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Development of methods and technologies for the specific inhibition of enzymes relevant for low colour stability of fruit juices and nectars.

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Abstract

This thesis explores the development of methods and technologies for the specific inhibition of enzymes relevant to the low colour stability of fruit juices and nectars. This research was carried out in collaboration with the HiStabJuice project, funded by the European Union's Horizon 2020 program.

This research project consists of five articles covering topics related to inhibiting enzymes and maintaining colour stability in strawberry nectars. One of these articles details how certain enzymes are deactivated and focuses on polyphenol oxidase, peroxidase, pectin methylesterase and polygalacturonase. It also explores methods, for inhibiting these enzymes.

Manuscript 2 analysed how thermal and nonthermal methods impact the quality of strawberry nectar in terms of factors, like viscosity, colour, enzymatic activity and microbial content. In the following, third, manuscript the comparison was extended over a six-week timeframe showing that processed nectars retained their colour better, than non-processed ones. A fourth manuscript studied the determinants of low colour stability in strawberry nectars by multilinear regression analysis. The results of the analysis revealed that storage time is the primary factor affecting the lightness of strawberry nectars and sugar content plays a key role in the preservation of red colour and overall colour saturation.

The final manuscript compared the energy consumption of thermal and non-thermal technologies in strawberry nectar production. Non-thermal treatments were less energy-consuming than thermal treatments, especially at increased packaging volume.

This thesis gives insights into the enzymes responsible for the low colour stability of strawberry nectars as well as other factors that contribute to the low colour stability of strawberry nectars. The findings inform how the product quality and shelf-life extension of strawberry nectars can be achieved.

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Preface and acknowledgements

This thesis was carried out as part of the HiStabJuice project, in collaboration with the International Fruit and Vegetable Juice Association (IFU) and the European Union's Horizon 2020 research and innovation program. The HistabJuice project combined expertise from professionals within European Universities, Academic Institutes, and International Food processing industries with a common goal of addressing issues related to the production and preservation of fruit juices and nectars. This research was supported by the education fund of Marie Skłodowska Curie which allowed her to showcase her work at conferences and engage, in technical summer schools as well as research activities at different academic institutions. Thanks to this opportunity I have made contributions to the realms of food science and engineering with a focus on sustainability. My aim is to impart my knowledge to the fruit juice sector by suggesting enhancements to existing food processing methods.

My heartfelt thanks go out to Professor Massimiliano for his guidance and support throughout my journey, as my supervisor and PhD course coordinator, at the University of Parma. His advice and support have greatly influenced this research as well as my development as a scientist. In addition, I would like to express my thanks to Luca Cattani from the Engineering Faculty of UNIPR, Luca Sandei- the director of SSICA, Carine Le-Bourvellec from INRAE and Stéphane Georgé from CTCPA for their guidance during my research. In addition, I want to express my gratitude to my coworkers Neamtallah Assaf, Andres Moreno, and Rohini Dhenge for their friendship and support during my academic career. Furthermore, I would like to thank the International Fruit and Vegetable Juice Association (IFU) for stepping forward to start this ETN.

I would like to further thank my fellow HiStabJuice ESRs for their teamwork, collaboration and advice over the last three years. I hope the collaboration will continue as we move forward with the next stages in careers. I am proud that the collaboration yielded a joint publication in the Journal of food science. This has also further raised my interest in both non-thermal food processing technologies and beverage studies. Currently, I lead a R&D project focused on colour and physical stability of beverages containing blue phycocyanin from microalgae. Besides that, I would like to share knowledge and expertise in order to develop healthy, attractive, and sustainable beverages.

Executive Summary

This report studied strawberry nectars treated by thermal and non-thermal technologies to study their impact on enzyme activity, colour stability, and other quality indicators, in addition to comparing their energy efficiency.

The first manuscript aimed to identify enzymes responsible for the low colour stability of strawberry juices and nectars. Thermal and non-thermal technologies were also studied for their impact on enzyme inactivation which led to the selection of four food processing technologies (two-thermal, one non-thermal and one combined). The results indicated that all treatments had varied effects on enzyme inactivation and colour. Non-thermal technologies showed promising results, comparable to thermal treatments at specific conditions. This work was complimented by a second manuscript which details the scientific article entitled the "the quality indicators of strawberry nectar stabilized by thermal and non-thermal (high-pressure processing) treatments". The latter study found that viscosity, colour stability, enzymatic activity, and microbial load differed depending on the specific treatment conditions. All treatments resulted in decreased enzymatic activity. Samples subjected to high-pressure treatment showed an increase in overall colour difference throughout the shelf life.

Following the learnings from the manuscript one and two, the third manuscript focused on comparing the effects of thermal treatment and non-thermal processing on colour stability over an extended shelf-life. Non-thermal processing at varied conditions was applied and compared with traditional thermal treatment. Quality indicators such as pH, brix, and colour were evaluated over six weeks. Throughout this extended shelf-life, the results suggested that thermally treated nectars had better colour stability over time when compared to non-thermal treated samples. To fully comprehend the impact of processing conditions on colour and quality parameters, further examination was considered necessary.

A multiple linear regression analysis is described in Manuscript four which investigated the determinants of colour in strawberry nectar, including factors like process (thermal and non-thermal treatments) and other quality indicators. Not surprisingly, the results showed that storage time was the primary factor affecting changes in brightness. The amount of sugar was important for preserving the intensity of the red colour and overall colour saturation. While not statistically significant, enzymatic activity tended to have a detrimental effect on colour parameters. Most unexpectedly, processing techniques

were shown not to significantly impact colour stability over storage, once the effect of enzymatic activity and other factors were accounted for in the statistical model. Sample size and assumptions may have influenced the results. Further evaluation using larger sample sizes is recommended for future research.

While some processing techniques may not differ significantly in the case of strawberry nectars from a quality perspective, they have a significant impact on the environment. Therefore, it was important to compare thermal and non-thermal based processes based on their energy consumption. Manuscript 5 compared the energy consumption of strawberry nectars treated by thermal and non-thermal treatments. Non-thermal treatments consumed significantly less energy than thermal treatment in the production of strawberry nectars and this result was strengthened at higher packaging volumes. The study highlighted that the selection of new process technologies, packaging sizes and treatment conditions may impact energy consumption.

In conclusion, this research explored technologies that could be applied to fruit juice and nectars. Thermal and non-thermal technologies may play a role in product quality. However, storage time and sugar content may also be significant factors affecting the quality of strawberry nectar. Non-thermal technology may be considered a lower energy consuming technology although a more thorough study should be conducted considering the life cycle assessment of both thermal and non-thermal technologies.

1. Introduction

Fruit juices and nectars are high valued by consumers due to their bioactive compounds, in particular vitamins, mineral and antioxidants (McDougal et al., 2018). Red fruits such as strawberries and raspberries have a significantly higher antioxidant capacity range (10-20 g-l) than apples and white grapes (5 g-l) (Kevers et al., 2007). Red fruits are consumed both in fresh and in processed forms. While processing extends their shelf-life and improves their accessibility, conventional processing methods can lead to undesirable nutritional and quality changes.

Juices and nectars derived from red fruits, strawberries in particular, exhibit short shelf lives due to their low colour stability. Providing the public with healthy red fruit juices is often costly due to their low colour stability. Although the low colour stability has traditionally been associated with chemical reactions, recent studies have indicated it is likely due an incomplete inactivation of endogenous enzymes during processing (Gössinger et al., 2009). The residual activity of endogenous enzyme may contribute to colour degradation during the products shelf-life. Silva et al., 2004 and Petruzzi et al., 2017, described isoenzymes resistant to pasteurization or even higher temperature.

This chapter will therefore outline the objectives of the HiStabjuice project, a European funded project aimed at elucidating the causality of the low colour stabilities associated with red fruit juices, in particular the role played by enzymes and microbes in the change of colour during storage. A second major focus is placed on preservation technology. The introduction will detail the legislation governing fruit juice and nectar formulation and labelling, identify the enzymes responsible for low colour stability of strawberry nectars, and list the conventional and non-conventional methods used for the production of strawberry nectars, while assessing their effect on product quality and stability. Finally, the specific objectives for Early-stage researcher (ESR2) in developing methods and technologies for inhibiting enzymes relevant to low colour stability in fruit juices and nectars will be detailed.

1.1. HiStabJuice

Histabjuice is European-funded network committed to providing research and training to 11 Early-stage researchers (ESRs) in areas related to the food and beverage industry. The International Fruit and Vegetable Juice Association (IFU) designed the project by integrating expertise from 5 universities, 2 research institutes, and 10 food companies

from 7 EU countries. The commitment of industry engagement alongside public and private sector institutions guaranteed hands-on training and the resolution of real-world problems while giving ESRs technological, analytical, and transferable skills. The project aimed to develop recommended guidelines for harvesting red fruits, ripening time, and preservation techniques to enhance the colour and nutritional stability of fruit juices. By guaranteeing that the results will be publicly available, this project supports Horizon2020's Open Science goal and has the potential to transform the fruit juice market and advance the European sector in the years to come.

ESRs collaborated to determine factors that influence colour stability in fruit juices, focusing on raw materials and preservation techniques. More specifically, this project focused on elements such as thermostable enzymes naturally present in fruits, fruit variety, maturity, harvest timing, and both traditional and non-traditional preservation methods, like pasteurization and high-pressure processing. A 4D approach was followed considering microbiology, endogenous enzymes, nutrients, and chemical-physical parameters.

The impact of this project can have significant economic impacts due to the value of fruits, which are important crops with widespread impacts on nutrition, health and horticulture. Approximately 10 billion litres of fruit juice and nectar are annually consumed worldwide, with around 25% being consumed in Europe (AIJN 2018). In 2018, orange took the lead of new fruit juices products in the world, accounting for 16%, while red berries accounted for 11% of new products, thanks to the popularity in Europe (Ard Druids, 2018).

The antioxidant capacity of fruits varies notably; berries such as blueberries and blackcurrants demonstrate high antioxidant levels compared to alternative fruits (Skrovankova, 2015). However, the poor colour stability of red fruit juices cause problems and increases the cost of production and storage. In Europe, frozen processed fruits and berries cost around 300 euros per t for storage and shipment.

The establishment of new technologies to remove the need for freezing could provide 10 million € in annual savings, while reducing the environmental impact as well. This project aims therefore at elucidating the causality of the low colour stabilities associated with red fruit juices, in particular the role played by enzymes and microbes in the change of colour during storage. A second major focus is placed on preservation technology.

The HiStabJuice Project methodology and approach are characterized by a combined effort between academic and non-academic teams to address pressing technological issues in the fruit juice industry. The project is supported by three main pillars (Table 1):

Table 1. HiStab Juice Project Objectives,

HiStab Juice Project objectives		
To identify critical factors effecting colour stability and nutritional quality in fruit juices, including:		
1.Enzymatic activity effecting colour stability:	*2. Raw material characteristics:*	*3.Preservation techniques:*
Thermostable enzymes	Fruit variety Ripeness stages Harvest time	Conventional thermal preservation techniques High pressure pasteurization Pulsed Electric Fields Ohmic heating

Source: Own elaboration.

HiStabJuice combines academic and non-academic teams for the first systematic approach to tackle urgent technological problems of the juice industry and HiStabJuice is based on 3 columns:

1. Identifying key players for low colour stability with a major but not exclusive emphasis on enzymes

2. Systematically assessing raw material for optimal characteristic for the production of colour stable juices

3. Comparison, at comparable conditions with respect to microbial load and using same raw material, of conventional thermal preservation with three non-conventional methods: High Pressure Pasteurization, Pulsed Electric Fields, Ohmic Heating

1.2. Fruit juice and nectars and legislation governing composition and labelling

The term fruit juice and nectars are strictly regulated and according to Codex Alimentarius, fruit nectar is characterized as an unfermented product made from combining water, sugars and/or sweeteners with fruit juice or puree (FAO and WHO, 2005).

Fruit juice must include 100% fruit content and no added sugar, even if natural fruit sugars are present. Pure juice is made from freshly squeezed juice, whereas juice from concentrate is reconstituted (Rabenhorst, 2024).

Nectar consists of fruit juice or pulp, water, sugar, or honey. Depending on the fruit variety, it usually contains 25–50% less fruit than juice. When some fruit is too pulpy or acidic to be consumed as juice, nectar is used and this is the case for strawberries (Rabenhorst, 2024).

According to the Codex Alimentarius, fruit purée for juices/nectars is an unfermented product made by processing whole or peeled fruit without extracting juice. Fruit nectar is also an unfermented product made from combining water, sugars, and/or sweeteners with fruit juice or purée (FAO and WHO, 2005).

Strawberry nectars are difficult to process without sacrificing qualitative features, particularly colour and nutrients (Faedi, 2010). Strawberry nectar production begins with the manufacture of fruit purée, which requires many critical procedures. Strawberries are first precooled to roughly 2°C before being washed and sliced with a cold refiner that includes sieves. The sieves may have holes measuring 1.3 mm, enabling achenes to stay in the purée, or 0.8 mm, which removes the achenes to provide a smoother texture.

Table 2. Minimum Brix Level and Minimum Juice or Purée Content for Reconstituted Juice and Purée at 20°C.

Botanical Name	Fragaria x. Ananassa Duchense
Fruit's Common Name	Strawberry
Minimum Brix Level for Reconstituted fruit juice and reconstituted puree	7.5
Minimum Juice and/or Puree Content (% v/v) for Fruit Nectars	40.0

Source: (FAO, 2005). Own elaboration.

Strawberry purée is a semi-finished product used to make a variety of processed products, such as nectars and jams. The strawberry puree is pre-treated, often undergoing a short pasteurization treatment at 80°C, which is then followed by the purée being filled into aseptic packaging and stored at 15°C to stabilize it without altering its sugar content.

Fresh purée can be used immediately as a fresh component or stored in the refrigerator for subsequent use. The final nectar product is then created by combining the purée with water and additional ingredients like sugar or sweeteners. However, because the resulting nectar is not inherently stable or safe to consume, it must be processed further before it can be consumed.

Key Regulations governing the composition and labelling of fruit juices and nectars

The legislative framework that governs fruit juices and nectars is critical for maintaining product safety, quality standards, and customer transparency. Various rules specify the compositional standards for various items to safeguard consumers from misleading labelling tactics. The Fruit Juices and Fruit Nectars Regulations establish specified compositional guidelines for fruit juices intended for human consumption and govern both composition and labelling (Table 3).

Table 3. The Fruit Juices and Fruit Nectar Regulations.

The Fruit Juices and Fruit Nectar Regulations: areas covered e.g.
Composition (e.g., only authorised additives)
Labelling (e.g., distinguish 100% juice from reconstituted (diluted) concentrate)

Source: Own elaboration.

They specify that:

- Fruit juices must be branded "100% juice" if they contain no other components.
- Fruit nectars must state their fruit content percentage prominently on the container.

In addition, certain phrases are legally reserved for specific product kinds, such as:

- Only products that meet specified compositional specifications can be branded as "fruit juice" or "nectar."
- Products branded "from concentrate" must clearly state this on the packaging.

The regulations also limit what ingredients can be used in fruit juices:

- Only authorized additives can be used (Table 4).
- If more sugar or sweeteners are added than the stipulated amount, this must be stated.

Table 4. Standardized Additives for Fruit Nectar (update of 2005).

Food Category No. 14.1.3.1 Fruit nectar			
Additive group		**Max Level [mg/kg]**	**Notes**
Phosphate	Phosphoric acid, Monosodium p., Disodium p., Trisodium p.	1000	As Phosphorus Pentasodium triphosphate only, to enhance the effectiveness of benzoates and sorbates Subject to national legislation of the importing country
Saccharins	Sodium saccharin, Calcium saccharin, Potassium saccharin, Acid Saccharin	80	-
Sodium Ascorbate	-	GMP	-
Sorbates	Sorbic Acid, Potassium Sorbate, Calcium Sorbate	1000	As Sorbic Acid Singly or in combination: Benzoates and Sorbates Subject to national legislation of the importing country
Sucralose	-	300	-
Sulfites	Sulfur Dioxide, Sodium Sulfite, Sodium Bisulfite	50	As residual SO2 Subject to national legislation of the importing country
Tartrates	Tartaric Acid, Sodium Tartrates, Potassium Sodium Tartrate	4000	Tartaric acid only

Source: (Codex Alimentarius Commission (2005))

Ascorbic Acid is not reported since the dosage depends on the specific formulation requirements and does not have a standard or fixed maximum amount. Good Manufacturing Practiced should be followed.

Local authorities enforce these restrictions by ensuring that all marketed items fulfil legal composition and labelling standards.

1.3. Enzymes responsible for low colour stability of strawberry nectar

Recent research has shown that several enzymes, including PPO, POD, PME and PG, play an important role in the low colour stability of strawberry nectars. These enzymes cause colour loss by oxidative and pectinolytic processes, which might impair the visual quality of strawberry products (Table 5).

Table 5. Enzymes effecting colour stability and the mechanisms causing discolouration

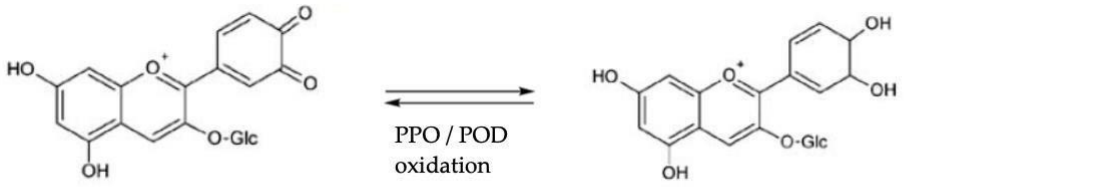
Enzymes effecting colour stability and the mechanisms causing discolouration	
Enzyme	*Mechanisms:*
PPO	Oxidation of phenolic substances
POD	Oxidation
PME	Alters pectin structure, effecting colour
PG	Alters pectin structure, effecting colour

Source: Own elaboration.

Polyphenol oxidase (PPO) and Peroxidase (POD)

Enzymatic browning is due to oxidation by molecular oxygen of phenolic compounds present in fruits and vegetables. This reaction is mainly catalyzed by PPO, which are present present in plants. PPO leads to the formation of quinones (Figure 1), as enzyme interact with anthocyanins and transform them into o-quinones when cells are broken up by chopping, crushing, or aging. Dark polymeric pigments are produced by the combination of these reactive substances (Dall'Asta, 2023). These products can then undergo oxidative polymerizations leading to the formation of pigments of generally brown colour, sometimes red or blue, responsible for browning. This is clearly visible in the apple (Hamdan et al., 2022). These enzymes are present in the mitochondria and plastids, two cell organelles in strawberries.

Figure 1. Anthocyanin oxidation.



Anthocyanin o-quinone Anthocyanin (i.e. Cyanidin-3-glucoside)

Source: (Krystian Marszałek, 2017)

Numerous factors impact the browning process, such as:

1. Active enzymes are present.

2. Availability of oxygen

3. The temperature

4. The pH

Several methods, such as oxygen removal and antioxidant administration, are employed to prevent enzymatic browning. However, in line with consumer desires for more natural food items, there is growing interest in natural and bioactive extracts from plant sources as safer substitutes for synthetic chemicals (Hamdan et al., 2022). Anthocyanin oxidation, which is accelerated by heating and typical working conditions and degrades product quality during storage, is catalysed by PPO and POD. This process, sometimes known as oxidation, uses oxygen from the surrounding environment including the air (Marszałek et al., 2017).

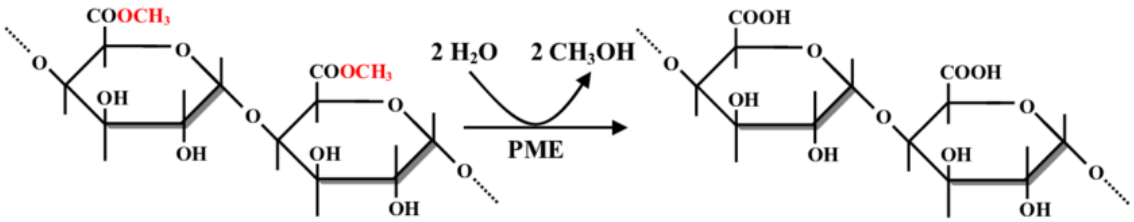
In order to prevent the oxidation phenomenon from happening, efforts have been attempted to inhibit PPO. In contrast to untreated samples, (Lacey et al.,2023) reported that heat pasteurization decreased PPO activity, which was associated with an improved colour retention in strawberry nectar. Protein unfolding and denaturing are thought to be the mechanism of inactivation in these cases (Terefe et al., 2014). According to the same study, strawberry nectar's original colour was preserved by inactivating POD activity during HPP, by preventing oxidative reactions that POD contributes to HPP's effectiveness in maintaining colour stability was demonstrated by the 600 MPa treatment, which inhibited PPO activity by 44% and POD activity by 4%. HPP causes conformational changes in enzyme structure, affecting secondary, tertiary, and quaternary

structures (Poojary et al., 2017). HPP inactivated PPO and POD in a variety of fruit products, with studies reporting rates of 25-60% inactivation in juices (Hernández & Cano, 1998).

Pectin methylesterase (PME)

PMEs are essential for the colour stability and cloud formation of fruit juices (Wicker et al., 2003). By eliminating methyl groups from the C6 position of galacturonic acid residues in the pectin chain, PME catalyses the demethylesterification of pectin (Micheli et al., 2001). Figure 2 represents the chemical reaction for demethylesterification of pectins by PME. The process produces methanol and protons as by-products, thereby converting the methoxyl (-COOCH3) groups to negatively charged carboxyl (-COOH) groups (Jolie et al., 2010). Interactions of the resulting demethylesterified pectin with calcium ions that induce gel formation and precipitation can lead to cloud destabilization and turbidity reduction in juices (Croak and Corredig, 2006).

Figure 2. Demethylesterification of pectins by PMEs (modified from: Micheli, 2001).



Source: Micheli, 2001.

This phenomenon influences the juice's flavour, texture, and overall quality in addition to its aesthetic appeal (Rodrigo et al., 2003). Controlling PME activity using different processing techniques like TT, HPP, or the use of natural inhibitors like those present in kiwifruit is essential to maintaining colour stability and cloud formation in fruit juices, particularly in citrus products (J). HPP was shown to be effective in control PME activity for citrus fruits, with treatments at HPP 600 MPa for 1 minute reducing PME activity by up to 92% depending on juice composition (Bull et al., 2004). PEF treatment have been shown to reduce PME by up 95% in strawberry and melon juices (Aguiló-Aguayo et al., 2010). Thermal treatment is widely used and requires temperature up to 90° C to completely inactivate PME (Demirdöven et al., 2014).

Polygalacturonase (PG)

PG enzymes hydrolyse pectin molecules found in fruit cell walls; they significantly contribute to the discolouration of fruit juices. Degradation of the cell wall a, subsequent colour pigment release and enhanced oxidation are some of the consequences of this process. In the assay method provided, the reducing groups generated by the PG enzymes - both endo-PG and exo-PG - during their hydrolysis of polygalacturonic acid - PGA - are determined using 2-cyanoacetamide. Pre-existing reducing groups are removed, the enzyme reaction is allowed to take place, and the liberated reducing groups are then measured spectrophotometrically. In this respect, PG enzyme activity becomes directly related to pectin breakdown and the resultant release and oxidation of pigments; it therefore becomes an important requirement for a proper understanding and control of colour loss in fruit juices during the course of processing and storage. In strawberry and watermelon juices, PEF treatments have shown some potential to inactivate enzymes, achieving 25–40% inactivation (Aguiló-Aguayo et al., 2008). According to Duvetter et al. (2006), combined techniques such high pressure and heat treatment have demonstrated potential, with up to 80% PG inactivation recorded in strawberry puree. Depending on the particular fruit product and processing settings, these methods efficacy varies.

In conclusion, colour stability of strawberry nectars is influenced by enzymatic activity, which include PPO, POD, PME and PG. These four enzymes can be further categorized into two groups based on their interactions which lead to colour changes of strawberry nectars, for example, PPO and POD are direct colour altering enzymes, responsible for oxidation reaction that led to melanin pigments being formed, browning and colour loss. PME and PG act indirectly, by altering the pectin structures and resulting in pigments leaching as well as cloudiness of the nectars which can be perceived lighter and less vibrant in colour. The combined action of these two enzyme groups can present a significant challenge for maintaining attractiveness of red fruit juices and nectars, which can be further enhanced throughout their shelf-life. It is important to further understand the specific roles and mechanisms of these enzymes in real-world applications and compare different processing strategies in comparable conditions.

1.4. Conventional and non-conventional treatments of juice and nectars

1.4.1. Conventional and non –conventional treatments for juice and nectars, in general.

Pasteurization is a thermal treatment, named after the French scientist Louis Pasteur which was designed to eliminate pathogenic bacteria in food and beverages to ensure food safety and shelf-life extension. The process involves heating foods or beverages below 100°C for an established duration of time and followed by rapid cooling (Lewis & Heppell, 2000). Pasteurization is applied to fruit juices and nectars at 80-98 °C (Renard et al., 2012). The exact temperature and time combination depends on the precise fruit type, acidity, sugar content, initial microbial load, and desired shelf life.

TT has long been the accepted procedure for commercial stability in fruit liquids, especially strawberry nectars. The objective of applying TT to strawberry nectars is to reduce the risk of microbial activity inactivating spoilage bacteria and in some cases inactivating enzymes that cause colour degradation (Holzworth et al., 2012). TT can be lethal enough to establish commercial sterility for foods with a pH below 4.6 and water activity below 0.92, such as strawberry juice (pH 3.0-3.6) (Brennan, 2006). Nonetheless, TT has undesirable effects on heat-sensitive components such as vitamins, minerals, volatiles, and colour pigments such as anthocyanins. This can result in unpleasant organoleptic alterations and lower nutritional quality (Odriozola-Serrano et al., 2008).

To maintain nutritional and organoleptic qualities of strawberry nectar, non-thermal technologies such as PEF and HPP are showing promise as alternatives to traditional thermal processing (Bevilacqua et al., 2018). Organoleptic qualities refer to the sensory properties of food, including taste, aroma, texture, and appearance.

However, non-thermal methods have limitations, especially when it comes to attaining long-term shelf stability for low-acid products, but they can be advantageous in terms of preserving nutrients and saving energy (Vignali et al., 2022). Factors like microbial reduction, enzyme inactivation, and nutritional compound retention should be considered when selecting a stabilization technique (Vignali et al., 2022).

Compared to conventional heat treatments, these alternative treatments have the potential to better retain the nutritional value, fresh-like qualities, and bioactive ingredients of strawberry nectars. Nevertheless, each application has individual characteristics which may have benefits depending on the applications.

Alternative technologies:

Table 6. Main Characteristics of Unconventional Treatment

Treatment	Key Characteristics	Advantages	Limitations
High Pressure Processing (HPP)	Pressure range: 100-1000 MPa Kills microorganisms and vegetative bacteria Inhibits oxidative enzymes	Preserves colours, flavours, and nutrients Uniform treatment In-package processing possible	Expensive equipment Requires ~40% free water for antimicrobial effect Batch processing
Pulsed Electric Fields (PEF)	Electric field intensity: 15-60 kV Energy input: 110-240 kJ kg^{-1} Destroys pathogenic bacteria and vegetative cells	Preserves colours, flavours, and nutrients Short treatment time	Only suitable for liquids or particles in liquids Scaling-up challenges
Ohmic Heating (OH)	Rapid and uniform heat transformation Efficient energy conversion (>90%)	Less colour degradation Absence of hot surfaces Suitable for continuous processing	Limited to electrically conductive foods
Ultrasonication (US)	Effective against vegetative cells, spores, and enzymes Can be combined with other processes	Reduces process times and temperatures Little adaptation required for existing plants	Potential unwanted modification of food structure Scaling-up challenges

High Voltage Cold Plasma (CP)	Generates reactive nitrogen and oxygen species Microbial and enzyme inactivation	Reduces anti-nutritional components and contaminants Improves final quality and stability	Limited penetration depth Potential oxidation of some food components
Pulsed Light (PL)	Very rapid process Low energy input	Suitable for dry foods Little or no changes to foods	Not effective against spores Possible resistance in some microorganisms
Irradiation (IR)	Excellent penetration into foods Suitable for packaged and frozen foods	Little or no heating of food Chemical preservatives can be avoided	High capital cost Potential oxidation of sensitive foods

Source: Own elaboration.

Table 6 summarizes the main characteristics of alternative technologies applied to food and beverages and their advantages and disadvantages. In total, seven alternative technologies were compared including HPP, PEF, OH, US, CP, PL and IR. Each method offers unique advantages in microbial inactivation, preservation of food quality, and processing efficiency.

HPP requires 40% free water for antimicrobial inactivation and in-package processing for food preservation, operating at pressures of between 100-1000 MPa (Huang et al., 2017). PEF can reduce pathogenic bacteria while preserving food qualities, with electric field intensities of 15-60 kV (Toepfl et al., 2014). OH, can uniformly and rapidly transfer heat with efficient energy conversion (Sakr & Liu, 2014). US can be combined with other technologies to reduce process time and enhance microbial inactivation although it can result in unwanted modification of texture by disrupting cell walls and altering the structure of proteins (Chemat et al.,2011). High Voltage Cold Plasma generates reactive nitrogen and oxygen species for microbial and enzyme inactivation (Misra et al., 2016). Pulsed Light is not effective against spores and some microorganisms may develop resistance (Oms-Oliu et al., 2010). For instance, IR provides treatment with minimal heating and is suitable for packaged and frozen foods (Farkas & Mohácsi-Farkas, 2011).

Table 7. Suitability of Technologies for Strawberry Nectar Treatment

Technology	Suitability	Key Advantages	Main Limitations
HPP	High	Effectively inactivates enzymes like PPO, POD, PME and PG. Preserves colour and flavour, suitable for liquids	High initial cost
PEF	Moderate	Effectively inactivates enzymes like PME and PG, Short treatment time, preserves colour and flavour, Suitable for liquids	Scaling challanges
OH	Moderate	Rapid uniform heating, Rapid inactivation of PME, less colour degradation compared to conventional methods, Suitable for liquids	Limited to electrically conductive foods, potential loss of fresh-like qualities
US	Moderate	Increased PME inactivation with combine with other thermal treatments, reduces processing times and temperatures	Potential unwanted modification of food structure, scaling-up challenges
CP	Low	Microbial inactivation without heat	Limited penetration depth, potential oxidation of sensitive components
PL	Low	Very rapid treatment process with low energy input	Limited effectiveness in liquids, not effective against spores or enzmes
IR	Low	Excellent penetration into packaged foods, can avoid chemical preservatives	Potential oxidation of sensitive components, high capital cost

Source: Own elaboration.

Table 7 describes the alternative technologies that are more suitable for fruit nectars. HPP is ranked the most suitable for this application as it worked well in applications with higher water content and was shown to be effective in reducing PPO and POD enzyme

activity (Lacey et al., 2023). HPP was also shown to reduce PME and PG (Hernández & Cano, 1998). PEF has been effective in reducing the activity of enzymes in particular PME and PG (Aguiló-Aguayo et al., 2008). PME inactivation was faster with OH than with conventional heating in orange juice(Leizerson & Shimoni, 2005). US when combined with heat treatment increased the inactivation rate of orange juice PME, while deterioration of quality parameters was observed through the shelf life (Agcam et al., 2016). The inactivation rate of PME was increased by 1.5–6 times and the inactivation rate of PG by 2.3–4 times in the temperature range of 60–75 °C, with the highest increase corresponding to the lowest temperature (Terefe et al., 2009). While CP shows promising effects in reducing microbial load in strawberries, this technology has limited penetration and is therefore not a suitable technology for fruit juices (Misra et al., 2014). PL and IR have limited effect in opaque or highly coloured juices and can adversely affect vitamins, and antioxidants and promote the formation of off-flavours while having a limited impact on enzymes (Koutchma et al.2009). HPP, PEF, and OH were the three technologies ranked highest due to their effectiveness in inhibiting enzymes. However, their mechanism of inactivation differs.

Table 8. Processing methods and their mechanism of inactivating enzymes.

