stability, probably by stabilizing phenolic compounds [44].



(A)



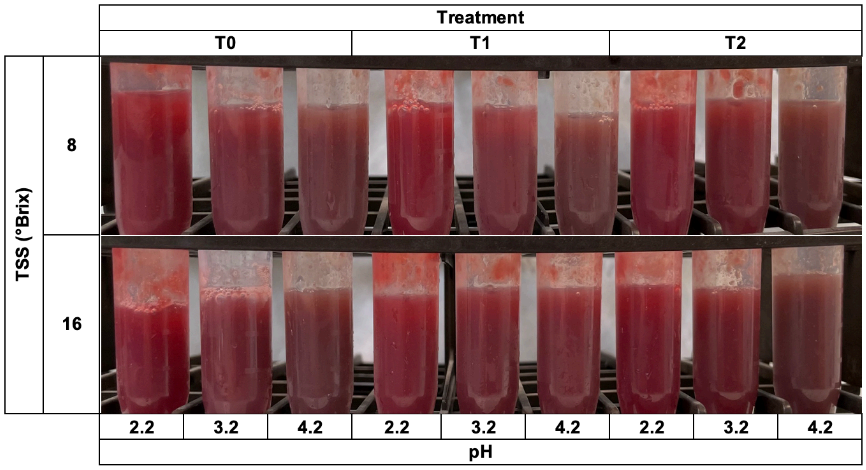
(B)

Figure 3.10. Effect of pH, total soluble solids (TSS), and treatment on total color difference (ΔE) in (A) sour cherry nectar and (B) strawberry nectar. (T0 = control, T1, T2)

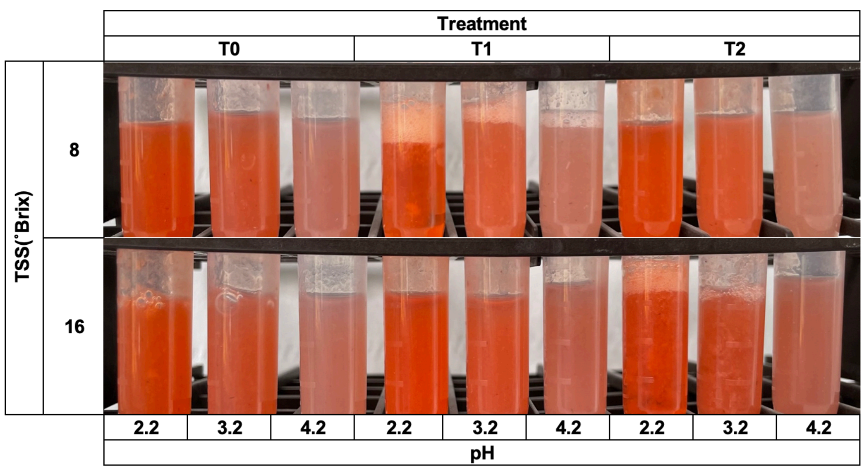
Treatments T1 and T2 have a significant impact on ΔE for both sour cherry and strawberry nectars, the untreated samples (T0) have the lowest ΔE values as expected, meaning the color remains closest to the original by the solely addition of sugar or citric acid. However, after treatment, especially T2 (90°C, 30 s), the total color difference increases significantly. This result suggests that temperature is a key factor in the color degradation of both products.

Strawberry nectar seems to have lower color stability than cherry nectar, it shows higher values of ΔE at the same changes in pH, TSS, and treatment than sour cherry. This could be due to the high phenolic content in sour cherries [44], compared to strawberries. The most significant ΔE, all together, is seen at pH 3.2, 8 °Brix, and after treatment T2 (90°C, 30 s). In Figure 3.11, panel (A) cherry nectar shows overall smaller color differences, indicating better color stability than strawberry nectar, which is supported by the ΔE values.

Pigments, like anthocyanins, appears to be more stable to pH and temperature changes. The TSS effect on ΔE is more prominent in this picture, as higher TSS clearly leads to lower visual change in color. In panel (B) for strawberry nectar, the samples display a similar trend to sour cherry nectar but with more pronounced visual changes. Color loss is evident at both lower and higher TSS, particularly at a higher pH (4.2) and following more intense treatments (T2). It can be notices that at lower TSS (8°Brix), there is more color loss, especially with higher pH (4.2). This reinforces the idea that higher sugar levels can help to stabilize the color.



(A)



(B)

Figure 3.11. Visual Representation of color changes in (A) sour cherry nectar and (B) strawberry nectar under different pH, TSS, and treatment conditions. (T0 = control, T1, T2)

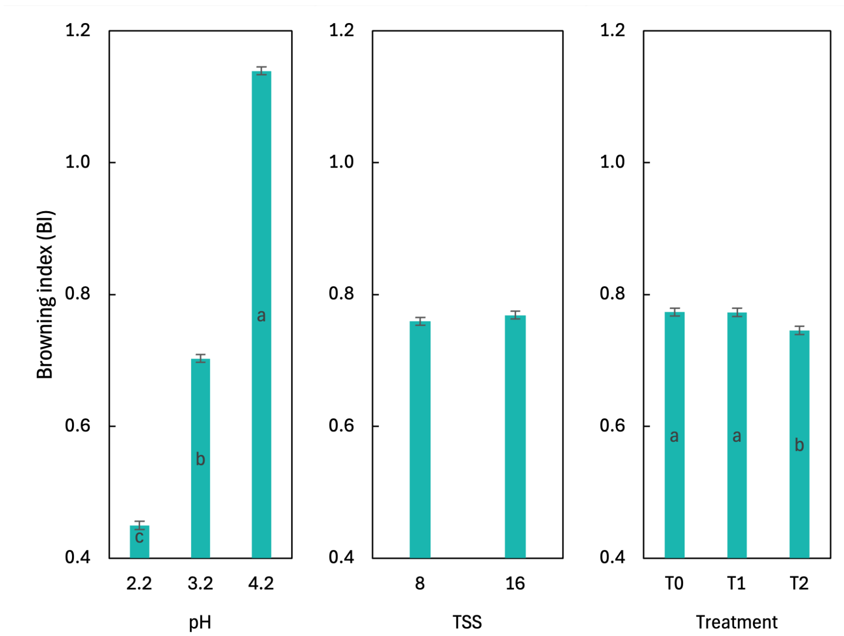
3.2.2.3 Effect of the Matrix on Browning Index (BI) of Strawberry and Sour Cherry Nectar

Figure 3.12 shows the effect of pH, TSS, on the BI in (A) sour cherry nectar and (B) strawberry nectar at three treatment levels: T0 (control), T1 (70°C, 3 min), and T2 (90°C, 30 s). Significant differences were found for all parameters except for TSS in cherry nectar.

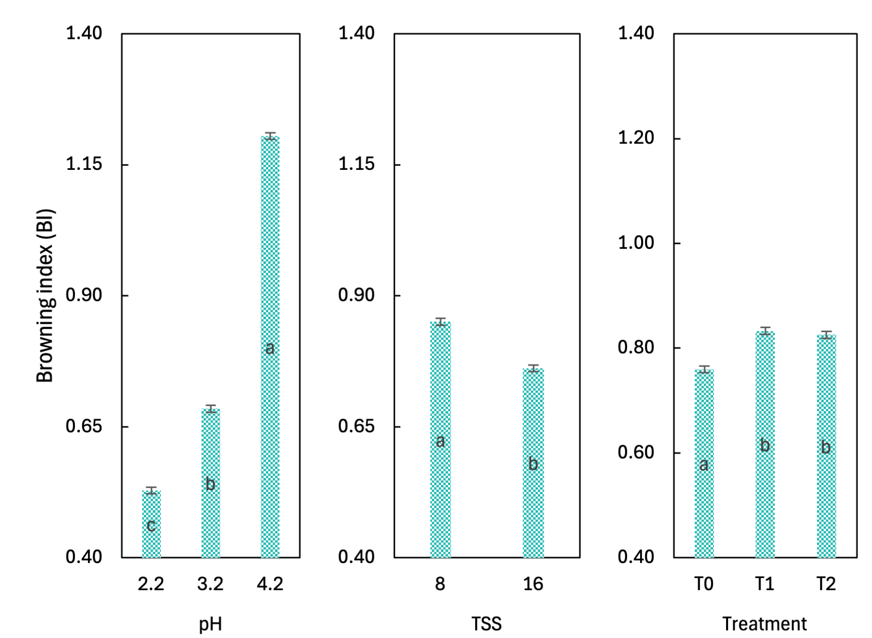
The ANOVA tables (Table A.2.4, Table A.2.5) for strawberry and sour cherry nectars reveal that, for sour cherry, TSS has no effect on the BI, with only pH having a significant effect on browning. In contrast, for strawberry nectar, TSS plays a significant role, together with two-way interactions between pH and TSS. Across both nectars, pH is the most critical factor, with lower pH values minimizing browning in the samples. This suggest that more acidic conditions help prevent browning reactions, by the stabilization of anthocyanins [10].

The effect of TSS on browning is less pronounced, with both 8 and 16 °Brix showing similar browning indices. Considering treatment, similar levels of browning are shown in between T1 and T2 respect to the control T0, this trend is consistent in both strawberry and cherry nectars, suggesting that pH in the key factors influencing the degree of browning, while TSS and treatment with less noticeable effect.

For cherry nectar, the browning index increased significantly at higher pH levels. This is consistent with previous studies that indicate pH plays a critical role in browning reactions, with higher pH accelerating non-enzymatic browning, including Maillard-type reactions and phenolic degradation [35,45]. In strawberry nectar, however, the browning index was affected by pH, TSS, and their interactions. As can be seen in the interaction plot (Figure A.2.4), higher TSS levels contributed to greater browning, at higher pH values, probably due to Maillard reactions, favored by higher pH [34].



(A)



(B)

Figure 3.12. Effect of pH, total soluble solids (TSS), and treatment on browning index (BI) in (A) sour cherry nectar and (B) strawberry nectar. (T0 = control, T1, T2)

3.2.2.4 Effect of Matrix on Antioxidant Activity by DPPH Scavenging Method on Strawberry and Sour Cherry Nectar

Figure 3.13 explains the Radical Scavenging Activity (RSA) as a measure of antioxidant capacity in (A) sour cherry nectar and (B) strawberry nectar, showing trends that are largely consistent in between the two products. For both, the RSA increases at mild pH (3.2) and higher TSS (16°Brix). However, an important difference between the two fruits is observed in the response to thermal treatment.

In sour cherry nectar (A), the RSA significantly increases with more intense treatment (T2), while in strawberry nectar (B), the antioxidant activity decreases under the same condition. This indicates a more pronounced degradation of antioxidant compounds with temperature in strawberry [46]. Additionally, the pH effect is more prominent in sour cherry nectar, with the highest RSA observed at pH 3.2 (53.7%). In contrasts with strawberry nectar where the impact of pH on RSA was less pronounced. This suggests that while similar patterns are observed, the matrix of each fruit plays a critical role in how antioxidants respond to processing conditions, with sour cherry being more stable under intense thermal treatment compared to strawberry nectar.

In summary, the key difference between the two fruits lies in the treatment effect, highlighting the fruit-specific response to processing. This effect can be explained as strawberry's antioxidants, likely vitamin C, are more sensitive to heat degradation [46,47].

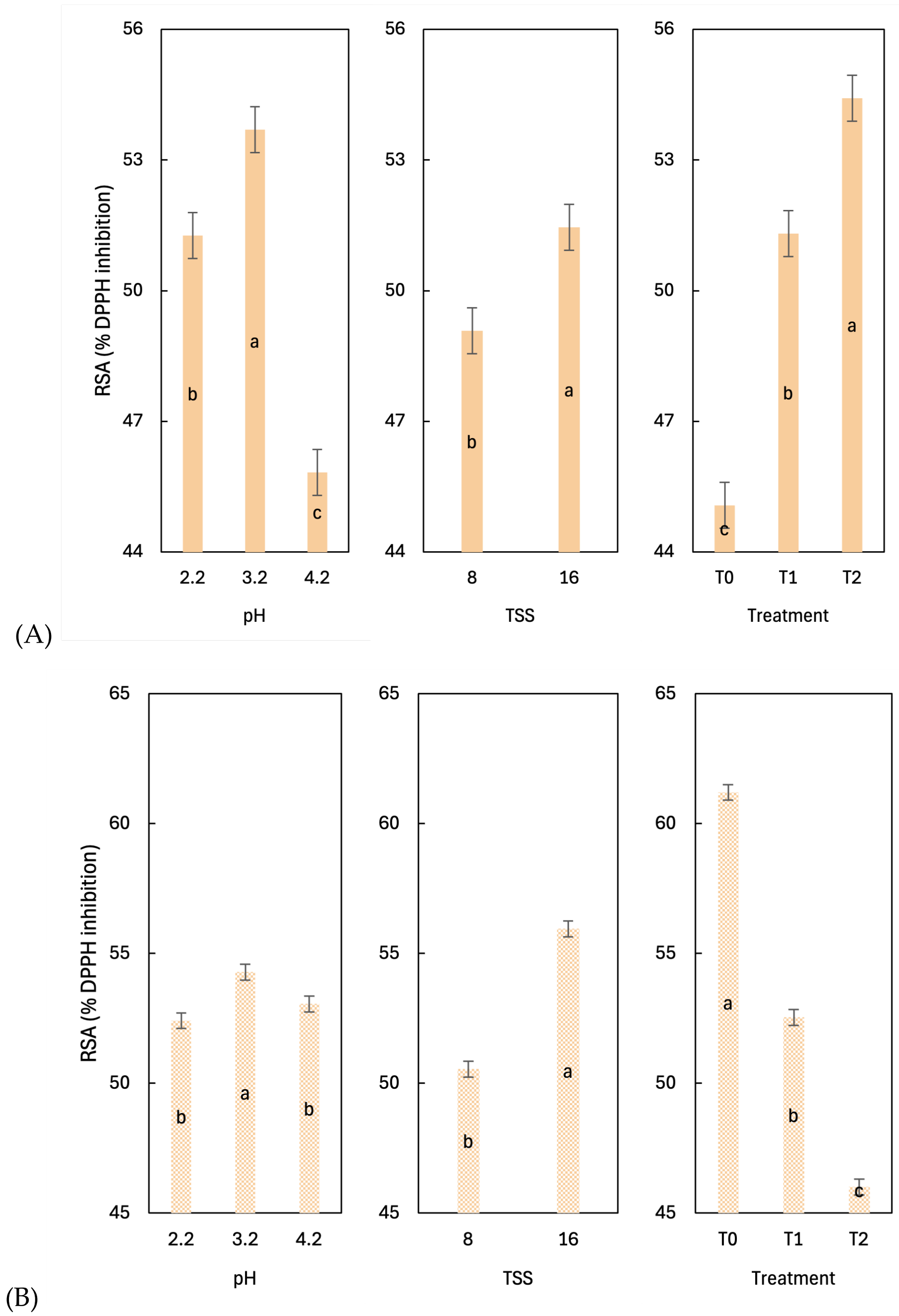


Figure 3.13. Effect of pH, total soluble solids (TSS), and treatment on Radical scavenging activity (RSA) in (A) cherry nectar and (B) strawberry nectar. (T0 = control, T1, T2)

3.2.2.5 Effect of the Matrix on Polyphenol-oxidase (PPO) Activity in Sour Cherry Nectar

The polyphenol oxidase (PPO) activity in the strawberry nectar was remarkably low, and no significant increase in absorbance could be detected at 420nm, even after 30 minutes of measurements. Due to this fact, the PPO activity for strawberry nectar was not reported in this study. Consequently, only the results for sour cherry nectar are discussed.

Figure 3.14 shows the effect of pH, TSS, and treatment of the relative activity of PPO in sour cherry nectar. The figure shows that pH, TSS, and treatment significantly impact the PPO residual activity. Lower pH leads to higher enzymatic inactivation, as PPO activity is reduced in acidic conditions [48]. Similarly, higher TSS (16°Brix) results in stronger inactivation compared to 8°Brix, likely due to osmotic effects [33]. In terms of the treatments, T1 results in a significant reduction in activity, but T2 shows complete inactivation. This clearly shows the effect of the temperature on the enzymatic reduction.

All two-way interactions showed significant differences, as indicated in ANOVA Table A.2.8. The interaction between pH and TSS plays an important role in PPO reduction. The combination of low pH and high TSS results in the greatest inactivation of PPO, while higher pH and lower TSS result in higher enzymatic activity. This suggests that both acidity and osmotic pressure contribute to the overall stability of PPO. In conclusion, pH, TSS, and treatment significantly influence PPO reduction, highlighting the importance of matrix conditions in enzyme inactivation.

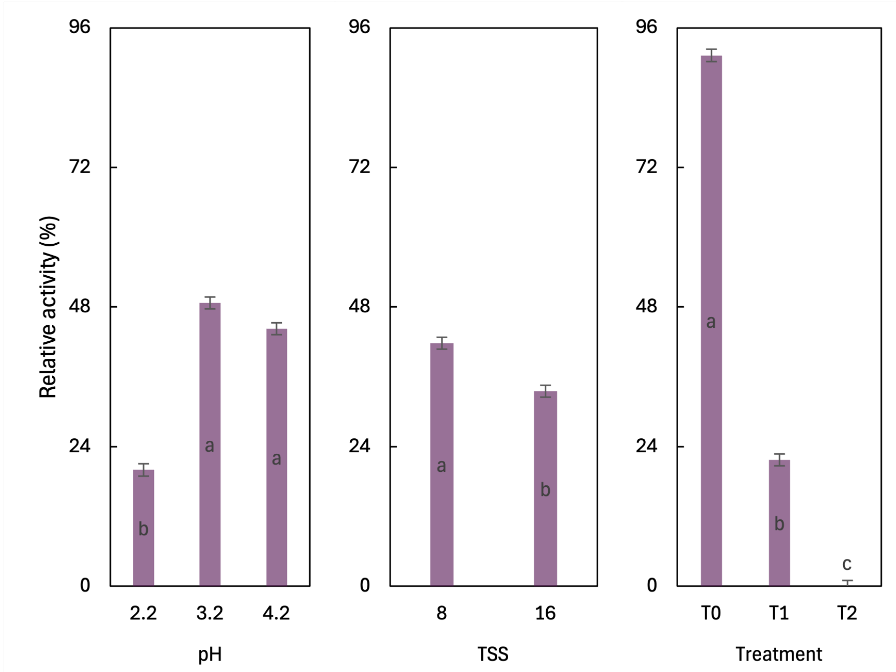


Figure 3.14. Effect of pH, total soluble solids (TSS), and treatment on Polyphenol oxidase (PPO) relative activity (%) in sour cherry nectar. (T0 = control, T1, T2)

Although T1 and T2 achieve the same log reduction in microbial inactivation, T1 was unable to completely inactivate PPO (relative activity of 21.73%), highlighting the difference in inactivation kinetics between enzymes and microbes. Additionally, changes in color and antioxidant activity varied between treatments, emphasizing the need for a more holistic approach when evaluating food processing methods. This "4D approach" should consider microbial reduction as the first dimension, alongside with enzyme inactivation, physical parameters like color, and nutritional aspects such as antioxidant activity, which are directly related to the content of vitamin C and phenolics [49,50]. This perspective ensures that all critical factors for product quality and safety are addressed.

Based on this approach, an optimization of parameters was performed for both strawberry and cherry nectars. For strawberry nectar, the objective was to maximize microbial reduction while minimizing changes in color and antioxidant activity. The optimal parameters identified were pH 2.2, TSS 16°Brix, and Treatment 1 (70°C, 3 min). For cherry nectar, in contrast, the focus shifted to maximizing enzyme inactivation and the optimal parameters for cherry nectar were pH 3.2, TSS 8°Brix, and Treatment 1 (70°C, 3 min). These results highlight two important points: (1) antioxidant activity, browning, and color degradation are not only dependent on pH and TSS, matrix but also on the product matrix, as the behavior differed between strawberry and cherry nectar; and (2) even if the two thermal treatments achieved equivalent microbial reduction (~4.75 log), they had varying effects on other components. In this case, a mild pasteurization (70°C, 3 min) was better for maintaining quality, reinforcing the importance of a 4D approach that considers microbial reduction, enzyme inactivation, physical parameters, and nutritional aspects.

3.2.3 Conclusions

The study on matrix effects on color, antioxidants, enzymes, and microbial safety in strawberry and sour cherry nectar revealed that Microbial reduction in strawberry nectar is mainly influenced by pH. Moreover, antioxidant activity, is highly influenced by pH, TSS, and treatment level, with strawberry nectar being more sensitive to thermal degradation. Higher TSS was found to help reducing ΔE, and lower values of pH were found to minimize browning. These results highlight the importance of optimizing treatment parameters based on pH, and treatment intensity, while also focusing on the specific fruit matrix, while pH is an important factor to control browning and overall product quality.

3.3 Impact of Thermal and High-Pressure Processing on the Viscosity and Color of Strawberry Nectar

The bright red color of strawberry nectar is an important visual quality attribute that significantly affects consumer preference of strawberry-based products [27]. This distinctive color is largely due to the presence of anthocyanins, which not only provide color but also contribute antioxidant properties to the nectar [13]. However, thermal processing often leads color degradation, due to anthocyanins loss during processing and Maillard reaction, which can occur during or after heat treatment. This color degradation can lead to a noticeable decrease in product quality [5]. Maintaining color stability is essential to ensure the sensory appeal and consumer acceptance of strawberry nectar [16].

Additionally, the rheological analysis of foods plays a crucial role in processing, quality control, and sensory evaluation [51]. Viscosity is an important quality factor in liquid foods, as it directly affects the mouthfeel, flavor perception and overall sensorial quality [52]. While the effects of thermal processing on nectar viscosity have been widely studied, there is limited research on how High-Pressure Processing (HPP) impacts the rheological properties of strawberry nectar. This research gap is particularly relevant in the context of quality of the product and later for long-term storage, where both viscosity and microbial stability are critical for product shelf life.

The main objective of this study was to assess the quality of strawberry nectar processed using HPP at three pressure levels (400, 500, and 600 MPa) and compare the outcomes with those from thermal pasteurization treatments at three temperatures (70°C, 80°C, and 90°C), as well as with an untreated control sample, focusing on color changes and rheology.

3.3.1 Materials and Methods

The strawberry puree, used as the raw material, and the processing procedure followed the methods outlined in the previous section (3.1.1.1 and 3.1.1.2.). For the thermal treatments, the nectar was placed in polyethylene (PE) bags (50 x 70 mm) with a zipper seal, containing approximately 12 mL of sample per bag. For the HPP treatments, the nectar was packaged in polyamide/polyethylene (PA/PE) bags (120 mm x 200 mm, 100 μm). Approximately 60 mL of nectar was placed inside each bag and sealed under vacuum. Three samples were prepared for each measurement point.

3.3.1.1 Thermal and High-Pressure Processing (HPP) Treatments

Thermal treatments were carried out in a thermostatic water bath (MPM Instruments, Italy) at temperatures of 75°C, 80°C, and 85°C for durations of 1 and 2 minutes, based on most common thermal treatments in fruit juices [19]. The heating process started immediately after placing the samples into the water, and after the designated treatment time, the samples were immediately cooled down in an ice bath to stop the thermal effects.

For the HPP samples the treatments were performed using a 300 L high-pressure plant unit (Avure Technologies Inc., Kentucky, United States) at HPP Italia Srl. Cold water at 4°C was used as the pressure transmission medium, hence the temperature rise during compression remained within 2 to 3°C per 100 MPa. The HPP treatments were conducted at pressure levels of 400, 500, and 600 MPa for 3 and 5 minutes. The pressure levels were selected to ensure microbiological safety and enzyme inactivation, as reported in previous studies [53,54]. The come-up time for each pressure level was approximately 80 seconds, and decompression occurred immediately after the treatment. The samples were prepared in triplicate for each treatment condition.

3.3.1.2 Rheological Measurements

Rheological parameters of the nectar samples were measured using an Anton Paar rheometer (MCR 702e, Anton Paar, Austria) equipped with a cylindrical probe of 26.7 mm diameter. The samples were subjected to increasing shear rates ranging from 10 s^{-1} to 300 s^{-1} at a constant temperature of 25°C, the values of shear stress were obtained from the measurements and the flow curves were calculated. Flow curves reveal non-Newtonian behavior of the samples. To model this behavior, the Ostwald-de Waele model was applied, as it is the most common and appropriate model for liquid foods [55]. Ostwald-de Waele model is expressed as:

$$\tau = \mathrm{K} \cdot \dot{\gamma}^{n} \tag{3.6}$$

where:

- τ is the shear stress (Pa),
- K is the consistency index ($Pa \cdot s^n$),
- $\dot{\gamma}$ is the shear rate (s^{-1}), and
- n is the flow behavior index (dimensionless).

With a linear regression in the model, the parameters n (flow behavior index) and K (consistency index) were determined for each sample.

3.3.1.3 Color Measurements

A Minolta colorimeter (CM 2600D, Minolta Co., Japan), equipped with a standard illuminant D_{65} was used to analyze the color of the samples. The nectar was poured into transparent plastic petri plates (100 mm x 15 mm), and measurements were taken at 9 different points on the surface. The

CIEL*a*b* color parameters (L*, a*, b*) were obtained, and the total color difference (ΔE) was calculated, as previously described (3.1.1.4). According to the calculated ΔE values, the differences in perceivable color were classified based on the study by Cserhalmi et al. [56] as follows:

- ΔE = 0 – 0.5: no noticeable difference,
- ΔE = 0.5 – 1.5: slightly noticeable difference,
- ΔE = 1.5 – 3.0: noticeable difference,
- ΔE = 3.0 – 6.0: well-visible difference,
- ΔE = 6.0 – 12.0: great difference.

