

Research Article

Preservation of Bioactive Compounds and Quality Parameters of Watermelon Juice Enriched with L-Citrulline through Short Thermal Treatment

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L-citrulline is a nonessential amino acid with demonstrated health benefits for humans, and watermelon is a fruit rich in this amino acid. The juice industry is developing functional beverages through the enrichment with external bioactive compounds; this kind of industry uses conventional pasteurization because of its efficiency and simplicity. In this experiment, the effects of pasteurization (80°C for 40 s or 90 s) and storage (4°C for 30 days) on different parameters were evaluated in a watermelon juice (3.68 g kg⁻¹ of natural L-citrulline) enriched with external L-citrulline (12 g kg⁻¹). Enzymatic activity (peroxidase, pectin methyl esterase, and polygalacturonase) was inactivated (74 to 89%, 89 to 90%, and 11 to 15%, resp.) with the pasteurization treatment, obtaining the highest degradation with the longest heating time. According to the rheology study, the juice's elasticity was mainly affected by type of heat treatment while its viscosity was more stable and affected by storage time. A reduction in bioactive compounds content, around 10–16% for lycopene and 19–20% for L-citrulline, was observed after the pasteurization treatments, with a higher decrease with increased treatment time. Storage time also induced a reduction in lycopene and L-citrulline. The shelf life was limited by sensorial parameters.

1. Introduction

At present, there is growing consumer interest on the benefits of foods with functional properties, as these foods have improved nutritional value, promoting better health and disease risk reduction. At this time, the food industry is promoting functional juices rich in vitamins, minerals, and antioxidants to help maintain good health. As a result, the market acceptance of watermelon juice is increasing worldwide due to its sensorial and health properties. Watermelon is very rich in bioactive compounds such as lycopene and L-citrulline, which makes it an extremely attractive product for consumers, with excellent growth prospects in the juice market [1, 2]. Lycopene is a carotenoid of great interest due to its antioxidant capacity and its role in the reduction of

coronary heart disease and some types of cancers such as prostate and kidney cancer [3]. L-citrulline is a nonessential amino acid that can be metabolized to L-arginine, an essential amino acid for humans, which produces nitric oxide (NO) [4], improving athletic performance and relieving muscle soreness [5]; it also has cardioprotective effects [6] among other properties. Thus, L-citrulline is commercially available in the form of pharmaceutical compounds or more recently as dietary supplement beverages such as juices. Watermelon juice enriched with L-citrulline can be defined as a functional food according to Goldberg [7]. A functional food is any food or food ingredient that has a positive impact on an individual's health, physical performance, or state of mind in addition to having nutritive value. Mandel et al. [1] described an increase in plasma citrulline and arginine

after the intake of 3.3 kg (fresh weight) of ripe watermelon. Moreover, Tarazona-Díaz et al. [5] observed that 500 mL of watermelon juice with 1.17 g or 6 g of L-citrulline helped to reduce the recovery heart rate and muscle soreness after 24 h.

Watermelon pulp contains from 1.1 to 4.7 g of L-citrulline kg^{-1} of fresh weight [2]. These variations could be due to preharvest and postharvest factors, which can affect the final concentration in juices. Therefore, watermelon juice should undergo an industrial process to guarantee its quality and safety during the shelf life of the product. One effective way of limiting microbial growth is to increase the juice's acidity by adding an acidifier in addition to other treatments such as high hydrostatic pressure pasteurization or conventional thermal treatments. Thermal treatments are still used by most of the fruit juice processing industries due to their simplicity and efficiency. Heat treatments are conventionally used to inactivate enzymes, ensure safety, and extend the shelf life of juices [8], but undesirable changes such as loss of bioactive compounds and sensory quality reduction are often induced [8, 9]. Reducing peroxidase (POD) activity in watermelon juice is important for avoiding color deterioration, off-flavor formation, and loss of nutrients [10]. On the other hand, decreasing the pectin methyl esterase (PME) and polygalacturonase (PG) activities could limit the degradation of pectins, decreasing the losses in viscosity and cloud stability [11]. Previously, Tarazona-Díaz and Aguayo [12] described that centrifugation and pasteurization (87.7°C for 20 s) of an acidified watermelon juice significantly reduced the red color, bioactive compounds (lycopene, antioxidant capacity, and total polyphenols), and sensory quality of the juice.

Hence, the creation of a noncentrifuged watermelon juice and the determination of the best timing and temperature conditions is important, as it could help maintain the attractive red color and sensorial quality, which are characteristic of watermelon juice, as well as the content of bioactive compounds, particularly lycopene and L-citrulline. In this experiment, we studied the effects of two different pasteurization treatments (80°C , for 40 s or 90 s) on shelf life under market storage conditions of a functional watermelon juice (3.68 g kg^{-1} of natural L-citrulline) enriched with external L-citrulline (12 g kg^{-1}).

2. Materials and Methods

2.1. Watermelon Juice Preparation. Seedless watermelons (*Citrullus lanatus* cv. "Fashion") were grown in open fields with a Mediterranean climate (Águilas, Murcia, Spain) and were harvested in late July at the commercial maturity stage ($>9.0^\circ\text{Brix}$). The fruits were transported (80 km) in a refrigerated vehicle to the laboratory in the Technical University of Cartagena (UPCT, Murcia, Spain) and kept at 10°C and 90% relative humidity (RH) in darkness until the next morning. Ten selected fruits, free from defects, with an average weight of $6.20 \pm 0.23 \text{ kg}$, were washed (100 mg L^{-1} sodium hypochlorite at pH 6.5 for 2 min) and dried. Afterwards, the pulp was separated from the skin and cut into sections (3 cm) and liquefied (Moulinex FruttiPro-BKA1, Spain), obtaining about 35 L of juice.

