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ORIGINAL PAPER

Combined Use of Ultrasound and Vanillin to Improve Quality Parameters and Safety of Strawberry Juice Enriched with Prebiotic Fibers

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Abstract In this work, a previously optimized preservation treatment (vanillin = 1.25 mg/mL; ultrasound = 7.5 min, 40 kHz, 180 W) was applied to strawberry juice enriched with inulin and oligofructose. The evolution of microbial, nutritional, and sensory parameters of treated juices was studied. It was confirmed that the inclusion of inulin and oligofructose had no negative implication regarding the quality of fresh juice. Furthermore, the prebiotic addition maintained sensory attributes of the product. The applied preservation treatment improved almost every quality attribute during storage, reducing microbial development, especially lactic acid bacteria and yeast and mold growth, which rapidly grew in untreated juices. Nutritional quality was also improved by the treatment as total polyphenol and total flavonoid content were increased and ascorbic acid content losses were reduced during storage, indicating higher antioxidant capacity. Overall, the evaluated sensory attributes of treated juices were deemed acceptable (>2.5). The addition of vanillin imparted pleasant flavor notes to the juice, compatible with the fruit product. Also, the performance of the treated juice was evaluated against postharvest contaminations with pathogens of interest in the food industry and of health concern (*Escherichia coli* O157:H7 and *Listeria monocytogenes*, evaluated through the surrogate *Listeria innocua*). The optimized treatment was able to reduce the counts of these microorganisms during storage reaching undetectable values after 7 days of storage. Thus, the combination of vanillin and ultrasound could be a feasible alternative to ensure safety and improve quality parameters of strawberry juice enriched with prebiotic fibers.

Keywords Strawberry product · Inulin and oligofructose · Natural antimicrobial · Non-thermal processing

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Introduction

In the last decades, the market for fresh, ready-to-eat, and nutritious foods such as fresh-cut fruit and unpasteurized fruit juices has grown in response to evolving consumer demands and a trend towards healthier foods (Mosqueda-Melgar et al. 2012; Rößle et al. 2011). Among fruits, strawberries are good sources of vitamins, minerals, fiber, and phytochemical compounds and have demonstrated many beneficial effects in human health and disease prevention (Duan and Zhao 2009; Rößle et al. 2011). Furthermore, strawberries are recognized as healthy food products and are frequently consumed by a significant percentage of consumers (Nazzaro et al. 2008).

For fulfilling today's market demand, the juice industry is now providing new innovative fruit juice products. Enhancement of these products with functional food ingredients such as prebiotics could provide producers with a product that satisfies consumer demands for foods with benefits beyond basic nutrition (Rößle et al. 2011). Therefore, many

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fruits, in the form of juice, have the capacity to act as a carrier for functional ingredients such as prebiotics like inulin and oligofructose. Prebiotics are functional ingredients that beneficially affect the host by selectively stimulating growth and activity of one or a limited number of beneficial colonic bacteria (Coussement 1999). While many products have claimed to deliver prebiotic effects, inulin and oligofructose have consistently shown to manipulate the composition of colonic microbiota (Keenan et al. 2011).

Alongside the need for new and innovative products containing functional ingredients, producers must address the necessity for products with "fresh-like" characteristics by considering processing techniques that can successfully extend shelf-life, ensure safety, and improve quality and consumer perception of the product without any adverse effect or damage to the nutritional quality (Keenan et al. 2011; Abid et al. 2013). Non-thermal processing techniques, such as ultrasound, have the potential to offset some of the unwanted reactions in foods resulting in undesirable organoleptic, texture, and nutritional effects often associated with thermally processed foods (Keenan et al. 2011). When ultrasound at low frequencies (20–100 kHz) propagates in liquid, cavitation (formation and collapse of bubbles) occurs. As a result, elevation of localized pressure and temperature ("tiny hotspots") provide the energy to alter the properties of food product either physically or chemically. Sonication is simple and reliable, thus providing reduced processing time and improved efficiency (Santhirasegaram et al. 2013). Several reports informed that ultrasound not only avoids quality losses from the product but also improves its nutritional quality and reduces the microbial load in fruit juices. Nonetheless, this technology appears to be more effective for preservation when is combined with other methods such as natural antimicrobials (Abid et al. 2013). These compounds found in nature are promising to accomplish increasing consumer demands for reduced-additive and synthetic preservative-free foods (Char et al. 2009). Vanillin (4-hydroxy-3-methoxybenzaldehyde), the major constituent of vanilla beans, is a phenolic compound that not only has demonstrated to have antimicrobial and antioxidant properties but also impairs pleasant flavor notes to a wide variety of products such as confectionery, beverages, and pharmaceuticals (Char et al. 2010).

In a previous study (Cassani et al. 2017), the effects and interactions between these two preservation technologies (application of ultrasound treatments and addition of vanillin) together with the addition of fiber (inulin/oligofructose) to strawberry juice were evaluated and the levels of each variable were optimized in order to minimize microbial counts and maximize sensory properties of the product at 14 days of storage. However, some issues remain unknown, such as the effect of this optimized treatment on the nutritional quality of the product or the performance of the treated product against possible contaminations with pathogenic microorganisms of interest in the food industry. Thus, the aims of this research were to study (1) the evolution of quality parameters (microbiological, nutritional, and sensory) of strawberry juice enriched with prebiotic fibers and treated with a previously optimized treatment (ultrasound and vanillin), (2) the performance of the optimized treatment to inactivate or control an eventual postharvest contamination with *Escherichia coli* O157:H7 and *Listeria innocua* on the fiber-enriched strawberry juice.

Materials and Methods

Juice Obtaining

Strawberries (*Fragaria x ananassa* Duch, cultivar Aromas) (pH 3.25 ± 0.02; 15.25 ± 0.01 °Brix) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. Fruits with defects were discarded and fruits with good quality were washed with tap water and the calyx was removed by hand. Then, fruits were squeezed by a commercial juice extractor and the fresh strawberry juice was collected in a glass jar. The juice was homogenized and bottled under hygienic conditions into 350-mL polyethylene terephthalate (PET) bottles and sealed with polyethylene (PE) caps to be subsequently used in the experiments.

