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Research Article

A Quality Assessment of Strawberry Nectar Stabilized by Thermal and High-Pressure Processing Conditions

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This study is aimed at evaluating the quality of strawberry nectar following high-pressure processing (HPP) at different pressure levels (400, 500, and 600 MPa for 3 minutes) and thermal pasteurization (TT) at 75°C for 15 s. Key parameters include total colour difference ($\Delta E*$), aparent viscosity, colour analysis via CIELAB coordinates, and pH. Additionally, residual enzymatic activity (RA%) of polyphenol oxidase (PPO) and peroxidase (POD) was measured. Strawberry nectar (12°Brix) was prepared and subjected to processing, with thermal treatment in 50 mL tubes and HPP in 40 mL polyethylene bags. Samples were stored under refrigeration during 21 days, with microbial analysis conducted to assess microbiological stability during storage. TT exhibited a more pronounced reduction in total aerobic microbial count (TAMC) compared to HPP, which showed no detectable yeast and mould. Apparent viscosity varied among samples, with HPP at 600 MPa displaying a significant increase of 52.1% compared to the control. The pseudoplastic behavior, characterized by n values lower than 1, was consistent across all treatments. TT led to a reduction in $L*$ brightness value during storage, whereas HPP-treated samples underwent colour changes over time with $\Delta E*$ of 3.1 for 400 MPa, 3 minutes, at 21 days. For PPO and POD, the 600 MPa treatment exhibited the highest inhibition (44% and 4% RA, respectively). This comprehensive study provides insights into how different HPP processing conditions affect the quality attributes, enzymatic activity, and microbiological stability of strawberry nectar during production and refrigerated storage. The research's significance lies in its holistic comparison of HPP with traditional thermal pasteurization, offering valuable information for both industry and consumers.

1. 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 [1]. Due to its appealing colour, pleasant perfume, and sweet-sour flavour, strawberry juice is one of the most consumed fruit juices [2]. Fruit juices, including from concentrates, and nectars are well-defined by European law. 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 from which it is derived [3]. Conversely, fruit nectars are produced through the addition of water to either fruit puree, fruit puree concentrate, or a combination of these materials, with or without the inclusion of sugars. Fruit nectars are notable for their substantial content of minerals, vitamins, bioactive phytocompounds, vitamin C, and other compounds endowed with antioxidant attributes. The quantities of these constituents exhibit variability on factors such as fruit quality, ripeness, harvesting

and storage techniques, and the employed technological processes [3, 4].

The attractive red colour is one of the visual quality attributes that greatly influence consumer appreciation of both fresh and processed strawberry fruit [5]. This is primarily attributed to the presence of anthocyanins, which are pigments found in fruit nectars that serve dual roles by imparting colour and contributing antioxidant properties to these beverages. 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 [6].

Polyphenol oxidase (PPO) is the primary enzyme responsible for enzymatic browning [7]. 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 nonenzymatic process to produce brown melanin pigments. Enzymatic browning causes colour changes and the breakdown of antioxidants due to the condensation of quinones with substances such as phenols, sugar, amino acids, and proteins. It also reduces the organoleptic quality and nutritional value of the product [8]. Peroxidase (POD) is an oxidoreductase enzyme that plays a significant role in the enzymatic browning process. This enzyme facilitates the conversion of diphenols into reactive intermediates, which subsequently participate in the browning reactions, leading to changes in the colour and quality of the nectars [9].

In addition to its role in microbial inactivation, thermal pasteurization is employed to deactivate fruit enzymes like PPO and POD, which contribute to the deterioration of quality in fruit products. Generally, the thermal pasteurization of fruit juices involves heat treatments higher than 60°C, to deactivate targeted microorganisms or enzymes [10]. Alternatively, juice preservation can be achieved using nonthermal methods like high-pressure processing (HPP) [11]. 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 endogenous microbiota and pathogens present in both solid and liquid foods are inactivated, ensuring that the product remains safe and microbiologically stable, without significantly modifying their sensory and nutritional characteristics [12]. Notably, the highest reduction in PPO activity has been found to be 51.5% at 600 MPa for 25 min for strawberry pulp [6]. Nevertheless, Garcia-Palazon et al. [13] reported that there was complete inactivation of PPO in strawberry fruits at 600 MPa for 15 minutes at room temperature. Regarding the POD enzyme, its activity exhibited a decline as the treatment time increased at both 400 and 500 MPa pressure levels. At 600 MPa for 5 minutes, the POD activity dropped to 35.7%, but it rebounded to 71.9% when the treatment time was 10 minutes. 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 [6].

Regardless of the treatment duration, Cao et al. [6] found that subjecting strawberry pulp to HPP at 400 MPa resulted in a noticeable darkening (lower $L*$ value) due to increased activity of PPO and POD enzymes. However, when applying pressures of 500 or 600 MPa, the colour of the strawberry pulp remained relatively stable. Additionally, the $a*$ parameter did not change after HPP treatments, except for a 5-minute duration treatment. The $b*$ parameter, on the other hand, increased significantly at 400 MPa but showed no significant changes at 500 or 600 MPa. Notably, h° and $C*$ values increased substantially at 400 MPa, except for the 5-minute treatment, while there were minimal changes at 500 and 600 MPa.

In addition to microbial and enzyme stability, viscosity is another important parameter for the production of fruit juices. It is essential to assess the viscosity of incoming liquid raw materials during production, as it directly impacts the mouthfeel of fruit juices for consumers [14]. Despite its importance, there is a limited number of research studying changes in rheological properties, together with microbial factors, and quality attributes of strawberry nectar following HPP treatment during storage. Consequently, further investigation is needed to comprehensively understand these treatment-related changes.

The primary objective of this study was to assess the quality of strawberry nectar after subjecting it to different processing methods, namely, high-pressure processing (HPP) at three pressure levels (400, 500, and 600 MPa for 3 minutes) and comparing them with thermal pasteurization at 75°C for 15 seconds. The quality evaluation was based on various key quality parameters: total colour difference (ΔE), CIE Lab values ($L*$, $a*$, and $b*$), apparent viscosity, and pH. Furthermore, the investigation involved an evaluation of the residual activity (RA%) of PPO and POD, both crucial for colour stability. The process involved sealing the nectar in hermetically sealed polyethylene bags and storing them under refrigeration. The quality indicators were measured at multiple time points: 0, 7, 14, and 21 days. In addition to the quality analysis, microbial examination was performed to gauge the microbiological aspects of the nectar over the entire storage duration. This comprehensive research is aimed at providing insights into how different HPP processing conditions impact the quality attributes, enzymatic activity, and microbiological stability of HPP-treated strawberry nectar postproduction and during storage under controlled refrigeration conditions. The study is significant because it considers numerous attributes and takes a more comprehensive approach to understanding the subject, by elucidating the multifaceted aspects of HPP in comparison to traditional thermal pasteurization, which can be of great importance to both industrial partners and consumers seeking for high-quality, nutritious products with extended shelf life.