The juice, which contained 3.68 g L^{-1} of natural L-citrulline, was enriched with 12 g L^{-1} of L-citrulline (Acofarma, Barcelona, Spain). The pH was adjusted to 3.80 ± 0.08 by adding citric acid (Panreac, Barcelona, Spain) [11]. The total volume of watermelon juice was subsequently separated into three groups as follows: (a) two pasteurization watermelon juice (PWJ) treatments at 80°C for 40 s (PWJ-40 s), at 80°C for 90 s (PWJ-90 s) and cooling conducted in a thermoresistometer (Mastia, Cartagena, Spain) [13] and (b) unpasteurized watermelon juice (UPWJ) used as control treatment. This equipment was provided with software that allowed for the recording of both the pasteurization and cooling temperatures. This consisted of a stainless-steel container where the watermelon juice sample was placed, heated by means of an electric resistance, and continuously stirred with the provided stirrer. After pasteurization at the set temperature and time, the thermoresistometer was programmed to automatically cool to 10°C , through a cold water bath ($1.4\text{--}2^\circ\text{C}$) that was connected to the equipment. The cooling rate was 7°C min^{-1} . Finally, when the cooling temperature was stabilized, the vessel containing the pasteurized juice was removed, and amber bottles, which were previously sterilized (121°C for 15 min), were rapidly filled under sterilized conditions and kept at 4°C for 30 days in a refrigerated incubator (Sanyo, Japan). For each pasteurization treatment, three rounds of pasteurization were performed. During the 30 days of storage time, samples were evaluated every 5 days, using three replicates per treatment (bottles of 150 mL) for each sampling day. Prior to collection of the data for the present work, two preliminary experiments were performed to observe changes and to establish the experimental conditions.

2.2. Analysis of Physicochemical Attributes. The analytical parameters pH (Crison 2001 pH meter, Crison Instruments SA, Barcelona, Spain) and solid soluble content (SSC) (digital refractometer, Atago Company Ltd., Tokyo, Japan) were measured as described by Tarazona-Díaz and Aguayo [12].

2.3. Color Measurements. Watermelon juice color at three equidistant points was determined using a Chroma meter (CR-300, Minolta, Ramsey, NY, USA) as described by Tarazona-Díaz and Aguayo [12]. The results are expressed as hue angle ($^{\circ}h = \tan^{-1}(b^*/a^*)$) and color index ($\text{CI} = [(a^*)(1000)]/[(L^*)(b^*)]$).

2.4. Biofunctional Compounds

2.4.1. Lycopene Content. Lycopene content was determined according to the methodology reported by Fish et al. [14] with some modifications [2]. Results are expressed as $\text{mg lycopene kg}^{-1}$ watermelon juice.

2.4.2. Citrulline Content. Citrulline analyses were carried out by HPLC-DAD-MS-APCI for the analysis of underivatized free amino acids in foods [15]. Total citrulline content was expressed as g kg^{-1} of watermelon juice.

2.5. Enzymatic Activity

2.5.1. Peroxidase (POD; EC 1.11.1.7). POD activity in watermelon juice samples (10 mL) was measured using the method described by Elez-Martínez et al. [16] with some modifications. POD activity was determined in the supernatant by using 96-well microplates (Greiner Bio-One, Frickenhausen, Germany) in a Tecan Infinite® 200 microplate reader (Grödig, Austria) at 509 nm for 10 min at 25°C. The reaction was started by adding 9 µL of the enzymatic extract to the well containing 243 µL of reaction medium described by Elez-Martínez et al. [16]. The enzyme activity unit was defined as the variation of 0.001 units of absorbance per min per mL of protein extract. The protein concentration was calculated according to the Bradford method [17].

2.5.2. Pectin Methylesterase (PME; EC 3.1.1.11). PME activity was measured using the method described by Kimball [11] and Anthon et al. [18] with some modifications. Watermelon juice (10 mL of each sample), previously warmed to 30°C in a water bath (JP Selecta, Barcelona, Spain), was mixed with pectin-salt substrate at 1% (40 mL) and incubated at 30°C and the pH 7 was adjusted with 2 N NaOH. Consumption of 0.05 N of NaOH (98% purity, Panreac, Spain) was recorded each minute during the reaction period of 10 min. The PME activity was expressed in units (U) PME per mL of watermelon juice.

2.5.3. Polygalacturonase (PG; EC 3.2.1.15). The determination of PG was carried out in watermelon juice samples (2.5 mL) using the method described by Aguiló-Aguayo et al. [19] with some modifications. The pellet dissolved in NaCl was shaken at 200 rpm (orbital shaker, Stuart SSL1, UK) at 4°C for 1 h. PG activity unit (U) was expressed as micromoles of galacturonic acid reducing equivalent per mL of watermelon juice per minute at 35°C. As a standard, α-D-galacturonic acid (≥98% purity, Sigma, Spain) was used.

2.6. Rheological Measurements. Stability was measured as described by Ramos and Ibarz [20]. Rheological experiments were executed using an AR G2 stress-controlled rheometer (TA Instruments, New Castle, US) equipped with a 40 mm aluminum plate. Samples (30 mL) were analyzed at 15°C. Frequency tests were performed within the linear viscoelastic region in a range of 100–0.01 Hz. The results of the oscillatory rheological measurements were expressed in terms of the storage modulus (G') in Pascals (Pa), where G' measured the material's ability to store energy. Three replicates of each treatment were analyzed in duplicate.

2.7. Microbial Analyses. Samples weighing 25 g were homogenized for 10 s in 0.1% sterile buffered peptone water (AES Laboratoire, Combours, France) (1:10 dilution). Standard enumeration methods were used to determine the microbial growth. For the enumeration of each microbial group (mesophilic, *Staphylococcus aureus*, enterobacteria, and yeasts and filamentous fungi), a dilution series were prepared in 9 mL of sterile, buffered peptone water (Scharlau

Chemie SA, Barcelona, Spain). The presence of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* was also evaluated according to the EU legislation (Regulation EC 1441/2007, 2007) [21]. The media and incubation conditions for each microbial group were previously described by Tarazona-Díaz and Aguayo [12]. Three samples (bottles of juice) of watermelon juice (UPWJ, PWJ-40 s, and PWJ-90 s) were analyzed in duplicate at day 0 and at every 5 days of storage. Microbiological counts were expressed as log colony forming units per mL of juice (log CFU mL⁻¹).