Optimized Treatment

Juice enrichment was carried out by adding 5.25 g of inulin/oligofructose mixtures (5:3 ratio) to each bottle. After that, vanillin (1.25 mg/mL) was added and the units were treated with ultrasound putting bottles into a 350-mL cylindrical vessel (diameter, 7 cm; height, 7.2 cm) and placing them in an inner tank of ultrasound bath cleaner (TestLab, Argentina) to be sonicated for 7.5 min at a constant temperature of 20 °C and 40 kHz in dark to avoid any interference of light. The power of ultrasound was 180 W transmitted from bottom to above. The amount of fiber added to strawberry juice was selected according to Cassani et al. (2016). The ratio inulin/oligofructose, vanillin concentration, and ultrasound conditions used in this study were established after carrying out an optimization study using response surface methodology which is explained in detail in our previous work (Cassani et al. 2017).

For each experiment (quality evolution and challenge test), two controls were kept and evaluated to study the effects of enrichment and preservation treatment applied. A batch of samples was enriched with fiber, as was previously described, but was not submitted to the optimized preservation treatment (control with fiber (CF)). Another batch of samples was simply strawberry juice without fiber addition or preservation treatment (control (C)). Samples were stored at 5 °C for 0, 3,

7, 10, and 14 days. At each storage time, the evolution of quality parameters was evaluated. Three replications (three juice bottle per storage day) were performed and the experiment was conducted twice.

Evolution of Quality Parameters During Storage

In the first experiment, strawberry juice submitted to enrichment and preservation treatment as was previously described, and their controls were evaluated during storage to determine the evolution of several quality indices in relation to microbiological, nutritional, and sensory aspects.

Microbiological Quality

Native microflora of strawberry juice was determined by mesophilic aerobic bacteria (MES), *Enterobacteriaceae* and total coliforms (ETC), lactic acid bacteria (LAB), and yeast and mold (YM) counts. Briefly, 10 mL of juice was sampled. Serial dilutions (1:10) of each sample were made in peptonated water (0.1% *w*/*v*) (Britania, Argentina) and surface spread by triplicate. The enumeration and differentiation of each particular microbial group were performed by using the following culture media and culture conditions: MES on plate count agar (PCA) incubated at 35 °C for 48 h and ETC in Mac Conkey agar incubated at 35 °C for 24 h. LAB were counted in Man, Rogosa, and Sharpe (MRS) medium incubated at 35 °C for 24 h. YM were counted in yeast-glucose-chloramphenicol (YGC) medium incubated at 25 °C for 5 days. All culture mediums were purchased from Britania, Buenos Aires, Argentina.

Nutritional Quality

Several parameters (ascorbic acid, total phenolic, total flavonoid contents, and antioxidant activity) associated with nutritional quality of strawberry were determined in samples during storage following the methodology described here.

For ascorbic acid content (AAC) determination, the titrimetric assay described by Goyeneche et al. (2015) was used. Briefly, 20 mL of strawberry juice samples was homogenized with 40 mL of 2% oxalic acid solution (Biopack, Argentina). This mixture was vacuum filtered through glass fiber. Aliquots (5 mL) of the filtrate were titrated with 2,6-dichloroindophenol (Anedra, Argentina). AAC is calculated as mg of reduced ascorbic acid/100 mL of juice.

Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity were determined on an extract of antioxidants from juice samples. The extraction of antioxidants was carried out homogenizing 2 mL of strawberry juice from each sample with 10 mL solution of ethanol (80% *v*/*v*) (Merck, Darmstadt, Germany). The homogenate was then centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was collected and filtered using Whatman filter paper no. 1. The final ethanolic extract was stored at −20 °C to be used in the determination of indices.

TPC was determined spectrophotometrically using the Folin–Ciocalteu reagent (FCR) (Biopack, Argentina) according to the methodology proposed by Viacava et al. (2015) with modifications. Extract samples properly diluted were added to 1000 μL of FCR (diluted 1:10). After 3 min of incubation at ambient temperature, 800 μL of 7.5% Na_2CO_3 (Merck, Darmstadt, Germany) solution was added and the reaction mixture was incubated for 2 h at the same temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (1601 PC UV-visible, Shimadzu Corporation, Kyoto, Japan) and TPC was calculated using gallic acid (Biopack, Argentina) as standard. Results were expressed as mg gallic acid equivalents (GAE)/100 mL of juice.

TFC was determined based on the method described by Viacava and Roura (2015). Extract samples (0.2 mL) were mixed with 1.280 mL of deionized H_2O and 0.06 mL of $NaNO_2$ (5% *w*/*v*) (Biopack, Argentina). After 5 min, 0.06 mL of $AlCl_3$ (10% *w*/*v*) (Anedra, Argentina) was added and, after 6 min, 0.4 mL of NaOH (1 M) (Biopack, Argentina) was incorporated. The mixture was stirred and absorbance was measured at 510 nm. TFC was expressed as mg of quercetin (Sigma-Aldrich, USA) equivalents (QE)/100 mL of juice.

The antioxidant capacity was studied by evaluation of the free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma-Aldrich, USA), according to the method described by Viacava et al. (2015). An ethanolic DPPH solution (100 μM) was used for determinations. Ethanol (0.1 mL) (Merck, Darmstadt, Germany) was mixed with 3.9 mL of DPPH (100 μM) to determine the initial absorbance of the DPPH solution. Then, 0.1 mL of sample extract was added to 3.9 mL of 100 μM DPPH solution. The mixture was shaken immediately and allowed to stand at ambient temperature in the dark. The decrease in absorbance at 517 nm was measured after 60 min. The radical scavenging activity was expressed as the inhibition percentage of the DPPH radical.

Sensory Quality

Instrumental color determination and sensory evaluation of samples were carried out to characterize sensory quality evolution of samples during storage.