Treatment	Mechanism	Effect on PPO	Effect on POD	Effect on PME	Effect on PG
HPP	Conformational changes	Generally resistant	Moderate inactivation	Variable response	More susceptible to inactivation
Thermal	Protein unfolding and denaturation	Resistant	More susceptible	More resistant than POD and PPO	Varies depending on source and conditions
PEF	Electrochemical effects and localized heating	Can inactivate or stimulate	Can inactivate or stimulate	Not well specified in sources	Limited information available
Ohmic	Rapid uniform heating with mild electroporation	Faster inactivation than conventional heating	Faster inactivation than conventional heating	Not well specified in sources	Potentially faster inactivation, but limited data

Source: Own elaboration

Table 8 compared the process methods and their mechanism for inactivation of enzymes relevant for low colour stability. Conformational changes induced by HPP affect the secondary, tertiary, and quaternary enzyme structures. According to Lopes et al. (2019), the effects of HPP differ among enzymes. Generally, PPO tends to be resistant to HPP; on the other hand, POD is usually slightly inactivated. While PG is more vulnerable to pressure inactivation, PME has variable responses and sometimes may become activated at certain pressures. Compared to the HPP, inactivation of enzymes through thermal treatment may be more effective, where the basic response usually involves the denaturation and unfolding of proteins (Lopes et al., 2019). Terefe et al., describe enzyme inactivation by HPP resulting in conformational changes that unlike thermal treatment do not break covalent bonds which can lead to reversible inactivation in some cases. While PG is generally more sensitive to inactivation by pressure, PME presents variable responses and sometimes activates at certain pressures, for example, PME can be activated at low pressure <300 MPa and inactivated at pressure (Terefe et al., 2014). Some food's components such as sugar can protect enzymes from pressure-induced inactivation.

Compared to HPP, enzyme inactivation due to thermal treatment is more effective, usually, because of the intense denaturation and unfolding of proteins. The heat disrupts hydrogen bonds and hydrophobic reactions (Terefe et al., 2014). OH, causes the protein to unfold similarly to thermal treatment, disrupting the secondary and tertiary structure. This exposed hydrophobic groups leading to a loss of activity. The electric field can further result in electroporation of the cell membrane which may enhance enzyme inactivation (Leizerson et al., 2005). According to Poojary et al. (2017), depending on the degree of treatment, either inactivation or increased enzyme activity can be achieved by PEF, though precisely how PEF affects PG is less well established. This method allows quick and homogenous heating of the whole product due to the synergy of thermal effects and mild electroporation. However, it can accelerate the inactivation of the enzyme more than conventional heating methods and may affect all enzymes including PG.

Table 9. Processing methods evaluated for their impact on anthocyanin levels and colour stability in strawberry products

Processing methods evaluated for their impact on anthocyanin levels and colour stability in strawberry products	
Methods	*Results*
TT	Optimal heat conditions vary by cultivar, affecting anthocyanin stability
HPP	Faster rate of decline due to incomplete inactivation of enzymes
PEF	Effectiveness can depend on the strength and duration of the treatment; can enhance stability
OH	Degradation can increase with both increasing voltage and increasing solids content

Source: Own elaboration.

Table 9 summarizes the methods and their impact on anthocyanin levels in strawberry products.

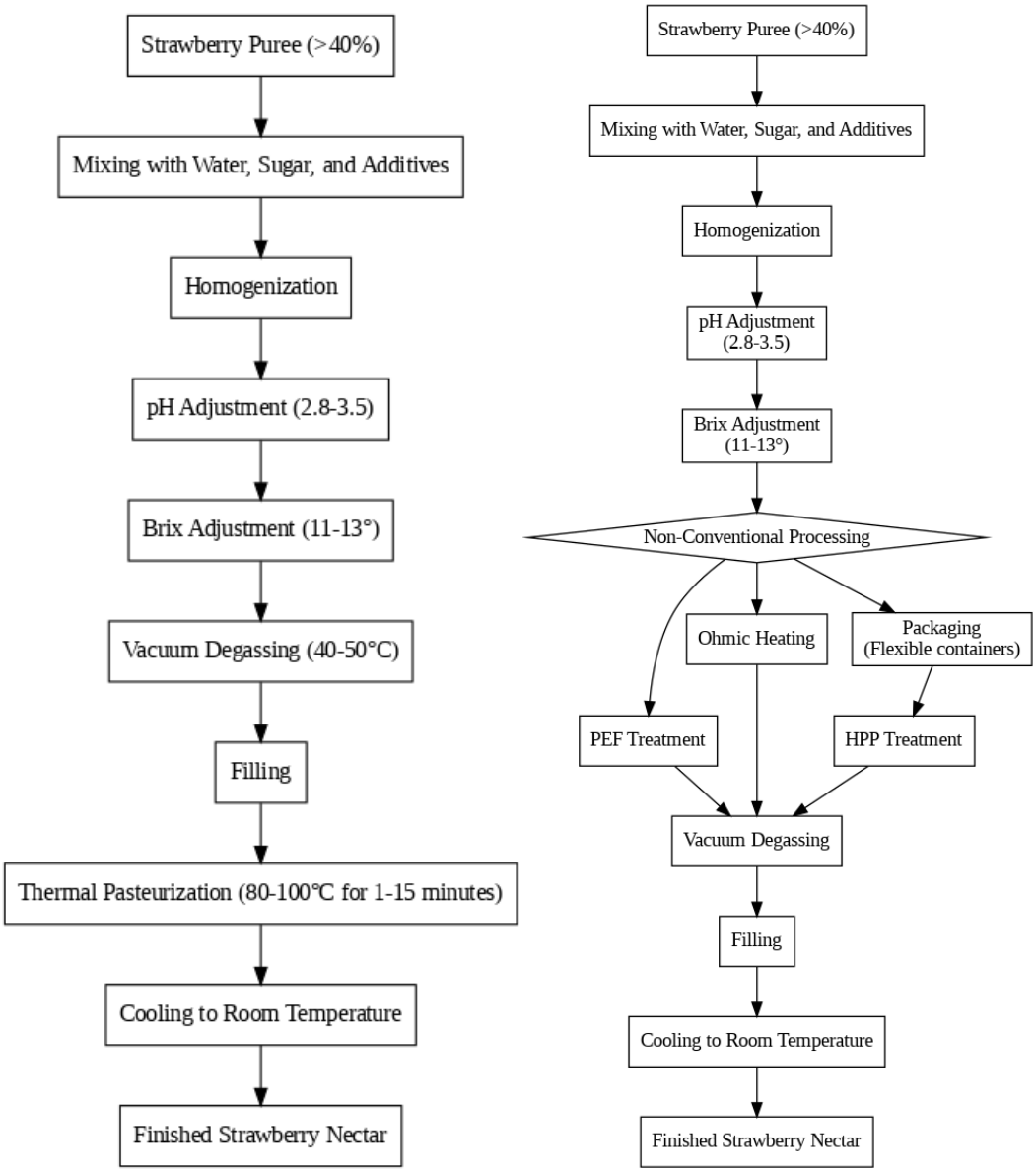
As mentioned earlier, anthocyanins are responsible for the red colour of strawberries. Buvé et al. (2018) studied the kinetics of colour changes in pasteurized strawberry juice during storage. Pasteurization resulted in an initial degradation of anthocyanin (90°C for 60 seconds) and this degradation continued during storage, following first-order kinetics. Amperawati et al., 2019, found that as temperature and time increased, anthocyanin and colour (a* and C*) thermally degraded less, whereas L*, b*, Ho, and ΔE increased. Furthermore, the study's findings demonstrated that longer storage times and higher temperatures led to a quicker rate of degradation (higher k-value) and a shorter shelf life (t1/2). Various studies have shown that anthocyanins present higher degradation rates at temperatures in the range of 65–90 °C for strawberry juice (Menelli et al. 2021).

In contrast, Aaby et al., 2018 did not determine any differences in the anthocyanin content of strawberry juices treated by HPP or TT. However, the concentration of anthocyanins declines faster in juices treated by HPP. This is thought to be due to an inefficient inactivation of PPO and regeneration of PPO or other degrading enzymes during the shelf-life.

Previously, it was found that anthocyanin content was largely dependent on the strength and duration of PEF treatment. Sarkis et al., 2013, evaluated anthocyanin degradation in pulp treated by ohmic heating compared to conventional treatment and found that when voltage levels were low, anthocyanin degradation was lower than

conventional treatment however the opposite was found when high electric fields were used.

Figure 3. Conventional and non- conventional processing methods for producing strawberry nectars.



Source: Own elaboration.

Based on the screening of suitable technologies for applying to strawberry nectars for effective inactivation of enzymes and promotion of colour stability, PEF, OH, and HPP were selected as the alternative processing methods for strawberry nectars. Figure 3, illustrates the process flow for conventional and non-conventional methods of production. The flow diagram highlights the initial processing steps that are shared by each approach starting with strawberry puree, mixing with other ingredients, homogenization, pH and

brix adjustment, and degassing. The key difference lies in the final processing stage, where conventional methods employ thermal sterilization, while non-conventional techniques utilize alternative technologies such as HPP, PEF, or OH. This comparison of both processes visually demonstrates how alternative technologies can be integrated into the production process of strawberry nectars, potentially improving the colour stability and nutritional and organoleptic properties of the final product.

1.5. Colour Stability Measurement Methods

Colour stability in strawberry products is critical to consumer approval and market viability. Recent research has investigated several approaches for evaluating colour stability which are listed in Table 10.

Table 10. Colour Stability assessment methods.

Colour stability assessment methods	
Stability Predictions value	SPV
Browning index	BI
Colour Acceptance factor	AF
Colourimetric analysis	CIELAB parameters (L*, a*, and b*)
Total Colour Difference	ΔE

Source: Own elaboration.

Various studies have employed colourimetric analysis using the CIELAB parameters (L*, a*, and b*) to determine quantitatively colour changes during storage. The "L" channel indicates lightness, while the "a" and "b" channels measure colours from green to red and colours from blue to yellow, correspondingly. This gives the stipulated basis for the measurement of colour changes quantitatively over time. Murray et al. (2024) introduced SPV as a predictive methodology for the investigation of colour retention in strawberry nectar. SPV was shown to predict colour stability with good accuracy for various processing methods, which in turn would allow the manufacturer to make a more knowledge-based decision about process parameters. Gössinger et al. (2009) showed that AF has a significant impact on the stability of anthocyanin in strawberry products. Knowledge of such processes allows producers to improve visual quality and marketability by striving to keep colour metrics above thresholds of 0.7. BI has been used in contexts targeting the level of colour change caused by enzyme activity or chemical reaction. In such literature, lower BI corresponds to increased visual attractiveness and quality in strawberry goods. For example, it has been demonstrated that BI can be used

to monitor the freshness quality of fresh-cut fruits and juices during the products' shelf life. Since the colour stability in strawberry products is complex, its measurement would require a holistic approach.

Irrespective of the many methods under study, a comparison study to find out the best method for colour stability assessment has been badly needed in the industry. CIELAB parameters provide a standardized, quantitative measure of colour and yield the component estimates for many of the other derived Colour measures-i.e., SPV and AF, and BI. However, this method may not consider all complexities of colour perception. The SPV proposed by Murray et al. (2023) is a promising predictive tool, but its reliability needs to be further validated across different methods of processing and storage. The colour stability could also be defined by methods such as BI, AF, and ΔE. This study will carry out a comparative study of colour stability in strawberry products using different techniques: colourimetric analysis, SPV, BI, AF, and ΔE. The study should take account of long-term predictive accuracy, including sensory results to correlate the instrumental measure with the perception of consumers. It should also test the feasibility and affordability of each procedure for industrial purposes. By comparing these methodologies under standardized conditions, it can be can establish the most accurate and reliable method for colour stability checks in strawberry products. The result will also allow processors to make appropriate decisions on technological parameters or storage conditions while offering increased product quality and consumer satisfaction within the strawberry industry.

1.6. Early-stage researcher (ESR2)

ESR2 focused on addressing the challenges associated with low colour stability of strawberry juices and nectars when different preservation/processing technologies are employed (Table 11).

Table 11. HiStab Juice Project: ESR2 Objectives.

HiStab juice project: ESR2 objectives		
To identify critical factors effecting colour stability in strawberry juices and nectar, with specific emphasis on controlling enzymatic activity using processing technologies, including:		
Inactivation of enzyme activity associated with colour deterioration:	*Optimizing processing technologies:*	*Colour measures:*
Polyphenol Oxidase (PPO) Peroxidase (POD) Pectin methylesterase (PME) Polygalacturonase (PG)	Conventional and non-conventional methods: High-Pressure Processing (HPP) Ohmic Heating (OH) Thermal treatment (TT) Pulsed Electric Field (PEF)	Browning index (BI) Colour Acceptance factor (AF) Colourimetric analysis, CIELAB parameters (L*, a*, and b*) Total Colour Difference (ΔE)

Source: Own elaboration.

The main objective was to identify methods for inactivating endogenous enzymes in strawberry nectars that cause undesirable colour change, including polyphenol oxidase.

In addition to enzyme inactivation, strawberry nectars were prepared with different technologies, and their shelf-life was assessed, with a particular emphasis on colour stability and other quality indicators.

Furthermore, ESR2's work was to identify processing parameters for conventional and non-conventional treatments that effectively render strawberry colour-degrading enzymes inactive. This research contributed to the development of improved processing methods that keep the colour quality of strawberry nectars over the duration of their microbiological shelf life.

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2. Manuscript 1: Enzymatic Activity Analysis of Strawberry Nectars: Comparing Conventional and Innovative Processing Technology

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2.1. Abstract

Anthocyanins give strawberry nectars their vivid red colour, albeit, they might lose colour when stored or processed. Numerous enzymes have been studied for their effect on colour stability, including polyphenol oxidase (PPO), peroxidase (POD), pectin methylesterase (PME), and polygalacturonase (PG). These enzymes start pectinolytic and oxidative processes that degrade strawberry products' aesthetic appeal.

With an emphasis on PPO and PME, this study sought to assess and contrast several approaches for enzyme inactivation in strawberry nectars, including, traditional thermal (TT) processing, ohmic heating (OH), high-pressure processing (HPP), pulsed electric field (PEF), and synergistic treatments combining PEF and heating. It was also evaluated for the effectiveness of these techniques in maintaining the colour after the treatments, besides enzyme inactivation in strawberry nectars.

The results indicated that new technologies such as HPP, PEF, and OH are promising alternatives to thermal processing for enhanced colour retention or improvement, with possibilities of customized enzyme-inactivation profiles.

2.2. Introduction

2.2.1. Introduction to Strawberry Nectar, Puree, and Juices

Strawberries (Fragaria × ananassa) are popular globally due to their delicious taste, vibrant colour, and rich vitamin and phytochemical content. The dietary intake of fruits and vegetables, strawberries included, has been reported in many epidemiological studies to be consistently associated with reduced risk of chronic diseases, such as obesity, infections, and cardiovascular, neurological diseases, and cancers. Strawberries have been particularly pointed out for their extremely high content of vitamin C, folates, and phenolic compounds bearing important antioxidant capabilities both in vitro and in vivo. Despite the many nutritional benefits that strawberries possess, there are their limits; such as loss of bioactive components because of processing and storage, which will reduce the nutritional value as well as sensory quality, Giampieri et al., 2012. Enzymatic browning of strawberries is a process that has Colour and nutrient losses undesirable. It results in unappealing fruit products to customers. Anthocyanins, the pigments that give strawberries their bright red colour, are especially prone to enzymatic deterioration during storage and processing (Holzwarth et al.2012). Polyphenol Oxidase (PPO), copper-containing enzymes, are critical in the breakdown of anthocyanins and the resulting browning. These enzymes catalyse the hydroxylation of monophenols to o-diphenols, which are then oxidised to o-quinones, which can react further to produce brown polymeric pigments. Because these reactions require oxygen, removing it is a possible, although not always realistic, strategy to delay browning (Holzwarth et al. 2012).

Several approaches have been studied to improve strawberry stability and inhibit the effects of enzymatic browning. Thermal treatment has been widely used for enzyme inactivation; however, the harsh treatment conditions can reduce the sensory and nutritional value of strawberries. Therefore, alternative non-thermal technologies have gained interest. Recent research has focused on several ways for assessing and improving colour stability. A deeper understanding of these factors is crucial for enhancing the visual appeal and market potential for strawberry nectars.

2.3. The objectives

To establish methods for the inactivation of enzymes relevant for low colour stability via thermal, non-thermal, and combined treatments.

2.4. Methods

2.4.1. Nectar ingredients

Sucrose, water, strawberry purée, and citric acid were used to prepare strawberry nectar. Citric acid of a concentration greater than 99.5% was obtained from Sigma-Aldrich®. Strawberry purée was from Sicoly®, France. The same batch of strawberries from the cultivar variety "Senga Sengana" that were gathered, puréed, and frozen in 2021 and kept at -18°C without breaking the cold chain are used for the purée.

2.4.2. Nectar preparation

Table 1. Final Nectar and Purée Specifications for the Strawberry Nectar

FINAL NECTAR SPECIFICATIONS		**PUREE SPECIFICATIONS**	
DRY SUBSTANCE [DSP]	12 °Brix	DRY SUBSTANCE [DSN]	6,8 °Brix
FRUIT PORTION [FP]	40%	ACIDITY [AP]	9,2 g/kg
ACIDITY DESIRED [AD]	5 g/kg	FACTOR TARTARIC ACID [FTA]	0,853
pH	3.1	DRY SUBSTANCE [DSN]	6,8 °Brix

Source: Own elaboration.

Table 2. Quantities and Ingredients for Strawberry for 5L of Nectar

STRAWBERRY NECTAR PREPARATION		
INGREDIENT	**FORMULA**	**QUANTITY**
FINAL NECTAR [N]	N = P / FP	5 Kg (l)
PUREE [P]	P = N x FP	2 Kg
CITRIC ACID [A]	A = (AD x N - P x AP) x FTA	5,63 g
SUCROSE [S]	S = (N x DSN)/100- (P x DSP)/100 - A	0,4 kg
WATER	W = N - S - A - P	2,5 kg (l)

Source: Own elaboration.

The components need to be mixed in a stainless-steel container, then blended and homogenized using an electric mixer. After the nectar formulation is finished, pour it into

40 ml centrifuge tubes, keep them chilled at 4 °C for the duration of the study, and remove them once a week to do the analysis. Before the nectar is put into sample tubes for HPP treatment, it is first treated in plastic bags. Within the test tubes, the nectar is instantly heated.

2.4.3. Stabilization Treatments

Table 3. Stabilization treatments examined

Test	Treatment	Strawberry Variety	Conditions	Production facility
1	Untreated (Control)	Senga sengana	No treatment after formulation	UNIPR
1	TT	Senga sengana	TT: 80° C, 5 minutes	UNIPR
1	HPP	Senga sengana	HPP 300 MPa, 3 minutes	HPP Italia
1	HPP	Senga sengana	HPP 300 MPa, 3 minutes	HPP Italia
1	HPP	Senga sengana	HPP 300 MPa, 3 minutes	HPP Italia
2	Untreated (Control)	Mixed Variety	No treatment after formulation	CTCPA
2	TT	Mixed Variety	TT: 65° C, 10 minutes	CTCPA
2	TT	Mixed Variety	TT: 80° C, 5 minutes	CTCPA
2	OH	Mixed Variety	OH: 80° C, 1 minute	CTCPA
3	Untreated (Control 1)	Senga sengana	Ambient temperature 20° C	UNIPR
3	TT- Mild heat	Senga sengana	40°C, 5 minutes	UNIPR
3	TT-Medium heat	Senga sengana	60°C, 5 minutes	UNIPR
3	TT	Senga sengana	80°C, 5 minutes	UNIPR
3	PEF	Mixed Variety	PEF 20°C, 45 Hz	UNIPR
3	PEF	Mixed Variety	PEF 40° C, 45 Hz	UNIPR
3	PEF	Mixed Variety	PEF 60°C, 45 Hz	UNIPR
3	PEF	Mixed Variety	PEF 80°C, 45 Hz	UNIPR

Source: Own elaboration.

Control

Each experimental treatment group's batch and starting raw materials were used to create a control sample. The control sample was left untreated to provide a baseline

against which the treated samples could be compared. This strategy ensures that any alterations noticed are due to the treatments used, not changes in the raw materials or preparation techniques.

TTs (Source UNIPR)

The samples underwent a 5-minute thermal treatment at 80°C in a water bath. To avoid additional heating after treatment, the samples were promptly cooled in an ice container. The Wisd Water Bath WB from WITEG Labortechnik was the water bath that was utilised, and it offered reliable temperature control. Three duplicates of each treatment were administered.

Ohmic and Thermal Treatments (Source CTCPA)

To ensure a 5-log reduction in the microbiological load and to produce nectar of mixed variety, 40% puree strawberry nectar was prepared at CTCPA and then exposed to ohmic and TTs. The ohmic therapy was carried out at 80°C for one minute. TTs were performed for 10 minutes at 65° C and 5 minutes at 80° C using a thermoresistometer.

PEF (Source UNIPR)

The nectar was treated with PEF using the EPULSUS®-BM1B-15 device, manufactured by Energy Pulse Systems. In this experiment, the "Multi Pulses" option with bipolar pulses was used. The voltage used was 1000V, the pulse width was +/-10μs, the relaxation time was 10 μs, and there were 25 pulses. The samples were treated at different temperatures (20, 60, and 80 °C) and a frequency of 45 Hz. For this aim, samples were kept in an incubator to guarantee exact temperature control. They were then transferred to a vacuum chamber to remove any remaining air before receiving treatment in the PEF box. Following the specifications of the settings, the capacitor begins to charge. The system allows pulses to be applied to the element being treated once the desired voltage is reached.

Table 4. Parameters of PEF treatment

Parameters	Limit range	Chosen	Description
Voltage	1500V to 15000V	10000 V	The pulse voltage to be applied to load in V
Pulse + width	2µs to 200µs	+10 µs	Positive pulse width in μs
Pulse - width	2µs to 200µs	-10 µs	Negative pulse width in μs
Relax time width	10µs to 99µs	10 µs	Relax time width between the positive and negative pulses in μs
Frequency	1Hz to 200Hz	45 Hz	Frequency of pulses on Hz
Number of pulses	2 to 220	25	-

Source: Own elaboration

HPP (Source HPP Italia)

Ohmic and Thermal Treatments (Source CTCPA)

To ensure a 5-log reduction in the microbiological load and to produce nectar of mixed variety, 40% puree strawberry nectar was prepared at CTCPA and then exposed to ohmic and TTs. The ohmic therapy was carried out at 80°C for one minute. TTs were performed for 10 minutes at 6HPP Italia used the Avure AV-50X equipment to conduct high-pressure processing testing. The primary characteristics of the high-pressure processing machine AVURE AV-20M, designed for large-scale food production, are displayed in Table 5. This industrial-grade device generates 1250–2400 kg/h, operates at pressures between 200 and 600 MPa, and has a vessel volume of 525 liters. It can handle up to 525 kg of product weight per cycle and process up to 525 liters of bottled juice at once.

To achieve the required pressure, the operational cycle included a 30-second decompression interval after around 2.5 minutes. It took ten minutes to complete the cycle, which included product handling, loading, pressurization, and decompression. Samples were prepared for the tests in 20 ml flexible plastic bags and sealed before being treated with HPP. When necessary, the machine's settings, including pressure (MPa) and treatment time, were changed. For three minutes each, treatments were carried out at pressures of 300, 450, and 600 MPa.

Table 5. AVURE AV-20M by JBT Specifications

AVURE AV-20M by JBT		
Number of high-pressure pumps (Each pump includes four high-speed intensifiers)	2	
Standardized Annual Capacity, 1-minute hold time	37.420.000 kg	82,500,000 lbs
Standardized Annual Capacity, 3-minute hold time	25.900.000 kg	57,100,000 lbs
Vessel STD Hourly Capacity, 1-minute hold time	4.797 kg	10,582 Ibs
Vessel STD Hourly Capacity (3-minute hold time)	3.321 kg	7,326 lbs
Utility requirements		
Process Water Cooling	300 L/min	58.1 U.S. gal/min
Power Supply	890 kVA / 3 ph. / 480V / 60Hz / 1070 A / 750 kW 850 kVA / 3 ph. / 400V / 50Hz / 1230 A / 725 kW	
Power Supply (3ph. / 400V / 50 Hz)	160 kVA	220 A 150 kW
Air Supply	6 bar 20 L/min	87 psi 0.7 cu ft/min
Pressure Vessel Data		
Diameter	471 mm	18.5"
Internal Length	3.000 mm	118.1"
Vessel Volume	525 L	138.7 U.S. gallons
Wire Length	277 km	172 miles
Wire Wound Frame	127 km	79.2 miles
Total Machine Weight	77.000 kg	170,000 Ibs
Recommended Input Water	4° - 29° C	39° - 84° F

Source: (JBT, 2023)

Analysis

- Within 24 hours after production, all samples were stored at 4°C and homogenised prior to analysis.
- Each therapy was assessed with three samples.
- Quality and colour indicators.

PH

The Portamess® 911 pH instrument from Knick Elektronische Messgeräte GmbH & Co. KG was used in this experiment to measure pH. This gadget has a pH-sensitive glass electrode for monitoring nectar pH, which requires stabilisation time to produce accurate findings.

Brix

Proster's Refractometer (101 ATC model) was used to determine the sugar content in Brix. Using a Pasteur pipette to ensure uniform distribution and removing air bubbles with a plate, sample drops were put on the prism surface of the refractometer in order to determine the Brix level. The instrument is then used to evaluate the reading, giving an exact measurement of the concentration of sugar.

Colour

Colour of strawberry nectar was characterized by the LCH CIELAB features: Lightness (L), Chroma (C), and (H). L, Lightness, that describes a colour brightness; its scale is from 0-black to 100-white. C, colour purity or vividness was expressed by a formula using a and b components. H is the abbreviation for tonality, expressed in circular grade within a range from 0 to 360 degrees. In addition, an acceptance factor calculated from CIELAB colour parameters was applied. The determination of the time that nectar takes to reach an unpleasant AF will provide an estimation of the shelf life. The CIELAB parameters were determined using the Konica Minolta® CM-2500d Spectrophotometer. This colour metric spectrophotometer targeted operation on solid surfaces by measuring the transmission or reflection of light at different wavelengths. The equipment measures the reflection or transmission spectrum and interprets the results using a proprietary program called SpectraMagicTMNX.

CIE Colour Space L*, a*, and b*

The colour characteristics of the samples were assessed using the CIE Lab* colour space. There are three parts to this colour space:

L*: Lightness, with 0 denoting black and 100 denoting whites.

a*: The green-red component, where greenness is indicated by negative values and redness by positive values.

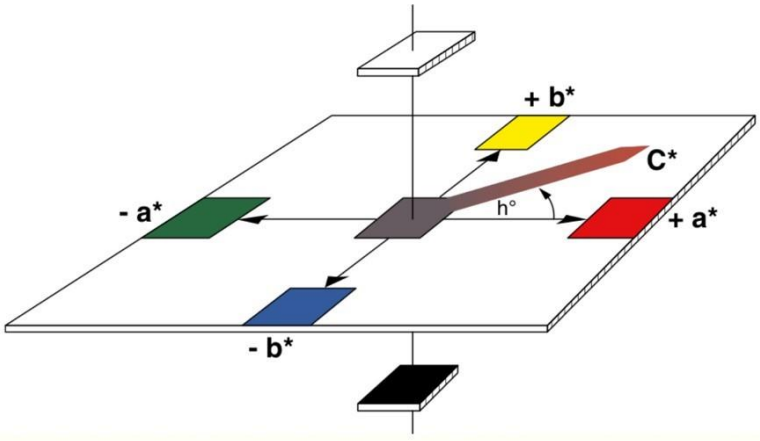
b*: The blue-yellow component, where blueness is indicated by negative values and yellowness by positive values.

The following formula was used to determine the C*:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

This calculation allows for the quantification of colour intensity.

Figure 1. CIE Lab Lch° graphical representation.



Source: (Hinoel Zamis Ehrenbring, 2022)

a. Calculation of ΔE

In a standardised colour space, ΔE quantified the total colour difference between samples and controls. Higher values imply considerable variations, whereas a ΔE value of 0 indicates that the colours are equal. This metric provides a thorough evaluation of colour stability during processing and storage by taking into account variations in brightness (L*), hue (H), and chroma (C*). Acceptable ΔE values were defined by

industry-specific thresholds, which typically ranged from 1 to 6, depending on application. This objective metric permitted accurate colour matching and quality control in a variety of sectors, including printing and product manufacture. The use of ΔE enabled standardised comparisons of colour variances, allowing for uniform evaluation of colour quality across samples and production batches.

ΔE was calculated using CIE Lab* values to measure colour variations between samples. To quantify colour changes across samples. The calculations were carried out using the following formula:

$$\Delta E_{ab} = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

b. AF

AF was calculated using the CIELAB colour components: a* (green-red), b* (blue-yellow) L* (lightness), from which C* (Chroma) and H (hue angle) are calculated. The AF is derived as follows: a*/h where "h" is the hue angle. The "h" angle = arctan(b*/a*).

Nectars with AF > 0.7 were considered excellent and those with AF < 0.4 were considered unacceptable (Gössinger et al., 2009).

c. BI Calculation

BI with colour values from CIE Lab:

Chromaticity was estimated for X:

x = 5.645L* + a* - 3.012b* / (a* + 1.75L*)

The BI formula was then modified to include the x value:

[100 * (x - 0.31)] = BI / 0.17

Where:

L* = Lightness value

a* = Redness value

b* = value of yellowness

This formula, which is extensively used in food science to quantify browning, is taken from the method given by Lunadei et al., 2011).

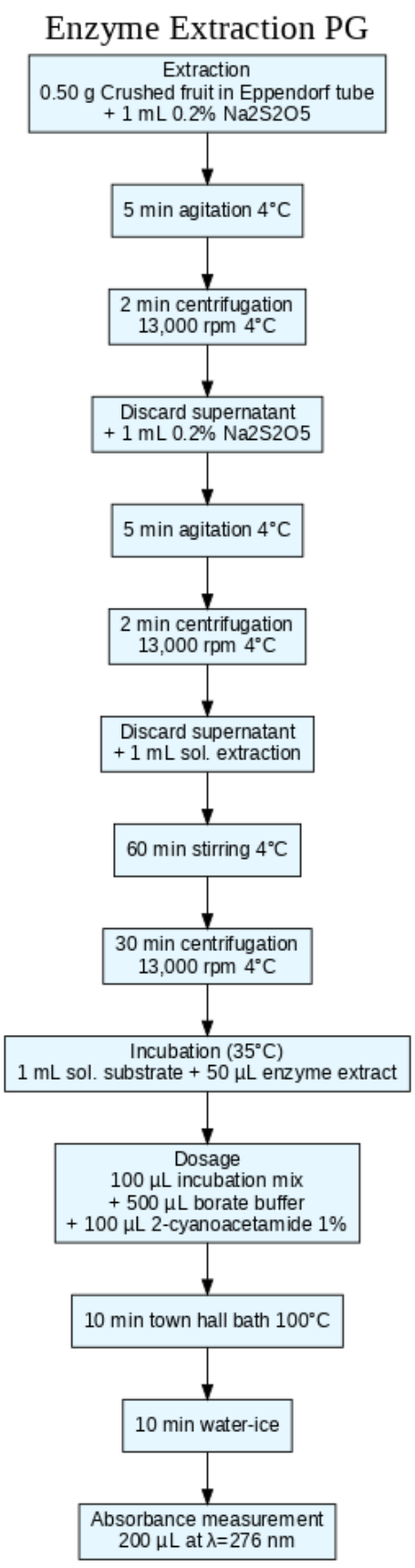
This index makes quality assessment and management easier by providing a numerical number that represents the level of browning in food products.

Enzymes

Polygalacturonase (PG) Activity

The release of D-galacturonic acid from the polygalacturonic acid substrate was used to assess PG activity. After homogenising 0.50 g of nectar sample in liquid nitrogen, 1 mL of extraction buffer (0.2 M phosphate, 1 M NaCl, pH 7) was added to create the enzyme extract. The mixture was centrifuged for 30 minutes at 13,000 rpm after being vortexed and stirred for two hours at 4°C. After collection, the supernatant was stored at -80°C until analysis. To measure PG activity, the enzyme extract was treated with a polygalacturonic acid substrate solution. Spectrophotometric measurements of D-galacturonic acid release were made at 520 nm. The amount of enzyme that, under normal conditions, produces 1 μmol of D-galacturonic acid per minute was considered to be one unit of PG activity (Adaption of Gross (1982), Hortscience 17:933-934). Because of technological constraints, PG was not carried out on samples that had been Ohmic or PEF treated.