2.8. Sensorial Analyses. Evaluation of the sensorial quality of watermelon juice (color, aroma, taste, and overall acceptability) was performed in a room at 20°C. The evaluations were performed by a panel of 15 judges (eight female and seven male; aged between 25 and 60 years) trained to recognize and score the quality attributes of watermelon juice using fresh and stored samples. Each juice was presented in a randomized order. Approximately 20 mL of sample juice in a clear plastic cup with a 3-digit code was given to each panel member. A nine-point hedonic scale was used: 1 = extremely dislike, not characteristic of the product, 5 = neither like nor dislike, limit of acceptance from the consumers' point of view, 9 = extremely like, very characteristic of the product [22]. The definition for this juice was natural product, 100% obtained from the edible part of ripe and fresh watermelons, obtained via a mechanical extraction procedure, followed by pasteurization as a preservation treatment, without added sugars, without preservatives. The color of the juice must be an intense red, with good appearance, characteristic watermelon aroma and taste, and good acceptance.

2.9. Statistical Analysis. Analysis of variance (ANOVA) was performed to compare the different pasteurization treatments and storage times at a significance level of $p \leq 0.05$ using SPSS Statistics 22 for Windows (SPSS Inc., Chicago, IL, USA). In some cases, when significant differences were observed, Tukey's HSD (Honestly Significant Difference) test was applied. In the rest of cases, the least significant differences (LSD) test at a significance level of $p \leq 0.05$ for pasteurization treatments and storage time was used, with the results shown in the figures and the table. Bilateral correlations were determined by Pearson's correlation coefficient with confidence intervals set at 95%.

3. Results and Discussion

3.1. Analysis of Physical and Chemical Attributes. The initial pH of the acidified UPWJ was 3.80 ± 0.08 . No significant differences were observed in pH or SSC with thermal treatment. The pH increased slightly with pasteurization to 3.86 and 3.89 for PWJ-40 s and PWJ-90 s, respectively. SSC had an average of $10.17 \pm 0.03^\circ$ Brix. However, the storage time significantly reduced the pH and SSC. After 30 days of storage, the pH was 3.68 ± 0.01 and SSC decreased to $10.07 \pm 0.03^\circ$ Brix, without differences between the thermal treatments. In other types of juices such as orange, pasteurization at 94.6°C for 30 s maintained pH and SSC during the 22 days of storage at 4°C,

TABLE 1: Effects of pasteurization treatment (80°C for 40 s or 90 s) and storage (4°C for 30 days) on lycopene and L-citrulline content of watermelon juice enriched with L-citrulline.

| Storage time (days) | Lycopene (mg kg ⁻¹) | | | L-citrulline (g kg ⁻¹) | | |
|---------------------|---------------------------------|-----------------------------|-----------------------------|------------------------------------|------------------------------|------------------------------|
| | UPWJ | PWJ-40 s | PWJ-90 s | UPWJ | PWJ-40 s | PWJ-90 s |
| 0 | | 11.72 ± 0.40 ^{Ba} | 10.91 ± 0.60 ^{Ca} | | 12.75 ± 0.03 ^{Ba} | 12.59 ± 0.16 ^{Ba} |
| 5 | | 11.70 ± 0.02 ^{Aa} | 10.95 ± 0.15 ^{Ba} | | 12.75 ± 0.03 ^{Aa} | 12.56 ± 0.03 ^{Ba} |
| 10 | | 11.72 ± 0.11 ^{Aa} | 10.92 ± 0.34 ^{Ba} | | 12.74 ± 0.03 ^{Aa} | 12.54 ± 0.05 ^{Bab} |
| 15 | 12.97 ± 0.08 ^A | 11.55 ± 0.11 ^{Aa} | 10.90 ± 0.13 ^{Ba} | 15.68 ± 0.05 ^A | 12.70 ± 0.01 ^{Aab} | 12.48 ± 0.03 ^{Bab} |
| 20 | | 11.44 ± 0.08 ^{Aa} | 10.70 ± 0.08 ^{Ba} | | 12.52 ± 0.01 ^{Aabc} | 12.40 ± 0.03 ^{Babc} |
| 25 | | 10.98 ± 0.12 ^{Aab} | 10.48 ± 0.17 ^{Bab} | | 12.49 ± 0.02 ^{Abc} | 12.33 ± 0.04 ^{Bbc} |
| 30 | | 10.51 ± 0.16 ^{Ab} | 10.13 ± 0.15 ^{Bb} | | 12.38 ± 0.03 ^{Ac} | 12.04 ± 0.09 ^{Bc} |

UPWJ, unpasteurized watermelon juice. PWJ-40 s, pasteurized watermelon juice for 40 s. PWJ-90 s, pasteurized watermelon juice for 90 s. Values correspond to measurements ($n = 3$) ± SE. Different capital letters in the same row show significant differences between treatments. Different lower case letters in the same column show significant differences between storage times. Control samples were only evaluated to test significant differences between pasteurized treatments at 0 days.

after which the microorganisms reduced the pH and °Brix values [23]. Esteve et al. [24] reported that the decrease in pH in refrigerated orange juices from Spain could indicate the start of spoilage or fermentation of the sample.

3.2. Color Measurements. The color of a product is the first quality factor that the consumer takes into account and has an important influence on the acceptance of a beverage. In this experiment, color was affected by pasteurization treatments and time of storage and by the interaction between both factors. The a/b ratio, which is frequently used to evaluate the redness of juices, showed a reduction without significant differences between pasteurization times (data not shown). Aguiló-Aguayo et al. [25] previously reported that watermelon juices subjected to heat treatment (90°C, 60 s) exhibited an increase in a and b values, which may be due to the formation of dark compounds causing browning of juices. The effects of pasteurization and storage time on h° and color index (CI) of thermal juices are shown in Figure 1. Chroma and h° from PWJ increased the value with respect to UPWJ, but CI decreased in thermal-treated juices. The PWJ-90 s treatment resulted in a juice with less red color (increased h° and decreased CI) than PWJ-40 s. The pasteurization time, 90 s versus 40 s, could induce a higher oxidation of thermolabile pigments that are responsible for the red color such as lycopene (Table 1). S. Srivastava and A. K. Srivastava [26] reported that under heat treatment lycopene is degraded via isomerization and oxidation.

In both thermal treatments, a red-orange color was observed with storage. The highest values in Chroma and h° were detected at 10 days of storage in both pasteurization treatments. These changes in color were probably associated with the loss of stability due to the residual enzymatic activity found in watermelon juices (Figure 2).