These categories were used to interpret the color changes after treatment.

3.3.1.4 Data Analysis

Data analysis was conducted using one-way ANOVA to evaluate the differences both between and within the treatments. When significant differences were found, post-hoc Tukey's tests were performed to identify specific group differences, with a significance level set at 0.05. All statistical analyses were conducted using JAMOVI software, version 2.3.28.

3.3.2 Results and Discussion

3.3.2.1 Impact of Treatment on Color Parameters

In the Figure 3.15, the ΔE of the samples are shown, the blue bars represent the samples treated with HPP while the green bars represent the samples subjected to thermal treatments. Firstly, it can be noticed that the thermally treated samples exhibited higher color differences compared to the HPP-treated ones. This difference can be attributed to Maillard-type reactions and vitamin C degradation, that are temperature dependent reactions [34,50], therefore occurring in thermally treated juices, as generally, no significant difference in anthocyanins or phenolic compounds reduction after treatment are reported between thermal and HPP treatments [57,58].

Statistical analysis also highlights the significance of these observations. The 85°C thermal treatment for 2 minutes showed the most significant color change (great difference), while HPP samples treated for 3 minutes and 75°C for 1 min, showed the least. This emphasizes that processing conditions, whether thermal or non-thermal, play a crucial role in color stability.

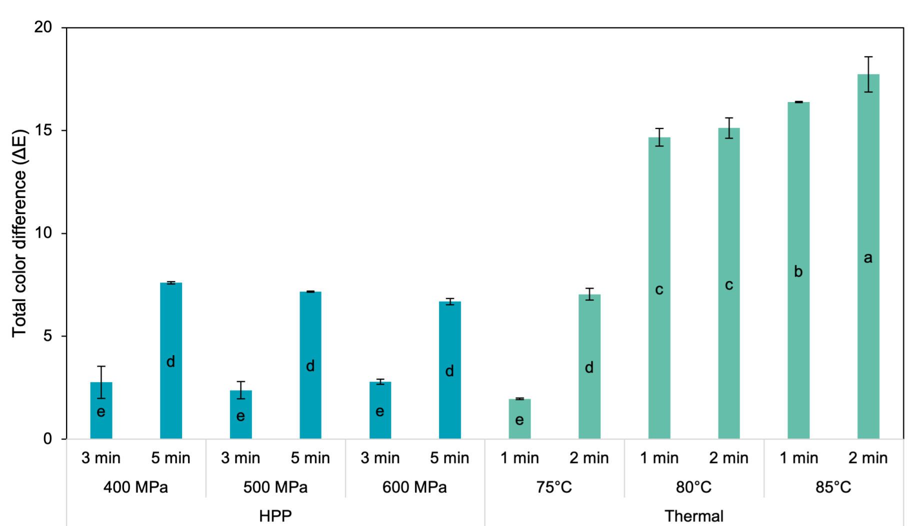


Figure 3.15. Total color difference (ΔE) in strawberry nectar after High pressure processing (HPP) and Thermal treatments. The lowercase letters on each bar indicate statistically significant differences between treatments ($p < 0.05$)

The ΔE values observed in the HPP-treated samples may be attributed to the intensified color caused by the extraction of anthocyanin pigments from the pulp into the intracellular juice under the influence of pressure [59]. Therefore, at higher pressures (600 MPa) the ΔE is higher.

While in this study HPP samples show lower ΔE than thermal treaded ones, sample difference was still ranked as noticeable differences, in contrast to the study of Marszałek et al. [59], where no noticeable difference was noted between control and HPP treated samples at 500 MPa for up to 15 min. This suggests that, although HPP is non-thermal, the pressure directly affects color by extracting pigments, such as phenolic compounds and anthocyanins, from the cell wall [60].

In addition to the ΔE, the a* value, which represents redness, was also studied. Since strawberry is a red fruit, redness (a*) is a key parameter to assess, as it plays a significant role in the visual quality and consumer perception of the product. Moreover, redness on strawberry have been related to bioactive compound levels [12].

Figure 3.16 shows the a* values of nectar samples after HPP and thermal treatments compared to a control. HPP-treated samples show a general reduction in a* values compared to the control. The reduction is more pronounced at longer times, with significant decreases observed at 5 minutes, in accord with the well visible difference showed in ΔE. Samples treated with thermal methods maintain a* values closer to the control, especially at lower temperatures (75°C and 80°C). At 85°C, both 1-minute and 2-minute treatments show a noticeable reduction in a* indicating more significant color loss, all these changes can be related to the stability of bioactive compounds, affected by higher temperatures and higher treatment times in HPP [12,61,62].

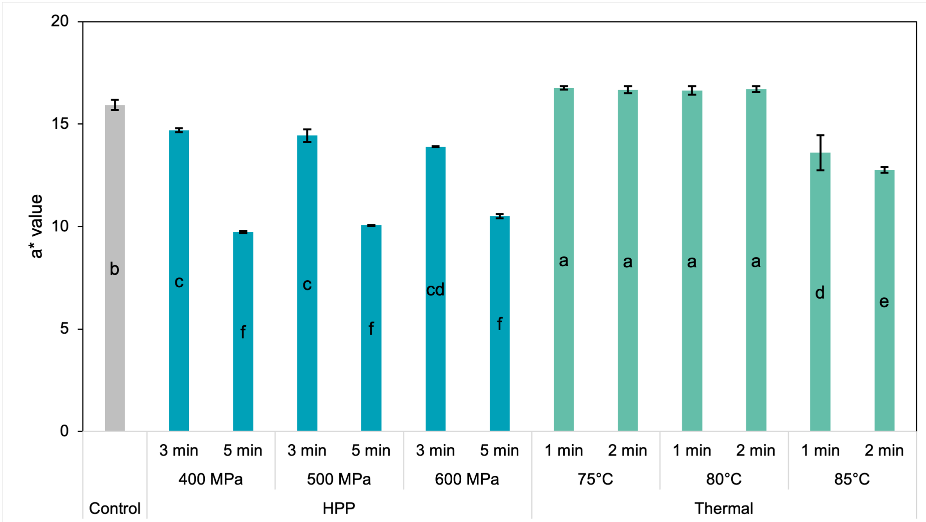


Figure 3.16. Redness (a) in strawberry nectar after High pressure processing (HPP) and Thermal treatments. The lowercase letters on each bar indicate statistically significant differences between treatments ($p < 0.05$)

3.3.2.2 Impact of Treatment on Rheological Parameters

The Figure 3.17 shows flow curves of selected samples representing the relationship between shear stress and shear rate for strawberry nectar treated with HPP and thermal treatments. The control sample exhibits the lowest shear stress values, while the HPP-treated samples are positioned in the upper region of the plot, with curves with higher pressures and longer times (e.g., 600 MPa, 5 min) showing the highest shear stress. Thermal treatments (80°C and 90°C) result in intermediate shear stress values. In general, the higher shear stress values for HPP-treated samples indicate that this treatment increases the viscosity of the nectar more significantly than thermal treatments, likely due to pressure-induced structural changes [63].

The increase in shear stress for HPP-treated samples compared to thermal and control samples suggests that HPP may induce structural changes in the nectar matrix, increasing its viscosity. This change can be explained by the cell wall disruption [18], or gelatinization of the pectin and other polysaccharides [64], which may increase the viscosity by increasing the soluble solids or by forming a more complex network in the matrix.

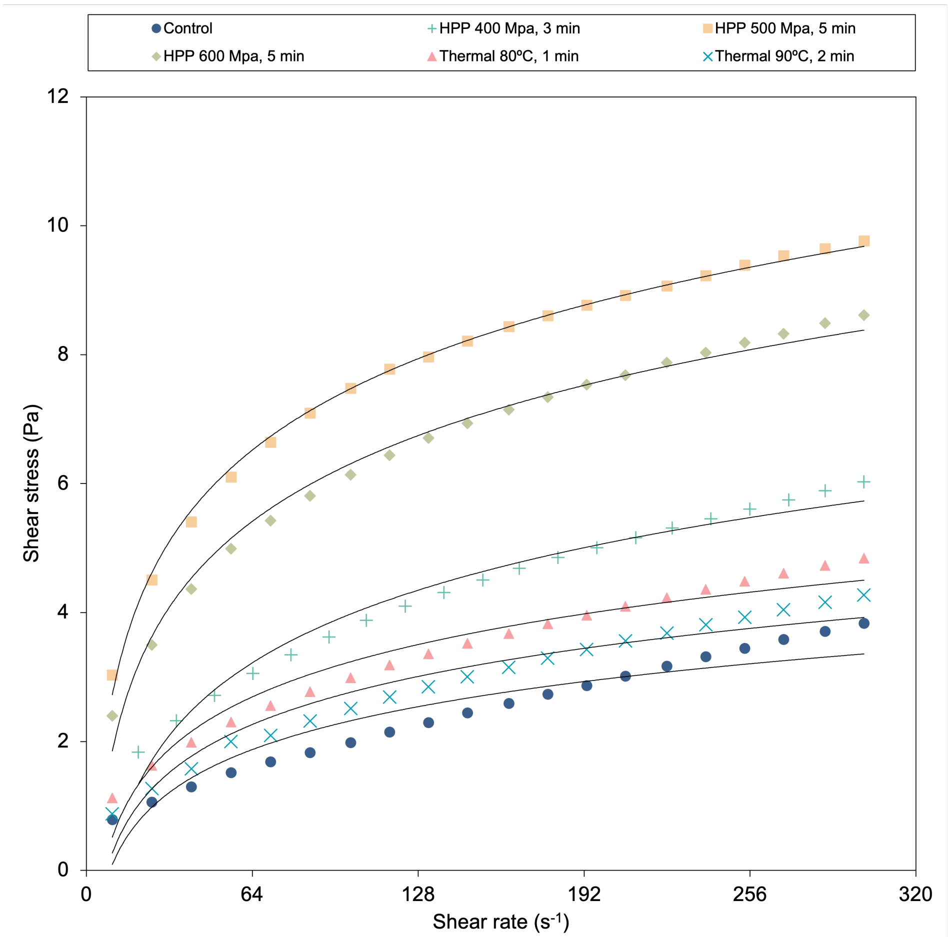


Figure 3.17. Flow curves of shear stress vs. shear rate for strawberry nectar selected samples treated by High pressure processing (HPP) and Thermal treatments with Ostwald-de Waele model regression. The different symbols represent specific treatments (as indicated in the legend).

For further analysis,

Table 3.2 presents the rheological parameters n (flow index) and K (consistency coefficient) of the Ostwald-de Waele model for strawberry nectar subjected to HPP and thermal treatments. These parameters describe how the nectar behaves under shear stress and offers insights into its viscosity. The flow index (*n*) indicates the degree of non-Newtonian behavior of the nectar [51]. The *n* values on the samples ranged from 0.24±0.02 to 0.61±0.07. Values less than 1 indicate pseudoplastic behavior (shear-thinning), meaning viscosity decreases with increasing shear rate. For HPP-treated samples, the values of n are generally lower than the control for the longer treatment times (5 min), especially at 400 MPa (0.24). These lower *n* values suggest more pronounced shear-thinning behavior, indicating structural changes. Consistency coefficient (K) and *n* values were in the range of those reported for fruit juices and purees [65].

Table 3.2. Rheological parameters (*n* and K) of Ostwald-de Waele model for strawberry nectar subjected to High pressure processing (HPP) and Thermal treatments

Treatment	Parameters	n*	K (Pa·sn)*
Control	—	0.53±0.02 a	0.18±0.02 d
HPP	400 Mpa, 3 min	0.61±0.07 a	0.14±0.04 d
	400 Mpa, 5 min	0.24±0.02 c	2.34±0.29 a
	500 Mpa, 3 min	0.60±0.07 a	0.15±0.04 d
	500 Mpa, 5 min	0.33±0.02 b	1.58±0.37 b
	600 Mpa, 3 min	0.55±0.01 a	0.35±0.00 d
	600 Mpa, 5 min	0.37±0.00 b	1.09±0.11 c
Thermal	75°C, 1 min	0.53±0.01 a	0.18±0.01 d
	75°C, 2 min	0.49±0.05 a	0.30±0.10 d
	80°C, 1 min	0.45±0.08 a	0.41±0.19 d
	80°C, 2 min	0.46±0.07 a	0.37±0.16 d
	85°C, 1 min	0.51±0.05 a	0.23±0.07 d
	85°C, 2 min	0.49±0.06 a	0.28±0.10 d

*Different lowercase letters in the same column indicate significant differences among treatments according to Tukey's test ($p < 0.05$).

Thermally treated samples show relatively higher *n* values, closer to the control. It has been reported in literature that thermal treatment often increases the value of *n* [51]. In this study no significant difference was found, suggesting that thermal processing does not alter the flow behavior.

The consistency coefficient reflects the thickness or viscosity of the nectar under shear stress. Higher K values indicate greater viscosity [55]. HPP at 400 MPa for 5 min (K = 2.34 Pa·s^n) and 500 MPa for 5 min (K = 1.58 Pa·s^n) show the highest. This is consistent with the flow curves shown in the previous figure, where higher shear stress is observed for these treatments. Thermal treatments had no significant difference with the control on the K values, indicating lower effect on rheology compared to HPP. In general, thermal processing maintain the viscosity, while HPP thickens the nectar, especially at longer times, thickens the nectar.

3.3.3 Conclusions

High-Pressure Processing (HPP) and thermal treatments both impact the color of strawberry nectar. HPP generally results in lower total color differences (ΔE). This suggests that HPP better preserves the color of the nectar, likely due to the absence of heat induced reactions. Rheological analysis shows that HPP increases the viscosity of strawberry nectar more than thermal treatments, due to pressure-induced structural changes. Longer HPP treatments further enhance viscosity, shown by higher consistency coefficient (K) values and lower flow behavior index (*n*).

3.4 References

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4 Comparative Analysis of Physicochemical Properties and Microbial Stability in Sour Cherry and Raspberry Juices Treated by High-Pressure Processing, Pulsed Electric Fields, and Thermal Treatment

Sour cherry (*Prunus cerasus)* and red raspberry (*Rubus idaeus*) juices are widely appreciated for their vibrant color, and high levels of antioxidant compounds, particularly anthocyanins and phenolics [1,2]. These compounds not only contribute to the antioxidant capacity of the juices but also play a crucial role in their sensorial properties, particularly color [3]. However, preserving these bioactive compounds during processing is challenging because methods that inactivate bacteria, like heat or pressure, can also degrade the juice's quality [4,5].

To meet consumer demands for safe and high-quality juices, advanced technologies like High-Pressure Processing (HPP) and Pulsed Electric Fields (PEF) have been adopted in the juice industry [5]. These technologies are particularly suited for fruit juices like sour cherry and raspberry, which are acidic products (pH < 4.5). In such acidic environments, pathogenic bacteria are less resilient, and there is no need to reach full sterility [6].

The goal is to achieve a 5-log reduction of pathogenic bacteria, which is considered sufficient to ensure microbial safety [7]. This makes HPP and

PEF ideal non-thermal treatments, as they can inactivate pathogens without the need of high-temperature processes that could negatively affect the juice's nutritional and sensorial properties [8].

Despite the research on PEF technology, the mechanism of action in PEF and the influence of processing parameters on microbial inactivation are not yet fully understood [9]. This presents a challenge for optimizing PEF in industrial applications. One of the keyways to improve the understanding of these technologies on microbial degradation is through kinetic modeling, which allows for the prediction of microbial inactivation under different processing conditions [10]. Kinetic models are crucial in food safety, as they help in designing processes that effectively reduce microbial loads while preserving product quality [11].

In contrast to thermal treatments, where microbial inactivation typically follows first order kinetics, the inactivation kinetics in HPP and PEF tend to be non-linear This non-linearity requires more advanced modeling approaches to accurately describe the microbial reduction process [12,13]. For thermal treatments, the Bigelow model has been widely used for over a century [14], but more refined models are necessary to address the complexity observed in modern processes, especially with non-thermal methods like PEF and HPP.

Previous comparative studies between technologies often did not apply the standard 5-log reduction criterion or use treatments commonly applied in commercial settings. Most studies focused on one technology without ensuring equivalent treatment conditions across all technologies, and thermal treatments are typically more severe than those applied for HPP and PEF [15–17]. As a result, these comparisons fail to provide a fair evaluation of thermal technologies or accurately reflect the true effectiveness of non-thermal methods. For a more meaningful assessment, it is essential to apply equivalent processing conditions across all technologies.

Beyond microbial safety, the quality attributes of the juice, such as phenolic content, anthocyanins, and antioxidant capacity, can be degraded during processing. Their degradation during processing can lead to diminished color intensity and reduced health benefits [2,18]. Therefore, it is essential to evaluate how different technologies affect these key quality parameters.

This chapter is divided into two main sections. The first section focuses on kinetic modeling of microbial reduction in sour cherry juice treated with HPP and thermal processes, and in both sour cherry and raspberry juices treated with PEF, to compare each technology's effectiveness in achieving a 5-log reduction of pathogens. The second section evaluates the physicochemical properties of the juices, with a focus on sensorial aspects like color, across the treatments, offering a thorough analysis of how each technology impacts juice quality and stability.

4.1 Microbial Inactivation Kinetics and Modelling in Sour Cherry and Raspberry Juices Treated by Thermal, High-Pressure Processing (HPP), and Pulsed Electric Fields (PEF) Process[1]

Effective microbial inactivation is essential for the safety and quality of acidic juices like sour cherry and raspberry, as harmful bacteria such as *E. coli*O157, *Salmonella sp.*, and *Listeria monocytogenes* can persist in acidic conditions. Despite their naturally acidity, these juices still require effective pasteurization techniques to eliminate potential spoilage organisms and pathogens that may survive in acidic environments [19,20].

Conventional thermal processing is widely used to achieve microbial inactivation, but non-thermal methods such as High-Pressure Processing (HPP) and Pulsed Electric Fields (PEF) are gaining attention for their ability to preserve the sensorial and nutritional properties of juices while ensuring microbial safety [5,21].

Microbial validation in juice processing often involves the use of surrogate microorganisms instead of directly working with the most pathogenic and resistant strains. Surrogates are non-pathogenic organisms that mimic the resistance characteristics of harmful pathogens, making them safer to handle in laboratory settings while providing meaningful data for microbial inactivation [22,23].

Kinetic modeling plays a crucial role in this process, as it helps in understanding how microorganisms, respond to different processing

[1] This section has been partially included in the unpublished paper: "Comparative Modelling of *E. coli* Inactivation in Sour Cherry and Raspberry Juices Under Pulsed Electric Field Treatments". Microbiol. Res. 2024, 15, Firstpage–Lastpage. https://doi.org/10.3390/xxxxx

conditions. Various models, such as Bigelow and Weibull models for thermal treatment and HPP [13,24] or even differential equation models for PEF and HPP [25], have been applied to predict and determine the necessary parameters to achieve 5-log reduction of pathogenic bacteria. Models provide insights into how different factors, such as temperature, pressure, and electric field strength, influence microbial inactivation. Offering a mathematical base for optimization of the processes [26].

In this study, the microbial inactivation of an *E. coli* surrogate (*E. coli* ATCC 8739) on sour cherry and raspberry juices were modeled using both linear and non-linear models to evaluate the effectiveness of different treatments. Additionally, response surface methodology was employed to analyze the interactions between key parameters and their impact on microbial reduction.

4.1.1 Materials and Methods

4.1.1.1 Preparation of the Juice

Pitted sour cherries (*Prunus cerasus*, variety 'Oblačinska') were sourced frozen from Miker Produkt (Serbia), and frozen red raspberries (*Rubus idaeus*) were obtained from Andreas Auer e.U. (Austria). Both frozen fruits were thawed at 4°C before extraction. The juices were then extracted using a 40L hydraulic fruit press (Speidel, Germany) and filtered through a 740 µm sieve. The filtered juice was then packed in aluminum bags and stored at -18°C. Before treatment, the juice bags were thawed at 4°C for one day, then gently warmed in lukewarm water to 20°C before the beginning of trials. For the thermal and HPP trials, only sour cherry juice was used. For PEF treatments, both sour cherry and raspberry juices were prepared and treated to assess the technology’s effectiveness across different juice types.

4.1.1.2 Microbial Preparation and Enumeration

The surrogate *Escherichia coli* ATCC 8739 was obtained from the Microbiology Lab, Department of Food and Drug, University of Parma. The frozen culture was stored at −80°C. Prior to use, the culture was thawed and transferred into (Luria-Bertani) LB broth, followed by incubation at 37°C for 24 hours to activate the microorganism. After this activation step, the culture was inoculated into fresh LB broth and incubated at the same temperature for an additional 16 hours, resulting in a population of approximately 10^8 CFU/mL.

For enumeration, the samples were serially diluted using peptone water as the diluent. The viable population of *E. coli* in the treated fruit juice samples was determined by plating 0.1 mL of the serial diluted samples onto LB agar plates. The plates were incubated at 37°C for 16-24 hours, following the method of Tuttle et al. [27]. Enumeration was performed by counting the colonies formed on the LB agar plates to determine the viable population.

The log reduction of *E. coli* was calculated for the modeling and results using the following equation:

$$Log\ reduction = \log\left(\frac{\mathrm{N}}{N_0}\right) \tag{4.1}$$

where, N_0 = Initial *E. coli* population (CFU/mL), and N = *E. coli* population after the treatment (CFU/mL).

4.1.1.3 Microbial Inoculation

The preparation of the inoculum was based on the total volume of juice used for the experiments. After culturing *E. coli* ATCC 8739 as described above, serial dilutions at a ratio of 1:10 were performed to achieve the desired volume of inoculum. The bacterial suspension was inoculated into the juice at a dilution of 1:10 to obtain an initial concentration of approximately 10^7 CFU/mL before treatment.

4.1.1.4 Processing Conditions

4.1.1.4.1 Thermal Treatment

The inoculated sour cherry juice was poured into 50 mL sterile PET bottles, and thermal treatments were carried out in a thermostatic water bath (MPM Instruments, Italy), with a control sample that remain untreated. A thermocouple was placed in the center of a reference bottle to monitor the temperature. The treatment time started once the center of the bottle reached the target temperature. After treatment, all samples were immediately cooled in an ice bath and stored at 4°C until further testing.

4.1.1.4.2 High-Pressure Processing (HPP)

The inoculated sour cherry juice was placed inside polyamide/polyethylene (PA/PE) bags (120 mm x 200 mm, 100 μm), with approximately 50 mL of juice in each bag, and then sealed. The HPP treatments were conducted using a 300 L high-pressure plant (Avure Technologies Inc., Kentucky, United States) at HPP Italia Srl, Traversetolo (Italy). Cold water at 4°C was used as the pressure transmission medium, with the temperature rising by 2 to 3°C per 100 MPa during compression. The come-up time for each pressure level was approximately 80 seconds, and decompression occurred immediately after the treatment.

4.1.1.4.3 *Pulsed Electric Fields (PEF)*

The PEF treatment was performed on sour cherry and raspberry juice using an ELEA PEF pilot system (Elea GmbH, Quakenbrück, Germany) at the HBLA und BA Klosterneuburg pilot plant (Austria). The PEF system was equipment equipped with two co-linear DN10 PEF treatment chambers, operating in continuous mode. The inlet temperature of the juice was maintained at 40°C using a coil heat exchanger during processing. The PEF processing parameters were set as follows: bipolar square wave pulse shape, a fixed pulse width of 7 μs, and an electrode gap of 10 mm. The pulse peak voltage (kV) and specific energy (kJ/L) were set according to the experimental design parameters. Following treatment, samples were filled into sterile containers, cooled in an ice bath, and stored at 4°C until further analysis.

4.1.1.4.4 *Experimental Design*

For the thermal treatments, a combination of three temperatures (75°C, 80°C, and 85°C) and three treatment times (30, 60, and 120 s) was used, resulting in a total of nine treatment combinations. For HPP treatments sour cherry juice was treated at three pressures (200, 400, and 600 MPa) and for three different times (2, 4, and 6 min), resulting in nine combinations. For the PEF treatments, the juice was subjected to four different electric field strengths (16, 18, 20, and 22 kV/cm) and two specific energy levels (100 and 120 kJ/kg), resulting in eight treatment combinations.

4.1.1.5 Data Analysis

The microbial inactivation data was analyzed using four different microbial reduction models. The models' parameters and indices were calculated in MATLAB (version R2024b, The MathWorks, Inc.). Models were compared by fitting the equations to the microbial reduction data for each treatment. The goodness-of-fit was evaluated by calculating the determination coefficient (R^2) and root mean square error (RMSE), to assess which model better describes the microbial inactivation. The response surface modeling (RSM) analysis was performed using R-Studio (version 2024.04.1, Posit Software, PBC), using the RSM package to explore interactions between variables, applying first- and second-order polynomial equations.

4.1.2 Mathematical Models and Parameters

For the analysis of microbial reduction, four kinetic models were applied, based on the most common used models in Thermal, HPP and PEF microbial reduction [12,13,24,28,29]. Bigelow and Weibull model were applied to describe the behaviors of thermal and HPP samples, while due to the complexity of PEF kinetics [12], three non-linear models were applied in PEF treated samples, Weibull, Peleg and Geeraerd.