2. Materials and Methods

2.1. Samples, Preparation, and Storage. Strawberry nectar was prepared from the frozen strawberry puree of the Senga Sengana strawberry variety 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. The nectar recipe consisted of 40% strawberry puree containing 7% total soluble solids; to form 1 kg of nectar 400 g of puree were necessary along with 90.36 grams of sugar, 1.64 gram of citric acid and 508 milliliters of water, based on the 7.7 g/kg acidity of the puree. This formulation results in a strawberry nectar of 12°Brix and pH range of 3.3-3.5. Subsequently, aliquots of 40 g of strawberry nectar were placed in high-density polyethylene plastic bags and sealed without headspace using a heat sealer. These sealed samples were stored at 4°C until they underwent the high-pressure processing (HPP) treatment the same day.

2.2. Thermal Treatment (TT). In this study, we employed a controlled heat treatment approach at the University of Parma, following the methodology described by Rinaldi et al. [15]. The thermal treatment (TT) involved maintaining a temperature of 75°C during 15 s, which is a mild treatment recommended by Tetra Pak for processing high-acid fruits and juices [16]. The procedure consist in the use of Eppendorf 50 mL plastic tubes, which one of them was equipped with a thermocouple at the center to ensure precise control of temperature and time during the process. These plastic tubes were chosen for their ability to withstand high temperatures while preserving the integrity of the enclosed samples. The nectar was filled up without headspace, and the tubes were tightly sealed to prevent liquid from evaporating. The samples within these tubes were exposed to a water thermostatic bath (MPM Instruments, Bernareggio, Italy). The application time began once the samples reached the desired temperature. After the treatment, the samples were quickly removed and cooled down in icy water. Each experiment was repeated three times.

2.3. High-Pressure Processing (HPP). High-pressure treatments were carried out using a 300 L high-pressure plant unit (Avure Technologies Inc., Erlanger, Kentucky, United States) and operated within the premises of HPP ITALIA SRL in Parma, Italy. In these experiments, cold water at 4°C served as the pressure transmission medium, ensuring that the temperature rise due to compression remained within the range of 2-3°C per 100 MPa. The HPP treatments were systematically applied at three distinct pressure levels: 400, 500, and 600 MPa, with each treatment lasting for 180 seconds (3 min). These pressure levels were selected to achieve microbiological safety and inactivation of enzymes, as supported by previous research on apple and carrot juices [17–19]. For each pressure level, the come-up time varied: approximately 60 seconds for 400 MPa, 80 seconds for 500 MPa, and 100 seconds for 600 MPa. The decompression occurred immediately after the designated treatment duration. Subsequently, the treated samples were stored under refrigerated conditions (4°C). HPP treatments were conducted in triplicate for each repetition.

2.4. Physical and Chemical Characterization of Strawberry Nectar

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

2.4.2. 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 (MCR 702e, Anton Paar, Graz, Austria) with a cylindrical probe, with a diameter of 26.66 mm. Samples were subjected to increasing shear rates from $10\,s^{-1}$ to $300\,s^{-1}$ at a constant temperature of 25°C based on the methodology of Liberatore et al. [17]. The analysis was performed on duplicate. The data obtained was recorded using the Anton Paar software RheoCompass. The variation of shear rate ($\dot{\gamma}$) and shear stress (τ) obtained from this measurement were used to determine the rheological properties of the different samples.

The samples were characterized by the power law model, also known as the Ostwald–de Waele model. The power law model is commonly employed due to its simplicity. This model describes viscosity (μ) as a function of shear rate ($\dot{\gamma}$), with constant values for the flow index (n) and consistency (k). A linear relationship between the logarithmic scale of viscosity, $\log(\mu)$, and shear rate, $\log(\dot{\gamma})$, is expected for a power law fluid in the shear thinning region [20]. The rheological parameters of the fluid in logarithmic scale can be calculated using

$$\log(\mu) = (n-1)\bullet\log(\dot{\gamma}) + \log(k), \tag{1}$$

$$\log(\tau) = n\bullet\log(\dot{\gamma}) + \log(k). \tag{2}$$

The power law index (n) and flow consistency (k) can be determined by fitting the rheometer measurements to equations (1) and (2). The slope of the entire region of the shear rate was chosen as a representative of the power law index of the fluid.

2.4.3. Colourimetric Analyses. The colour was analyzed using a Minolta Colorimeter (CM 2600D, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65, and the data was analyzed with the Spectramagic 3.6 software. Strawberry nectar colour changes were evaluated on 10 points on each samples, after treatment (0 day) and after 7, 14, and 21 days of refrigerated storage (4° C). The CIELAB coordinates $L*$, $a*$, and $b*$ were used for the evaluation. $L*$ 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 (2) by comparing the sample with the untreated control sample:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}, \tag{3}$$

where

$$\Delta L^{*2} = L^*_{\text{sample}} - L^*_{\text{control}},$$
$$\Delta a^{*2} = a^*_{\text{sample}} - a^*_{\text{control}}, \quad (4)$$
$$\Delta b^{*2} = b^*_{\text{sample}} - b^*_{\text{control}}.$$

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

(i) If $\Delta E = 0 - 0.5$, the difference is not perceptible

(ii) If $\Delta E = 0.5 - 1.5$, the difference is slightly perceptible

(iii) If $\Delta E = 1.5 - 3.0$, the difference is evident

(iv) If $\Delta E = 3.0 - 6.0$, the difference is clearly visible

(v) If $\Delta E = 6.0 - 12.0$, there is a big colour difference

2.4.4. Microbial Analysis. Microbial analyses were performed following standard plate count methodologies for total aerobic microbial count (TAMC) (UNI EN ISO 4833 : 2004) and total yeast and mould count (TYMC) (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.