3.3. Biofunctional Compounds

3.3.1. Lycopene Content. The initial lycopene content in untreated watermelon juice was 12.97 ± 0.08 mg kg⁻¹. After pasteurization, the content was reduced by around 10% for PWJ-40 s and around 16% for PWJ-90 s (Table 1). During

storage, no significant differences were observed between the lycopene contents at day 0 after the treatments and at 25 days of storage, with the samples pasteurized for 40 s maintaining the highest content (Table 1). In this study, lycopene content was more affected by pasteurization conditions (80°C, 40 s versus 90 s) than by storage conditions (4°C for 30 days), although significant losses at the end of storage were also observed. At 30 days of storage, the lycopene losses were around 10% for PWJ-40 s and 7% for PWJ-90 s. Similar results were reported for watermelon juice, which showed higher losses after thermal treatment (88°C for 20 s) than during the 30 days of storage time [12]. Additionally, a previous study detected a loss of about 40% in the lycopene content after conventional sterilization of tomato puree [27]. However, Knockaert et al. [28] only measured a significant decrease (20–30%) in the lycopene content of tomato puree after thermal sterilization (117°C for 1.5 min or 3 min), while no significant differences between tomato puree treated at 90°C for 10 mins and 60°C for 1 min and untreated samples were observed.

The degradation of lycopene is influenced by factors such as oxygen, light, reaction medium, temperature, physical state, and environmental conditions. Lee and Chen [29] studied the stability of lycopene by heating standard lycopene at 50°C, 100°C, and 150°C. For 50°C, there was no significant change in all-*trans*-lycopene found within the first 12 hours. For 100°C the levels of all-*trans*-lycopene decreased by 78% after 120 minutes of heating. The mono-*cis* forms of lycopene showed a decreasing trend in concentration, and for 150°C not all-*trans* lycopene was detected after 10 min. The levels of all the mono-*cis* forms of lycopene showed the same trend. These authors concluded that due to the increasing temperature and heating time, degradation dominated over isomerization. Moreover, Maillard reactions and Strecker degradation products were detected in the thermal treatment (74°C for 45 s) of watermelon juice [30]. Lycopene was positively correlated with Chroma (0.540**) and PG (0.900*), while showing a negative correlation with h° (-0.811**), CI (-0.704**), and POD (-0.986**). These results can help the beverage industry to understand how to work with

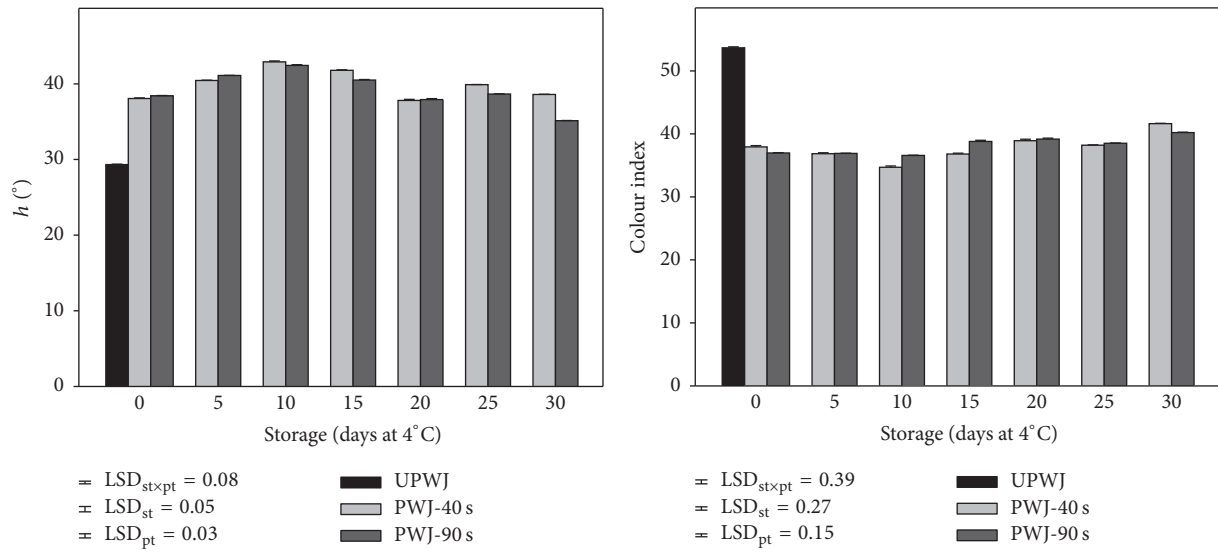


FIGURE 1: Effect of pasteurization treatment (80°C for 40 s or 90 s) and storage (4°C for 30 days) on hue (h°) and color index (CI) of watermelon juice enriched with L-citrulline. LSD values are shown for interaction and both factors. st, storage time; pt, pasteurized treatment. UPWJ, unpasteurized watermelon juice. PWJ-40 s, pasteurized watermelon juice for 40 s. PWJ-90 s, pasteurized watermelon juice for 90 s.