4.1.2.1 Thermal and High-Pressure Processing (HPP) Kinetics on Cherry Juice

4.1.2.1.1 Bigelow Model

The Bigelow kinetics model, is a liner reduction model, that is widely used to describe microbial reduction based on log reduction, following the equations [14]:

$$Log\ reduction = \frac{t}{D} \tag{4.2}$$

$$Log\ reduction = \frac{t}{D_P} \tag{4.3}$$

where,

- t = treatment time (min),
- D = decimal reduction time, defined as the time required to reduce the microbial population by one log cycle (CFU/mL),
- D_P = the equivalent of the D-value for HPP. D_P-value represents the time required at a specific pressure to achieve 1-log reduction in microbial population.

4.1.2.1.2 *Weibull Model*

The Weibull model accounts for non-linear inactivation behavior, and is more appropriate for microbial reduction in HPP treatments [24,30]:

$$Log\ reduction = \left(\frac{t}{\delta}\right)^p \tag{4.4}$$

where,

- t = treatment time (min),
- δ = scale parameter (time for the first significant log reduction),
- p = shape parameter, indicating whether the curve is concave or convex.

4.1.2.2 Pulsed Electric Fields (PEF) PEF Kinetics on Sour Cherry and Raspberry Juices

4.1.2.2.1 *Weibull Model – PEF*

The Weibull model has been successfully applied to describe microbial inactivation kinetics of fruit juices treated by PEF [29]:

$$Log\ reduction = \left(\frac{E}{\delta_E}\right)^p \quad (4.5)$$

where,

- E is the applied electric field strength (kV/cm),
- δ_E = scale parameter, as the amount of field strength needed to initiate significant microbial reduction,
- p = shape parameter, indicating whether the curve is concave or convex.

4.1.2.2.2 *Peleg model*

Some microorganisms exhibit sigmoid-shaped survival curves after PEF. Peleg [31] developed a microbial inactivation model, which describes the relationship between field strength and survival fraction:

$$\frac{N}{N_0} = \frac{1}{1 + e^{\frac{E-E_C}{k}}} \quad (4.6)$$

where,

- E is the applied electric field strength (kV/cm),
- E_C is critical field strength (kV/cm), as the minimum electric field strength required to initiate microbial inactivation,
- k is a parameter that indicates the steepness of survival curve around E_C.

4.1.2.3 Geeraerd – Log-Linear with Shoulder

For PEF treatments that show minimal or no reduction at lower electric field strengths, a more suitable model might be a log-linear reduction with a shoulder, based on the model proposed by Geeraerd [32]. This model accounts for the initial resistance to reduction at lower field strengths, before a more significant decrease in microbial survival occurs:

$$\log(N) = \log(N_0) - \frac{k_{max} \cdot \mathrm{E}}{\ln(10)} + \log\left(\frac{\mathrm{e}^{k_{max} \cdot L_S}}{1 + (\mathrm{e}^{k_{max} \cdot L_S} - 1) \cdot \mathrm{e}^{-k_{max} \cdot \mathrm{E}}}\right) \tag{4.7}$$

Where:

- E is the applied electric field strength (kV/cm),
- L_S is the shoulder length (length of the minimal reduction zone) in specific energy units (kV/cm),
- k_{max} is the rate constant in the significant reduction zone in $(\mathrm{kV/cm})^{-1}$.

4.1.3 Thermal and HPP Results and Discussion (Sour Cherry Juice)

The thermal treatment levels were based on the most common temperature range used for High-Temperature Short-Time (HTST) processing of juices [4]. For HPP, it is known that microbial inactivation of *E coli.* becomes effective at pressures of 400 MPa [19]. However, to allow for a more comprehensive modeling approach, pressure values starting from 200 MPa were included in this study.

The parameters for the Bigelow kinetics and Weibull models, along with the determination coefficient (R^2) and RMSE values for the cherry juice, are presented in

Table 4.1. This table provides a detailed comparison of the D-values from the Bigelow model, as well as the δ and p parameters from the Weibull model.

Table 4.1. Bigelow kinetics and Weibull model parameters, R^2, and RMSE Values for sour cherry juice treated by Thermal, HPP and PEF technologies

Treatment	Condition	Bigelow			Weibull			
		D (min)*	RMSE	R^2	δ (min)	*p*	RMSE	R^2
Thermal	75°C	0.83±0.02	0.198	0.952	0.46±0.01	0.62±0.02	0.036	0.998
	80°C	0.37±0.01	1.077	0.775	0.02±0.01	0.39±0.01	0.524	0.947
	85°C	0.33±0.00	2.344	0.476	0.02±0.00	0.48±0.04	0.479	0.739
HPP	200 MPa	4.77±0.40	0.119	0.939	4.57±0.03	0.91±0.07	0.119	0.939
	400 MPa	1.21±0.01	0.721	0.867	0.07±0.00	0.37±0.03	0.036	0.999
	600 MPa	0.87±0.01	1.011	0.865	0.06±0.00	0.43±0.01	0.399	0.979

* In HPP, D refer to D_P-value.

In thermal treatments, D and Z values are commonly used for comparison, as they are frequently referenced in the literature. The Z-value was determined from the calculated D-values, a value of 24.96°C was found. This indicates that an increase of 24.96°C would be required to achieve a tenfold reduction in the D-value. D and z values are on the typical range for *E. coli* reduction in fruit juices and nectars [4,33,34]. Is important to account the importance of the matrix on the different values of these parameters. For example, a study on different acidic juices (pH 3.30–4.73), using the same surrogate (*E. coli* ATCC8739), reported D-values ranging from 0.09 to 0.207 minutes at 73°C. These variations were attributed to differences in the matrix and physicochemical properties of the fruit juices [35].

Remarkably, when comparing the fit of predictive models, the Bigelow model showed a moderate fit, with values of R^2 ranging from 0.476 to 0.952, and relatively high RMSE values in thermal treatments, indicating some deviation from the expected behavior. On the other hand, the Weibull model provided a better fit, with R^2 values consistently above 0.9, except for 85°C. Due to the nature of the data, where bacterial inactivation occurs rapidly, the Bigelow model (linear) was not the best fit, whereas the Weibull model provided a more accurate representation.

In HPP, the Bigelow model shows a good fit at 200 MPa in this matrix. At 200 MPa, bacteria are less affected by pressure [19], showing only a moderate, more linear reduction. However, at higher pressures, 400 MPa and above, the inactivation behavior becomes more complex and non-linear. In these cases, the Weibull model provides a significantly better fit. The higher R^2 values and lower RMSE values confirm that the Weibull model is more suited for predicting microbial inactivation under HPP.

Additionally, the comparison of D-values (from the Bigelow model) and δ-values (from the Weibull model) highlights that at higher pressures, there is a faster microbial inactivation. The comparison of D-values (from the Bigelow model) and δ-values (from the Weibull model) demonstrates that higher pressures lead to faster microbial inactivation. Lower D and δ values indicate that *E. coli* is inactivated more quickly at pressures like 400 MPa compared to lower pressures. The decrease in δ values at higher pressures highlights the rapid microbial reduction, underscoring the Weibull model's ability to capture this accelerated inactivation rate.

Moody et al. [36] found that in HPP-treated apple juice, a pressure of 300 MPa produced a moderate reduction in *E. coli* population. In contrast, at 400 MPa, complete inactivation of *E. coli* (10^7 CFU/mL) was achieved within 3 minutes.

Overall, the findings suggest that while the Bigelow model is effective for modeling thermal and HPP data, the Weibull model offers superior flexibility and accuracy, particularly for non-linear microbial inactivation behavior in HPP treatments [13]. This is consistent with other studies that indicate that the Weibull model is more appropriate for microbial inactivation that does not follow a simple linear pattern [24,37].

The Figure 4.1 compares the microbial inactivation behavior of *E. coli* using two different treatments modeled with Weibull kinetics. The thermal treatment (80°C) shows a steeper linear decline, reaching 5-log reduction before two minutes (1.3 min). This rapid decrease suggests that *E. coli* is more sensitive to thermal treatment. While, for HPP at 400 MPa, the inactivation curve is less steeper showing a gradual inactivation up to 6 minutes, reaching 5 log reduction at 3.8 minutes. This indicates that HPP is effective over a prolonged time, achieving a more consistent and sustained microbial reduction. As discussed earlier, the Weibull model tends to better capture inactivation trends in HPP, due to its ability to model non-linear behaviors.

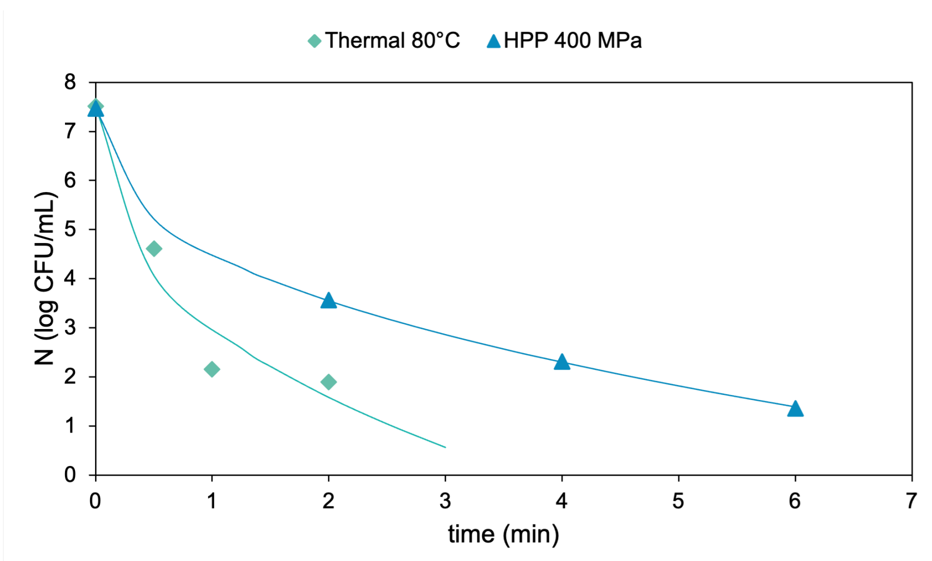

Figure 4.1. Comparison of microbial log reduction curves for thermal (75°C), experimental points (◆), and HPP (400 MPa), experimental points (▲), with Weibull model regression (lines)

4.1.3.1 Response Surface Modeling (RSM) Analysis for Sour Cherry Thermal and HPP Treatments

Thermal and HPP microbial inactivation in sour cherry juice was evaluated through RSM. The objective was to understand the influence of processing parameters (temperature and time for thermal; pressure and time for HPP) on microbial reduction. Modeling microbial inactivation through log reduction, rather than log count, provides a clearer assessment of process effectiveness. Log reduction values directly indicate the extent of microbial reduction, and are independent of initial counts, thus facilitating the interpretation and comparison [4].

Various regression models were tested, with linear regression providing the best fit for the data on thermal treatment (R^2 = 0.845). Additional ANOVA tables and fitted vs observed plots are presented in Appendix B.1. The resulting model equation is presented as follows:

$$Log\ reduction = -33.033 + 2.30068 \cdot t + 0.4309 \cdot T \quad (4.8)$$

where, t is the time in min, and T is the temperature in °C.

The model suggests that both time and temperature significantly contribute to microbial inactivation, though temperature has a slightly smaller coefficient. The observed linear model aligns well with thermal inactivation kinetics for microbial cells. Many studies report that log reductions increase linearly with both temperature and treatment time [4,38]. The relatively strong influence of time (coefficient of 2.30068) compared to temperature (0.4309) might indicate that, at these temperatures (75–85°C), sour cherry juice responds more to prolonged exposure times rather than relying on temperature. This behavior is consistent with the D-values showed previously, where higher temperatures reduce the time needed for microbial reduction.

Figure 4.2 shows the response surface graph for thermal treatment in sour cherry juice indicating the log reduction of microbial counts as a function of time and temperature in the range of the study. As can be seen, higher temperatures and longer treatment times lead to increased microbial inactivation, shown at the top right of the graph, representing higher log reductions. With an R^2 value of 0.845, the model explains around 85% of the variation in microbial reduction data, indicating a reasonably good fit. The RMSE of 1.51 indicates some variability around the predicted values, likely due to slight experimental or process inconsistencies, which is typical in thermal inactivation studies.

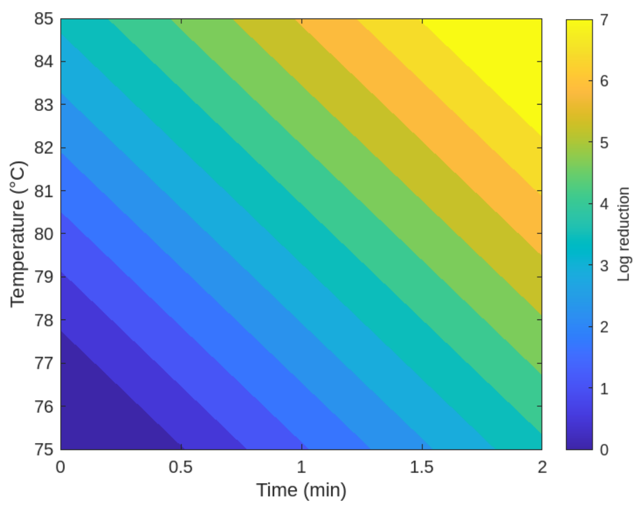

Figure 4.2. Thermal treatment response surface for sour cherry juice microbial inactivation as log reduction

Figure 4.3 shows the response surface graph for HPP treatment on sour cherry juice microbial log reduction as a function of pressure (200 to 600 MPa) and time (0 to 6 minutes). Like in the thermal treatment graph, the color gradient moves from cooler colors (blue) at lower pressures and shorter times, indicating lower log reductions, to warmer colors (yellow) at higher pressures and longer times, indicating greater microbial inactivation.

The model equation calculated for this graph, showed a second-order interaction effect between pressure (P) in MPa and time (t) in minutes.

$$Log\ reduction = -0.3931 - 0.2077 \cdot t + 0.0026 \cdot P + 0.00236 \cdot \mathrm{P} \cdot t \qquad (4.9)$$

This equation highlights a clear interaction effect between pressure and time (P·t), as confirmed by the ANOVA table (Appendix B.2) with a significant p-value (< 0.05). This interaction suggests that microbial inactivation in HPP depends on the combined effect of both pressure and duration. HPP processing literature shows that where sufficient pressure-time combinations are necessary to achieve microbial stability. Literature on HPP processing indicates that achieving microbial stability requires an effective combination of both pressure and processing time [13,39,40].

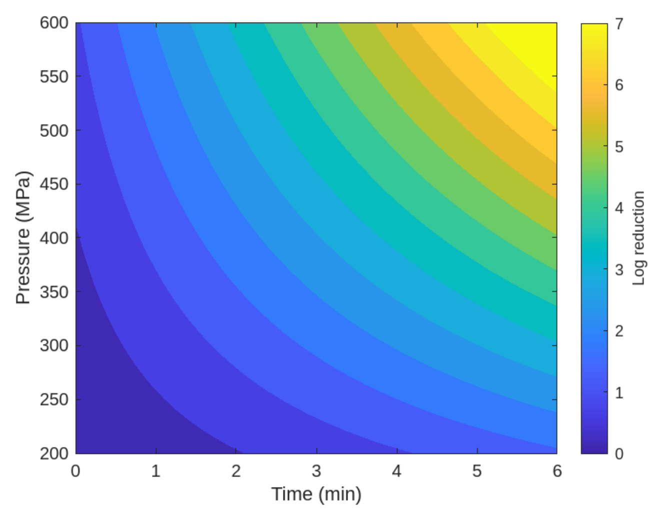

Figure 4.3. High-Pressure Processing (HPP) response surface for sour cherry juice microbial inactivation as log reduction

With an R^2 value of 0.899, this model captures around 90% of the variability in microbial reduction, indicating a strong fit. The RMSE of 0.986 reflects a low error margin, suggesting the model accurately predicts microbial inactivation outcomes within this pressure and time range.

This interaction model aligns with HPP research, showing that microbial inactivation is most effective at pressures above 400 MPa. Microbial cells become highly susceptible to inactivation as these high pressures disrupt cell membranes [39]. Additionally, the effectiveness of HPP depends on the combination of pressure and time. In general, longer exposure at high pressures enhances microbial reduction, ensuring better stability in the treated product [13].

HPP can achieve substantial log reductions at higher pressures with moderate times, corroborating its use as a non-thermal preservation technique that minimizes the loss of nutrients and bioactive compounds compared to thermal treatments [40].

4.1.4 PEF Results and Discussion (Sour Cherry and Raspberry Juice)

PEF parameters were selected based on findings from previous trials. The pre-trials were conducted using a different PEF unit with the same juice and bacteria. Electric field strengths ranging from 10 to 15 kV/cm were applied, achieving a maximum log reduction of 1.06 log CFU/mL at 12.5 kV/cm, with a frequency of 20 Hz and a specific energy of 121.7 kJ/kg. Other studies have corroborated that a minimum electric field strength greater than 10 kV/cm is necessary for microbial reduction [41], and typical PEF pasteurization processes ranged from 15 kV/cm up to 40 kV/cm, that is the strength necessary for irreversible electroporation [42,43].

Additionally, since electric field strength is recognized as the most important parameter for assessing microbial inactivation and PEF efficiency [44], microbial inactivation in this study was modeled using electric field strengths starting from 16 kV/cm.

In Table 4.2 the parameters for the Weibull, Peleg and Geeraerd models describing the reduction of the *E. coli* surrogate are presented. In PEF treatments, the microbial reduction kinetics using Weibull, Peleg and Geeraerd models showed varying results. But in general, the Peleg and the Geeraerd models provided a better fit (R^2 higher than 0.85 for sour cherry juice and R^2 higher than 0.94 for raspberry juice). The Peleg and Geeraerd models have been proposed as a better fit for PEF data [31,32].

At a specific energy level of 100 kJ/L, similar kinetic parameters were observed between sour cherry and raspberry juices, indicating comparable rates of microbial inactivation across both juices. However, at 120 kJ/L, the kinetic parameters began to differ, showing that raspberry juice generally exhibited higher parameters that translate in a faster microbial reduction than sour cherry juice. This trend was experimentally validated, highlighting that raspberry juice may respond more effectively to higher energy inputs in PEF treatments. The observed difference in microbial reduction rates between sour cherry and raspberry juices at higher energy levels, such as 120 kJ/L, may be attributed to variations in the physicochemical properties of the juices, such as pH, sugar content, and conductivity [45], which was higher in raspberry juice.

Critical electrical field strength (E_C) in both juices was found from 8.25 kV/cm to 13.44 kV/cm, with higher values for sour cherry juice. In the study by Grahl et al. [46] the critical electrical field for *E. coli* reduction by PEF in various food products was found from 11.9 kV/cm to 14 kV/cm.

The L_S parameter, in Geeraerd model, that represent the inactivation limit, is higher for the 100 kJ/L treatment (11.17±0.6 in sour cherry and 11.45±0.11 in raspberry) compared to 120 kJ/L (2.02±0.07 in sour cherry and 5.91±1.24 in raspberry). This suggests that microbial cells inactivate more quickly at higher specific energy levels, likely due to enhanced electric field distribution or a greater thermal effect [47,48]. Additionally, k_{max} values, which indicates the maximum inactivation rate, are also higher at 120 kJ/L than at 100 kJ/L. This shows that microbial inactivation occurs more rapidly at higher energy level, as expected.

Table 4.2. Parameters for the inactivation model by Peleg, R^2, and RMSE values for PEF treated sour cherry juice.

Models	Parameters and indices	Sour cherry		Raspberry	
		100 (kJ/L)	120 (kJ/L)	100 (kJ/L)	120 (kJ/L)
Weibull	δ_E	5.02±0.58	0.69±0.31	4.23±0.05	2.29±0.14
	p	14.18±0.54	2.49±2.05	13.78±0.08	9.75±0.43
	RMSE	1.543	0.500	1.181	0.662
	R^2	0.445	0.925	0.664	0.877
Peleg	k	0.63±0.05	0.94±0.18	0.77±0	1.08±0.07
	E_C	13.44±0.72	9.64±1.75	12.53±0.07	8.25±0.72
	RMSE	0.734	0.666	0.420	0.336
	R^2	0.852	0.868	0.945	0.968
Geeraerd	L_S	11.17±0.6	2.02±0.07	11.45±0.11	5.91±1.24
	k_{max}	1.12±0.05	0.57±0.01	1.11±0.01	0.77±0.07
	RMSE	0.621	0.469	0.388	0.305
	R^2	0.894	0.935	0.953	0.974

Based on the Peleg model, an effective 5-log reduction in sour cherry juice is achieved at 20.50 kV/cm with 120 kJ/L, and at 20.75 kV/cm with 100 kJ/L, and in raspberry at 21.01 kV/cm at 100 kJ/L and 21.46 kV/cm at 120 kJ/L. Given the RMSE values in both models, it can be concluded that a 5-log reduction of the *E. coli* surrogate can be reliably achieved from 20 kV/cm and 100 kJ/L in both juices.

The Weibull model fails to accurately fit the data, as indicated by its lower R^2 values and higher RMSE. In contrast, both the Peleg and Geeraerd models show similar but improved R^2 and RMSE values. Among these, the Peleg model provides the best fit for the behavior of the PEF data, as also noted in Figure 4.6 that shows the microbial log reduction curves for raspberry juice PEF treatment at 120 kJ/L with the three models. Peleg model also have the best fit in previous research by Singh et al. [12] in carrot juice. As seen in the figure, the Peleg model is more appropriate for PEF data. In contrast, the Weibull model, that is also non-linear, did not accurately capture the real behavior of the sample.

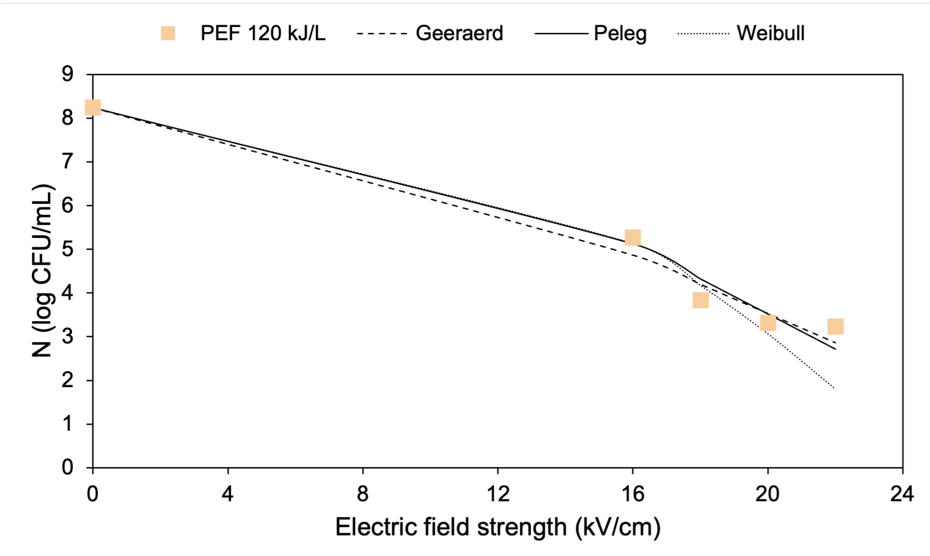

Figure 4.4. Microbial log reduction curve for raspberry juice PEF treatment at 120 kJ/L.

4.1.4.1 Response Surface Modeling (RSM) Analysis for Sour Cherry and Raspberry Juices PEF Treatment

In this analysis, various models were tested to fit the PEF data, with a focus on identifying the most accurate model for predicting microbial inactivation. For the RSM, treatment time (μs) was chosen as one of the main parameters, together with electric field strength (kV/cm), as it is commonly used in PEF kinetics modeling and allows for a broader dataset [10]. All parameters from the trials, including the calculation of treatment time, are detailed in Appendix B.3, along with ANOVA tables and model parameters for both sour cherry and raspberry juices (Appendix B.4).

The behavior of *E. coli* inactivation in sour cherry juice in response to PEF treatment is illustrated in the Figure 4.5. The color gradient represents log reduction, with higher electric field strengths and longer treatment times resulting in greater microbial inactivation, as indicated by the shift from blue to yellow. As can be deducted from the figure, an effective 5-log reduction is achieved from around 20 kV/cm and 40 μs of treatment time.