2.4.5. Protein Extraction. For the extraction of the enzymes, 100 grams of frozen strawberry nectar was 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°C, and the supernatant was subjected to precipitation of ammonium sulphate (80%). The suspensions were then stored at 4°C.

The sample was then prepared for the enzymatic activity tests. 1 mL of the homogeneous suspension was centrifuged for 5 minutes at 4°C, 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.

2.4.6. PPO and POD Activity. 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 (RA) was referred to the untreated control sample, measured on the first day of storage, and calculated as RA (%) = enzyme activity in the sample/enzyme activity in the control sample × 100.

2.5. Statistical Analysis. IBM SPSS statistical software (v. 27.0, SPSS Inc., Chicago, USA) was used. Comprehensive analysis encompasses 2-way and 1-way ANOVA (with standard and log-transformed data). To assert statistically significant differences among groups, ANOVA and post hoc testing using Tukey's method were performed. Colour data was analyzed using MANOVA, and significant differences were assessed by ANOVA and Tukey as a post hoc test.

2.5.1. Process Comparison. To compare the process with the control, the closeness of five quality markers including total colour difference (ΔE), CIE Lab parameters ($L*$ and $a*$), apparent viscosity, pH, and Brix were measured. The data obtained for processed samples (TT and HPP) was plotted against the control on an XY scatter plot. R^2 provides a measure of closeness (the closest to 1, the closer that the quality parameters are to the control). 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. The sum squared regression is the sum of the residuals squared, and the total sum of squares is the sum of the distance that the data is away from the mean all squared. As it is a percentage, it will take values between 0 and 1.

3. Results and Discussion

3.1. Physicochemical Analyses. Evaluation of pH changes in the untreated (control), TT, and HPP (400, 500, 600 MPa) on strawberry nectar were evaluated. All samples remained within pH 3.1 and 3.5 after treatment and in storage conditions. Constant pH during refrigerated storage indicates microbiological stability [21]. In the study of Aaby et al. [22], there were also no observable alterations in strawberry puree and juice samples, whether they underwent high-pressure processing (HPP) or thermal treatment (TT), regardless of the storage duration.

3.2. Microbiological Analyses. The presence of mesophilic aerobic bacteria and yeast and moulds was evaluated. The evaluation was performed on untreated nectar (control), and samples of heat and nonheat treatments.

As can be seen in Table 1 for day 0 (D0) and day 21 (D21) of refrigerated storage, the TT resulted in a 2-log reduction in TAMC compared to the control postprocessing, while all the HPP-treated samples resulted in approximately 1-log reduction. While there is a reduction in the HPP-

Table 1: Microbial count analysis: total aerobic microbial count (TAMC) and yeast and mould count (TYMC) log cfu/g for thermal treatment (TT) and three high-pressure processing (HPP) treatments.

Treatments	TAMC		TYMC	
	D0	D21	D0	D21
Control	3.23	—	1.47	—
TT 75°C 15 s	1.00	2.48	<1	3.26
HPP 400 MPa 3 min	2.08	2.67	<1	<1
HPP 500 MPa 3 min	1.70	2.11	<1	<1
HPP 600 MPa 3 min	2.00	2.40	<1	<1

treated samples, it is not as significant as the TT treatment. All samples increase TAMC after 21 days of storage. The presence of yeasts and moulds (TYMC) was not detectable on the HPP treatments, indicating effective control of yeast and mould.

The result obtained for TT agrees with Timmermans et al. [23] who used a heat treatment of 72°C for 20 seconds on orange juice (pH 3.3-3.4), obtaining a microbial load < 3 log and <1 log of TAMC.

On the other hand, HPP-treated samples did not perform as effectively in terms of microbial reduction compared to the other studies. In the study of Aaby et al. [22] for strawberry juice, on day 0, the bacterial growth (total aerobic count or TAB) remained below 2.0 log cfu/g even in the control sample, only isolated colonies were observed, and there were no notable alterations postprocessing. The control sample displays yeast and mould (1.7 log cfu/g). However, after undergoing processing, no yeast and mould were detectable in any treatment. Throughout the storage period, the bacterial levels in the processed samples remained stable. Instances of yeast and mould were infrequent, with the exception of the 400 MPa juice stored for 49 days.

Some scientific literature suggests that treatments at pressures higher than 500 MPa are necessary to ensure microbial safety in certain food processing applications [18, 24]. However, in the specific case of our study, it is apparent that this level of microbial safety was not achieved. One possible cause for this outcome could be related to the treatment parameters or conditions used in the HPP process, such as time. Additionally, it is important to note that the TT employed in the study can be considered a mild treatment, which may not lead to complete microbial inactivation. The choice of treatment method, in this case, should be carefully considered in light of the specific microbial safety requirements of the product and the intended shelf life. In our study, the primary aim was to compare the efficacy of TT and HPP treatments, rather than reach safety levels, and it is important to note that different treatments and processing times may achieve varying levels of reduction. Further research and experimentation may be necessary to optimize treatment conditions and achieve the desired level of microbial safety in the processed food product.

3.3. Rheological Behavior. The apparent viscosity (Pa·s) of samples during the storage period was evaluated. A 2-way ANOVA was performed (log-transformed data) considering differences between processes and storage period. From the statistical analysis, it emerges that the different treatments determine a significant difference ($p < 0.01$) in the apparent viscosity of the sample. On the other hand, the storage period and the interaction between treatment and storage period were not significant. On day 0, the apparent viscosity of the HPP 400 and HPP 500 MPa samples were 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 at day 0. As can be seen in Figure 1, there are significant differences in the apparent viscosity measures between the samples treated at different pressures and the control.

Liberatore et al. [17] show that viscosity varied in apple juices among cultivars after TT and HPP. These differences may be linked to pulp composition and pectin content. For example, the Golden Delicious variety has high galacturonic acid and pectin levels that resulted in increased viscosity with HPP in comparison with the other varieties. Similar viscosity changes were observed in carrot juice due to factors like particle size and enzyme inactivation [25].

The rheological behavior 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 [26]. The characteristic property of pectin is the ability to form a gel in the presence of Ca^{2+} 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 [27].