Color determination was carried out using a LoviBond colorimeter RT500 (Neu-Isenburg, Germany) with an 8-mm diameter measuring area, calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$, $y = 0.3192$). Color of strawberry juices was determined by measuring CIE L^*, a^*, and b^*

chromaticity coordinates (Illumination 1978). Hue angle ($h°$) was calculated according to Eq. (1).

$$h° = \text{arctg}\left(\frac{b^*}{a^*}\right) \quad (1)$$

On the other hand, a quantitative descriptive analysis was used to evaluate sensory attributes of strawberry juice samples. At each storage time, treated and controls strawberry 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 strawberry juices. The attributes evaluated were color, odor, acid, and sweet taste and overall visual quality (OVQ) of the beverages. Evaluations were performed immediately after juice removal from storage conditions. The coded (three-digit) samples were presented one at a time in random order to the panel members, who sat at a round table and made independent evaluations. The intensity of the attributes evaluated was quantified on an unstructured intensity scale from 0 to 5. OVQ was scored from 0 (highly deteriorated aspect) to 5 (fresh aspect). Color was rated from 0 (deteriorated color) to 5 (typical color), odor from 0 (intense off-odors) to 5 (fresh), and sweet and acid taste from 0 (extremely dislike) to 5 (extremely like). The limit of acceptance was 2.5, indicating that score below 2.5 for any of the attributes evaluated was deemed to indicate end of shelf-life (Alvarez et al. 2013).

Performance Against *E. coli* O157:H7 and *L. innocua* Contaminations

In the second experiment, enriched strawberry juice was inoculated with *E. coli* O157:H7 and *L. innocua*, simulating a contamination with pathogens and then submitted to preservation treatment as was previously described. As was stated before, two controls were also used here, CF and C, both inoculated with the corresponding microorganism but not submitted to the preservation treatment, with and without fiber, respectively. After inoculation and treatment, samples were stored at 5 °C for 0, 3, 7, 10, and 14 days. At each storage time, the evolution of pathogenic microbial counts was followed during storage.

Strain and Inoculum Preparation

L. innocua, non-pathogenic specie, is usually used as a biological indicator for *Listeria monocytogenes* because of its similar response to physical, chemical, or thermal treatments. *L. innocua* (CIP 8011, CCMA 29, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina) and *E. coli* O157:H7 non-toxigenic (FP 605/03, Malbran Institute, Buenos Aires, Argentina) were used. A stock culture was maintained in tryptic soy broth (Britania, Argentina) at 4 °C. Before use, *E. coli* O157:H7 and *L. innocua* were cultured in brain heart infusion (BHI) broth (Britania, Argentina) for 24 h at 37 °C. Aliquots (0.1 mL) of the cultures were transferred to 9.9 mL of BHI broth at two consecutive 24-h intervals followed by incubation at 37 °C, before each experiment, to obtain cells in stationary growth phase. Two bacterial suspensions (approximately 10^8 colony forming units/mL (CFU/mL)) were prepared by adding 10 mL of the *E. coli* and *L. innocua* cultures to 90 mL of sterile peptonated water (0.1% *w*/*v*) (Britania, Argentina).

Sample Inoculation

Inoculation was carried out by adding the bacterial suspension to fresh strawberry juice to obtain the final desired concentration of cells (10^5–10^6 CFU/mL approximately). Finally, all inoculated samples were stored at 5 ± 1 °C until analysis. A batch of samples without inoculation was followed and evaluated to verify the absence of endogenous pathogens in the juice.

Microbial Counts

E. coli and *L. innocua* counts were determined according Tomadoni et al. (2015a). Briefly, 10 mL of juice was sampled. Serial dilutions (1:10) of each sample were made in peptonated water (0.1% *w*/*v*) (Britania, Argentina). *E. coli* counts were determined using eosin methylene blue (EMB) agar (Britania, Argentina), and the colonies were counted after incubation at 37 °C for 24–48 h. EMB is a selective medium that allows the characterization of typical *E. coli* colonies; those that were dark centered, flat with a metallic sheen were taken into account. Oxford Agar (base) with Oxford Selective Supplement (BS003) (Biokar Diagnostics, France) was used for differentiation, isolation, and enumeration of *Listeria*. Olive-green colonies surrounded by a black halo were counted after incubation at 37 °C for 24–48 h.

Statistical Analysis

A completely randomized design was used for each experiment. Results reported in this work are mean values accompanied by their standard errors. Experimental data were analyzed using R, software version 2.12 (R Development Core Team, 2011). Analysis of variance (ANOVA, $p < 0.05$) was performed and Tukey-Kramer comparison test was used to estimate significant differences between treatments and through storage time ($p < 0.05$).

Results and Discussion

Evolution of Quality Parameters During Storage

Microbiological Quality

Table 1 shows the evolution of MES, ETC, LAB, and YM populations in strawberry juices during storage. In every population analyzed, no significant differences were observed between counts of controls (CF and C), indicating that added fibers themselves did not exert any effect on native microflora, as it was found in previous studies (Cassani et al. 2016). Immediately after treatment application, no significant effect was registered on native microflora, as treated samples presented similar microbial counts compared to controls. However, during the whole storage, mesophilic bacteria growth rate was significantly higher in both untreated samples (CF and C) than in treated ones, indicating that the applied preservation treatment exerted a significant inhibitory effect on this population. In fact, at the end of storage, MES counts of treated strawberry juice resulted ~2 log cycles lower than controls. For ETC, a reduction in their counts was registered along storage in all juices samples (treated and controls). This behavior could be associated with the low pH of the samples and the increase in the population of lactic acid bacteria which constitutes a barrier for ETC, a microbial population sensible to low pH (Kim and Beuchat 2005). However, it can be observed that ETC reductions were greater in treated samples than in controls reaching undetectable levels from 7 days until the end of storage. Otherwise, LAB found an ideal growth media in untreated strawberry juices (CF and C) as their counts continuously increased reaching values around 8 log cycles CFU/mL at the end of storage. The preservation treatment applied on enriched juice was highly effective to control this microbial population as inhibited its growth during the first 7 days of storage and reduced their counts afterwards, reaching noticeable differences of approximately 6 log cycles with respect to control samples at the end of storage.