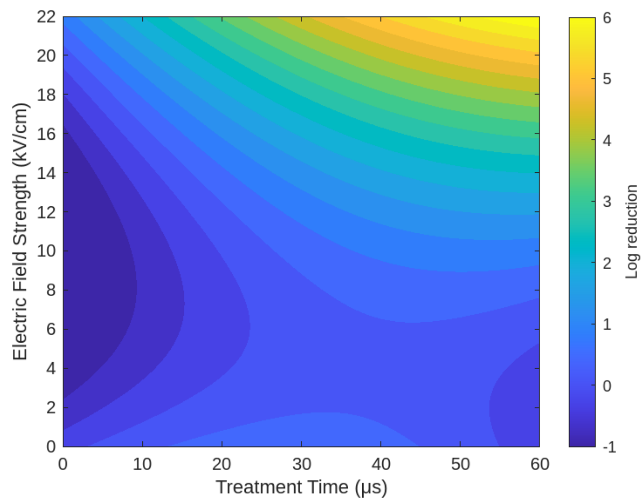

Figure 4.5. Pulsed Electric Fields (PEF) response surface for sour cherry juice microbial inactivation as log reduction

For sour cherry juice, the selected model is a second-order polynomial with the following parameters:

$$\begin{aligned} Log\ reduction = & -0.085249 + (-0.288901 \cdot E) + (0.052209 \cdot t) \\ & + (0.004278 \cdot E \cdot t) + (0.015307 \cdot E^2) \\ & + (-0.000901 \cdot t^2) \end{aligned} \qquad (4.10)$$

where, *E* is the electric field strength in kV/cm and *t* the treatment time in μs. This model yielded an R^2 value of 0.880 and an RMSE of 0.701, indicating a good fit with a relatively low error. The figure show that increasing both the electric field strength and treatment time enhances microbial inactivation in sour cherry juice, following the expected trend for PEF applications.

For sour cherry juice, the behavior observed is like that of cherry juice, as reflected in both the graphical trend and model parameters. The selected model is also a second-order polynomial, with the following equation:

$$Log\ reduction = -0.269176 + (-0.219414 \cdot E) + (0.101881 \cdot t) + (0.003528 \cdot E \cdot t) + (0.012540 \cdot E^2) + (-0.001662 \cdot t^2) \quad (4.11)$$

This model achieved an R^2 value of 0.9161 and an RMSE of 0.5781, indicating a strong fit to the experimental data, comparable to the cherry juice model. Figure 4.6 illustrates the log reduction of microbial cells as a function of electric field strength (kV/cm) and treatment time (µs). As with cherry juice, higher electric field strengths combined with longer treatment times result in increased microbial inactivation, moving from blue (low log reduction) to yellow (high log reduction). This trend underscores the effectiveness of PEF at high energy inputs, where electric field strength and exposure time synergistically enhance microbial reduction [46,49].

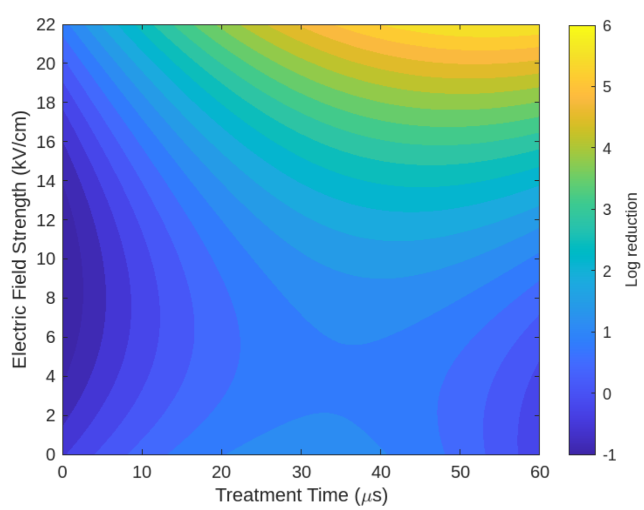

Figure 4.6. Pulsed Electric Fields (PEF) response surface for raspberry juice microbial inactivation as log reduction

4.1.5 Conclusions

In conclusion, the microbial inactivation kinetics in sour cherry juice under PEF, thermal, and HPP treatments show distinct patterns that require different modeling approaches. For thermal and HPP treatments, microbial inactivation was rapid at higher intensities, with the Weibull providing a better fit for both treatments. For PEF treatments, microbial reduction was faster at higher energy levels, with a 5-log reduction occurring at around 20-21 kV/cm. The Peleg model offered the best fit, capturing the non-linear inactivation behavior. Overall, the Peleg and Weibull models showed superior performance compared to the Bigelow classical method.

The RSM results emphasize the importance of selecting optimal PEF parameters for effective microbial inactivation. The model's accuracy, as shown by the high R^2 and low RMSE values, supports the suitability of the different models in capturing the inactivation behavior in sour cherry and raspberry juices. These results demonstrate the importance of selecting the appropriate models based on treatment intensity and inactivation behavior.

4.2 Comparative Physicochemical Evaluation of Sour Cherry and Raspberry Juices under Thermal, HPP, and PEF Treatments

This subchapter presents a comparative analysis of the physicochemical properties of sour cherry (*Prunus cerasus*) and red raspberry (*Rubus idaeus*) juices subjected to thermal, high-pressure processing (HPP), and pulsed electric fields (PEF) treatments. Each treatment was designed to achieve a 5-log reduction in pathogens, ensuring microbial safety [50]. The study evaluates key quality indicators, including color parameters, browning, viscosity, and anthocyanins, to assess the physicochemical stability of the juices following treatment. Multivariate analysis, particularly Principal Component Analysis (PCA), was employed to investigate the relationships between these parameters across different processing methods. By examining how each treatment impacts the quality and stability of the juices, this analysis offers valuable insights into the potential of non-thermal technologies as alternatives to conventional thermal methods, balancing quality preservation with microbial safety[21].

4.2.1 Materials and Methods

4.2.1.1 Sour cherry and Red Raspberry Juice Preparation

Sour cherry and red raspberry juices were prepared following the procedure outlined in section 4.1.1.1. As previously described, the juices were thawed at 4°C for 24 hours before the trials, to ensure even defrosting and maintain their physicochemical properties.

4.2.1.2 Juice Processing

The juices were processed using three different technologies: thermal treatment (TT), high-pressure processing (HPP), and pulsed electric fields (PEF). The details of each process were explained in section 4.1.1.4. In brief, thermal treatment was conducted in a thermostatic water bath with temperature monitored via thermocouple, and samples were cooled in an ice bath after the treatment. HPP was performed in a 300 L high-pressure system with cold water (4°C) as the pressure medium. PEF treatments were carried out using a continuous flow system with co-linear chambers, operating at set pulse width and energy levels. After treatment, all samples were immediately cooled and frozen at -18°C for anthocyanin compound analysis, while the rest were stored at 4°C for further analysis.

4.2.1.3 Viscosity (μ)

The viscosity of the juice samples was measured using a concentric cylinder geometry in two different rheometers. For thermal and HPP-treated samples, measurements were performed using a rheometer (MCR 702e, Anton Paar) at University of Parma (Italy), and or PEF-treated samples at BOKU University (Vienna, Austria), with a rotational rheometer (Kinexus PRO, Malvern). In both cases, the flow curve, shear rate, and shear stress were recorded across a range of 1 to 100 s^{-1}.

4.2.1.4 Browning Index (BI)

The browning index (BI) was calculated as the ratio between the absorbance at 420 nm and 510 nm, following the method of Suh et al. [51], using a spectrophotometer (6600 UV-VIS, photoLab). Prior to the measurements, the samples were centrifuged in a centrifuge (Thermo

Scientific) at 10 000 g for 3 minutes, and the supernatant was diluted 1:20 with water for the absorbance measurements.

4.2.1.5 Color Measurements

For color measurements, a colorimeter (CM-A286, Konica Minolta) was used in transmittance mode with Specular Component Included (SCI). The color parameters were measured in the CIELab color space, including L* (lightness), a* (red-green), and b* (yellow-blue). The total color difference (ΔE) was calculated as the geometrical distance between the treated samples and the untreated control in the CIELab color space, providing a quantitative assessment of color changes.

4.2.1.6 Anthocyanins

The anthocyanin measurement data for cherry juices was generously provided by researcher Alema Puzović from the Department of Agronomy at the University of Ljubljana (Slovenia). While the author did not perform direct experimental measurements, the data provided enabled a detailed analysis based on the original researchers' method.

The analysis of total anthocyanins in the juice was conducted at 25°C, following the method of Mikulic-Petkovsek [52] on a high-performance liquid chromatography (HPLC) system (Finnigan Surveyor, Thermo Fischer Scientific) equipped with a photodiode array (PDA) and mass spectrometer (MS). Total anthocyanins were expressed in mg/L of juice.

4.2.1.7 Experimental and data analysis

Based on previous microbial validation and kinetic study (0), only treatments that achieved at least a 5-log reduction in microbial counts were used in this assay. For sour cherry juice, the thermal treatments were set at 80°C and 85°C for 60 and 120 seconds. The HPP treatments included 400 MPa and 600 MPa for 4 and 6 minutes. The PEF treatments were applied at 20 and 22 kV/cm with specific energy levels of 100 and 120 kJ/L. For red raspberry juice, thermal treatments were applied at 80°C and 85°C for 30 and 60 seconds. The HPP treatments were conducted at 500 MPa and 600 MPa for 2 and 6 minutes, and same PEF treatment levels as in sour cherry juice.

A one-way ANOVA was conducted for each variable and fruit type to evaluate the differences between treatments and control samples. Additionally, to identify grouping patterns and underlying relationships between the treatments and parameters, a Cluster analysis was performed using k-means clustering and hierarchical clustering methods. A dendrogram was generated using Euclidean distance and Ward's D2 method. Principal Component Analysis (PCA) was performed for both fruits and all measured parameters. The ANOVA and post-hoc Tukey tests were performed using R Studio (version 2024.04.1, Posit Software, PBC), while the Cluster and PCA analyses was carried out using Jamovi software (Version 2.3.28.0, The jamovi project).

4.2.2 Results and Discussion

4.2.2.1 Analysis of Total Color Difference (ΔE) in Sour Cherry and Raspberry Juice

Figure 4.7 shows the comparison of total color difference (ΔE) measurements for sour cherry (A) and raspberry (B) juices subjected to (1) High-Pressure Processing (HPP), (2) Pulsed Electric Fields (PEF), and (3) Thermal treatments (TT) at different processing conditions. Lowercase letters within sour cherry juice (A) and uppercase letters within raspberry juice (B) indicate significant differences ($p < 0.05$) between conditions and control samples. The data highlights the variations in color stability between the different technologies. In general, sour cherry juice samples show significantly higher ΔE values, with a maximum of 16.80±0.20, compared to raspberry juice, with a maximum of 4.65±0.01, both for a thermal treatment.

As seen in Figure 4.7, In HPP-treated samples (1), the ΔE in both sour cherry and raspberry juices is generally lower, compared to PEF and thermal treatments. In the case of sour cherry there are significant differences among the samples, as indicated by the grouping labels. Samples labeled with 'a' differ significantly from those labeled with 'bc,' while samples sharing 'ab' show some overlap, suggesting partial similarity but still distinct from 'bc' and 'a' group (HPP 600 MPa) which presents the lowest level of color difference. In raspberry, the post hoc test results indicate partial differences in ΔE among samples, with 'b' groups (6 min) distinct from the 'ab' groups (2 min). The low variation in color differences with HPP treatment is due to the minimal temperature fluctuation compared to thermal and PEF treatments, as HPP relies on high pressure, not heat, for microbial inactivation [40]. In other comparative studies HPP samples also present the lower variation in ΔE, compared with the other technologies [15,53,54].

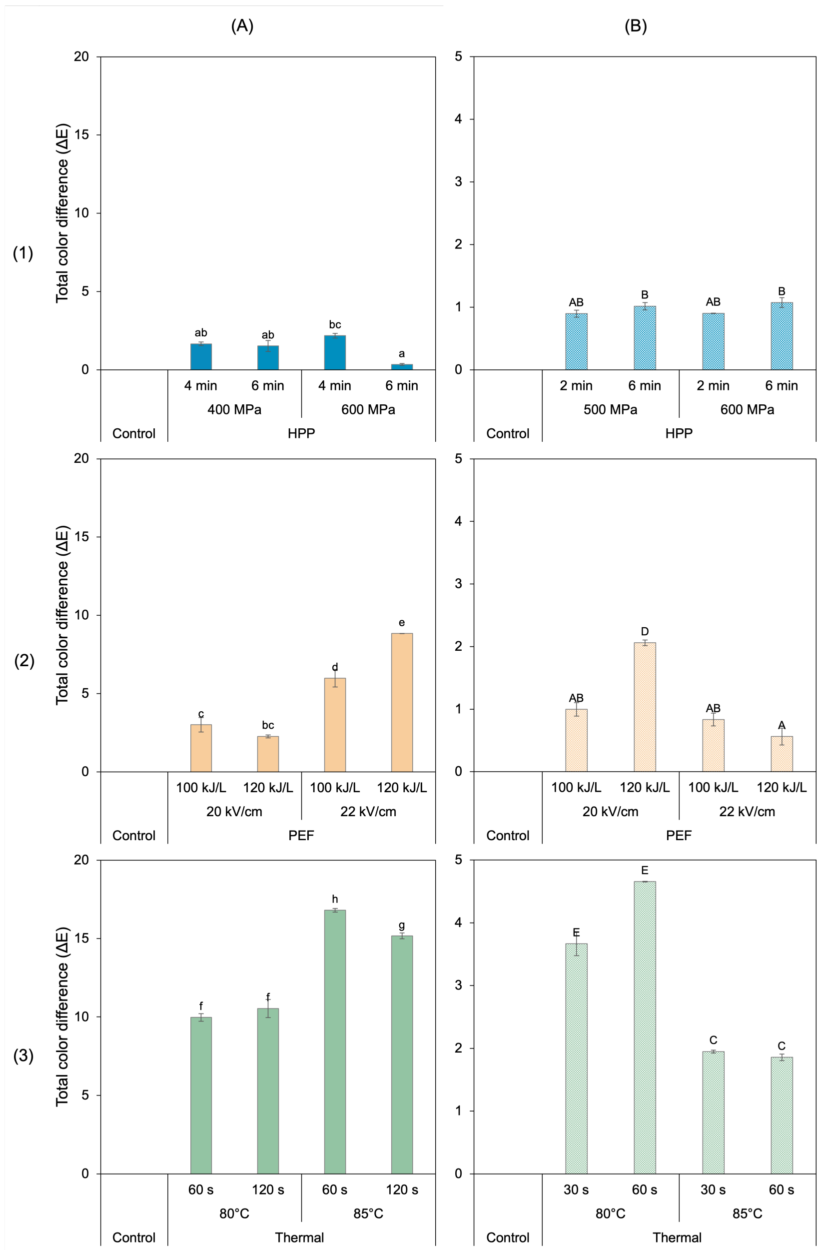

Figure 4.7. Comparison of Total color difference (ΔE) in sour cherry (A) and raspberry (B) juices treated by (1) High-pressure processing (HPP), (2) Pulsed electric fields (PEF), and (3) Thermal processing at different conditions

The observed increase in ΔE at 22 kV/cm and 120 kJ/L in the PEF-treated sour cherry samples (2-A), is likely due to the ohmic heating effect, this heating effect increases with specific energy input, rather than directly with electric field strength [47]. This effect can also be seen in the raspberry sample at 20 kV/cm and 120 kJ/L. In these samples, the final temperature reached was approximately 64°C with a ΔT increase of more than 20°C, indicating that a thermal effect was present. The TT samples of sour cherry had the highest ΔE at 85°C. This heat-induced degradation is well known when compared to non-thermal treatments [55,56].

In raspberry juice samples (B), a higher ΔE was observed at 20 kV/cm, whereas samples treated at 22 kV/cm displayed a lower difference. This pattern contrasts with cherry juice samples, where higher field strengths result in greater color changes. The unexpected ΔE at 22 kV/cm in raspberry juice may be due to differences in how specific energy is distributed within the sample. At 20 kV/cm, energy distribution may lead to localized heating, causing pigment degradation and higher color differences, while at 22 kV/cm may allow for more uniform energy dispersal [9]. In TT raspberry juice samples (3-B), a lower ΔE was observed at 85°C compared to 80°C. This result could be attributed to the water bath's come-up time, as higher temperatures might increase the heating rate [4].

Literature on color changes in fruit juices supports these findings, with several studies indicating that HPP generally preserves color better than thermal methods due to the minimal heat involved [57,58]. PEF, while non-thermal, can still induce color changes when high specific energies are applied [59]. In the study by Timmermans et al. [60] on orange juice, HPP treatment did not cause a significant increase in ΔE, while both thermal and PEF treatments did. Thermal processing resulted in a less red and more yellow hue, whereas PEF treatment produced a significantly darker color.

The change in ΔE can also be linked to the increase in phenolic content. Treatments like HPP and PEF enhance phenolic levels, which may contribute to a more intense color in the final beverage and result in noticeable color differences. [61]. On the other hand, TT, especially at higher temperatures or with prolonged heating times, are well-known to cause color degradation, as color-sensitive compounds in fruit juices are more thermolabile [2].

Figure 4.8 shows the visual differences in red raspberry juice after control, HPP (400 MPa for 6 min), PEF (22 kV/cm, 120 kJ/L), and TT (80°C, 60 s). In the figure, the color of the red raspberry juice samples appear quite similar across treatments, with no obvious visual distinctions between the control, HPP, PEF, and TT. While the ΔE values indicate that TT (ΔE = 4.65) should have the most significant impact on color, this difference is not strongly, PEF sample (ΔE = 0.56) appear very close in color to the control, corroborated by the ΔE value (0.56), and compared with HPP (ΔE = 1.02). Overall, based on visual assessment alone, the samples appear very similar, reinforcing the findings that HPP and PEF have minimal impact on color.

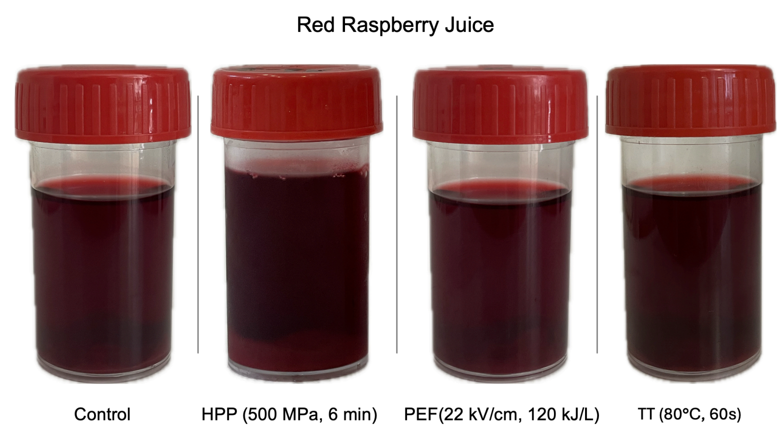

Figure 4.8. Visual comparison of red raspberry juice samples: Control, HPP (500 MPa, 6 min), PEF (22 kV/cm, 120 kJ/L), and TT (80°C, 60 s)

4.2.2.2 Analysis of Browning Index (BI) in Sour Cherry and Raspberry Juice

Figure 4.9 shows the comparison of BI between the different treatments and conditions for sour cherry and raspberry juices. Lowercase letters within sour cherry juice (A) and uppercase letters within raspberry juice (B) indicate significant differences ($p < 0.05$) between conditions and control samples. As seen in the figure for sour cherry (A), there were no significant differences in the BI on all the treatments, which maintained similar browning levels to the control. This lack of significant browning differences suggests that the applied treatments did not substantially affect the browning degradation reactions in cherry juice. According to the literature, browning effects in fruit juices are often more closely related to prolonged storage time rather than the immediate impact of processing methods [62]. Browning typically results from enzymatic reactions, such as the activity of polyphenol oxidase, which can intensify over time, particularly during storage [63]. Thus, the absence of BI variation may indicate that processing conditions alone are not sufficient to generate noticeable browning changes.

Regarding the raspberry juice samples (B), there was no significant variation in BI between control, PEF and thermal treatments. However, a significant reduction in BI was observed in the HPP-treated samples, with an average of 30.15% decrease from the control. This reduction in browning may be attributed to the strong negative correlation between BI and anthocyanin content in anthocyanin-rich fruits like raspberries [64]. As anthocyanins increase during HPP treatments [61], there will be a corresponding decrease in BI.

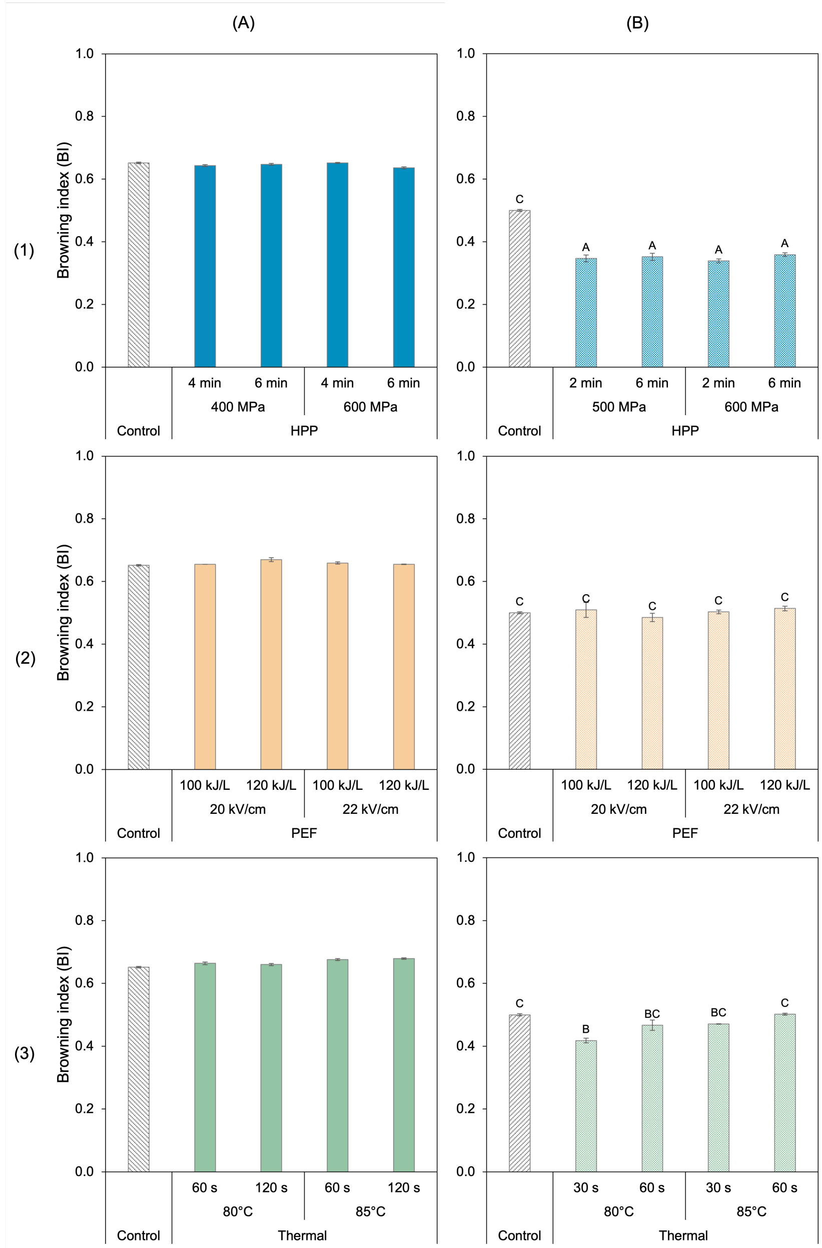

Figure 4.9. Comparison of Browning index (BI) in sour cherry (A) and raspberry (B) juices treated by (1) High-pressure processing (HPP), (2) Pulsed electric fields (PEF), and (3) Thermal processing at different conditions

4.2.2.3 Analysis of Viscosity in Sour Cherry and Raspberry Juice

Figure 4.10 shows the viscosity of cherry and raspberry juice samples treated with different technologies (HPP, PEF, and thermal) at different treatment conditions and intensities. Lowercase letters within sour cherry juice (A) and uppercase letters within raspberry juice (B) indicate significant differences ($p < 0.05$) between conditions and control samples.