The fact that there was no significant difference in viscosity compared to the control at 400 MPa and 500 MPa, but a noticeable increase at 600 MPa, can be explained by the nonlinear relationship between pressure and its effects on the rheological properties of the strawberry nectar. At 400 MPa and 500 MPa, the pressure might not have been strong enough to significantly affect the strawberry's structure and properties, so viscosity remained stable; however, at 600 MPa, the pressure could have reached a critical point where it started affecting viscosity more. It is known that some proteins and enzymes may need higher pressure to be affected [28, 29].

A study conducted on guava juice has shown that viscosity increases at pressures greater than 500 MPa, due to interactions between pectin and other components in the juice [30]. In a sensorial test of strawberry juice, it was observed that higher viscosity was only perceived in samples treated at 600 MPa [22].

The increase in apparent viscosity of strawberry nectar when treated with HPP technology at higher pressures, such as 600 MPa, can be attributed to several factors such as cell wall disruption, as high pressures are known to cause significant disruption in the cell structure of vegetables. This disruption results in the release of intracellular components,

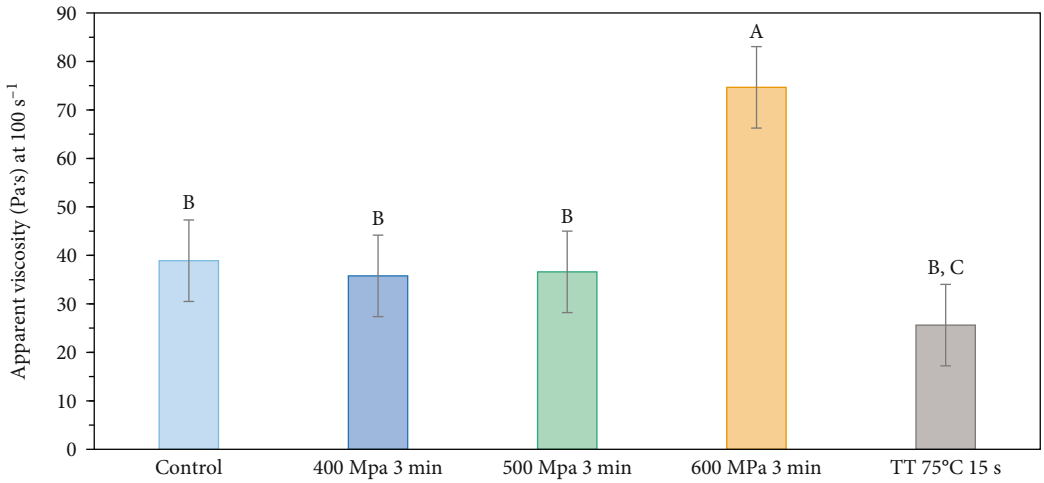

Figure 1: Apparent viscosity values determined at 100 s^{-1} in strawberry nectar samples at day 0. The means with different letters are significantly different ($p < 0.05$).

including pectin, into the nectar, leading to an increase in viscosity, and protein denaturation, as under high pressures, proteins present in strawberries can undergo denaturation and aggregation. This altered protein structure can contribute to the formation of gel-like structures within the nectar, thereby increasing its viscosity [29, 31].

The rheological analysis of the strawberry nectar samples yielded interesting insights into their behavior under different treatment conditions. Regardless of the treatment applied, all samples exhibited a consistent trend in their rheological profiles, as illustrated in Figure 2. Specifically, they displayed a pseudoplastic behavior, which means that the apparent viscosity decreased as the shear rate increased. This characteristic was consistent across all treatments, reflecting the inherent nature of strawberry nectar. However, a notable variation emerged when considering the treatment at 600 MPa for 3 minutes. In this case, the nectar exhibited significantly higher viscosity at all data points in comparison to the other treatments (Figure 2).

The results align with the parameters obtained from the power law model, as summarized in Table 2. The "n" values for all treatments were found to be less than 1, indicating shear-thinning behavior. Although specific "n" values were relatively similar across treatments, they collectively confirmed the pseudoplastic nature of the strawberry nectar. On the other hand, the "k" values showed expected values for the behavior seen before. Higher "k" values were associated with samples possessing greater viscosity, being the highest 22.80 ± 0.65 for 600 MPa 3-minute treatment, in comparison of the 7.90 ± 0.41 value for the control sample.

Notably, all fitted functions showed statistical significance. Additionally, the residual analysis affirmed the adequacy of these models in describing the observed rheological behavior of the strawberry nectar samples under various treatment conditions.

Understanding these rheological changes is crucial for optimizing processing parameters and ensuring the desired consistency and quality of strawberry-based products. Further exploration of these relationships may yield additional insights for industrial applications and product development.

3.4. Colour Analysis. Table 3 shows the results related to the CIE Lab parameters $\boldsymbol{L*}$, $\boldsymbol{a*}$, $\boldsymbol{b*}$, $\boldsymbol{h}$ °, and $\boldsymbol{C*}$ evaluated on day 0 and over 21 days of refrigerated storages, compared with the control sample. The statistical analysis suggests that, in general, all the parameters have significant differences ($\boldsymbol{p < 0.05}$) both concerning the type of treatment and the storage period and the interaction between the two. All the parameters of the treated samples evaluated on day 0 are statistically different from the control sample. However, $\boldsymbol{L*}$ parameter showed no significant differences ($\boldsymbol{p > 0.05}$) between treatments at day 0.

In the case of storage time, the brightness value ($L*$) changed during 21 days of refrigerated storage (4°C). $L*$ shows a slight increase in the case of the thermally treated samples. A similar behavior was noted on a pasteurized apple juice at 60-75°C for 15-20 seconds, attributed to the partial precipitation of unstable and suspended particles in the juice [32].

This behavior 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 [33].