Finally, YM counts presented a similar behavior than LAB, with a continuous increase in control samples (CF and C) during storage, reaching values higher than 8 log cycles at 14 days. Also in this case, a high efficiency to control YM development was shown by the preservation treatment not only inhibiting its growth during the first days of storage but also reducing their counts by approximately 3 log cycles from day 0 to day 14 of storage. It is important to note that control samples presented fermentation, gas production, off-odors, and increased turbidity, which are associated to YM growth.

In a previous work (Tomadoni et al. 2017), the effect of ultrasound alone on native microflora of strawberry juice

Table 1 Native microflora evolution (log CFU/mL) in strawberry juice samples during refrigerated storage

Samples	Storage time (day)				
	0	3	7	10	14
Mesophilic bacteria					
T	4.65 ± 0.15^{aA}	2.79 ± 0.06^{bB}	3.40 ± 0.08^{aB}	2.77 ± 0.21^{bB}	2.70 ± 0.00^{bB}
CF	5.08 ± 0.17^{aA}	4.38 ± 0.10^{aB}	3.96 ± 0.15^{aB}	4.11 ± 0.17^{aB}	5.09 ± 0.10^{aA}
C	4.83 ± 0.15^{aA}	4.32 ± 0.06^{aB}	3.67 ± 0.07^{aC}	4.17 ± 0.1^{aB}	5.11 ± 0.09^{aB}
Enterobacteriaceae and total coliform					
T	4.47 ± 0.20^{aA}	2.61 ± 0.22^{bB}	ND	ND	ND
CF	4.95 ± 0.12^{aA}	4.30 ± 0.14^{aAB}	4.01 ± 0.25^{aAB}	3.82 ± 0.22^{aAB}	3.16 ± 0.44^{aB}
C	4.89 ± 0.15^{aA}	4.52 ± 0.11^{aA}	3.72 ± 0.18^{bB}	3.45 ± 0.16^{aB}	3.39 ± 0.16^{aB}
Lactic acid bacteria					
T	5.28 ± 0.08^{aA}	5.51 ± 0.17^{bA}	5.07 ± 0.08^{bA}	3.09 ± 0.06^{bB}	2.25 ± 0.11^{cB}
CF	5.32 ± 0.08^{aC}	6.48 ± 0.14^{aB}	6.72 ± 0.12^{aB}	6.80 ± 0.08^{aB}	8.27 ± 0.16^{aA}
C	5.18 ± 0.10^{aC}	6.24 ± 0.16^{aB}	7.00 ± 0.10^{aAB}	6.68 ± 0.09^{aAB}	7.53 ± 0.24^{bA}
Yeasts and molds					
T	5.29 ± 0.06^{aA}	5.29 ± 0.01^{bA}	4.41 ± 0.09^{bA}	2.94 ± 0.17^{bB}	2.39 ± 0.09^{bB}
CF	5.29 ± 0.07^{aB}	6.32 ± 0.07^{aB}	7.54 ± 0.14^{aA}	8.22 ± 0.19^{aA}	8.06 ± 0.14^{aA}
C	5.27 ± 0.09^{aB}	6.05 ± 0.16^{bB}	7.40 ± 0.18^{aA}	8.37 ± 0.20^{aA}	8.16 ± 0.13^{aA}

Data is shown as means of 3 determinations ± standard deviation. Values with different lowercase letter in the same column indicate significant differences ($p < 0.05$) between treatments and values with different capital letter in the same row indicate significant differences ($p < 0.05$) trough storage time

T treated samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) and treated with vanillin (1.25 mg/mL) and ultrasound (7.5 min, 40 kHz, 180 W); *CF* control with fiber samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) without preservation treatment; *C* control samples: strawberry juice having neither fibers nor preservation treatment; *ND* non-detectable level (<2 logs CFU/mL)

stored at 5 °C was investigated. The effect of ultrasound was also more pronounced on yeast and molds than mesophilic bacteria and the decrease in the growth rate was higher when ultrasound was applied for 30 min (2 log cycles) than 10 min (<1 log cycle). On the other hand, the antimicrobial effects of vanillin alone on native microflora of strawberry juice enriched with prebiotic fibers and stored at 5 °C were also previously studied in Cassani et al. (2016). Significant reductions in every population of treated samples were observed (4 to 6 log cycles) when compared to untreated juices but the presence of vanillin introduced small sensory changes to the enriched samples. In the present work, a combined treatment previously optimized (Cassani et al. 2017) achieved better results with lower vanillin concentration and less ultrasound time. Also, Tomadoni et al. (2015b) reported that when ultrasound is applied in combination with chemicals, they improve the efficiency of the sanitizing agents. This phenomenon may be due to the high pressure generated during ultrasound treatments that weakens the cell wall, facilitating the penetration of the antimicrobials (in this study, vanillin) through the cellular membrane (Tomadoni et al. 2015b). Lanciotti et al. (2004) reported that the cell membrane is the primary target of bioactive aroma compounds. Phenolic compounds (including vanillin) have a lipophilic nature and could accumulate in the lipid bilayer of the cell, disturbing and sensitizing the membrane to ultrasound (Gómez et al. 2011). In addition, the low pH of strawberry juice seemed to favor the effectiveness of vanillin (Fitzgerald et al. 2004).

Summarizing, the combined preservation treatment resulted highly effective to control native microbial population of enriched strawberry juice. In fact, shelf-life of the beverage was mainly dependent on LAB and YM growth since they were the predominant microflora in control samples. According to the Spanish Regulation, 7 log CFU/mL is the maximum limit of microorganisms (at expiry date) in minimally processed foods (BOE 2001). Therefore, from the seventh day of storage, samples without preservative treatment (CF and C) would not be commercially accepted. However, when the optimized preservative treatment was applied to strawberry juice, spoilage was delayed and, as a consequence, microbiological shelf-life was extended for at least seven more days.