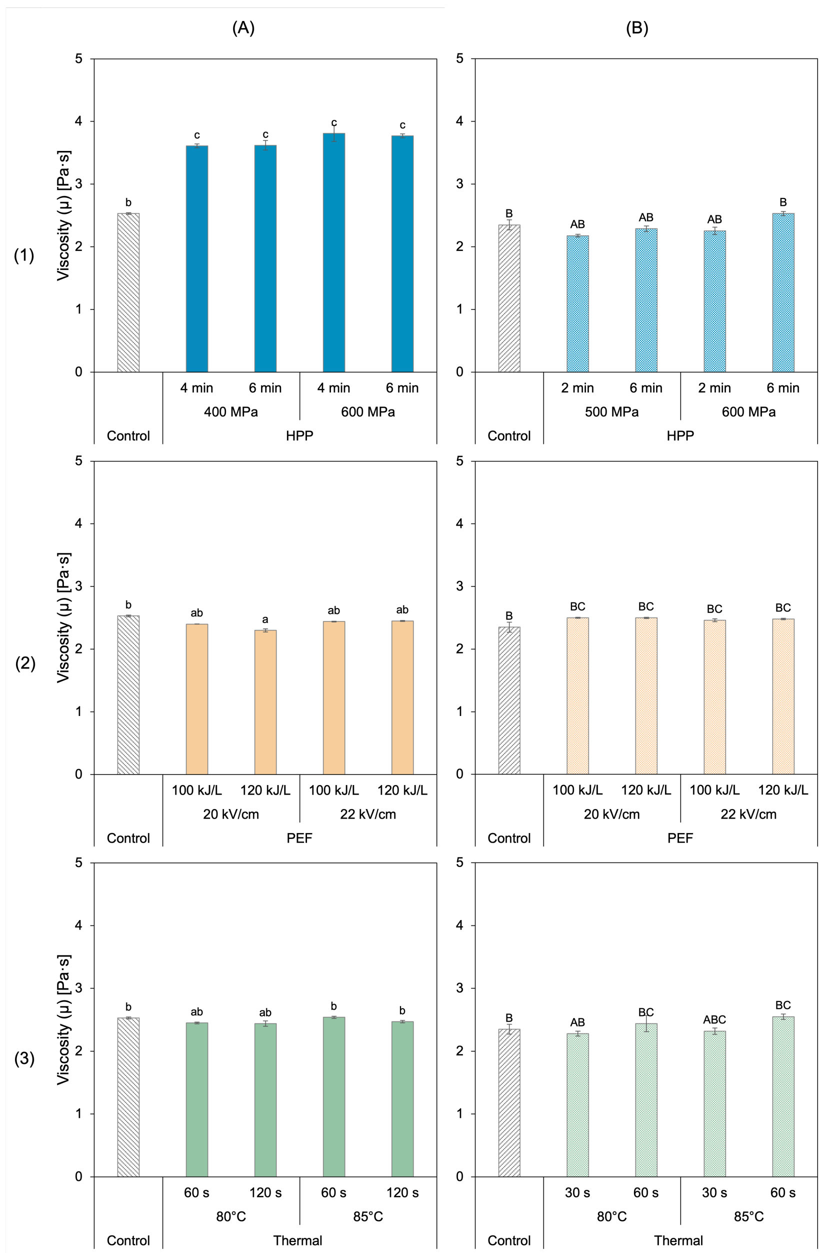

Figure 4.10. Comparison of Viscosity (μ) in sour cherry (A) and raspberry (B) juices treated by (1) High-pressure processing (HPP), (2) Pulsed electric fields (PEF), and (3) Thermal processing at different conditions

In sour cherry juice samples (A), HPP treatment induces a noticeable increase in viscosity compared to the control (1-A). This rise in viscosity could be due to structural changes in the juice components, such as the solubilization of pectin or cell wall disruption [59,65], as well as the increment of pectin methyl esterase (PME) activity, which has been observed in red and black raspberries treated with HPP at pressures from to 400 MPa [61]. However, in raspberry juice samples, the viscosity remained generally the same as the control, indicating no significant structural effects from HPP. This could suggest that raspberry juice has a more stable matrix compared to sour cherry juice, making it less susceptible to pressure induced disruptions.

In contrast, both PEF (2) and thermal treatments (3) appear to have little to no significant effect on viscosity for either fruit. This could be explained by the fact that neither PEF nor thermal processes induce structural breakdown. According to the literature, thermal processing tends to have a minimal effect on of the viscosity in fruit juices [66,67]. PEF treatments primarily target microbial inactivation through electroporation, which disrupts cell membranes without causing significant molecular changes to juice components. This minimal effect on molecular structure explains the slight changes in viscosity observed with PEF treatments [45,49].

4.2.2.4 Analysis of Total Anthocyanins in Sour Cherry Juice

As can be seen in Figure 4.11, the total anthocyanin content (TAC) significantly increased in sour cherry samples treated with HPP at 600 MPa. This observation is consistent with previous studies, which have reported that pressures above 500 MPa can enhance the extraction of anthocyanins and other phenolic compounds from the plant matrix [68,69]. In the study by Tenuta et al. [70], sour cherry juice subjected to HPPat 600

MPa for 3 minutes showed a significant 24% increase in phenolic content. However, despite the rise in phenolics, there was no statistically significant change in anthocyanin content after processing. The increase in phenolic content is important because phenolic compounds are closely associated with the antioxidant capacity and overall quality of fruit juices [58].

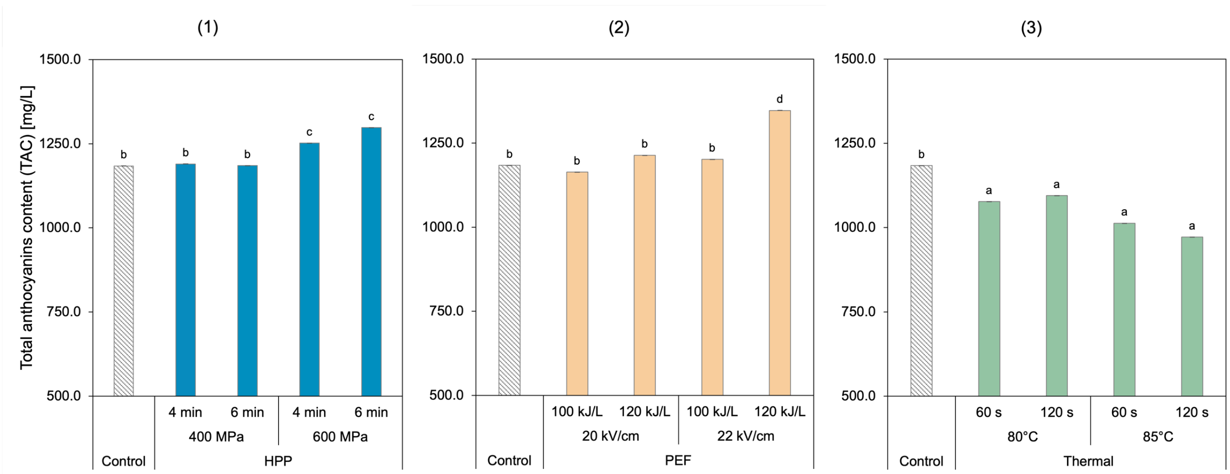

Figure 4.11. Comparison of Total Anthocyanin Content (TAC) in sour cherry juice treated by (1) High-pressure processing (HPP), (2) Pulsed electric fields (PEF), and (3) Thermal processing at different conditions

On the other hand, the thermal treatments in sour cherry juice resulted in a significant reduction in TAC, with a decrease of 12.2% across all temperature-time combinations. This reduction is in line with findings in the literature, which generally report losses of anthocyanins in fruit juices due to thermal degradation [15]. Phenolic compounds, like anthocyanins, are sensitive to heat, and a prolonged exposure to elevated temperatures can lead to their breakdown or oxidation [71], which diminishes their antioxidant activity and health-promoting properties [72].

PEF treatments show no significant difference in anthocyanin content compared to control samples, except for the 22 kV/cm and 120 kJ/L, were a aignificant 12.1% increase in phenolic content was observed compared to the control. PEF treatments generally show no significant change o slight increases in anthocyanin content when compared to untreated samples, even under field strengths like 22 kV/cm [73]. Nevertheless, in the study by Buitinea-Cantúa et al. [61], both HPP and PEF treatments increasing the TAC. HPP conditions (400-600 MPa for 10 minutes) resulted in a 2% to 30% increase, while PEF treatment (200 Hz, 168.4 kJ/L) led to even greater improvements, rising by up to 79%. The reduction or increase in anthocyanins content may also negatively impact the overall quality and sensory attributes of the juice, as anthocyanins contribute to flavor and color in berries [74].

4.2.2.5 Multivariate Analysis of Color, Viscosity, Browning Index, and Polyphenols in Sour Cherry and Raspberry Juices Across Multiple Treatment Levels

In the multivariate analysis for sour cherry data, anthocyanin levels were included, while this variable was omitted in raspberry data. The analysis involved k-means clustering and Principal Component Analysis (PCA). K-means clustering groups data points into clusters based on their similarities, minimizing variance within each cluster. Meanwhile, PCA reduces data dimensionality by identifying principal components (directions of maximum variance) allowing for a simple visualization and interpretation of complex datasets [75]. Together, these methods reveal patterns and relationships in sour cherry and raspberry juice characteristics.

4.2.2.5.1 *Cluster Analysis*

The dendrogram and heatmap in Figure 4.12 presents a hierarchical clustering analysis of sour cherry (CH) and raspberry (RB) juices based on color parameters: L (lightness), a (redness), b (yellowness), total color difference (dE), and BI, for HPP, PEF, and TT. The clustering analysis shows two distinct blocks, one for raspberry (RB group) (on the left side of the figure) and one for sour cherry (CH group) (on the right side).

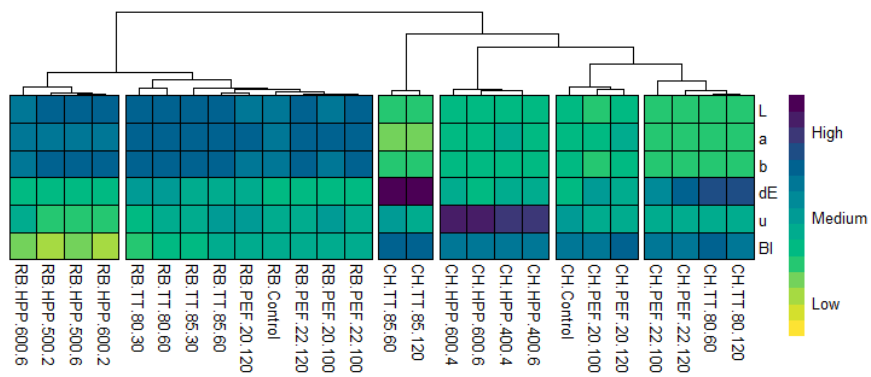

Figure 4.12. Cluster analysis and dendrogram of color Parameters (L, a, and b), total color difference (dE), viscosity (u), anthocyanins, total phenolic compounds (TPC), and browning index (BI) in sour cherry (CH) and raspberry (RB) juices Treated by HPP, PEF, and Thermal (TT) Processes

The heatmap (color coding) indicates the relative magnitude of each parameter across treatments (low, medium and high). The separation between raspberry (RB) and sour cherry (CH) juice samples appears to be driven predominantly by the color parameters (L, a, and b). Additionally, raspberry (RB) samples show a more uniform and lower range of values for ΔE (dE) and BI. This is indicative of a relatively uniform color profile across all treatments for raspberry.

The k-clustering analysis revealed six distinctive groupings in the dataset (showed by the separation in the dendrogram). For the raspberry samples, two clear clusters were identified: one group for the HPP-treated samples and another group consisting of the control, PEF, and thermal-treated samples. The primary factor driving this separation was the BI. In the previous analysis on BI of raspberry samples, in HPP treated samples, BI values were observed to be lower than those in control samples, indicating reduced browning, while most other treatment effects showed minimal or no change in BI.

On the other hand, sour cherry samples exhibited four distinct clusters. One cluster was formed by the HPP treated samples driven mainly by the high viscosity, consistent with previous analyses that showed an increase in viscosity due to the treatment. Another cluster grouped the thermal-treated samples at 85°C, driven by their high ΔE values. A third cluster consisted of samples treated with lower PEF intensities (20 kV/cm) and the control sample. The final cluster included the samples treated with higher PEF intensities (22 kV/cm) and low thermal treatments (80°C), showing similar profiles in terms of the measured parameters. The formation of separate clusters suggests that HPP specifically affects viscosity, creating a unique profile, while thermal treatments at 85°C significantly impact color (ΔE values). PEF treatments further stratify samples: lower-intensity PEF treatments align closely with control samples, indicating minimal effect, whereas higher-intensity PEF treatments combined with mild thermal processing create another profile, suggesting similar influences on juice attributes.

The separation between raspberry and cherry juices could be attributed to intrinsic differences in their color profiles and how their pigments respond to various treatments. Sour cherry juices are known to have more complex anthocyanin profiles [76], which may be more sensitive to processing conditions, especially at higher pressures or temperatures. Raspberry juice color parameters appear to be more stable across treatments, suggesting that this juice matrix is less sensitive to the processing conditions.

4.2.2.5.2 Principal Component Analysis (PCA)

The complete PCA analysis, eigen values and description of axes in both fruit juices are presented in the Appendix C. The PCA plot on individuals for sour cherry juice (Figure 4.13) represents the distribution of cherry juice samples subjected to various treatments: TT (black), Control (red), HPP (blue), and PEF (green). The two dimensions account for a significant portion of the variance, with Dim 1 explaining 74.52% and Dim 2 explaining 10.71%. Dim 1 is related with color attributes and color changes, based on the eigenvalues and cosine of variables. ΔE and BI are located at the left side, and L*, a* and b* are on the right side of the axis. Dim 2, on the other side is related with viscosity, anthocyanins and TPC.

HPP-treated samples (blue) form a distinct group on the right-downside of the plot, indicating that HPP significantly influences the physicochemical properties of sour cherry juice, separating these samples from the other treatments. This could be due to structural changes, such as increased viscosity and phenolic content, as previously discussed.

PEF-treated samples (green) are clustered closer to the control samples, especially along the positive side of Dim 1. However, they still exhibit some differentiation from the other treatments, particularly PEF samples treated at 100 kJ/cm and 20 kV/cm. This suggests that PEF treatments induce moderate changes in certain parameters, such as color or anthocyanin content, but these changes are less pronounced than in HPP-treated samples. The proximity of some control samples to PEF-treated samples may indicate that PEF treatments do not drastically alter the juice's properties in comparison to untreated samples, unlike HPP or thermal treatments.

TT samples (black) are grouped on the far-left side of the plot. This clustering suggests that thermal treatments have a strong impact con color parameters and BI, possibly due to the degradation of heat-sensitive compounds, such as anthocyanins [77]. The TT samples show a clear separation from both HPP and PEF, which aligns with the previous results and literature where TT tends to reduce anthocyanins and total phenolic compounds and, therefore, impact on the color parameters [58].

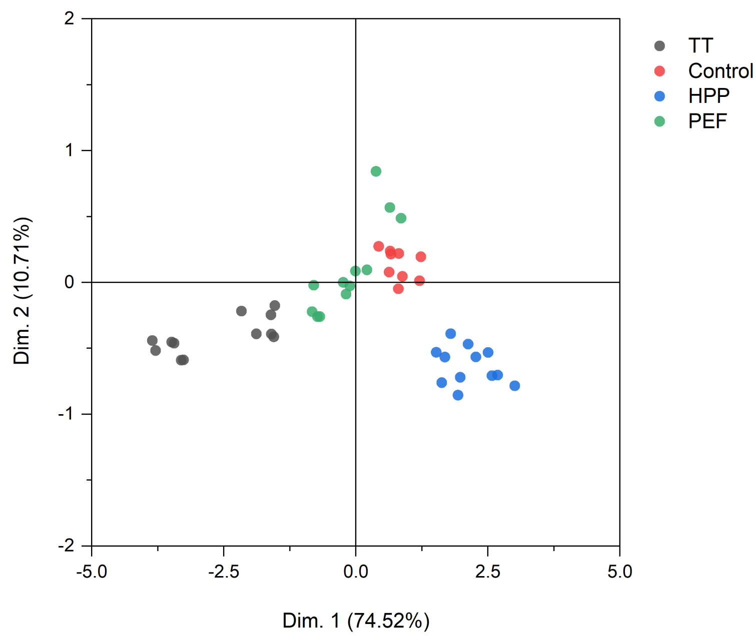

Figure 4.13. Principal Component Analysis (PCA) – individuals plot for sour cherry juice samples treated by HPP, PEF, and TT

The PCA plot for raspberry juice samples shows the distribution of individuals (samples) based on the key physicochemical parameters, with different treatments: thermal (black), control (red), HPP (blue), and PEF (green). The two dimensions, Dim 1 (55.27%) and Dim 2 (33.22%), together explain 88.49% of the total variance, providing a good representation of the data. Viscosity and BI are strongly associated with Dim 2, indicating that these variables contribute significantly to the separation along this axis. Color parameters (L, a, b) and ΔE are grouped together along Dim 1, implying that these attributes are highly correlated and primarily drive the variation in this dimension. Changes in lightness (L), in red-green axis (a), and in ΔE are closely related.

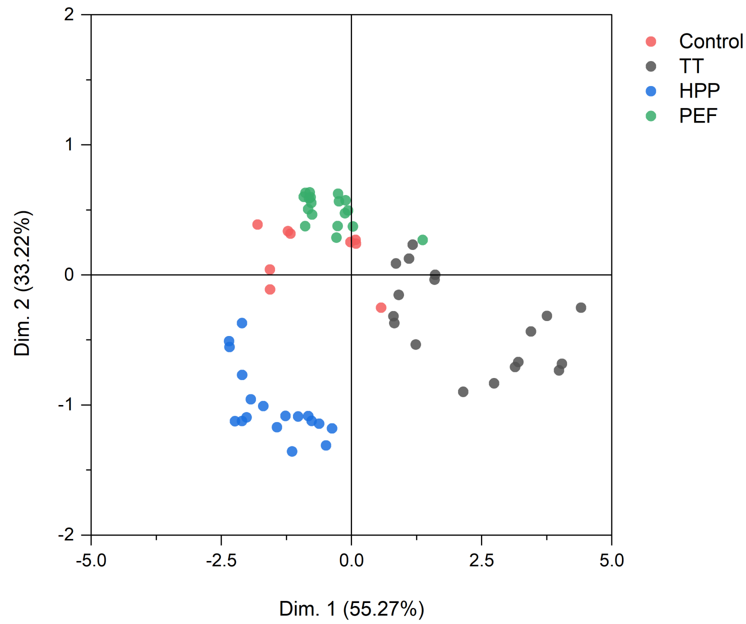

Figure 4.14. Principal Component Analysis (PCA) – individuals plot for raspberry juice samples treated by HPP, PEF, and Thermal treatments

HPP-treated samples (blue) are clustered on the lower left side of the plot, indicating that high-pressure processing leads to a distinct grouping of these samples. The behavior is similar as the one seen in sour cherry juice. PEF-treated samples (green) are located mostly in the upper right quadrant of the plot. The separation between PEF samples and the control or HPP samples suggests that PEF treatments induce moderate changes in raspberry juice, likely affecting variables such as ΔE and BI.

Finally, TT samples (black) are spread across the right side of the plot, indicating that thermal treatments create a different set of effects on the physicochemical properties of raspberry juice. This diversity may reflect varying degrees of color changes, viscosity, and browning across different temperatures and times. Control samples (red) are located near the center of the plot, with some overlap with PEF-treated samples. This one more suggests that PEF treatments may not induce changes on the measured parameters as HPP or TT does.

In summary, the PCA highlights that HPP, PEF, and TT lead to distinct effects on raspberry and cherry juice properties, with HPP samples forming the most distinct cluster, while PEF and thermal samples exhibit more variability. This separation reflects how different processing methods impact the structure, color, and stability of the juices. This may also indicate that ΔE is a significant discriminator for both fruits, especially for thermal-treated samples, but the degree and type of processing may affect each juice. The color parameters (L, a, b) and ΔE are consistently important in both juices, but they are primarily driving the separation along Dim 1, in raspberry juice, while in cherry juice, they contributed less significantly. This may indicate that raspberry juice is more sensitive to color changes, while viscosity and phenolics are more critical in sour cherry juice.

4.2.3 Conclusions

This study compares raspberry and sour cherry juices under HPP, PEF, and TT revealing unique responses for each. Sour cherry juice exhibited significant color changes, especially under thermal and PEF treatments, while raspberry retained color better, particularly with HPP. Viscosity increased notably in cherry juice with HPP, likely due to structural changes, while raspberry viscosity stayed stable. Anthocyanins content increased in cherry juice with HPP but declined under thermal treatments. PCA analysis showed that viscosity and ΔE primarily separate the grouping in cherry juice samples, while color parameters alone were more influential in raspberry juice. Results highlight the need to select processing methods based on each juice's unique qualities.

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5 Ohmic Heating of Strawberry Nectar: Modeling, Microbial Validation, and Nutrient Retention

Ohmic heating (OH) is an emerging thermal processing technology that offers several advantages over conventional heat treatments for liquid foods [1]. This method generates heat by passing an electrical current directly through the food product, using the electrical conductivity of the medium. The energy dissipation due to electrical resistance produces uniform and rapid heating [2].

Strawberry nectar is an ideal candidate for the application of OH due to its composition, as it is a liquid food and a uniform product. However, achieving the desired balance between microbial inactivation and nutrient retention, particularly for vitamins such as ascorbic acid (Vitamin C), requires careful consideration of the process parameters [3]. The efficiency of OH depends on various factors, including the electrical conductivity of the medium, the applied voltage, frequency, and temperature. These factors influence both the rate of microbial inactivation and the degree of nutrient degradation, which are crucial to the quality and safety of strawberry nectar [2].

This chapter focus on a comprehensives study on OH process applied to strawberry nectar, focusing on mathematical calculations, microbial validation, and nutrient retention. The first part of this study involves developing a mathematical model to describe the OH behavior during the process. This model accounts for the electrical conductivity, and energy requirements necessary to heat the nectar within a continuous flow system. By predicting the temperature profiles and necessary energy inputs, this model serves as the foundation for optimizing the heating process. The second part focus on the reduction of microorganisms, using an *Escherichia coli* surrogate for pathogenic bacteria, to ensure that the process meets food safety standards. Kinetic models were developed based on time-temperature profiles to describe the microbial inactivation and vitamin C reduction and to compare these results with conventional thermal kinetics. These findings provide valuable insights into the efficacy of OH in achieving microbial stability while minimizing nutrient loss.

5.1 Ohmic Heating Calculations and Modeling in Strawberry Nectar

This section provides a detailed analysis of the OH process applied to strawberry nectar, focusing on the calculations and modeling necessary to predict temperature distribution and energy requirements within a continuous flow system. The objective was to calculate the energy required to heat the nectar from an initial temperature of 20°C to a target temperature of 70°C, given specific juice properties and a known electric field applied across a pipe of 200 cm. The study accounts for factors such as temperature-dependent conductivity, mass flow rate, and continuous heating dynamics.

5.1.1 Methodology and Equations

5.1.1.1 Conductivity measurement

To account for the temperature-dependent behavior of the conductivity, conductivity measurements at various temperatures were taken. These measurements were taken in a fixed-volume cylinder using a conductivimeter (Hanna Instruments, HI9635). The data obtained allowed for the development of a temperature-conductivity linear relationship, used in the subsequent calculations.

5.1.1.2 Energy Requirement Calculation

The theoretical energy required (Q) to achieve the desired temperature change in the nectar, assuming ideal conditions with no losses, was calculated using the formula, :

$$Q = \dot{m} \cdot c_p \cdot \Delta T \tag{5.1}$$

where:

- $\dot{m}$ is the mass flow rate (350 kg/h or 0.0972 kg/s),
- c_p is the specific heat capacity (3.31 kJ/kg·K for strawberry juice[4]), and
- ΔT is the temperature increase (50°C).

However, due to the dynamic nature of OH, the actual energy requirement may varies along the pipe as the temperature and conductivity change.

5.1.1.3 Ohmic Heating Power Calculation

For each segment of the pipe, the heat dissipated (Q_{seg}) was determined, based on the Joule effect using the relationship:

$$Q_{seg} = \frac{V_{seg}^2}{R_{seg}} \quad (5.2)$$

where, V_{seg} is the voltage across each segment, calculated based on an electric field strength of 50 V/cm (or 5000 V/m), considering a generator of 10 000 V and the 200 cm of the pipe, and R_{seg} is the resistance of each segment, calculated as:

$$R = \frac{L}{\sigma \cdot A} \quad (5.3)$$

With σ being the temperature-dependent conductivity and A the cross-sectional area of the pipe.

5.1.1.4 Temperature Profile and Residence Time

Given the flow rate and pipe geometry, the residence time (t) for each segment is calculated as the segment length over the linear velocity, with linear velocity derived from the flow rate and cross-sectional area of the pipe. The temperature increase dT in each segment is then calculated iteratively as:

$$dT = \frac{P \cdot t}{m_{seg} \cdot c_p} \tag{5.4}$$

where m_{seg} represents the mass of juice in each segment.

5.1.2 Results

Conductivity of the strawberry nectar was calculated as a function of temperature by the equation:

$$\sigma(T) = 0.0369 \cdot T + 0.6954 \tag{5.5}$$

This equation was later used to calculate the resistance and the power of each section, a summary of the power required and process parameters for OH calculation are provided in the Appendix D.

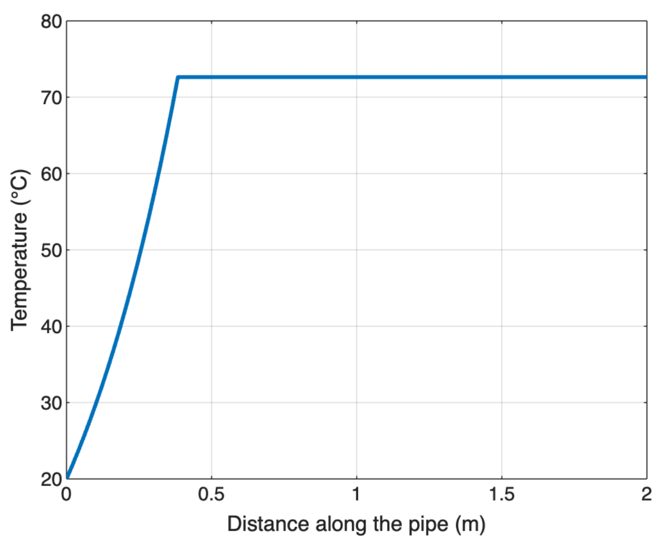

Figure 5.1. Temperature profile along the pipe length during ohmic heating of strawberry nectar

5.1.2.1 Discussion

The final temperature profile in Figure 5.1 shows a rapid increase in temperature at the beginning of the pipe, with the strawberry nectar reaching the target temperature of 70°C before 0.5 meters. Since the juice reaches 70°C within the first section of the pipe, the system likely uses less energy over time, as it only needs to maintain the temperature rather than increase it along the entire pipe.

