Green ultrasound-assisted processing for extending the shelf-life of prebiotic-rich strawberry juices

Lucía Cassani[1*], Bárbara Tomadoni[2], María del Rosario Moreira[1]

[1] Grupo de Investigación en Ingeniería en Alimentos (GIIA), Instituto en Ciencia y Tecnología de alimentos y Ambiente (INCyTAA), Facultad Ingeniería, Universidad Nacional de Mar del Plata (UNMdP) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), J.B. Justo 4302, Mar del Plata (7600), Argentina.

[2] Grupo de Materiales Compuestos Termoplásticos (CoMP), Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA), Facultad de Ingeniería, Universidad Nacional de Mar del Plata (UNMdP) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Colón 10850, Mar del Plata (7600), Argentina.

Corresponding author:

*Lucía Cassani

Telephone: +54-223-4816600

E-mail: lcassani@fi.mdp.edu.ar

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.10604

Running title: Effect of ultrasound and storage on overall quality of prebiotic-rich juices

Abstract

BACKGROUND: Adding value to conventional fruit juices by including prebiotic compounds into their formulation and preserving them using non-thermal, eco-friendly and safe technologies represents interesting and strategic approaches to diversify the healthy and innovative food products offer. In this context, the effect of ultrasound-assisted processing (for 15 and 30 min, 40 kHz, 180 W) on microbiological, physicochemical, nutritional and sensory quality of prebiotic-rich strawberry juices was investigated during storage (14 days, 5 ºC).

RESULTS: Compared to untreated samples, the applied preservation treatments enhanced the microbiological and nutritional quality of samples by significantly reducing native microflora counts (reductions up to 1 log CFU mL^{-1} at day 14) and increasing the total phenolic content (by more than 25% in comparison to controls at day 14) leading to higher antioxidant capacity of prebiotic-rich strawberry juices. Ultrasound processing and prebiotic enrichment had no negative effect on sensory attributes of enriched samples, suggesting that this non-thermal technique allowed to successfully retain the fresh-like attributes of strawberry juices during their shelf-life, contributing to the good sensory stability of juices. In addition, ultrasound treatments had no detrimental impact on physicochemical quality and ascorbic acid content of enriched samples, showing similar stability to control samples during storage.

CONCLUSION: Based on our results, ultrasound processing appears to be a promising non-thermal technique to ensure a stable product from both microbiological and sensory points

of view with improved antioxidant capacity and unaltered physicochemical quality while offering a healthier, nutritive and valuable food alternative.

Keywords: eco-friendly technology, prebiotic enrichment, fruit-based product, quality, storage.

1. Introduction

The development of functional food products is a growing sector of the food industry concerned at fulfilling the demand of increasingly exigent consumers. In particular, the systematically growing number of vegans, having special difficulties to achieve the daily requirements of certain nutrients (*e.g.*, vitamins and minerals), underlines the need for formulating healthy innovative food products. In this context, prebiotic compounds such as inulin and oligofructose, constitute natural ingredients with well-demonstrated health beneficial properties, appreciated for the formulation of functional foods.[1]

Over the last years, eating habits have been changed since worldwide consumers are more conscious and informed about the role of nutrition in health promotion and prevention of some chronic disease (*e.g.*, obesity, diabetes, cardiovascular, etc.).[2] The net result is a steadily growing demand for so-called ‘real foods’, a term that is difficult to define by any formal standards of identity, but refers to foods typically perceived as fresh, clean (free of artificial additives), beneficial and less processed. For example, fruit-based products meet all these requirements as they have fresh-like characteristics and a high nutrition profile while being microbiologically safe with an extended shelf-life.[3] Strawberry is one of the most popular fruits used in the juice industry due to its significant nutritional value, attractive organoleptic attributes and interesting antioxidant compounds composition.[4] Thermal pasteurization is the most common practice used in the food industry to prolong fruit juices' shelf-life by inactivating deteriorating microorganisms and enzymes.[1] However, this popular preservation technique has received many doubts not only for unavoidably leading to the worse quality and nutritional value of fruit juice (resulting in lower consumer preference) but also for consuming a lot of energy and large volumes of water.[3] In this regard, the growing awareness in environmental sustainability has encouraged the food industry to adopt more sustainable, green and innovative processing technologies increasing production efficiency by reducing the use of water, energy and time. Ultrasound-assisted processing has been demonstrated to be a potential alternative to thermal pasteurization of fruit juices since it allowed to inactivate deteriorating and pathogenic microorganisms of fruit juices while enhancing their bioactive compounds composition.[5] Furthermore, ultrasound is reliable, environmentally friendly and easy to scale-up, suggesting that it is more feasible to implement at commercial scale than other expensive non-thermal techniques.[4]

So far, many studies have reported the effect of ultrasound mainly on microbiological and antioxidant properties of fruit juices (immediately evaluated after application, day 0).[6-9] However, it is important to analyze the evolution of overall quality parameters, including sensory analysis of sonicated prebiotic-rich strawberry juices during long-term storage. To fill this gap, the main objective of this work was to study the effects of ultrasound-assisted processing (for 15 and 30 min, 40 kHz, 180 W) on microbiological, physicochemical, nutritional and sensory quality of prebiotic-rich strawberry juices during storage (14 days, 5 ºC).

2. Materials and Methods

2.1 Obtaining prebiotic-rich strawberry juices

Strawberries (*Fragaria* x *ananassa* Duch, cv. Aromas) were provided by a local producer and were subjected to inspection, washing and squeezing according to the procedure proposed in our previous work.[1] Then, prebiotic compounds (inulin and oligofructose) were directly incorporated into each strawberry juice at 15 g L^{-1} and stirred until total dissolution. The prebiotic concentration was selected according to our previous studies. Once homogenized, juices were bottled under hygienic conditions to be then used in the experiments.

