LWT - Food Science and Technology 72 (2016) 475–484

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

# Optimization of ultrasound, vanillin and pomegranate extract treatment for shelf-stable unpasteurized strawberry juice

B. Tomadoni [a, b, *], L. Cassani [a, c], A. Ponce [a, b], M.R. Moreira [a, b], M.V. Agüero [b, d]

[a] Grupo de Investigación en Ingeniería de Alimentos, Facultad de Ingeniería, UNMdP, Juan B. Justo 4302, Mar del Plata, 7600, Argentina
[b] Consejo Nacional de Ciencia y Tecnología (CONICET), Argentina
[c] Agencia Nacional de Promoción Científicas y Tecnológica (AGENCIA), Argentina
[d] Laboratorio de Microbiología Industrial: Tecnología de Alimentos, INTECIN, Facultad de Ingeniería, Universidad de Buenos Aires, Argentina

ARTICLE INFO

*Article history:*
Received 3 March 2016
Received in revised form
30 April 2016
Accepted 7 May 2016
Available online 9 May 2016

*Keywords:*
Unpasteurized juice
Hurdle technology
Simultaneous optimization
Biopreservatives

ABSTRACT

Optimum combination of ultrasound, vanillin and pomegranate extract to improve quality of strawberry juice was determined using response surface methodology. Samples were stored at 5 °C for 14 days. The optimal conditions to simultaneously minimize native microflora, maximize nutritional parameters and minimize the impact on sensory quality resulted in: 7.5 min of ultrasound treatment, pomegranate extract concentration of 360 μg/mL and vanillin concentration of 0.925 mg/mL. A new batch of strawberry juice was treated at these optimal conditions and stored for validation of the optimization and to evaluate the performance of the optimum treatment on quality parameters throughout storage. Furthermore, a second batch of juice was inoculated with *Escherichia coli* O157:H7 and processed at optimal conditions to evaluate the effectiveness of the treatment on the pathogen survival. The native microflora of the juice, as well as inoculated pathogen, decreased significantly using the proposed hurdle technologies, with no impact on sensory parameters. Ascorbic acid retention was slightly decreased by the optimum treatment; however, DPPH and polyphenolic compounds were significantly higher than those in untreated sample. Overall, a combination of ultrasound, vanillin and pomegranate extract showed interesting potential to enhance quality and safety of strawberry juice, extending the shelf-life of the product.



## 1. Introduction

Strawberry juice is one of the most popular fruit juices consumed around the world due to their appreciable organoleptical attributes such us attractive color, good aroma and taste of sweet and sour mouth. Additionally, both cloudy and clear juices are considered healthy strawberry products and they are consumed as natural antioxidant drinks (Cao et al., 2012).

In the fruit juice industry, inactivation of microorganisms and enzymes is usually carried out through thermal processing generally at 70–121 °C for 30–120 s (Cao et al., 2011; Zhou, Wang, & Liao, 2009). It is widely known that thermal treatments seriously affect the quality of juices, presenting loss of nutritional components and undesirable changes in sensorial quality. In particular, strawberry juice is highly susceptible to this processing (Cao et al., 2012). Therefore, the application of non-thermal techniques to strawberry juice is gaining popularity. This tendency is also motivated by the increasing consumer demand for minimally processed food products with sensory and nutritional characteristics similar to fresh product.

Sonication is a non-thermal food preservation technique, which uses ultrasound for inactivating food spoilage microorganisms and enzymes, generally at a frequency of 20–40 kHz. The physical phenomenon of cavitation that occurs when applying ultrasound is considered the main cause of microbial inactivation (Vercet, Sanchez, Burgos, Montañés, & López, 2002). Cavitation can break molecules or particles through different mechanisms that can occur individually or combined, generating free radicals in the water sonolysis ($H^+$ and $OH^-$), that are responsible of producing oxidative damage, leading to microbial inactivation (Mañas & Pagan, 2005).

Another emerging non-thermal technology is the use of natural compounds as food preservatives, in response to consumers concern about the safety of synthetic compounds used in food.

* Corresponding author. Grupo de Investigación en Ingeniería de Alimentos, Facultad de Ingeniería, UNMdP, Juan B. Justo 4302, Mar del Plata, 7600, Argentina.
*E-mail address:* bmtomadoni@fi.mdp.edu.ar (B. Tomadoni).

http://dx.doi.org/10.1016/j.lwt.2016.05.024
0023-6438/

Among natural compounds, vanillin (4-hydroxy-3-methoxybenzaldehyde) is a non-toxic and GRAS phytochemical used as food flavoring agent and obtained from vanilla beans. Furthermore, it is known to be antimycotic and bacteriostatic (Fitzgerald, Stratford, & Narbad, 2003). Like many other low-molecular weight phenolic compounds, vanillin displays antioxidant and antimicrobial properties and hence has the potential for use as a food preservative (Tomadoni, Cassani, Moreira, & Ponce, 2015). Fruit extracts have also been identified as novel candidates in the search of natural compounds with antimicrobial properties. In particular, many research studies have focused on the antimicrobial activity of pomegranate (*Punica granatum*) extracts.

The use of these natural compounds as food antimicrobials may have a negative effect on the sensory characteristics of the final product, because of their strong flavor when applied at high concentrations. As a solution to this problem, hurdle technology have been proposed (Leistner, 2000) allowing to reduce the intensity of each hurdle in order to avoid the loss of nutritional and sensory value while maintaining the stability and safety of the product.

In order to optimize the levels of various hurdles, multivariate statistical techniques such as response surface methodology (RSM) have been suggested. RSM is a powerful mathematical tool that presents the advantage of efficiently exploring a particular region on selected ranges of independent variables at low cost, reducing the number of experimental runs (Kuehl, 2000). However, when several responses must be optimized at the same time, the independent optimization of each one can lead to conflicting results, i.e., improving one response may have an opposite effect on another one, failing in the finding of the best solution for all responses simultaneously (Costa, Lourenço, & Pereira, 2011). For these cases, the Desirability function could be a complementary tool to resolve this conflict, allowing finding the optimal experimental conditions to successfully satisfy the optimization of all responses (Costa et al., 2011).

Therefore, the objectives of this study were: (a) to optimize the intensity or levels of three different non-thermal hurdle technologies (vanillin, pomegranate extract and ultrasound) applied on strawberry juice in order to simultaneously improve microbiological, sensory and nutritional quality; (b) to evaluate the effect of the optimum treatment on quality parameters of strawberry juice throughout storage time; and (c) to study the performance of the optimum treatment against a contamination with *Escherichia coli* O157:H7.

## 2. Materials and methods

### 2.1. Plant material and juice extraction

Strawberries (*Fragaria x ananassa*) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. The strawberries were destemmed and the juices were prepared with a commercial juice extractor. Once the treatments were applied, the strawberry juices were stored for 14 days in sterile polypropylene flasks at 5 °C.

### 2.2. Treatments and experimental design

The application of hurdle technology on strawberry juice was studied by combining 3 different non-thermal preservation techniques: ultrasound, pomegranate extract and vanillin.

The ultrasound treatments were performed at 40 kHz frequency, using an ultrasonic cleaning bath (TestLab, Argentine), with a rectangular container (290 × 150 × 150 mm) and a maximal tank capacity of 6.5 L. The 40 kHz transducers at the bottom transmit ultrasound waves of 180 W from bottom to above. Temperature in the ultrasonic bath was monitored at 20 ± 1 °C. The juice level in the flasks was 2 cm below the water surface in the ultrasonic bath. The height of the bottom surface of the flasks from the bottom surface of the tank (face of transducers) was 4 cm.

Pomegranate extract used in this study was purchased from PureBulk, USA (35% ellagic acid, 19% gallic acid, 10% punicalagin A, 5% punicalagin B, 2% caffeic acid). Vanillin (>97%) was purchased from Sigma Aldrich (St. Louis, MO, USA). The natural preservatives were applied directly into the juice samples.