This profile indicates that the OH system efficiently delivers the necessary energy to reach the target temperature quickly and sustains it with minimal additional power input [5]. Nevertheless, the additional time that the strawberry nectar spends at the target temperature within the pipe may have some detrimental effects. Ideally, for optimal product quality preservation, the process should be designed so that the target temperature of 70°C is reached at the end of the pipe.

This adjustment would allow the product to experience only the minimum necessary exposure to high temperatures, reducing thermal damage while still ensuring microbial safety. Subsequent calculations were adjusted to reduce energy input until the desired exit temperature at the pipe's exit was near the objective temperature. The optimal energy input was determined to be 22.5 V/cm. Future optimization could focus on refining power delivery and flow dynamics to achieve this precise end-point heating.

The fast initial heating observed here is typical of OH, where electrical energy is directly converted to heat within the liquid [6]. This direct heating mechanism reduces the need for prolonged heat transfer times, allowing the product to reach the target temperature over a shorter distance, so that the final residence time, summing up the holding phase and cooling phase, will be shorter compared to conventional thermal [7].

This efficiency minimizes the time the juice spends at high temperatures, which is beneficial for preserving heat-sensitive qualities, such as color, flavor, and nutrients, in the strawberry nectar [5]. By minimizing the residence time at high temperatures, the system reduces potential thermal degradation of sensitive compounds, like vitamin C, in the strawberry nectar.

5.2 Validation of Ohmic Heating Pilot Plant for Vitamin C Retention and *E. coli* Surrogate Inactivation on Strawberry Nectar[1]

Ensuring microbial safety and maintaining the nutritional integrity of fruit-based products are critical objectives in food processing. Ohmic heating offers potential advantages in minimizing the degradation of bioactive compounds [1]. Ohmic heating has emerged as a promising thermal technology due to its potential for rapid and uniform heating, based on the Joule effect [2]. It has been extensively studied and used in the industry for its effectiveness in the pasteurisation of fruit juices, leading to minimal nutrient loss and preservation of the sensory attributes of the product [8,9].

In the context of strawberry nectar, achieving the necessary 5-log reduction of the most heat-resistant pathogen, without compromising product quality is a significant challenge. This nectar is rich in vitamin C, a compound that is highly sensitive to heat. Traditional thermal processing frequently causes undesirable changes in the quality of the final product, such as loss of Vitamin C and alterations in color [10,11]. Literature showed that ohmic heating can significantly reduce the loss of thermolabile nutrients, such as vitamin C, anthocyanins, and polyphenols, during processing [6]. Therefore, the objective of ohmic heating processing is not only to meet food safety standards but also to maintain the nutritional

[1] This section is based on the unpublished paper: Darío J. Pavón-Vargas, Vincenzo Alfonsi, Stephane Georgé, Mario Gozzi, Sara Rainieri, Luca Cattani. Validation of Ohmic Heating Pilot Plant for Vitamin C Retention and *E. coli* Surrogate Inactivation on Strawberry Nectar. Foods 2024, 13, x. https://doi.org/10.3390/xxxxx

value of the nectar. Achieving this balance is essential to meet both microbial safety standards and consumer demand for high-quality, nutrient-rich foods.

Based on thermal kinetics and safety assessments, *E. coli* ATCC 8739 has emerged as a suitable surrogate for validating heat treatments in fruit nectar production [12], as it offers similar resistance to temperature as pathogens like *E. coli* O157:H7, which is a common cause of food-borne diseases outbreaks linked to fruit juices [13]. Consequently, the primary objective of this study was to achieve the required 5-log reduction of the surrogate *E. coli* ATCC 8739 while simultaneously evaluating the retention of vitamin C using a pilot-scale unit. The study focused on modelling the thermal kinetics of vitamin C degradation alongside microbial inactivation.

Kinetic models were developed from laboratory trials using a thermoresistometer, which enabled a fast-heating rate [14]. These models were then applied to pilot-scale experiments. By comparing the observed reduction in ohmic heating with those predicted in the lab models, the goal was to verify whether the kinetics were comparable. This approach aimed to provide a more precise understanding of nutrient preservation and microbial safety during ohmic processing.

Given that both, thermal and ohmic, are thermal processes, it is important to compare them, as the degradation mechanisms in both methods are believed to be the same [3], and literature presents conflicting results regarding the electric field effects on the degradation of bioactive compounds during ohmic heating, when the treatment time is equivalent [15].

While previous research often focused on either microbial reduction or nutrient retention independently, and usually on a laboratory scale, this study attempts to bridge these gaps. By adopting a synergistic approach, the aim was to evaluate both critical factors—microbial inactivation and nutrient preservation - in a pilot-scale environment. This investigation not only addresses the need for more comprehensive validations of emerging technologies but also expand the understanding of how ohmic heating can be optimized. By combining pilot plant validation with thermal kinetics modelling, the study aims to develop optimized processing parameters that ensure both the safety and quality of food products treated with ohmic heating.

5.2.1 Materials and Methods

5.2.1.1 Reagent, Nectar, Buffer, and Model Solution

The reagents used in this study included HPLC-grade water (Thermo Fisher), anhydrous citric acid from Fisher BioReagents (≥ 99.5%), and HPLC-grade acetonitrile from Acros Organics. All other chemicals were of analytical grade and were purchased from Sigma-Aldrich.

McIlvaine's buffer solution (BS), with an initial pH of 3.5, was prepared by mixing 0.1 M citric acid and 0.2 M disodium phosphate solutions [16]. To achieve the final pH of 3.14, corresponding to that of the strawberry nectar, a 5% v/w citric acid solution was gradually added until the desired pH was reached. The BS was then stored at 4°C until use. Strawberry Nectar (SN) was prepared using frozen untreated strawberry puree (*Fragaria x ananassa*), purchased from SVZ International (Breda, The Netherlands). Sucrose and citric acid were added to the puree, along with filtered tap water. The final formulation contained 40% (w/w) strawberry puree, sucrose added to achieve 12% of total soluble solids (TSS), and citric acid added to reach an acidity level of 5.0 g/kg. Finally, a model solution was formulated to minimize the use of strawberry puree during the pilot trials, which would require approximately 400 L/h. This solution replicated the conductivity, vitamin C concentration, and the pH of SN. It contained citric acid, sucrose, and ascorbic acid. The pH was adjusted to 3.14 using a 1 M disodium phosphate solution, and the conductivity was adjusted by adding an appropriate amount of salt (NaCl).

5.2.1.2 Initial Physicochemical Parameters

The initial parameters were evaluated in booth the puree and in the SN, and later used to calculate and adjust the MS to match these same values. pH was measured at 20°C using a pH meter (FiveEasy pH/mV, Mettler Toledo). Electrical conductivity was calculated at 20°C using the electrical resistance in a fixed-volume cell. Total soluble solids (TSS) were determined with a digital refractometer (DBX-55, Atago). Acidity, expressed as citric acid, was measured by the titration using a potentiometric titrator (916 Ti-Touch, Metrohm) with a 0.1N NaOH solution.

5.2.1.3 Vitamin C Reduction in Thermoresistometer

The kinetics of vitamin C reduction was analysed using a Mastia® thermoresistometer in a 400 mL stirred vessel operating in isothermal mode. The system and operational setup, described by [17], allowed for uniform heating of the media to specific temperatures (65°C, 85°C, and 105°C). Samples were collected at regular intervals (5, 10, and 30 minutes) until 180 minutes treatment. BS was evaluated under the same conditions as the SN for a comparative analysis of degradation rates.

5.2.1.4 Microbial Inactivation in Thermoresistometer

Microbial inactivation in SN was evaluated using *E. coli* ATCC 8739 as a surrogate pathogen. A frozen stock culture of *E. coli* ATCC 8739 was thawed and reactivated in Tryptic Soy Broth (TSB) at 37°C for 16 hours. A portion of this reactivated culture was then diluted in acidified TSB to match the pH of the nectar and incubated for further 24 hours at 37°C. This medium, with an initial concentration of approximately 10^9 CFU/mL, was then inoculated into the SN and BS at a ratio of 1:100, resulting in an approximate concentration of 10^7 CFU/mL. Inoculation was carried out directly into the thermoresistometer at 60°C, 65°C, and 70°C, following the same procedure as for the vitamin C trials. Viable counts were taken at three specific times for each temperature to assess the rate of microbial inactivation. On this assay, the BS was adjusted to 12% TSS by adding sucrose. Both, SN and BS were sterilized prior to inoculation by heating to 145°C for 2 minutes in the thermoresistometer.

5.2.1.5 Vitamin C Analysis

The concentration of ascorbic acid in the samples was measured using a modified version of the procedure by [18]. Five grams of sample was mixed with 50 mL of 4% metaphosphoric acid (MPA). To quantify both ascorbic acid and dehydroascorbic acid, a reduction step was performed using tris(2-carboxyethyl)-phosphine hydrochloride (TCEP). The solution was then filtered through a Whatman™ cellulose acetate (CA) syringe filter (0.45 μm) and transferred to HPLC vials for analysis.

Vitamin C analysis was performed using a 1260 Agilent Infinity LC coupled with a 1290 Agilent diode-array detector (DAD) from Agilent Technologies. A C18 ACE column (250 × 4.6 mm, 5 μm particle size) was employed. The mobile phase consisted of 100%, 0.01% sulfuric acid (0.1 mL of concentrated sulfuric acid in 1 L of HPLC-grade water), with a flow rate of 0.8 mL/min. The column temperature was maintained at 30°C and 100 μL of each sample was injected for analysis. Data acquisition was conducted at 245 nm. Quantification was performed using a calibration curve based on ascorbic acid standards, and results were reported as total ascorbic acid content.

5.2.1.6 Microbial Analysis

Microbiological analysis was conducted using samples for each test condition, with viable counts based on duplicate plates prepared from appropriate dilutions. Samples were plated on TSA (Tryptic Soy Agar). The plates were incubated at 37°C for 24 hours to allow colony development and determination of viable counts.

5.2.1.7 Kinetics of Vitamin C degradation

The degradation of ascorbic acid in fresh strawberry juice was evaluated using first-order kinetics [12]:

$$[AA] = [AA]_0 \cdot \exp\,(-k \cdot t) \tag{5.6}$$

where [AA] is the ascorbic acid (AA) concentration (mg AA/100 mL) at time t, $[AA]_0$ the ascorbic acid concentration at time 0, and k the ascorbic acid degradation rate constant for the first order (s^{-1}). The temperature dependence of AA degradation was expressed using the activation energy (*Ea*) described by Arrhenius kinetic.

5.2.1.8 Kinetics of *E. coli* surrogate inactivation

The inactivation kinetics of *E. coli* ATCC 8739 were modelled using the Bigelow model, which assumes first-order kinetics [16]. This approach was applied to both the strawberry nectar and model solution. The model is expressed by the equation:

$$N_t = N_0 \cdot \exp(-k \cdot t) \quad (5.7)$$

Where N_t represents the number of microorganisms at time t, N0 is the initial number of microorganisms, and k is the first-order rate constant (s^{-1}). This equation can be rearranged to relate the k constant to the decimal reduction time D by the equation:

$$D = \frac{2.303}{k} \quad (5.8)$$

The temperature dependence of D is described by the z-value and can be related to Ea by:

$$z = \frac{2.303 \cdot R \cdot T^2}{Ea} \quad (5.9)$$

5.2.2 Pilot Trials in Ohmic Heating Unit

5.2.2.1 Microbial Inoculation

A volume of 300 L of MS was inoculated with *E. coli* ATCC 8739 at an initial con-centration of approximately 107 CFU/mL. The inoculum was prepared by serial dilution of E. coli cultures grown in TSB medium, with a final volume of 3 L. The solution was then processed using ohmic heating, and the microbial inactivation results were com-pared with the kinetics obtained from thermoresistometer experiments.

5.2.2.2 Ohmic Heating Treatment

The product was processed using a 60-kW pilot ohmic heating system (CFT SpA, Parma, Italy) operating at 1-5 kV and 25 kHz. The holding phase was conducted through six pipes (25 mm internal diameter, 4 m length) arranged to maintain the desired temperature for the required time.

After treatment, the product was aseptically packaged in 2.5 L aseptic bags using a Macropack F1 aseptic filler (CFT SpA, Parma, Italy). The MS was processed at 65°C with a holding time of 3 minutes to achieve a 5-log reduction based on previously calculated kinetics. Vitamin C degradation and microbial inactivation were monitored, with triplicate samples collected for analysis at the end of the process for analysis. Untreated samples were also collected as controls.

A schematic representation of the methodology applied in the MS is shown in Figure 5.2. This visual approach outlines the key steps followed during the solution process, treatment conditions, and analysis considered in the model's development. Following the model solution trials, the SN was processed using the same ohmic heating system at 85°C and one minute treatment. Samples were collected for vitamin C analysis, and the results were compared with those from both the thermoresistometer trials and the model solution tests.

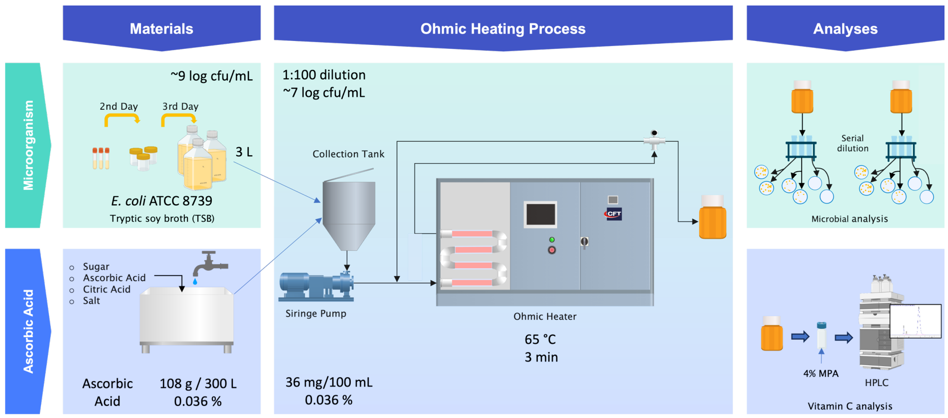

Figure 5.2. Schematic Representation of the Methodology Applied in the Model Solution

5.2.2.3 *Data* Analysis

Statistical differences were assessed using the Student's t-test, with Levene's test to confirm homogeneity of variance. A significance level of $p = 0.05$ was used to determine statistical significance. Hypothesis testing was conducted to compare the mean values of the experimental data with the modelled values. P-values and standardized residuals were calculated to evaluate significant deviations from model predictions. Additionally, models were evaluated based on their coefficient of determination (R^2) values. All statistical analyses were performed using IBM SPSS software version 29.0.1.0, while regression analysis was carried out in Microsoft® Excel.

5.2.3 Results and Discussion

The physicochemical parameters of the strawberry puree, SN, and MS measured in the laboratory are presented in Table 1. These parameters include pH, total soluble solids (TSS), electrical conductivity, ascorbic acid content, acidity, and the initial microbial count.

Electrical conductivity is an important parameter for the ohmic heating process, as it directly affects the efficiency of heat generation [19]. The model solution (MS) was specifically designed to replicate the electrical conductivity of the strawberry nectar (SN) as closely as possible to ensure accurate comparison. However, it is important to acknowledge that other properties of the MS, such as heat capacity and viscosity, may cause variations in heating behaviour compared to the actual nectar.

Table 5.1. Physicochemical properties of strawberry puree (SP), strawberry nectar (SN), and model solution (MS) with p-values from t-test comparison between SN and MS.

Properties	**SP**	**SN**	**MS**	**p-value***
Total soluble solids (TSS) [°Brix]	7.9±0.1	11.9±0.1	11.80±0.1	0.553
pH at 20°C	3.40±0.01	3.16±0.02	3.14±0.03	0.519
Acidity as citric acid [g/kg]	7.49±0.08	5.23±0.11	5.15±0.04	0.448
Electrical conductivity [mS/cm]	-	1.07±0.03	1.04±0.07	0.675
Total ascorbic acid [mg/100 g]	89.6±0.2	34.2±1.0	36.0±1.2	0.242
Total aerobic count [LOG cfu/mL]	4.92	2.31	<1	-

* Two sample t-test confidence interval of 95%. $p < 0.05$ indicates significant differences between strawberry nectar (SN) and model solution (MS).

As it can be seen in Table 5.1, there were no statistically significant differences between the physicochemical parameters of the SN evaluated at the laboratory level and the MS used at the pilot scale. The pH, TSS, and electrical conductivity values were particularly similar, supporting the validity of using the model solution to simulate the behaviour of the actual nectar during ohmic heating. The data from these laboratory evaluations provide the basis for comparing the thermal and ohmic heating responses of the different matrices, ensuring that the model system could accurately represents the nectar.

The physicochemical values for the strawberry puree (SP) and SN, with pH values between 3.1 and 3.4, and ascorbic acid content of 63.6 - 89.0 mg/100g for puree and 39.8±1.1 mg/100g for pasteurized nectar are consistent with previous studies [13,20,21].

5.2.3.1 Kinetics of Vitamin C Reduction and Microbial Inactivation

Table 2 presents the kinetic parameters for the degradation of ascorbic acid and the reduction of *E. coli* ATCC 8739 in both SN and MS, providing a comparison of their behaviour under the conditions tested. The degradation of vitamin C in both SN and BS was evaluated using first-order kinetics [22]. The results showed that temperature played a crucial role in the degradation rate, with higher temperatures leading to accelerated loss of vitamin C (higher k value). The first-order model provided a clear description of the degradation (R-squared >0.98) indicating a strong representation of the degradation process. Bigelow kinetic parameters were calculated from the values of the reaction kinetics and activation energy (Ea), to be use later in the model.

Although the results for SN and BS were generally comparable, there was a noticeable deviation. This suggests that although the rate of degradation (k value) of vitamin C in the nectar may be slower, it is more sensitive to temperature changes (Z value). Additionally, when plotting rate constants (k) for both the BS and SN processes against the reciprocal of absolute temperature to calculate the frequency factor (k_0), the degradation kinetics of SN showed a higher factor (k_0 = 0.98 s^{-1}) compared to the strawberry puree (k_0 = 0.31 s^{-1}). This is probably due to the different matrix composition and the nature of ascorbic acid in the nectar versus the solution, affecting heat transfer and degradation behaviour.

Table 5.2. Kinetic parameters for the degradation of ascorbic acid and the reduction of E. coli ATCC 8739 in strawberry nectar (SN) and buffer solution (BS).

Parameter	Matrix	T (°C)	k x 10^{-3} (s^{-1})	Ea (kJ/mol])	D-value (s)	z-value (°C)	R^2
Vitamin C	BS	65	0.51±0.02	25.8±0.4	4496.6±199.0	93.8±1.7	0.998
		85	0.66±0.05		3495.6±251.1		0.996
		105	1.37±0.05		1684.5±63.9		0.986
	SN	65	0.13±0.00	31.0±2.6	18181.6±239.2	80.1±6.8	0.997
		85	0.36±0.05		6359.0±877.9		0.995
		105	0.40±0.01		5757.5±208.7		0.980
E. coli ATCC 8739	BS	60	23.76±1.17	150.6±2.8	97.06±4.77	14.5±0.3	0.959
		65	59.80±0.64		38.52±0.42		0.865
		70	115.72±2.31		19.91±0.40		0.857
	SN	60	18.61±0.25	108.3±3.3	123.78±1.65	20.2±0.6	0.991
		65	32.97±1.61		69.95±3.41		0.978
		70	58.20±1.22		39.58±0.83		0.823

The degradation of compounds is generally faster in real matrices compared to model systems [23,24]. In the study by Van Bree et al. [25], vitamin C degradation kinetics presents higher k-values in imitation fruit juice compared to commercial juice, but the model accurately predicted the behavior. Therefore, despite this variation, the BS still provides a reasonable approximation, supporting its use for simulating the nectar during ohmic heating.

The kinetics of vitamin C degradation observed in this study are consistent with those reported in the literature for thermal degradation in strawberry products [3,26]. While the results are focus on thermal degradation kinetics, it is important to note that studies comparing ohmic and conventional heating on strawberries indicate similar degradation behaviours for ascorbic acid in both methods [3]. For example, Castro et al. reported rate constants (k_0) of 0.15 s^{-1} with activation energies (E_a) of 21.36

kJ/mol with no significant statistical differences [27]. This suggests that the presence of an electric field during ohmic heating does not significantly alter the degradation behaviour of vitamin C, a key consideration to the later evaluation of ohmic heating in pilot scale. Bigelow kinetic parameters were calculated from the values of the reaction kinetics and activation energy (Ea), to be use later in the model.

Microbial inactivation was modelled using the Bigelow model, which assumes first-order kinetics. The D and z values for *E. coli* ATCC 8739 were calculated in both SN and BS. The results indicated that *E. coli* inactivation followed a first-order model, with faster inactivation observed at higher temperatures. The microbial inactivation kinetics in both, SN and BS, were comparable, confirming that the model solution can represent the inactivation behaviour of the nectar. The z-values ranged from 14.54 to 20.02, indicating high thermal resistance.

In the literature, D-values for *E. coli* at 60°C range from 13.2 to 300 s, which is in good agreement with our findings [28]. A study on different strains of *E. coli* surrogates, including ATCC8739, reported D-values between 13.8 and 99.6 s, and z-values between 19.66 and 24.89°C in the same temperature range for different fruit nectars [12]. Similarly, another study reported a D_{50} of 324.6 s, a D_{60} of 95.4 s, and a z-value of 18.78±1.44°C for *E. coli* O157 in orange juice. These comparable results further validate the use of *E. coli* ATCC 8739 as an appropriate surrogate for heat treatments in acidic fruit-based products like strawberry nectar.

The selection of 65°C for the pilot trials was based on kinetic parameters aimed at achieving a 5-log reduction of *E. coli* while minimizing vitamin C loss. Typically, thermal pasteurization in juices result in a vitamin C loss of minimum 5% [29]. Nevertheless, different heating methods, even at similar temperatures, can result in different levels of vitamin C degradation [30]. According to the model, the 5-log reduction in *E. coli* at 65°C corresponds to a treatment time of three minutes, which was further validated during the pilot scale trials.

Figure 5.3 presents the mathematical predictions using the Bigelow model for achieving a 5-log reduction of *E. coli* ATCC 8739 (dashed lines), alongside the modelled degradation of ascorbic acid (dot-dashed lines) for both the model solution (A) and strawberry nectar (B).

In panel (A), the blue dot-dashed line represents a 9% loss of ascorbic acid, which line with the experimental treatment point of 65°C for 3 minutes. Similarly, panel (B) shows the modelled ascorbic acid degradation curve (green dot-dashed line), corresponding to a 2.7% loss. This curve intersects with the treatment point chosen for strawberry nectar at 85°C for 2 minutes, which resulted in a significantly higher microbial reduction. The treatment for strawberry nectar was selected to validate the model, considering that ascorbic acid is more resistant in nectar, the limitations of the pilot plant and the raw material available.

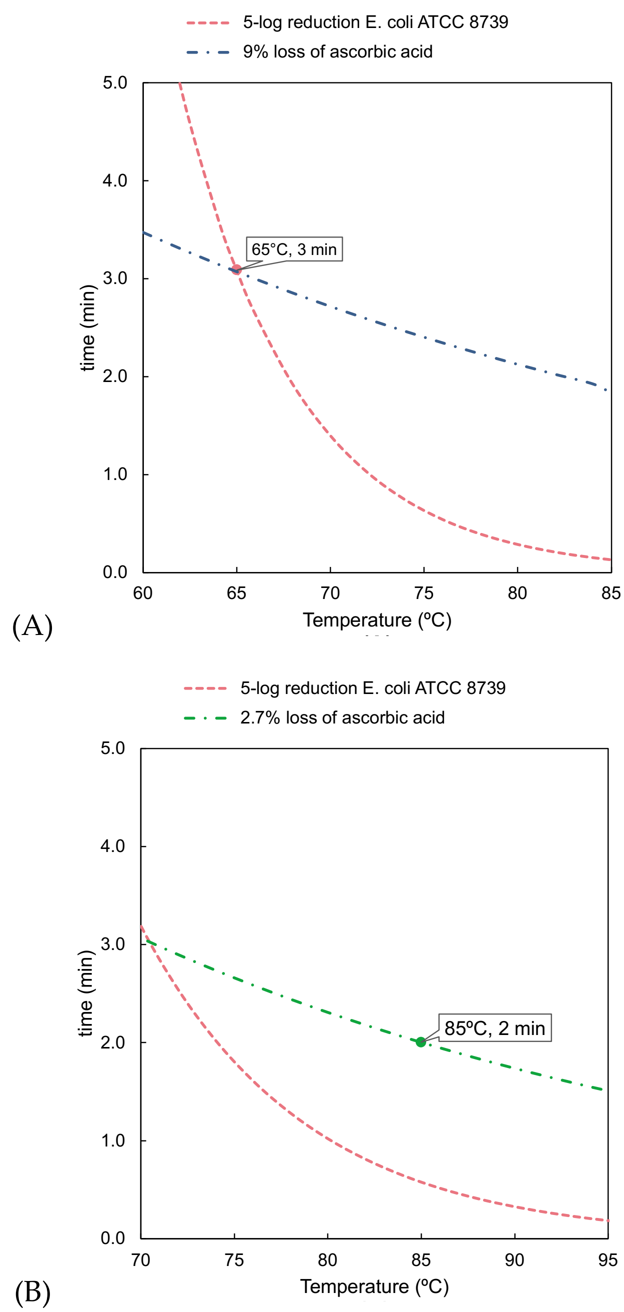

Figure 5.3. Temperature-time chart for mathematical predictions (Bigelow model) of 5-log E. coli ATCC 8739 reduction and ascorbic acid loss in model solution (A) and strawberry nectar (B) at selected treatment points.