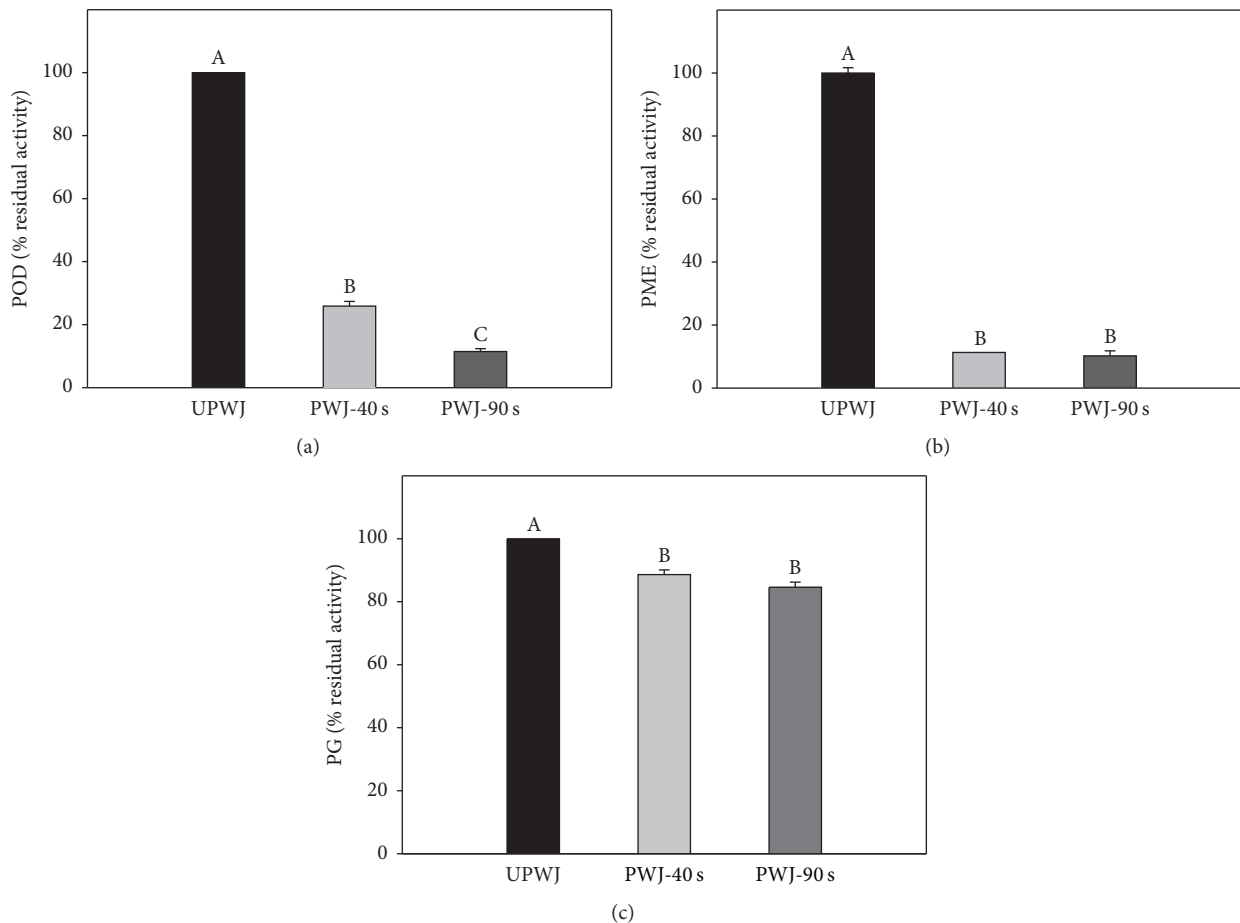


FIGURE 2: Effect of pasteurization treatment (80°C for 40 s or 90 s) with respect to unpasteurized juice (UPWJ) at day 0 on enzymatic activity (POD, PME, and PG) of watermelon juice enriched with L-citrulline. Mean values denoted with the same letter do not significantly differ statistically, $p \leq 0.05$. UPWJ, unpasteurized watermelon juice. PWJ-40 s, pasteurized watermelon juice for 40 s. PWJ-90 s, pasteurized watermelon juice for 90 s.

these parameters so that the lycopene contents of the juices are better retained. On the other hand, the presence and stability of other antioxidants such as polyphenols or thermal inactivation oxidizing enzymes (such as POD) could promote the lycopene stability [31].

3.3.2. Citrulline Content. The natural L-citrulline content in watermelon juice was of 3.68 g kg^{-1} . It was supplemented with 12 g kg^{-1} previous to heat treatments, so the initial L-citrulline content in enriched watermelon juice was 15.68 g kg^{-1} . Amino acids are highly important components of the daily diet, even at relatively low levels. In addition, as citrulline supplementation increases plasma concentration of both arginine and citrulline, the enriched watermelon juice could be considered a functional beverage, as it enhances sports nutrition in humans [32] and can be used to prevent arginine and nitric oxide deficiencies under various physiological and pathological conditions [33].

After heat treatments, L-citrulline was degraded around 19% (for PWJ-40 s) and 20% (for PWJ-90 s) without significant differences between heat treatments (Table 1). L-citrulline content showed significant reduction at 30 days of storage (3% loss in PWJ-40 s and 4% loss in PWJ-90 s). As with lycopene, the heat treatment affected the L-citrulline content more than the storage conditions. These results could be related to the thermal treatment that modified the structure of amino acids. Sereewatthanawut et al. [34] have said that, with increasing temperature, some amino acids are degraded to form low molecular weight carboxylic acids such as formic, acetic, and propionic acids, resulting in a reduction of the total amino acid concentration. On the other hand, in fermented orange juice, the amino acid profile, including citrulline, was not modified by pasteurization (85°C for 30 s); however, the fermentation time (9 days) significantly increased the total amino acid content [35].

3.4. Enzymatic Activity. Most of the undesirable changes in the characteristic red color, viscosity, and taste of watermelon juice are catalyzed by enzymes such as peroxidase (POD), pectin methylesterase (PME), and polygalacturonase (PG). These enzymes were analyzed at day 0.

3.4.1. POD Activity. POD activity was reduced after the application of heat treatments and was dependent on the duration of the treatment. The residual POD activity was around 26% in PWJ-40 s samples and 12% in PWJ-90 s samples (Figure 2(a)). Similarly, Aguiló-Aguayo et al. [36] described a similar residual POD activity after the application of thermal treatments (90°C for 40 s or 60 s). It was rapidly reduced in the first week of storage and remained constant until day 30. POD showed a positive correlation with h° (0.955^{**}) and a negative correlation with Chroma (-0.967^{**}), CI (-0.848^*), and lycopene (-0.986^{**}). Therefore, the inactivation of this enzyme is very important for the maintenance of the characteristic red color of watermelon juice and its lycopene content.