Nutritional Quality

Ascorbic Acid Content Figure 1 displays the evolution of AAC of strawberry juice samples during storage. The inclusion of prebiotics had no significant effect on the ascorbic acid content of strawberry juice, since the difference detected in AAC initial values between CF and C was not statistically significant. Also, the application of the preservative treatment was not detrimental for this parameter, since AAC initial value in treated samples was similar to those registered in controls. In fact, initial AAC values were between 41.92 and 46.42 mg/100 mL of juice. These values are within the range observed in Klopotek et al. (2005), who reported that vitamin C contents were between 37 and 69 mg/100 mL in different strawberry juices. This is an interesting result since it has been demonstrated that heat pasteurization (the conventional preservation treatment used in juice industry) detrimentally affects AAC, diminishing the nutritional quality of juices (Klopotek et al. 2005).

During the first week of storage, AAC in every sample significantly increased but no significant differences among samples were detected. Thereafter, a slight decrease was observed and, at the end of storage, losses of AAC in treated samples were not statistically significant (approximately 3.4%) compared to the controls (CF and C). This fact demonstrated that no oxidative processes took place since the retention of AAC was almost 100%.

Losses of over 50% are typical for vegetables but are significantly lower for most fruits, particularly acid fruits because of the stabilizing effects of low pH conditions, as it is the case of strawberry juice (pH 3.25) (Davey et al. 2000).

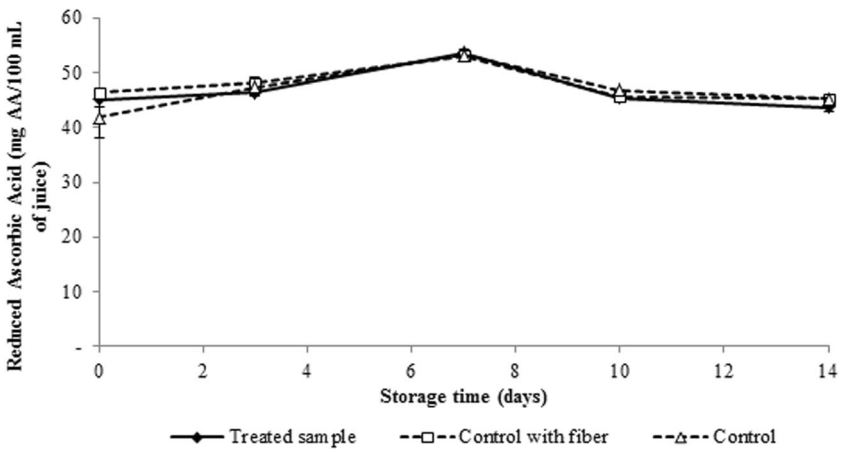

Fig. 1 Effect of vanillin combined with ultrasound on ascorbic acid content of strawberry juice enriched with prebiotic fibers during storage. *Bars* indicate standard errors

Total Polyphenol Content, Antioxidant Activity, and Total Flavonoid Content Figure 2a–c) displays total phenolic content, DPPH· radical scavenging activity, and total flavonoid content of strawberry juice samples during storage. The addition of oligofructose and inulin in the formulation significant increased TFC of strawberry juices, but no significant differences in TPC and DPPH were detected between controls (CF and C).

Immediately after the application of preservative treatment, TPC almost doubled the content found in untreated samples (Fig. 2a). This was an expected result given that vanillin is a phenolic compound and this effect was also reported in previous works (Tomadoni et al. 2016). Also, disruption of cell wall due to cavitation as a result of rapid change in pressures of the liquid by shear forces exerted during sonication might lead to the release of some chemically bound polyphenolic phytonutrients and increased their availability in the juice as was reported by Abid et al. (2013). During storage, the difference found in TPC between treated and controls samples remained unchanged, i.e., TPC in treated samples resulted twofold greater than controls at 14 days. Similar behavior was reported by Tomadoni et al. (2016) when evaluated TPC of strawberry juice treated with vanillin at two concentrations (2.5 and 5 mg/mL) stored at 5 °C. These authors