Figure 2. Summary diagram of the experiment:

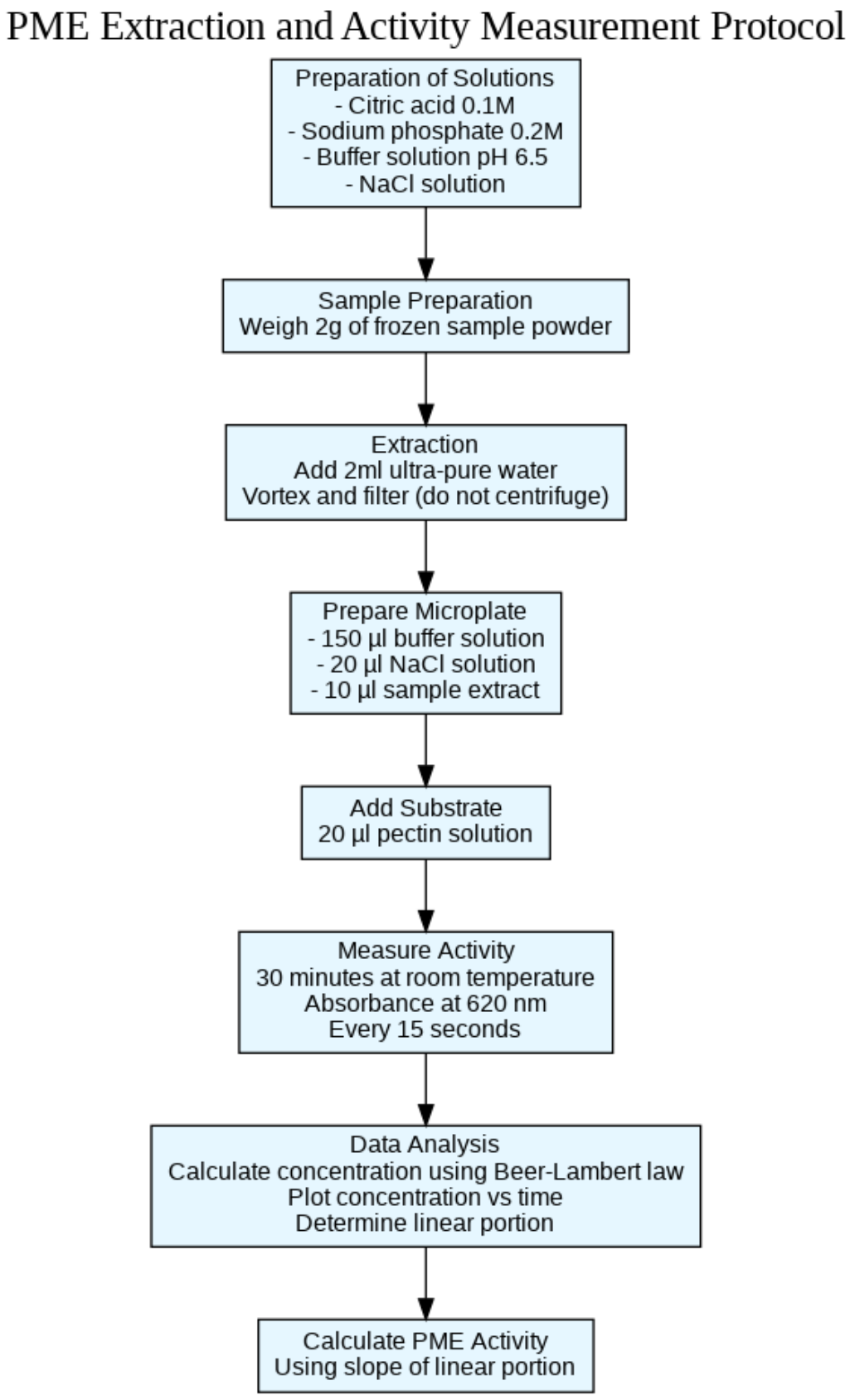


Source: Own elaboration.

Pectin Methylesterase (PME) Activity

Measurement of PME activity by titrimetry is detailed below in figure 3. PME catalyses the degradation of methyl-ester groups of polygalacturonic acid, releasing methanol, a carboxyl group, and acidifying the incubation medium. The titration measures the amount of 0.1 N NaOH added per minute to balance the acidification produced by PME activity. The de-esterification of methyl groups is important for texture modifications, either for the subsequent activity of the polygalacturonase enzyme (which degrades demethylated polygalacturonic acid chains), or for the creation of ionic bridges between ionized groups (with the participation of Ca2+ or other ions). Inrae internal protocols (2023) defined one unit of PME activity as the quantity of enzyme releasing 1 µmol of carboxyl groups per minute at pH 7.0.

Figure 3. PME Extraction and Activity Measurement Protocol.

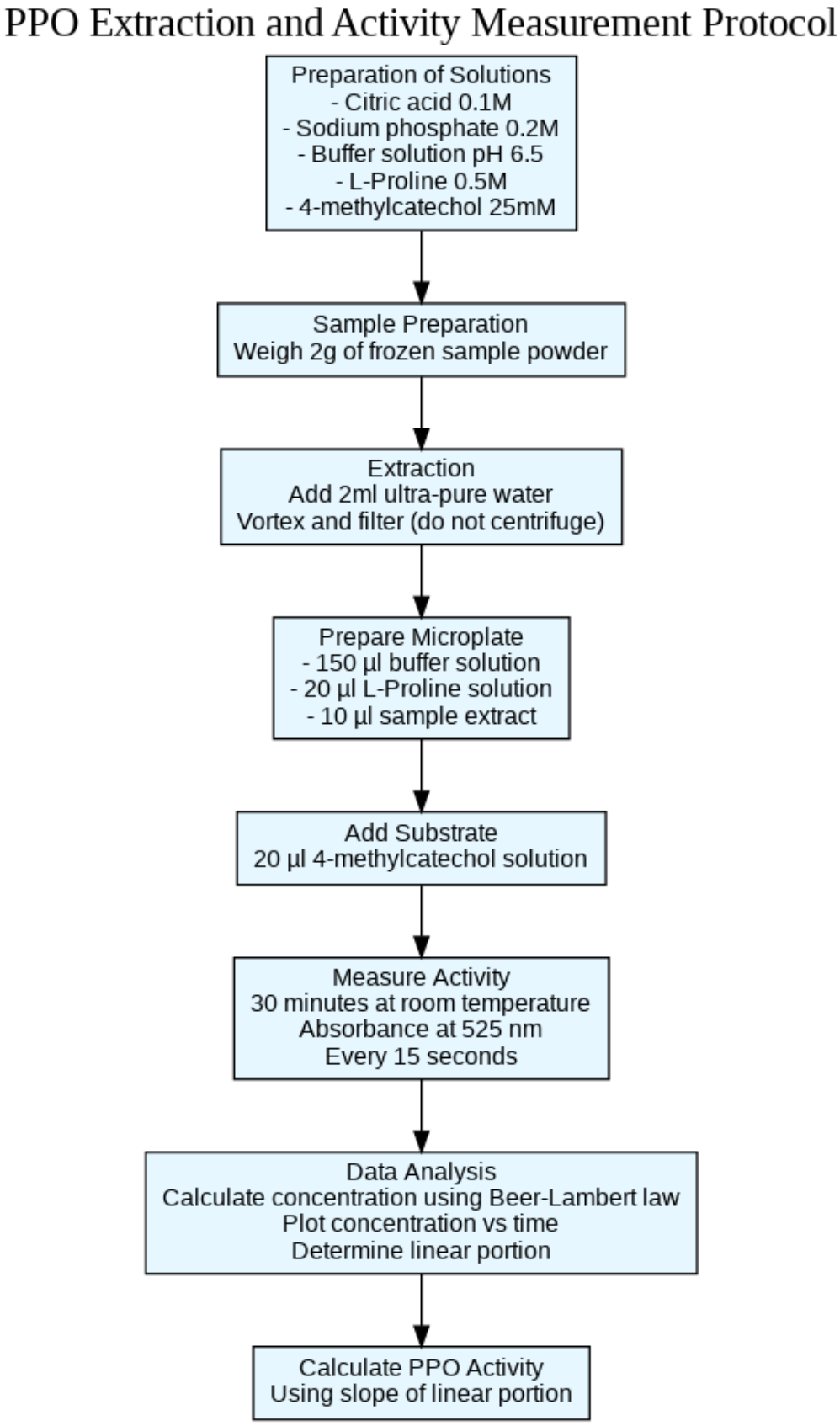


Source: Own elaboration

Polyphenoloxidase (PPO) Activity

PPO activity was evaluated by analysing catechol oxidation rates. The process is summarised in figure 4. The experiment was carried out by incubating the enzyme extract with catechol as a substrate. The reaction was observed spectrophotometrically at 525 nm over time. According to Reinkensmeier et al. (2016), PPO activity was calculated by measuring the rise in absorbance per minute, where one unit represents the quantity of enzyme needed to oxidise one μmol of catechol per minute.

Figure 4. PPO Extraction and Activity Measurement Protocol.



Source: Own elaboration

Enzyme activity expression

Enzyme activity was expressed in terms of NKatal/G, where G refers to grams of enzyme (table 6). This expression indicates the catalytic efficiency of an enzyme normalized to its mass. Specifically, it allows for comparison between different enzymes or different preparations of the same enzyme by standardizing their activity relative to their concentration (Schilling, 2008; Schilling, 2016)

Table 6. Measurement and Calculation

Measurement	Definition	Formula
Enzyme Activity	Quantifies the amount of substrate converted by an enzyme per unit time	1 NKatal = 1 nmol substrate converted per second.
Relative Activity	Compares observed enzyme activity to maximum activity under optimal conditions	Relative Activity = (Observed Activity nkat / Maximum Activity nkat) × 100
Specific Activity	Measures enzyme activity per unit mass of enzyme	Specific Activity = Activity nkat / Mass of Enzyme g

Source: Own elaboration.

Statistical Analysis

The data was examined with IBM SPSS Statistics 27 software, which reported mean values as standard deviations. A General Linear Model (Univariate) and one-way analysis of variance (ANOVA) were employed to assess experimental factors and sample differences, respectively. Tukey's post hoc test was helpful for pairwise comparisons, with letters indicating significant differences between groups. This analyses technique ensures that deviations are accurately recognized and evaluated, resulting in more robust statistical results.

The nomenclature is as follows: letters of the alphabet are allocated based on the numerical order of the means (i.e., a>b>c>d). In addition, means assigned different letters are significantly different from each other, whereas means assigned the same letter are not significantly different,

2.5. Results and discussion

HPP vs Thermal treatment

Colour and quality analysis.

Table 7. Colour and quality results for samples treated by HPP and Thermal

Parameter	*n*	*Control*	*300 MPa*	*450 MPa*	*600 MPa*	*TT*	*P*
L*	3	37.53 ± 0.01 a	36.45 ± 0.01 c	37.50 ± 0.03 a	36.18 ± 0.01 d	37.01 ± 0.00 b	S
a*	3	16.51 ± 0.03 c	17.88 ± 0.01 a	17.19 ± 0.05 c	17.75 ± 0.01 b	17.92 ± 0.02 a	S
C*	3	18.75 ± 0.08 e	20.70 ± 0.00 a	19.65 ± 0.07 d	20.57 ± 0.01 b	20.43 ± 0.02 c	S
ΔE	3	0.00 ± 0.00 d	2.34 ± 0.09 a	0.93 ± 0.16 c	2.38 ± 0.08 a	1.77 ± 0.06 b	S
AF*	3	0.58 ± 0.00 b	0.59 ± 0.00 b	0.59 ± 0.00 b	0.59 ± 0.00 b	0.62 ± 0.00 a	S
BI*	3	31.86 ± 0.07 d	35.51 ± 0.02 a	33.19 ± 0.08 c	35.54 ± 0.01 a	30.18 ± 0.01 b	S
TSS, Brix	3	12.27 ± 0.06 a	12.37 ± 0.06 a	12.27 ± 0.06 a	12.20 ± 0.10 a	12.27 ± 0.06 a	NS
pH	3	3.24 ± .06 a	3.23 ± .06 a	3.23 ± .06 a	3.22 ± .10 b	3.24± .06 a	S

Source: Own elaboration

Statistical significance is indicated by letters (a, b). Values with the same letter within each enzyme category are not significantly different from each other ($p > 0.5$), while different letters indicate significant differences ($p < 0.5$). S = Significant differences were found and NS = No significant differences were found.

Table 7 HPP at different pressures and TT effects on colour parameters and quality attributes. Significant differences among treatments were observed in most parameters, except TSS at $p < 0.05$.

L* values had a significant effect, where the 600 MPa treatment had the lowest L* value of 36.18 ± 0.01. No significant difference could be established between levels post 450MPa and control, at 37.53 ± 0.01 and 37.50 ± 0.03, respectively. At the same time, levels post TT and 350Mpa were lower compared to the control but not as low as those post 600MPa.

A significant treatment effect was detected with a* levels.

a* levels increased significantly compared with control for 300MPa, 600MPa and TT but not for 450MPa. Highest levels were observed after TT (17.92±0.02) compared to Control (16.51±0.03)

C* values were significantly enhanced by all treatments, with 300 MPa resulting in the highest value (20.70 ± 0.00) compared to the control (18.75 ± 0.08).

ΔE was most pronounced in the 600 MPa and 300 MPa treatments (2.38 ± 0.08 and 2.34 ± 0.09, respectively), indicating substantial colour changes from the control.

A significant treatment effect was detected for AF*. This was contributed by a significant increase in AF* post TT with no significant differences in AF compared with control for other treatments.

A significant treatment effect was detected with BI levels.

BI* significantly increased with HPP treatments, especially for 300 MPa and 600 MPa (35.51 ± 0.02 and 35.54 ± 0.01), as compared to the control and TT at 31.86 ± 0.07 and 30.18 ± 0.01, respectively.
No significant differences among the treatments have been presented for the TSS values so far.

A significant treatment effect was detected with pH.

The pH values exhibited small but significant differences, with a slightly lower pH for HPP 600Mpa compared to control.

These results demonstrate that HPP treatments, particularly at higher pressures, can induce significant colour changes comparable to or exceeding those of TTs, while maintaining other quality attributes such as TSS.

Enzyme activity

Table 8. Enzyme Activity of Polyphenol Oxidase (PPO), PME, and PG in Strawberry Nectar Following Various Processing Treatments**

Enzyme	*Treatment*	*Activity (nkatal/g) (Mean ± SD)*	*Remaining Activity (%)*	*P Value*
PPO	Control	5.73 ± 1.31 a	100	NS
	HPP 300	5.02 ± 0.50 a	87.5	-
	HPP 450	5.02 ± 0.50 a	87.6	-
	HPP 600	3.73 ± 1.24 a	65.0	-
	TT	2.15 ± 1.72 a	37.5	-
PME	Control	5.70 ± 1.65 ab	100	S
	HPP 300 MPa	5.84 ± 0.20 ab	102.47	-
	HPP 450 MPa	0.10 ± 0.1 b	1.78	-
	HPP 600 MPa	7.30 ± 0.09 a	128.18	-
	TT	4.05 ± 0.09 a	71.01	-
PG	Control	0.64 + 0.0 b	100	S
	HPP 300	0.35 ± 0.09 b	54.1	-
	HPP 450	0.46 ± 0.05 b	72	-
	HPP 600	0.51 ± 0.13 b	8	-
	TT	1.03 ± 0.23 a	159.9	-

Source: Own elaboration

Statistical significance is indicated by letters (a, b). Values with the same letter within each enzyme category are not significantly different from each other ($p > 0.5$), while different letters indicate significant differences ($p < 0.5$). S = Significant differences were found.

The enzymatic activities of PPO, PME, and PG in strawberry nectar were evaluated following various HPP treatments and TT (Table 8). The effects of these treatments on enzyme activities were found to be enzyme-specific and pressure-dependent.

No differences were observed in treatments for PPO activity, according to ANOVA ($p > 0.05$), but there was a trend showing the decrease in activity with pressure. The HPP treatments at 300 and 450 MPa inactivated the PPO activity to the same level of about 87.5%, while the treatment of 600 MPa resulted in a higher reduction of this enzyme activity to about 65% of the control and the thermal treatment promoted the highest reduction to about 37.5% of the control.

In contrast, the activity of PME was evidently affected by treatments with $p < 0.05$. Interestingly, HPP at 300 MPa slightly increased the activity of PME up to 102.47% from the control, while 450 MPa lowered it drastically to 1.78%. Surprisingly, HPP at 600 MPa viii caused a great increase in PME activity to 128.18 % of the control. TT brought an evident moderate decrease in PME activity to 71.01% from that of the control.

PG activity also presented significant differences between treatments ($p < 0.05$). All HPP treatments reduced PG activity with respect to the control, although the most effective was 300 MPa with an activity of 54.1%. HPP at 450 and 600 MPa gave residual activities of 72% and 8%, respectively. On the contrary with the trend described above for PPO and PME, TT increased the PG activity up to 159.9% of the control.

The above results give evidence for the diversity and complexity in the response of various enzymes to HPP and TTs in strawberry nectar. HPP could be used as a tool for selective enzyme inactivation, enabling a specific processing strategy targeting specific enzymatic profiles in fruit products.

Ohmic vs Thermal treatment

Table 9. Colour and quality results for samples treated by Ohmic and thermal methods.

Parameter	**Control 13,06**	**65°C 10 mins 15,06**	**80°C 5 mins 15/06**	**ohmic 80°C 1 min 11,07**	
L*	26.82 ± 0.14 a	27.32 ± 0.08 a	28.07 ± 0.08 a	26.24 ± 0.16 a	NS
a*	10.59 ± 0.11 b	11.42 ± 0.17 ab	10.94 ± 0.02 ab	14.32 ± 0.11 a	S
C*	12.47 ± 1.25 b	13.69 ± 1.37 ab	13.81 ± 1.38 ab	17.13 ± 1.71 a	S
ΔE	N/A b	1.36 ± 0.10 ab	2.25 ± 0.11 ab	4.70 ± 0.20 a	S
AF*	0.33 ± 1.07 b	0.34 ± 2.01 ab	0.29 ± 0.30 ab	0.43 ± 0.01 a	S
TSS, Brix	16.2 ± 3.24 a	16.0 ± 3.2 a	16.2 ± 3.24 a	16.2 ± 3.24 a	NS
pH	3.11 ± 0.31 a	3.2 ± 0.32 a	3.22 ± 0.322 a	3.2 ± 0.32 a	NS

Source: Own elaboration

Statistical significance is indicated by letters (a, b). Values with the same letter within each enzyme category are not significantly different from each other ($p > 0.5$), while different letters indicate significant differences ($p < 0.5$). S = Significant differences were found and NS = No significant differences were found

The effects of various TTs, including conventional heating and OH, on the colour parameters and quality attributes of a fruit product were investigated (Table 9).

Colour parameters analysed were L*, a*, and C*, and ΔE, AF*, TSS, and pH. By statistical analysis, it was noticed that there is a significant difference, at $p < 0.05$, for the values of a*, C*, and ΔE among treatments, while for L*, AF*, TSS, and pH, there were no significant treatment effects.

L* values varied between 26.24 and 28.07 and were not statistically significant among treatments. On the other hand, in a* and chroma C*, significant treatment effects were obtained. Treatment with OH at 80°C for 1 min had the highest a* of 14.32 ± 0.11 and C* of 17.13 ± 1.71, representing a brighter red colour when compared to other treatments.

Therefore, treatments significantly affected ΔE. Among them, OH had the highest Δ E value that was significantly different from the control with a value of 4.70 ± 0.20. Conventional heating at 65°C for 10 minutes and 80°C for 5 minutes gave ΔE values of 1.36 ± 0.10 and 2.25 ± 0.11, respectively.

AF* oscillated from 0.29 to 0.43 without any significant difference among the treatments. In the same way, TSS remained at the same level in all treatments, between 16.0 and 16.2 °Brix, and pH values did not very much differ from one another, going from 3.11 to 3.22. No significant difference was obtained.

These results thus show that OH at 80°C for 1 min, though it provoked the most colour changes, in particular, in the aspect of redness and overall colour differences, did not provoke any significant changes in other characteristics like TSS and pH. The present study also proves that OH can be an effective method to enhance the colour attributes of products made from fruit with the retention of other quality characteristics.

Enzyme Activity Summary

Table 10. Enzyme Activity of Polyphenol Oxidase (PPO), PME in Strawberry Nectar Following Various Processing Treatments.

Treatment	*PPO Activity (nkatal/g) ± SD*	*RA% (PPO)*	*P value*	*PME Activity (nkatal/g) ± SD*	*RA% (PME)*	*P value*
Control	4.87 ± 0.50 a	100	NS	5.92 ± 1.48 a	100	S
65°C 10 mins (15.06)	3.73 ± 0.50 a	76.47	-	0.00 ± 0.00 b	0	S
80°C 5 mins (15/06)	3.87 ± 1.55 a	79.41	-	0.00 ± 0.00 b	0	S
Ohmic 85°C 1 min (11.07)	4.59 ± 0.66 a	94.12	-	0.00 ± 0.00 b	0	S

Source: Own elaboration

Statistical significance is indicated by letters (a, b). Values with the same letter within each enzyme category are not significantly different from each other ($p > 0.5$), while different letters indicate significant differences ($p < 0.5$). S = Significant differences

The activity of PPO has taken place without showing any statistical difference among treatments ($p > 0.05$). The PPO activity was highest in a control sample (4.87 ± 0.50 nkatal/g). TTs of 65°C for 10 minutes and 80°C for 5 minutes reduced the PPO activity to 76.47% and 79.41% of the control, respectively. Surprisingly, OH at 85°C for 1 min caused the least reduction, having 94.12% remaining activity.

PME activity was significantly affected by all treatments ($p < 0.05$). The control sample showed a PME activity of 5.92 ± 1.48 nkatal/g. Notably, all TTs, including conventional heating at 65°C for 10 minutes, 80°C for 5 minutes, and OH at 85°C for 1 minute, completely inactivated PME activity.

These results evidence the differential effects of TTs on both activities of PPO and PME in strawberry nectar. Although PPO exhibited resistance to thermal inactivation, where all treatments resulted in relatively high remaining activities, PME was highly susceptible to TTs, with complete inactivation occurring for all processing conditions.

Results obtained are suggestive that the treatments applied, including the new OH treatment, are able to drastically reduce PME activity. This might favorably affect strawberry nectar stability and quality by preventing pectin breakdown. On the other hand, the residual activity of PPO, especially after OH, underlines the need for additional

interventions in order to control enzymatic browning of strawberry products during processing.

PEF vs Thermal

Table 11. Colour and quality results for samples treated by PEF and thermal methods

SAMPLE	L*	A*	C*	ΔE	AF*	BI*	TSS, BRIX	PH
C.20°C	36.05 ± 0.00 b	14.80 ± 0.01 c	16.89 ± 0.00 c	2.38 ± 0.05 de	0.51 ± 0.00 c	29.90 ± 0.01 a	13,03 ± 0.01 c	3,19 ± 0.01 a
C.40°C	35.39 ± 0.01 e	14.69 ± 0.01 d	17.01 ± 0.01 b	1.46 ± 1.16 bcd	0.49 ± 0.00 f	30.37 ± 0.03 a	13,13 ± 0.01 bc	3,2 ± 0.01 a
C.60°C	35.40 ± 0.00 f	14.50 ± 0.00 f	16.33 ± 0.00 f	1.76 ± 1.21 ac	0.53 ± 0.00 g	29.70 ± 0.00 a	13,06 ± 0.01 c	3,19 ± 0.01 a
C.80°C	34.73 ± 0.00 g	13.49 ± 0.01 h	15.38 ± 0.01 g	2.25 ± 1.79 a	0.47 ± 0.00 a	28.38 ± 0.02 a	13,1 ± 0.01 bc	3,19 ± 0.01 a
PEF20_45HZ	36.35 ± 0.00 a	14.86 ± 0.00 b	16.99 ± 0.01 b	2.21 ± 0.13 ce	0.51 ± 0.00 c	29.80 ± 0.00 a	13.03 ± 0.06 c	3.19 ± 0.01 a
PEF40_45HZ	35.63 ± 0.00 d	14.62 ± 0.01 e	16.79 ± 0.01 e	1.46 ± 1.08 bcd	0.50 ± 0.00 d	29.96 ± 0.01 a	13.07 ± 0.06 bc	3.18 ± 0.01 a
PEF60_45HZ	36.34 ± 0.00 a	16.07 ± 0.01 a	18.76 ± 0.01 a	1.26 ± 0.24 a	0.52 ± 0.00 b	32.32 ± 0.01 a	13.17 ± 0.06 ab	3.19 ± 0.01 a
PEF80_45HZ	34.56 ± 0.00 h	14.31 ± 0.01 g	16.38 ± 0.00 g	2.04 ± 1.48 ab	0.49 ± 0.00 e	30.18 ± 0.01 a	13.23 ± 0.06 a	3.20 ± 0.01 a
SIGNIF.	S	S	S	S	S	NS	S	NS

Source: Own elaboration

Statistical significance is indicated by letters (a, b). Values with the same letter within each enzyme category are not significantly different from each other ($p > 0.5$), while different letters indicate significant differences ($p < 0.5$). S = Significant differences were found and NS = No significant differences were found

Table 11 presents the colour and quality parameters of strawberry samples treated by PEF and thermal methods. Significant differences ($p < 0.05$) were observed in the L*, redness a*, chroma C*, and ΔE values across treatments. L* values ranged from 34.56 to 36.35, with the PEF20_45Hz treatment resulting in the highest lightness. The a* values varied from 13.49 to 16.07, with PEF60_45Hz showing the highest redness, while C* values ranged from 15.38 to 18.76, again with PEF60_45Hz exhibiting the highest chroma. The ΔE values indicated noticeable colour changes across treatments, highlighting the effectiveness of PEF in altering colour attributes.

AF* also showed significant differences among treatments, ranging from 0.47 to 0.53, with the highest value observed in the C.60°C treatment and the lowest in C.80°C

treatment. In contrast, no significant differences were noted in the BI*, suggesting that the processing methods did not significantly impact browning reactions. TSS values varied significantly among treatments, ranging from 13.03 to 13.23 °Brix, with the PEF80_45Hz treatment yielding the highest TSS value.

However, pH values remained consistent across treatments, showing no significant differences and ranging from 3.18 to 3.20. In summary, PEF and TTs significantly affected most colour parameters and AF, with PEF60_45Hz generally resulting in the most intense colour attributes. These findings suggest that PEF technology, particularly at 60 kV/cm and 45 Hz, could be an effective method for enhancing specific quality attributes in strawberry products while maintaining others such as pH and browning index stability.

Table 12. Enzyme Activity of PPO and PME in Strawberry Nectar Following Various Processing Treatments

Treatment	PPO Activity (nkatal/g)	PPO RA%	P Value	PME Activity (nkatal/g)	PME RA%	P Value
20°C	5.73 ± 1.31 a	100.0	NS	7.46 ± 2.12 a	100.00	NS
40°C	4.52 ± 0.30 a	78.8	-	6.50 ± 1.82 a	87.13	-
60°C	2.15 ± 0.61a	37.5	-	5.52 ± 2.13 a	73.99	-
80°C	1.29 ± 1.22 a	22.5	-	3.77 ± 1.08 a	50.54	-
20°C, 45 Hz	3.01 ± 0.00 a	52.5	-	2.77 ± 0.34 a	37.13	-
40°C, 45 Hz	0.86 ± 0.61 a	15.0	-	2.01 ± 2.84 a	26.94	-
60°C, 45 Hz	2.44 ± 0.66 a	42.5	-	3.53 ± 4.99 a	47.32	-
80°C, 45 Hz	2.15 ± 2.62 a	37.5	-	10.61 ± N/A a	142.22	-

Source: Own elaboration

Letters a and b denote statistical significance. Within each enzyme category, values sharing the same letter are not significantly different at $p > 0.5$ while different letters indicate significant differences at $p < 0.5$.

Table 12 illustrates the enzyme activity results of PPO and PME of strawberry nectar subjected to several thermal and PEF treatments. Results are presented as enzyme

activity expressed in nkatal/g and the RA% for both enzymes in different processing conditions.

For the PPO activity, all the treatments tended to reduce the enzymatic activity with respect to the control, though there were no significant differences among them ($p > 0.05$). In general, the TTs showed a trend of reducing the PPO activity as temperature increased; in this respect, the treatment at 80°C produced the lowest remaining activity, 22.5%. Among the PEF treatments, the greatest reduction of PPO activity was observed in the treatment at 40°C with 45 Hz, which obtained an activity of 15.0% remaining activity.

On the other hand, PME activity was also lowered by most treatments, although no significant differences were established. TTs showed a steady trend of reduction in the activity of PME with the rise in temperature; the treatment at 80°C lowered the activity to 50.54% of the control. Furthermore, PEF treatments at 20°C/45 Hz, 40°C/45 Hz, and 60°C/45 Hz caused more losses in PME activity compared to their corresponding thermal treatments. However, the 80°C PEF treatment has an unexpected increase in PME activity, accounting for 142.22% of remaining activity. Of course, without a standard deviation, it may be an outlier or something to be further researched. Overall, since both thermal and PEF treatments tended to reduce the activities, great variability in results was obtained; thus, there was no statistical significance between treatments. The data thus indicates that PEF treatment, at the lower range of temperatures in particular, may prove more effective at lowering PME activity than thermal treatment times alone, therefore providing for potential means of enzyme inactivation that might not as much affect other quality parameters of strawberry nectar.

Discussion:

The data obtained from Tables 7-12 report the effects that different methods of processing - namely HPP, TT, OH, and PEF - have on colour. The increase in redness and chroma values for the HPP-treated samples could be because of the pressure-induced injury that may have caused the release of anthocyanins from cell structures, as reported by Terefe et al. (2009).

These findings have important implications for the food processing industry, particularly in the development of minimally processed fruit products with improved quality retention. The outcome of this work contributes to the development of new

techniques to improve flavour and the aesthetic appeal not only of strawberry nectars but also of other fruit products.

Colour and Quality Parameters:

All the processing methods significantly changed colour parameters L*, a*, C*, and ΔE from control samples. HPP treatments, especially at 300 and 600 MPa, were more effective in changing the colour of the jam than the TT, which was reflected by the higher a*, C*, and ΔE values obtained in HPP-treated samples (Table 7). These are in agreement with Patras et al. (2009), who stated that high-pressure treatment increases the colour intensity of strawberry purées.

OH at 80°C for 1 min had the maximum a* and C* values, indicating it had the most intense red colour among all treatments. The same treatment also recorded the largest ΔE among all treatments. This result is in agreement with a previous study where Mercali et al. (2013) demonstrated that OH can improve colour traits in fruit products much better compared to conventional heating. Furthermore, during ohmic treatment, rapid and uniform heating takes place; this could be one of the reasons for higher colour retention and improvement.

Colour parameters were also significantly modified by PEF treatments, being PEF60_45Hz in the group of highest values of a* and C* in comparison with the rest of PEF treatments (Table 11). This agrees with the observations of Odriozola-Serrano et al. (2008), which mentioned that PEF could improve colour characteristics in fruit juices. Indeed, the cell permeabilization induced by the electric field may favour the release of colour compounds, increasing the general intensity of colour.

Moreover, changes in colour were significant, while the other quality parameters such as TSS and pH remained fairly constant under the different treatments. It therefore follows that such processing methods may improve attributes like colour without major variation in the basic compositional characteristics of the product, which is critical to the overall quality and consumer acceptance of strawberry nectar.

Activities of the Enzymes:

The sensitivity of the enzymes on the other side varied to kinds of enzymes and different forms of treatments. To be specific, it was found that PPO had a resistance to inactivation by all treatments. There was no significant difference within the same

treatment also, as can be seen from Tables 7 and 9. However, thermal and ohmic treatments are supposed to have an advantage in reducing its activity compared to HPP. This resistance of PPO to various methods of processing has similarly been reported by a number of researchers and makes the control of enzymatic browning in fruits difficult. The responses of PME activity were more divergent.

HPP treatments resulted in variable responses, where some treatments enhanced the activities, while others drastically reduced it (Table 8). Variability in the response of PME to HPP has been reported in the literature, which could be due to the complex structure and isoforms of PME present, as cited by Terefe et al., 2009. In contrast, thermal and ohmic treatments caused marked inactivation of PME. Indeed, complete inactivation of PME was attained for all the treatments conducted, as shown in Table 9. This is in line with the fact that PME is usually more heat labile than PPO, as stated in the literature. While all HPP treatments lowered the PG activity, it increased with TT (Table 8). Observations like this have been recorded in numerous studies and can be considered to be a result of the release of bounded PG during the thermal processing. This increase in PG activity with TT may increase pectin degradation and can alter product texture over time. Implications and Future Directions:

These results evidence that the different methods of processing have their particular effects on colour, quality attributes, and enzyme activities in strawberry nectar. The method of processing should therefore be chosen according to the quality objectives to be attained in the product. For instance, OH or HPP at higher pressures could be advisable if the achievement of better colour is among the main targets. On the contrary, thermal treatments or ohmic treatments would be more suitable if the objective is the inactivation of enzymes, especially PME.