$L*$ 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 0 and day 21. The decrease in the $L*$ parameter is associated with the residual enzymatic activity that, during the shelf life, brings about the degradation of anthocyanins. This is following the hypothesis of López-Serrano and Ros Barceló [7] to justify why a pressure at 400 MPa, regardless of the

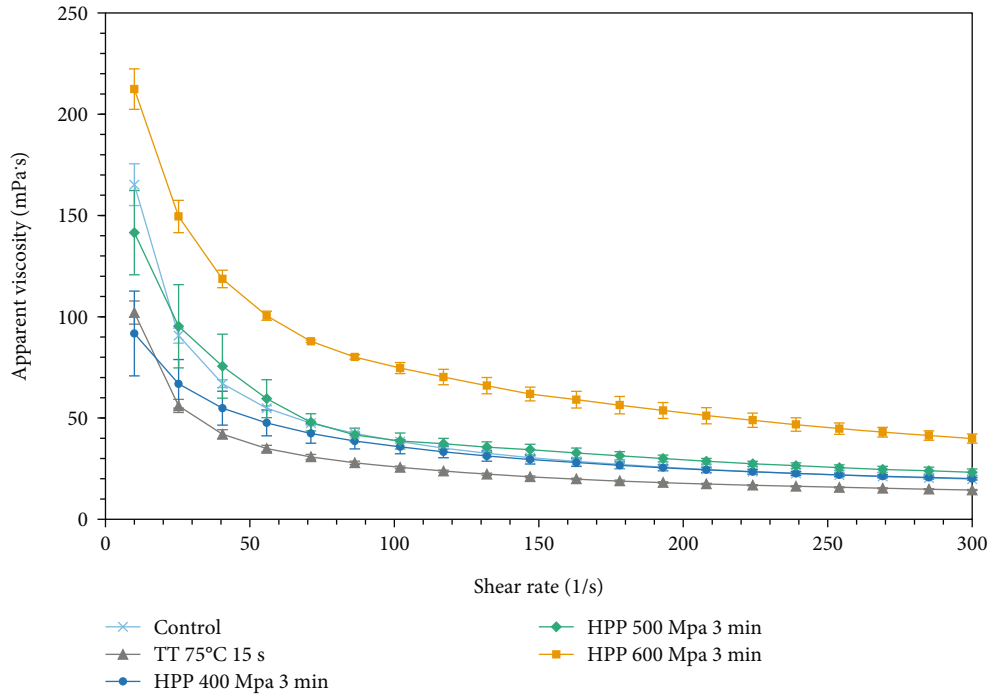

Figure 2: Viscosity curves of strawberry nectar (12°Brix) at day 0 measured at 25°C.

Table 2: Consistency index (k) and flow index (n) of strawberry nectar samples at different treatments.

Treatments	k	n
Control	7.90 ± 0.41	0.42 ± 0.01
TT 75°C 15 s	4.76 ± 0.22	0.47 ± 0.22
HPP 400 MPa 3 min	9.92 ± 1.83	0.38 ± 0.04
HPP 500 MPa 3 min	16.43 ± 1.83	0.29 ± 0.02
HPP 600 MPa 3 min	22.80 ± 0.65	0.34 ± 0.00

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 redness; concerning this, both treatment and the duration of storage have a noticeable impact. The highest $a*$ value is associated with the heat treatment. Conversely, the $a*$ value remains consistent for samples treated with high pressures. Regardless of the treatment, the $a*$ values consistently decrease as the storage period extends. It is possible to associate this decrease with the residual activity of the enzymes [6]. $b*$ value indicates the yellowish component and is higher in the TT sample, which disagrees with studies conducted on strawberry and peach puree, which attributed this result to browning caused by nonenzymatic reactions [24]. Additionally, $C*$ differs according to the type of treatment and during storage and always shows higher values in the case of the heat-treated sample. This result agrees with the study conducted on strawberry and peach puree where the $C*$ value for the samples subjected to heat treatment has a different colour intensity compared to the other samples. The values of h° were also different between treatments and vary over the storage period, again presenting the highest value for TT samples, therefore a red tint that is closer to yellow than the others.

Total colour difference ($\Delta E*$) 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 [34]. TT does not vary considerably and remained below the value of 3 (Figure 3). Also, in the strawberry puree and juice study by Aaby et al. [22], it was found that the colour of heat-treated products was more stable during storage than the colour of samples treated with high pressures. In this case, it is associated with the fact that during storage, there is an activation of the PPO in the HPP samples but not in the pasteurized ones [21].

The $\Delta E*$ values of all the samples, after the treatment, were found between 1.5 and 3 indicating that there is a perceptible difference in colour between the treated samples and the control. However, in all the samples subjected to high pressures, the value of $\Delta E*$ tends to increase during the storage period, up to day 21, to values greater than 3, which results in a noticeable colour difference compared to the control and TT samples as can be seen in Figure 3.

In general, other studies on strawberry juices have also shown a decrease in parameters such as $L*$, $a*$, and $b*$ and an increase in $\Delta E*$ during storage [35, 36]. 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 due to nonenzymatic 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 [36]. In addition, different reactions

Table 3: Colour changes in samples compared to control during 21 days of refrigerated storage.

	Control					HPP 400 MPa 3 min					HPP 500 MPa 3 min					HPP 600 MPa 3 min					TT 75 C 15 s				
	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^*
D0	33.74 ± 0.68 c	16.05 ± 0.23 b	7.97 ± 0.49 a	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 ± 0.1c. 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
D7						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
D14						33.92 ± 0.15 c.C	13.21 ± 0.06 d.C	6.13 ± 0.21 b.c.B	24.92 ± 0.63 a.A	14.56 ± 0.14 c.C	34.27 ± 0.24 b.B	14.01 ± 0.11 b.C	6.2 ± 0.29c. 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 ± 0.2c	22.63 ± 0.46 c.	14.62 ± 0.23 c	35.15 ± 0.11 a.A.B	14.91 ± 0.19 a.B	6.9 ± 0.3b. B	24.81 ± 0.69 a.B	16.43 ± 0.3 a.B
D21						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 ± 0.2 6c	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

Different letters indicate significant differences ($p < 0.05$) among days (small caps) and sample treatments (capital letters) by post hoc Tukey's test. Rows = difference between processes ($p < 0.01$); columns = difference between measures ($p < 0.01$).

