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INDIVIDUAL AND COMBINED EFFECTS OF POMEGRANATE EXTRACT AND ULTRASONIC TREATMENTS ON KIWIFRUIT JUICE QUALITY PARAMETERS

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ABSTRACT

The objectives of this study were to report the effectiveness of ultrasound and pomegranate extract, individually and combined, on kiwifruit juice quality attributes. Fruit juices were treated with 180 μg/mL pomegranate extract and sonicated for 10 and 30 min (180 W, 40 kHz). Microbial populations, ascorbic acid retention, sensory attributes and color parameters were evaluated through refrigerated storage. By the end of storage, individual ultrasound treatments were able to reduce yeast and molds counts compared with that in control. Furthermore, combined treatments of ultrasound and pomegranate extract showed synergism, with higher reductions on yeast and molds. None of the treatments were able to increase the ascorbic acid retention through storage time. Color parameters evaluated showed that the treatments applied had significant differences from that of control. At Day 2, the treatments showed an improvement in color, with total color difference (ΔE) lower than that of untreated sample, indicating less color difference from fresh kiwifruit juice. Sensory attributes were not significantly affected by the treatments. However, a significant effect of storage time was perceived by the panelists, with a decrease in overall visual quality score throughout time, indicating the end of shelf-life of the product at Day 7 of refrigerated storage.

PRACTICAL APPLICATIONS

Ultrasonic processing technology and the application of natural antimicrobials are continuously being explored as alternatives to traditional thermal treatments in the food industry. It is necessary to study the impact of these technologies both individually and combined, in terms of microbiological stability and quality preservation. In practice, it is important to not only decrease the microbial growth on food products, but also minimize the loss of their nutritional and sensory attributes. This study suggests some processing and preservation conditions for kiwifruit juice, demonstrating the potential use of ultrasound in combination with the application of pomegranate extract as a biopreservative. This hurdle technology could be easily introduced in the fruit and vegetable juice industry, replacing thermal treatments that compromise the nutritional and sensory quality of the final product.

INTRODUCTION

Kiwifruit has long been called "the king of fruits" because of its high vitamin C content and balanced composition of minerals, dietary fiber and other metabolites beneficial to human health (Stonehouse *et al.* 2013; Tang *et al.* 2015). Furthermore, kiwifruit has a strong antioxidant capacity because of its wide

number of phytonutrients, including carotenoids, lutein, phenolics, flavonoids and chlorophyll (Cassano *et al.* 2006).

In the last centuries, thermal treatment has been the most commonly used technology to preserve food products because of its ability to inactivate enzymes and destroy microorganisms. Nonetheless, it is well known that fruit juices that undergo heat treatments, such as thermal pasteurization or sterilization, tend to change color and lose some of its aromas and vitamins during the thermal process (Choi and Nielsen 2005).

However, unpasteurized kiwifruit juice is highly perishable, susceptible to spoilage and deterioration, because of the activity of enzymes, and the exposure to oxygen and microorganisms. Thus, if juices do not receive the proper treatment, enzymatic, microbial, chemical and physical deterioration can take place, reducing considerably its nutritional and sensory quality, shortening the shelf-life of the product (Bates *et al.* 2001).

Therefore, there has been a growing interest in the search of novel technologies that can avoid the application of thermal treatments required for the stabilization of food products. This search has led to new physical treatments, such as ultrasound, becoming a promising alternative to traditional food preserving methods. Ultrasonic treatment would maintain the nutritional value and the sensory characteristics of the fresh product, reducing the loss of flavor, color and nutrients (Gonzalez 2005).

Another emerging technology is the application of certain bioactive compounds from fruits and vegetables as potential antimicrobial and antioxidant compounds, such as pomegranate fruit (*Punica granatum*) extract. Recent interests in these technologies are not only to obtain high-quality food with "fresh-like" characteristics, but also to provide food with improved functionalities by the addition of bioactive compounds (Zenker *et al.* 2003).

To improve the microbial stability and the nutritional quality of food products, as well as their sensory properties, the different preservation technologies could be combined as hurdle technology in the search of synergism between them. The main purpose of these combined treatments is to reduce the concentration of each hurdle in order to avoid the loss of nutritional and sensory value while maintaining the stability and safety of the product (Leistner 2000).

The objective of this work was to evaluate the individual and combined effects of pomegranate extract and ultrasound treatments on quality parameters of kiwifruit juice during refrigerated storage.

MATERIALS AND METHODS

Juice Preparation

Kiwifruits (*Actinidia deliciosa* cv. Hayward) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentina. Kiwifruits were peeled off, and the juices were prepared with a commercial juice extractor. Once the treatments were applied, the kiwifruit juices were stored in sterile glass flasks (250 mL of juice in each flask) at 5C for 7 days, and the evolution of microbial populations, color parameters, sensory attributes and ascorbic acid (AA) retention were studied.