3.4.2. PME Activity. Heat treatments reduced the PME activity, without significant differences between both treatments.

PWJ-40 s samples showed a residual PME activity of around 11% and PWJ-90 s of around 10% (Figure 2(b)). These values were lower than those obtained by Aguiló-Aguayo et al. [36] in watermelon juice that had been thermally treated at 90°C for 60 s or 30 s, which obtained 25% and 38% of PME activity, respectively. Other results in clementine tangerine juice, after pasteurization at 74°C for 10 s, reduced the initial value of PME activity to 7% [37]. Changes in the percentage of inactivation could be due to the differences in plant material [18]. In addition to these results, previous authors observed that a conformational change of the enzyme could explain the high percentage of inactivation reached with mild temperatures [25]. PME is an enzyme that is known to participate in vegetable softening processes and the catalysis of the hydrolysis of methylester groups of cell wall pectin. From an industrial point of view, a juice is commercially stable when the residual enzymatic activity remains below 10% [19]. According to our results, both pasteurized treatments could be considered commercially stable.

3.4.3. PG Activity. After thermal treatment, the residual PG activity was around 89% in PWJ-40 s and around 85% in PWJ-90 s samples, being the most thermoresistant enzyme analyzed in our study (Figure 2(c)). These values were lower than those obtained by Aguiló-Aguayo et al. [36] in watermelon juice at 90°C for 60 s or 30 s. This author did not observe significant differences between thermally treated and nontreated juices. The incomplete reduction of PME and PG could explain the loss of stability in watermelon juice depending on the duration of the heat treatment [25]. A previous research stated that the PG present in whole tomato occurs in two forms: one stable in heat and another that is thermolabile, and therefore, the low inactivation of the PG could be attributed to the resistance of the heat-stable form of PG [19]. Therefore, the high residual PG activity obtained in our study could be due to the presence of the heat-stable PG isoform.

3.5. Rheological Features. The rheology of fruit juices is very useful in quality control and sensory evaluation and for predicting storage stability. For all the treatments, the storage modulus (G') was always higher than the loss modulus (G'') in the oscillatory frequency (ω) range evaluated (Figure 3). This indicates that the elastic properties of watermelon juice are dominant, rather than the viscous ones. The magnitudes of both increased with frequency. However, the dependence of G'' on the oscillatory frequency was greater than for G' , especially at high frequencies. Moreover, the differences between G' and G'' decreased with the storage time, and an intersection between both curves was observed depending on treatment and day of storage. The tangent of phase angle (δ) showed the lower capacity of UPWJ to maintain integrity rather than PWJ, as well as the time of storage being affected in PWJ-90 s before PWJ-40 s (Figure 3).

At day 0, G' and G'' increased with an increase in the oscillatory frequency of up to 80 Hz, except in control samples, where G' started to decrease at 40 Hz and was crossed over with G'' at 19.95 Hz as shown by the tangent (δ) (Figure 3). The dynamic viscosity of the watermelon

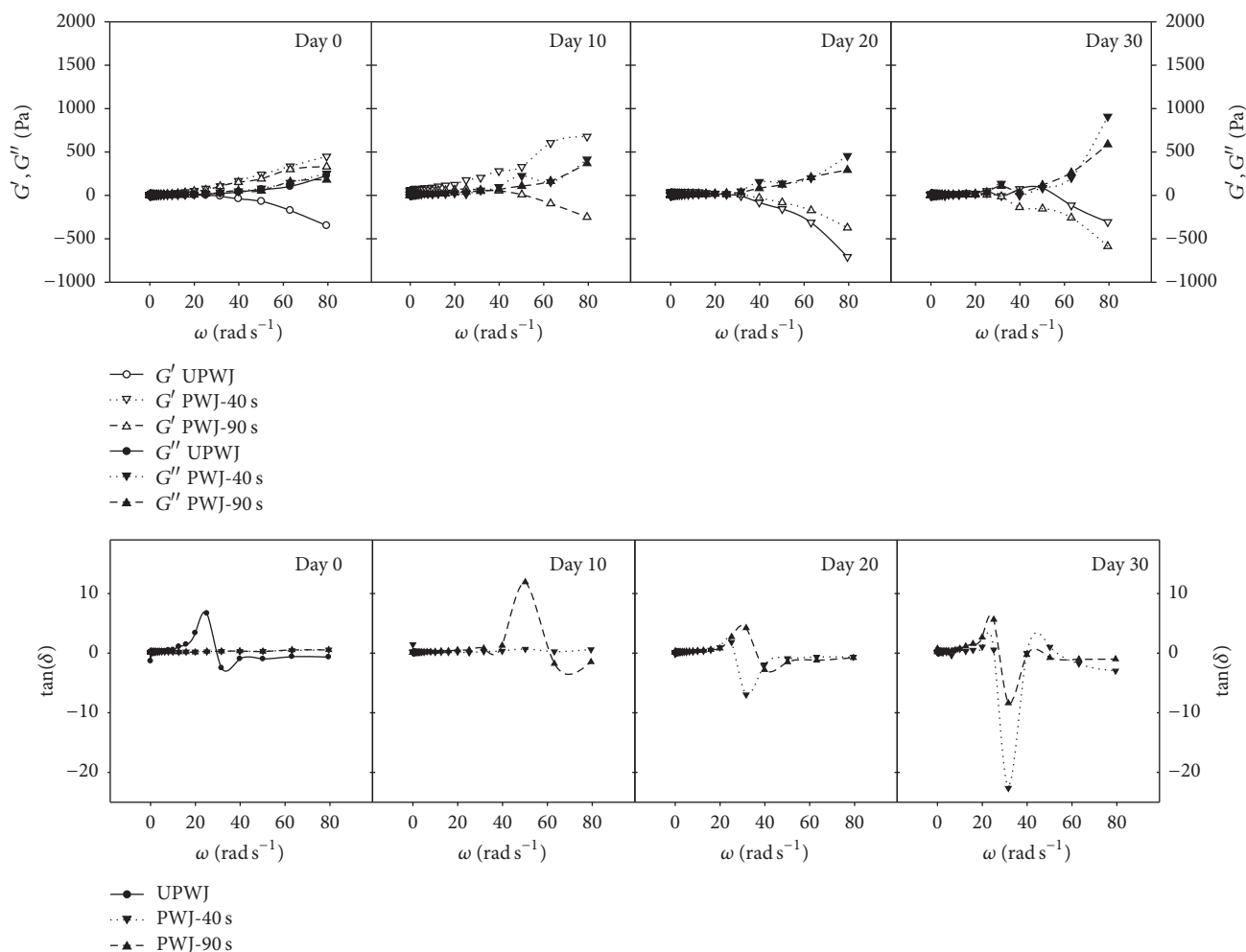


FIGURE 3: Effect of pasteurization treatments (80°C for 40 s or 90 s) during storage at 4°C for 30 days on viscoelastic behaviour and loss tangent of watermelon juice enriched with L-citrulline. UPWJ, unpasteurized watermelon juice. PWJ-40 s, pasteurized watermelon juice for 40 s. PWJ-90 s, pasteurized watermelon juice for 90 s.