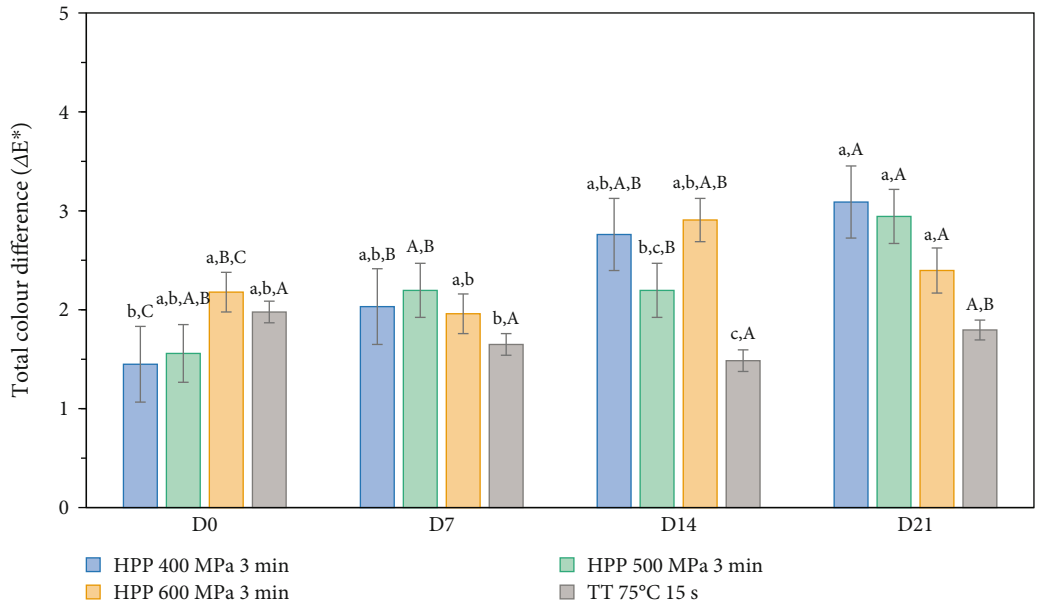

Figure 3: Total colour difference $\Delta E*$ of samples during the storage period. Different letters show a 2-way ANOVA (log-transformed data), and the error bars are the standard deviation (± SD).

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 [37].

3.5. Remaining PPO and POD Activity. As can be seen in Figures 4 and 5, the results of the statistical analysis allow us to state that the type of treatment to which the strawberry nectar has been subjected was not significant in the inhibition of PPO and POD, although some differences could be observed among samples.

The large variability in the data is believed to be due to the low concentration of enzymes in the raw material, and a more sensitive method should be developed. HPP 400, 500, and 600 MPa resulted in a RA of 21, 14, and 4%, respectively. TT resulted in a RA of 12%. On the other hand, the RA of POD of samples HPP 400, 500, and 600 MPa was 102, 52, and 44%, respectively, 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 the processes and the control, suggesting that the colour instability observed was due to nonenzymatic browning. 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% [6]. Other studies indicated that there was a 35% inhibition of PPO on strawberry puree following treatment at 30°C, 600 MPa for 5 minutes [37]; additionally, 11%, 12%, and 30% reduction at 400 MPa, 500 MPa, and 600 MPa for 3 minutes at 20°C, respectively, was observed [21]. As for the heat treatments, it was shown that 85°C for 2 min inhibited PPO activity by 25% compared to the control [21]. Other studies on strawberry fruit state that the enzyme is almost completely inactivated after 10 min at 65°C [38]. It is known that a temperature above 80°C is required to ensure the inactivation of PPO in fruit juices [39].

The enzymatic results obtained on the strawberry nectar of this study reveal a lower 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 have been attributed to differences in fruit variety of interest and growth conditions. The effectiveness of HPP in inactivating PPO is also associated with treatment conditions such as pressure, time, and temperature [39]. In general, an enzyme is more stable in intact tissue or in a homogenate matrix, where it is protected from the presence of other materials such as proteins and carbohydrates [40]. Nevertheless, activation of latent forms of the enzyme can also occur during treatment. It is possible that treatments (including thermal treatments) can cause an increase in the release of membrane-bound PPOs, counteracting the inactivating effect of the treatment itself [39].

Aditionally, the nectar recipe contains citric acid as an ingredient, which has also been reported to effectively inhibit PPO [37]. Its inhibitory effect is due to the chelation of copper located in the active site of PPO and the lowering of the pH [40]. 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.

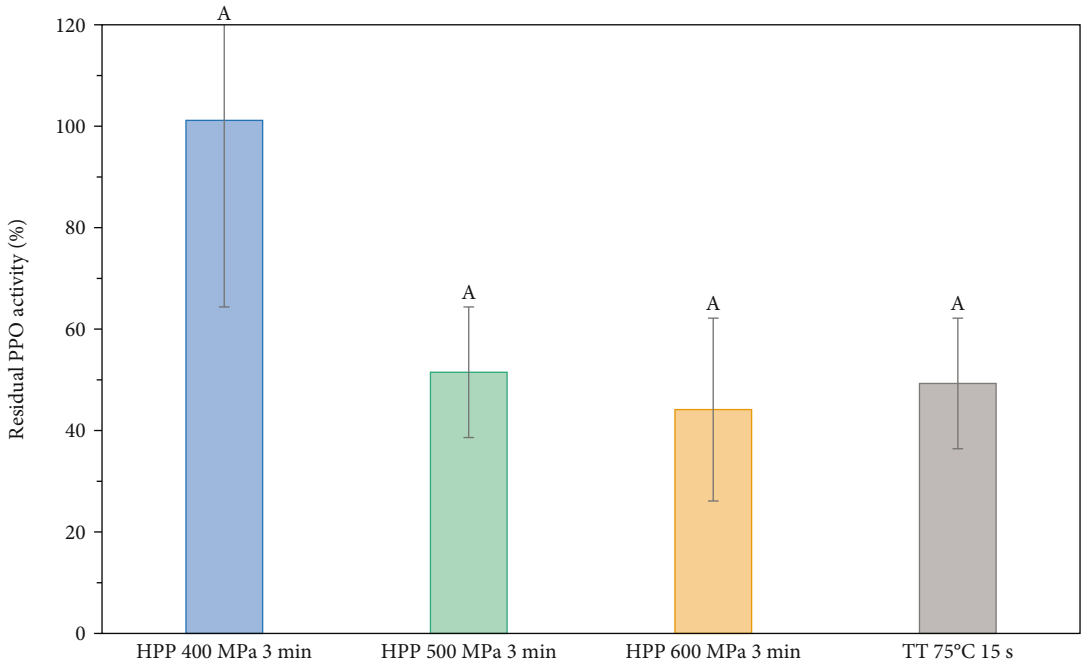

Figure 4: Residual activity (%) of PPO following HPP treatments and heat treatments. Letters indicate the difference between processes ($p < 0.01$), and the error bars are the standard deviation ± SD.