Treatments

Pomegranate extract was purchased from PureBulk, Roseburg, OR. The extract was applied to kiwifruit juice at 180 μg/mL, according to a previous *in vitro* study by Alvarez *et al.* (2012).

On the other hand, the ultrasound treatments were performed at 40 kHz frequency, using an ultrasonic cleaning bath (TestLab, Buenos Aires, Argentina). The ultrasonic bath is a rectangular container (290 $\times$ 150 $\times$ 150 mm) with the maximal tank capacity of 6.5 L. The 40-kHz transducers at the bottom transmit ultrasound waves of 180 W from bottom to above. Temperature in the ultrasonic bath was monitored at 20 $\pm$ 1C. The juice level in the flasks was 2 cm below the water surface in the ultrasonic bath. The height of the bottom surface of the flasks from the bottom surface of the tank (face of transducers) is 4 cm. The processing time was 10 and 30 min.

Finally, in order to test the combined effects of both treatments (physical and chemical), and to evaluate the potential synergic effects between them, the combinations of ultrasound and pomegranate extract were tested as double-hurdles. First, the pomegranate extract was applied to the juice. After that, the samples were treated in the ultrasonic bath, for the corresponding amount of time.

The following terms were used to describe the different treatments in this study: untreated (fresh kiwifruit juice with no treatment or control); US10 (ultrasound treatment for 10 min); US30 (ultrasound treatment for 30 min); PE180 (pomegranate extract 180 μg/mL); US10+PE180 (combination of US10 and PE180 treatments); US30+PE180 (combination of US30 and PE180 treatments). Every treatment was applied in triplicate.

Characterization of Pomegranate Extract

In order to quantify the main phenolic compounds present in the pomegranate extract, a high performance liquid chromatography (HPLC) analysis was performed.

Standard Solutions. Caffeic acid, gallic acid (GA), coumaric acid, ellagic acid (EA) and punicalagin A and B standards were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions of each standard were prepared in a 50:50 (v/v) methanol: water mixture.

Sample Preparation. Pomegranate extract solution 1:100 was prepared using a 50:50 (v/v) methanol: water mixture. Then, a 1:10 dilution was made in order to perform the analysis.

Equipment. The HPLC system consisted of an HP 1100 HPLC with quaternary pump. The chromatographic separation of the compounds was achieved using a Varian C18 (4.6 mm I.D. × 250 mm, 5 μm) with column oven temperature maintained at 30C. The mobile phase consisted of a methanol: water mixture. The mobile phase flow rate was 1 mL/min with gradient elution from 10% to 90% methanol. The injection volume was 20 μL, and the UV detection wavelength was set at 210–380 nm range.

Sensory Evaluation

Quantitative descriptive analysis was used to evaluate sensory attributes of kiwi fruit juice samples at 0, 2 and 7 days of refrigerated storage. At each storage time, treated and untreated kiwi fruit juices were subjected to a panel of testers to evaluate the sensory quality of the beverages. Ten judges, aged 25–50 years, with sensory evaluation experience were trained in descriptive evaluation of kiwi fruit juices. Evaluations were performed immediately after juices removal from storage conditions. Samples labeled with three-digit code numbers were randomly provided. Water was provided to panelists for eliminating the residual taste between samples.

The sensory attributes evaluated were: overall visual quality (OVQ), odor, acid and sweet taste of the beverages. Unstructured line scales (5 cm) anchored at the ends with terms related with minimum and maximum intensities were used to evaluate each attribute. OVQ was scored from 0 (highly deteriorated aspect) to 5 (fresh aspect). Odor was rated from 0 (intense off-odors) to 5 (fresh) and intensity of sweetness and acidity from 0 (low) to 5 (high). The limit of acceptance was 2.5, indicating that score below 2.5 for OVQ and odor attributes was deemed to indicate end of shelf-life (Alvarez *et al.* 2013).

Microbiological Studies

The microbial stability of kiwifruit juices was evaluated through the determination of total aerobic mesophilic bacteria (MES) and yeast and molds (YM) populations. A 10-mL aliquot of juice from each treatment was sampled at different times of refrigerated storage (0, 2 and 7 days). Serial dilutions (1:10) of each sample were made in peptonated water (0.1% w/v) and surface spread in duplicate. The enumeration of the microbial populations was performed according to Ponce *et al.* (2008) by using the following culture media and culture conditions: mesophilic aerobic bacteria on Plate Count Agar incubated at 30–32C for 48–72 h; molds and yeast on Yeast-Glucose-Chloramphenicol medium incubated at 25C for 5 days. Culture mediums were purchased from Britania, Buenos Aires, Argentina. Microbial counts were performed in duplicate, in three independent experimental runs, and expressed as log CFU/mL.