Each technology was evaluated at 3 different levels according to the conditions established in the experimental design (Table 1). The ultrasonic processing times selected for this study were: 0, 15 and 30 min, according to previous unpublished experiments. Pomegranate extract was applied at 0, 180 and 360 μg/mL of strawberry juice, while vanillin was applied at 0, 0.625 and 1.25 mg/mL of juice. These concentrations were selected according to Tomadoni, Viacava, Cassani, Moreira, and Ponce (2016).

Response Surface Methodology (RSM) with a Box-Behnken (BB) design was used to establish the effects of the three independent processing parameters namely ultrasonication time ($X_1$, min), pomegranate concentration ($X_2$, μg/mL) and vanillin concentration ($X_3$, mg/mL) on the selected response variables. Fifteen experimental runs were carried out combining the 3 levels of each variable as shown in Table 1, as is suggested by the BB design. Samples were stored at 5 °C for 14 days. On day 14, responses were measured for each trial and a second-order polynomial model (Eq. (1)) was fitted to each response variable using the least-squares regression method.

$$Y_n = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=2, j>i}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad (1)$$

where $Y_n$ is the predicted response ($Y_1$:yeast and molds counts, $Y_2$: psychrophilic bacteria counts, $Y_3$: DPPH radical scaveging activity, $Y_4$: total polyphenol content, $Y_5$: ascorbic acid, $Y_6$: hue angle, $Y_7$: off-odor), $\beta_0$ is the model constant, $\beta_i$ is the linear coefficient, $\beta_{ii}$ is the quadratic coefficient, $\beta_{ij}$is the coefficient for the interaction effect, and $X_i$ is a dimensionless coded value of the independent variable, $x_i$.

### 2.3. Response variables

The effects of combined treatments using ultrasound, vanillin and pomegranate extract applied on strawberry juice were

**Table 1**
Box-Behnken experimental design matrix.

| Exp no. | Independent variables | | | Coded independent variables | | |
|---|---|---|---|---|---|---|
| | $x_1$ US (min) | $x_2$ PE (μg/mL) | $x_3$ V (mg/mL) | $X_1$ | $X_2$ | $X_3$ |
| 1 | 0 | 180 | 0 | −1 | 0 | −1 |
| 2 | 0 | 180 | 1.25 | −1 | 0 | 1 |
| 3 | 30 | 180 | 0 | 1 | 0 | −1 |
| 4 | 30 | 180 | 1.25 | 1 | 0 | 1 |
| 5 | 15 | 0 | 0 | 0 | −1 | −1 |
| 6 | 15 | 360 | 0 | 0 | 1 | −1 |
| 7 | 15 | 0 | 1.25 | 0 | −1 | 1 |
| 8 | 15 | 360 | 1.25 | 0 | 1 | 1 |
| 9 | 0 | 0 | 0.625 | −1 | −1 | 0 |
| 10 | 30 | 0 | 0.625 | 1 | −1 | 0 |
| 11 | 0 | 360 | 0.625 | −1 | 1 | 0 |
| 12 | 30 | 360 | 0.625 | 1 | 1 | 0 |
| 13 | 15 | 180 | 0.625 | 0 | 0 | 0 |
| 14 | 15 | 180 | 0.625 | 0 | 0 | 0 |
| 15 | 15 | 180 | 0.625 | 0 | 0 | 0 |

simultaneously evaluated through several quality indices associated with microbiological, nutritional and sensory quality. These indices were assessed at day 0 and after 14 days of refrigerated storage.

*2.3.1. Microbiological quality*

Yeast and molds, and psychrophilic bacteria were selected to characterize the microbiological quality as they are the main native microflora populations associated to strawberry juice (Tomadoni et al., 2016). The enumeration of these microbial populations was performed according to Ponce, Agüero, Roura, del Valle, and Moreira (2008). Briefly, 10 mL aliquot of juice from each treatment was sampled. Serial dilutions (1:10) of each sample were made in sterile peptonated water (0.1% w/v) and surface spread by duplicate by using the following culture media and culture conditions: psychrophilic bacteria (PSY) on Plate Count Agar (PCA) incubated at 7 °C for 7 d; and yeast and molds (YM) on Yeast-Glucose-Chloramphenicol (YGC) medium incubated at 25 °C for 5 d. All culture mediums were purchased from Britania, Buenos Aires, Argentine. Microbial counts were performed by duplicate and expressed as log CFU/mL.

*2.3.2. Nutritional quality*

*2.3.2.1. Antioxidant activity and total phenolic compounds content.* Extraction of antioxidant and phenolic compounds was carried out homogenizing 2 mL of strawberry juice was homogenized with 10 mL solution of ethanol (80% v/v) in a vortex. The homogenate was then centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was collected and filtered using Whatman filter paper #1. The ethanolic extract was stored at −20 °C to be used in the determinations of total phenolic content (TPC) and antioxidant activity by DPPH method.

The radical scavenging activity was measured in terms of hydrogen-donating or free radical-scavenging using the DPPH methodology proposed by Brand-Williams, Cuvelier, and Berset (1995). An ethanolic DPPH solution (100 μM) was used for determinations. Ethanol (0.1 mL) was mixed with 3.9 mL of DPPH (100 μM) to determine the initial absorbance of the DPPH solution. Next, 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 capacity was expressed as the percentage of the DPPH radical inhibition (%RSC) and was measured by duplicate.

TPC was determined spectrophotometrically using the Folin–Ciocalteu reagent (FCR) according to the methodology proposed by Singleton, Orthofer, and Lamuela-Raventos (1999) 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% $Na_2CO_3$ 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 as standard. Results of TPC were expressed as mg gallic acid equivalents (GAE)/100 mL of juice and were measured by duplicate.

*2.3.2.2. Determination of ascorbic acid.* Ascorbic acid (AA) content was determined by the titrimetric assay described by Moreira, Roura, and del Valle (2003). For the AA determination, 20 mL of strawberry juice from each treatment were homogenized with 40 mL of 2% w/w oxalic acid solution (Mallinckrodt, USA). This mixture was vacuum filtered through glass fiber. 10 mL aliquots of the filtrate were titrated with 2,6 dichloroindophenol (Anedra SA, Argentine). Determinations of AA in strawberry juice were performed by triplicate. Ascorbic acid contents are reported as a percentage of retention ($AA/AA_0$). Where AA is the content of ascorbic acid at a certain time of refrigerated storage, and $AA_0$ is the concentration of ascorbic acid on the strawberry juice immediately before applying the corresponding treatment.

*2.3.3. Sensory quality*

*2.3.3.1. Instrumental color.* Color determination was carried out using a colorimeter (Lovibond, RT Series, England) with an 8 mm diameter measuring area, calibrated with a standard white plate ($L^* = 97.63$, $a^* = 0.3133$, $b^* = 0.3192$). Measurements were done in triplicate for each sample. Color of strawberry juice was recorded using the CIE − L * a* b* uniform color space, where L* indicates lightness (whiteness or brightness/darkness), a* indicates chromaticity on a green (−) to red (+) axis, and b* indicates chromaticity on a blue (−) to yellow (+) axis (CIE., 1977). Numerical values L*, a*, b* were used to estimate hue angle (Eq. (2)), according to:

$$Hue = arctg(b^*/a^*) \quad (2)$$

*2.3.3.2. Sensory evaluation.* The samples were subjected to sensory quality evaluation by ten trained panelists. Samples labeled with 3 digit code numbers were randomly provided. Water was provided to panelists for eliminating the residual taste between samples. The attributes evaluated were: overall visual quality (OVQ), off-odor, vanillin odor, sweetness, acidity and bitterness. Unstructured line scales (5 cm) anchored at the ends with terms related with minimum and maximum intensities were used to evaluate each attribute. OVQ was rated using 0 = excellent and 5 = poor. Off-odor, vanillin odor, sweetness, acidity and bitterness were rated using 0 = low and 5 = high.