The results also stressed that complete quality retention could be achieved with a combination of different techniques. For example, a product treated with HPP to increase colour could receive a short TT in order to inactivate the enzymes.

Further research is needed to investigate such combinations of treatments for optimal colour improvement and enzyme inactivation. Such studies on sensorial quality and shelf-life will also be helpful in establishing how these processing-induced changes influence consumer acceptance and stability of the products during storage. It goes without saying that these treatments may affect the nutritional components, mainly

anthocyanins and other bioactive compounds, and further studies are required in order to ensure that the nutritional quality of the product is preserved besides its sensory attributes.

Conclusion

The general comparison done in the present work between new and conventional technologies applied to strawberry nectar processing will be of extreme usefulness for the food industry when choosing or optimising techniques for fruit products. In fact, these results imply that novel technologies, like HPP, PEF, and OH, are promising alternatives to traditional thermal treatments when processing fruit juices, providing improved colour retention/enhancement and new opportunities regarding specific enzyme inactivation profiles.

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3. Manuscript 2: Evaluation of quality indicators of strawberry nectar stabilized by thermal and non-thermal (high-pressure processing) treatments

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3.1. Abstract

Strawberry nectar was formulated with 40% fruit puree, water, sugar, and citric acid and evaluated based on 5 quality parameters including total colour difference (ΔE), colour parameters L*and a*, apparent viscosity, and pH and Brix. Nectar was subjected to HPP (400, 500, and 600 MPa for 3 mins) and TP (75 °C 15 s). All samples were measured at 0, 7, 14 and 21 days, an untreated sample was used as control. The biggest difference observed among the samples was apparent viscosity (25.62 ± 0.65 for TP and 74.66 ± 2.72 for 600 MPa). Enzymatic activity was reduced after all treatments, with the lowest reduction after HPP 600 MP 3 mins. In the samples subjected to HPP, the value of ΔE tends to increase during the shelf life, with values greater than 3 on day 21. Further studies are needed to evaluate different combinations that could give better stability.

Keywords: Thermal processing; high-pressure processing; colour stability; polyphenol oxidase; peroxidase; rheology

3.2. Introduction

The Rosaceae family of plants includes strawberries, which are widely consumed both as fresh fruit and in processed forms including jams, juices, and nectars (Albuquerqueet al., 2018). Due to its appealing colour, pleasant perfume, and sweet-sour flavour, strawberry juice is one of the most consumed fruit juices (Wang and Jiao, 2000). Fruit juices, including fruit juices from concentrates, and fruit nectar are defined in Annex 1 and 2 to Council Directive 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption (OJ L 10, 12.1.2002, p. 58). Fruit juice is defined as the fermentable but unfermented product obtained from the edible part of healthy fruit that is ripe, fresh, or preserved by refrigeration or freezing, belonging to one or more species, and having the distinctive colour, aroma, and taste of the fruit juices from which it is derived (Directive 2012/12/EU of the European Parliament and of the Council of April 19, 2012). However, when the fermentable but unfermented substance is created by adding water to fruit puree, fruit puree concentrate, or a mixture of these substances, with or without the addition of sugars and/or honey, it is referred to as nectar. Nectars contain significant amounts of minerals, vitamins, and bioactive phyto-compounds, vitamin C, and other substances with antioxidant properties. The quantities of these substances vary depending on the fruit's quality, degree of ripeness, methods of harvesting and storage, and technological processes used, as well as the fruits' quality and degree of ripeness (SINU).

The attractive red colour is one of the visual quality attributes that greatly influence consumer appreciation of both fresh and processed strawberry fruits (Mollov et al., 2007). This is mainly due to the presence of anthocyanins which, in addition to being antioxidants, are a group of water-soluble pigments. Studies have shown that a key role in colour degradation is attributed to the enzymatic browning of phenolic compounds, the degradation of anthocyanins, and the products of the Maillard reaction during or after thermal processing. The loss of colour contributes significantly to the loss of quality (Cao et al., 2011).

PPO is the primary enzyme responsible for enzymatic browning (López-Serrano & Ros Barceló, 2002). PPO is an intracellular diphenol oxidase that contains copper and catalyses the conversion of polyphenolic substrates to quinone groups when oxygen is present. The quinones then undergo a non-enzymatic process to produce brown melanin pigments. Due to the condensation of quinones with substances including phenols, sugar,

amino acids, and proteins, enzymatic browning not only results in the colour change and destruction of antioxidants but also losses in organoleptic quality and nutritional value (Jiang, 1999). POD is another oxidoreductase enzyme involved in enzymatic browning since diphenols can function as reducing substrates in its reaction. (Aaby, Wrolstad, Ekeberg & Skrede, 2007).

In addition to microbiological inactivation, TP is used to inactivate fruit enzymes such as PPO and POD which are responsible for qualitative decay. Generally, the TP of fruit juices involves heat treatments higher than 60 ° C, to destroy target microorganisms or enzymes (AĞÇAM, Erdal et al, 2018). Alternatively, non-thermal methods of juice preservation can be employed such as high-Pressure treatment (HPP) (Krystian Marszałek et al., 2017). The HPP process is an innovative technology that subjects packaged foods to hydrostatic pressures thousands of times higher than atmospheric pressure (up to 6,000 bars). In this way, devoid of heat input, the alternative microorganisms and pathogens present in both solid and liquid foods are inactivated to make the treated products microbiologically stable for longer and safer, without significantly modifying their sensory and nutritional characteristics (HPP Italy). Notably, the highest reduction in PPO activity was 51.5% at 600 MPa for 25 min (Cao et al., 2011). However, Garcia- Palazon et al. reported that there is complete inactivation of PPO in strawberry fruits at 600 MPa for 15 minutes at room temperature. As for the POD enzyme, at 400 or 500 MPa, the POD activity decreased with increasing treatment time. POD activity was reduced to 35.7% at 600 MPa for 5 min but returned to 71.9% for 10 min. Monomeric anthocyanins and total monomeric anthocyanins showed no significant changes after HPP treatments regardless of treatment pressures or times, indicating that monomeric anthocyanins were well retained after HPP treatments (Cao Xiamin et al., 2011).

Without regard to the length of the treatment, Cao Xiamin et al. (2011) showed that HPP at 400 MPa caused a considerable drop in L * of strawberry pulp, leading to browning of the pulp. This was connected to greater PPO and POD activity at 400 MPa in the residual state. At 500 or 600 MPa, strawberry pulp's L* did not significantly decrease. After HPP treatments, the strawberry pulp's a* parameter exhibited no change. Except for 5 minutes, parameter b * did not change at 500 MPa or 600 MPa but significantly increased at 400 MPa. In contrast to b *, H ° and C * dramatically increased

to 400 MPa apart from 5 minutes, but there were no appreciable changes at 500 and 600 MPa.

In addition to microbial and enzyme stability, viscosity is an important parameter for the production control of fruit juices. Also, incoming liquid raw materials must be controlled by checking their viscosity. The customers' mouthfeel of a fruit juice depends on the "thickness" of the product and influences the taste. However, only a few studies have focused on changes in the rheological characteristics, microorganisms, and quality attributes of strawberry nectar after HPP during storage, and, therefore, this treatment and the relevant changes require further investigation.

3.3. Objective

Therefore, the purpose of this experimental study was to evaluate different processing methods on quality indicators of strawberry nectar: HPP (400, 500, and 600 MPa for 3 minutes) and TT (TT 75 °C 15s). Nectar was formulated with 40% fruit puree, water, sugar, and citric acid and evaluated based on 5 quality parameters including colour difference (ΔE), CIE Lab parameters (L*, A*, B*), apparent viscosity in addition to pH. In addition to this analysis, the residual activity (RA) of enzymes relevant for low colour stability was also evaluated on all samples at 0 days. All quality indicators were measured at 0,7,14 and 21 days while the untreated nectar (control) was analysed only at day 0 due to its short shelf life.

3.4. Materials and Methods

Samples, preparation, and storage

Strawberry Nectar was prepared from the frozen strawberry puree of the Senga Sengana strawberry variety and was purchased from SAS SICA SICODIS (Saint Laurent d'Agny - FRANCE).

The unpasteurized frozen strawberry puree was thawed at 4◦C overnight, followed by 30 minutes at room temperature. Water, sugar, and citric acid were added to the puree and mixed, thus obtaining strawberry nectar of 12 Brix and pH 3.3-3.5. Subsequently, aliquots of 40 g of strawberry nectar were placed in plastic bags and immediately sealed with a heat sealer. Sealed samples were kept at 4◦C until treated by HPP and TT within 10 hours. The Strawberry nectar was subjected to two different treatments: TT and high

hydrostatic pressure (HPP). All the samples were stored at a refrigerated temperature (+4 ± 1°C) until the analyses were performed.

TT

TT was carried out in a pilot plant at the University of Parma laboratories as reported by Rinaldi et al. (2018). The samples were heat treated in a thermostatic bath until reaching an internal temperature of 75 ◦ C and were kept at this temperature for 2 minutes. The samples were then immediately cooled to room temperature in an ice water bath. The selected heat treatment is in line with Tetrapak commercial guidelines for low-pH juices.

HPP

High-pressure treatments were conducted in a 300 L high-pressure plant unit manufactured by JBT Avure Technologies (Erlanger, Kentucky, United States) in HPP ITALIA SRL (Parma, Italy). An indirect method for the generation of high isostatic pressure using means of cold water (4 °C) was used, and the temperature increase due to compression was not higher than 2-3 °C/100 MPa. HPP treatments were conducted at 400-600 MPa for 180 s, chosen based on the goal of inactivation of PPO in strawberry nectar (Cao et al., 2011) and to be microbiologically safe. The treated samples were then stored at a refrigerated temperature (+4 °C). HPP treatments were conducted in triplicate and for each repetition.

pH

The pH was measured in triplicate using a pH meter (Model 3150, Jenway, UK).

Rheology

The apparent viscosity of the strawberry nectar was evaluated immediately after preparation and after 1,7,14 and 21 days of storage at refrigeration temperature (4 ° C). The analysis was performed using an Anton Paar rheometer of the MCR series (modular compact rheometer, mod. 102) with a cylindrical probe, with a diameter of 26.66 mm, applying a "flow curve" type test. The type of probe chosen is by the type of sample to be analysed, that is a low-viscosity liquid whose relationship between shear stress and shear rate is to be evaluated through the execution of rotational movements on the sample. The various samples were subjected to increasing shear rates from 10 s -1 to 300 s -1 at a constant temperature of 25 ° C. The analysis was performed on two identical samples but

taken from two different bags. The data obtained was recorded using the Anton Paar software RheoCompass.

Colourimetric analyses

Strawberry nectar colour changes were evaluated on the sample immediately after preparation and after 1,7,14 and 21 days of storage at refrigeration temperature (4 ° C). The colour was analysed with the Konica Minolta colour meter and the data analysed with the Spectramagic3.6 software. The CIELAB coordinates L *, a *, b * were evaluated. L * indicates brightness and has a value between 0 (black) and 100 (white), a * and b * indicate the direction of the colour, in particular: + a * is the direction of red, -a * is the direction of green, + b * is the direction of yellow and -b * is the direction of blue.

The total colour difference was determined with equation (1) by comparing the sample with the untreated control sample:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

where is it:

- $\Delta L^* 2$ = L * sample - L * control.
- $\Delta a^* 2$ = a * sample - a * control.
- $\Delta b^* 2$ = b * sample - b * control.

The perceptible colour difference between the sample and the untreated control was interpreted based on the following classification:

- If ΔE = 0 - 0.5 the difference is not perceptible.
- If ΔE = 0.5 - 1.5 the difference is slightly perceptible.
- If ΔE = 1.5 - 3.0 the difference is evident.
- If ΔE = 3.0 - 6.0 the difference is clearly visible.
- If ΔE = 6.0 - 12.0 there is a big colour difference.

The C *, calculated according to equation (2), was calculated to determine the colour saturation of the samples perceived by the human eye by comparing the transition from grey (low values of C *) to pure colour (high values of C *):

$$C^* = \sqrt{\left(a^2 + b^2\right)} \quad (2)$$

q (h °) which indicates the relative amount of redness (0 ° or 360 °), yellow (90 °), green (180 °) or blue (270 °) was also calculated; the equation (3) used was the following:

$$h° = \tan -1 (b^* / a^*) \quad (3)$$

Microbial analysis

Microbial analyses were performed following standard plate count methodologies for total mesophilic charge (TMC) (UNI EN ISO 4833:2004) and moulds and yeasts (ISO 21527-1:2008). Samples of 1 mL of fresh and treated strawberry nectar were diluted with 0.1% peptone water (Bactro, Sparks, MD) to ten-fold serial dilutions. From each dilution, two samples were plated. Plates were incubated at 32 °C for 48 h. Analyses were performed after treatments on all samples.

Protein extraction

For the extraction of the enzyme 100 grams of frozen strawberry nectar were homogenized. The homogenized nectar was mixed with citric acid buffer (0.1 M, containing 25 mM sodium ascorbate, pH 6.5), in a ratio of 1: 1.5 (w: v). After centrifugation (5450 g, 4 ° C) the pellet was taken on which a second extraction with citric acid buffer (0.1 M, with 25 mM of sodium ascorbate, pH 6.5) was carried out containing the 4 % of Triton X-100 (v / v) in a ratio of 1: 4 (w v) for 1 hour at 4 ° C, under stirring. The mixture was centrifuged at 5450 g for 30 minutes at 4 ºC and the supernatant was subjected to precipitation of ammonium sulphate (80%). The suspensions were then stored at 4 ºC.

The sample was then prepared for the enzymatic activity tests. 1 ml of the homogeneous suspension was centrifuged for 5 minutes at 4 ºC, 14000 g, and the liquid was carefully removed while maintaining the floating pellet. The pellet was resuspended in 200 µl 100 mM MES buffer, pH 6.5. After centrifugation (5 min, 14000 g, 4 ° C) of the insoluble protein pellet, 100 µl was used for the assay.

PPO and POD activity test

Finally, the assay for enzymatic activity was prepared. To determine the activity of the PPO 150 ul of extract was mixed in 2.5 ml of 100 Mm MES buffer (pH 6.6) and 150 ul

of 1 M of pyrocatechol was added. The increase in absorbance was measured spectrophotometrically at 25 ° C and 420 nm for 10 minutes; the slope of the linear absorbance curve concerning time was considered as the enzymatic activity. To determine the activity of PODs, 100 ul of enzyme extract was added to 2.25 ml of 100 mM MES buffer containing hydrogen peroxide (1.5% w/v) at pH 6.5, and 600 ul of p-Phenylenediamine 1.67% (w/v). POD activity was defined as the amount of enzyme that caused an increase in absorbance at 485 nm per minute. The increase in absorbance was measured spectrophotometrically at 25 ° C and 485 nm for 10 minutes.

The residual activity was referred to the untreated control sample, measured on the first day of storage, using the following equation:

RA = Enzyme activity in the sample / Enzyme activity in the control sample × 100 (%)

Statistical analysis

SPSS (v. 27.0, SPSS Inc., Chicago, USA) was used to calculate means, standard deviations. A 2-way and 1-way ANOVA were performed (with standard and log-transformed data), rows = difference between processes ($p < 0.01$), columns = difference between measures ($p < 0.01$).

Process comparison

To compare the process with the control the closeness of five quality markers including total colour difference (ΔE), CIE Lab parameters (L*, A*), apparent viscosity in addition to pH and Brix (for treatment vs control measured on Day 21 (log-transformed data). The data obtained for processed samples (TT and HPP) was plotted against the control on an XY scatter plot. R^2 provides measure of closeness (the Closest to 1 the closer that the quality parameters are to the control ones). The coefficient of R^2 is a measure that provides information about the goodness of fit of a model. In the context of regression, it is a statistical measure of how well the regression line approximates the actual data. It is therefore important when a statistical model is used either to predict future outcomes or in the testing of hypotheses. There are a number of variants although the one presented here is widely used

$$R^2 = 1 - \frac{\text{sum squared regression (SSR)}}{\text{total sum of squares (SST)}},$$
$$= 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}.$$

The sum squared regression is the sum of the residuals squared, and the total sum of squares is the sum of the distance the data is away from the mean all squared. As it is a percentage it will take values between 0 and 1.

3.5. Results and discussion

Physio-chemical analyses

Evaluation of pH changes in the untreated (control), TT, HPP (400, 500, 600 MPa), and strawberry nectar were evaluated. All samples remained within a suitable range after treatment and in shelf life: pH = 3.1 - 3.5; constant pH during refrigerated storage indicates microbiological stability (Sulaiman et al., 2017). Samples presented no noticeable changes either depending on the treatment or shelf life (Aaby et al., 2018).

Microbiological analyses

The presence of mesophilic aerobic bacteria and yeasts and moulds was evaluated. The evaluation was performed on untreated puree, untreated nectar (control), and samples within 24 hours of heat and non-heat treatment.

The TT and the HPP 600 MPa 3 min treatments resulted in a 2-log reduction in MABs compared to the control; the presence of yeasts and moulds was not detected. As for the 400 MPa, 500 MPa, 600 MPa treatments for 3 min, they demonstrated a slightly lower reduction although not significantly different in MABs; also, in this case yeasts and moulds were not detected.

The result obtained from the heat treatment agrees with Timmermans RAH et al. (2022) who used a heat treatment of 72 ° C for 20 seconds on an orange juice (pH 3.3-3.4), obtaining a microbial load <3 log and <1 log of MAB and yeasts and moulds respectively.

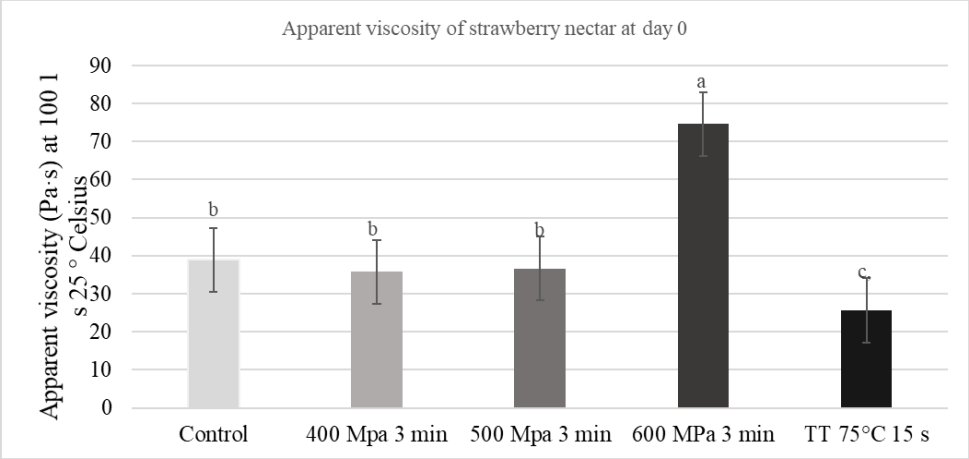
The results obtained from the high-pressure treatments agree, both for MABs and yeasts and moulds, with those obtained by Aaby K. Et al. (2018) on strawberry puree also treated at 400 MPa, 500 MPa and 600 MPa for 3 min obtaining respectively 2 logs UFC

/ g, 1.6 log UFC / g, and 1.7 logs UFC / g: undetectable moulds and yeasts. Studies on strawberry nectar on which yeasts, moulds, and lactic bacteria have been inoculated have shown that treatment of 600 MPa for 3 min is required to inactivate them; while on uninoculated nectar samples, 500 MPa are sufficient for microbial stabilization (Rovere P. et al., 1996).

TT is a stabilization technique that is generally coupled with refrigerated storage and sterility is generally not achieved by this treatment.

Viscosity behaviour

Figure 1. The effect of processing on rheological behaviour of strawberry puree.



Source: Own elaboration

The apparent viscosity (Pa·s) of samples during shelf life was evaluated. A 2-way ANOVA was performed (log-transformed data) considering differences between processes and shelf-life ($p < 0.01$), the error bars are ± SD (standard deviation). From the statistical analysis, it emerges that the different treatments determine a significant difference in the apparent viscosity of the sample. On the other hand, the shelf life and the interaction between treatment and shelf life are not significant. On day 1 the apparent viscosity of the HPP 400 and HPP 500 MPa samples was not significantly different from the control; higher values, on the other hand, were shown for the samples subjected to 600 MPa; in the TT sample, the value was slightly lower than the control but not significantly different from HPP 400 MPa and 500 MPa during the shelf life. In all cases, the rheological behaviour of the samples, therefore, remained almost unchanged constant during the 21 days of refrigerated storage (4°C). There is significant difference in the apartment viscosity measures between the samples treated at different pressures (Figure

1). As the applied pressure increases, viscosity increases. The rheological behaviour of fruit juices is generally attributable to pectin. Pectin is a complex polysaccharide having functions in the growth, morphology, development, and defence of plants and above all also acts as a gelling and stabilizing polymer in various food products (Mohnen, 2008). The characteristic property of pectin is the ability to form a gel in the presence of Ca2 + ions or sugar and acid. Fruit drinks are known to be a blend of carbohydrates, proteins, pigments, and organic and mineral acids. Interactions between these molecules, in particular pectin and proteins, can influence the consistency of the products (Thakur et al, 1997). It has been suggested that HPP increases gel firmness compared to a raw sample as it improves interactions involving polysaccharide-based structures (Dervisi et al., 2011). Studies conducted on guava juice have shown that viscosity increases at pressures greater than 500 MPa, due to interactions between pectin and other components in the juice (Yen et Lin, 1998).

Colour analysis

Different letters indicate significant differences (p < 0.05) among days (small caps) and sample treatments (capital letters) by post hoc Tukey test

Table 1. Colour changes in samples compared to control during 21 days of refrigerated storage.

	Control					HPP 400 MPa 3 minutes					HPP 500 MPa 3 minutes					HPP 600 MPa 3 minutes					TT 75C 15s				
	L*	a*	b*	h°	C*	L*	a*	b*	h°	C*	L*	a*	b*	h°	C*	L*	a*	b*	h°	C*	L*	a*	b*	h°	C*
T1	33,74 ± 0,68c	16,05 ± 0,23 b	7,97 ± 0,49a	26.36 ± 1.15 b	17,92 ± 0,39 b	34,66 ± 0,23 a,A	14,85 ± 0,27 c,A	6,9 ± 0,27 c,C	24.92 ± 0.51 c,A	16,37 ± 0,35 c,A	34,78 ± 0,11 a,A	14,43 ± 0,2 d,A	6,49 ± 0,33 c,A	24.18 ± 0.80 c,A	15,83 ± 0,32 d,A	34,14 ± 0,22 bc	13,96 ± 0,06 d	6,26 ± 0,2 b	24.12 ± 0.69 b,c,	15,3 ± 0,1 c,d	34,96 ± 0,34 a,B, C	16,74 ± 0,13 a,A	9,33 ± 0,39 c,C	29.11 ± 0.97 a,A	19,17 ± 0,27 a,A
T7						34,18 ± 0,19 c,B	13,85 ± 0,23 c,B	6,33 ± 0,33 b,c,A	24.52 ± 0.80 b,A	15,23 ± 0,33 c,d,B	34,18 ± 0,32 c,B	13,61 ± 0,2 d,B	6,13 ± 0,56 c,d,A ,B	24.10 ± 1.60 b,A	14,93 ± 0,4 d,B	34,53 ± 0,19 b	14,03 ± 0,11 c	6,25 ± 0,3 d	24.01 ± 0.86 b,	15,36 ± 0,21 c	34,84 ± 0,05 a,C	14,81 ± 0,05 b,B	6,7 ± 0,14 a,A	24.29 ± 0.40 b,B	16,26 ± 0,1 b,B
T14						33,92 ± 0,15 c,C	13,21 ± 0,06 d,C	6,13 ± 0,21 b,c,B	24.92 ± 0.63a , A	14,56 ± 0,14 c,C	34,27 ± 0,24 b,B	14,01 ± 0,11 b,C	6,2 ± 0,29 c,A, B	23.89 ± 0.86 b,A	15,32 ± 0,2 b,C	34,47 ± 0,08 b	13,49 ± 0,17 c	5,63 ± 0,2 c	22.63 ± 0.46 c,	14,62 ± 0,2 3c	35,15 ± 0,11 a,A, B	14,91 ± 0,19 a,B	6,9 ± 0,3 b,B	24.81 ± 0.69 a,B	16,43 ± 0,3 a,B
T21						33,82 ± 0,22 c,d,C	12,92 ± 0,12 c,D	5,85 ± 0,25 b,B	24.35 ± 0.860 a,b\, A	14,18 ± 0,18 b,D	33,61 ± 0,05 c,d,C	13,22 ± 0,12 b,c,D	5,76 ± 0,19 b,B	23.55 ± 0.57 b,A	14,42 ± 0,17 b,D	34,01 ± 0,26 c	13,24 ± 0,31 b,c	5,89 ± 0,38 c	23.95 ± 0.92 a,b,	14,49 ± 0,43 b	35,3 ± 0,34 a,A	14,92 ± 0,62 a,B	6,88 ± 0,56 a,B	24.70 ± 0.86 a,B	16,43 ± 0,8 a,B

\ 0

Table 1 shows the results related to the CIE Lab parameters L*, a*, b*, h° and C evaluated on day 1 and compared with the control sample. Furthermore, the parameters of the treated samples were evaluated over 21 days of refrigerated storage.

The analysis of the general linear model suggests that, in general, all the parameters have significant differences ($p < 0.05$) both concerning the type of treatment and the shelf life and the interaction between the two. However, it highlights a non-significance ($p > 0.05$) of the treatment concerning the L* parameter. All the parameters of the treated samples evaluated on day 1 are statistically different from the control sample.

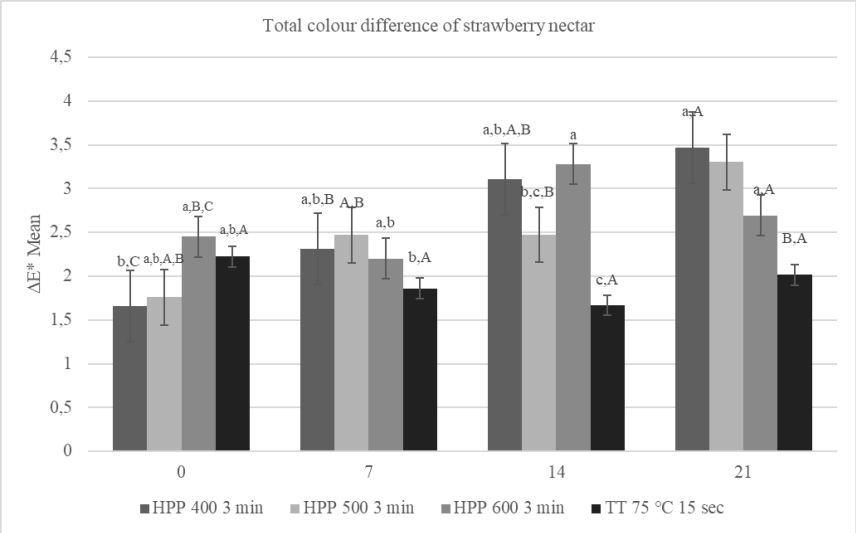
In the case of brightness, although the applied treatment is not significant, the shelf life is significant: the L* parameter changes during 21 of refrigerated storage (4 ° C). L* shows a slight increase in the case of the thermally treated sample; a similar behaviour was noted on a pasteurized apple juice at 60-75 ° C for 15-20 seconds, attributing the slight increase in brightness to the partial precipitation of unstable and suspended particles in the juice (Genovese et al., 1997). This behaviour can also be explained considering that anthocyanins tend to go towards a lighter colour when heated because the balance of the anthocyanins shifts towards the colourless carbonyl base and the chalcone forms (yellow pigments); however, the original colour could be recovered following a sufficient cooling to allow the reconversion of the chalcones (Bodelón et al., 2013).

L* decreases slightly in the case of the treated samples at 400 MPa and 500 MPa respectively. Even in the case of the two samples subjected to pressures of 600 MPa, the brightness has slight variations during storage but there do not seem to be significant differences between day 1 and day 21. The decrease in the L* parameter is associated with the RA that during the shelf life brings about the degradation of anthocyanins. This is following the hypothesis of Cao et al. (2011) to justify why a pressure at 400 MPa, regardless of the treatment time, had induced a significant decrease in the L* of strawberry pulp with consequent browning.

Another important parameter is a* which indicates the direction of red, concerning which both treatment and shelf life are significant. The highest value of a* is that relating to the heat treatment to indicate a more saturated red, while it is the same in the case of samples treated with high pressures. In all cases a* decreases over the shelf life; also, in this case, it is possible to associate this decrease with the residual activity of the enzymes. b * indicates the yellowish component and is higher in the sample TT 75 ° C 15 sec, which

disagrees agreement with studies conducted on strawberry and peach puree which attributed this result to browning caused by non-enzymatic reactions (Bleoanca et al., 2021). Also, C* differ according to the type of treatment and during storage and always shows higher values in the case of the heat-treated sample; also, this agrees with the study conducted on strawberry and peach puree where the chroma values (C*) for the samples subjected to heat treatment indicated a colour intensity significantly perceived by the human eye compared to the other samples. The value of h° is also different between treatments and varies over the shelf life, again presenting the highest value for TT 75 ° C 15 sec, therefore a red tint that is closer to yellow than the others.

Figure 2. ΔE* of samples during shelf life.



Source; Own elaboration.

A 2-way ANOVA was performed (log-transformed data), rows = difference between processes, columns = difference between measures ($p < 0.01$), the error bars are ± SD (standard deviation)

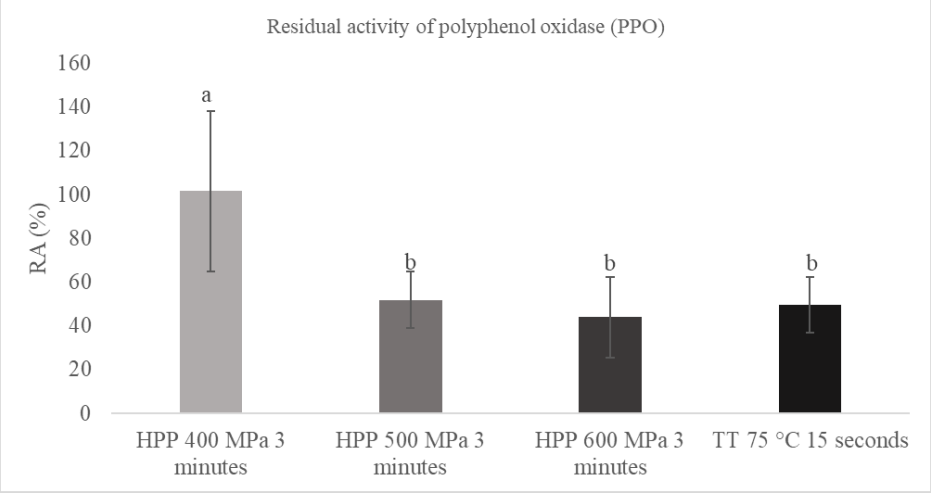
ΔE can be used as an indicator to check whether colour changes can be perceived by humans: if it exceeds the value of 3.0, the colour difference should be visible to consumers (Cserhalmi et al., 2006). TT does not vary considerably and remains below the value of 3 (Figure 2). Also, in the strawberry puree and juice study by Aaby et al. (2018), it was found that the colour of heat-treated products is more stable during storage than the colour of products treated with high pressures. In this case, it could be associated with the fact that during storage there was an activation of the PPO in the HPP samples but not in the pasteurized ones (Aaby et al., 2018).

The ΔE value of all the samples, after the treatment applied to them, is between 1.5 and 3 to indicate that there is a perceptible difference in colour between the samples and the control. However, in all the samples subjected to high pressures, the value of ΔE tends to increase during the shelf life up to, on day 21, values greater than 3 which results in a noticeable colour difference compared to the control (Figure 2).

In general, other studies on strawberry juices have also shown a decrease in parameters such as L *, a *, and b * and an increase in ΔE during storage (Cao et al, 2012). From the literature it is known that the degradation of anthocyanins during storage is not only due to enzymatic reactions, but also to condensations with other compounds (such as amino acids) or to non-enzymatic oxidation reactions: in the latter case, oxygen can react with anthocyanins through a direct oxidative mechanism, or it can oxidize other compounds which then interact with anthocyanins to form colourless compounds (Buvé et al., 2018). In addition, different reactions may occur that lead to colour degradation such as phenol polymerization, sugar degradation, ascorbic acid degradation, and the Maillard reaction which involves the formation of dark pigments (Bleoanca et al., 2021).