Determination of AA

AA content was determined by the titrimetric assay described by Moreira *et al.* (2003). For the AA determination, a 20-mL aliquot of juice from each treatment was sampled at 0, 2 and 7 days of refrigerated storage, in each independent run. Twenty milliliters of kiwifruit juice was homogenized with 40 mL of 2% w/w oxalic acid solution (Mallinckrodt, St. Louis, MO). This mixture was vacuum filtered through glass fiber. Ten-milliliter aliquots of the filtrate were titrated with 2,6-dichloroindophenol (Anedra SA, Buenos Aires, Argentina). Determinations of AA in kiwifruit juice were performed in triplicate.

AA contents are reported as a percentage of retention (AA/AAo). Where AA is the content of AA at a certain time of refrigerated storage, and AAo is the concentration of AA on the kiwi juice immediately before applying the corresponding treatment.

Color Determination

Color determination was carried out using a colorimeter (Lovibond, RT Series, England) with an 8 mm diameter measuring area, calibrated with a standard white plate ($L^\star = 97.63$, $a^\star = 0.3133$, $b^\star = 0.3192$). Measurements were done in triplicate for each sample.

Color of kiwifruit juice was recorded using the CIE–$L^\star$ $a^\star$ $b^\star$ uniform color space ($L^\star$ $a^\star$ $b^\star$), where $L^\star$ indicates lightness (whiteness or brightness/darkness), $a^\star$ indicates chromaticity on a green (−) to red (+) axis and $b^\star$ indicates chromaticity on a blue (−) to yellow (+) axis (CIE 1977). Numerical values $L^\star$, $a^\star$, $b^\star$ were used to estimate hue angle (Eq. 1), total color difference (ΔE) (Eq. 2) and chroma (Eq. 3), according to:

$$\text{Hue} = arctg\left({}^{b*}/_{a*}\right) \quad (1)$$

$$\Delta E = \sqrt{(a* - a_0)^2 + (b* - b_0)^2 + (L* - L_0)^2} \quad (2)$$

$$\text{Chroma} = \sqrt{a*^2 + b*^2} \quad (3)$$

Total color difference (ΔE) is generally used to acknowledge the difference between two colors according to the following scale: Trace level difference $\Delta E^\star = 0$–0.5, slight difference $\Delta E^\star = 0.5$–1.5, noticeable difference $\Delta E^\star = 1.5$–3.0, appreciable difference $\Delta E^\star = 3.0$–6.0, large difference $\Delta E^\star = 6.0$–12.0 and very obvious difference $\Delta E^\star > 12.0$ (Chen and Mujumdar 2008). Chroma is a measure of color

TABLE 1. MAIN PHENOLIC COMPOUNDS CONCENTRATIONS IN POMEGRANATE EXTRACT

Component	Concentration (mg/g of extract)
Ellagic acid	345 ± 7
Gallic acid	184 ± 4
Punicalagin A	98 ± 2
Punicalagin B	47 ± 1
Caffeic acid	19.0 ± 0.4
Coumaric acid	ND

Note: Data are shown as means ± standard deviations.
ND, not detected.

intensity or saturation, which varies from dull (low value) to vivid (high value) (Goyeneche *et al.* 2014).

Statistical Analysis

A completely randomized design was used. Three independent runs were performed. Data obtained were analyzed using R v. 2.12.2. (R Development Core Team 2011). Results reported in this article are mean values accompanied by their standard errors (Kuehl 2001). Analysis of variance was performed and Tukey–Kramer comparison test was used to estimate significant differences between treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Characterization of Pomegranate Extract

Quantification of pomegranate extract's main phenolic compounds is shown in Table 1. The most important component in the pomegranate extract is EA (345 mg/g of extract). EA is a potent antioxidant, acting as a scavenger of oxygen species produced by hydrogen peroxide treatment, and as a protector of the DNA double helix from alkylating agent injury. Moreover, EA has been recorded as having antimicrobial activity (Choi *et al.* 2011).

The next compound present in the extract is GA (184 mg/g of extract). GA is a simple phenolic acid possessing a single aromatic ring. It is a well-known antioxidant, and has been previously reported to possess strong antimicrobial activity (Chanwitheesuk *et al.* 2007).

Finally, punicalagin A and B (98 and 47 mg/g of extract, respectively) are important phenolic compounds characteristic of pomegranate fruit extracts. Punicalagins are also found to be the major component responsible for pomegranate juice antioxidant activity. They are known to hydrolyze into smaller phenolic compounds such as EA (Marzouk *et al.* 2002).