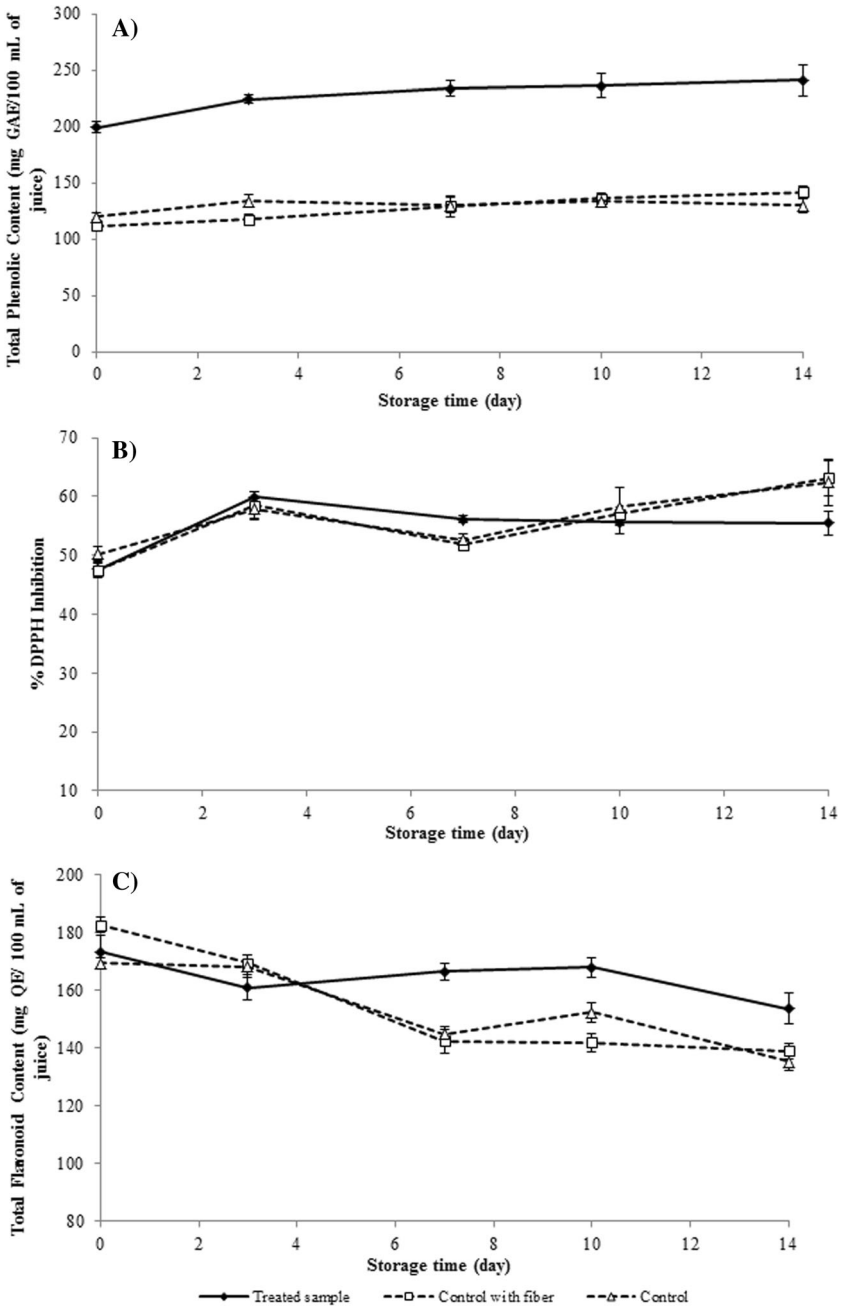

Fig. 2 Effect of vanillin combined with ultrasound on antioxidant capacity of strawberry juice enriched with prebiotic fibers during storage. **a** Total phenolic content. **b** DPPH radical scavenging activity. **c** Total flavonoid content. *Bars* indicate standard errors

reported a significant increment in TPC of treated samples and its maintenance throughout refrigerated storage.

Figure 2b shows the DPPH radical scavenging antioxidant activity of strawberry juice samples. The antioxidant capacity of all samples showed a similar behavior during storage with no significant differences in their values. A slight increase in this parameter was detected in every sample during the first 3 days of storage. Afterwards, all samples exhibited fluctuations in the percentage of DPPH inhibition. Similar to our findings, Piljac-Žegarac et al. (2009) observed some fluctuations in antioxidant capacity of dark fruit juices in refrigerated storage. In our previous work (Cassani et al. 2016), the application of vanillin did not display any effect on the antioxidant capacity of strawberry juices enriched with prebiotics. Besides, Bortolomeazzi et al. (2007) demonstrated that antioxidant capacity of vanillin depends on the pH of the medium. At low pH (3.25), antioxidant capacity of this natural preservative significantly decreases.

Figure 2c shows the evolution of TFC of strawberry juices samples during storage. Up to 7 days of storage, a noticeable decrease in TFC was detected in control samples (CF and C), with values significantly lower than those registered in treated ones. Treated samples also showed a decrease in TFC values during storage, however at a lower rate than control ones. Thereafter, the preservation treatment applied contributed to retain TFC during storage, achieving a product with higher nutritional quality during storage.

Sensory Quality

Instrumental Color Table 2 shows the evolution of L^* and $h°$ of the strawberry juice samples during storage. The addition of oligofructose and inulin in the formulation did not cause any change in L^* and $h°$ parameters as their values were similar between control samples (CF and C). L^* parameter was also not affected by the application of vanillin and ultrasound treatment, indicating that luminosity of treated samples remained similar to control ones. On the other hand, $h°$ parameter was slightly reduced after treatment; however, $h°$ value of treated sample resulted really close to those of control ones and it is probably that this little but statistically difference was not perceived by the human eye. These are interesting results since color is a visual indicator to judge the quality of fruit juices and plays an important role in consumer satisfaction and acceptability of the product. During storage, L^* and $h°$ values decreased in every sample, with no significant differences among treated and control samples. Similar results were found by Tomadoni et al. (2017) who observed that when ultrasound treatments (for 10 and 30 min) were applied to strawberry juice, L and $h°$ values decreased during storage with no differences respect to control samples.

Sensory Evaluation Table 3 displays results of sensory evaluation (color, OVQ, odor, and acid and sweet taste) of strawberry juice samples at 0, 7, and 14 days of storage. The addition of prebiotic fibers into the formulation did not significantly modify any of the sensory attributes studied. It was an unexpected result since the incorporation of oligofructose usually enhances the sweet taste of the juice because its sweetness is 35% higher than sucrose and, as a result, this prebiotic fiber is used as a sugar substitute in many foods (Franck 2002). The possible explanation of this result can be attributed to the strawberries used in this study which presented higher intensity

Table 2 Color evolution in treated and untreated strawberry juice samples during refrigerated storage

Samples	Storage time (day)				
	0	3	7	10	14
L^*					
T	34.12 ± 1.39^{aA}	34.78 ± 1.02^{bA}	28.94 ± 1.14^{aB}	25.92 ± 0.75^{aB}	25.66 ± 0.68^{aB}
CF	37.70 ± 0.76^{aA}	38.43 ± 0.65^{aA}	26.52 ± 1.33^{abB}	25.75 ± 0.59^{aB}	25.23 ± 0.78^{aB}
C	34.09 ± 0.75^{aA}	33.69 ± 1.13^{bA}	23.92 ± 0.47^{bB}	24.57 ± 0.63^{bB}	24.28 ± 0.61^{aB}
$h°$					
T	39.25 ± 0.24^{bA}	36.63 ± 0.34^{bAB}	34.07 ± 1.34^{aB}	35.65 ± 0.49^{aB}	36.10 ± 0.67^{aAB}
CF	40.55 ± 0.19^{aA}	38.34 ± 0.21^{aAB}	36.61 ± 1.15^{aB}	36.34 ± 0.43^{aB}	36.78 ± 0.60^{aB}
C	40.32 ± 0.20^{aA}	37.66 ± 0.36^{abAB}	36.70 ± 0.50^{aB}	36.69 ± 1.28^{aB}	35.57 ± 0.40^{aB}