*2.4. Simultaneous optimization and validation*

A simultaneous optimization was carried out using the Desirability function (D). For this purpose, predicted values obtained from each model ($Y_n$) were transformed to a dimensionless desirability scale ($d_n$). The desirability scale ranges from 0 to 1, where d = 0 for an unacceptable response value, and d = 1 for a completely desirable one. The individual desirability functions from the considered responses are then combined to obtain the overall desirability D, defined as the geometric average of the individual desirability. An algorithm is then applied to this function in order to determine the set of values that maximize it (Almeida Bezerra, Erthal Santelli, Padua Oliveira, Silveira Villar, Escaleira, 2008).

In order to test the reliability of the simultaneous optimization, a new set of experiments using optimal operating conditions obtained with the Desirability function were performed. The experimental and predicted values of the response variables were compared in order to determine the validity of the model.

*2.5. Performance of the juice treated at optimal conditions during storage*

*2.5.1. Shelf-stability of strawberry juice*

Strawberry juice treated at optimal conditions as predicted with the Desirability function (optimum treatment), and strawberry juice with no treatment (untreated or control) were stored under refrigeration (5 °C) for 14 days. Samples were studied at 0, 3, 7, 11 and 14 days of storage in order to evaluate the effect of the optimum treatment on the quality parameters of strawberry juice throughout storage time.

#### 2.5.2. Challenge test

Additionally, a challenge test was carried out in order to assess the effectiveness of the optimum treatment to inactivate an eventual contamination with *Escherichia coli* O157:H7, a food pathogen of great concern in the fruit juice industry. For this test, a second batch of strawberry juice was prepared and inoculated with the pathogen. Once inoculated, the optimum treatment was applied and an untreated inoculated juice was used as control.

*Escherichia coli* O157:H7 non-toxigenic (FP 605/03, Malbran Institute, Buenos Aires, Argentina) was used. A stock culture was maintained in tryptic soy broth (Britania, Buenos Aires, Argentine) at 4 °C. Before use, *Escherichia coli* O157:H7 was cultured in brain heart infusion (BHI) broth (Britania) for 24 h at 37 °C. A 0.1 mL aliquot of the culture was transferred to 9.9 mL of BHI broth at two consecutive 24 h intervals followed by incubation at 37 °C before each experiment. A bacterial suspension was prepared by adding 10 mL of the *E. coli* culture to 90 mL of sterile peptonated water (0.1% w/v). To inoculate the samples, 100 μL of the bacterial suspension previously prepared were added to 10 mL of fresh strawberry juice to reach a final pathogen concentration of approximately 5 log CFU/mL.

Viable *E. coli* counts were monitored as follows: 0.1 mL aliquot of each sample were spread on the surface of eosin methylene blue (EMB, Britania, Buenos Aires, Argentine) agar plates 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 and with a metallic sheen were taken into account. Randomly, selected *E. coli* colonies were confirmed using an *E. coli* chromogenic test kit (Chromobrit, Britania). Microbial counts were performed by duplicate and expressed as log CFU/mL.

### 2.6. Statistical analysis

Results reported are mean values accompanied by their standard errors (Kuehl, 2000). Data for RSM were analyzed using the STATISTICA 7.1 (Statsoft Inc., Tulsa, U.S.A., 2004). The statistical analysis was performed using the analysis of variance (ANOVA) including the F-ratio, which established the model global significance, and the determination coefficient $R^2$. The Lack of Fit test was performed for each model with a 95% confidence level. In addition, experimental and predicted values for each dependent variable were compared. The significance of fitted coefficients for each model describing relationship among dependent variables and independent ones were established according to the Student t-test with a 95% confidence level (Kuehl, 2000).

Data for shelf-stability test was analyzed using R v. 2.12.2. (R Development Core Team, 2011). Analysis of variance ANOVA was performed and Tukey-Kramer comparison test was used to estimate significant differences between treatments and storage time ($p < 0.05$).

## 3. Results and discussion

### 3.1. Model fitting, simultaneous optimization and validation

Initial mean values and after 14 d of refrigerated storage for all responses obtained for untreated samples (control) and treated samples under different experimental conditions, are presented in Table 2.

The experimental data were used to calculate the coefficients of the second order polynomial equations (Eq. (1)). The estimated coefficients, along with their standard errors, t-value, and p-value, as well as the adjusted correlation coefficient ($adjR^2$) for each response variable are presented in Table 3.

Even though this study is primarily focus on the simultaneous optimization of the response variables, the analysis of the effects of the different treatments on each of the response variables individually is also of great interest. In this sense, the regression analysis showed that yeast and molds, and psychrophilic bacteria were significantly affected by vanillin concentration, as linear and quadratic terms associated to this independent variable ($X_3$ and $X_3^2$) were significant for these models. Furthermore, psychrophilic bacteria was also significantly affected by the interaction between both natural compounds tested ($X_2{}^*X_3$). On the other hand, ultrasound ($X_1$) and pomegranate extract ($X_2$) affected both AA and off-odor. DPPH and Hue were affected by ultrasound ($X_1$) and pomegranate extract ($X_2$), respectively, while vanillin ($X_3$) significantly affected every response variable evaluated.

The results of the simultaneous optimization indicated that the levels of the optimized conditions were 7.5 min of ultrasonic processing time, a pomegranate extract concentration of 360 μg/mL and a vanillin concentration of 0.925 mg/mL, with a Desirability value of 0.79 (Fig. 1). The predicted optimum responses of the optimization were: 1.93 and 2.15 log CFU/mL for yeast and molds, and psychrophilic counts respectively; 33.49 for °Hue, 78.06% RSC, 289.83 mg GAE/100 mL, and 39.91% of ascorbic acid retention; and an off-odor score of 0.58.

In order to test the reliability of the models in predicting optimal responses, validation experiments were carried out at the optimal levels. The results indicated that the confidence intervals (95%) for experimental values after 14 d of storage were 1.29–3.66 and 2.89–4.06 log CFU/mL for yeast and molds, and psychrophilic counts respectively, 29.91–32.40 for Hue, 63.67–74.88%RSC, 277.62–373.56 mg GAE/100 mL, 26.62–22.08% of ascorbic acid retention, and an off-odor score of 0.92–4.67 at the selected optimum conditions of ultrasonic treatment times, pomegranate extract and vanillin concentrations. It is worth noting that predicted values for psychrophilic counts and off-odor were lower than those obtained in the validation experiment. These results could be associated to the fact that strawberry samples used for validation presented higher initial microbial load that grew during storage reaching higher values at day 14 of storage. These higher counts could be also responsible for the higher score in off-odor during validation. In spite of this behavior, it is noteworthy that psychrophilic as well as off-odors scores during validation were all within the acceptability range and significantly lower in the treated sample than in the control. On the other hand, predicted DPPH and Hue values resulted slightly higher than those obtained during validation test. In spite of this, relative errors between predicted and measured responses were in the order of 10% for almost all responses.

### 3.2. Effect of optimum treatment on shelf-stability and safety of strawberry juice

#### 3.2.1. Nutritional quality

Evolution of DPPH radical scavenging capacity (%RSC), total poliphenol content (TPC) and ascorbic acid retention (AA) through storage are shown on Fig. 2(a–c). Initial values of DPPH and TPC were 64.39%RSC and 130.54 mg GAE/100 mL, respectively. At day 0, a significant increase on both DPPH and TPC was found on strawberry juice treated at optimal conditions compared to control, with values of 75.91%RSC and 227.22 mg GAE/100 mL of juice, respectively. This increment may be due to the addition of pomegranate extract and vanillin which are polyphenolic compounds. Also, the application of ultrasound could be responsible for these increments as it has been proved that this treatment improves the extraction yield of bioactive compounds and antioxidant molecules in several food products (Vilkhu, Mawson, Simons, & Bates, 2008).