2.2 Ultrasound treatments

350-mL cylindrical vessels (diameter, 7 cm; height, 7.2 cm) containing the prebiotic-rich juice samples were treated with ultrasound by placing them in an inner tank of ultrasound bath cleaner (dimensions: 15 x 29 x 15 cm, capacity: 6.5 L) (TestLab, Argentine) at 40 kHz frequency and 180 W (transmitted from bottom to above) in the dark, to avoid any light interference. The temperature during ultrasound treatments was controlled at 20 ± 1 ºC using a thermometer. Processing time was set at 15 and 30 min and was selected according to Zinoviadou *et al*. [10]

Samples were coded for easy identification throughout the manuscript as follows: Ol+US15 and In+US15 (refers to oligofructose or inulin-rich juice treated with ultrasound during 15 min); Ol+US30 and In+US30 (refers to oligofructose or inulin-rich juice treated with ultrasound during 30 min). Three extra juice samples were used as control: Control_In (refers to strawberry juice enriched with inulin without any treatment), Control_Ol (refers to strawberry juice enriched with oligofructose without any treatment) and control (refers to strawberry juice without any prebiotic addition or treatment).

The evolution of microbiological, physicochemical, nutritional and sensory parameters of juice samples was thoroughly analyzed during 14 days of storage at 5 ºC.

2.3 Microbiological quality

The native microflora of juice samples was evaluated through the determination of total aerobic mesophilic bacteria (MES), psychrophilic bacteria (PSY) and yeast and molds (YM) populations. For this purpose, 10 mL of each treated juice was sampled at 0, 3, 7, 10 and 14 days of storage. Then, serial dilutions (1:10) were made in peptonated water (1 g kg^{-1}) and surface spread by duplicate. The enumeration of microbial population was determined as described in our previous work.[1] Microbial counts were expressed as log CFU mL^{-1}.

2.4 Physicochemical parameters

The physicochemical analysis included the determination of different parameters, i.e. total soluble solids (TSS), titratable acidity (TA) and instrumental color, during cold storage according to the methodologies described below.

TSS is an important quality parameter in both harvest (as strawberry should have a minimum TSS to be harvested), and postharvest stage since TSS is related to sensory properties of the fruit. In this regard, TSS was measured through the ºBrix scale using an Atago refractometer (Abbe 1T74T, Tokyo, Japan).

TA is also an important quality parameter since organic acids may affect the sensory attributes of the fruit. TA was determined as described according to the methodology proposed by Sadler and Murphy.[11] TA was expressed as g citric acid 100 mL^{-1} of juice.

Color is the first sensory attribute which consumers perceive and may influence the product acceptability. Color measurement 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 juice samples was determined by measuring the L*, a*, b* coordinates of the CIE-Lab scale. In addition, a* and b* values were used to calculate huge angle (hº) according to the equation described in our previous work.[1]

2.5 Nutritional quality

Nutritional quality of juice samples was evaluated through different parameters (including the ascorbic acid content, total phenolic compounds and antioxidant activity) during refrigerated storage according to the methodologies described below.

The ascorbic acid (AA) content is an important nutritional parameter of fruit products since AA is more sensitive to food processing and long-term storage than other nutrients. This suggests that if the AA content remains unchanged during processing and storage, other nutrients would also do.[12] In this regard, the determination of AA content was carried out according to the procedure proposed in our previous study.[13]

Strawberry is a rich source of phytochemical compounds contributing to its nutritional and sensory properties.[14] Extracts of juice samples were obtained to determine the total phenolic content (TPC) and antioxidant capacity. For this purpose, 2 mL of each juice sample were homogenized with 10 mL of aqueous ethanol (0.8 L L^{-1}) (Merck, Darmstadt, Germany). Then, extracts were centrifuged at 13 000 × *g* (15 min, 4 °C) and the supernatant was collected and filtered using Whatman filter paper no. 1. Finally, all extracts were stored at -20 °C to be used in the subsequent experiments.

For TPC determination, the spectrophotometric Folin–Ciocalteu assay was carried out according to the method described by Viacava and Roura[15] with some adaptations. TPC was expressed as mg gallic acid equivalents (GAE) 100 mL^{-1} of juice.

The antioxidant capacity of juice samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to the method proposed by

Viacava and Roura[15] with some modifications. Results were expressed as mg of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents 100 mL^{-1} of juice.

2.6 Sensory quality

Analyzing sensory attributes of food products is frequently used during the development of functional foods or the re-formulation of original products to detect possible changes in organoleptic parameters in comparison to the original counterpart. In this context, sensory characteristics of juice samples were evaluated by a panel comprising of eight members, ages ranged from 25 to 50 years, with sensory experience in fruits and juices. Panelists discussed the meaning of each attribute after observing a set of strawberry juices with different degree of spoilage. From these meetings, sensory attributes were defined as follows:

Overall visual quality (OVQ): associated to the general aspect of juice sample, including freshness, color, consistency and presence of suspended particles.

Odor: related to the fresh and characteristic strawberry odor.

Off-odor: resulted from the growth of microorganisms and other non-characteristic strawberry odor.

Sweet taste: refers to basic taste of sweetness.

Acid taste: refers to basic taste of acidity.

Juice samples (30 mL) were served and randomly presented in small plastic cups coded with three-digit numbers. Mineral water was provided for panelists to rinse their mouths between samples. The intensity of each attribute was indicated by placing a mark on a 5 cm unstructured linear scale, anchored with the legends ‘low intensity’ and ‘high intensity’. In this sense, OVQ was rated from 0 = ‘highly deteriorated appearance’ to 5 = ‘excellent’; odor was marked from 0 = ‘not perceptible’ to 5 = ‘very perceptible’ (fresh), off-odor, sweet and acid taste were scored from 0 = ‘not perceptible’ to 5 = ‘intense’.

Considering that the consumers purchase decision is mainly influenced by OVQ of the product, this attribute was selected to determine the sensory shelf-life of juice samples. In this sense, it is important to highlight that special emphasis on ensuring that panelists could adequately discriminate between a ‘highly deteriorated appearance’ (value 0), ‘acceptable’ (value 2.5) and ‘excellent’ (value 5) was put it on. Thus, the sensory shelf-life limit was established at an OVQ score of 2.5. Those juices that showed a lower score than the established one, indicated the end of their sensory shelf-life.

2.7 Statistical analysis

A completely randomized design was used and two independent runs were performed. Data was analyzed using R v. 2.12.2 (R Development Core Team, 2011). Results reported in this work are mean values accompanied by their standard errors. 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 ($p < 0.05$).

3. Results and discussion

3.1 Microbiological quality

Figure 1a-c depicts the evolution of MES, PSY, and YM counts of treated and untreated samples during storage. The application of ultrasound for 15 and 30 min, showed no immediate effect on reducing the initial native microflora counts of prebiotic-rich juices since no significant differences in comparison to controls were observed (day 0).