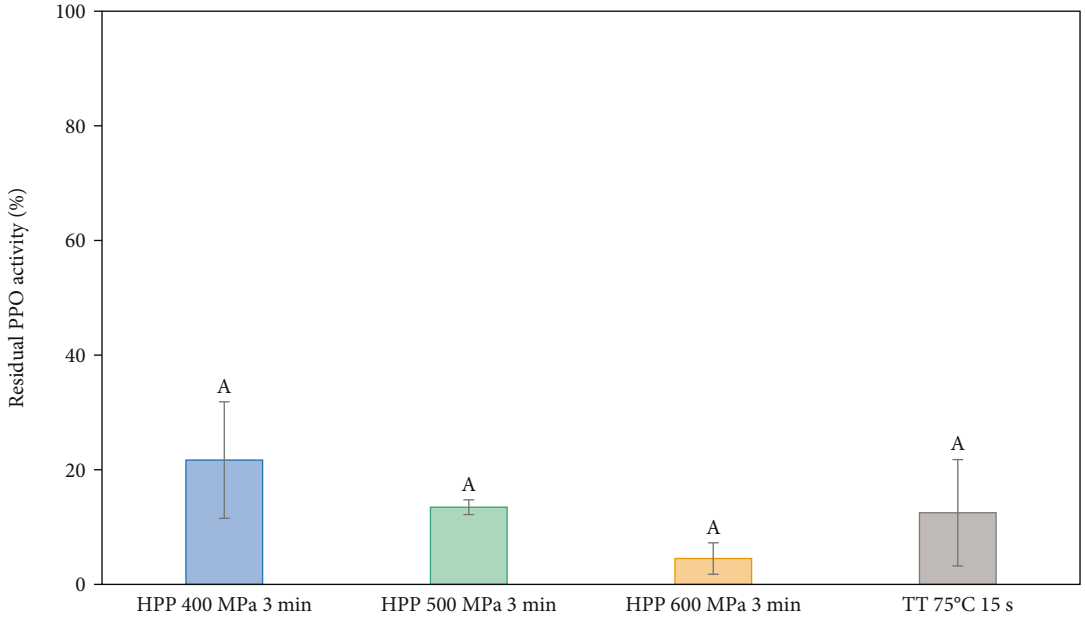

Figure 5: Residual activity (%) of POD following HPP treatments and heat treatments. Letters indicate the difference between processes ($p < 0.01$), and the error bars are the standard deviation ± SD.

3.6. Process Comparison. At day 0, the process that was most positively correlated with the control sample was HPP 400 MPa and 3 minutes ($R^2 = 0.9998$), meaning that the quality parameters of the treated nectar are more similar to the control, as shown in Figure 6(a).

It is important to notice that all treatments showed a high level of correlation with the control sample at day 0 and at day 21. However, at day 0, the correlation with the control of the samples at 600 MPa for 3 minutes was relatively lower ($R^2 = 0.9921$), even though the difference was small (Figure 6(c)).

On the other hand, at day 21, as shown in Figure 7, the treatment that exhibited the strongest positive correlation with the control sample was the TT, conducted at 75°C for 15 seconds ($R^2 = 0.9998$). This result is likely due to the fact that this treatment reduced enzyme activity, which in turn

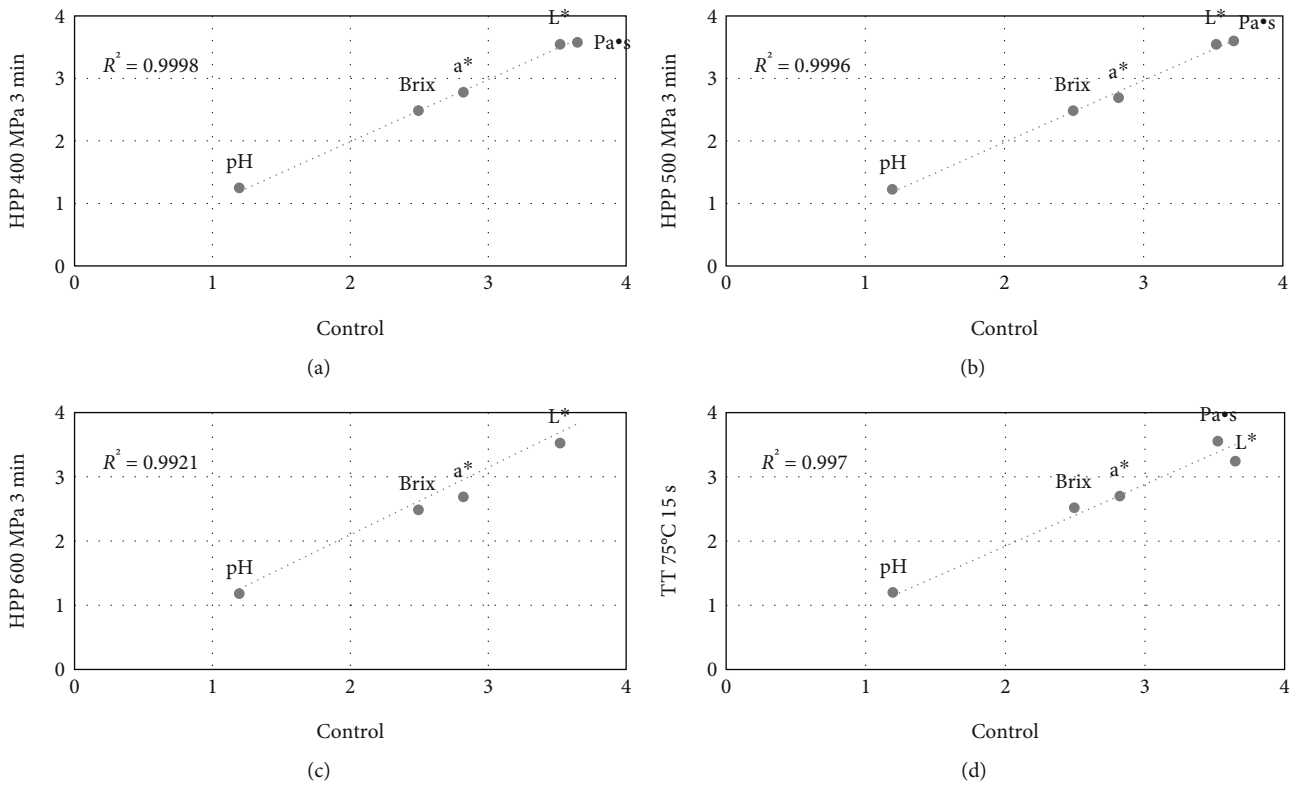

Figure 6: Closeness of five quality markers: pH, Brix, $a*$, $L*$, and viscosity (Pa·s), for treated samples vs control, measured on day 0 (log-transformed data). (a) 400 MPa 3 min, (b) 500 MPa 3 min, (c) 600 MPa 3 min, and (d) TT 75 C 12 s.