Sensory Analysis

Figure 1 shows the effects of individual and combined treatments of pomegranate extract and ultrasound on sensory attributes of kiwifruit juice through storage time. At Day 0, no significant differences were found between the treatments and the control sample in any of the evaluated attributes, with mean scores of 4.79 and 4.88 in OVQ and odor parameters, and 2.83 and 3.55 in sweetness and acidity, respectively. These results indicate that the tested treatments have no impact on the sensory quality of kiwifruit juice and would be a feasible alternative to traditional thermal treatments.

At Days 2 and 7 of refrigerated storage, the samples (both treated and untreated) showed no significant differences from each other. However, a significant effect of storage time was perceived by the panelists, with a decrease in OVQ attribute throughout time (3.76 and 1.66, at Days 2 and 7 respectively), indicating the end of shelf-life of the product.

Microbiological Analysis

Native microflora evolution through refrigerated storage is shown in Fig. 2. The mean initial populations of MES (Fig. 2a) and YM (Fig. 2b) in untreated kiwifruit juice were 2.97 and 2.87 log CFU/mL, respectively. The initial counts found in this study are similar to those found by Fernández-Sestelo *et al.* (2013), who observed initial MES counts of 3.44 log CFU/mL in kiwifruit puree.

At Day 0, no significant differences were found between MES counts in treated and untreated samples (2.80–3.12 log). Throughout storage, none of the treatments evaluated showed a significant reduction in mesophilic counts compared with that of control. Furthermore, no significant differences were found in MES through storage time, with counts of 2.50–2.72 log by Day 7. Some European countries, such as France and Spain, have limited the microbial load in minimally processed foods to 5 log CFU/mL to consider the product commercially acceptable (BOE 2001). Therefore, the low mesophilic counts present in the kiwi juice (mean value of 2.74 log CFU/mL throughout storage) are not considered a risk for human health.

The low MES counts observed in both treated and untreated kiwi juice along storage time could be due to effects such as storage temperature (5C) and significant acidity present in kiwifruits. Mesophilic bacteria grow best around neutral pH values (6.5–7.0), and kiwifruit is classified as a "high-acid" food, because of its important content of quinic, citric and malic acids. Depending on its state of ripeness, the pH value of a kiwifruit varies between 3.10 and 3.96.

The evolution of YM counts throughout storage in kiwifruit juices treated with pomegranate extract and ultrasound is shown in Fig. 2b. At Day 0, no significant differences were found on YM counts between treated and untreated samples (2.63–3.06 log). No significant changes were observed in YM counts between Day 0 and Day 2 of refrigerated storage.

However, at Day 7 of refrigerated storage, untreated kiwi juice showed an important increase in YM counts, reaching