juice after thermal treatment was probably similar due to the nonsignificant differences in PME and PG residual activity in the different pasteurized juices at day 0. Similar results were previously described in watermelon juices treated with thermal, ultraviolet-C, and high pressure treatments at the same temperature (60°C) where a correlation between PME activity and dynamic viscosity has been described [38]. In agreement with our results, Aguiló-Aguayo et al. [36] did not observe a significant change in viscosity at day 0 between treatments, detecting a lower viscosity during the storage in samples pasteurized for 30 s with respect to 60 s, maintaining the highest viscosity values during the storage period. In previous studies, changes in color and viscosity of tomato juice were assigned to the activity of the enzymes POD, PME, and PG [19]. In our study, at 10 days of storage, an intersection point at 31.62 Hz was observed in the PWJ-90 s treatment, while at 20 days this occurred at 19.95 Hz for both treatments. Also, a similar point was detected at 30 days for PWJ-40 s and at 12.59 Hz for PWJ-90 s. These differences represent a greater degree of gel strength in PWJ-40 s as compared to PWJ-90 s and the changes in rheological characteristics showed

by tangent (δ) could be due to changes in the concentration of pulp in suspension or a break in the microstructure of watermelon juice. These results show that pasteurization for 40 s provided more stability than at 90 s, with the elastic behaviour principally affected by heat treatment and then by storage time, while viscosity was more stable and affected by storage time at higher frequencies.

3.6. Microbiological Analysis. In the present study, mesophilic aerobic bacteria, enterobacteria, yeast and filamentous fungi, *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., and *Listeria monocytogenes* were analyzed. Pathogenic bacteria were not detected in any pasteurized watermelon juice. Likewise, UPWJ samples showed a *S. aureus* count close to 2 log CFU mL⁻¹, but after heat treatment *S. aureus* was not detected. Microbial counts for mesophilic aerobic bacteria, enterobacteria and yeast and filamentous fungi are shown in Figures 4(a)–4(c).

After heat treatments, the mesophilic aerobic bacteria counts were reduced between 1.2 and 1.4 logarithmic cycles (Figure 4(a)), while an enterobacteria reduction of

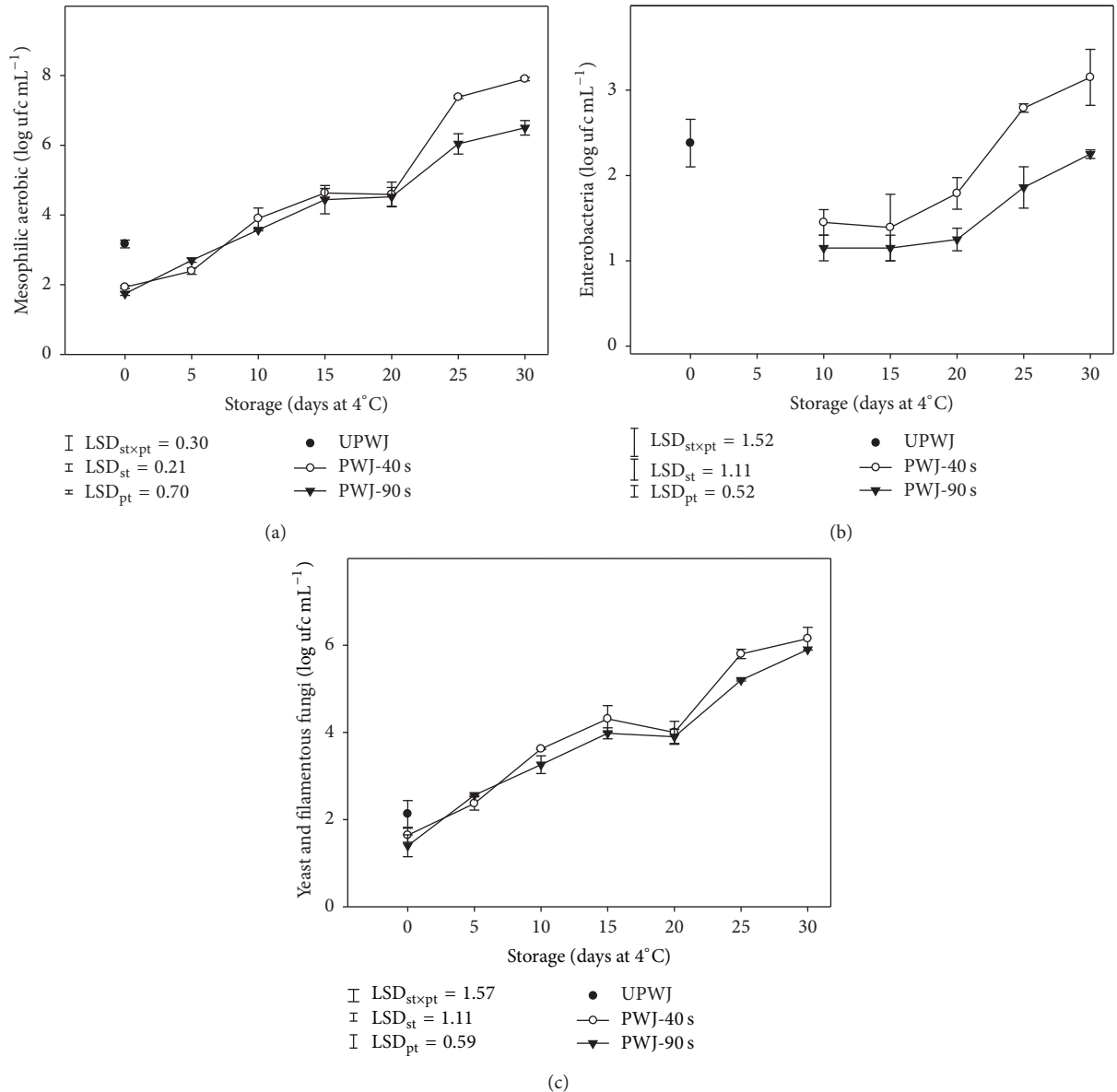


FIGURE 4: Effect of pasteurization (80°C for 40 s or 90 s) and storage (4°C for 30 days) on microbial counts (log CFU mL⁻¹) of watermelon juice enriched with L-citrulline. LSD values are shown for interaction and both factors. st, storage time; pt, pasteurized treatment. UPWJ, unpasteurized watermelon juice. PWJ-40 s, pasteurized watermelon juice for 40 s. PWJ-90 s, pasteurized watermelon juice for 90 s. Enterobacteria counts remained under the detection limit (<1 log cfu g⁻¹) in pasteurized juices during the first 5 days of storage.