Data is shown as means of 3 determinations ± standard deviation. Values with different lowercase letter in the same column indicate significant differences ($p < 0.05$) between treatments and values with different capital letter in the same row indicate significant differences ($p < 0.05$) trough storage time

T treated samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) and treated with vanillin (1.25 mg/mL) and ultrasound (7.5 min, 40 kHz, 180 W); *CF* control with fiber samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) without preservation treatment; *C* control samples: strawberry juice having neither fibers nor preservation treatment

Table 3 Impact of combined treatment and storage time on sensory attributes of strawberry juices at days 0, 7, and 14 of refrigerated storage

Time (day)	Samples	Sensory attribute				
		Overall visual quality	Color	Odor	Sweet taste	Acid taste
0						
T		5.00 ± 0.00[a]	5.00 ± 0.00[a]	4.33 ± 0.21[b]	2.11 ± 0.42[a]	1.73 ± 0.38[a]
CF		4.93 ± 0.06[a]	5.00 ± 0.00[a]	4.96 ± 0.03[a]	2.94 ± 0.19[a]	1.17 ± 0.45[a]
C		4.95 ± 0.05[a]	5.00 ± 0.00[a]	4.92 ± 0.06[a]	2.53 ± 0.13[a]	1.31 ± 0.41[a]
7						
T		3.91 ± 0.19[a]	4.48 ± 0.09[a]	3.36 ± 0.31[a]	2.03 ± 0.72	2.00 ± 0.14
CF		3.68 ± 0.12[a]	4.17 ± 0.22[ab]	0.45 ± 0.16[b]	NE	NE
C		3.48 ± 0.19[a]	3.90 ± 0.10[b]	0.16 ± 0.08[b]	NE	NE
14						
T		3.61 ± 0.23[a]	3.87 ± 0.24[a]	3.16 ± 0.22[a]	1.94 ± 0.01	2.73 ± 0.01
CF		1.77 ± 0.25[b]	3.86 ± 0.29[a]	0.50 ± 0.09[b]	NE	NE
C		1.43 ± 0.39[b]	3.78 ± 0.28[a]	0.33 ± 0.22[b]	NE	NE

Data is shown as means ± standard deviation. Values with different letters in the same column indicate significant differences ($p < 0.05$) between treatments

NE not evaluated. Sweet and acid taste at 7 and 14 days of storage, in EC and C samples, were not evaluated due to their high microbial load; *T* treated samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) and treated with vanillin (1.25 mg/mL) and ultrasound (7.5 min, 40 kHz, 180 W); *CF* control with fiber samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) without preservation treatment; *C* control samples: strawberry juice having neither fibers nor preservation treatment

of sweet taste than those found in previous studies (Cassani et al. 2016; Tomadoni et al. 2016). Sugar and acid content are strongly affected by different factors, such as genetic and environmental conditions (Crespo et al. 2010 and Kallio et al. 2000), which lead to significant differences in the taste of strawberries among experimental runs. The application of the preservation treatment on enriched strawberry juice did not affect initial scores of the sensory parameters evaluated, except for odor scores which resulted slightly lower in treated samples than in controls. This is probably due to the application of vanillin which was perceived by panelists, slightly changing the initial odor of the juice. However, this flavor was found acceptable and compatible with the fruit product and, therefore, did not abate over the course of the experiment.

After the first week of storage, untreated samples (CF and C) were only visually evaluated as they presented signs of fermentation and microbial growth. During storage, odor and overall visual quality of these samples significantly decreased obtaining scores below 2.5 also associated with fermentation, gas production, off-odors, and increased turbidity observed in these samples, which could be attributed to the important growth of yeast and molds registered. On the contrary, treated samples maintained higher scores of odor and OVQ than untreated ones during storage and acceptable values for sweet and acid taste. Moreover, up to 14 days of storage, no visible deleterious effects were detected on treated samples, presenting good appearance and showing an OVQ score higher than the acceptability level. With respect to color and in accordance with results found for instrumental color determination, the inclusion of prebiotics and the applied preservative treatment did not affect the typical color of the strawberry juices, maintaining its scores during the entire evaluation period.

In a previous work, 1.87 mg/mL of vanillin applied to strawberry juice enriched with different prebiotic fibers (inulin, oligofructose, and apple fiber) was able to maintain sensory characteristics over the acceptability level during the entire refrigerated storage period (Cassani et al. 2016). Similar results were found by Tomadoni et al. (2016) who evaluated the application of vanillin (at 2.5 and 5 mg/mL) in strawberry juice stored at 5 °C. In their work, Tomadoni et al. (2016) found that odor and taste attributes were significantly affected by incorporation of vanillin in comparison to untreated samples. Furthermore, no significant differences were observed on strawberry juice color between treated and untreated samples. Besides, Tomadoni et al. (2015b) evaluated the application of ultrasound at 15 and 30 min (40 kHz; 180 W) to kiwifruit juice and they found that the applied treatments had no negative impact on the sensory quality.

Summarizing, the applied preservative treatment did not introduce deleterious effects on sensory parameters of enriched strawberry fruit and allowed to retain their attributes for a longer period extending its sensory shelf-life.