During the first week of storage, a significant decrease ($p < 0.05$) in every microbial population counts of prebiotic-rich juices treated with ultrasound when compared to controls was observed. In addition, the antimicrobial effect was higher when ultrasound was applied for 30 min, suggesting that the higher ultrasound processing time, the higher decrease in microbial counts. In contrast, native microflora found an ideal growth media in untreated juices as their counts continuously increased during storage. Samples enriched with prebiotics and treated with US (at both processing times) also showed an increase in their counts during the second week of storage, but the growth rate was much lower than that observed in controls. In fact, up to day 10 of storage, microbiological counts of untreated samples were greater than 7.0 log CFU mL^{-1} (the maximum limit established by the Spanish Regulation of allowed mesophilic total counts of minimally processed foods at expiry). Considering 10^7 CFU mL^{-1} as a maximum limit for all the studied populations, untreated juices would not be commercially accepted from the 10th day of storage. However, when ultrasound was applied to prebiotic-rich juices, spoilage was delayed and thus, the microbiological shelf-life was extended for 4 more days.

Possibly, the ultrasound treatment caused sublethal damage on the native microflora of prebiotic-rich juices. In other words, the application of ultrasound showed no immediate effect in reducing initial counts of the microbial populations but delayed cell reproduction during the first days of storage. The antimicrobial activity of ultrasound is attributed to the intracellular cavitation phenomenon, which causes micro-mechanical shocks resulting from the formation and continuous rupture of microscopic bubbles, induced by pressure changes during ultrasound application. This process may be responsible for causing several damages in structural cell components and cellular functions leading to cell lysis or death. Furthermore, free radical formation, namely $O^{\cdot 2}$, $OH^{\cdot}$, $HOO^{\cdot}$, have also been considered as an important mechanism that may explain the antimicrobial activity of ultrasound.[16] These radicals produce oxidative damages due to their high reactivity, injuring microorganisms' cell walls and affecting mitochondria activity.

Many studies have been carried out using this non-thermal technology to enhance microbiological stability of fruit juices at day 0. For example, ultrasound was used to control native microflora growth of pear juice,[9] guava juice,[17] minimally processed strawberry,[8] apple juice,[6] blueberry juice,[18] and tomato juice.[7] In contrast, few scientific studies focused on the native microflora evolution of fruit juice during storage (particularly strawberry juice) has been reported. In this regard, Gómez-López *et al.*[19] studied the application of ultrasound (8 min, 10 ºC, 20 kHz) on microbiological stability of orange juice with added calcium stored at 4 ºC and observed that MES and YM counts were significantly lower in comparison to control during storage.

3.2 Physicochemical changes

Table 1 shows the evolution of the total soluble solids content and titratable acidity of prebiotic-rich juices treated or not with ultrasound during storage. As it was expected, the addition of prebiotics significantly increased ($p < 0.05$) the initial TSS of juices (day 0). Then, the application of ultrasound treatments showed no effect on TSS and TA of prebiotic-rich juices, since no significant differences in these parameters between treated and control samples (neither at day 0 nor during storage) were observed. These results highlight the advantages of using ultrasound as preservation treatment since this technique successfully maintained the same TSS and TA values as control sample during the product's shelf-life. In contrast, storage time had a significant impact ($p < 0.05$) on TA of all studied samples (treated and controls), as a gradual increase in this parameter was observed during storage. A possible explanation of this result may be related to the production of metabolites (namely lactic acid, acetic acid, and ethanol) due to the high microbial load observed in all samples (treated and controls) at the end of storage. Similarly, Khandpur and Gogate[20] reported a significant increase in TA of carrot juice treated or not with ultrasound (15 min, 20 kHz) during storage (10 weeks, 4 ºC).

Table 2 depicts changes in L* and hº parameters of prebiotic-rich juices treated or not with ultrasound during storage. The addition of prebiotics significantly increased ($p < 0.05$) L* values of strawberry juices (Control_Ol and Control_In) in comparison to control (day 0). However, hº value was not affected by the prebiotics enrichment (day 0). Then, ultrasound treatments had no effect on color parameters of prebiotic-rich juices, since no differences in L* and h° values between treated and untreated samples (neither at day 0 nor during storage) were observed. These results suggest that ultrasound processing was able to successfully retain the typical color of strawberry juices during their shelf-life, contributing to the desired color stability of juices.

The luminosity of all studied samples significantly decreased ($p < 0.05$) in comparison to control as storage time increased, possibly attributed to oxidative browning reactions. In line with these results, h° values of all juices also decreased during storage. However, possibly these small variations of color parameters of all juices during storage would be barely perceptible to the consumers' eye. Similarly, Khandpur and Gogate[20] observed color changes of sweet lime and carrot juice treated with ultrasound (15 min, 20 kHz) during storage (10 weeks, 4 ºC) attributed to the degradation of pigments (carotenoids, anthocyanins), vitamins and other components.

Based on our results, ultrasound processing of prebiotic-rich strawberry juices appears as a promising non-thermal technique to improve quality attributes of juices without affecting the physicochemical parameters of strawberry juices.

3.3 Nutritional quality

Figure 2a shows the effect of ultrasound treatments on the ascorbic acid content of prebiotic-rich samples during storage. Both ultrasound treatments had no initial effect on the AA content of the inulin-enriched samples since no significant differences with respect to control (Control_In) were observed (day 0). In contrast, when ultrasound was applied to samples enriched with oligofructose, a significant decrease ($p < 0.05$) on the initial AA content in comparison to untreated samples (Control_Ol) was observed (day 0). However, minor differences in the AA content between samples were detected. In fact, the ascorbic acid values of all samples were within the range reported by other authors.[21] It is important to mention that the AA concentration may differ between experimental runs and results reported in the literature due to the fact that this parameter can fluctuate according to many different factors, such as variety, harvest conditions, environmental factors, extraction and quantification method, etc.