**Table 2**
Initial values of fresh strawberry juice and mean values of all responses of untreated samples and samples treated under different experimental conditions after 14 d of storage at 5 °C.

| Days of storage | Sample | Experimental responses | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | YM (log CFU/mL) | PSY (log CFU/mL) | DPPH (% inhibition) | TPC (GAE/100 mL) | AA (%) | Hue | Off-odor |
| 0 | Fresh sample | 4.54 | 3.59 | 60.90 | 115.19 | 100.0 | 40.35 | 0.00 |
| 14 | Run 1 | 8.11 | 6.59 | 62.10 | 136.32 | 44.64 | 34.09 | 3.65 |
| | 2 | 2.50 | 2.70 | 65.29 | 255.12 | 41.11 | 33.02 | 0.57 |
| | 3 | 8.46 | 6.41 | 67.22 | 126.90 | 57.20 | 34.70 | 3.33 |
| | 4 | 2.65 | 2.70 | 58.52 | 253.18 | 27.30 | 33.29 | 0.37 |
| | 5 | 8.51 | 5.01 | 63.57 | 138.96 | 54.62 | 35.42 | 3.58 |
| | 6 | 8.53 | 6.81 | 69.59 | 165.72 | 54.17 | 34.04 | 3.55 |
| | 7 | 2.80 | 2.70 | 61.75 | 220.36 | 40.46 | 34.40 | 0.37 |
| | 8 | 2.50 | 2.70 | 70.89 | 255.55 | 39.72 | 32.62 | 1.13 |
| | 9 | 2.65 | 2.70 | 64.05 | 208.80 | 38.36 | 34.92 | 0.33 |
| | 10 | 2.74 | 2.70 | 78.45 | 236.98 | 41.83 | 34.76 | 0.67 |
| | 11 | 2.65 | 2.70 | 72.99 | 262.44 | 42.51 | 34.04 | 0.85 |
| | 12 | 2.95 | 2.70 | 74.36 | 344.79 | 36.18 | 33.88 | 0.50 |
| | 13 | 2.50 | 2.70 | 82.99 | 245.67 | 34.05 | 34.29 | 0.38 |
| | 14 | 2.85 | 2.70 | 80.03 | 266.43 | 38.40 | 34.50 | 0.43 |
| | 15 | 2.85 | 2.70 | 81.99 | 295.37 | 36.34 | 35.08 | 0.27 |
| | *Untreated sample* | 8.20 | 6.65 | 60.00 | 146.88 | 47.31 | 35.81 | 3.90 |

**Table 3**
Statistical parameters of RSM analysis of response variables.

| Response[a] | Term[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Intercept* | $X_1$ | $X_1^2$ | $X_2$ | $X_2^2$ | $X_3$ | $X_3^2$ | $X_1{*}X_2$ | $X_1{*}X_3$ | $X_2{*}X_3$ |
| **YM ($adjR^2 = 0.99$; $p < 0.001$)** | | | | | | | | | | |
| Coefficient | 2.733 | 0.112 | −0.069 | −0.009 | 0.084 | −2.895 | 2.767 | 0.053 | −0.051 | −0.079 |
| SE | 0.092 | 0.056 | 0.083 | 0.056 | 0.083 | 0.056 | 0.083 | 0.079 | 0.079 | 0.079 |
| *t*-value | 29.783 | 1.987 | −0.836 | −0.162 | 1.015 | −51.518 | 33.458 | 0.670 | −0.639 | −0.995 |
| *p*-value | <0.001[e] | 0.104 | 0.441 | 0.878 | 0.357 | <0.001[e] | <0.001[e] | 0.533 | 0.551 | 0.366 |
| **PSY ($adjR^2 = 0.96$; $p < 0.001$)** | | | | | | | | | | |
| Coefficient | 2.700 | −0.022 | 0.148 | 0.226 | −0.148 | −1.753 | 1.753 | 0.000 | 0.044 | −0.452 |
| SE | 0.198 | 0.121 | 0.178 | 0.121 | 0.178 | 0.121 | 0.178 | 0.171 | 0.171 | 0.171 |
| *t*-value | 13.640 | −0.183 | 0.831 | 1.863 | −0.831 | −14.466 | 9.827 | 0.000 | 0.259 | −2.635 |
| *p*-value | <0.001[e] | 0.862 | 0.444 | 0.121 | 0.444 | <0.001[e] | <0.001[e] | 1.000 | 0.806 | 0.046[c] |
| **DPPH ($adjR^2 = 0.81$; $p = 0.0181$)** | | | | | | | | | | |
| Coefficient | 81.672 | 1.765 | −6.188 | 2.500 | −3.018 | −0.752 | −12.202 | −3.256 | −2.973 | 0.782 |
| SE | 1.981 | 1.213 | 1.785 | 1.213 | 1.785 | 1.213 | 1.785 | 1.715 | 1.715 | 1.715 |
| *t*-value | 41.232 | 1.455 | −3.466 | 2.061 | −1.690 | −0.620 | −6.834 | −1.898 | −1.733 | 0.456 |
| *p*-value | <0.001[e] | 0.205 | 0.018[c] | 0.094 | 0.152 | 0.563 | 0.001[e] | 0.116 | 0.144 | 0.668 |
| **TPC ($adjR^2 = 0.74$; $p = 0.0383$)** | | | | | | | | | | |
| Coefficient | 269.157 | 12.398 | −4.086 | 27.925 | −1.817 | 52.040 | −72.191 | 13.543 | 1.872 | 2.108 |
| SE | 18.298 | 11.205 | 16.494 | 11.205 | 16.494 | 11.205 | 16.494 | 15.847 | 15.847 | 15.847 |
| *t*-value | 14.710 | 1.106 | −0.248 | 2.492 | −0.110 | 4.644 | −4.377 | 0.855 | 0.118 | 0.133 |
| *p*-value | <0.001[e] | 0.319 | 0.814 | 0.055 | 0.917 | 0.006[d] | 0.007[d] | 0.432 | 0.911 | 0.899 |
| **AA ($adjR^2 = 0.93$; $p = 0.0016$)** | | | | | | | | | | |
| Coefficient | 36.263 | 0.361 | 0.264 | −0.335 | 3.193 | −6.944 | 7.786 | −2.450 | −4.968 | −0.071 |
| SE | 1.130 | 0.692 | 1.018 | 0.692 | 1.018 | 0.692 | 1.018 | 0.978 | 0.978 | 0.978 |
| *t*-value | 32.099 | 0.521 | 0.259 | −0.485 | 3.136 | −10.038 | 7.646 | −2.504 | −5.078 | −0.073 |
| *p*-value | <0.001[e] | 0.624 | 0.806 | 0.648 | 0.026[c] | <0.001[e] | 0.001[e] | 0.054 | 0.004[d] | 0.945 |
| **Hue ($adjR^2 = 0.75$; $p = 0.0365$)** | | | | | | | | | | |
| Coefficient | 34.619 | 0.071 | −0.284 | −0.615 | 0.063 | −0.617 | −0.561 | 0.000 | −0.087 | −0.097 |
| SE | 0.225 | 0.138 | 0.203 | 0.138 | 0.203 | 0.138 | 0.203 | 0.195 | 0.195 | 0.195 |
| *t*-value | 154.020 | 0.517 | −1.402 | −4.467 | 0.309 | −4.480 | −2.768 | −0.001 | −0.444 | −0.496 |
| *p*-value | <0.001[e] | 0.627 | 0.220 | 0.007[d] | 0.770 | 0.007[d] | 0.039[c] | 1.000 | 0.675 | 0.641 |
| **Off-odor ($adjR^2 = 0.99$; $p < 0.0001$)** | | | | | | | | | | |
| Coefficient | 0.358 | −0.068 | 0.026 | 0.135 | 0.203 | −1.459 | 1.593 | −0.171 | 0.031 | 0.196 |
| SE | 0.076 | 0.046 | 0.068 | 0.046 | 0.068 | 0.046 | 0.068 | 0.066 | 0.066 | 0.066 |
| *t*-value | 4.737 | −1.462 | 0.382 | 2.924 | 2.979 | −31.507 | 23.361 | −2.608 | 0.477 | 2.990 |
| *p*-value | 0.005[d] | 0.204 | 0.718 | 0.033[c] | 0.031[c] | <0.001[e] | <0.001[e] | 0.048[c] | 0.653 | 0.030[c] |

[a] Each response is informed with ANOVA parameters.
[b] Coefficients with *p*-value lower than 0.05 were retained in the models.
[c] Significant with $p < 0.05$.
[d] Significant with $p < 0.01$.
[e] Significant with $p < 0.001$.