Remaining activity of PPO and POD

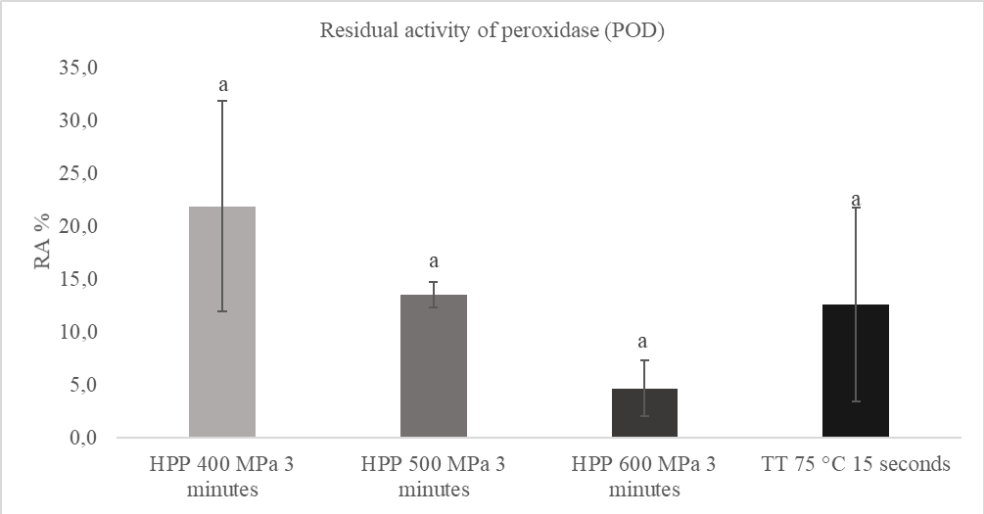
Figure 3. Residual activity (%) of PPO following HPP treatments and heat treatments



Source; Own elaboration.

A 2-way ANOVA was performed (log-transformed data), rows = difference between processes ($p < 0.01$), columns = difference between measures ($p < 0.01$), the error bars are ± SD (standard deviation).

Figure 4. Residual activity (%) of POD following HPP treatments and heat treatments.



Source: Own elaboration.

A 2-way ANOVA was performed (log-transformed data), rows = difference between processes ($p < 0.01$), columns = difference between measures ($p < 0.01$), the error bars are ± SD (standard deviation).

The results of the statistical analysis allow us to state that the type of treatment to which the strawberry nectar has been subjected is significant in the inhibition of POD. HPP 400, 500 and 600 MPa resulted in a RA of 21, 14 and 4% respectively. TT resulted in a RA of 12%. Albeit there was no significant difference between processes. On the other hand, the RA of PPO of samples HPP 400, 500 and 600 MPa was 102, 52 and 44% with TT results in a RA of 50%. As expected, PPO activity gradually decreased with increasing pressure levels. In this case there was a significant difference between processes. The literature suggests that an HPP treatment of 600 MPa for 25 min (at room temperature, 25 ° C) on strawberry pulp inhibits PPO by 51.5% (Cao X. et al., 2011). There is a 35% inhibition of PPO on strawberry puree following treatment at 30 ° C, 600 MPa for 5 minutes (Tinello F. and Lante A., 2018). Other studies on strawberry puree show that HPP treatments at 400 MPa, 500 MPa, and 600 MPa for 3 minutes at 20 ° C, resulted in an inhibition of PPO activity of 11%, 12%, and 30% respectively (Aaby K. Et al., 2018). As for the heat treatments, it was shown that 85 ° C for 2 min inhibited PPO activity by 25% compared to the control (Aaby K et al., 2018). Studies on strawberry extract state that the enzyme is almost completely inactivated after 10 min at 65 ° C (Dalmadi I.et al., 2006). A temperature above 80 ° C is required to ensure the inactivation of PPO in fruit juices (Terefe et al., 201The results obtained on the strawberry nectar

object of this study reveal a greater inactivation than the results reported in the literature. However, it should be noted that the comparison is made with studies conducted on a matrix other than the one in question (purees, extracts, or juices), as no study has been carried out on strawberry nectar. The stability of enzymes and their susceptibility to inactivation by high pressure or other treatment depends on intrinsic factors such as the source of the enzyme, the pH, and the composition of the matrix in which it is present: the same types of enzymes from different sources differ remarkably in their stability towards pressure inactivation. The variation in reported inactivation kinetic parameters of the same types of enzymes from similar sources in the literature can be attributed to differences in fruit variety of interest and growth conditions (Terefe NS et al., 2014). The effectiveness of HPP in inactivating PPO is also associated with treatment conditions such as pressure, time, and temperature (Tinello F. and Lante A., 2018). In general, an enzyme is more stable in intact tissue or in a homogenate where it is protected from the presence of other materials such as proteins and carbohydrates than in its purified form (Whitaker, 1972). However, activation of latent forms of the enzyme can also occur during treatment. It is also possible that treatments (including TTs) can cause an increase in the release of membrane-bound PPOs, counteracting the inactivating effect of the treatment itself (Terefe NS et al., 2014).

Nectar contains citric acid as an ingredient which has also been reported to effectively inhibit PPO. Its inhibitory effect is due to the chelation of copper located in the active site of PPO and the lowering of the pH (Holzwarth M. et al., 2013). Therefore, it is possible to attribute a small part of the inhibition to this compound added in the formulation of the product before the treatments.

Figure 5. *Closeness of five quality markers for treatment vs control measured on Day 1 (log-transformed data)*

Figure 5.1

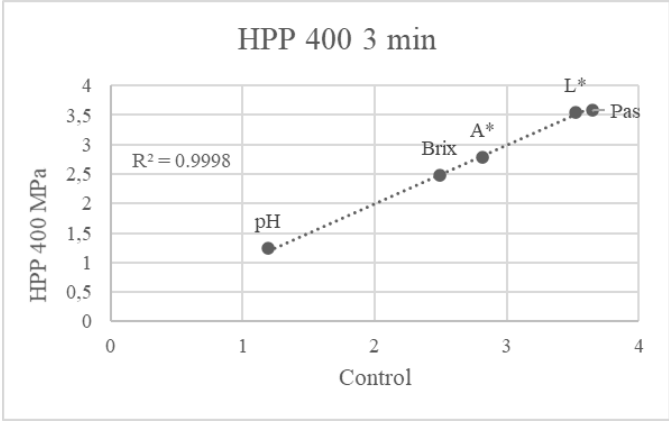


Figure 5.2

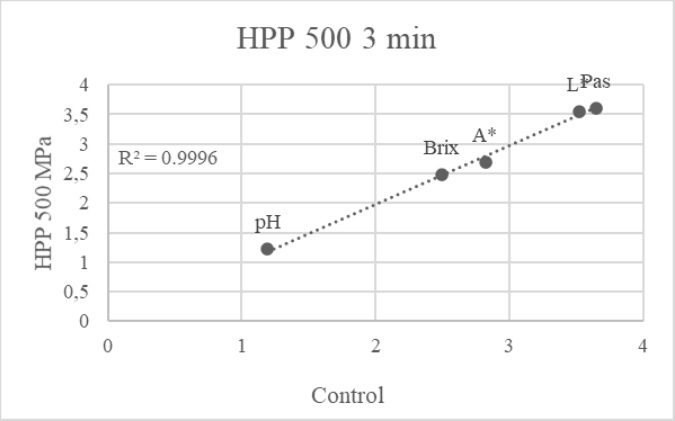


Figure 5.3

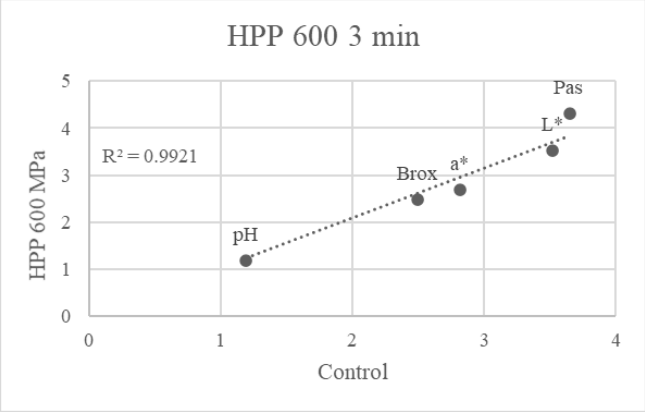
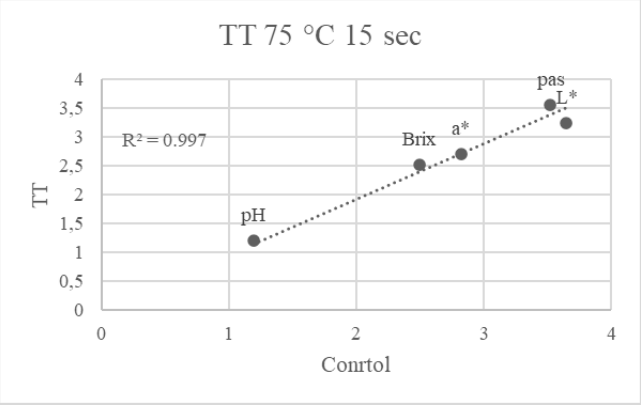


Figure 5.4



Source: Own elaboration

Figure 6. *Closeness of five quality markers for treatment vs control measured on Day 21 (log-transformed data)*

Figure 6.1

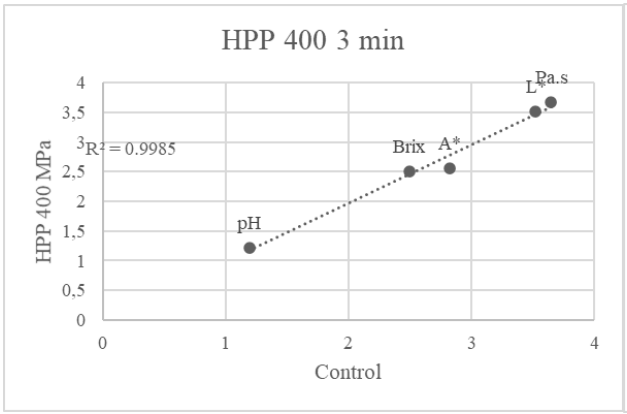


Figure 6.2

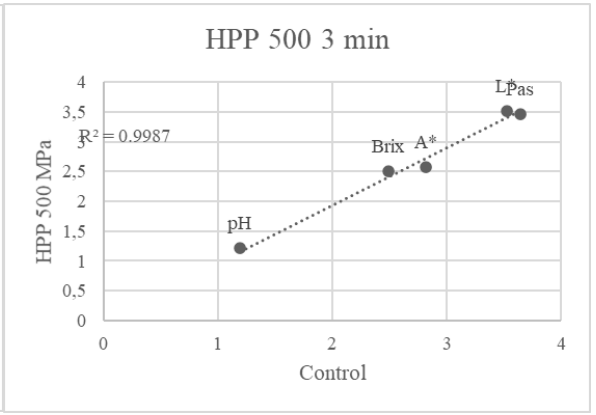


Figure 6.3

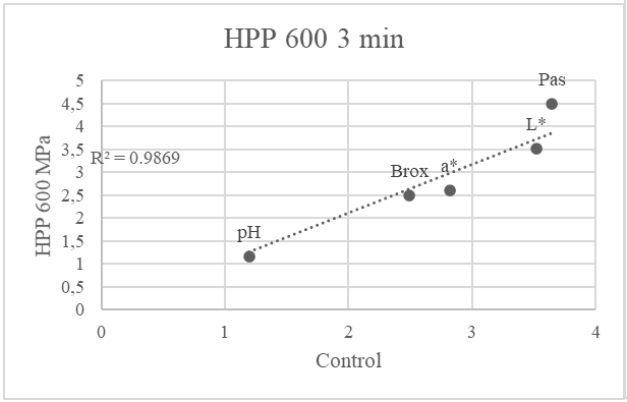
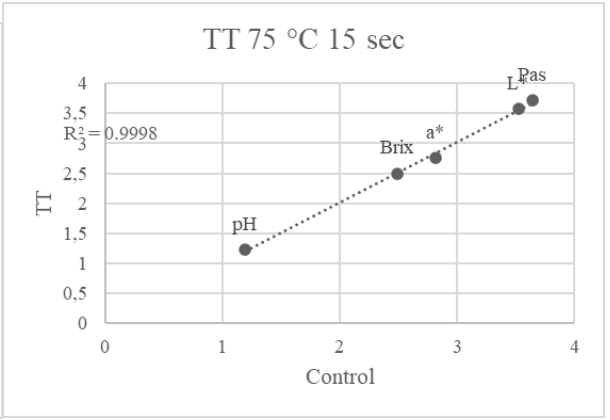


Figure 6.4



Source: Own elaboration.

At Day 1 the process that was most positively correlated with the control was HPP 400 MPa and 3 minutes (R^2 = 0.9998) and at Day 21 the process that was most positively correlated with the control was TT 75 °C 15 sec (R^2 = 0.9998). This result is likely due to the reduced enzyme activity in TT samples that lead to colour stability and the apparent viscosity which remained relatively stable in comparison to the control sample.

3.6. Conclusions

On day 1, values for the 5 quality indicators for samples treated by HPP 400 MPa more closely approximated those for the control sample, however, after 21 days, it appears that TT was the most effective treatment for maintaining quality attributes through shelf life. The greatest difference observed among those samples treated by TT and HPP was apparent viscosity. However, the rheological behaviour of the samples remained stable during the shelf life. PPO activity was reduced after all treatments, with the lowest RA after HPP 600 MPa 3 mins, which resulted in an inactivation of 64.63%. For POD, however, the most effective treatment was also HPP 600 MPa 3 min with inactivation of 95%. In the samples subjected to high pressures, the value of ΔE tends to increase during the shelf life up to, on day 21, values greater than 3 which results in a noticeable colour difference compared to the control. In conclusion, it would be of interest to investigate this aspect further by subjecting the samples to stress tests or longer shelf-life studies and evaluating a wider range of quality attributes. Furthermore, the changes in viscosity after HPP treatment should also be studied further. It is suggested that application of HPP could be considered for marketing purposes as the TT is the method most applied within the fruit juice industry nowadays.

Conflict of interest

The Authors declare that there are no conflicts of interest

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4. Manuscript 3: Colour kinetics of strawberry nectars treated by high pressure processing and thermal treatment

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4.1. Abstract

This study determined how thermal treatment (TT) and high-pressure processing (HPP) affect strawberry nectar colour stability over time. HPP was applied at three different pressure levels: 300, 450 and 600 MPa and, the colour dynamics and quality variations were examined during a six-week storage period at 4°C.

To evaluate the impact of processing techniques on product quality, colour parameters (L*, a*, b*, ΔE, AF, and BI) and total soluble solids (TSS) were tracked. To assess the influence of processing methods on quality, colour parameters (L*, a*, b*, ΔE, AF, and BI) and total soluble solids were monitored. For the first time, this work provides an in-depth comparison of the measurement reliability, kinetics of degradation, and colour stability of strawberry nectars submitted to HPP versus heat processing.

The results indicated that while TT always showed better colour stability for a wide range of parameters, HPP at 600 MPa had the potential to replace thermal treatment as a non-thermal alternative in order to maintain colour quality in strawberry nectar. Other quality aspects, energy aspects and specific product requirements would also drive decision-making between the two technologies.

Keywords: Thermal processing; high-pressure processing; colour stability; polyphenol oxidase; pectin methylesterase; kinetics

4.2. Introduction

Colour is an important quality attribute of strawberry products and plays a major role in consumer acceptance and perceived freshness. The complex composition of strawberry nectars, however, makes them very susceptible to colour degradation during processing and storage due to the constant threat of enzymatic browning. In general, due to the growing consumer demand for minimally processed fruit products, the interest is focused on novel processing technologies which can retain the vivid red colour of strawberry nectars while guaranteeing microbiological safety and increasing shelf-life. HPP is among the most promising non-thermal technologies used so far to preserve fruit juices and nectars. Unlike traditional thermal pasteurization, HPP may inactivate microorganisms and enzymes with minimal alteration of colour, flavour, and nutritional compounds. However, in these complex systems, such as strawberry nectars, the impact of HPP on colour stability during storage, in particular compared with Thermal Treatments, is not well understood. The changes in colour of strawberry products are mainly due to some chemical reactions and enzymatic activities, especially those catalyzed by PPO and POD. The kinetics of such colour changes may yield useful information with regard to the mechanisms of degradation and allow prediction of colour stability as a function of time. It is very important to understand the colour kinetics due to different processing methods for the optimization of treatment parameters and storage conditions in relation to maximal colour retention. Several measurement systems are used for a complete evaluation of the changes in colour. CIE Lab* colour space gives objective measures of Lightness (L*), red-green axis (a*), and blue-yellow axis (b*).

The Colour AF is an example of a derived metric for consumer acceptance, but BI can quantify colour changes caused by enzymatic and non-enzymatic browning. ΔE allows for overall colour change comparisons across samples or over time.

However, their reliability and sensitivity may vary according to the nature and characteristics of strawberry nectars and their various processing methods. For instance, Gössinger et al. (2009) found that AF was particularly successful in predicting consumer acceptance of strawberry nectars, with AF values above 0.7 indicating excellent quality.

4.3. Objectives

This work also compared the colour kinetics of strawberry nectars subjected to different levels of pressure (300, 450 and 600 MPa) against those conventionally treated.

Colour parameters (L*, a*, b*, ΔE, AF and BI) together with quality parameters such as Total Soluble Solids-TSS-will be followed up for a period of 6 weeks during storage at 4°C. Importantly, this work has been the first comparative study of reliability and sensitivity of different colour measurement techniques for strawberry nectars. This study analyses the consistency of different colour parameters along with their predictive power for various processing conditions and storage times in order to find the most robust and informative colour measurements for quality control and shelf-life prediction in strawberry nectar production. The work comparatively approached the colour stability, degradation kinetics, and reliability of measurements using HPP and TTs to provide comprehensive information with a view toward driving the development of optimized processing strategies and quality assessment methodologies that produce high-quality strawberry nectars possessing improved colour retention.

4.4. Materials and Methods

Sucrose, water, strawberry purée, and citric acid were used in the elaboration of strawberry nectar. Citric acid was purchased from Sigma-Aldrich® with more than 99.5% purity. Strawberry purée came from Sicoly®, a French Company manufacturing high-quality semi-finished product, made from the "Senga Sengana" cultivar. Strawberries had been picked, pureed, and frozen in 2021 and stored at -18°C by strict cold chain.

The specifications for the nectar and purée were laid down to harmonize parameters such as dry substance content, proportion of fruit, acidity, and pH. Quantitative composition was provided for 5L of strawberry nectar with formulae for each of its constituents in arriving at the actual quantity needed.

Preparation consisted of assembling all the ingredients in large stainless-steel containers and homogenizing by means of an electric mixer. The nectar, after formulation, was aliquoted to 50 ml aseptic pouches. Samples were stored at 4°C throughout the study period with weekly removals for analysis. This approach has ensured standardized and controlled preparation of strawberry nectar for further analyses and treatment protocols. Treatment was prepared according to what was described in table 1.

Sample Preparation and Storage

Table 1. Stabilization treatments examined

Treatment	Strawberry Variety	Conditions	Production facility
Untreated (Control)	Senga sengana	No treatment after formulation	UNIPR
TT	Senga sengana	TT: 80 C, 5 minutes	UNIPR
HPP	Senga sengana	HPP 300 MPa, 3 minutes	HPP Italia
HPP	Senga sengana	HPP 300 MPa, 3 minutes	HPP Italia
HPP	Senga sengana	HPP 300 MPa, 3 minutes	HPP Italia

Source: Own elaboration

All samples were homogenized immediately after production and stored at 4°C for a period of 6 week.

Sampling and Analysis Schedule

Analyses were conducted at 7-day intervals over the 6-week storage period. For each sampling point, 3 samples were removed from storage, thoroughly mixed, and analysed within 24 hours.

Physicochemical Analysis

– *pH Measurement*

pH was measured using a Portamess® 911 pH meter (Knick Elektronische Messgeräte GmbH & Co. KG) equipped with a pH-sensitive glass electrode.

– *Total Soluble Solids*

TSS (°Brix) were determined using a Proster Refractometer (model 101 ATC). Samples were applied to the prism surface using a Pasteur pipette, ensuring uniform distribution and removal of air bubbles.

– *Colour Analysis*

Colour parameters were measured using a Konica Minolta® CM-2500d Spectrophotometer and analysed with SpectraMagicTMNX software. The following

colour attributes were calculated based on CIE Lab* Colour Space, L*: Lightness (0 = black, 100 = white) a*: Green-red component b*: Blue-yellow components.

Table 2. Colour parameters and their methods of measure

Colour Parameter	Method of measure	Reference
Chroma (C*)	$C^* = \sqrt{(a^{*\wedge}2 + b^{*\wedge}2)}$	Itle et al., (2009)
ΔE	$\Delta E = \sqrt{[(\Delta L^*)^{\wedge}2 + (\Delta a^*)^{\wedge}2 + (\Delta b^*)^{\wedge}2]}$ The colour difference in the results can be interpreted as follows: – $0 < \Delta E^* < 1$—the difference in colour is visually nonrecognizable by a standard observer; – $1 < \Delta E^* < 2$—the difference is visually recognizable only by an experienced observer; – $2 < \Delta E^* < 3.5$—the difference can be visually recognized by an inexperienced observer; – $3.5 < \Delta E^* < 5$—every observer can easily see the difference; – $\Delta E^* > 5$—an observer recognizes two different colours	(Pielak et al., 2024)
AF	$AF = (a^* / h°)$ *Nectars with AF > 0.7 were considered excellent, while those with AF < 0.4 were deemed unacceptable*	(Gössinger et al., 2009).
Browing Index	$x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$ $BI = [100 * (x - 0.31)] / 0.17$	(Lunadei et al., 2011).

Source: Own elaboration

Kinetic Models and Statistical Methods

– *Zero-Order and First-Order Reaction Kinetics*

This study focuses on the kinetic analysis of zero-order and first-order reactions. The aspects of both are described in table 3 below.

Table 3. Derivation of parameter values in zero order and first order models

Aspect	Zero-order reaction	First-order reaction
Rate law	Rate = k	Rate = k[A]
Integrated rate law	$[A] = [A]_0 - kt$	$\ln[A] = \ln[A]_0 - kt$
Plot for linear fit	[A] vs. t	ln[A] vs. t
Slope	Using Excel's SLOPE function: $m = (\Sigma (x_i - \bar{x})(y_i - \bar{y})) / (\Sigma (x_i - \bar{x})^2)$	Same as Zero-Order
Units of k	concentration/time	$time^{-1}$
Half-life	$_{1/2} = [A]_0 / (2k)$	$t_{1/2} = 0.693 / k$
R^2 Calculation	1 - (SSres / SStot)	Same as Zero-Orde
Standard Error (SE)	$SE = s / \sqrt{n}$	Same as Zero-Order
RMSE	$RMSE = \sqrt{[(\Sigma(yi - f(xi))^2) / n]}$	Same as Zero-Order

Source: Own elaboration

Where:

- [A] is the concentration at time t
- $[A]_0$ is the initial concentration
- k is the rate constant
- t is time
- m is the slope
- x_i and y_i are individual data points
- $\bar{x}$ and $\bar{y}$ are means
- SSres is the sum of squared residuals
- SStot is the total sum of squares
- RMSE is the root mean square error
- s is the standard deviation
- n is the sample size
- yi are observed values
- f(xi) are predicted values

In a zero-order reaction, the rate of change of concentration is constant and independent of the reactant concentration. A first-order reaction depends on the concentration of only one reactant. The rate of reaction changes linearly with changes in this reactant's concentration. Understanding zero-order and first-order kinetics provides insight into chemical reaction mechanisms and their sensitivity to reactant concentrations. Using measures like slope, R^2, SE, and RMSE, researchers may accurately evaluate model correctness/reliability and anticipate behaviour under different scenarios.

4.5. Results and discussion

Parameter a*

Table 4: Zero order a*

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.21	-0.14	-0.14	-0.14	-0.11
k (Day^{-1})	0.15	0.18	0.16	0.21	0.06
r2	0.84	0.81	0.86	0.73	0.59
Media	12.35	13.96	13.40	13.52	16.13
RMSE	1.57	1.15	0.72	1.59	1.55
St.dev	3.22	1.73	1.91	1.80	2.16
SE	1.32	0.71	0.78	0.73	0.88

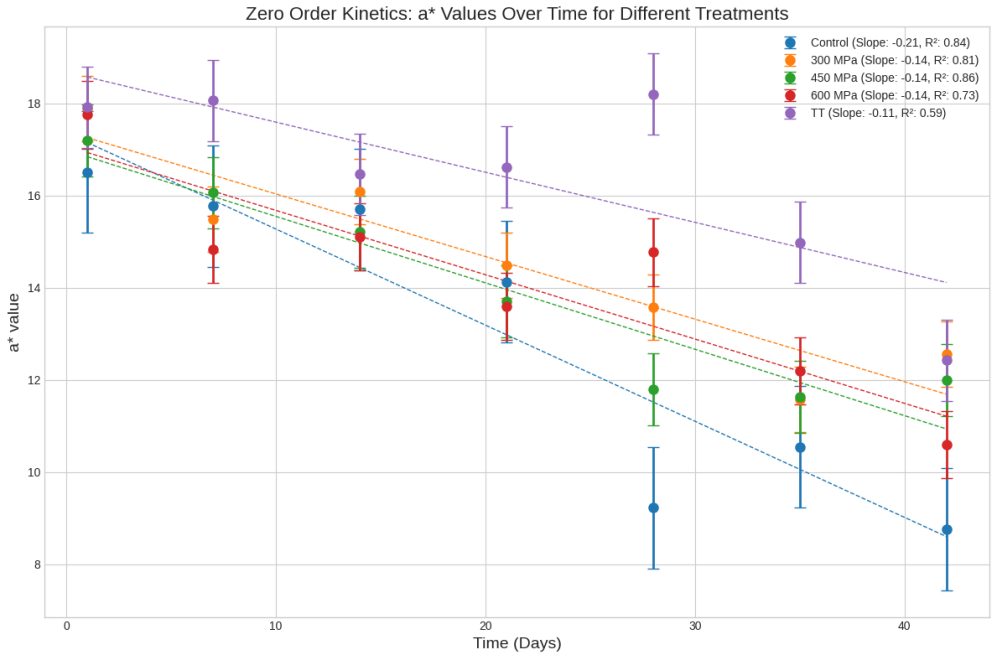
Source: Own elaboration

Table 5: First order measured LN(a*)

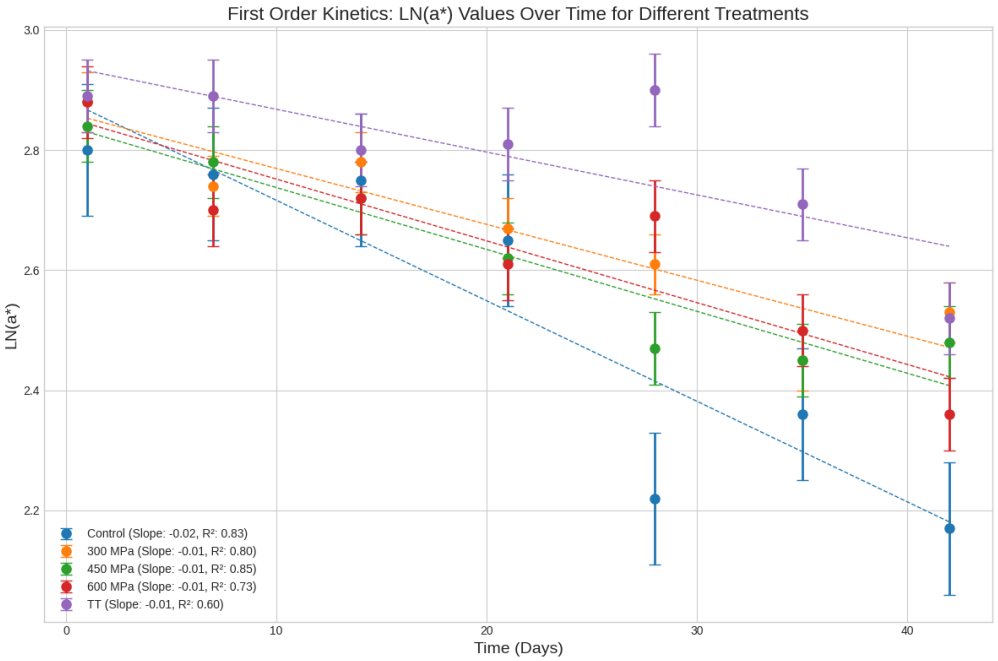
	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.02	-0,01	-0.01	-0.01	-0.01
k (Day^{-1})	0.01	0.01	0.01	0.01	0.00
r2	0.83	0.80	0.85	0.73	0.60
Media	2,48	2.63	2.59	2.60	2.77
RMSE	0.17	0.06	0.06	0.07	0.11
St.dev	0.27	0.13	0.14	0.14	0.14
SE	0.11	0.05	0.06	0.06	0.06

Source: Own elaboration

Figure 1 and Figure 2. Zero order and First order assessment for colour parameter a*

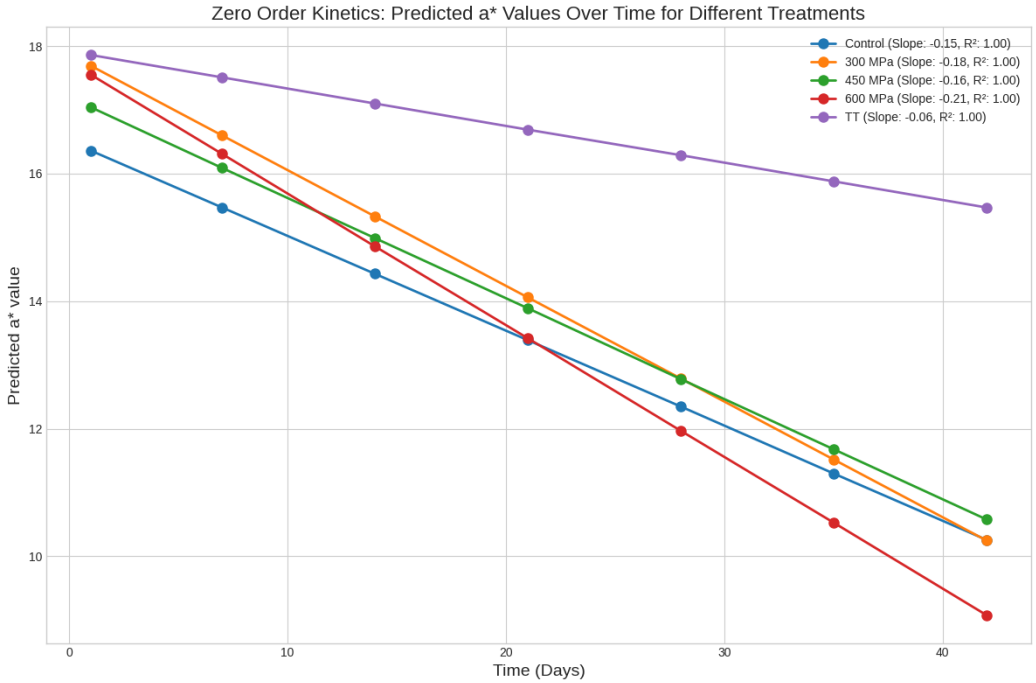


Source: Own elaboration

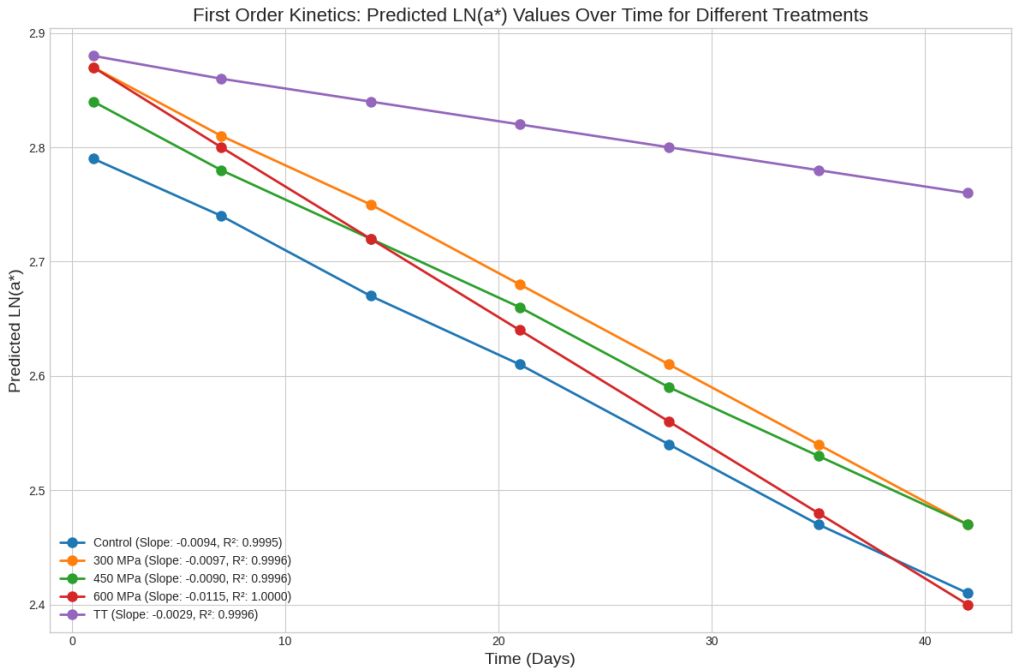


Source: Own elaboration

Figure 1.1 and Figure 2.1. Observed s predicted values for Zero order model and First order model for a* and Ln a*, respectively.