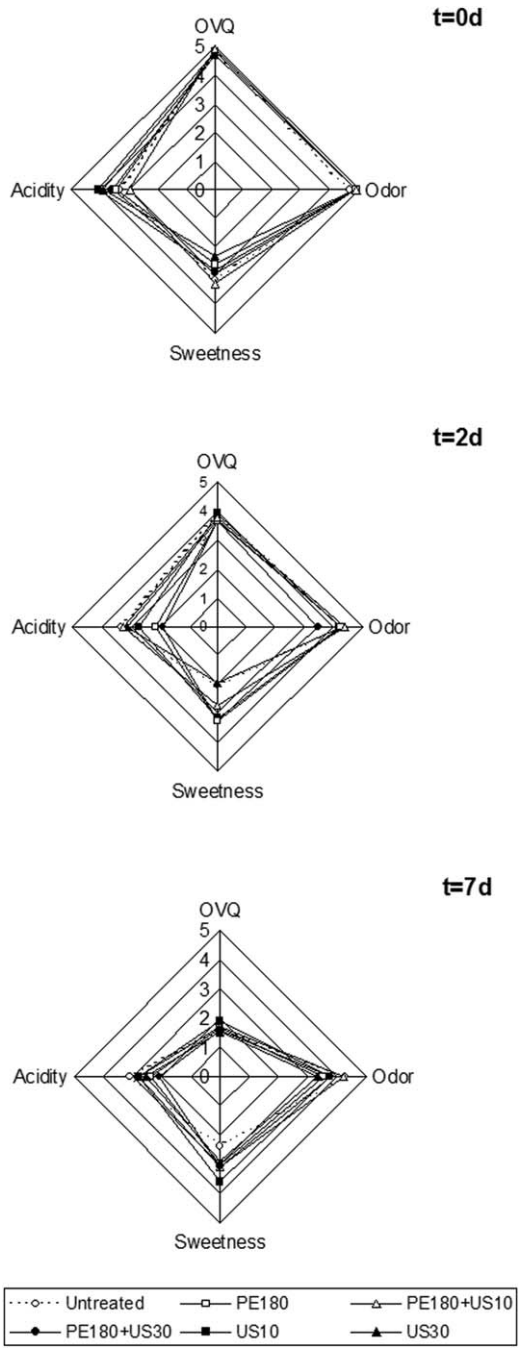

FIG. 1. INDIVIDUAL AND COMBINED EFFECTS OF POMEGRANATE EXTRACT AND ULTRASOUND TREATMENTS ON SENSORY ATTRIBUTES OF KIWIFRUIT JUICE REFRIGERATED AT 5C. PE180: 180 μg/mL OF POMEGRANATE EXTRACT; US10: ULTRASOUND TREATMENT FOR 10 MIN; US30: ULTRASOUND TREATMENT FOR 30 MIN

values of 4.48 log CFU/mL. Ultrasound treatments applied individually (US10 and US30) showed significant reductions on YM counts with respect to control sample (0.96 log and 1.40 log reductions, respectively). On the other hand, PE180 applied individually was not effective in reducing YM compared with control sample, with counts of 4.54 log by Day 7 of storage.

At Day 7 of storage, the combinations of ultrasonic treatment with the application of pomegranate extract (US10+PE180 and US30+PE180) showed significant reductions compared with that of control (1.46 and 2.13 log reductions, respectively). Moreover, the addition of a second hurdle technology to the ultrasonic treatments increased its effectiveness, by incrementing the reduction compared with the ones achieved with US10 and US30 as mono-hurdles.

At Day 7 of refrigerated storage, there were no significant differences between the antifungal effects of US30 and US10+PE180. This result implies that a shorter ultrasound treatment time could be applied to kiwifruit juice when combined with pomegranate extract, achieving the same

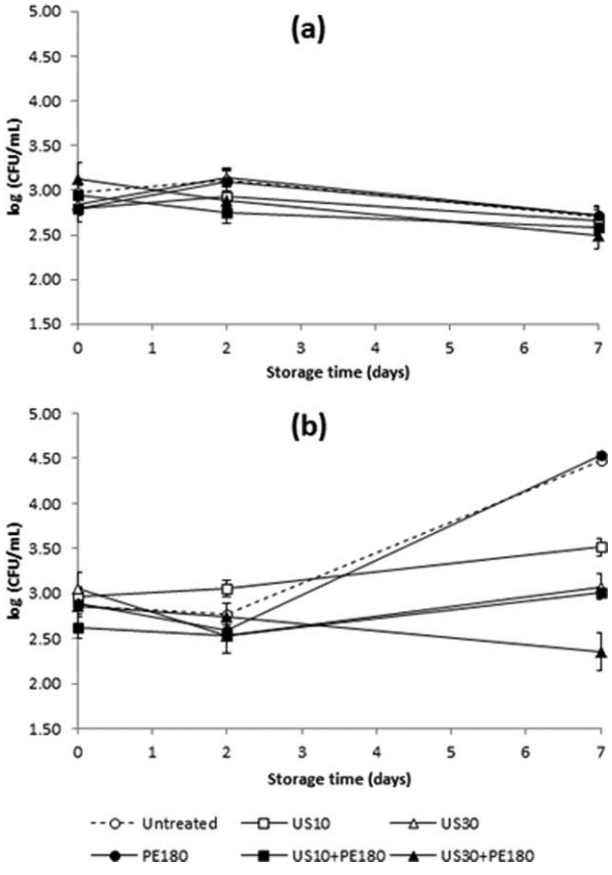

FIG. 2. INDIVIDUAL AND COMBINED EFFECTS OF POMEGRANATE EXTRACT AND ULTRASOUND TREATMENTS IN THE GROWTH OF MESOPHILIC BACTERIA **(A)** AND YEAST AND MOLDS **(B)** IN KIWIFRUIT JUICE REFRIGERATED AT 5C. BARS INDICATE STANDARD DEVIATIONS. PE180: 180 μg/mL OF POMEGRANATE EXTRACT; US10: ULTRASOUND TREATMENT FOR 10 MIN; US30: ULTRASOUND TREATMENT FOR 30 MIN

reductions in YM counts, but with less impact on the quality attributes of the product.

The reductions in YM counts observed for those samples treated with ultrasound could be attributed to the physical phenomenon of cavitation that occurs when applying ultrasound (Vercet *et al.* 2002). Cavitation can break molecules or particles through different mechanisms that can occur individually or combined, and free radicals are generated in the water sonolysis. Mañas and Pagan (2005) noticed the inhibitory effect of ultrasound treatments in the growth of microorganisms, attributing this effect to the water cavitation and the generation of radicals H^+ and OH^-. These radicals produce oxidative damage because of their high reactivity, leading to the microbial inactivation. In accordance with our results, Cao *et al.* (2010) applied 10 min ultrasound treatments at 40 kHz to strawberries and found significant reductions in YM counts through storage.