With regards to AA (Fig. 2c), untreated fresh strawberry juice presented a content of 45.21 mg of AA/100 mL of juice. Immediately after the application of the optimum treatment (day 0), a significant decrease in AA content was observed compared to control, with

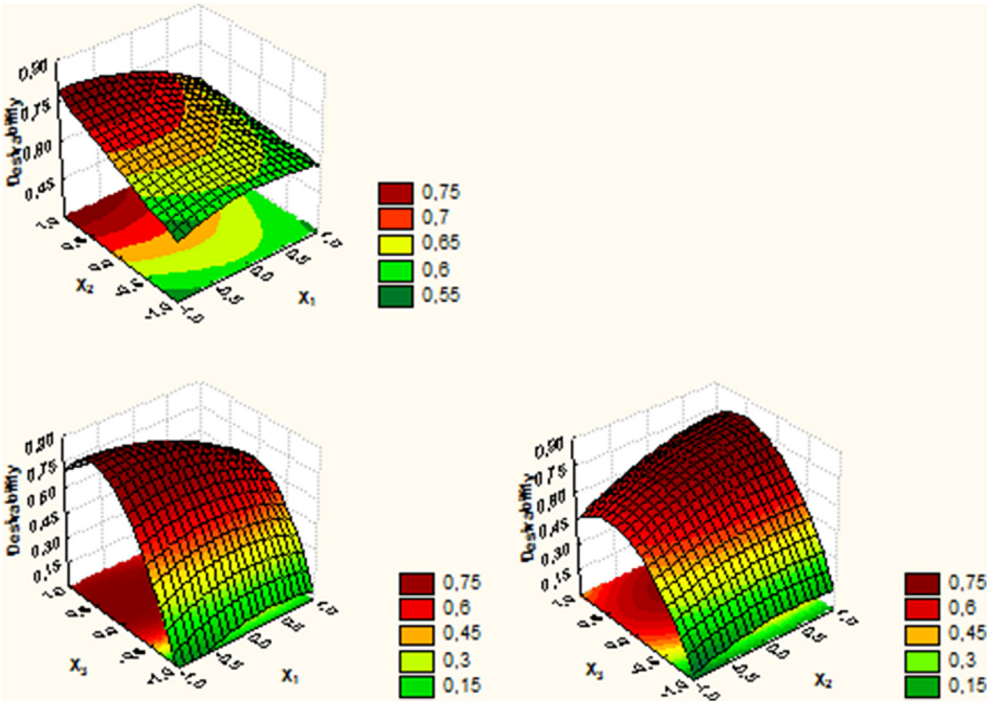


**Fig. 1.** Response surface plots showing the combined effect of pomegranate extract concentration and ultrasound time (A), vanillin concentration and ultrasound time (B), and vanillin and pomegranate extract concentration (C) on the Desirability function with other variables constant at middle level.

90.43% of AA retention. Through storage, samples treated at optimal conditions showed significantly lower AA retention percentages, reaching values of 28.53% of AA retention by the end of storage (day 14), while control sample showed 39.70%. These results may be attributed to the application of ultrasound. The ascorbic acid degradation during ultrasonic processing may follow one or both of the following pathways: (1) thermolysis inside the bubbles, triggering the Maillard reaction; (2) reaction with hydroxyl radicals produced by cavitation, producing oxidative products on the bubbles surface (Feril & Kondo, 2004). However, radical production has been considered the most probable mechanism that explains the degradation of ascorbic acid (Vercet, Burgos, & Lopez-Buesa, 2001).

A significant increase of phenolic compounds through storage time was observed on both samples. The changes that caused the senescence and decomposition of the cell structure and thus the liberation of free phenolic acids and free amino acids, may contribute to the increase in total polyphenols in the untreated sample (Puttongsiri and Haruenkit, 2010). While TPC increased, AA decreased significantly on treated and untreated samples through storage. Both ascorbic acid and polyphenolic compounds contribute to the antioxidant capacity of the product, therefore, their opposite effects may have influenced in the behavior of DPPH, which showed no significant differences through time on neither treated nor untreated juice samples.

### 3.2.2. Sensory analysis

The results of the sensory evaluation are shown on Fig. 3. At day 0, no significant differences were found on overall visual quality (OVQ) between treated and untreated samples (OVQ: 0-excelent), concluding that the application of the optimum treatment had no initial effect on the appearance of the product. Contrary to that result, odor attributes were significantly affected ($p < 0.05$) by the treatment, since significantly higher scores were obtained in comparison with untreated samples. Panelists perceived vanillin odor with a mean value of 1.16 on the treated sample, and an off-odor score of 0.73. With regards to taste attributes at day 0, no significant differences were found between the treated sample and the control on sweetness (2.06 and 2.18, respectively), acidity (2.04 and 1.55, respectively) and bitterness scores (0.56 and 0.14, respectively).

At day 7, panelists found significantly higher Off-odor on control compared to the optimum-treated sample (3.53 and 1.67, respectively). This may be a result of the fermentation, given the important microbial load on the control sample by day 7 of storage. On the other hand, vanillin odor on treated samples increased with respect to day 0 (vanillin odor score: 2.17). Taste attributes at day 7 were not carried out on untreated sample given its important microbial load. However, strawberry juice treated at optimal conditions was evaluated, and no significant differences were found on sweetness, acidity and bitterness scores between day 0 and day 7 (2.43, 2.37 and 0.37, respectively).

Storage time showed an important effect on OVQ and off-odor since statistically significant differences ($p < 0.05$) were found between the values obtained at 0, 7 and 14 days, indicating that changes throughout storage time appeared to be perceived by the panelists. Untreated sample scores for OVQ increased from 0 at the beginning of storage, to a score of 4.58 at day 14, while optimum-treated sample reached a score of 3.40. With regards to off-odor, untreated sample scores increased from 0.19 to 4.75 at the end of storage, while treated sample reached a score of 2.8. Given the high scores on both treated and untreated samples on OVQ and off-odor, taste attributes were not evaluated on day 14 on neither of the samples.