Source: Own elaboration



Source: Own elaboration

Tables 4 and 5 show the zero order and first-order kinetic models of colour parameter a* of strawberry nectar as a function of various treatments applied in the course of 42 days storage.

As for the zero-order model (Table 4), R^2 values are the following: control, R^2 = 0.84; 300 MPa, R^2 = 0.81; 450 MPa, R^2 = 0.86; 600 MPa, R^2 = 0.73; and TT, R^2 = 0.59. A treatment of 450 MPa gave the best fit since most variability in the data was explained in it, while TT gave the poorest fit.

Also, the first-order model (Table 5) showed R^2 values for all treatments: Control (R^2 = 0.83), 300 MPa (R^2 = 0.80), 450 MPa (R^2 = 0.85), 600 MPa (R^2 = 0.73) and TT (R^2 = 0.60). Again, the 450 MPa treatment presented the best fit and TT the poorest. Figure 1 and Figure 2 present the results for zero order and first order evaluation for colour parameter a*.

The R^2 values indicate no improvement of fit with First order versus zero order models so the untransformed data models are used for the parameter estimates and prediction purposes discussed in more detail below.

Calculations of rate constants (k) in the zero-order model gave evidence that the lowest decline rate, k = 0.06 Day^{-1} was for TT treatment, while similar decline rate constant, k = 0.16 Day^{-1} was for both treatments of 300 MPa and 450 MPa, followed by Control, k = 0.15 Day^{-1} and the highest decline rate constant was for 600 MPa, k = 0.21 Day^{-1} which suggested that colour red in strawberry nectar was best retained during storage by TT.

These differences between the rate constant k and the negative slope of the change in concentration could be due to various inherent factors in the reaction kinetics. Further, there is a variation in R^2 values ranging from 0.59 to 0.86 across the treatments, which indeed may suggest that the system is adequately complex not to allow a simple interpretation in every respect. Besides this, the method of calculation and unit conversions might be the cause for alteration in the relationship between k and slope. Besides, the variability of conditions in various experiments and the effects of treatments increase this gap. These results show how important the use of multivariable is in the interpretation of kinetics for complex systems. These results evidence that, although all treatments produced a loss of red colour over time, TT provided the best behaviour of the colour, but the model is able to represent just 60% of the variability. HPP treatments, especially at 450 MPa, have been relatively effective in maintaining redness; however, it presents higher decay rates compared to TT.

All treatments follow the downtrend of a* values in the storage days, representing the loss of red colour during storage. From the data provided for a* values of strawberry nectar submitted to different kinds of treatment, it was shown that, over a period of 42 days in storage, it is possible to infer that all treatments follow the downtrend of a* values in the storage days-meaning, the loss of red colour during storage. Correspondingly, it reflects the normal degradation of colour in strawberry products, as reported by Murray et al., 2024, with regard to colour stability in strawberry nectars.

Parameter L*

Table 6. Zero order model (L*)

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
k (Day-$^{1)}$	-0.02	-0.10	-0.05	-0.08	-0.05
Slope	0.21	0.21	0.22	0.20	0.14
r2	0.81	0.84	0.71	0.92	0.98
Media	39.77	40.23	40.58	39.34	39.28
RMSE	3.87	2.42	3.84	2.33	1.81
St.dev	3.98	3.41	4.11	3.21	2.25
SE	1.63	1.39	1.68	1.31	0.92

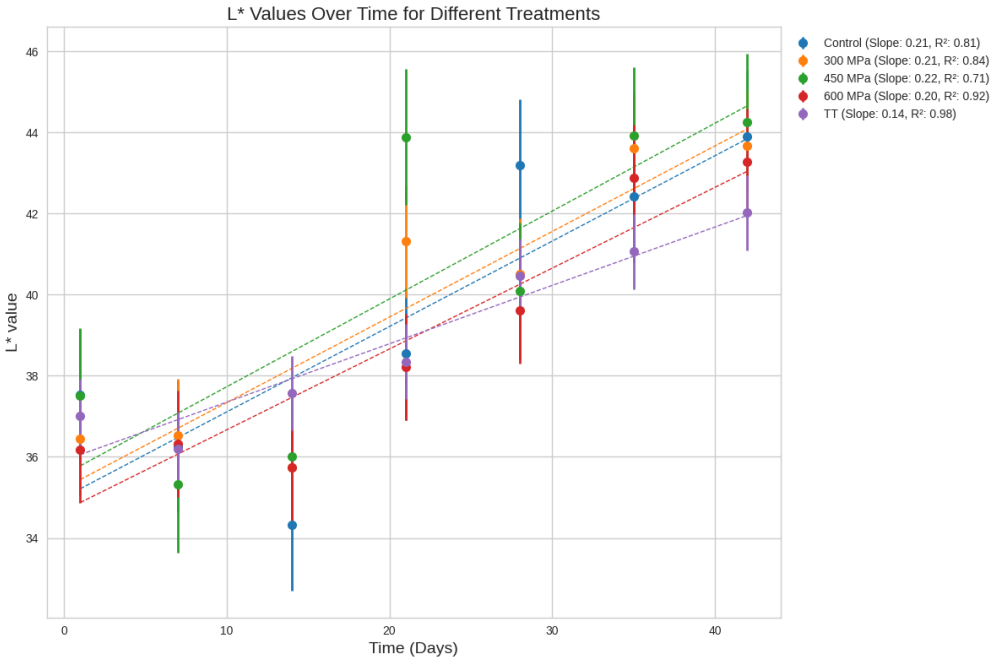
Source: Own elaboration

Table 7. First order model LN(L*)

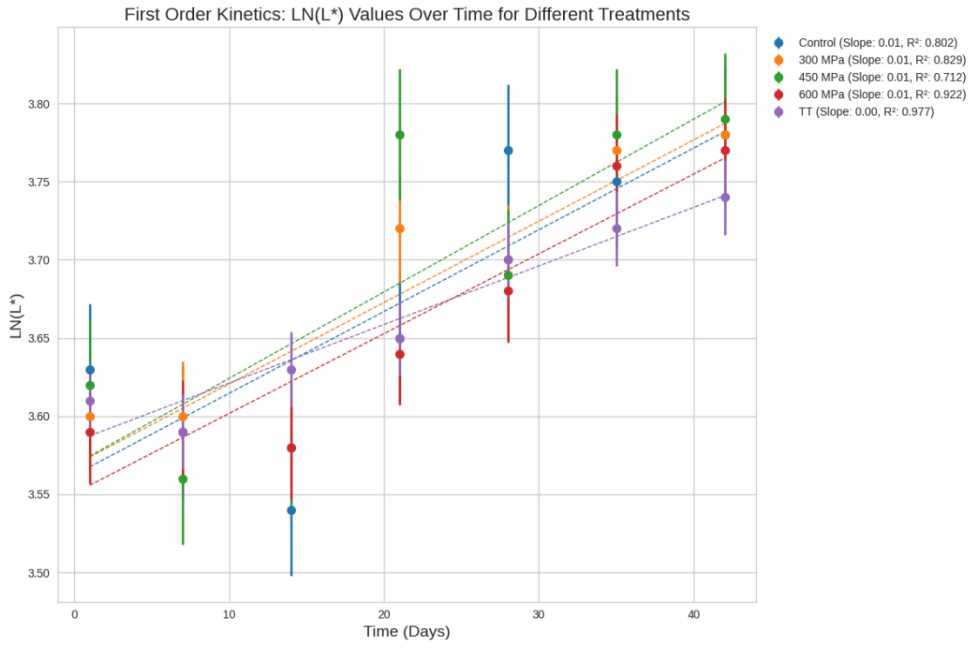
	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
k (Day^{-1})	0.00	0.00	0.00	0.00	0.00
Slope	0.01	0.01	0.01	0.01	0.00
r2	0.80	0.83	0.71	0.92	0.98
Media	3.68	3.69	3.70	3.67	3.67
RMSE	0.11	0.10	0.11	0.09	0.07
St.dev	0.10	0.09	0.10	0.08	0.06
SE	0.04	0.04	0.04	0.03	0.02

Source: Own elaboration

Figure 3 and Figure 4. Zero order and First order assessment for colour parameter L*

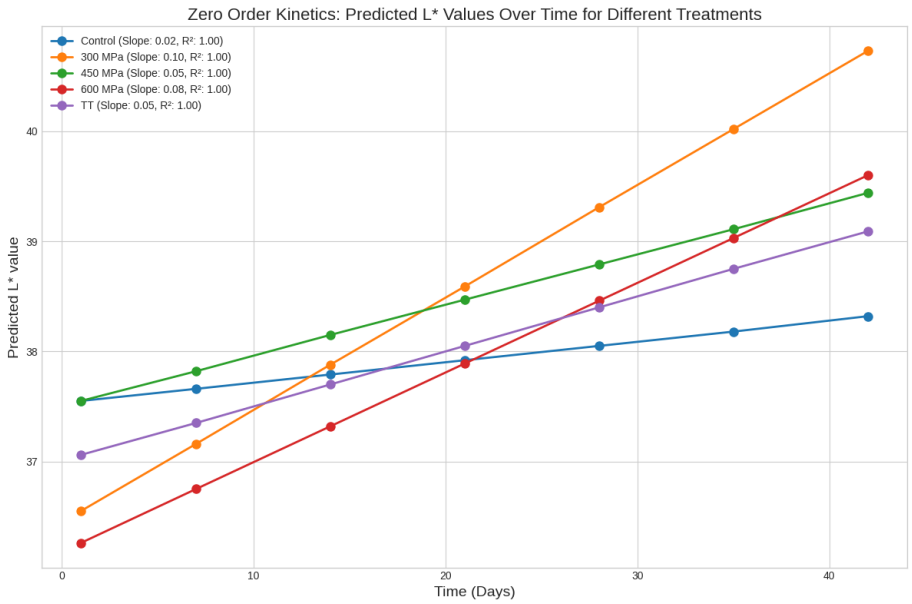


Source: Own elaboration



Source: Own elaboration

Figure 3.1. Observed versus predicted values for zero order model for L*



Source: Own elaboration

Tables 6 and 7 present the zero-order and first-order kinetic models for the colour parameter L* in strawberry nectar treated with different processing methods over 42 days of storage. For the zero-order model (Table 6), Control (R^2 = 0.81), 300 MPa (R^2 = 0.84), 450 MPa (R^2 = 0.71), 600 MPa (R^2 = 0.92), and TT (R^2 = 0.98).

The first-order model (Table 7) showed similar trends in R^2 values, with all treatments Control (R^2 = 0.80), 300 MPa (R^2 = 0.83), 450 MPa (R^2 = 0.71), 600 MPa (R^2 = 0.92), and TT (R^2 = 0.98). The logarithmic transformation for first order kinetics did not improve R^2 for the treatments so, for the purpose of parameter estimation and prediction, the estimates will be taken using the zero order models. Among these, the zero-order models showed the best fit of the TT, which was closely followed by the 600 MPa treatment. The latter models presently afford reliable predictions in respect to L* changes over time.

By considering the k values obtained in the zero-order model (Figure 3), Control had the lowest rate of change in lightness, k = -0.02 Day^{-1}, followed by 450 MPa and TT that had equal values of k = -0.05 Day^{-1}, 600 MPa at k = -0.08 Day^{-1}, and 300 MPa at k = -0.10 Day^{-1}. These results would, therefore, indicate that Control and TT were most effective in maintaining lightness values during storage. The slopes of the zero-order model refer to an increasing trend of L* during time for all treatments, showing TT with the smallest slope (0.14) and 450 MPa with the greatest slope (0.22). Similar trends could be observed in figure 4 using the first order model. Either model could be reliably used

for colour changes prediction of strawberry nectars except for the nectars treated by HPP 450 MPa. The upward trend in L* value suggests that the samples lighten with time of storage, which may be an indication of colour degradation. The findings support a recent study on the importance of initial processing conditions for colour stability of strawberry nectars by Murray et al., 2024. The results ensure that kinetic models are applicable in the prediction of colour changes during storage and were discussed by Lacey et al. (2023), where the quality assessment of strawberry nectar was done in different conditions of processing. Overall, although the increase of lightness, L* values against the time for all treatments, TT proved to be most effective in maintaining consistent lightness with the lowest rate of change and best model fit. HPP treatments at especially 600 MPa demonstrated some promise in the context of colour stability; however, the rates of change were higher than those of TT.

Table 8. Zero order (ΔE)

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	0.26	0.16	0.19	0.14	0.10
k (Day^{-1})	-0.24	-0.07	-0.21	-0.05	-0.06
r2	0.85	0.68	0.52	0.76	0.60
Media	6.13	5.05	6.06	4.27	3.32
RMSE	1.34	2.14	2.08	2.07	1.33
St.dev	3.71	2.85	3.21	2.70	1.98
SE	1.51	1.16	1.31	1.10	0.81

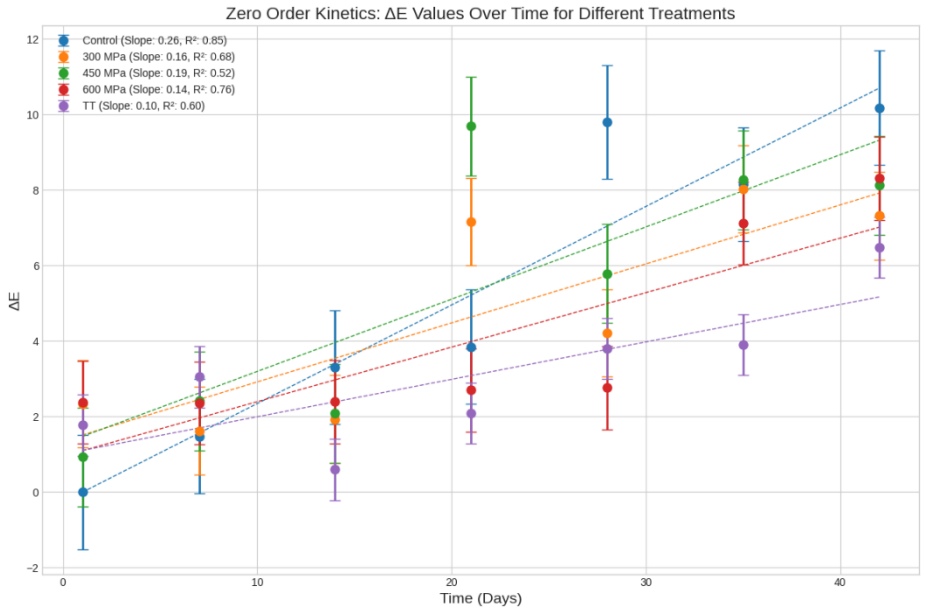
Source: Own elaboration

Table 9. First order measured LN (ΔE)

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	0.06	0.04	0.05	0.03	0.03
k (Day-^{1})	-0.05	-0.01	-0.02	-0.01	N.D.
r2	0.86	0.72	0.60	0.79	0.42
Media	1.61	1.44	1.64	1.30	0.98
RMSE	0.49	0.56	1.38	0.47	N.D.
St.dev	0.76	0.71	0.67	0.58	0.82
SE	0,31	0,29	0,27	0,24	0,34

Source: Own elaboration

Figure 5 and Figure 6. Zero order and First order assessment for colour parameter ΔE



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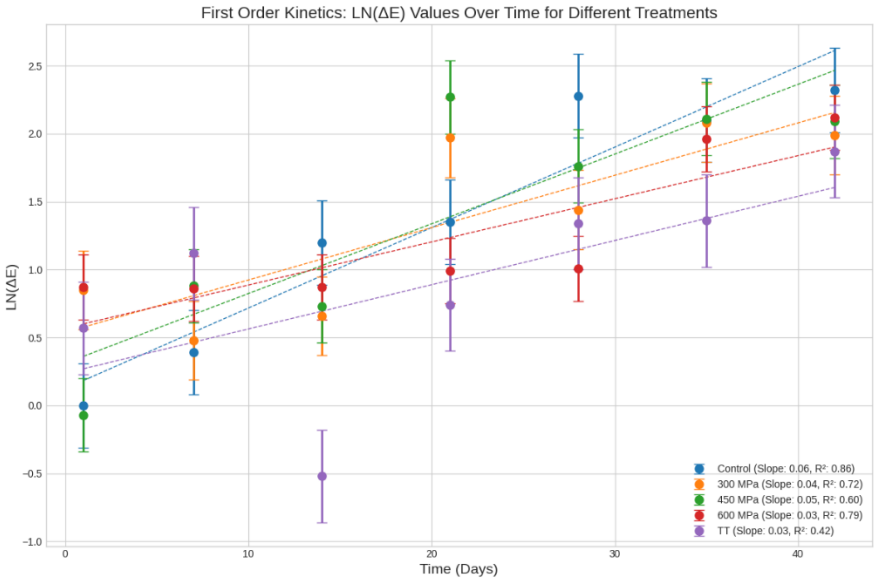
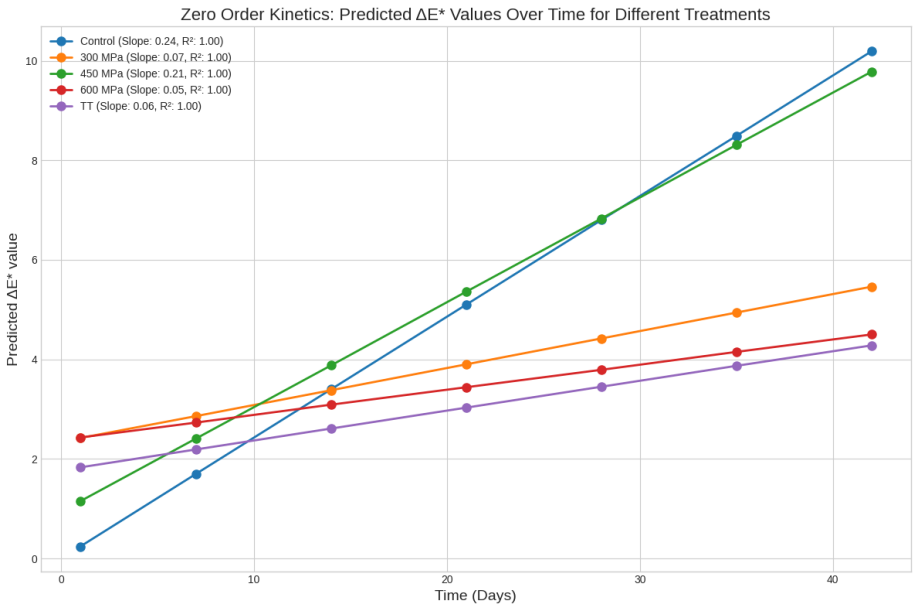


Source: Own elaboration

Figures 5.1. Observed versus predicted values for zero order for parameter ΔE



Source: Own elaboration

Parameter ΔE

Tables 8 and 9 present the zero-order and first-order kinetic models for the total colour difference parameter ΔE in strawberry nectar treated with different processing methods over 6 weeks of storage.

For the zero-order model (Table 8), the Control showed the highest R^2 value (0.85), indicating the best fit among all treatments. The 600 MPa treatment had the second-highest R^2 value (0.76), followed by 300 MPa (0.68), TT (0.60), and 450 MPa (0.52). The R^2 for the first order models did not improve the fit significantly so for the purpose of parameter estimation and prediction, the results from the untransformed zero order model will be used. (Table 9).

Examining the rate constants (k) in the zero-order model, the Control showed the highest rate of change in ΔE ($k = -0.24\ Day^{-1}$), while 600 MPa showed the lowest rate of change ($k = -0.05\ Day^{-1}$). The order of treatments from highest to lowest rate of change was: Control > 450 MPa > 300 MPa > TT > 600 MPa

The slopes in the zero-order model (figure 5) indicate an increasing trend in ΔE values over time for all treatments, with TT showing the smallest slope (0.10) and control the largest (0.26).

Mean ΔE values were highest for Control and 450 MPa treatments at 6.13 and 6.06, respectively, while that of TT had the lowest value of 3.32 from the zero-order model.

These results showed that there was colour stability to a lesser or higher degree in the different treatments, with more stable colour changes over time evinced by HPP treated at 600 MPa and the TT samples, while the greatest changes were exhibited by the Control and 450 MPa HPP treatment.

Parameter AF*

Table 10. Zero order (AF)

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.01	-0.01	-0.01	-0.01	0.00
k (Day^{-1})	0.01	0.00	0.01	0.00	0.00
r2	0.67	0.62	0.51	0.76	0.38
T1/2	52.36	76.26	56.09	63.90	69.73
Media	0.43	0.48	0.47	0.47	0.53
RMSE	0.10	0.07	0.09	0.06	0.05
St.dev	0.16	0.11	0.13	0.11	0.06
SE	0.07	0.05	0.05	0.04	0.02

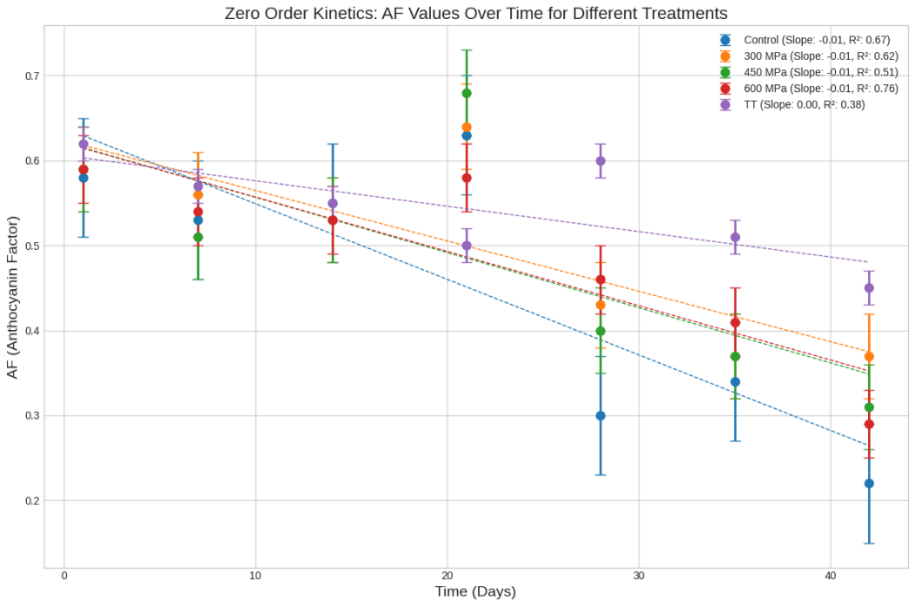
Source: Own elaboration

Table 11. First order LN (AF)

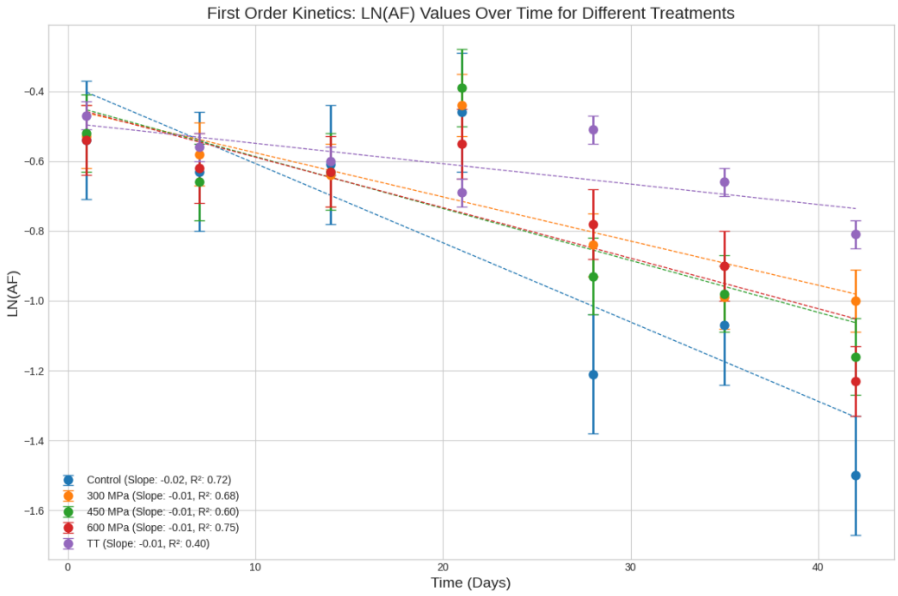
	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.02	-0.01	-0.01	-0.01	-0.01
k (Day^{-1})	0.01	0.01	0.01	0.01	0.01
r2	0.72	0.68	0.60	0.75	0.40
T1/2	63.53	103.93	74.81	88.02	103.48
Media	-0.91	-0.75	-0.79	-0.79	-0.64
RMSE	0.29	0.16	0.19	0.17	0.08
St.dev	0.41	0.23	0.28	0.25	0.11
SE	0.17	0.09	0.11	0.10	0.04

Source: Own elaboration

Figure 7 and Figure 8. Zero order and First order assessment for colour parameter AF

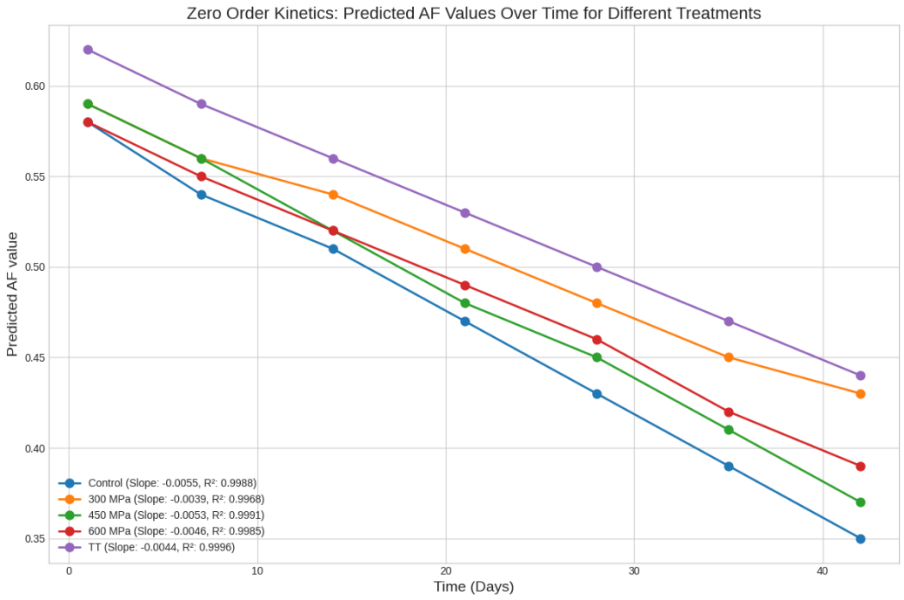


Source: Own elaboration



Source: Own elaboration

Figure 7.1 and Figure 8.1. Observed versus predicted values for Zero order and First order assessment for colour parameter AF



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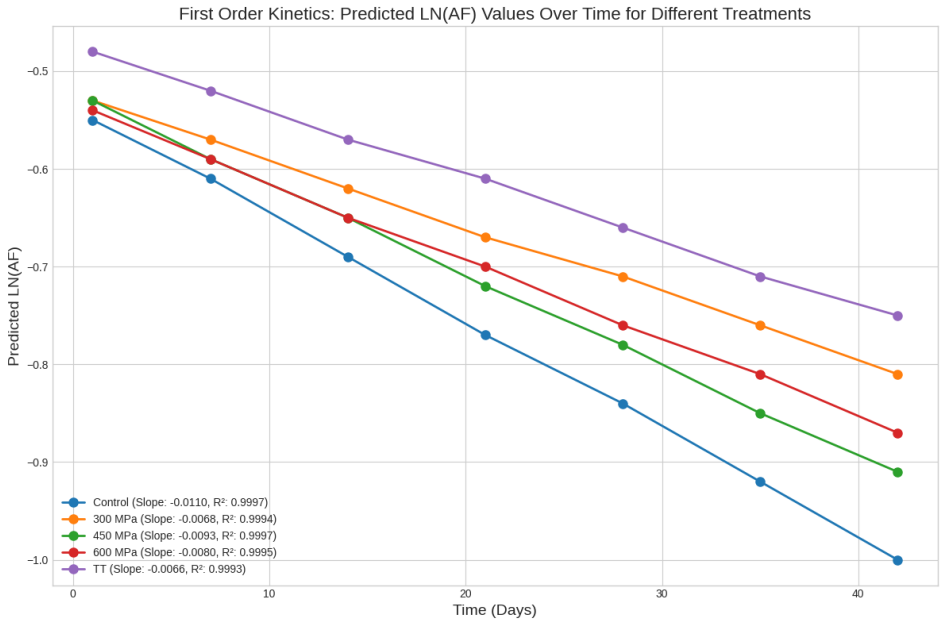


Source: Own elaboration

Tables 10 and 11 present the zero-order and first-order kinetic models for the AF in strawberry nectar treated with different processing methods over 42 days of storage.

Starting from the zero-order model, the 600 MPa treatment had the best description of all the treatments with the highest R2 value of 0.76. This was followed by the Control, R2 = 0.67; 300 MPa, R2 = 0.62; 450 MPa, R2 = 0.51, and TT, R2 = 0.38. The R^2 for the

first order models did not show significant improvements in fit so for the purposes of parameter estimation and predicting, the zero-order model using untransformed data will be used (Table 11, Figures 7 and 8).

Examining the rate constants (k) in the zero-order model, the Control and 450 MPa treatments showed the highest rate of change in AF ($k = 0.01\ Day^{-1}$), while 300 MPa, 600 MPa, and TT showed lower rates ($k = 0.00\ Day^{-1}$).

The slopes in Figure 8 for zero order and first order were all negative for all treatments, meaning a decreasing trend of AF values as a function of time. The slope for all treatments, with the exception of TT in the zero-order model, was -0.01; for TT it was 0.

The half-life (T1/2) values in the zero-order model ranged from 52.36 days for the Control to 76.26 days for the 300 MPa treatment.

The mean AF values in the zero-order model were highest for TT (0.53) and lowest for the Control (0.43).

These results show the different levels of stability of AF for the different treatments, and from the R^2 values, HPP at 600 MPa showed the most consistent changes in AF over time. TT maintained the highest mean AF value but gave the poorest fit to both models, suggesting more complex changes in AF over time for this treatment.