5.2.3.2 Pilot Trials with Model Solution

During the pilot-scale Ohmic heating trials, the model solution was processed to assess both vitamin C degradation and microbial inactivation. The results from these trials were consistent with those observed in the thermoresistometer experiments. The model, which was derived from Bigelow kinetics for a 5-log reduction in *E. coli* and a 9% loss of AA, provides the basis for comparison with the pilot-scale ohmic heating results. As can be seen in Figure 5.4, the comparison between the experimental results from ohmic heating and the model-based kinetics (†) shows no significant differences in ascorbic acid (AA) retention and microbial inactivation, aligning with the model predictions. The comparison between the laboratory-scale and pilot-scale trials showed that the ohmic heating system maintained similar kinetic behaviour, supporting the scalability of the process.

As shown in Figure 5.4, the reduction after thermal treatment at 65°C for 3 minutes resulted in a significant reduction of 9.71%, which is in good agreement with the expected results from the model. A two-sample t-test comparing the ohmic treated samples with the modelled reduction (derived from the mean control value) shows a t-statistic of -1.44 and a p-value of 0.287, indicating no significant difference between the two. The vitamin C content in the ohmic treated sample was 32.53±1.02 mg/100g, while the model predicted a value of 33.14 mg/100g.

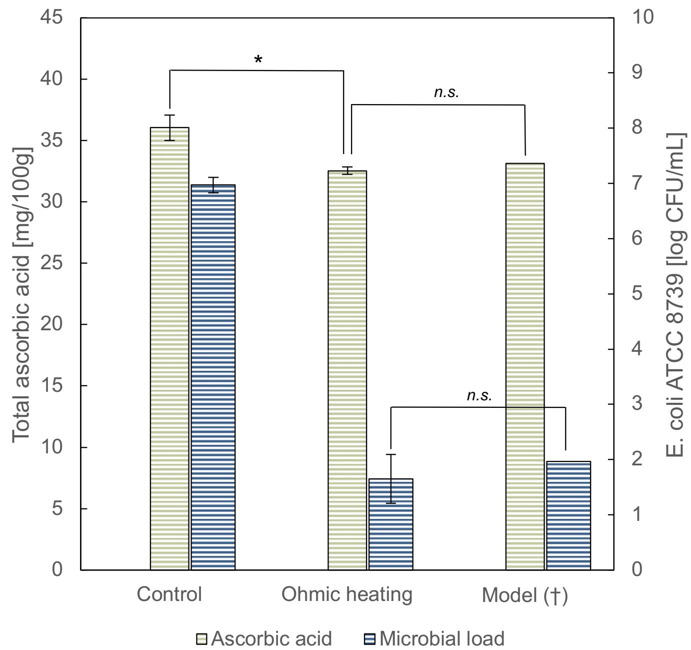

Figure 5.4. Comparison of ascorbic acid content [mg/100g] (Left axis) and microbial load (*E. coli* ATCC 8739) [log CFU/mL] (Right axis) after ohmic heating at 65°C-3 min, in a model system. Bigelow kinetics model predictions (†), control (before treatment), and ohmic heating (after treatment)

In terms of microbial validation, no significant difference was found between the model and the ohmic treated samples (p-value of 0.343). The log reduction for the Ohmic treatment was 5.32, compared to the model's predicted value of 5. These results suggest that the model can accurately describe the behaviour of *E. coli* surrogates and vitamin C reduction in the pilot unit, indicating that the kinetics of thermal treatments closely match those observed in ohmic heating. This corroborates the findings from other studies which have demonstrated similar kinetics for these processes [3,8,15].

5.2.3.3 Pilot Trials with Strawberry Nectar

Figure 5.5 shows the comparison of ascorbic acid content [mg/100g] in strawberry nectar treated at 85°C – 2 min in ohmic heating plant. In this

Figure, ascorbic acid retention is compared across Control 1 (immediately after mixing), Control 2 (just before ohmic processing), ohmic heating, and model predictions based on Bigelow kinetics. Control 1 and Control 2 were measured to assess not only the effect of Ohmic processing but also the impact of the overall pilot plant operation on vitamin C levels. In the pilot trials with actual strawberry nectar, the results showed a 19.45% difference in microbial reduction compared to the values predicted by the modelled system and thermal kinetics observed in the laboratory, indicating some inconsistency between the two.

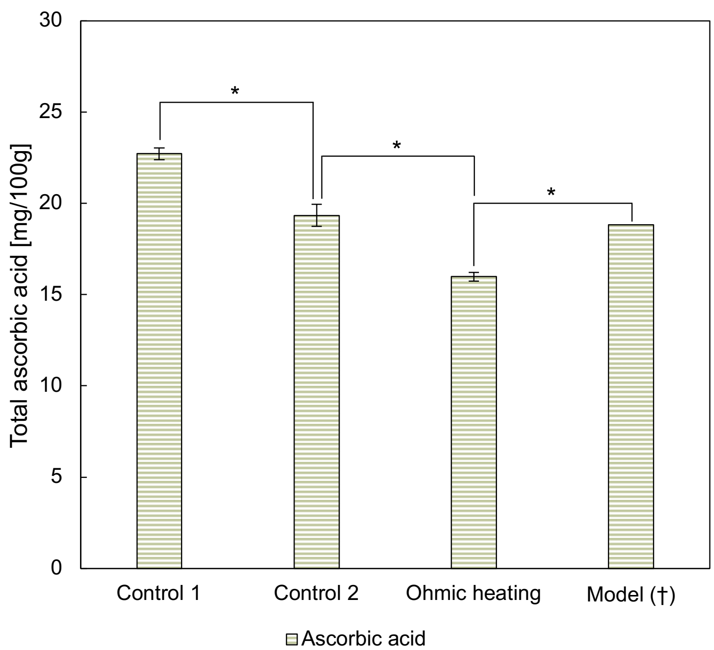

Figure 5.5. Comparison of ascorbic acid content [mg/100g] in strawberry nectar treated at 85°C-2 min in ohmic heating plant. Control 1 (immediately after mixing), Control 2 (just before ohmic processing), ohmic heating, and model predictions based on Bigelow kinetics (†)

An important observation is the low value of ascorbic acid in Control 1 (22.71 mg/100g), which is considerably lower than the lab-measured value of 36.03 mg/100g. This discrepancy may be attributed to the extensive defrosting of the nectar prior to processing, which could have led to the degradation of vitamin C. Additionally, industrial factors such as exposure to oxygen and prolonged residence in tanks could further contribute to this lower initial value. Control 2, sampled just before Ohmic heating, showed an additional loss in ascorbic acid compared to Control 1. This suggests that handling processes in the pilot plant have a cumulative effect on nutrient degradation.

As shown in Figure 3, the ohmic heating process resulted in a statistically significant lower retention of ascorbic acid than expected, probably due to factors such as pre-processing losses and time in aseptic tank or packaging. The Bigelow kinetics model predicted a higher retention, there is a 15% difference when comparing the model value to the ohmic-treated samples, highlighting that models may not fully account for pilot condition with real product. While the model predicted a reduction of 2.7% (to 18.81 mg/100g), the actual degradation after treatment was 17.38%, yielding 15.97 mg/100g. Overall, a 29.64% loss of ascorbic acid occurred from the fresh nectar (Control 1). In the literature, the degradation of vitamin C during ohmic heating ranged from 3.08% to 10.63% [31], and in orange juice (12°Brix), conventional heating caused 22.45% degradation, while Ohmic heating resulted in 20.03% [32].

Ohmic heating can lead to electrolysis and electrode corrosion, increasing oxygen levels and accelerating ascorbic acid oxidation. However, using stainless-steel electrodes significantly reduces this effect [11,27]., Industrial stainless-steel electrodes operating at 25 kHz were used in this experiment. Studies have shown that at frequencies above 100 Hz, the rate constants for conventional and Ohmic heating are similar, suggesting that the Ohmic effect has minimal effect on the samples, with oxidation and temperature being the primary degradation mechanisms [25].

Ohmic heating may cause electrolysis and electrode corrosion, which can increase oxygen and accelerate ascorbic acid oxidation. However, the use of stainless-steel electrodes minimizes this effect [3,33]. Additionally, the rate constant for conventional and ohmic heating is similar at frequencies above 100 Hz [31]. In this experiment, industrial stainless-steel electrodes operating at 25 kHz were used, indicating that ohmic effect does not affect the samples and, therefore, oxidation and thermal effect were the main degradation mechanisms.

5.2.4 Conclusions

The objective of this study was to validate an ohmic heating pilot plant for the retention of vitamin C and the inactivation of an *E. coli* surrogate in strawberry nectar, using thermal kinetics as a predictive model. The pilot-scale validation of the model solution aligned with the thermal kinetics model calculated in the laboratory. The 5-log reduction of *E. coli* was achieved at 65°C for 3 minutes, aligning with the kinetics calculation, supporting the scalability of the process for industrial applications. Meanwhile, in the strawberry nectar test, ohmic heating resulted in more ascorbic acid degradation than predicted, possibly due to factors like localized overheating or non-uniform energy distribution, and other factors inherit from the pilot trial itself. These deviations likely appear from the pilot scale, which lacks the precise control of laboratory conditions. However, vitamin C reduction is still on the range of conventional thermal treatments.

5.3 References

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6 Conclusions

This research provides a comprehensive analysis of the impact of different processing technologies, high-pressure processing (HPP), pulsed electric fields (PEF), thermal treatments (TT), and Ohmic Heating (OH) on the quality and safety of fruit juices and nectars, focusing on strawberry, sour cherry, and raspberry matrices. Each technology demonstrates unique influences on microbial safety, antioxidant activity, color stability, and physicochemical properties of the juices, suggesting that no single method suits all juice types or desired quality outcomes. Optimizing factors such as pH, total soluble solids (TSS), temperature, and treatment intensity for each juice matrix is essential for maximizing quality while achieving microbial safety. This general conclusion integrates the findings of studies on matrix effects, color stability, antioxidant preservation, viscosity, and microbial inactivation kinetics.

The **Matrix Effect on Antioxidant Activity and Color in Strawberry Nectar** underscores the significant influence of pH, TSS, and temperature on quality attributes. Lower pH levels and moderate TSS proved optimal for color stability and antioxidant activity, while temperature increases tended to reduce overall quality. Notably, the matrix's composition, including pH and sugar content, affects both antioxidant stability and microbial safety, with strawberry nectar exhibiting greater sensitivity to pH, especially under higher values, compared to cherry nectar. This study indicates that optimizing these parameters can help in preserving the desired qualities of each juice type.

Similarly, the **Impact of Thermal and HPP Treatments on Strawberry Nectar** demonstrates that HPP outperforms thermal processing in preserving color while thermal methods are more effective for texture retention and reducing enzymatic activity, such as polyphenol oxidase (PPO) activity. Particularly, HPP increases nectar viscosity due to pressure-induced matrix changes, whereas thermal treatments have limited impact on viscosity but require optimized pH and sugar levels to minimize degradation.

In parallel, the studies on **Microbial Inactivation Kinetics and Modelling in Sour Cherry Juice Treated by HPP, PEF, and Thermal Processes** contribute valuable insights into the safety aspects of these technologies. Each method achieves a 5-log reduction in pathogen load, but with distinctive kinetic profiles. For thermal inactivation, the non-linear Weibull model provided a better fit than the traditional Bigelow model, capturing the kinetics more accurately. In the case of HPP, non-linear patterns were also observed, particularly under higher pressures (≥400 MPa), while PEF treatments required even more advanced models (e.g., Peleg and Geeraerd) to capture the complex inactivation kinetics. PEF's efficacy depends primarily on electric field strength and specific energy, with inactivation increasing at higher field strengths (around 20 kV/cm) and specific energies (100-120 kJ/L). These findings highlight the need for distinct kinetic models to accurately describe microbial inactivation across processing methods.

The **Comparative Physicochemical Evaluation of Sour Cherry and Raspberry Juices under HPP, PEF, and Thermal Treatments** showed that HPP optimally maintains color, phenolic content, and browning index (BI) without sacrificing microbial safety, making it ideal for sour cherry and raspberry juices. Although PEF treatments showed some color degradation due to the ohmic heating effect at higher energy levels, multivariate analyses, including PCA and cluster analysis, confirmed that PEF-treated samples still closely resembled the control in physicochemical profiles. This part of the study emphasizes the distinct responses of different juices to each processing technology: sour cherry juice undergoes more noticeable color and viscosity changes than raspberry juice, whose color and viscosity remain stable.

Finally, in the study on **Ohmic heating validation** the degradation kinetics of vitamin C and the inactivation of an *E. coli* surrogate to assess whether OH could provide similar efficacy to traditional thermal treatments. The findings indicate that OH maintained consistent kinetic behavior in both vitamin C degradation and microbial reduction, aligning closely with the expected outcomes from conventional thermal methods. This consistency in kinetic response demonstrates that OH is an effective method for microbial inactivation, comparable to thermal processing, while also offering a promising alternative for applications requiring precise control over nutrient retention and microbial safety.

In summary, the findings confirm that each technology offers unique advantages and challenges in preserving juice quality and ensuring safety. While HPP retain color and nutritional quality, particularly increasing anthocyanins content and browning stability, thermal treatments are more effective for enzymatic control and texture maintenance. PEF treatments, especially under controlled electric field strengths and specific energies, provide an alternative with minimal physicochemical disruption, yet require advanced modeling for accurate microbial inactivation analysis.

The overarching conclusion is that a customization approach, which considers the processing parameters and the unique attributes of each fruit juice, is critical. Such customization can significantly enhance juice quality by selectively applying HPP, PEF, or TT according to the specific matrix.

Appendix A ANOVA for Statistical Analysis on Strawberry and Cherry Nectar Matrix Effect

A.1 ANOVA Tables for the Study on the Matrix Effect on Antioxidant Activity and Color in Thermal Treated Strawberry Nectar

Table A.1.1. ANOVA results for the effect of the matrix on the Total Color Difference (ΔE) on strawberry nectar samples

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	1	2.30919	2.30919	2.77227	0.10414
TSS	1	2.09669	2.09669	2.51716	0.12090
Temp	1	8.83501	8.83501	10.60676	0.00237
pH*pH	1	0.07118	0.07118	0.08545	0.77163
TSS*TSS	1	7.44692	7.44692	8.9403	0.00487
Temp*Temp	1	3.97866	3.97866	4.77653	0.03508
pH*TSS	1	1.10966	1.10966	1.33219	0.25562
pH*Temp	1	0.96549	0.96549	1.1591	0.28844
TSS*Temp	1	0.31857	0.31857	0.38245	0.53998
Error	38	31.65249	0.83296		
Lack of fit	3	13.17971	4.39324	8.32378	2.60411
Pure Error	35	18.47278	0.52779		
Total	47	58.78385			

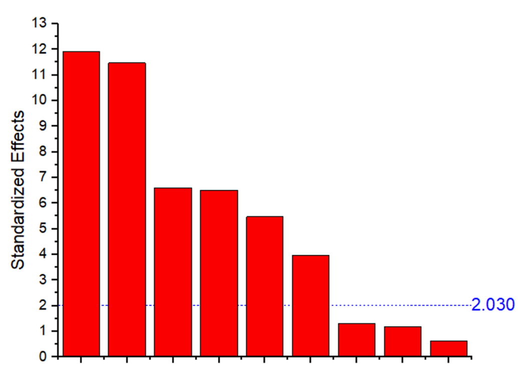

Figure A.1.1. Standardized effects plot graph of the matrix effect on the total color difference (ΔE) on strawberry nectar samples. For main and two-way interactions of pH, Total Soluble Solids (TSS) and Temperature (Temp)

Table A.1.2. ANOVA results for the effect of the matrix on the Browning Index (BI) on strawberry nectar samples.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	1	0.09438	0.09438	25.9709	9.8279E-6
TSS	1	0.37151	0.37151	102.2336	2.50944E-12
Temp	1	0.0168	0.0168	4.6234	0.03797
pH*pH	1	0.005	0.005	1.3765	0.248
TSS*TSS	1	0.16333	0.16333	44.94694	6.19673E-8
Temp*Temp	1	0.01394	0.01394	3.83611	0.05753
pH*TSS	1	0.19026	0.19026	52.35679	1.18236E-8
pH*Temp	1	0.00241	0.00241	0.66274	0.42067
TSS*Temp	1	0.04284	0.04284	11.78915	0.00145
Error	38	0.13809	0.00363		
Lack of fit	3	0.1217	0.04057	86.62714	2.88141
Pure Error	35	0.01639	4.68286E-4		
Total	47	1.03856			

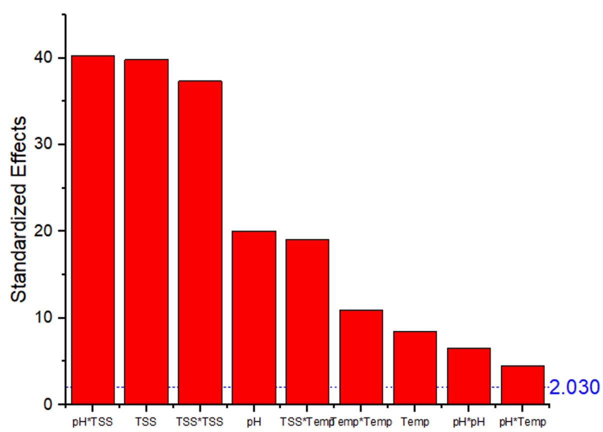

Figure A.1.2. Standardized effects plot graph of the matrix effect on the browning index (BI) on strawberry nectar samples. For main and two-way interactions of pH, Total Soluble Solids (TSS) and Temperature (Temp). Significant threshold line ($p<0.05$) in blue.

Table A.1.3. ANOVA results for the effect of the matrix on the Radical Scavenging Activity (RSA%) on strawberry nectar samples.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	1	0.09767	0.09767	33.65714	1.06589E-6
TSS	1	0.00118	0.00118	0.40527	0.5282
Temp	1	0.0108	0.0108	3.72017	0.06125
pH*pH	1	0.01463	0.01463	5.04179	0.03064
TSS*TSS	1	0.04539	0.04539	15.64118	3.22754E-4
Temp*Temp	1	0.01505	0.01505	5.18722	0.02847
pH*TSS	1	0.01033	0.01033	3.5583	0.06691
pH*Temp	1	4.6875E-4	4.6875E-4	0.16154	0.68999
TSS*Temp	1	1.33333E-4	1.33333E-4	0.04595	0.83141
Error	38	0.11027	0.0029		
Lack of fit	3	0.06201	0.02067	14.99092	1.93503
Pure Error	35	0.04826	0.00138		
Total	47	0.3059			

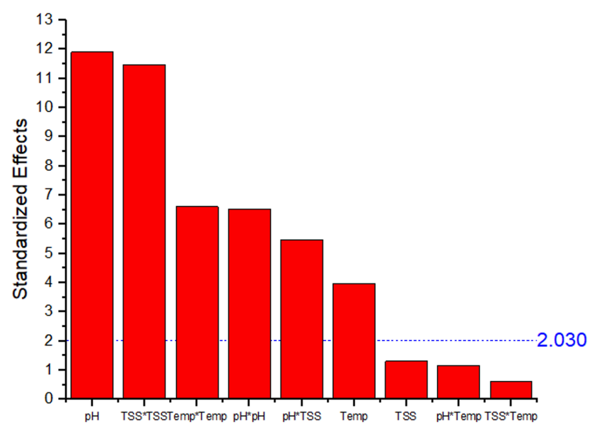

Figure A.1.3. Standardized effects plot graph of the matrix effect on Radical Scavenging Activity (RSA%) on strawberry nectar samples. For main and two-way interactions of pH, Total Soluble Solids (TSS) and Temperature (Temp). Significant threshold line (p<0.05) in blue.

A.2 ANOVA Tables on the Effects of the Matrix on the Stability of Color, Antioxidants, Enzymes, and Microbial Safety in Strawberry and Sour Cherry Nectar

Table A.2.1. ANOVA results for the effect of the matrix on the reduction of *E. coli* ATCC8739 in strawberry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	10.88543	5.44271	54.78206	1.20E-11
TSS	1	33.70614	33.70614	339.25943	6.56E-20
Treatment	2	160.6581	80.32905	808.52887	1.21E-30
pH*TSS	2	1.8603	0.93015	9.36216	5.32E-04
pH*Treatment	4	5.56763	1.39191	14.00984	5.49E-07
TSS*Treatment	2	16.90697	8.45349	85.08612	2.28E-14
pH*TSS*Treatment	4	1.90438	0.4761	4.792	0.00334
Error	36	3.57668	0.09935		
Total	53	235.06563			

Table A.2.2. ANOVA results for the effect of the matrix on the total color difference (ΔE) of strawberry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	13.64863	6.82431	60.97374	2.7556E-12
TSS	1	4.45367	4.45367	39.79254	2.69799E-7
Treatment	2	41.85017	20.92509	186.96103	9.65654E-20
pH*TSS	2	3.50954	1.75477	15.67849	1.26636E-5
pH*Treatment	4	8.03219	2.00805	17.94146	3.48571E-8
TSS*Treatment	2	0.8333	0.41665	3.72268	0.03392
pH*TSS*Treatment	4	2.62167	0.65542	5.85602	9.78038E-4
Error	36	4.0292	0.11192		
Total	53	78.97838			

Table A.2.3. ANOVA results for the effect of the matrix on the total color difference (ΔE) of cherry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	4.6848	2.3424	150.90762	3.14209E-18
TSS	1	4.78827	4.78827	308.48074	3.07534E-19
Treatment	2	20.99752	10.49876	676.37521	2.79389E-29
pH*TSS	2	1.83903	0.91951	59.23895	4.11008E-12
pH*Treatment	4	5.19455	1.29864	83.66379	1.02051E-17
TSS*Treatment	2	2.24381	1.12191	72.27797	2.47989E-13
pH*TSS*Treatment	4	1.61965	0.40491	26.08616	3.32478E-10
Error	36	0.5588	0.01552		
Total	53	41.92641			

Table A.2.4. ANOVA results for the effect of the matrix on the browning index (BI) of strawberry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	4.51603	2.25801	1988.02048	1.4219E-37
TSS	1	0.10663	0.10663	93.87627	1.43511E-11
Treatment	2	0.05856	0.02928	25.78078	1.12668E-7
pH*TSS	2	0.05534	0.02767	24.36038	2.03999E-7
pH*Treatment	4	0.05729	0.01432	12.6092	1.63564E-6
TSS*Treatment	2	0.06633	0.03317	29.20159	2.90861E-8
pH*TSS*Treatment	4	0.02889	0.00722	6.35846	5.61137E-4
Error	36	0.04089	0.00114		
Total	53	4.92995			

Table A.2.5. ANOVA results for the effect of the matrix on the browning index (BI) of cherry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	4.37946	2.18973	2269.63168	1.33646E-38
TSS	1	0.00119	0.00119	1.23833	0.27317
Treatment	2	0.00911	0.00456	4.72301	0.01508
pH*TSS	2	4.66926E-4	2.33463E-4	0.24198	0.78634
pH*Treatment	4	0.01972	0.00493	5.11106	0.00229
TSS*Treatment	2	0.00477	0.00238	2.4706	0.09875
pH*TSS*Treatment	4	0.00725	0.00181	1.87865	0.13542
Error	36	0.03473	9.64796E-4		
Total	53	4.45671			

Table A.2.6. ANOVA results for the effect of the matrix on the radical scavenging activity (RSA%) of strawberry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	32.38605	16.19302	6.45158	0.00403
TSS	1	394.44738	394.44738	157.15462	1.07185E-14
Treatment	2	2092.42261	1046.2113	416.82857	1.27403E-25
pH*TSS	2	155.16726	77.58363	30.91065	1.53336E-8
pH*Treatment	4	14.54657	3.63664	1.4489	0.23805
TSS*Treatment	2	74.61447	37.30723	14.86384	1.96777E-5
pH*TSS*Treatment	4	26.93444	6.73361	2.68279	0.04687
Error	36	90.35755	2.50993		
Total	53	2880.87633			

Table A.2.7. ANOVA results for the effect of the matrix on the radical scavenging activity (RSA%) of cherry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	585.63859	292.81929	39.05567	9.58356E-10
TSS	1	76.35045	76.35045	10.18347	0.00294
Treatment	2	815.22558	407.61279	54.3666	1.32806E-11
pH*TSS	2	51.09849	25.54924	3.40771	0.04412
pH*Treatment	4	98.70371	24.67593	3.29123	0.02131
TSS*Treatment	2	124.00747	62.00373	8.26994	0.00111
pH*TSS*Treatment	4	107.80911	26.95228	3.59484	0.01448
Error	36	269.90947	7.49749		
Total	53	2128.74285			

Table A.2.8. ANOVA results for the effect of the matrix on the Polyphenol oxidase (PPO) relative activity (%) on cherry nectar