### 3.2.3. Color parameters

The evolution of lightness ($L^*$) and hue angle of both treated and untreated samples during storage is shown on Table 4. At day 0, strawberry juice treated at optimal conditions showed no significant difference in $L^*$ value compared to control. However, this

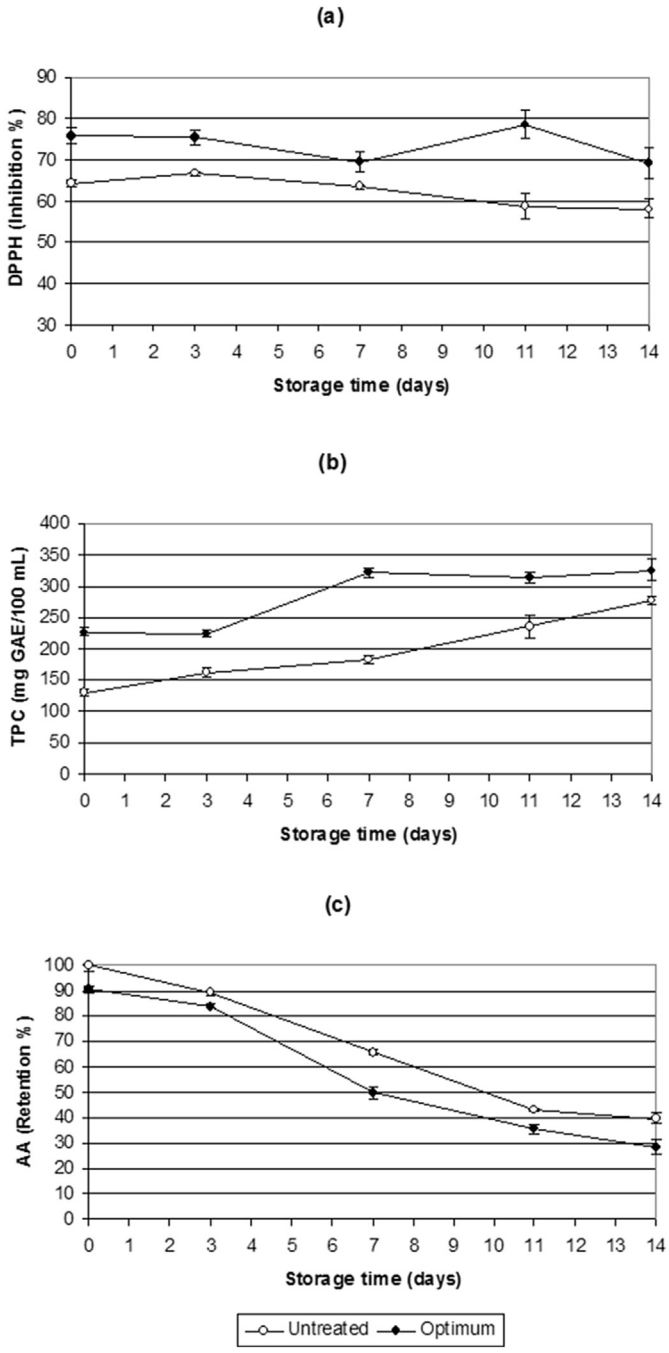


**Fig. 2.** Effect of optimum treatment on nutritional parameters of strawberry juice through refrigerated storage at 5 °C. Bars indicate standard errors. (a) DPPH (b) TPC (c) AA.

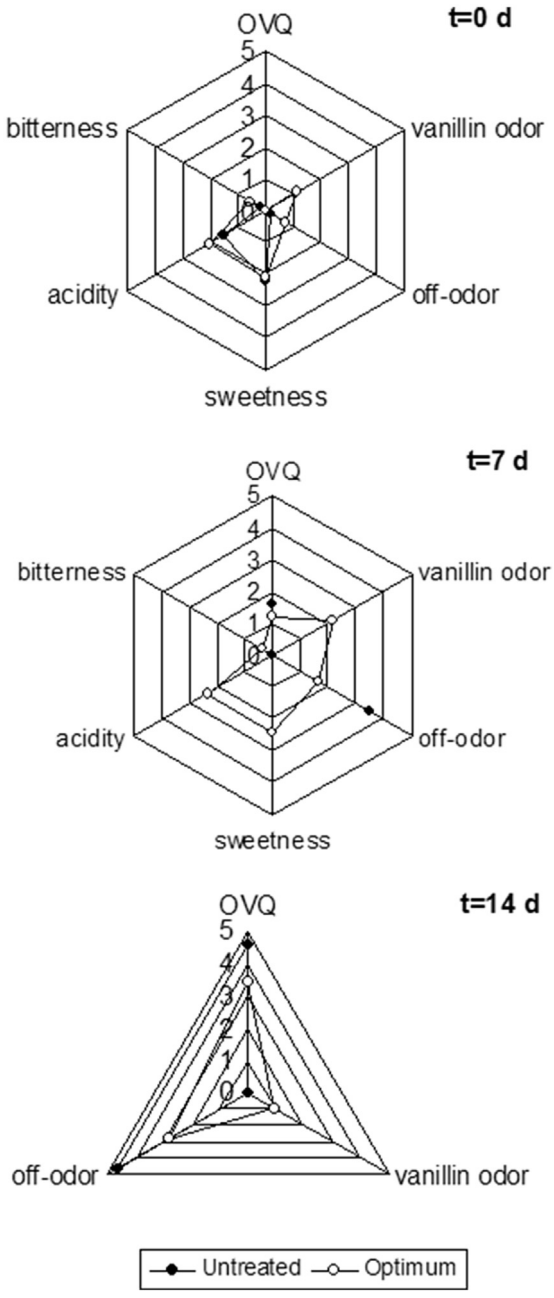


**Fig. 3.** Effect of optimum treatment on sensory evaluation of strawberry juice. At day 7 taste attributes (Bitterness, Sweetness and Acidity) in untreated strawberry juice were not carried out due to their high microbial load. At day 14, neither treated nor untreated samples were tasted.

parameter decreased during storage for both treated and untreated samples, and by the end of refrigerated storage, no significant differences were found between both samples. A decrease of the L* value of juice indicates browning, which results in a darkening color (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008).

The hue angle of the fresh strawberry juice indicates that the juices had a red color. At day 0, significant differences ($p \leq 0.05$) were found between untreated and treated samples, where strawberry juice treated at optimal conditions showed lower Hue values compared to control. This may be due to an extraction effect of phenolic compound due to the ultrasound treatment (Mohideen et al., 2015). As well as L* value, this parameter significantly decreased during storage for both the treated and untreated samples, maintaining the initial significant difference from control.

In accordance to these results, color degradation in sonicated fruit juices was reported by Tiwari et al. (2008) and Santhirasegaram, Razali, and Somasundram (2013). Color changes or darkening in juices could be explained by accumulation of unstable particulate fractions (Ugarte-Romero, Feng, Martin, Cadwallader, & Robinson, 2006). In addition, changes in color could be due to cavitation which governs various physical, chemical or biological reactions, accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or breakdown of susceptible

**Table 4**
Effect of optimum treatment on lightness (L*) and hue angle of strawberry juice through refrigerated storage at 5 °C.

| Storage time (days) | Treatments | |
|---|---|---|
| | Control | Optimum |
| **L*** | | |
| 0 | $46.12 \pm 0.98^{Aa}$ | $48.77 \pm 0.92^{Aa}$ |
| 3 | $29.81 \pm 1.17^{Ba}$ | $33.18 \pm 1.36^{Ba}$ |
| 7 | $28.09 \pm 0.85^{Ba}$ | $28.12 \pm 0.79^{Ca}$ |
| 11 | $26.73 \pm 0.64^{Ba}$ | $27.10 \pm 0.57^{Ca}$ |
| 14 | $27.16 \pm 0.59^{Ba}$ | $27.26 \pm 0.69^{Ca}$ |
| **Hue°** | | |
| 0 | $36.28 \pm 0.35^{Aa}$ | $34.89 \pm 0.23^{Ab}$ |
| 3 | $35.60 \pm 0.60^{ABa}$ | $31.83 \pm 0.51^{Bb}$ |
| 7 | $33.30 \pm 0.40^{BCa}$ | $31.23 \pm 0.33^{Bb}$ |
| 11 | $34.43 \pm 0.34^{Ca}$ | $32.81 \pm 0.36^{Bb}$ |
| 14 | $33.40 \pm 0.48^{Ca}$ | $31.16 \pm 0.53^{Bb}$ |

Data is shown as mean value ± Standard error. Different low case letters in the same row indicate significant differences ($p < 0.05$) between treatments. Different upper case letters in the same column indicate significant differences ($p < 0.05$) between storage time.

particles such as enzymes and microorganisms (Adekunte, Tiwari, Cullen, Scannell, & O'Donnell, 2010).