Parameter C*

Table 12. Zero order (C*)

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.21	-0.14	-0.14	-0.14	-0.13
k (Day^{-1})	0.17	0.23	0.18	0.26	0.04
r2	0.80	0.56	0.50	0.42	0.62
Media	14.12	15.96	15.40	15.40	18.72
RMSE	1.56	2.09	1.58	2.76	2.25
St.dev	3.25	1.85	2.20	1.94	2.73
SE	1.33	0.76	0.90	0.79	1.11

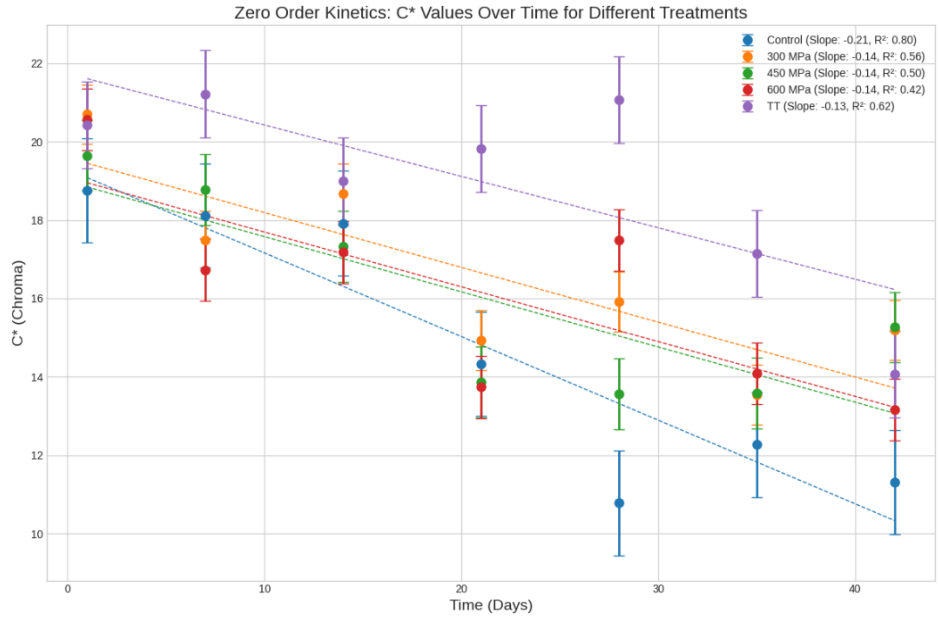
Source: Own elaboration

Table 13. First order (C*)

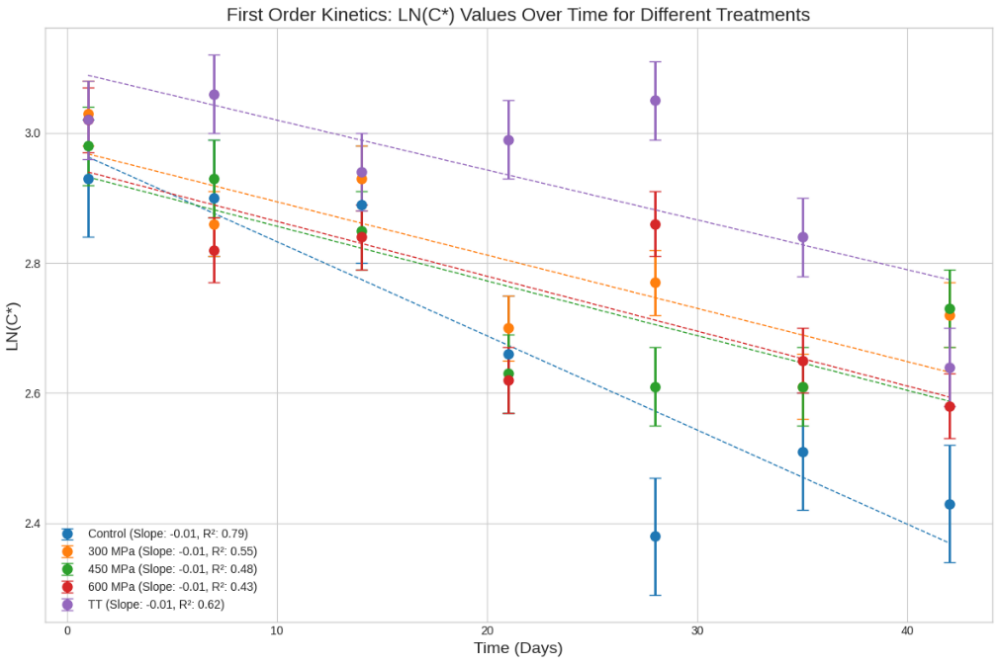
	Control	*300 MPa*	*450 MPa*	*600 MPa*	*TT*
Slope	-0.01	-0.01	-0.01	-0.01	-0.01
k (Day^{-1})	0.01	0.01	0.01	0.01	0.00
r2	0.79	0.55	0.48	0.43	0.62
Media	2.63	2.76	2.73	2.73	2.92
RMSE	0.14	0.09	0.10	0.12	0.13
St.dev	0.23	0.12	0.14	0.13	0.16
SE	0.09	0.05	0.06	0.05	0.06

Source: Own elaboration

Figure 9 and Figure 10. Zero order and First order assessment for colour parameter C*

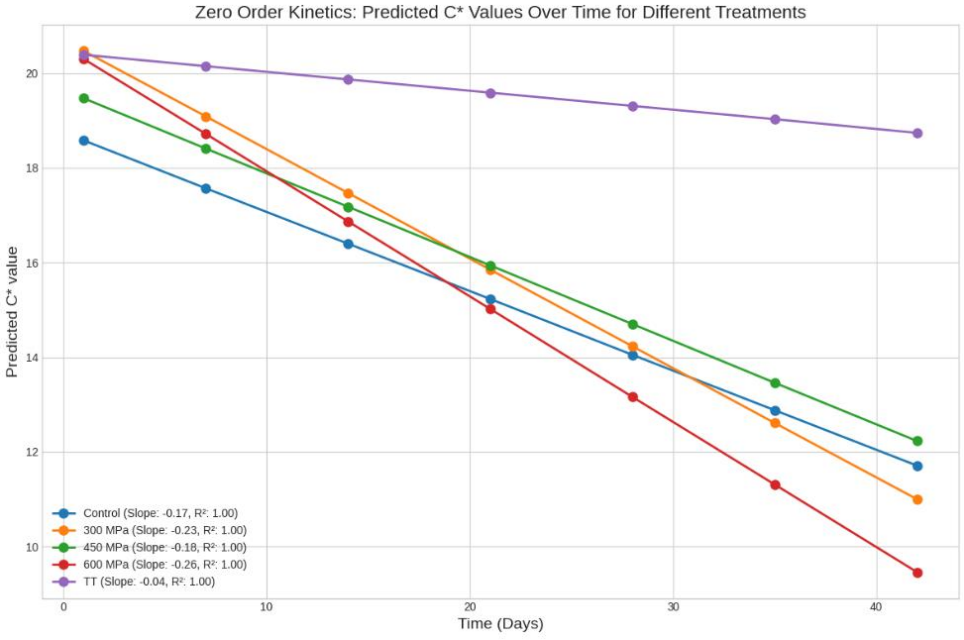


Source: Own elaboration

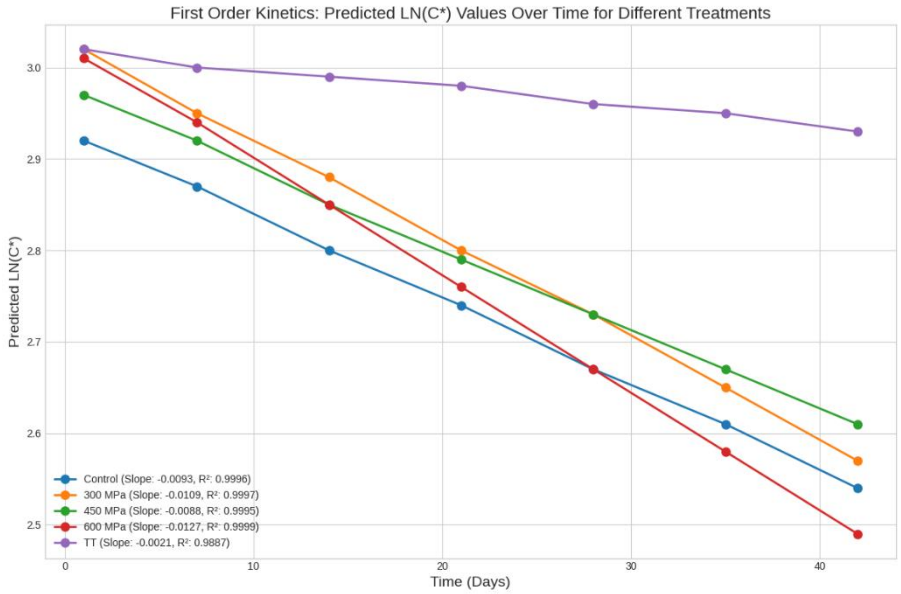


Source: Own elaboration

Figure 9.1 and Figure 10.1. Observed versus predicted values for Zero order and First order assessment for colour parameter C*



Source: Own elaboration



Source: Own elaboration

Table 12 and 13 and Figure 9 and Figure 10 present the results for the zero order and first order evaluation for the colour parameter C*.

Tables 12 and 13 present the zero-order and first-order kinetic models for the chroma (C*) in strawberry nectar treated with different processing methods over 42 days of storage. For the zero-order model (Table 12), the Control showed the highest R^2 value (0.80), indicating the best fit among all treatments. This was followed by TT (R^2 = 0.62), 300 MPa (R^2 = 0.56), 450 MPa (R^2 = 0.50), and 600 MPa (R^2 = 0.42). In the first-order model (Table 13), a similar trend was observed with the Control showing the highest R^2 value (0.79), followed by TT (R^2 = 0.62), 300 MPa (R^2 = 0.55), 450 MPa (R^2 = 0.48), and 600 MPa (R^2 = 0.43). The R^2 for the First order models were not significantly improved from those with the zero order models so for parameter estimation and prediction, the zero order models using untransformed data will be used.

Examining the rate constants (k) in the zero-order model, the 600 MPa treatment showed the highest rate of change in C* (k = 0.26 Day^{-1}), while TT showed the lowest rate of change (k = 0.04 Day^{-1}). The order of treatments from highest to lowest rate of change was: 600 MPa > 300 MPa > 450 MPa > Control > TT.

The slopes in the zero-order (Figure 9) model indicate a decreasing trend in C* values over time for all treatments, with TT showing the smallest slope (-0.13) and control the largest (-0.21).

In the zero-order model, the highest mean C* values belonged to TT with the value of 18.72 and the lowest to Control with 14.12.

The RMSE values were generally higher in zero order compared to the first order, with 600 MPa showing the highest RMSE value of 2.76 in zero order.

These results evidence that, in fact, there is a difference in chroma stability among the various treatments. From the R^2 values, it was the time course for Control that had varied most consistently, and TT maintained its highest mean C* value throughout the period of storage

Parameter TSS*

Table 14. Zero order (TSS*)

	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.11	-0.11	-0.12	-0.12	-0.11
k (Day^{-1})	0.05	0.06	0.06	0.06	0.04
r2	0.90	0.88	0.91	0.91	0.77
T1/2	112.49	105.98	94.65	95.34	141.28
Media	10.48	10.51	10.14	10.15	10.73
RMSE	1.09	1.13	1.18	1.13	1.43
St.dev	1.74	1.83	1.93	1.89	1.95
SE	0.71	0.75	0.79	0.77	0.80

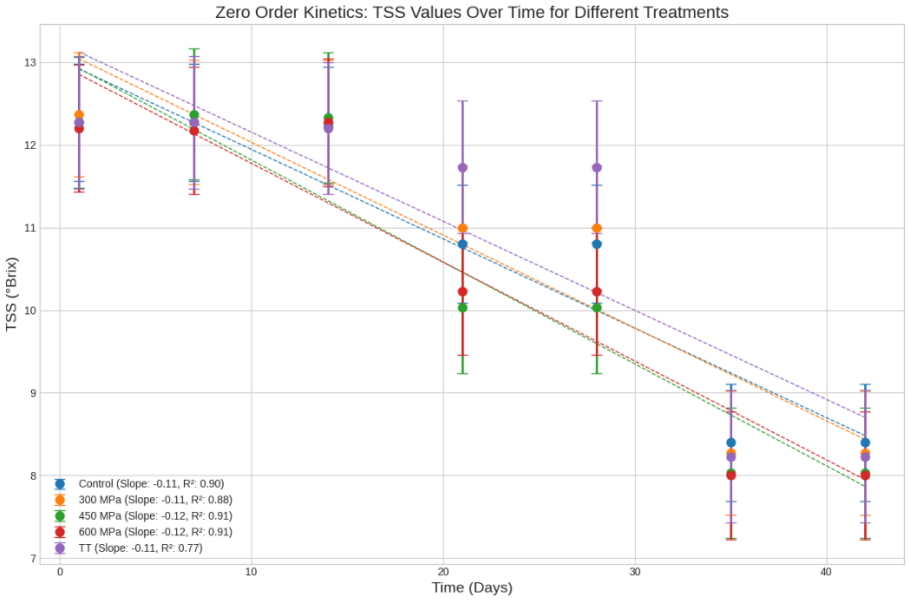
Source: Own elaboration

Table 15. First order (TSS*)

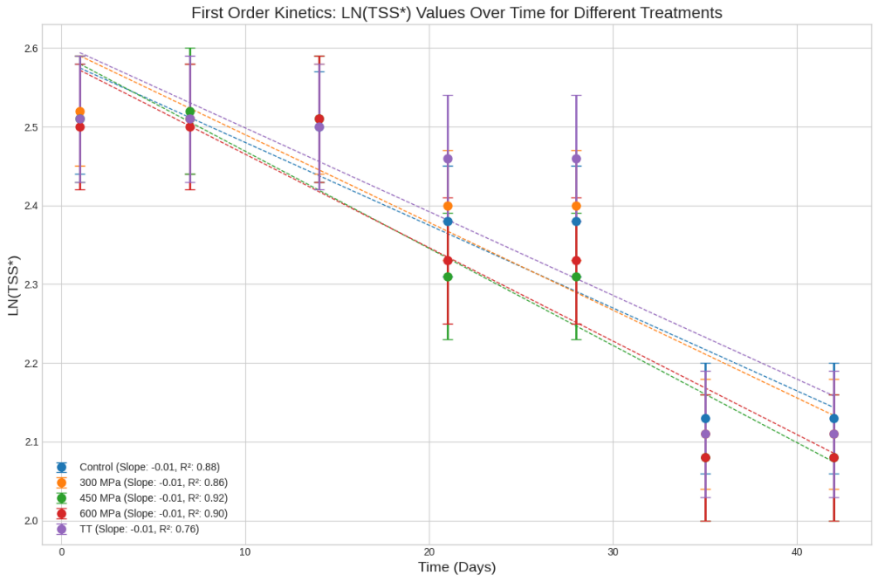
	Control	***300 MPa***	***450 MPa***	***600 MPa***	***TT***
Slope	-0.01	-0.01	-0.01	-0.01	-0.01
k (Day^{-1})	0.00	0.00	0.01	0.01	0.00
r2	0.88	0.86	0.92	0.90	0.76
T1/2	158.32	148.82	129.80	131.98	194.21
Media	2.34	2.34	2.30	2.30	2.36
RMSE	0.13	0.13	0.14	0.14	0.15
St.dev	0.17	0.18	0.19	0.19	0.19
SE	0.07	0.07	0.08	0.08	0.08

Source: Own elaboration

Figure 11 and Figure 12. Zero order and First order assessment for colour parameter TSS*

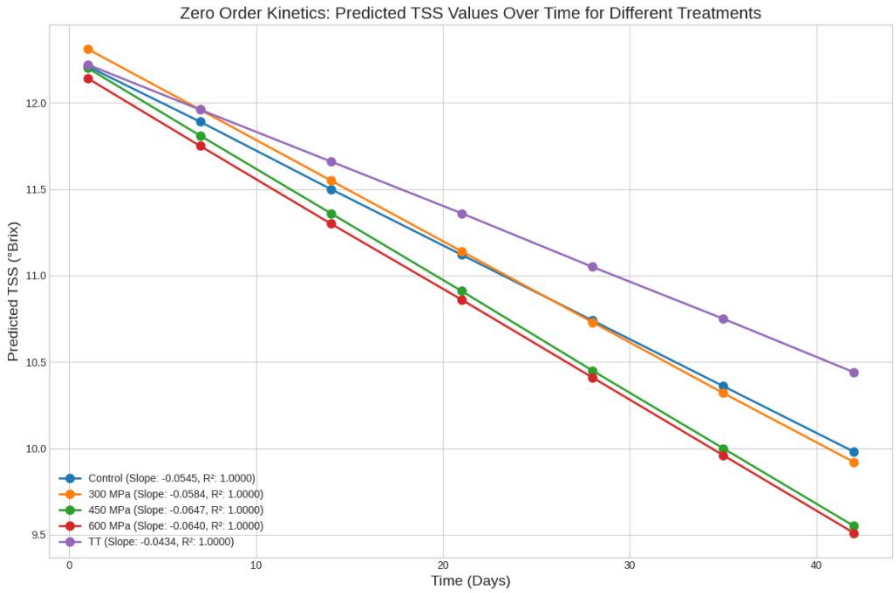


Source: Own elaboration

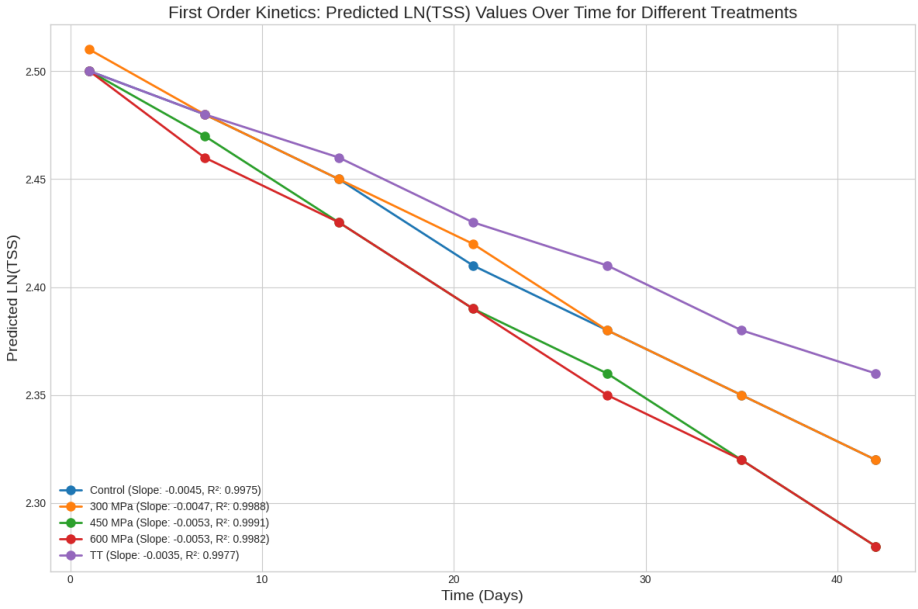


Source: Own elaboration

Figure 11.1 and Figure 12.1. Observed and predicted values for Zero order and First order assessment for colour parameter TSS



Source: Own elaboration



Source: Own elaboration

Parameter TSS*

Tables 14 and 15 present the zero-order and first-order kinetic models for the Total Soluble Solids (TSS*) in strawberry nectar treated with different processing methods over 42 days of storage.

For the zero-order model (Table 14):

The 450 MPa and 600 MPa treatments showed the highest R^2 values (both 0.91), indicating the best fit among all treatments. This was followed closely by the Control (R^2 = 0.90), 300 MPa (R^2 = 0.88), and TT (R^2 = 0.77).

For the first-order model (Table 15):

The 450 MPa treatment showed the highest R^2 value (0.92), followed by 600 MPa (R^2 = 0.90), Control (R^2 = 0.88), 300 MPa (R^2 = 0.86), and TT (R^2 = 0.76).

The R^2 for the First order models were not significantly improved compared with the zero order models, so for the purposes of parameter estimation and prediction, the estimates from the zero order models using untransformed data were used.

Based on the zero-order model, the slopes were similar across all treatments, ranging from -0.11 to -0.12, indicating a consistent decrease in TSS* over time.

The k values also showed equality, except for those treatments that applied 300 MPa, 450 MPa, and 600 MPa, displaying the highest rate of change, k = 0.06 Day^{-1}, while TT presented the lowest rate, k = 0.04 Day^{-1}.

Half-life (T1/2) ranged from 94.65 days in the case of 450 MPa to 141.28 days in the case of TT.

Mean TSS* was higher for TT, showing a value of 10.73, whereas for 450 MPa, it showed the minimum, 10.14.

Slopes were uniform for all the treatments and stood at -0.01.

From the rate constant (k), it is observed that 450 MPa and 600 MPa had a slightly higher rate, 0.01 Day^{-1}, compared to the rest of the treatments, 0.00 Day^{-1}.

These results show that, with the exception of one or two minor increases in TSS*, TSS* for all treatments generally decreased with time; however, rates of change were rather greater with high-pressure treatments of 450 MPa and above, giving the best fit to both zero-order models. The TT maintained the highest mean TSS* throughout but also gave the poorest fit to either model, indicating more complex changes in TSS* over time for that treatment.

Discussion

This research investigates the colour stability of strawberry nectar processed under different conditions by HPP at different pressures and TT, during 42 days of storage. For this purpose, parameters such as redness (a*), lightness (L*), ΔE, and AF were taken into consideration.

Redness values (a*) showed a declining tendency in all treatments. This is consistent with earlier researches on strawberry products that showed similar colour degradation behaviour Cao et al., 2012. TT showed the best retention of redness, and therefore presented the slowest decrease rate ($k = 0.06\ Day^{-1}$ in the zero-order model). This infers that thermal processing may be superior in the complete inactivation of enzymes responsible for anthocyanin degradation, as supported by Patras et al. (2009). All the treatments increased in L* value during storage, meaning that the samples became lighter with time. This may be due to the breakdown of anthocyanins and the formation of colourless or brown-coloured compounds. The best fit models were the following: for TT and HPP at 600 MPa, the R^2 values were 0.98 and 0.92, respectively.

ΔE analysis showed that the samples treated with HPP at 600 MPa and thermal treatment were those showing the least colour instability with time. This observation compares favourably with similar work done by Terefe et al. (2013), who observed that HPP could preserve colour in strawberry puree. The maximum colour change rate was shown by the control samples, which have again pointed to the need for processing in maintaining colour in strawberry products.

In the AF degradation kinetics, the HPP with a moderate pressure of 300 MPa showed the longest half-life, followed by that of TT. It suggests that both the moderate pressure HPP and thermal processing can be used for preserving anthocyanin content in these fruits. Results are in corroboration with those reported by Marszałek et al. 2017, who concluded that moderate pressure treatments may enhance the stability of anthocyanins in fruit products.

These results, on the effectiveness of various HPP pressure levels in the preservation of colour parameters, suggest that optimization of the HPP processing parameters is quite crucial to enhance the maximum retention of colour in strawberry nectar. This observation agrees with Suthanthanjai et al. (2020), who stated that optimization of the HPP conditions is one of the important considerations in the preservation of fruit juice.

Colour parameter changes were satisfactorily fitted by both zero-order and first-order kinetic models with, in general, $R^2 > 0.70$ for most treatments. In this respect, any of the models would be usable in a trustworthy form to predict colour changes in strawberry nectar during storage, concludes Mercali et al. (2013), in a study related to kinetic degradation of anthocyanins.

Conclusion

Although TT was superior for most of the colour stability parameters studied, HPP at 600 MPa showed a promising result as an alternative non-thermal treatment for strawberry nectar colour quality retention.

Choosing which one is better over the other would depend upon several aspects like other quality aspects, energy consideration, and specific product requirement. Future research work should focus on the optimization of HPP parameters that understand the mechanism of colour preservations as a function of the processing conditions and the synergistic effects of combined treatments for further colour stability enhancement in strawberry nectar products.

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5. Manuscript 4: A multiple linear regression analysis examining the determinants of colour change over time in storage in strawberry nectar treated by different stabilization processes

5.1. Abstract

This analysis examined the factors that influence colour stability in strawberry nectar during a 42-day storage period after thermal, HPP treatments and no processing treatment (i.e. control). Multiple regression analyses were performed on a range of colour parameters (L*, a*, C*, and AF*) in order to evaluate the effects of storage duration, total soluble solids (Brix), enzymatic activity (PME, PPO, and PG), and processing techniques on colour changes.

The product gets lighter over time, with storage time being the main factor influencing variations in lightness (L*). The rate of change in overall colour saturation (C* and AF*) and red colour intensity (a*) depended significantly on the sugar content (Brix), when all other factors were accounted for. While not statistically significant, enzymatic activities show- especially PPO-a trend to negatively affect colour parameters. Process type does not show a significant effect when other factors have been accounted for.

Although this analysis was limited by sample size and assumptions, the information derived gives the food sector some indication, showing that control of storage conditions and maximization of sugar content should be considered to preserve colour stability in strawberry nectar.

Future research should focus on analysing larger sample sizes to ensure that all statistically significant determinants of colour change with time can be established in a more robust analysis. Investigations of the interaction of factors may also be carried out for a fuller understanding of the mechanisms involved in fruit nectar colour stability.

5.2. Introduction

There are a number of potential factors which will influence the rate of colour change in strawberry nectar as described in Chapters 1 and 2. These include, for example, levels of enzymatic activity responsible for colour change and the effect of stabilization processes on these enzymes. The availability of data on residual enzymatic activity, post process, in addition to colour assessments and quality indicators, for 42 days in storage, post process, for a number of processes, allowed an analysis of the relative impact of these factors on colour change over time in storage. It was anticipated that the analysis would help identify the relative influence of the different potential determinants on colour change over the storage duration of 42 days.

5.3. Objective

The Objective was to examine the determinants of colour change over time of strawberry nectar treated by different stabilization treatment and none (i.e., control)

5.4. Materials and Methods

Data and statistical methods

The strawberry nectar was exposed to four separate stabilization processes as described in Manuscript 1, Thermal, and High-pressure processing at pressures of 300MPa, 450MPa and 600MPa. A control sample was not treated to any stabilization processes. All samples were placed on storage for 42 days with evaluations of the following parameters post process Day 1, Day 7, Day 14, Day 21, Day 28, Day 35 and Day 42:

Quality indicators

- pH
- Brix (TSS) sugars

Colour indicators:

- L*
- a*
- C*
- AF*
- DE*

– BI

In addition, enzymatic activity of PPO, PME and PG were evaluated, post process on day 1 for control and other processed samples. % Residual activity (RA) for each of these enzymes was calculated for samples exposed to thermal and HPP processes by comparison with enzyme activity in the control samples (arbitrarily assigned 100% for RA). 3 samples from the same batch were measured for each parameter at each time point and the mean parameter value for the 3 samples estimates was used in the model.

A separate multiple linear regression analysis was conducted for each measure of colour as it is unclear which of these is the "gold standard" for colour evaluation in samples of strawberry puree.

The analysis was of the form:

Colour = linear function (Time, Process, pH, TSS, PPO RA, PME RA, PG RA) + constant

As enzymatic activity was determined at only one time point, it was necessary to assume that this level of residual enzyme activity remained stable for the 42-day period (Table 1).

Table 1. Parameter values included in the multiple regression analysis of the determinants on colour change in strawberry nectar over 42 days storage

Day	Process	PME RA%	PPO RA%	PG RA%	L *	a*	C*	AF*	DE*	BI	pH	TSS, Brix
1	0	100.00	100.00	100.00	37.53	16.51	18.75	0.58	0.001	31.86	3.24	12.27
7	0	100.00	100.00	100.00	36.26	15.77	18.12	0.53	1.48	31.65	3.30	12.27
14	0	100.00	100.00	100.00	34.33	15.70	17.91	0.55	3.31	33.10	3.26	12.23
21	0	100.00	100.00	100.00	38.54	19.25	22.37	0.63	3.85	36.17	3.26	12.23
28	0	100.00	100.00	100.00	43.19	9.23	10.78	0.30	9.80	16.12	3.37	10.80
35	0	100.00	100.00	100.00	42.43	10.55	12.27	0.34	8.15	18.65	3.24	10.80
42	0	100.00	100.00	100.00	43.90	8.76	11.32	0.22	10.18	15.52	3.24	8.40
1	1	102.46	87.50	54.69	36.45	17.88	20.70	0.59	2.34	35.51	3.23	12.37
7	1	102.46	87.50	54.69	36.54	15.49	17.48	0.56	1.62	30.71	3.28	12.27
14	1	102.46	87.50	54.69	35.74	16.09	18.67	0.53	1.94	32.80	3.44	12.27
21	1	102.46	87.50	54.69	41.31	20.83	24.64	0.65	7.16	36.70	3.44	12.27
28	1	102.46	87.50	54.69	40.50	13.58	15.92	0.43	4.21	24.90	3.27	11.00
35	1	102.46	87.50	54.69	43.59	11.58	13.54	0.37	8.03	19.91	3.26	11.00
42	1	102.46	87.50	54.69	43.68	12.56	15.19	0.37	7.32	21.70	3.26	8.27
1	2	1.75	87.50	71.88	37.50	17.19	19.65	0.59	0.94	33.19	3.23	12.27
7	2	1.75	87.50	71.88	35.32	16.06	18.78	0.51	2.41	33.20	3.29	12.37
14	2	1.75	87.50	71.88	36.01	15.22	17.33	0.53	2.08	30.71	3.44	12.33
21	2	1.75	87.50	71.88	43.89	21.87	25.89	0.68	9.69	36.31	3.44	12.33
28	2	1.75	87.50	71.88	40.09	11.79	13.57	0.40	5.79	21.84	3.29	10.03
35	2	1.75	87.50	71.88	43.92	11.63	13.59	0.37	8.27	19.84	3.23	10.03
42	2	1.75	87.50	71.88	44.26	12.00	15.28	0.31	8.12	20.82	3.23	8.03
1	3	128.13	65.00	80.21	36.17	17.76	20.57	0.59	2.38	35.54	3.22	12.20
7	3	128.13	65.00	80.21	36.32	14.84	16.73	0.54	2.36	29.64	3.29	12.17
14	3	128.13	65.00	80.21	35.73	15.11	17.18	0.53	2.39	30.71	3.41	12.27

21	3	128.13	65.00	80.21	38.21	18.08	21.15	0.58	2.70	34.46	3.41	12.27
28	3	128.13	65.00	80.21	39.62	14.77	17.49	0.46	2.76	27.64	3.34	10.23
35	3	128.13	65.00	80.21	42.88	12.20	14.09	0.41	7.12	21.18	3.23	10.23
42	3	128.13	65.00	80.21	43.27	10.60	13.16	0.29	8.31	18.74	3.23	8.00
1	4	70.99	37.50	160.94	37.01	17.92	20.43	0.62	1.77	34.90	3.16	12.27
7	4	70.99	37.50	160.94	36.20	18.06	21.22	0.57	3.05	36.27	3.26	12.27
14	4	70.99	37.50	160.94	37.58	16.46	18.99	0.55	0.60	31.91	3.14	12.20
21	4	70.99	37.50	160.94	38.34	16.62	19.82	0.50	2.09	31.94	3.14	12.20
28	4	70.99	37.50	160.94	40.46	18.20	21.07	0.60	3.81	32.75	3.05	11.40
35	4	70.99	37.50	160.94	41.06	14.98	17.15	0.51	3.90	26.77	3.25	11.40
42	4	70.99	37.50	160.94	42.03	12.43	14.08	0.45	6.48	21.84	3.25	8.23

Source: Own elaboration

Process 0 = control, process 1 = HPP 300MPa, process 2 = HPP 450MPa, process 3 = HPP 600MPa and process 4 = Thermal; DE was arbitrarily assigned a value of 0.001 as the analysis required non zero values.

5.5. Results

Determinants of colour as measured by L*

Table 2 summarises the results for the multiple regression where L* is the dependent variable.

The model explained 77% of the variability in the data. The constant and time(days) were the only statistically significant determinants of L*.

Whilst the enzymatic RA%s were not statistically significant, the slopes suggested a negative relationship with L*, reducing as RA% increased, and with PPO having slightly more influence than the other enzymes.

Table 2. Results of multiple regression analysis for determinants of L* over time

Overall model R^2	0.773	
Parameters:	**Statistical Significance**	**Coefficients of interest**
Constant	P<0.005	49.3316
Day	P<0.0005	0.1929
Process	NS	-0.2994
PME RA%	NS	-0.0092
PPO RA%	NS	-0.0131
PG RA%	NS	-0.0113
pH	NS	-3.1535
Brix	NS	-0.0237

Source: Own elaboration

Determinants of colour as measured by a*

Table 3 summarises the result for the multiple regression where a* is the dependent variable.The model explained 63% of the variability in the data. Brix (TSS) was the only statistically significant explanatory parameter.

Whilst the enzymatic RA%s were not statistically significant, the slopes suggested a negative relationship, with a* reducing as RA% increased, and with PPO having more influence than the other enzymes.

Table 3. Results of multiple regression analysis for determinants of a* over time

Overall model R^2	0.626	
Parameters:	**Statistical Significance**	**Coefficients of interest**
Constant	NS	6.1663
Day	NS	-0.0322
Process	NS	-1.1233
PME RA%	NS	-0.0158
PPO RA%	NS	-0.1327
PG RA%	NS	-0.0232
pH	NS	3.2917
Brix	P<0.05	1.2918

Source: Own elaboration

Determinants of colour as measured by c*

Table 4 summarises the result for the multiple regression where c* is the dependent variable.

The model explained 55% of the variability in the data. Brix (TSS) was the only statistically significant explanatory parameter.