During storage, the AA content of prebiotic-rich samples treated with ultrasound showed no significant differences with respect to controls, indicating that ultrasound processing had no detrimental effect on this nutritional parameter. This is an interesting result since pasteurization (the conventional preservation treatment used in the food industry) has shown to produce several losses of this functional indicator, decreasing the nutritional quality of juices. Storage time had a significant impact on this parameter, as the AA content of all samples (treated and control) reduced as storage time increased, reaching losses of 30% at day 14 (compared to the initial AA values). During storage, juice samples are exposed to oxidation processes which lead to ascorbic acid degradation. The mechanism of AA degradation occurs through either aerobic or anaerobic pathways depending on storage conditions, packaging, and the processing method employed.[22] In this way, storage at a cold temperature (5 ºC), as well as, the low pH of juices probably aided to delay the degradation reactions of ascorbic acid.

Numerous works have studied the effect of ultrasound treatments on the AA content in fruit juices and contradictory results, differing mainly in operational conditions (processing time, frequency, temperature) and food matrix have been reported. Ascorbic acid stability has been studied in various matrices including strawberry juice (2-10 min, 25 °C, 20 kHz),[21] orange juice (2-10 min, 25 °C, 20 kHz),[23] tomato juice (2-10 min, 32-45 °C, 20 kHz) [7] and watermelon juice (2-10 min, 25-45 °C, 20 kHz).[24] In these works, degradation of ascorbic acid occurred as ultrasound processing time increased and this effect was attributed to oxidation reactions as a result of interaction between AA and free radicals formed during treatment[7]. In contrast, Aadil *et al.*[25] reported a significant increase in the AA content of sonicated grapefruit juices during 30, 60 and 90 min (20 °C, 28 kHz). Also, Cheng *et al*.[17] showed that this nutritional parameter significantly increased in guava juice treated with ultrasound for 30 min (20 °C, 35 kHz) with respect to control. These authors attributed the improvement in AA content to the oxygen removal due to the cavitation phenomenon.[25]

In this context, considering the instability of this nutritional parameter against various factors, it is important to study each individual case.

Figure 2b displays the evolution of the total phenolic content of prebiotic-rich samples treated (or not) with ultrasound during storage. Ultrasound processing significantly enhanced ($p < 0.05$) the TPC of prebiotic-rich samples in comparison to the control group (day 0). During storage, the TPC of juice samples treated with US were maintained at higher levels than controls (regardless of processing time) and remained stable during the entire evaluation period. In line with these results, Wang *et al*.[4] reported that ultrasound treatment enhanced the catechin, gallic acid, and ellagic acid (the main phenolic compound phenolic compounds present in strawberry) content in strawberry juice. Also, Abid *et al.*[26] reported a significant increase ($p < 0.05$) in TPC of apple juice (in particular, chlorogenic acid, caffeic acid, and catechin) when compared to control, after sonication for 30 min (20 ºC, 25 kHz).

Phenolic compounds are a complex group of secondary metabolites present mainly in the plant cell vacuoles, either in free or bound form with the cell wall components (namely polysaccharides). Thus, the improvement in the extraction yield of polyphenolic compounds in sonicated strawberry juices can be attributed to the mechanical disruption of cell walls leading to the release of phenolic compounds as the result of rapid change in pressures of the liquid by shear forces applied during sonication.[27]

Figure 2c shows the evolution of the antioxidant capacity of prebiotic-rich samples treated (or not) with ultrasound during storage. Ultrasound processing was effective in enhancing the initial antioxidant capacity of prebiotic-rich juices as significant differences ($p < 0.05$) in comparison to controls were observed (day 0). During storage, the antioxidant capacity of juice samples treated with US (regardless of processing time) significantly decreased ($p < 0.05$), however, these reductions did not exceed 20% compared to the initial values. Despite this, antioxidant capacity of treated samples remained at higher levels than control ones throughout the entire period of evaluation. The enhancement of antioxidant capacity of prebiotic-rich samples treated with ultrasound is related to the improvement in the release of bound phenolic compounds due to the cavitation mechanism. Similarly, Abid *et al*.[6] reported an increase in antioxidant capacity of apple juice when ultrasound treatment was applied for 30 min (25 ºC, 25 kHz). Higher antioxidant capacity values were reported in raspberry puree when ultrasound was applied for 30 min.[28]

In this context, ultrasound processing has demonstrated to be a promising technique to retain or enhance the concentration of nutritionally-relevant bioactive compounds and along with the prebiotic enrichment, turn the strawberry juice into a healthier and safer alternative.

3.4 Sensory quality

Table 3 shows the impact of applying ultrasound on sensory attributes of prebiotic-rich juices during storage. The incorporation of prebiotic compounds had no undesirable effect on sensory attributes evaluated in strawberry juices, indicating that it can be a feasible strategy to add value to the product. Ultrasound treatments (regardless of processing time) had no initial effect on the sensory attributes scores since no significant differences in comparison to the control group were observed (day 0).

During the first week of storage, OVQ of all samples significantly decreased ($p < 0.05$) and no differences between treated and untreated samples were observed, indicating that neither ultrasound treatment nor prebiotic enrichment had a detrimental effect on OVQ of strawberry juices. Regarding typical odor, a clear decrease in this attribute was recorded in control samples, while prebiotic-rich juices treated with ultrasound showed a more gradual decrease in typical odor, reaching scores significantly higher ($p < 0.05$) than the control group. Consequently, off-odors of all samples significantly increased ($p < 0.05$) during the first week of storage, with a more pronounced increment in untreated samples. Concerning taste attributes, the intensity of sweet taste significantly decreased ($p < 0.05$) in all samples throughout storage and no differences between treated and control juices were registered. On the other hand, acidity of all samples remained constant during the evaluation period (until day 3 for the control group and day 10 for the treated ones due to the high microbial load) and no significant differences between samples were found.

During the second week of storage, the OVQ scores of all samples gradually decreased, but at a lower rate in those prebiotic-rich samples treated with ultrasound. The typical odor of all samples (treated and controls) showed a similar trend to that observed in OVQ, reaching lower scores at day 14. Consequently, a strong increase in off-odor of all samples (treated or not with ultrasound) was observed and no significant differences between samples were reported.

Considering an OVQ score of 2.5 as the limit of the juices' shelf-life (from a sensory point of view), the control group showed a sensory shelf-life of 7 days while those prebiotic-rich samples treated with ultrasound (regardless processing time) retained the maximum sensory quality for a longer time period extending their sensory shelf-life (three more days).