1.3 log CFU mL⁻¹ was obtained (Figure 4(b)); it remained under 1 log cfu g⁻¹ for the thermal samples during the first 5 days of storage. Lastly, within filamentous fungi and yeasts, a reduction of 0.5 to 0.7 logarithmic cycles was obtained, depending on pasteurization time (Figure 4(c)). The storage time showed a clear increase in the growth of mesophilic aerobic bacteria, enterobacteria, and filamentous fungi and yeast observed in the PWJ, yielding a higher growth of mesophilic bacteria and enterobacteria in PWJ-40 s juice as compared to PWJ-90 s juice. At 30 days of storage at 4°C, there was no detection of pathogenic growth and the microbial quality was tolerable in all of the thermal treatments. Therefore, the shelf life according to the microbial acceptability could reach

30 days for both types of heat treatments. These microbial counts were higher than previously detected in watermelon juice acidified and pasteurized at 87.7°C for 20 s [12].

3.7. Sensory Evaluation. The overall quality of the PWJ was significantly lower than that of the UPWJ (Figure 5). In addition, a significant reduction with the time of pasteurization and storage was detected, but there was no interaction between these factors. Mainly, taste and aroma followed by color were the parameters that were more affected by heat treatment time (data not shown). After 15 days of storage, PWJ-90 s was close to the limit of acceptability due to the low score in overall quality (Figure 5). In line with these

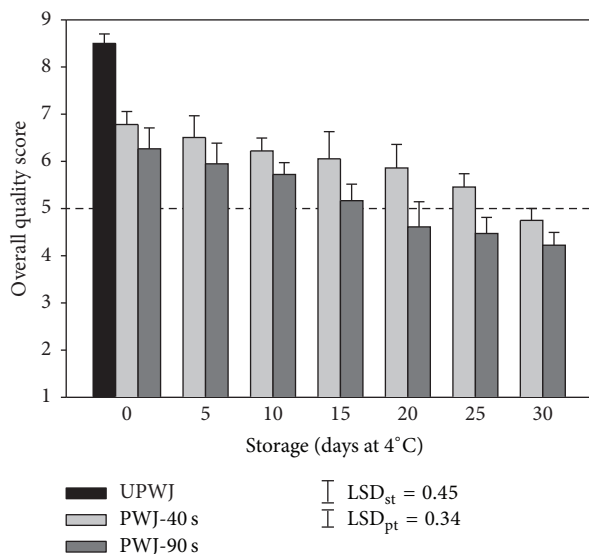


FIGURE 5: Effect of pasteurization (80°C for 40 s or 90 s) and storage (4°C for 30 days) on overall quality of watermelon juice enriched with L-citrulline. Data were based on a hedonic scale where 1 is extremely dislike, 5 is limit of consumption, and 9 is extremely like. LSD values are shown for storage time (st) and pasteurized treatment (pt). UPWJ, unpasteurized watermelon juice. PWJ-40 s, pasteurized watermelon juice for 40 s. PWJ-90 s, pasteurized watermelon juice for 90 s.

results, the best color (determined by CIElab and sensorial analyses) and overall quality rating corresponded to PWJ-40 s, which also had the lowest lycopene degradation rates. The overall quality at day 25 of PWJ-40 s samples was scored close to the limit of acceptability but suffered a decrease in quality at day 30, resulting in a shelf life of one month. The lowest scores in sensorial parameters were taste followed by aroma. The reduction in sensorial quality parameters has been previously described by Aguiló-Aguayo et al. [36], who observed reductions in taste compounds when watermelon juice was thermally treated (90°C, 60 s) and stored for up to 56 days at 4°C.

4. Conclusions

The implementation of a pasteurization treatment is necessary in order to obtain a safe watermelon juice, but this treatment needs to be balanced with a proper treatment time to maintain functional and sensory parameters, which are easily thermodegraded. The main parameter that limits the shelf life was the sensorial quality. Thus, watermelon juice pasteurized at 80°C for 40 s (PWJ-40 s) had a shelf life that was less than 30 days and when the pasteurization was for 90 s (PWJ-90 s), it was less than 20 days. The use of a short thermal treatment, 80°C for 40 s, incurred lycopene losses of 10% and 19% of L-citrulline after the pasteurization treatment. Storage time also induced a slight but significant reduction in both bioactive compounds. Polygalacturonase was the most thermoresistant enzyme. Knowing the quality degradation due to the pasteurization and storage period, it would be

possible to establish the type of market for a functional watermelon juice and the quantity of enrichment if necessary.

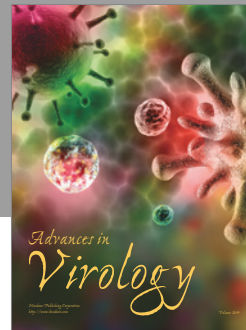
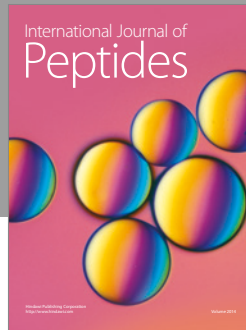
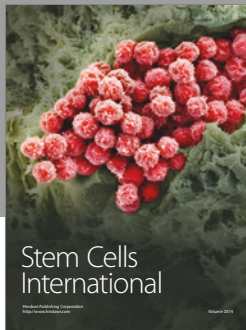
Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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