Even though the application of pomegranate extract had no inhibitory effect on kiwifruit juice native microflora when applied individually, it showed a synergic effect when combined with ultrasound. Several studies have shown the effects of ultrasound treatments (40 kHz) combined with different chemical sanitizers (Chen and Zhu 2011; Sagong *et al.* 2011; São José and Dantas Vanetti 2012). In these studies, the combination of chemical treatments and ultrasounds resulted in a greater reduction of the contaminant population on fruits and vegetables. Gogate and Kabadi (2009) also concluded that when ultrasound treatments are applied in combination with chemicals, they could increase the efficiency of sanitizing agents. This phenomenon may be due to the intense pressure generated during ultrasound treatments that contributes to the penetration of the chemical sanitizers (in this study, pomegranate extract) through the cellular membrane, and the cavitation process may assist in the disaggregation of the microorganisms, which culminates in an increased efficiency of the sanitization treatment (Gogate and Kabadi 2009).

AA Retention

Determination of AA content in fruits and vegetables is highly important because of its major role in human nutrition, and also because its degradation can favor nonenzymatic browning, leading to the development of off-flavors (Bernhardt 1997). Furthermore, ascorbate as a thermolabile vitamin is an important indicator of quality, as its presence can indicate that other nutrients can be preserved in the product (Harder *et al.* 2009).

Because of its antioxidant activity, AA plays an important role in many metabolic pathways, which have a direct impact on the oxidative stability of fruits (Lurie 2003). The loss of AA is the main limiting factor of nutritional quality (Ahvenainen 1996); therefore, it is regarded as a quality indicator

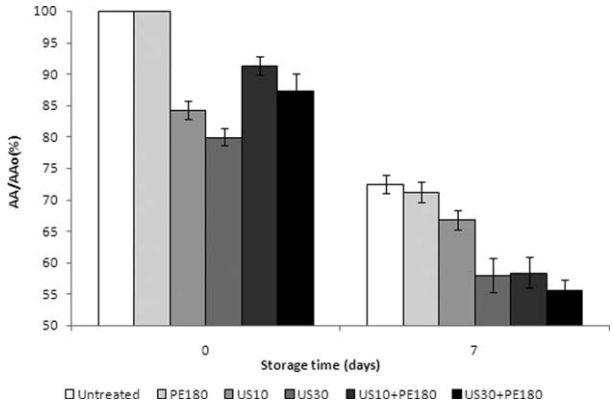

FIG. 3. INDIVIDUAL AND COMBINED EFFECTS OF POMEGRANATE EXTRACT AND ULTRASOUND TREATMENTS IN THE RETENTION OF ASCORBIC ACID IN KIWIFRUIT JUICE REFRIGERATED AT 5C. BARS INDICATE STANDARD DEVIATIONS. PE180: 180 μg/mL OF POMEGRANATE EXTRACT; US10: ULTRASOUND TREATMENT FOR 10 MIN; US30: ULTRASOUND TREATMENT FOR 30 MIN

for minimally processed fruits and vegetables. In kiwifruit, vitamin C is considered the major antioxidant compound, contributing to about 40% of the total antioxidant activity (Tavarini *et al.* 2008).

The AA retention for treated and untreated samples at different times of refrigerated storage is shown in Fig. 3. The results are expressed as a percentage of AA retention, in order to eliminate the biovariability between samples. The mean initial AA content found in this study was 69.3 mg/100 mL of kiwi juice. This result is in agreement with the values found by Barberis *et al.* (2015), who measured an AA content of 69.89–73.09 mg/100 mL of kiwi juice.

At Day 0 (immediately before the application of the treatments), samples treated with ultrasound individually or combined with pomegranate extract showed significant decrease in AA retention. However, the decrease in AA content was attenuated by the addition of pomegranate extract (Fig. 3): US10 and US30 applied individually showed AA retentions of 84.30% and 79.96%, respectively, whereas the combinations with pomegranate extract, US10+PE180 and US30+PE180, showed AA retentions of 91.32% and 87.26%, respectively. Therefore, the addition of pomegranate extract to ultrasound-treated juice can improve the retention of AA immediately before treated compared with those samples that were only sonicated. This may be due to the antioxidant capacity of the main components of pomegranate extract.

By the end of refrigerated storage, the retention of AA in the control sample was of 72.54%, with no significant differences from the retention achieved in the sample treated with PE180 individually. None of the treatments where ultrasound was applied (individually or combined) were able to increase the AA retention compared with that of the control sample.