4. Conclusion

Ultrasound application was effective in controlling native microflora of strawberry juices enriched with prebiotics during storage, extending their microbiological shelf-life for four more days compared to untreated samples, without affecting their physicochemical parameters.

The applied preservation treatment also improved the bioactive compounds extraction, as total phenolic content of prebiotic-rich juices was increased, indicating a higher antioxidant capacity of the formulated product. Furthermore, ultrasound treatments had no negative effect on the ascorbic acid content of prebiotic-rich samples and its stability during storage was similar to untreated ones.

In general, the applied preservation treatment and the inclusion of prebiotic compounds had no undesirable effects on sensory attributes of juice samples, showing a better sensory stability during storage than the control group.

Based on our results, ultrasound processing appears as a promising non-thermal preservation technique to ensure a stable product from a microbiological and sensory point of view with improved antioxidant capacity and unaltered physicochemical quality while offering a healthier, nutritive and valuable food alternative.

Acknowledgment

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Project PICT/2016/0723), and Universidad Nacional de Mar del Plata (UNMDP). L.C., B.T.

and M.R.M are members of the research career of the Argentinean Research Council (CONICET).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Cassani L, Tomadoni B, Viacava G, Ponce A and Moreira M, Enhancing quality attributes of fiber-enriched strawberry juice by application of vanillin or geraniol. *LWT-Food Sci and Technol* **72**:90-98 (2016).

2. Slawson DL, Fitzgerald N and Morgan KT, Position of the Academy of Nutrition and Dietetics: the role of nutrition in health promotion and chronic disease prevention. *J Acad Nut Diet* **113**:972-979 (2013).

3. Wang J, Liu Q, Xie B and Sun Z, Effect of ultrasound combined with ultraviolet treatment on microbial inactivation and quality properties of mango juice. *Ultrason Sonochem* **64**:105000 (2020).

4. Wang J, Wang J, Ye J, Vanga SK and Raghavan V, Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control* **96**:128-136 (2019).

5. Nadeem M, Ubaid N, Qureshi TM, Munir M and Mehmood A, Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrason Sonochem* **45**:1-6 (2018).

6. Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S, Zhang X and Zeng X, Effect of ultrasound on different quality parameters of apple juice. *Ultrason Sonochem* **20**:1182-1187 (2013).

7. Adekunte A, Tiwari B, Cullen P, Scannell A and O'Donnell C, Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem* **122**:500-507 (2010).

8. Gani A, Baba WN, Ahmad M, Shah U, Khan AA, Wani IA, Masoodi F and Gani A, Effect of ultrasound treatment on physico-chemical, nutraceutical and microbial quality of strawberry. *LWT-Food Sci and Technol* **66**:496-502 (2016).

9. Saeeduddin M, Abid M, Jabbar S, Wu T, Hashim MM, Awad FN, Hu B, Lei S and Zeng X, Quality assessment of pear juice under ultrasound and commercial pasteurization processing conditions. *LWT-Food Sci and Technol* **64**:452-458 (2015).

10. Zinoviadou KG, Galanakis CM, Brnčić M, Grimi N, Boussetta N, Mota MJ, Saraiva JA, Patras A, Tiwari B and Barba FJ, Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Res Int* **77**:743-752 (2015).

11. Sadler GD and Murphy PA, pH and titratable acidity, in *Food Analysis*. Springer, pp 219-238 (2010).

12. Özkan M, Kırca A and Cemeroğlu B, Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. *Food Chem* **88**:591-597 (2004).

13. Cassani L, Tomadoni B, Moreira MR and Agüero MV, Improving quality parameters of functional strawberry juices: Optimization of prebiotic fiber enrichment and geraniol treatment. *Food and Bioprocess Tech* **11**:2110-2124 (2018).

14. Tulipani S, Mezzetti B, Capocasa F, Bompadre S, Beekwilder J, De Vos CR, Capanoglu E, Bovy A and Battino M, Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J Agr Food Chem* **56**:696-704 (2008).

15. Viacava GE and Roura SI, Principal component and hierarchical cluster analysis to select natural elicitors for enhancing phytochemical content and antioxidant activity of lettuce sprouts. *Sci Hort* **193**:13-21 (2015).

16. Takahashi M, Chiba K and Li P, Free-radical generation from collapsing microbubbles in the absence of a dynamic stimulus. *J Phy Chem B* **111**:1343-1347 (2007).

17. Cheng L, Soh C, Liew S and Teh F, Effects of sonication and carbonation on guava juice quality. *Food Chem* **104**:1396-1401 (2007).

18. Mohideen FW, Solval KM, Li J, Zhang J, Chouljenko A, Chotiko A, Prudente AD, Bankston JD and Sathivel S, Effect of continuous ultra-sonication on microbial counts and physico-chemical properties of blueberry (*Vaccinium corymbosum*) juice. *LWT-Food Sci Technol* **60**:563-570 (2015).

19. Gómez-López V, Orsolani L, Martínez-Yépez A and Tapia M, Microbiological and sensory quality of sonicated calcium-added orange juice. *LWT-Food Sci Technol* **43**:808-813 (2010).

20. Khandpur P and Gogate PR, Evaluation of ultrasound based sterilization approaches in terms of shelf life and quality parameters of fruit and vegetable juices. *Ultrason Sonochem* **29**:337-353 (2016).

21. Tiwari B, O'Donnell C, Patras A and Cullen P, Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *J Agr Food Chem* **56**:10071-10077 (2008).

22. Tiwari B, O'Donnell C, Muthukumarappan K and Cullen P, Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. *LWT-Food Sci Technol* **42**:700-704 (2009).

23. Valdramidis V, Cullen PJ, Tiwari B and O'donnell C, Quantitative modelling approaches for ascorbic acid degradation and non-enzymatic browning of orange juice during ultrasound processing. *J Food Eng* **96**:449-454 (2010).

24. Rawson A, Patras A, Tiwari B, Noci F, Koutchma T and Brunton N, Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. *Food Res Int* **44**:1875-1887 (2011).

25. Aadil RM, Zeng X-A, Han Z and Sun D-W, Effects of ultrasound treatments on quality of grapefruit juice. *Food Chem* **141**:3201-3206 (2013).

26. Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S and Zeng X, Sonication enhances polyphenolic compounds, sugars, carotenoids and mineral elements of apple juice. *Ultrason Sonochem* **21**:93-97 (2014).

27. Rojas ML, Miano AC and Augusto PE, Ultrasound processing of fruit and vegetable juices, in *Ultrasound: Advances for food processing and preservation*. Elsevier, pp 181-199 (2017).

28. Golmohamadi A, Möller G, Powers J and Nindo C, Effect of ultrasound frequency on antioxidant activity, total phenolic and anthocyanin content of red raspberry puree. *Ultrason Sonochem* **20**:1316-1323 (2013).

Figure captions

Figure 1. Changes in native microflora of prebiotic-rich samples treated or not with ultrasound during storage (14 days, 5 ºC). (a) mesophilic bacteria (MES); (b) psychrophilic bacteria (PSY); (c) yeast and molds (YM). Bars indicate standard error. **Ol+US15** and **In+US15**: refers to oligofructose or inulin-rich juice treated with ultrasound for 15 min; **Ol+US30** and **In+US30**: refers to oligofructose or inulin-rich juice treated with ultrasound during 30 min. **Control_In**: refers to strawberry juice enriched with inulin without any treatment; **Control_Ol**: refers to strawberry juice enriched with oligofructose without any treatment; **Control**: refers to strawberry juice without any prebiotic addition or treatment.

Figure 2. Changes in nutritional parameters of prebiotic-rich samples treated or not with ultrasound during storage (14 days, 5 ºC). (a) ascorbic acid content; (b) total phenolic content (TPC); (c) antioxidant capacity. Bars indicate standard error. **Ol+US15** and **In+US15**: refers to oligofructose or inulin-rich juice treated with ultrasound for 15 min; **Ol+US30** and **In+US30**: refers to oligofructose or inulin-rich juice treated with ultrasound during 30 min. **Control_In**: refers to strawberry juice enriched with inulin without any treatment; **Control_Ol**: refers to strawberry juice enriched with oligofructose without any treatment; **Control**: refers to strawberry juice without any prebiotic addition or treatment.

FIGURE 1

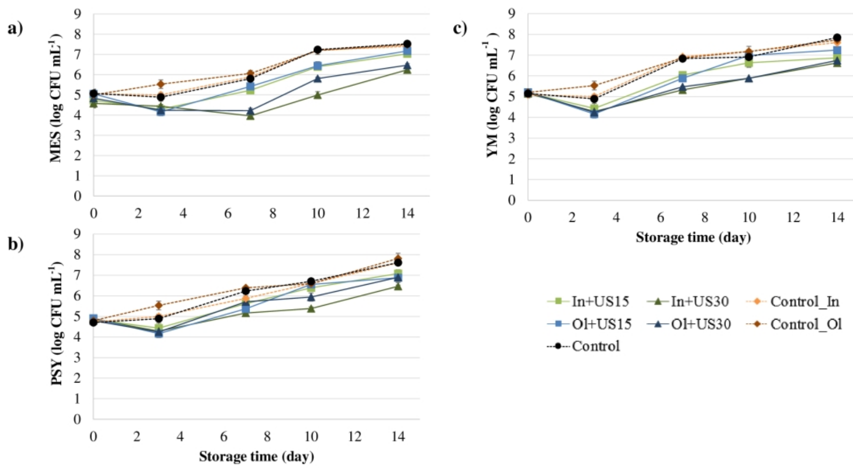

Figure 1

FIGURE 2

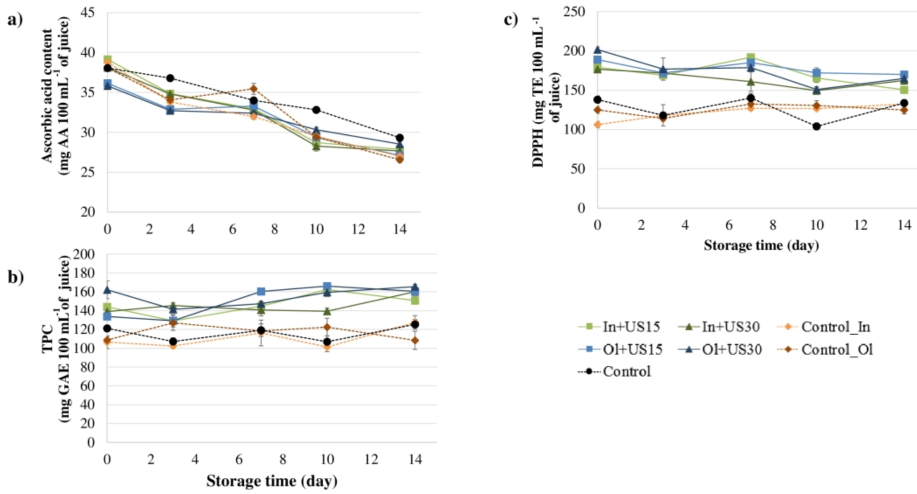

Figure 2

Table 1: Evolution of total soluble solids and tritatable acidity of prebiotic-rich strawberry juices treated or not with ultrasound during storage (14 days, 5 ºC).