Whilst the enzymatic RA%s were not statistically significant, the slopes suggested a negative relationship, with c* reducing as RA% increased, and with PPO having more influence than the other enzymes.

Table 4. Results of multiple regression analysis for determinants of c* over time

Overall model R^2	0.55	
Parameters:	**Statistical Significance**	**Coefficients of interest**
Constant	NS	8.5405
Day	NS	-0.0285
Process	NS	-1.3262
PME RA%	NS	-0.0193
PPO RA%	NS	-0.1551
PG RA%	NS	-0.0286

pH	NS	3.2851
Brix	P<0.05	1.3973

Source: Own elaboration

Determinants of colour as measured by AF*

Table 5 summarises the result for the multiple regression where AF* is the dependent variable.

The model explained 75% of the variability in the data. Brix (TSS) was the only statistically significant explanatory parameter.

Whilst the enzymatic RA%s were not statistically significant, the slopes suggested a negative relationship with AF*, reducing as RA% increased, and with PPO having more influence than the other enzymes.

Table 5. Results of multiple regression analysis for determinants of AF* over time

Overall model R^2	0.748	
Parameters:	Statistical Significance	Coefficients of interest
Constant	NS	0.0987
Day	NS	-0.0021
Process	NS	-0.0333
PME RA%	NS	-0.0004
PPO RA%	NS	-0.0041
PG RA%	NS	-0.0006
pH	NS	0.1209
Brix	P<0.01	0.0447

Source: Own elaboration

Determinants of colour as measured by DE*

Table 6 summarises the result for the multiple regression where DE* is the dependent variable.

The model explained 71% of the variability in the data. Time (days) was the only statistically significant explanatory parameter.

Whilst the enzymatic RA%s were not statistically significant, the slopes suggested a positive relationship with DE*, increasing as RA% increased, for PPO and PG.

Table 6. Results of multiple regression analysis for determinants of DE* over time

Overall model R^2	0.713	
Parameters:	**Statistical Significance**	**Coefficients of interest**
Constant	NS	-11.8856
Day	P<0.001	0.1587
Process	NS	0.1589
PME RA%	NS	-0.0037
PPO RA%	NS	0.0468
PG RA%	NS	0.0059
pH	NS	3.2212
Brix	NS	-0.1474

Source: Own elaboration

Determinants of colour as measured by BI*

Table 7 summarises the result for the multiple regression where BI* is the dependent variable.

The model explained 76% of the variability in the data. Time (days) and Brix (TSS) were the only statistically significant explanatory parameters.

Whilst the enzymatic RA%s were not statistically significant, the slopes suggested a negative relationship with BI*, reducing as RA% increased, with PPO having more influence than the other enzymes.

Table 7. Results of multiple regression analysis for determinants of BI* over time

Overall model R^2	0.761	
Parameters:	**Statistical Significance**	**Coefficients of interest**
Constant	NS	11.2873
Day	P<0.05	-0.1938
Process	NS	-1.7505
PMERA%	NS	-0.0200

PPO RA%	NS	-0.2162
PGRA%	NS	-0.0321
pH	NS	6.9226
Brix	P<0.05	2.0476

Source: Own elaboration

When the analysis was repeated using log-transformation of the dependent variable data (i.e., the different measures of colour), very similar results were obtained to those given above based on the analysis of the untransformed data.

Discussion

The various multiple linear regression analyses carried out on different colour parameters L*, a*, C*, and AF* of strawberry nectar during a 42-day storage period - show some interesting results about what the determinants of colour change in this product are.

The model for L* explained 77% of the variability and was thus reasonably substantial. Curiously, the only statistically significant determinants were time - in days - along with the constant. This implies that the lightness of the strawberry nectar is modified practically with the storage time and regardless of the type of processing or enzymatic activity. The positive coefficient for time, 0.1929, involves that during the period of storage the nectar is lighter, probably because of some degradation of pigments or any other time-dependent chemical reactions. The negative coefficients derived from the enzyme residual activities, though not significant, would indicate a trend that higher enzyme activities tend to yield products with a slightly darker hue.

This is in line with their known activities within browning reactions and breakdown of cell wall components that would influence the light-scattering properties of the nectar. The negative, albeit non-significant, coefficients of determination for the enzyme activities, particularly for PPO, agree with expectations. PPO is believed to catalyze browning reactions that could reduce redness and, for that matter, overall colour intensity. In both a* and C*, the proportion of variability explained by the models was lower, 63% and 55%, respectively, than for L*. Surprisingly, Brix, being the measure of total soluble solids, was the only statistically significant parameter for both.

The positive coefficients of 1.3 for a* and 1.4 for C* express that higher sugar content is positively related to more intense red colour and overall colour saturation. Maybe due to the protective effects of sugars to anthocyanins, which are the primary responsible red pigments in strawberries (Wrolstad et al., 2005). The AF* model accounted for 75% of the variability, having Brix once again the only significant parameter. This further confirms the influence of sugar content on maintaining colour intensity in strawberry nectar. On the other hand, all colour parameters recorded negative relationships with all three enzymes, though without statistical significance.

This trend suggests that colour degradation may be related to higher residual enzyme activities. Among these three enzymes that have been studied, PPO apparently exerts the strongest effect. This is interesting, because there were no statistical significances for the different processing methods and treatments of thermal versus various HPP; however, when other factors accounted for were modelled, process was not significant. If the process effect is mediated through effects on the other parameters, e.g., enzymatic activity or brisk levels, then, after accounting for these in the model, there may have been no significant independent effects of process. It may be a problem in that the relationship of the parameters may impinge upon being able to detect differences among parameters.

In this analysis, there was a series of limitations concerning the sample size and also the need to assume RA did not change with storage. Notwithstanding this caveat, it gives some interesting insights into the factors that influence colour stability in strawberry nectar over storage time. The most important things it brought out were that storage time is one of the main drivers in the change in lightness L*, whereby the product will get lighter with an increase in time of storage. Sugar content, expressed in Brix, turned out to be a suitable predictor for the intensity of red colour (a*) and overall colour saturation parameters, which are C* and AF*. Enzymatic activities-particularly PPO demonstrated a trend to negatively affect colour parameters, although not significantly in this study.

The type of initial processing the tomato puree underwent does not seem to independently affect colour change when considering enzyme activity and other quality indicator values. These findings have important implications for the food industry. Attempts to preserve colour stability in strawberry nectar should be focused particularly on storage conditions and optimization of sugar content. Although enzyme inactivation remains important, other factors could be involved in long-term colour stability. This may indicate that a choice between thermal and HPP treatments, other than for colour stability,

could be based on nutritional retention or energy efficiency. Barba et al., 2012. Larger sample sizes should be considered in future studies to enable the identification of all the statistically significant determinants of colour change with time in more robust analyses. The possibility of considering factor interactions for a better comprehension of mechanisms which affect

The investigation presented has brought out the complexity of colour stability in processed fruit products and has managed to emphasize the fact that there can indeed be no one-dimensional approach for maintaining the visual quality of strawberry nectar during storage.

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6. Manuscript 5: Energy assessment of thermal and on-thermal technologies

6.1. Abstract

In order to develop food processes, it is important to understanded the energy requirements to operate such a unit. This study compared energy usage in heat pasteurisation and High-Pressure Processing (HPP) for the production of strawberry nectars. The study covers basic energy consumption principles in both technologies and identifies elements that affect efficiency, like process parameters and equipment design.

A comparative case study showed that HPP at 600 MPa for 5 minutes used much less energy (5.62-9.3 Wh/L) than thermal treatment (TT) at 80°C for 5 minutes (64.76 Wh/L). HPP showed better energy efficiency, especially at larger densities of packaging. Although TT has it's advantageous, particularly for quality assurance of food products, its high energy consumption makes HPP appear like a more environmentally friendly processing option.

In order to contribute to a more energy efficiency food system, processing parameters and packaging techniques should be considered when selecting processing methods. This study suggest further study into non-thermal food processes should be explored, balancing energy efficiency and product quality.

6.2. Introduction: Energy Consumption Evaluation

The basic principles of energy usage in thermal and non-thermal food processing technologies are different in nature, based on different physical mechanisms. Thus, thermal pasteurization relies on a heat transfer phenomenon to inactivate microorganisms and enzymes; the energy consumption is mainly driven by the need to raise the temperature of the product to a target level and hold it for a specified time, followed by rapid cooling of the product (Pereira and Vicente, 2010). These may be influenced by specific heat capacity, thermal conductivity, convection, and the loss of heat to the environment. In contrast, HPP inactivates microorganisms under hydrostatic pressure. Generally, the energy use in HPP is controlled by factors such as pressure-volume work, adiabatic heating, pump efficiency, pressure holding time, and cooling systems (Huang et al., 2017). Energy consumption in HPP is mainly related to the generation and maintenance of pressure rather than to direct heating of the product.

TES pertains to energy management of food processing. The properties to be decided on energy utilization in TES include properties of storage media, temperature differentials, insulation efficiencies, and charge-discharge rates. In general, TES would increase the overall energy efficiency of a food processing operation by offering better utilization of renewable energy sources that are intermittent in nature and recovery of residual heat

Understanding such basic principles is important for optimization in energy use and the development of more sustainable methods of food processing.

Factors Influencing Energy Efficiency

Energy efficiency in industry and food processing depends on many factors, from the design features of the equipment to the operational parameters of processes. Understanding such factors would go a long way in optimizing energy consumption and coming up with greener methods of processing (Patel and Sharma, 2019).

Equipment design

Design is a key role of processing equipment in relation to energy efficiency. Good thermal insulation of equipment reduces heat loss and hence maximizes overall thermal efficiency. According to Pereira and Vicente, 2010, good insulation helps in the reduction of heat dissipation during processing. Besides, the use of designed heat recovery systems

reduces energy wastes that take place as a result of developed heat during processes. Such heat recovery systems include heat exchangers and regenerative systems Aganovic et al., 2017. It is also of essence to employ high-efficiency motors together with frequency drives since these could cut down electrical energy use significantly. Furthermore, equipment that is appropriately sized will operate at peak efficiency and, consequently cannot waste energy as would have been witnessed with oversized systems (Ramaswamy and Marcotte, 2005). Equipment designed for easy maintenance ensures it keeps on running efficiently for quite a long period (Toepfl et al., 2006).

Process parameters

Besides, process parameters influence energy consumption and efficiency directly. In general, the higher the processing temperature, the higher the energy input; thus, great energy savings may result from temperature profile optimization (Bermúdez-Aguirre and Barbosa-Cánovas, 2021. Such will be the case for HPP, where the level of pressure has a direct influence on energy consumption; therefore, this element's optimization in each product will enhance its efficiency (Huang et al., 2017). Longer times of processing generally result in higher energy usage; thus, optimization of cycle times is an integral component in order to reduce general energy consumption (Pankaj and Keener, 2017). Product flow rate matched with equipment capacity is another important factor that assures efficient operation (Ramaswamy and Marcotte, 2005). Moreover, the physical properties and thermal properties of the product being processed can be a factor in the overall energy requirements (Singh and Heldman, 2014).

Operational practices

Operational practices are another crucial element in the pursuit of energy efficiency. This involves regular maintenance schedules that can help equipment run at their best efficiency. Aganovic et al. (2017) installed advanced process control systems to achieve optimisation of energy use in real-time and well-trained operators are capable of operating equipment with higher efficiency according to Patel and Sharma, 2019. Besides, the optimization of production scheduling can reduce the time of idling and hence the waste of energy.

External Factors

Energy efficiency in the process is also dictated to a great extent by the ambient factors of the external environment. For example, the ambient temperature of the environment can influence heat losses and gains while processing food items (Singh and Heldman, 2014), while humidity may affect those processes that have evaporation or condensation steps involved in it (Ramaswamy and Marcotte, 2005). Finally, the layout configuration also plays an important role in how well energy will be distributed within the processing environment (Bermúdez-Aguirre and Barbosa-Cánovas, 2021).

Clearly, the optimization of these factors may result in surprisingly good improvements in energy efficiency. However, the share of the relative contribution of each of the listed factors can change in regard to a particular process and context of an industry. Only continued monitoring and analysis of energy consumption patterns locate chances for improvement to achieve long-term energy efficiency (Pankaj and Keener, 2017).

6.3. Objectives

The objective of this work was to analyse energy use in thermal and non-thermal processing technologies for strawberry nectar production.

6.4. Methods

6.4.1. Thermal treatment (TT)

Heat transfer and electric power consumption:

The quantification of energy requirements for thermal pasteurization employed a basic equation flow from the first law of thermodynamics. It is at the core of all heat transfer problems in food engineering, since accurate calculations are therefore possible of how much thermal energy is required to increase the temperature of a known quantity of food product.

The heat transfer equation was expressed as

$$Q = m * Cs * \Delta T$$

Where Q is the heat energy transferred in joules, m is the mass of the substance in kg, Cs is the specific heat capacity in J/kg°C, and ΔT is the change in temperature in °C. This equation, therefore, acts as a base in the carrying out of energy calculations in

thermal food processing, enabling an engineer or any food scientist to optimize heating processes and allow for energy efficiency studies.

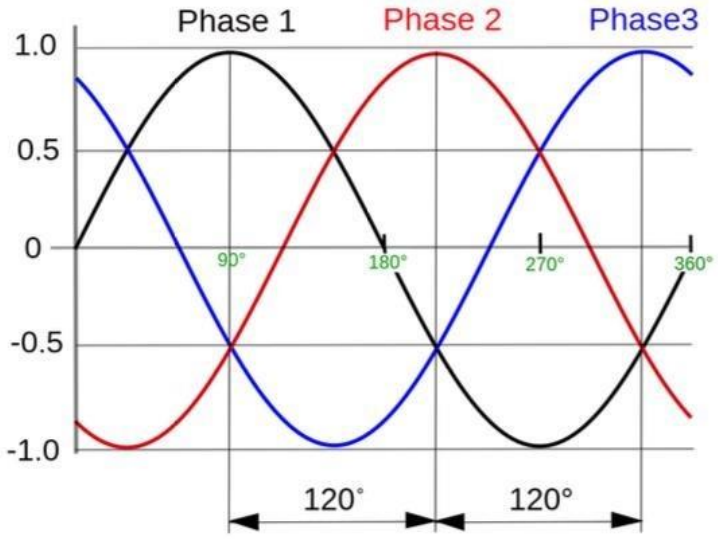
Cs can be taken as approximately 3600 J/kg°C for the pasteurization of juice or nectar. In this case, all energy requirement estimations can be precisely made for different eventualities of the process.

6.4.2. High Pressure Process

Energy Consumption: After the overview of basic principles ruling energy use both in thermal and non-thermal food processing technologies, it is relevant to proceed with the specific case of energy consumption assessment regarding HPP. In fact, HPP systems have more complex energy requirements compared to thermal pasteurization; this aspect has relevance not only concerning the pressure generation system but also the connected refrigeration circuits.

The calculation of the energy consumption by HPP must therefore include both pure electric power input to the major processing machinery and total energy use by auxiliaries such as refrigeration. The major component of the HPP system typically comprises a 3-phase electric power motor, which is generally more efficient and effective than a single-phase motor. In a 3-phase system, there is an alternating current with a phase shift of 120° between each other. Figure 1.

Figure 1. Phase Electric Power Motor



Source: Park (2023).

Energy Consumption:

The electric power consumed by such a motor is given by the formula:

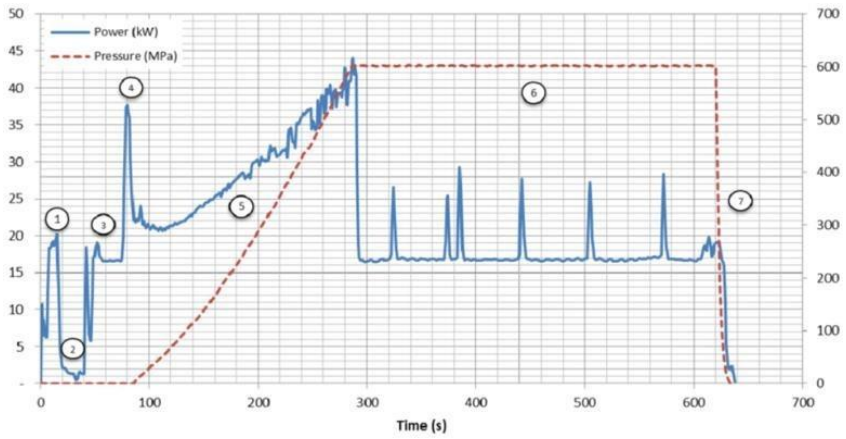
$$P = \sqrt{3} \times V \times I \times Pf$$

Where:

- P - power in watts
- V - voltage in volts
- I - current in amperes
- Pf - power factor

This equation therefore gave the exact amount of electrical energy that would be fed into the HPP system. However, this is only part of the energy consumed. Further consideration of the efficiency of pressure generation, maintenance, and cooling systems' power consumption is required to correctly assess the overall energy used. Taken all together, these factors describe the total energy profile of the HPP process and as such serve to provide a much more valuable means of comparison against other methods of food preservation. The target pressure will be achieved by a distribution of power as illustrated in the sample shown in Figure 2.

Figure 2. Energy consumption during high pressure cycle (600 MPa, 5 min) including different steps and pressure.

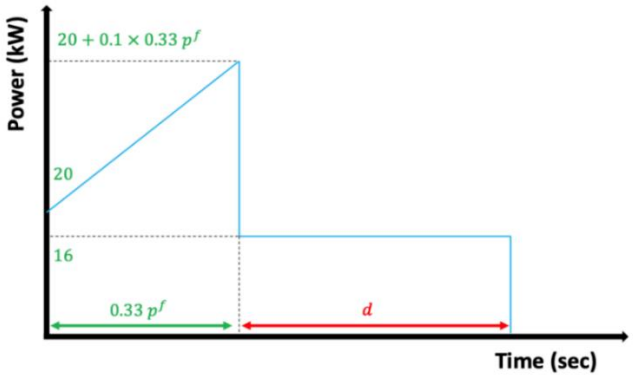


Source: (Aganovic, 2017)

Figure 3 represents the profile of power consumption during the pressurization phase of the HPP. This figure gives a good overview of power usage in the building-up pressure and thus is an integral part of the energy dynamics understanding for HPPs. Consumption

in HPP treatments is not directly derived from the parameters that form part of the electric power equation. Thus, instead of using P = √3 × V × I × Pf, it does relate in this case to pressure and time. Real energy consumption would be obtained by integrating power over time, which refers to the area under the curve, as represented in the figure.

Figure 3. Example of Energy Consumption Diagram



Source: Park (2023).

Figure 3 illustrates this relationship, whereby the energy to be used for an HPP treatment can be calculated by using the following equation:

$$Ehpp = 1/2 \times (20 + 20 + 0.033p) \times 0.33p + 16d$$

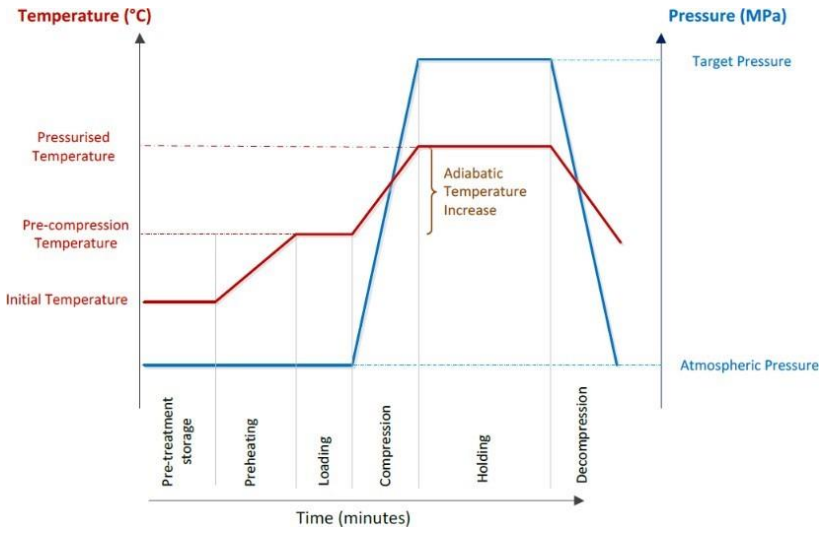
Where:

Ehpp is the energy consumed during the HPP treatment

- p is the pressure applied (MPa)
- d is the holding time (s)

This equation took into consideration the energy needed for pressurization, holding at target pressure, and the base energy consumption of the system. It has given a practical method for estimating the energy requirements of HPP treatments based on the two key operational parameters: pressure and holding time. This understanding of the relationship between these factors aids in the optimization of HPP processes through which operators will be capable of predicting energy use and allowing management according to the pressure and time requirements of different food products under various respective standards for safety.

Figure 4. Temperature Changes During HPP



Source: Koutsoumanis, 2022

In general, adiabatic temperature rise is one of the most important considerations in HPP, depending on several parameters such as pre-compression temperature, packaging material, food type, and target pressure. Food temperature increases owing to a phenomenon called adiabatic heating due to the rise in pressure during the process. This can easily be explained with Boyle's Law (PV=RT), where the relationship among gas pressure and volume is combined with temperature. This relationship points out the significance of pre-compression temperature because the latter has a major effect on the food product as well as on the pressure medium prior to the pressurizing. In the holding phase the food is kept for specific time at constant pressure. During decompression, the temperature of the food goes down and could drop below the initial pre-compression temperature in case heat dissipation occurred during the holding phase.

The temperature increase that results is usually fairly uniform for homogeneous foods, and it relates to the pre-compression temperature as well as properties specific to the material being pressurized. All commercial batch pressure vessels are normally fitted with sensors to measure the temperature of the pressure medium during processing outside of the food. Yet, this is not available in all systems, and it does not show exactly what happens inside the food product. Therefore, in general, the industry performs measurements of the temperature of food products before and after the process of

pressurization. Temperature and pressure data for analytical purposes will be extracted from the HPP machine's output.

The data obtained from this will then be further graphed using GeoGebra software, hence giving a clear analysis of the temperature-pressure relationship along the HPP cycle. The approach can be further used in the better understanding of how these variables interact and directly influence the overall effectiveness of the HPP process. Temperature monitoring and analysis are some of the ways to ensure food safety and maintain product quality, as also process optimization in HPP. It allows full comprehension of thermal dynamics occurring during high-pressure treatment, necessary for the development of effective HPP protocols for a variety of foods.

Table 1 reports the typical temperature variation per 100 MPa at a pre- compression temperature of 25°C. Fatty foods, with higher compressibility and lower specific heat capacity, undergo more compression heating. For water-based products, temperature increase aligns with pressure rise; however, fatty products might take longer to reach peak temperature post-pressurization.

Table 1. Temperature Change during Pressurization for Different Foods

Substance initially at 25°C	Temperature change per 100 MPa
Water, juice, milk (2% fat)	3.0
Egg albumin, Mashed potato	3.0
Tofu, Yoghurt	3.1
Honey, Salmon	3.2
Chicken fat	4.5
Beef fat	6.3
Olive oil	8.7 to 6.3 *(Decreased temperature rise as pressure increased)*
Water / glycol (50/50)	4.8 to 3.7 *(Decreased temperature rise as pressure increased)*
Silicone oil	18.5
Metal	0
Polypropylene polymer	~ 4.0

Source: (Balasubramaniam, 2015)

For juices and nectars, the temperature change is 3°C, this means that in the case of a treatment of 600 MPa, the temperature increases by approximately 18°C.

6.4.3. Optimization Objectives

The objective was to minimize colour difference, enzymatic activity, reduction in nutrients, and energy consumption by controlling the decision variables that are:

Also, constraints should be defined:

Final temperature should not exceed maximum ($tpre + 0.03pf$)

Enzyme/microbial activity after treatment should be reduced to a certain level ($fm, i\ (tpre, pf, d, x) \leq \overline{Mi}\ \forall i$)

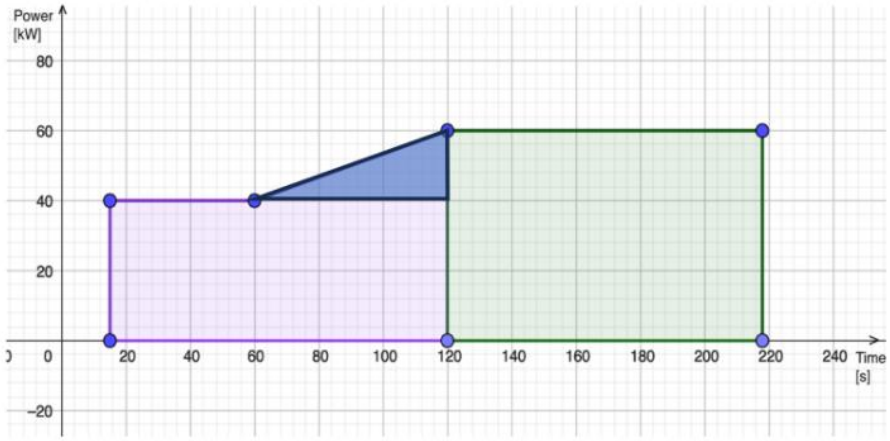
Target pressure level cannot exceed machine specifications ($pf \leq \overline{P}$)

Non-negativity of variables ($tpre, pf, d \geq 0$)

Binary condition for choice of inhibitor ($x \in \{0,1\}$)

6.4.4. Energy Consumption Analysis

Figure 5. Schematization of the Energy Consumption



Source: Geogebra®

As explained earlier, the energy is represented by the area under figure 5 (the integration of power to time).

$$Ehpp = purple\ area + blue\ area + green\ area$$

$$Ehpp = (120 - 15)s\ x\ 40kW + \frac{(120 - 60)sx(60 - 40)kW}{2} + (217 - 120)s\ x\ 60kW = 10620kWs$$

$$Ehpp = 10620\ kWs\ x\frac{1h}{3600s} = 2.95\ kWh$$

6.4.5. Product volume considerations

To be able to compare these results with other technologies involving heat transfer, it is necessary to select a mass of the treated product. Since HPP is a non-continuous treatment, it is necessary to understand the quantity of product processed per cycle. In HPP, the product is usually treated in its final packaging, which in our case could be single-serving plastic bottles of 200mL, TetraPak® bricks, or even larger bricks or bottles that are inserted into the vessel. In this way, since the product is already in its final packaging, the available volume inside the vessel is not entirely occupied due to its cylindrical shape. For this reason, plastic bags have been introduced, creating an inner skin of the vessel that can be entirely filled with liquid and then sealed tightly, maximizing the quantity of processed product for each cycle. However, after the treatment is completed, bottling will have to be carried out. The plastic accessory case is the most ideal scenario. However, if, for example, the single-serving TetraPak® brick is considered, imagining overturning the bricks randomly in the pre-loading vessel, it is necessary to deal with bulk materials in their solid form. Bulk density is defined as the ratio between the mass of the solid and the total volume occupied by it, and one must consider the quantity of juice that can actually be processed per cycle. Bulk density depends on many factors, including the shape of the container, and the size, and shape of the items to be packaged. Estimating that the items are placed randomly in the container, by an operator (as is the case with HPP Italia), the packaging density could be around 60-70% of the total volume of the container.

Therefore, in the case of the plastic bag or keg

$Vnectar \approx Vvessel$ = 525 L (3.

In the case of a 200 ml brick, it can be considered a 65% packaging density:

$Vnectar \approx 65\% \, Vvessel$ = 315 L

In the same way, it is possible to calculate the energy consumption for the different temperature's differences:

In the samples treated at 80°C with a water bath:

$$Q1 = 525L \times 1.08\frac{L}{kg} \times 3600\frac{J}{kg \cdot °C} \times (80°C - 20°C) = 122472kJ$$

$$Eth1 = \frac{122472kj}{1sx3600\frac{s}{h}} = 34kWh$$

$$Q2 = 315L\ x\ 1.08\frac{kg}{L}x3600\frac{J}{kg°C}x(80°C - 20°C) = 73483kj$$

$$Eth2 = \frac{73483kJ}{3600\frac{J}{Wh}} = 20{,}4kWh$$

In the same way, it is possible to calculate the energy consumption for the different temperature's differences (Table 2):

Results

Table 2. Energy Consumption Evaluation for HPP treatments vs TT treatments.

Case 1: 525 L		
Treatment	**Energy [kWh]**	**Energy per L [Wh/L]**
Hpp 600 MPa 5 mins	2.95	5.62
TT at 80°C, 5 mins	34	64.76
Case 2: 315 L		
Treatment	**Energy [kWh]**	**Energy per L [Wh/L]**
Hpp 600 MPa 5 mins	2.95	9.3
TT at 80°C, 5 mins	20.4	64.76

Source: Own elaboration

Table 2 Energy comparison between HPP and TT for two volumes of liquid. In Case 1, HPP at 600 MPa for 5 min requires only 2.95 kWh equivalent to 5.62 Wh/liter. At 80°C for the same time, however, energy consumption is much higher: 34 kWh in total, that is 64.76 Wh per litter. In Case 2, with a volume of 315 litters, HPP consumes as much as before, 2.95 kWh; because of the small batch size, energy consumption per liter rises to 9.3 Wh. On the contrary, TT consumes 20.4 kWh, hence again giving a constant energy consumption of 64.76 Wh per liter. Overall, these results show that in both cases HPP is more energy-efficient than that of TT and therefore can be an effective long-term solution

towards greener processing of voluminous liquid materials with much greater energy gains compared to conventional thermal technologies.

Discussion

HPP consistently used significantly less energy than the TTs for every temperature difference and nectar volume tested. This energetic advantage of HPP is exacerbated if one considers more realistic packaging densities, because HPP uses vessel capacity optimally to minimize wasted energy.

TTs-especially those of the high temperatures, like 80°C-rely on much larger energy inputs. With this, although TT still works for some of the uses, its energy use highlights the reasons why more attention is thrown on HPP among other methods in terms of energy efficiency. Packaging type and density are among the most crucial issues to decide upon energy efficiency for both the pressure technologies HPP and TTs. As with the use of plastic bags in HPP, this may be further optimized by maximizing packaging density to increase the amount of product processed per cycle. In general, our results confirm that HPP is one of the most promising technologies for energy-efficient food processing and a potentially sustainable solution for nectar manufacturing and possibly other beverages. Nevertheless, deeper research and development are required to optimize the processing parameters for maximizing the energy efficiency for various applications and product types. As well, because these were laboratory tests where it was not possible to take into consideration all the surrounding environmental conditions, an LCA was not done.

An LCA study has not been conducted, although it has the potential for giving very useful information about the environmental impacts of the studied processes. LCA implementation requires much effort, expertise, and time, and this may become a challenge to many organizations, especially small ones. Besides, uncertainties due to data quality and methodological choices may affect the LCA results' reliability. More importantly, setting the boundary of analysis may be complicated and lead to incomplete or biased assessments. Since most HPP services manufacture products for third parties, the lack of conducted LCA study underlines the need to take into consideration diverse factors, such as issues related to transportation logistics, packaging materials, and others, in order to further enable a full understanding of the environmental implications. Conclusion

This paper provides a detailed analysis of the energy utilization in TT and HPP applied in the manufacture of strawberry nectar, pointing out remarkable differences between the two methods.

Indeed, compared to TT treatment at 80°C for 5 minutes, energy consumption was very low: 64.76 Wh/L versus 5.62-9.3 Wh/L following HPP at 600 MPa for 5 minutes-long treatments, which points out the potential of HPP as being more energy-efficient.

The research demonstrated that, besides the creation and maintenance of pressure and associated temperature effects, the energy aspects of HPP are not at all straightforward, and juices and nectars are typically subjected to an adiabatic temperature rise of 3°C for every 100 MPa. Indeed, HPP presented an increase in energy efficiency with higher package densities and, therefore, advantages in large-scale industrial processes. Energy savings are significant, yet TT still maintains its advantages concerning generally accepted quality assurance procedures.

Conclusion

In order to produce strawberry nectar, this study offers a thorough examination of the energy used in thermal treatment (TT) and high-pressure processing (HPP), highlighting notable variations in energy efficiency. When compared to TT at 80°C for 5 minutes (64.76 Wh/L), HPP at 600 MPa for 5 minutes showed much less energy use (5.62-9.3 Wh/L), suggesting HPP's potential as a more energy-efficient solution. In addition to pressure creation, maintenance, and related temperature changes, the research demonstrated complex energy dynamics in HPP, with juices and nectars experiencing an adiabatic temperature increase of 3°C every 100 MPa. At higher package densities, HPP demonstrated increased energy efficiency, indicating advantages for large-scale industrial processes. Energy conservation is important, but TT still has advantages when it comes to accepted quality assurance procedures.

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