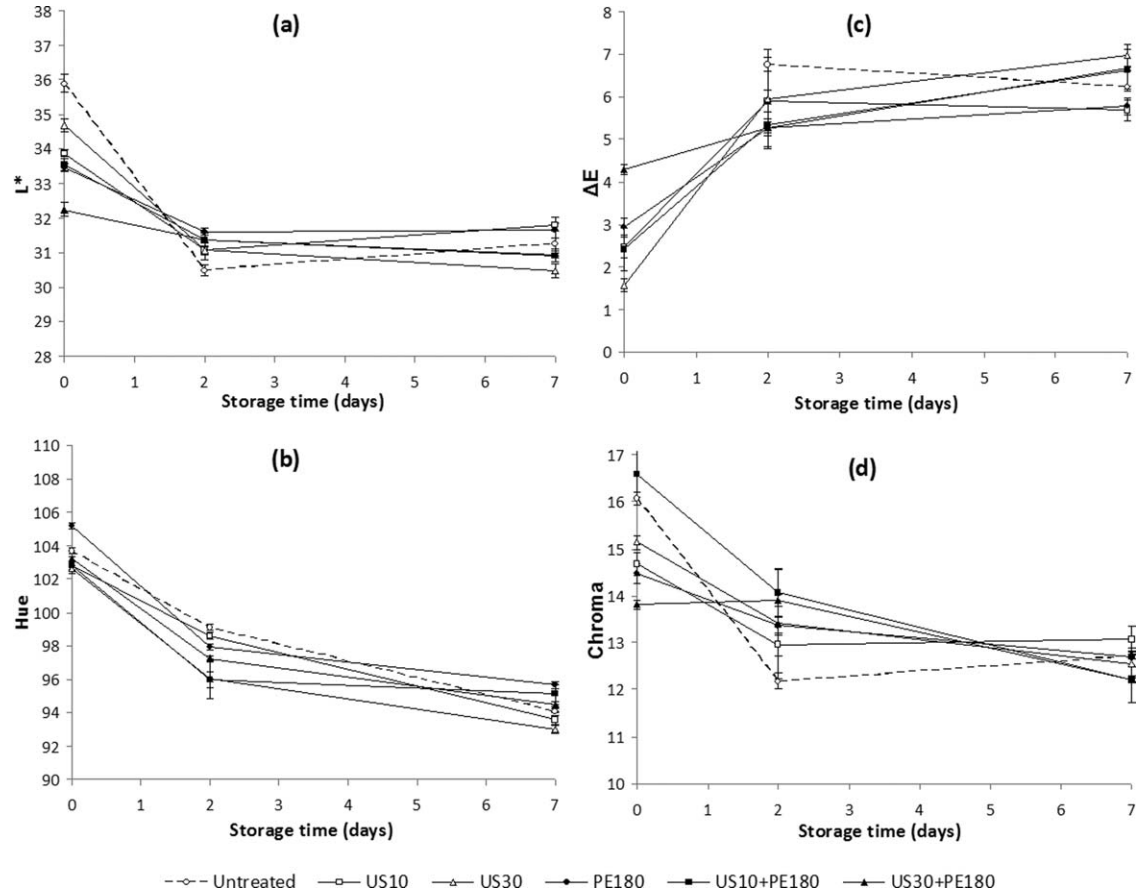

FIG. 4. INDIVIDUAL AND COMBINED EFFECTS OF POMEGRANATE EXTRACT AND ULTRASOUND TREATMENTS ON COLOR PARAMETERS OF KIWIFRUIT JUICE REFRIGERATED AT 5C. **(A)** LIGHTNESS (L^*); **(B)** HUE ANGLE; **(C)** TOTAL COLOR DIFFERENCE (ΔE); **(D)** CHROMA. BARS INDICATE STANDARD DEVIATIONS. PE180: 180 μg/mL OF POMEGRANATE EXTRACT; US10: ULTRASOUND TREATMENT FOR 10 MIN; US30: ULTRASOUND TREATMENT FOR 30 MIN

These results may be attributed to various mechanisms that can occur simultaneously when ultrasound is applied; for example, thermal effects produced by bubble implosion, microstreaming and implosion shock waves produced by mechanical stresses and free radical production. However, radical production has been considered the most probable mechanism that explains the degradation of AA (Vercet *et al.* 2001). Sonication cavities can be filled with water vapor and gases dissolved in the juice such as O_2 and N_2. The interactions between AA and free radicals may occur at the gas–liquid interfaces.