Sample	Storage time (day) 0	3	7	10	14
Total soluble solids (ºBrix)					
Control	11.37±0.12^{bA}	11.37±0.12^{bA}	11.62±0.12^{bA}	11.37±0.12^{bA}	11.50±0.00^{bA}
Control_Ol	12.37±0.12^{aA}	12.50±0.00^{aA}	12.37±0.12^{aA}	12.50±0.00^{aA}	12.37±0.12^{aA}
Control_In	12.37±0.12^{aA}	12.35±0.15^{aA}	12.37±0.12^{aA}	12.37±0.12^{aA}	12.37±0.12^{aA}
Ol+US15	12.37±0.12^{aAB}	12.25±0.00^{aB}	12.37±0.12^{aAB}	12.75±0.00^{aA}	12.50±0.00^{aAB}
Ol+US30	12.37±0.12^{aA}	12.37±0.12^{aA}	12.50±0.00^{aA}	12.62±0.12^{aA}	12.37±0.12^{aA}
In+US15	12.37±0.12^{aA}	12.37±0.12^{aA}	12.37±0.12^{aA}	12.37±0.12^{aA}	12.50±0.00^{aA}
In+US30	12.50±0.00^{aA}	12.37±0.12^{aA}	12.50±0.00^{aA}	12.37±0.12^{aA}	12.37±0.12^{aA}
Tritatable acidity (g citric acid 100 mL^{-1} of juice)					
Control	0.92±0.01^{aB}	0.90±0.01^{aB}	0.96±0.01^{aB}	0.97±0.01^{aB}	1.04±0.01^{aA}
Control_Ol	0.89±0.01^{abB}	0.88±0.00^{aB}	0.93±0.01^{abcB}	1.01±0.01^{aA}	1.03±0.01^{aA}
Control_In	0.87±0.01^{abD}	0.90±0.01^{aCD}	0.96±0.00^{aBC}	1.01±0.01^{aAB}	1.06±0.01^{aA}
Ol+US15	0.85±0.01^{bC}	0.91±0.01^{aBC}	0.94±0.01^{abcB}	0.97±0.01^{aAB}	1.01±0.01^{aA}
Ol+US30	0.85±0.01^{bC}	0.92±0.01^{aB}	0.94±0.01^{abAB}	0.98±0.01^{aAB}	0.99±0.01^{aA}
In+US15	0.85±0.01^{bD}	0.92±0.01^{aBC}	0.88±0.01^{cCD}	0.98±0.01^{aAB}	1.03±0.01^{aA}
In+US30	0.88±0.01^{abC}	0.92±0.01^{aBC}	0.89±0.01^{bcBC}	0.95±0.00^{aB}	1.05±0.01^{aA}

Data is shown as means of 3 determinations ± standard error. Values with different lower case letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different capital letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **Control**: untreated strawberry juice; **Control_Ol**: untreated strawberry juice enriched with oligofructose; **Control_In**: untreated strawberry juice enriched with inulin; **Ol+US15**, **Ol+US30**: oligofructose-rich juice treated with ultrasound for 15 and 30 min, respectively; **In+US15**, **In+US30**: inulin-rich juice treated with ultrasound for 15 and 30 min, respectively.

Table 2: Changes in color parameters (L* and hº) of prebiotic-rich samples treated or not with ultrasound during storage (14 days, 5 ºC).

Sample	Storage time (day) 0	3	7	10	14
L*					
Control	25.08±2.08^{bAB}	27.65±0.76^{abA}	25.49±0.94^{cAB}	21.19±0.19^{cB}	23.26±0.50^{abAB}
Control_Ol	33.99±0.45^{aA}	26.76±0.78^{abBC}	28.76±0.48^{abB}	25.22±0.55^{aCD}	22.84±1.04^{bD}
Control_In	34.04±0.22^{aA}	25.76±0.53^{bB}	25.90±1.04^{bcB}	24.69±0.49^{abB}	23.02±0.91^{bB}
Ol+US15	32.68±0.62^{aA}	28.25±0.27^{abB}	27.30±0.31^{abcB}	22.86±0.31^{bcC}	22.57±0.93^{bC}
Ol+US30	30.88±0.18^{aA}	27.76±1.57^{abAB}	26.24±0.49^{bcB}	25.05±0.39^{abBC}	21.63±0.58^{bC}
In+US15	30.78±0.84^{aA}	28.81±0.90^{abAB}	29.53±0.36^{aA}	24.61±0.55^{abC}	26.39±0.30^{aBC}
In+US30	34.73±0.08^{aA}	29.97±0.34^{aB}	29.36±0.41^{aB}	25.05±0.54^{abC}	24.57±0.06^{abC}
hº					
Control	41.60±0.67^{aA}	39.61±0.48^{abAB}	38.33±1.07^{bB}	38.54±0.19^{abAB}	38.66±0.57^{aAB}
Control_Ol	42.52±0.70^{aA}	40.25±0.71^{abAB}	38.83±0.23^{abB}	39.03±0.24^{abB}	39.98±0.52^{aB}
Control_In	40.44±0.82^{aA}	39.85±0.51^{abA}	38.63±0.87^{abA}	39.02±0.27^{abA}	38.24±1.35^{aA}
Ol+US15	42.50±0.24^{aA}	39.53±0.64^{abB}	40.06±0.20^{abB}	38.75±0.27^{abB}	39.97±0.66^{aB}
Ol+US30	42.18±0.15^{aA}	38.28±0.57^{bB}	40.16±0.41^{abAB}	39.65±0.26^{aB}	39.07±0.69^{aB}
In+US15	40.66±0.40^{aAB}	41.67±0.33^{aA}	41.20±0.12^{aAB}	38.21±0.24^{bC}	40.44±0.27^{aB}
In+US30	42.48±0.07^{aA}	39.85±0.51^{abB}	40.72±0.31^{abB}	39.51±0.41^{abB}	39.89±0.35^{aB}

Data is shown as means of 3 determinations ± standard error. Values with different lower case letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different capital letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **Control**: untreated strawberry juice; **Control_Ol**: untreated strawberry juice enriched with oligofructose; **Control_In**: untreated strawberry juice enriched with inulin; **Ol+US15**, **Ol+US30**: oligofructose-rich juice treated with ultrasound for 15 and 30 min, respectively; **In+US15**, **In+US30**: inulin-rich juice treated with ultrasound for 15 and 30 min, respectively.

Table 3: Changes in sensory attributes of prebiotic-rich samples treated or not with ultrasound during storage (14 days, 5 ºC).