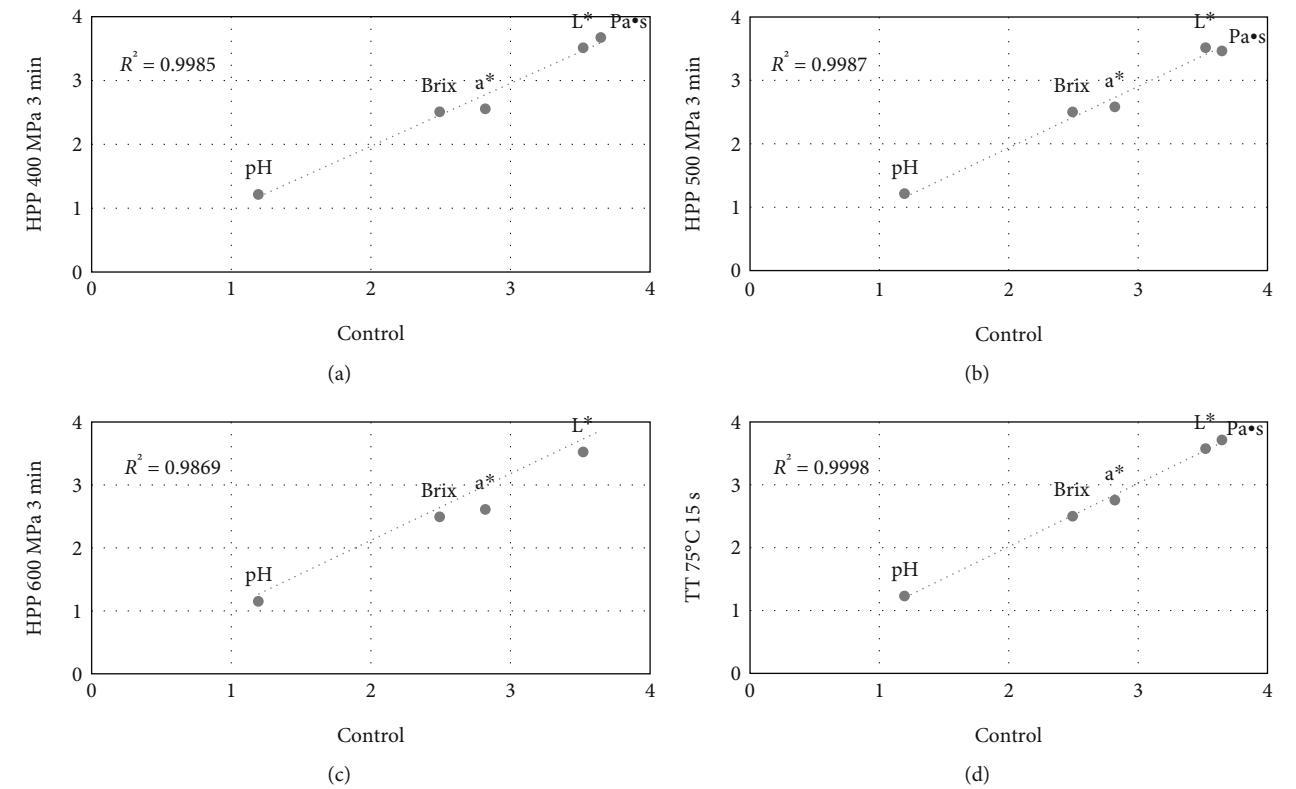

Figure 7: Closeness of five quality markers: pH, Brix, $a*$, $L*$, and viscosity (Pa·s), for treated samples vs control, measured on day 21 (log-transformed data). (a) 400 MPa 3 min, (b) 500 MPa 3 min, (c) 600 MPa 3 min, and (d) TT 75 C 12 s.

led to better colour stability and maintained relatively stable viscosity. In contrast, the samples treated at 600 MPa for 3 minutes showed a lower correlation with the control sample (Figure 7(c)). This divergence is likely a result of a significant increase in viscosity for these samples.

These findings align with the individual results that we obtained for colour analysis and viscosity. At day 0, the most noticeable difference in the sample treated at 600 MPa for 3 minutes was in viscosity. Similarly, even though there was no significant difference between day 0 and day 21, viscosity remained significantly different from the control. Additionally, at day 21, changes in the $L*$ and $a*$ parameters compared to the control sample contributed to a reduction in the correlation coefficient value.

4. Conclusions

In conclusion, the results and discussion provide valuable insights into the effects of different processing methods on strawberry nectar. Physicochemical analyses revealed that both thermal treatment (TT) and high-pressure processing (HPP) treatments maintained the pH within a stable range (3.1-3.5) during storage, indicating microbiological stability. Microbiological analyses demonstrated that TT was more effective in reducing bacterial counts (2-log reduction) compared to HPP treatments (1-log reduction), though HPP effectively controlled yeast and mould growth. The rheological behavior of the nectar varied with processing methods, with HPP at 600 MPa leading the increase in viscosity after treatment, with 75.1 Pa·s compared with the 39.2 Pa·s of the control sample. This effect was attributed to cell wall disruption and protein denaturation. Colour analysis showed that while TT samples exhibited better colour stability during storage, HPP-treated samples, especially at 600 MPa, showed perceptible colour changes ($\Delta E* > 3$). Enzyme activity analysis indicated that PPO and POD were inhibited without significant differences within treatments, with some variation among samples. Process comparison revealed that HPP at 400 MPa for 3 minutes closely resembled the control at day 0, while TT performed best in maintaining similarity to the control at day 21. Overall, this study highlights the importance of selecting the appropriate processing method based on specific product quality requirements and the need for further research to optimize treatment conditions for nectar production.

Data Availability

The experimental data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest between the authors and the funding body.

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