It is known that hydrogen ions (H^+), free radicals (O^-, OH^- and HO_2^-) and hydrogen peroxide (H_2O_2) are formed during the sonolysis of water molecules (Feril and Kondo 2004) present in juice samples. The AA degradation during ultrasonic processing may follow one or both of the following pathways: (1) thermolysis inside the bubbles, triggering the Maillard reaction; (2) reaction with hydroxyl radicals produced by cavitation, producing oxidative products on the bubbles surface (Feril and Kondo 2004).

Color Parameters Evolution

Color is one of the most important attributes of food and beverages; in kiwifruit juices, a bright green color is desired. Significant differences in color were recorded between the control and the treatments (individual and combined), as shown in Fig. 4. Furthermore, an effect of storage time was observed in every parameter in both treated and untreated samples.

The L^* value observed for untreated kiwifruit juice ($L^* = 35.9$) was similar to the values found by Carpi *et al.* (1997), and slightly lower than those found by Talens *et al.* (2001). At Day 0, a significant decrease in L^* value was observed in the treated samples compared with control, especially in those treatments with PE180 (Fig. 4a). These differences diminished during storage period. In any case, this parameter decreased during storage for both treated and untreated samples. A decrease in the L^* value of juice indicates browning, which results in a dark color.

Hue angles of 0°, 60°, 120°, 180°, 240° and 300° indicate red, yellow, green, cyan, blue and magenta color, respectively. In our study, the hue angle of the fresh kiwifruit juice indicates that the juices had a green color (Hue = 103.7°). Hue values were similar to those found for *A. deliciosa* Hayward kiwifruit pureé by Fernández-Sestelo *et al.* (2013). At Day 0, no significant differences were found between untreated and treated samples (102.7°–103.7°), except for the PE180 juice sample, which had a hue value of 105.2° (Fig. 4b). However, at Day 2 of storage, sonicated juice samples showed significantly ($P \leq 0.05$) lower Hue values compared with those of untreated sample. This may be due to an extraction effect of phenolic compound due to sonication (Mohideen *et al.* 2015). As well as L^* value, this parameter significantly decreased during storage for both the treated and untreated samples.

Total color difference (ΔE) is shown in Fig. 4c. According to the classification of Chen and Mujumdar (2008), every sample but US30+PE180 fell within the "noticeable" color change range. The change in color increased over time in every sample, although this effect was stronger for the control juice. At Day 2, an effect of storage time was observed, where every sample showed "appreciable" color changes ($3.0 < \Delta E < 6.0$) while the untreated juice showed "large differences" ($\Delta E > 6$) from the fresh kiwifruit juice. Likewise, color degradation in sonicated fruit juices was reported by Tiwari *et al.* (2008) and Santhirasegaram *et al.* (2013). Color changes or darkening in juices could be explained by accumulation of unstable particulate fractions (Ugarte-Romero *et al.* 2006). In addition, changes in color could be due to cavitation, which governs various physical, chemical or biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or breakdown of susceptible particles, such as enzymes and microorganisms (Adekunte *et al.* 2010).

Moreover, at Day 0 a slight decrease in chroma values of treated samples was observed compared with that of untreated ones (Fig. 4d). Chroma values indicate saturation (Gonnet 1998), and higher values of chroma indicate a more vivid color. Therefore, the treated juices showed less vivid color immediately after being treated. However, at Day 2, the untreated sample showed a significant decrease in the chroma value, and every treated sample showed a more vivid color. There is a correlation between chroma values at Day 2 and the rest of the measured color parameters, where L^* values were higher for treated samples and ΔE and Hue angles were lower compared with that of control.

In general, even though at Day 2 the treatments showed an improvement in color parameters compared with that of control sample, the effect of storage time prevailed, with noticeable color degradation for both treated and untreated samples compared with that of fresh kiwifruit juice. Juice browning and severe astringency are common technical problems in the kiwifruit juice industry. A high content of polyphenol substances in kiwifruit juice is the main factor contributing to these phenomena (Gao *et al.* 2013).

Even though the native microflora was below the established limits by the Spanish regulations, the important changes in color made the product undesirable by Day 7, and therefore, the experiment was ended after 1 week of storage.

CONCLUSIONS

This work allows a better understanding of the complex physicochemical mechanism of nonthermal technologies and their effects on the technological and functional properties of exotic fruits and their products that would contribute to reinforce their industrial application.

Ultrasound treatment combined with pomegranate extract application could be presented as a potential technology able to reduce the YM populations present in unpasteurized fruit juices. Therefore, it presents a viable natural alternative from thermal treatments to significantly reduce the YM population present in kiwifruit juice and to control the native microflora during refrigerated storage without affecting the sensory attributes compared with those of fresh control. This hurdle technology could be easily introduced in the fruit juice industry, replacing thermal treatments, such as pasteurization, that compromise the nutritional and sensory quality of the final product.

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