Sample	Storage time (day)				
	0	**3**	**7**	**10**	**14**
Overall visual quality					
Control	4.86 ± 0.08^{aA}	3.86 ± 0.15^{aB}	2.76 ± 0.19^{aC}	0.61 ± 0.11^{bD}	0.58 ± 0.06^{abD}
Control_Ol	4.98 ± 0.01^{aA}	3.82 ± 0.19^{aB}	2.60 ± 0.26^{aC}	0.50 ± 0.10^{bD}	0.00 ± 0.00^{bD}
Control_In	4.28 ± 0.69^{aA}	4.00 ± 0.13^{aA}	2.38 ± 0.20^{aB}	0.53 ± 0.12^{bC}	0.33 ± 0.22^{abC}
Ol+U15	4.93 ± 0.04^{aA}	4.22 ± 0.28^{aAB}	3.38 ± 0.40^{aBC}	2.13 ± 0.31^{aCD}	1.06 ± 0.35^{aD}
Ol+U30	4.85 ± 0.08^{aA}	4.24 ± 0.25^{aAB}	3.55 ± 0.35^{aBC}	2.83 ± 0.28^{aC}	0.75 ± 0.26^{abD}
In+U15	4.88 ± 0.08^{aA}	3.96 ± 0.22^{aAB}	3.13 ± 0.41^{aBC}	2.45 ± 0.33^{aC}	0.31 ± 0.22^{abD}
In+U30	4.90 ± 0.08^{aA}	3.52 ± 0.24^{aB}	3.70 ± 0.39^{aAB}	3.01 ± 0.34^{aB}	0.71 ± 0.27^{abC}
Odor					
Control	4.65 ± 0.23^{aA}	2.74 ± 0.18^{bB}	0.88 ± 0.14^{bC}	0.61 ± 0.08^{cC}	0.41 ± 0.11^{aC}
Control_Ol	4.88 ± 0.08^{aA}	3.04 ± 0.22^{abB}	0.88 ± 0.09^{bC}	0.50 ± 0.16^{cCD}	0.18 ± 0.12^{aD}
Control_In	4.76 ± 0.19^{aA}	2.86 ± 0.16^{abB}	0.91 ± 0.08^{bC}	0.45 ± 0.15^{cC}	0.28 ± 0.12^{a}
Ol+U15	4.88 ± 0.09^{aA}	3.72 ± 0.23^{abB}	2.18 ± 0.10^{aC}	1.81 ± 0.08^{bC}	1.03 ± 0.27^{aD}
Ol+U30	4.86 ± 0.07^{aA}	4.20 ± 0.39^{aA}	2.40 ± 0.11^{aB}	1.76 ± 0.09^{bBC}	1.15 ± 0.43^{aC}
In+U15	4.90 ± 0.08^{aA}	3.64 ± 0.25^{abB}	2.31 ± 0.09^{aC}	2.58 ± 0.25^{aC}	0.60 ± 0.27^{aD}
In+U30	4.70 ± 0.07^{aA}	3.10 ± 0.50^{abB}	2.43 ± 0.12^{aB}	2.11 ± 0.24^{abB}	0.53 ± 0.29^{aC}

Table 3 (cont.): Changes in sensory attributes of prebiotic-rich samples treated or not with ultrasound during storage (14 days, 5 ºC).

	Storage time (day)				
Sample	**0**	**3**	**7**	**10**	**14**
Off-odor					
Control	0,05±0,01^{aD}	1,78±0,21^{aC}	2,96±0,09^{aC}	3,65±0,08^{aA}	3,58±0,25^{aA}
Control_Ol	0,01±0,00^{aE}	1,66±0,21^{abD}	2,91±0,08^{aC}	3,75±0,21^{aB}	4,48±0,21^{aA}
Control_In	0,01±0,00^{aE}	1,26±0,13^{abcD}	2,66±0,15^{aC}	3,70±0,09^{aB}	4,54±0,22^{aA}
Ol+U15	0,05±0,01^{aC}	0,32±0,09^{cdC}	1,46±0,17^{bcB}	1,95±0,18^{bB}	3,54±0,49^{aA}
Ol+U30	0,08±0,01^{aD}	0,08±0,02^{dD}	0,85±0,21^{cC}	1,95±0,12^{bB}	3,76±0,29^{aA}
In+U15	0,08±0,02^{aC}	0,20±0,13^{dC}	1,71±0,09^{bB}	2,13±0,13^{bB}	3,74±0,43^{aA}
In+U30	0,28±0,05^{aC}	0,72±0,41^{bcdBC}	1,43±0,12^{bcBC}	1,85±0,16^{bB}	3,78±0,48^{aA}
Sweet taste					
Control	2,68±0,31^{aA}	1,36±0,10^{aB}	NE	NE	NE
Control_Ol	2,82±0,31^{aA}	1,48±0,09^{aB}	NE	NE	NE
Control_In	2,64±0,49^{aA}	1,12±0,26^{aB}	NE	NE	NE
Ol+U15	2,56±0,27^{aA}	2,06±0,32^{aAB}	1,65±0,26^{aAB}	1,48±0,12^{aB}	NE
Ol+U30	2,70±0,45^{aA}	2,06±0,33^{aAB}	1,55±0,13^{aAB}	1,36±0,25^{aB}	NE
In+U15	2,72±0,49^{aA}	2,04±0,12^{aAB}	1,70±0,34^{aAB}	1,11±0,26^{aB}	NE
In+U30	2,28±0,18^{aA}	1,74±0,45^{aA}	1,71±0,18^{aA}	1,33±0,11^{aA}	NE

Table 3 (cont.): Changes in sensory attributes of prebiotic-rich samples treated or not with ultrasound during storage (14 days, 5 ºC).

Sample	Storage time (day) 0	3	7	10	14
Acid taste					
Control	1,45±0,45^{aA}	2,52±0,41^{aA}	NE	NE	NE
Control_Ol	1,56±0,49^{aA}	2,50±0,37^{aA}	NE	NE	NE
Control_In	1,40±0,56^{aA}	2,66±0,18^{aA}	NE	NE	NE
Ol+U15	1,56±0,52^{aA}	1,66±0,51^{aA}	1,73±0,25^{aA}	2,03±0,32^{aA}	NE
Ol+U30	1,41±0,43^{aA}	1,90±0,54^{aA}	1,20±0,24^{aA}	2,00±0,39^{aA}	NE
In+U15	1,38±0,42^{aA}	1,96±0,63^{aA}	1,75±0,44^{aA}	2,30±0,18^{aA}	NE
In+U30	1,26±0,38^{aA}	1,46±0,43^{aA}	1,76±0,35^{aA}	2,08±0,50^{aA}	NE

Data is shown as means of 3 determinations ± standard error. Values with different lower case letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different capital letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **NE**: not evaluated due to high microbial load. **Control**: untreated strawberry juice; **Control_Ol**: untreated strawberry juice enriched with oligofructose; **Control_In**: untreated strawberry juice enriched with inulin; **Ol+US15**, **Ol+US30**: oligofructose-rich juice treated with ultrasound for 15 and 30 min, respectively; **In+US15**, **In+US30**: inulin-rich juice treated with ultrasound for 15 and 30 min, respectively.