Both color parameters evaluated in optimum-treated samples showed a similar trend through storage time to those indices in control samples. This indicates the absence of overall color degradation between untreated and treated samples.

### 3.2.4. Native microflora

The effects of the optimum treatment on strawberry juice native microflora are shown on Fig. 4(a–b). Initial populations of yeast and molds, and psychrophilic bacteria on untreated strawberry juice were 5.40 and 5.38 CFU/mL, respectively. At days 0 and 3, the optimum treatment showed no initial effect on the native microflora of the strawberry juice, with no significant differences in any of the evaluated populations. However, from day 7 of refrigerated storage, significant reductions in every population studied were observed in the treated sample compared to control. The reductions in native microflora increased until the end of storage (day 14), where untreated samples showed microbial counts of 8.33 and 8.30 log CFU/mL in yeast and molds, and psychrophilic bacteria, respectively; whereas the sample treated at optimal conditions reached microbial counts of 2.48 and 3.48 log CFU/mL, respectively (Fig. 4a–b).

According to the Spanish regulation for hygienic processing, distribution and commerce of prepared meals, the maximum limit of allowed mesophilic total count at expiry allowed is $10^7$ CFU/g (BOE, 2001). Considering $10^7$ CFU/mL as a maximum limit for all the populations, the optimum treatment was able to extend strawberry juice microbiological shelf-life (>14 days) compared to the untreated sample (<7 days).

The significant reductions in native microflora may be due to the interaction between the antimicrobial agents and microbial populations during storage. Vanillin, like other phenolic compounds present in pomegranate extract, interacts with the phospholipid bilayer of the cell membrane, causing an increase of permeability and leakage of vital intracellular constituents or impairment of bacterial enzyme systems (Ponce, Del Valle, & Roura, 2004). In previous studies, vanillin has shown significant antimicrobial activity evaluated through *in vitro* (Tomadoni et al., 2015) and *in vivo* when applied in strawberry juice at concentrations of 2.5 and 5 mg/mL (Tomadoni et al., 2016). A study conducted by Fitzgerald et al. (2003) suggests that the aldehyde moiety plays a key role in the antifungal activity of vanillin.

Furthermore, several studies have shown the effects of

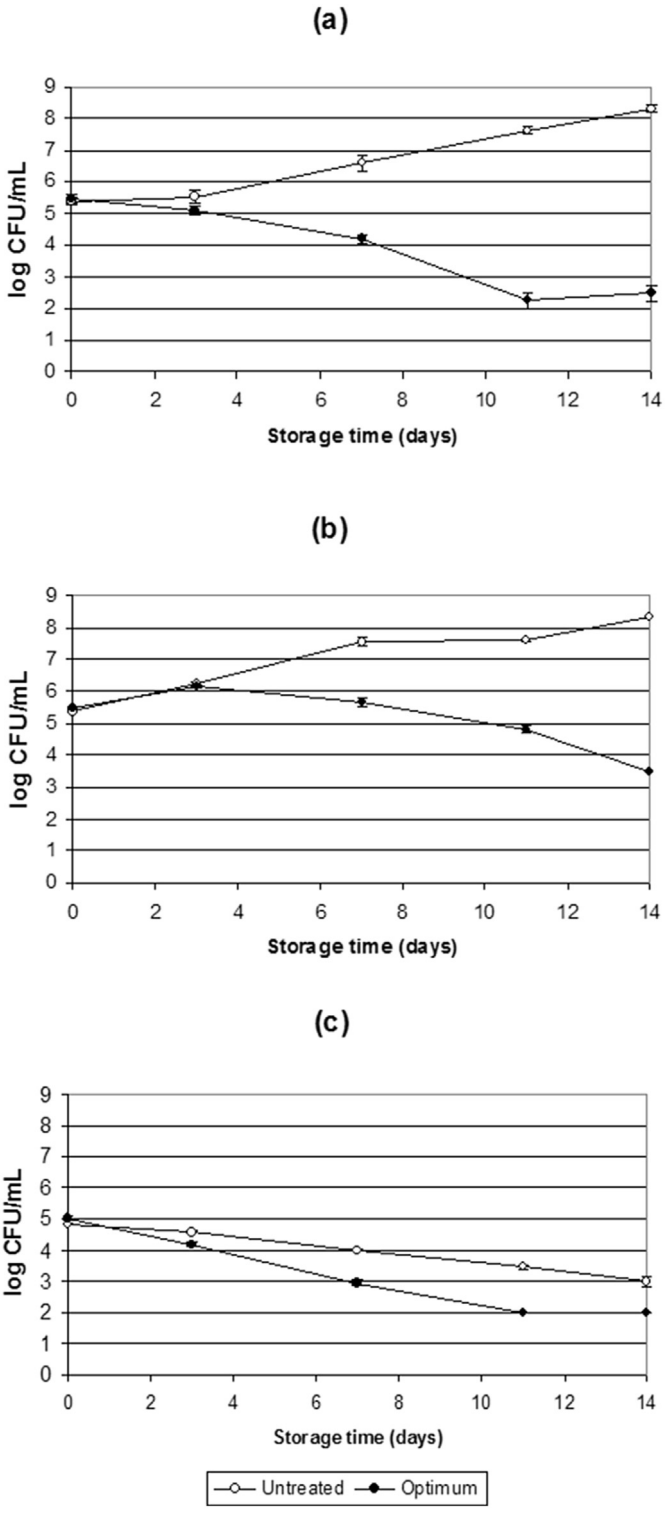


**Fig. 4.** Effect of optimum treatment on native microflora of strawberry juice and inoculated *E. coli* through refrigerated storage at 5 °C. Bars indicate standard errors. (a) yeast and molds; (b) psychrophilic bacteria; (c) inoculated *E. coli*.

ultrasound treatments (40 kHz) combined with different chemical sanitizers (São José & Dantas Vanetti, 2012; Chen & Zhu, 2011; Sagong et al., 2011). In these studies, a greater reduction of the contaminant population on fruits and vegetables was found when chemical treatments were combined with ultrasound. This

phenomenon may be due to the intense pressure generated during ultrasound treatments that contributes to the penetration of the chemical sanitizers through the cellular membrane. Also, cavitation process may assist in the disaggregation of the microorganisms, which culminates in an increased efficiency of the sanitization treatment (Gogate & Kabadi, 2009).

### 3.3. Effect of the optimum treatment against E. coli inoculated on strawberry juice

Despite of the inherent acidity of unpasteurized fruit juices, which is lethal to most bacterial species likely to contaminate such products, several outbreaks of infections caused by *Escherichia coli* O157:H7 have occurred caused by the consumption of unpasteurized fruit juices (Keller & Miller, 2006). In reported outbreaks of diseases involving fruit juices, *E. coli* O157:H7 and enterotoxigenic *E. coli* strains were indicated to be the most common causative agents, followed closely by *Salmonella* spp. (Vojdani, Beuchat, & Tauxe, 2008).

In order to evaluate the effectiveness of the proposed optimum treatment in the inactivation of *E. coli* O157:H7 in strawberry juice, samples were inoculated with the pathogen. Fig. 4c shows the effect of optimum treatment on *E. coli* survival throughout refrigerated storage at 5 °C. The initial count of *E. coli* on untreated sample was 4.84 log CFU/mL.

Immediately after the application of the treatment (day 0), no initial effect on *E. coli* counts was observed, with no significant differences from control. From day 3 until the end of storage significant differences were observed, with reductions in *E. coli* counts from 0.40 log reduction on day 3 to 1.05 and 0.99 log compared to control on days 11 and 14, respectively.