	DF	Sum of Squares	Mean Square	F Value	Prob>F
pH	2	4.6848	2.3424	150.90762	3.14209E-18
TSS	1	4.78827	4.78827	308.48074	3.07534E-19
Treatment	2	20.99752	10.49876	676.37521	2.79389E-29
pH*TSS	2	1.83903	0.91951	59.23895	4.11008E-12
pH*Treatment	4	5.19455	1.29864	83.66379	1.02051E-17
TSS*Treatment	2	2.24381	1.12191	72.27797	2.47989E-13
pH*TSS*Treatment	4	1.61965	0.40491	26.08616	3.32478E-10
Error	36	0.5588	0.01552		
Total	53	41.92641			

Appendix B Response Surface Modelling (RMS) for Microbial Inactivation in Sour Cherry and Raspberry Juices

B.1 RSM Model and ANOVA Table for Sour Cherry Juice Thermal Treatment

- Linear regression model (Linear):
  Log reduction ~ 1 + t + T

Table B.1.1. Estimated coefficients for the linear regression model of sour cherry juice log reduction – Thermal treatment RSM model

Term	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-33.033	10.517	-3.1409	0.01191
Time (t)	2.3068	0.72349	3.1884	0.011035
Temperature (T)	0.43094	0.13105	3.2883	0.009404
T·t	-33.033	10.517	-3.1409	0.01191

Number of observations: 12, Error degrees of freedom: 9
Root Mean Squared Error: 1.51
R-squared: 0.854, Adjusted R-Squared: 0.842
F-statistic vs. constant model: 10.5, p-value = 0.00445

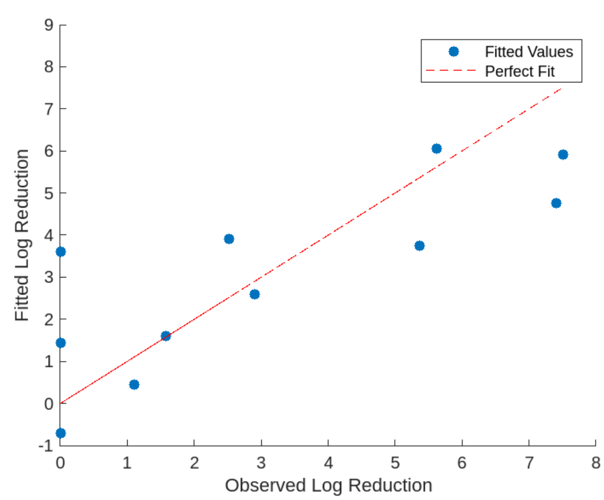

Figure B.1.1. Log reduction fitted vs observed values in sour cherry juice thermal treatment RSM

B.2 RSM Model and ANOVA Table for Sour Cherry Juice High-Pressure Processing (HPP) Treatment

- Linear regression model (second order interaction):
  Log reduction ~ 1 + t + P + P· t

Table B.2.1. Estimated coefficients for the linear regression model of sour cherry juice log reduction – HPP RSM model

Term	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-0.39308	1.26060	-0.31182	0.76315
Time (t)	-0.20774	0.33691	-0.61662	0.55462
Pressure (P)	0.00255	0.00292	0.87397	0.40761
P·t	0.00234	0.00078	2.99540	0.01719

Number of observations: 12, Error degrees of freedom: 8
Root Mean Squared Error: 0.986
R-squared: 0.899, Adjusted R-Squared: 0.862
F-statistic vs. constant model: 23.9, p-value = 0.00001

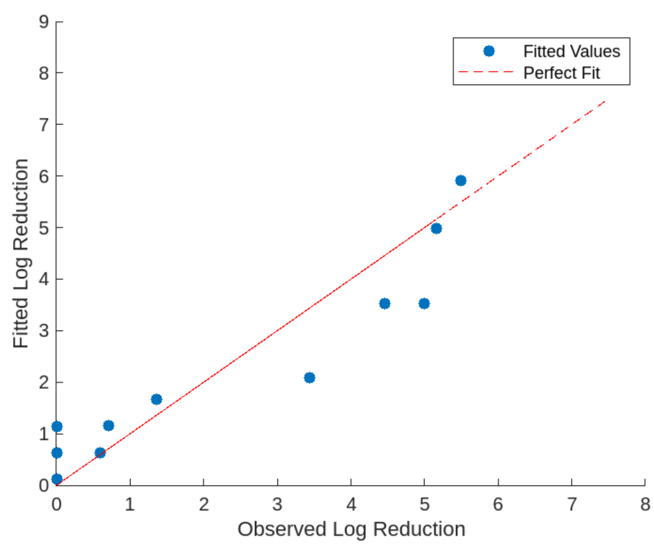

Figure B.2.1. Log reduction fitted vs observed values in sour cherry juice HPP treatment RSM

B.3 PEF Measured and Calculated Parameters in the Microbial Inactivation Trials for Sour Cherry and Raspberry

In this section, the measured and calculated parameters from the microbial inactivation trials in the PEF Pilot Dual machine (ELEA, Quakenbrück, Germany) (Figure B.3.1) are presented. A datalogger included in the machine recorded the parameters every 10 seconds, and the average values from these measurements were used to calculate the other parameters.

The first calculated parameter was the residence time, determined using the flow rate of 50 L/h and the chamber geometry of 10x10 mm. The residence time (t_{res}) was calculated as:

$$t_{res} = \frac{Volume}{Flow\ rate} = \frac{chamber\ area\ \times\ chamber\ length}{Flow\ rate}$$

where:

- Flow rate: 50 L/h
- Chamber diameter: 10 mm
- Chamber length: 10 mm

So the residence time was calculated at 113.04 ms. Next, the number of pulses was calculated by multiplying the pulse frequency by the residence time. Finally, the treatment time by multiplying the number of pulses by the pulse width, which was maintained at 7 μs. Table B.3.1 summarizes all the measured and calculated parameters, including data for both sour cherry and raspberry.

Figure B.3.1. PEF Pilot Dual machine model used during the trials (elea-technology.com)

Table B.3.1. Summary of Pulsed Electric Field (PEF) Treatment Parameters and Microbial Reduction Results for Sour Cherry and Raspberry Juices

Juice	E (kV/cm)	W (kJ/L)	Total # of pulses	Pulse width (μs)	Frequency (Hz)	Treatment time (μs)	Log reduction
raspberry	0	0	0.00	0	0	0.00	0.0000
raspberry	0	0	0.00	0	0	0.00	0.0000
raspberry	16	100	8.82	7	78	61.72	1.7518
raspberry	16	100	8.82	7	78	61.72	1.6966
raspberry	16	120	9.38	7	83	65.68	3.0625
raspberry	16	120	9.38	7	83	65.68	2.8911
raspberry	18	100	5.65	7	50	39.56	3.7757
raspberry	18	100	5.65	7	50	39.56	3.6882
raspberry	18	120	6.90	7	61	48.27	4.4747
raspberry	18	120	6.90	7	61	48.27	4.3498
raspberry	20	100	4.63	7	41	32.44	4.4078
raspberry	20	100	4.63	7	41	32.44	4.3608
raspberry	20	120	5.54	7	49	38.77	4.9204
raspberry	20	120	5.54	7	49	38.77	4.9518
raspberry	22	100	3.96	7	35	27.69	4.7343
raspberry	22	100	3.96	7	35	27.69	4.7088
raspberry	22	120	4.63	7	41	32.44	4.9306
raspberry	22	120	4.63	7	41	32.44	5.1161
sour cherry	0	0	0.00	0	0	0.00	0.0000
sour cherry	0	0	0.00	0	0	0.00	0.0000
sour cherry	16	100	8.03	7	71	56.18	1.4365
sour cherry	16	100	8.03	7	71	56.18	1.8837
sour cherry	16	120	9.38	7	83	65.68	3.5035
sour cherry	16	120	9.38	7	83	65.68	3.2816
sour cherry	18	100	6.44	7	57	45.10	3.7697
sour cherry	18	100	6.44	7	57	45.10	4.1761
sour cherry	18	120	7.69	7	68	53.81	4.9246
sour cherry	18	120	7.69	7	68	53.81	4.7163
sour cherry	20	100	5.31	7	47	37.19	5.0144
sour cherry	20	100	5.31	7	47	37.19	4.9972
sour cherry	20	120	6.10	7	54	42.73	3.7765
sour cherry	20	120	6.10	7	54	42.73	4.0641
sour cherry	22	100	4.41	7	39	30.86	4.4065
sour cherry	22	100	4.41	7	39	30.86	4.7076
sour cherry	22	120	5.09	7	45	35.61	5.0386
sour cherry	22	120	5.09	7	45	35.61	5.1847

B.4 RSM Coefficient and ANOVA Tables for Sour Cherry and Raspberry Juices Treated by PEF

Table B.4.1. Coefficient for the second-order polynomial RSM regression in sour cherry juice

Term	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-0.08525	0.563528	-0.15128	0.881648
Electric Field Strenght (E)	-0.2889	0.150158	-1.92398	0.072333
Time (t)	0.05221	0.039805	1.311646	0.20815
E · t	0.004278	0.001172	3.648693	0.002165
E^2	0.015307	0.006622	2.311633	0.034448
t^2	-0.0009	0.000547	-1.64584	0.119296

Table B.4.2. ANOVA results for the second-order polynomial RSM regression in sour cherry juice

Term	Df	Sum²	Mean²	F.value	Pr..F.
FO(E, t)	2	58.18438	29.09219	43.09604	3.61E-07
TWI(E, t)	1	8.861799	8.861799	13.12753	0.002284
PQ(E, t)	2	12.34984	6.17492	9.147288	0.002245
Residuals	16	10.80088	0.675055		
Lack of fit	7	10.475	1.4964	41.2658	3.94
Pure error	9	0.326	0.0363		

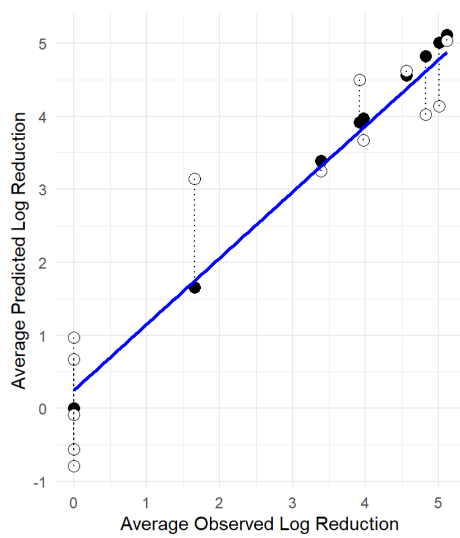

Figure B.4.1. Log reduction average observed vs average predicted values in sour cherry juice PEF treatment RSM

Table B.4.3. Coefficient for the second-order polynomial RSM regression in raspberry juice

Term	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-0.26918	0.462713	-0.58173	0.568852
Electric Field Strenght (E)	-0.21941	0.12436	-1.76434	0.096754
Time (t)	0.101881	0.030007	3.395296	0.003697
E · t	0.003528	0.000971	3.631244	0.002246
E^2	0.01254	0.005495	2.28197	0.036516
t^2	-0.00166	0.0004	-4.15111	0.000752

Table B.4.4. ANOVA results for the second-order polynomial RSM regression in sour cherry juice

Term	Df	Sum^2	$Mean^2$	F.value	Pr..F.
FO(E, t)	2	54.16322	27.08161	58.94223	4.16E-08
TWI(E, t)	1	5.800184	5.800184	12.62391	0.00265
PQ(E, t)	2	20.27843	10.13921	22.06766	2.51E-05
Residuals	16	7.351364	0.45946		
Lack of fit	7	7.304	1.0435	199.901	3.81
Pure error	9	0.047	0.0052		

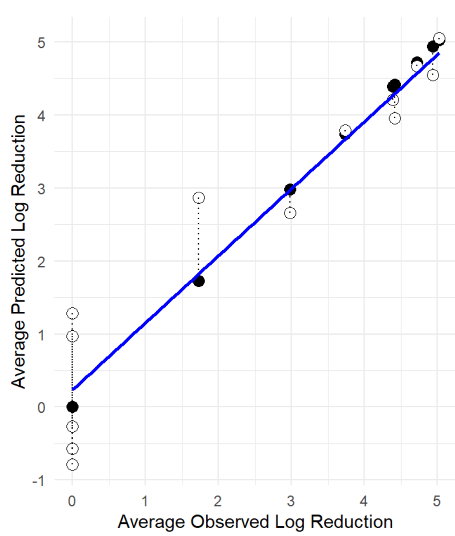

Figure B.4.2. Log reduction average observed vs average predicted values in raspberry juice PEF treatment RSM

Appendix C Principal Component Analysis (PCA) Results on Comparative Physicochemical Evaluation of Sour Cherry and Raspberry Juices

C.1 Principal Component Analysis on Raspberry Physicochemical parameters treated by TT, HPP, and PEF treatments

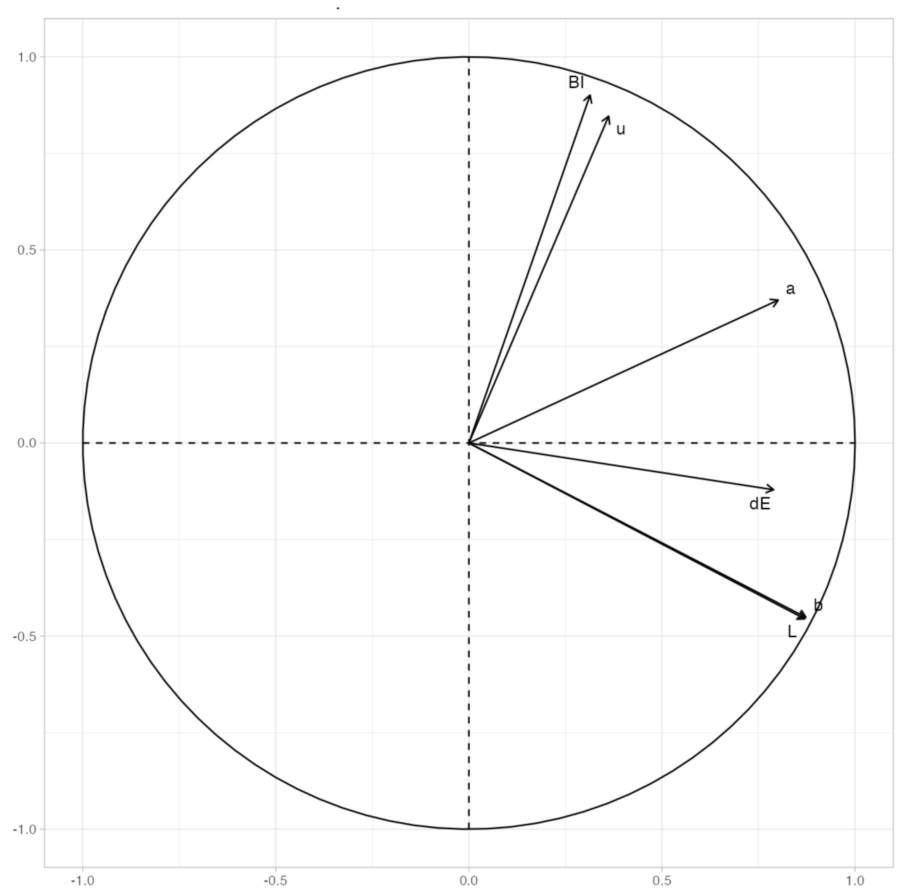

Figure C.1.1. Principal Component Analysis (PCA) – Variables plot for raspberry juice samples treated by HPP, PEF, and Thermal treatments. Browning Index (BI), Vsicosity (u), Color parameters (L, a, b), Total color difference (dE)

Table C.1.1. Eigenvalue and (Cumulative) Percentage of Variance on the Dimensions (Dim.) – raspberry

	Eigenvalue	**% of the variance**	**Cumulative %**
Dim. 1	3.012	74.52353	74.5
Dim. 2	2.091	10.71026	85.2
Dim. 3	0.571	9.52111	94.7
Dim. 4	0.189	3.15650	97.9
Dim. 5	0.136	2.26640	100.0
Dim. 6	3.12e-4	0.00520	100.0

Table C.1.2. Contributions of Variables to the first 3 Dim. – raspberry

	Dim.1	**Dim.2**	**Dim.3**
L	25.12	9.917	1.83
a	21.30	6.553	26.09
b	25.29	9.750	2.48
dE	20.67	0.700	55.99
u	4.35	34.265	11.28
BI	3.27	38.815	2.32

Table C.1.3. Cosine values of Variables on the first 3 Dim. – raspberry

	Dim.1	**Dim.2**	**Dim.3**
L	0.7566	0.2074	0.0104
a	0.6417	0.1370	0.1490
b	0.7618	0.2039	0.0142
dE	0.6226	0.0146	0.3199
u	0.1310	0.7164	0.0645
BI	0.0985	0.8116	0.0133

C.2 Principal Component Analysis on Sour Cherry Physicochemical parameters treated by TT, HPP, and PEF treatments

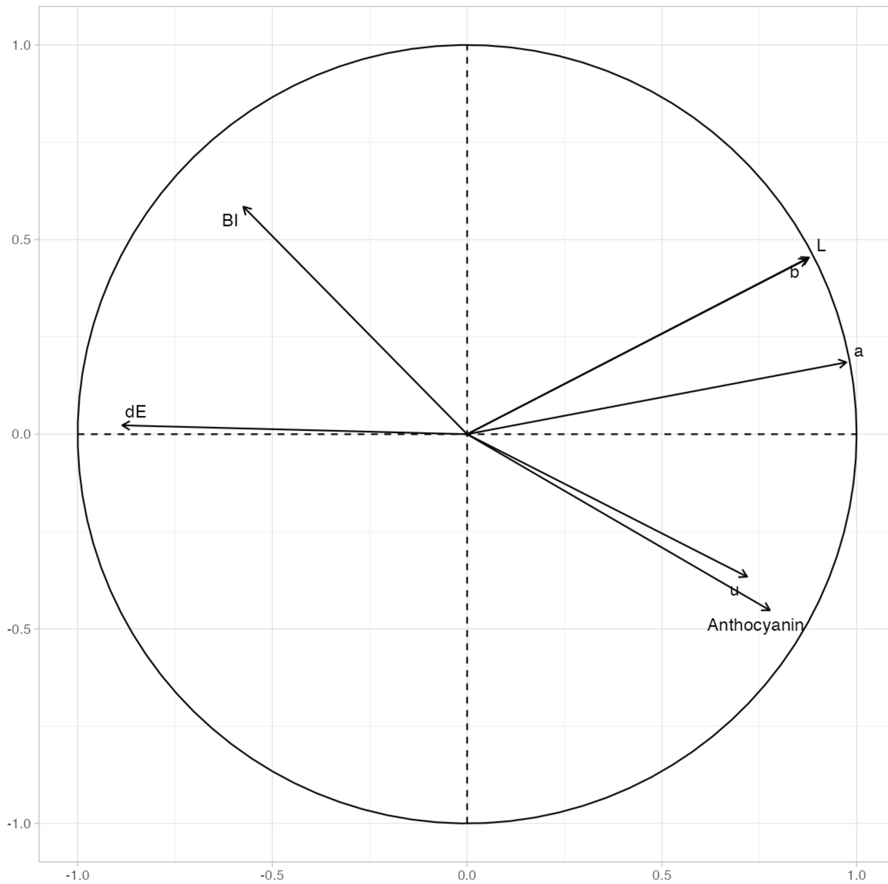

Figure C.2.1. Principal Component Analysis (PCA) – Variables plot for sour cherry juice samples treated by HPP, PEF, and Thermal treatments. Browning Index (BI), Viscosity (u), Color parameters (L, a, b), Total color difference (dE), and Total Anthocyanins (Anthocyanin)

Table C.2.1. Eigenvalue and (Cumulative) Percentage of Variance on the Dimensions (Dim.) – sour cherry

	Eigenvalue	% of the variance	Cumulative %
Dim. 1	4.71569	55.2736	55.3
Dim. 2	1.12353	33.2205	88.5
Dim. 3	0.60657	8.6652	97.2
Dim. 4	0.31700	4.5285	100.0
Dim. 5	0.21571	3.0816	100.0

Table C.2.2. Contributions of Variables to the first 3 Dim. – sour cherry

	Dim.1	Dim.2	Dim.3
dE	16.64	0.0464	4.9892
u	10.96	11.9357	42.4923
L	16.35	18.3503	0.0141
a	20.11	3.0367	4.98e-4
Anthocyanin	12.81	18.2102	6.5439
b	16.13	18.0091	0.7425
BI	7.00	30.4116	45.2175

Table C.2.3. Cosine values of Variables on the first 3 Dim. – sour cherry

	Dim.1	Dim.2	Dim.3
dE	0.784	5.21e-4	0.03026
u	0.517	0.1341	0.25774
L	0.771	0.2062	8.58e-5
a	0.948	0.0341	3.02e-6
Anthocyanin	0.604	0.2046	0.03969
b	0.761	0.2023	0.00450
BI	0.330	0.3417	0.27427

Appendix D Ohmic heating in a pipe: Temperature profile calculation in MATLAB

This appendix details the calculations and MATLAB code used to simulate the ohmic heating process of a liquid product flowing through a pipe. First the necessary electrical current to heat a liquid product from an inlet temperature of 20 °C to a target temperature of 70 °C was calculated. Then, the code computes and plots the temperature profile along the length of the pipe and the corresponding temperature-time profile. The summary of the parameters calculated and used in the model are shown in Table D.1.1.

Table D.1.1. Summary of power and process parameters for OH calculations

Parameter		Value	Units
mass flow rate	$\dot{m}$	350	kg/h
density	ϱ	1040	kg/m³
Volumetric flow	$\dot{V}$	336.54	l/h
specific heat	c_p	3.31	kJ/kg·K
Initial temperature	T_{in}	20	°C
Final temperature	T_{out}	70	°C
Required thermal power	Q	16.09	kW
Electrical efficiency of the generator	η_{el}	95%	
Electrical power required	Q_{el}	16.94	kW
Electric Field Strength	E	50	kV/cm

D.1 Explanation of the Code

There are 100 segment on the pipe and the calcualtions are done with an iterative approach. The temperature increase for each segment is calculated using the fixed power input, then the residence time is calculated in each segment, using the linear velocity, the residence time in each segment, which represents the time a particular segment of juice is exposed to the heating power.Then the code calculate the temperature increase for each segment, calculate the temperature increase based on the energy imput and residence time. There is a stop heating hondition once the temperature in any segment reaches the target temperature of 70°C, and the subsequent segments maintain the last temperature achieved.

D.2 MATLAB code

```
% Parameters
% Define Constants
density = 1040; % kg/m^3
cp = 3.31; % kJ/kg*K
cp = cp * 1000; % convert to J/kg*K
initial_temp = 20; % Initial temperature in °C
target_temp = 70; % Target temperature in °C
pipe_diameter = 0.032; % diameter in meters
pipe_length = 2.0; % length in meters
flow_rate_kg_per_h = 350; % Flow rate in kg/h
% Convert flow rate to kg/s
flow_rate = flow_rate_kg_per_h / 3600; % Flow rate in kg/s
% Electric field strength
electric_field = 50 * 100; % 50 V/cm converted to V/m
% Conductivity Equation (temperature-dependent)
conductivity = @(T) 0.0369 * T + 0.6954; % Conductivity as a function of
temperature
% Calculate Pipe and Mass Properties
pipe_area = pi * (pipe_diameter/2)^2; % Cross-sectional area of the pipe (m^2)
linear_velocity = flow_rate / (density * pipe_area); % Linear velocity in m/s
dz = pipe_length / 100; % Segment length in meters
z = linspace(0, pipe_length, 100); % Position along the pipe
% Initialize Temperature Profile
T_profile = initial_temp * ones(1, 100); % Initial temperature at each segment
total_energy_joules = 0; % Total energy counter
% Heating Loop with Residence Time
heating_active = true;
for i = 2:100
    if heating_active
        % Calculate voltage applied across this segment
        V_segment = electric_field * dz; % Voltage in this segment (V)
        % Calculate local conductivity and resistance
        sigma = conductivity(T_profile(i-1)); % Conductivity at previous
segment's temp
        R_segment = dz / (sigma * pipe_area); % Resistance of this segment
        % Calculate power dissipated in this segment using V^2 / R
        segment_power = V_segment^2 / R_segment; % Power in Watts for this
segment
        % Calculate residence time in each segment
        residence_time = dz / linear_velocity; % Time in seconds for each
segment
        % Temperature increase in this segment due to segment power
        segment_energy = segment_power * residence_time; % Energy in Joules
        segment_mass = flow_rate * residence_time; % Mass of juice in this
segment
        dT = segment_energy / (segment_mass * cp); % Temperature increase
        % Update temperature profile
        T_profile(i) = T_profile(i-1) + dT;
        % Stop heating if target temperature is reached
        if T_profile(i) >= target_temp
            heating_active = false;
        end
    else
        % Maintain temperature after reaching target
        T_profile(i) = T_profile(i-1);
    end
end
% Display total energy used
fprintf('Final Temperature at Pipe Outlet: %.2f °C\n', T_profile(end));
% Plot Temperature Profile
figure;
plot(z, T_profile, 'LineWidth', 2);
xlabel('Distance along the pipe (m)');
ylabel('Temperature (°C)');
grid on;
```