Table 4 *E. coli O157:H7* and *L. innocua* survival (log CFU/mL) in inoculated strawberry juice samples during refrigerated storage

Samples	Storage time (day)				
	0	3	7	10	14
E. coli 0157:H7					
T	4.41 ± 0.29^{aA}	2.23 ± 0.00^{cB}	ND	ND	ND
CF	4.73 ± 0.14^{aA}	4.21 ± 0.03^{bAB}	3.63 ± 0.16^{aBC}	3.27 ± 0.19^{aC}	2.07 ± 0.07^{bD}
C	4.72 ± 0.11^{aA}	4.61 ± 0.04^{aA}	3.99 ± 0.05^{aB}	3.20 ± 0.16^{aC}	2.52 ± 0.12^{aD}
L. innocua					
T	3.80 ± 0.06^{bA}	2.35 ± 0.35^{aB}	ND	ND	ND
CF	4.03 ± 0.16^{abA}	2.75 ± 0.07^{aB}	2.35 ± 0.35^{aB}	2.82 ± 0.18^{aB}	2.70 ± 0.00^{aB}
C	4.33 ± 0.17^{aA}	3.06 ± 0.15^{aB}	2.61 ± 0.19^{aB}	2.33 ± 0.20^{aB}	2.35 ± 0.35^{aB}

Data is shown as means of 3 determinations ± standard deviation. Values with different lowercase letter in the same column indicate significant differences ($p < 0.05$) between treatments and values with different capital letter in the same row indicate significant differences ($p < 0.05$) trough storage time

T treated samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) and treated with vanillin (1.25 mg/mL) and ultrasound (7.5 min, 40 kHz, 180 W); *CF* control with fiber samples: strawberry juice enriched with inulin and oligofructose (5:3 ratio) without preservation treatment; *C* control samples: strawberry juice having neither fibers nor preservation treatment; *ND* non-detectable level (<2 logs CFU/mL)

Performance Against Pathogen Contamination

Table 4 shows the survival of *E. coli* O157:H7 and *L. innocua* in inoculated strawberry juice samples during storage. Presence of endogenous *L. innocua* and *E. coli* in non-inoculated juices was studied during the whole storage and no colonies of either pathogen were detected. The incorporation of fibers in the juice formulation did not affect the pathogens behavior in inoculated samples. The application of the preservation treatment on enriched strawberry juice did not reduce initial counts of *E. coli*, since counts in treated samples were not statistically different from those registered in controls (CF and C). On the other hand, the treatment produced a slight decrease in the initial *L. innocua* counts.

During storage, the preservation treatment had a noticeable effect in reducing *L. innocua* and *E. coli* counts compared to controls (CF and C). In fact, no colonies were detected (detection limit, 2 log CFU/mL) in treated samples after 7 days of storage. It is important to note that control samples presented also a decrease in *L. innocua* and *E. coli* counts during storage, reaching values between 2 and 3 log CFU/mL up to day 14. Those observed reductions in control samples along storage could be due to storage temperature (5 °C), low pH (3.25), and competitive native microflora, which meant multiple barriers that affected *E. coli* and *L. innocua* growth. In spite of this, observed reductions through storage time in treated samples were even greater, and this additional effect is attributed to the applied preservation treatment (Raybaudi-Massilia et al. 2008). Thus, the applied preservation treatment was not sufficient to immediately inhibit *E. coli* or *L. innocua* growth but probably exerted a deleterious effect on these microorganisms, leaving these populations more susceptible to other barriers such as low temperatures or low pH.

Similar results were found in previous works. A study conducted by Fitzgerald et al. (2004) suggests that the inhibitory activity of vanillin resides primarily in its ability to detrimentally affect the integrity of the cytoplasmic membrane, with the resultant loss of ion gradients, pH homeostasis, and inhibition of respiratory activity. Ferrante et al. (2007) reported that the overall inactivation effect observed in *L. innocua* when ultrasound treatment was combined with the addition of antimicrobials, depended on several factors, such as the considered antimicrobial agent and its concentration, the medium, and the microorganism studied. For instance, Tomadoni et al. (2015a) studied the efficacy of vanillin (2.5 and 5 mg/mL) in reducing *E. coli* O157:H7 on strawberry juice and they found that the juice sample with the lowest vanillin concentration had no initial effect in *E coli* counts, while the highest one showed a significant reduction (~2 log cycles) in comparison to untreated sample. Similar results were found by Corte et al. (2004) who studied the survival of *L. innocua* in apple juice (pH 3.3) supplemented with vanillin (1.5 or 3 mg/mL). They registered a significant reduction (~5 log cycles) when 3 mg/mL of vanillin was applied. However, 1.5 mg/mL was significantly less effective in inactivating *L. innocua.* In the present work, 1.25 mg/mL of vanillin combined with 7.5 min of ultrasound treatment at 40 kHz might not be enough to show significant differences immediately after treatment application. Nonetheless, the effect was observed after 7 days of storage, when *L. innocua* and *E. coli* counts were not detected in fiber-enriched sample treated with ultrasound and vanillin. Furthermore, it is recognized that if vanillin is to be used as a preservative at higher concentrations than those established in this work, it will impart its flavor characteristics to the food product and hence its organoleptic quality will be compromised, which is of major relevance (Fitzgerald et al. 2004).

Conclusions

The evolution of quality parameters (microbiological, nutritional, and sensory) of strawberry juice enriched with prebiotic fibers and treated with a previously optimized treatment (ultrasound and vanillin) was studied during 14 days of storage at 5 °C. From these results, it can be concluded that the addition of the mix of prebiotics had no negative implication regarding the quality of fresh juice and maintained sensory attributes of the product. The applied preservation treatment improved almost every quality attribute during storage, reducing microbial growth, especially in the main spoilage flora of strawberry (lactic acid bacteria and yeast and molds). Total polyphenol and total flavonoid content were increased and ascorbic acid content losses were reduced by the treatment during storage, indicating higher antioxidant capacity. Overall, the evaluated sensory attributes of treated juices were deemed acceptable (>2.5). The addition of vanillin imparted pleasant flavor notes to the juice compatible with the fruit product. Also, the optimized treatment was able to reduce *E. coli* O157:H7 and *L. innocua* counts (pathogens of interest in the food industry and with health concern) during storage reaching undetectable values after 7 days of storage.

Combination of vanillin with ultrasound, at levels previously optimized, showed to be a good alternative to traditional thermal treatment and chemical sanitizers in extending the shelf-life as well as increasing safety of strawberry juice enriched with prebiotic fibers. Therefore, the inclusion of prebiotics to strawberry juice could appeal to health conscious consumers who are looking for healthier and nutritive food products. Besides, the combination of vanillin and ultrasound is a feasible alternative technology to deliver a microbiologically stable product while improving its quality parameters.

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