Significant reductions ($p < 0.05$) of *E. coli* population were also observed through the storage time for both treated and untreated samples. *E. coli* counts on untreated samples decrease from initial counts of 4.84 log to 2.99 log on day 14, while *E. coli* counts on treated juice were below detection limit (<2 log) from day 11 until the end of storage. Those reductions observed in untreated strawberry juice along storage time could be due to effects such as storage temperature (5 °C), pH of the strawberry juice and competitive microflora (native flora), whereas the reductions observed through time in treated strawberry juice could be also attributed to combined effects of ultrasound and antimicrobial compounds in the optimum treatment.

## 4. Conclusions

This study optimized the intensity of three different non-thermal technologies applied to strawberry juice (ultrasound as a physical treatment in combination with vanillin and pomegranate extract as natural preservatives), simultaneously improving microbiological, sensory and nutritional quality of the product. The optimum treatment obtained by response surface methodology was able to significantly reduce the native microflora of strawberry juice compared to control, while improving the nutritional quality of the product without affecting its sensory properties. Furthermore, the optimal combination of non-thermal treatments was effective to reduce inoculated *E. coli* O157:H7. The results obtained in this work may be used as baseline information for further establishment of non-thermal processes for fruit juices.

## Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas, Agencia Nacional de Promoción Científica y Tecnológica and Universidad Nacional de Mar del Plata.

## References

Adekunte, A. O., Tiwari, B. K., Cullen, P. J., Scannell, A. G. M., & O'Donnell, C. P. (2010). Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chemistry, 122*(3), 500–507.

Almeida Bezerra, M., Erthal Santelli, R., Padua Oliveira, E., Silveira Villar, L., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76*, 965–977.

Boletín Oficial del Estado (BOE). (2001). Normas de higiene para la elaboración, distribución y comercio de comidas preparadas. *Orden del 29 de diciembre del 2000. BOE, 11*, 1435–1441.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*, 25–30.

Cao, X. M., Bi, X. F., Huang, W., Wu, J. H., Hu, X. S., & Liao, X. J. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science & Emerging Technologies, 16*, 181–190.

Cao, X. M., Zhang, Y., Zhang, F. S., Wang, Y. T., Yi, J. Y., & Liao, X. J. (2011). Effects of high hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps. *Journal of the Science of Food and Agriculture, 91*, 877–885.

Chen, Z., & Zhu, C. (2011). Combined effects of aqueous chlorine dioxide and ultrasonic treatments on postharvest storage quality of plum fruit (*Prunus salicina* L.). *Postharvest Biology and Technology, 61*, 117–123.

CIE. (1977). Recommendations on uniform color spaces, Color difference equations, and metric color terms. *Color Research & Application, 2*(1), 5–6.

Costa, N. R., Lourenço, J., & Pereira, Z. L. (2011). Desirability function approach: a review and performance evaluation in adverse conditions. *Chemometrics and Intelligent Laboratory Systems, 107*, 234–244.

Feril, L. B., & Kondo, T. (2004). Biological effects of low intensity ultrasound: the mechanism involved and its implications on therapy and on biosafety of ultrasound. *Journal of Radiation Research, 45*, 479–489.

Fitzgerald, D. J., Stratford, M., & Narbad, A. (2003). Analysis of the inhibition of food spoilage yeasts by vanillin. *International Journal of Food Microbiology, 86*, 113–122.

Gogate, P. R., & Kabadi, A. M. (2009). A review of applications of cavitation in biochemical engineering/biotechnology. *Biochemical Engineering Journal, 44*(1), 60–72.

Keller, S. E., & Miller, A. J. (2006). Microbiological safety of fresh citrus and apple juices. In G. M. Sapers, J. R. Gorny, & A. E. Yousef (Eds.), *Microbiology of fruits and vegetables* (pp. 211–230). Boca Raton, Florida: CRC Press, Taylor and Francis Group.

Kuehl, R. O. (2000). *Design of experiments: Statistical principles of research design and analysis*. Pacific Grove, CA: Duxbury/Thomson Learning.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology, 55*, 181–186.

Mañas, P., & Pagan, R. (2005). Review Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology., 98*, 1387–1399.

Mohideen, F. W., Mis Solval, K., Li, J., Zhang, J., Chouljenko, A., Chotiko, A., et al. (2015). Effect of continuous ultra-sonication on microbial counts and physicochemical properties of blueberry (*Vaccinium corymbosum*) juice. *LWT - Food Science and Technology, 60*, 563–570.

Moreira, M. R., Roura, S. I., & del Valle, C. E. (2003). Quality of Swiss Chard produced by conventional and organic methods. *LWT- Food Science & Technology, 36*, 135–141.

Ponce, A. G., Agüero, M. V., Roura, S. I., del Valle, C. E., & Moreira, M. R. (2008). Dynamics of indigenous microbial populations of butter head lettuce grown in mulch and on bare soil. *Journal of Food Science, 73*, 257–263.

Ponce, A., Del Valle, C., & Roura, S. (2004). Shelf life of leafy vegetables treated with natural essential oils. *Journal of Food Science and Technology, 69*, 550–556.

Puttongsiri, T., & Haruenkit, R. (2010). Changes in ascorbic acid, total polyphenol, phenolic acids and antioxidant activity in juice extracted from coated kiew wan tangerine during storage at 4, 12 and 20 C. *Natural Science, 44*, 280–289.

R Development Core Team. (2011). *R: A language and environment for statistical computing*. Vienna, Austria: the R Foundation for Statistical Computing, ISBN 3-900051-07-0. http://www.R-project.org/.

Sagong, H. G., Lee, S. Y., Chang, P. S., Heu, S., Ryu, S., Choi, Y. J., et al. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology, 145*(1), 287–292.

Santhirasegaram, V., Razali, Z., & Somasundram, C. (2013). Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. *Ultrasonics Sonochemistry, 20*(5), 1276–1282.

São José, J. F. B., & Dantas Vanetti, M. C. (2012). Effect of ultrasound and commercial sanitizers in removing natural contaminants and *Salmonella enteric* Typhimurium on cherry tomatoes. *Food Control, 24*(1–2), 95–99.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Meth Enzymology, 299*, 152–178.

StatSoft, Inc. (2004). *STATISTICA (data analysis software system), version 7*. www.statsoft.com.

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. (2008). Effects of sonication on the kinetics of orange juice quality parameters. *Journal of*

*Agricultural and Food Chemistry, 56*(7), 2423–2428.
Tomadoni, B., Cassani, L., Moreira, M. R., & Ponce, A. (2015). Efficacy of vanillin and geraniol in reducing *Escherichia coli* O157:H7 on strawberry juice. *LWT-Food Science and Technology, 64*(2), 554–557.
Tomadoni, B., Viacava, G., Cassani, L., Moreira, M. R., & Ponce, A. (2016). Novel biopreservatives to enhance the safety and quality of strawberry juice. *Journal of Food Science and Technology, 53*(1), 281–292.
Ugarte-Romero, E., Feng, H., Martin, S. E., Cadwallader, K. R., & Robinson, S. J. (2006). Inactivation of *Escherichia coli* with power ultrasound in apple cider. *Journal of Food Science, 71*, 102–108.
Vercet, A., Burgos, J., & Lopez-Buesa, P. (2001). Manothermosonication of foods and food-resembling systems: effect on nutrient content and non enzymatic browning. *Journal of Agricultural and Food Chemistry, 49*(1), 483–489.
Vercet, A., Sanchez, C., Burgos, J., Montañés, L., & López, P. (2002). The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties. *Journal of Food Engineering, 53*, 273–278.
Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry – a review. *Innovative Food Science and Emerging Technologies, 9*, 161–169.
Vojdani, J. D., Beuchat, L. R., & Tauxe, R. V. (2008). Juice-associated outbreaks of human illness in the United States, 1995 through 2005. *Journal of Food Protection, 71*, 356–364.
Zhou, L. Y., Wang, Y. Y., & Liao, X. J. (2009). Effect of high pressure carbon dioxide on the quality of carrot juice. *Innovative Food Science & Emerging Technologies, 10*, 321–327.