Food and Bioprocess Technology (2018) 11:2110–2124
https://doi.org/10.1007/s11947-018-2170-x

ORIGINAL PAPER

Improving Quality Parameters of Functional Strawberry Juices: Optimization of Prebiotic Fiber Enrichment and Geraniol Treatment

Lucía Cassani [1,2] · Bárbara Tomadoni [1,2] · María R. Moreira [1,2] · María V. Agüero [3]

Received: 21 March 2018 / Accepted: 20 August 2018 / Published online: 27 August 2018

Abstract

Enrichment of strawberry juices with prebiotic fiber (inulin and oligofructose) and their preservation treatment (with ultrasound and geraniol) were optimized by response surface methodology with a Box-Behnken design in order to simultaneously maximize microbiological, nutritional, and sensory quality of juices after 2 weeks of refrigerated storage. The optimal conditions were inulin/oligofructose proportion of 5:3, 0.225 μL/mL of geraniol and ultrasound time equal to 0, with a desirability value of 0.77. After that, strawberry juices were treated at optimal conditions to investigate changes on microbiological (native microflora), nutritional (including ascorbic acid content and antioxidant capacity indicators), sensory quality and safety (simulating an eventual postharvest contamination with *Escherichia coli* O157:H7 and *Listeria innocua*) during storage. Finally, a study evaluating the performance of the optimized treatment on stability of prebiotics added to strawberry juices was carried out. The optimized treatment was highly effective to reduce native microflora counts, as well as, to inhibit those inoculated pathogens in juices. Treatment at optimal conditions did not induce any negative effect neither on antioxidant capacity indicators nor on ascorbic acid content of juices. Furthermore, the optimized treatment ensured the stability of inulin and oligofructose added to juices during storage. The simultaneous optimization allowed lower concentrations of geraniol, reducing the impact on sensory quality. Therefore, the enrichment and treatment of strawberry optimized and proposed in this work could be a feasible alternative to thermal pasteurization to ensure the microbiological quality and safety in juices, as well as, to improve nutritional and sensory quality.

Keywords Natural antimicrobial · Response surface methodology · Functional ingredients · Fruit-based product · Storage

Introduction

Consumers' demand for high nutritional quality food products with "fresh-like" characteristic without added chemical additives such as unpasteurized fruit juices have increased in the last decade (Mosqueda-Melgar et al. 2012). In response to this demand, the market of strawberry juices has become more popular, not only for the noticeable organoleptic attributes ascribed to the fruit but also for its nutritional properties, since strawberry is a valuable source of compounds with potential health benefits (Aday et al. 2013). To attend the demand of healthier foods, food products can be enriched with functional ingredients, such as prebiotics like inulin and oligofructose, thus increasing their nutritional properties (Keenan et al. 2011). Inulin and oligofructose are among the most studied and well-established prebiotics. These compounds are considered fibers because they are not digested in the gastrointestinal due to their structure. Among their nutritional attributes, these substances stimulate beneficial gut microflora and relieve constipation, as well as, improve calcium availability (Zuleta and Sambucetti 2001). Besides, these compounds have important technological properties; for example, inulin has a remarkable capacity to replace fat and improve the stability of foams and

✉ Lucía Cassani
lcassani@fi.mdp.edu.ar

[1] Grupo de Investigación en Ingeniería en Alimentos, Facultad Ingeniería, UNMdP, J.B. Justo 4302, Mar del Plata 7600, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

[3] Universidad de Buenos Aires (UBA), Consejo Nacional de Investigaciones Científica y Técnicas (CONICET), Instituto de Tecnologías y Ciencias de la Ingeniería (INTECIN), Laboratorio de Microbiología Industrial: Tecnología de alimentos, Av. Int. Guiraldes 2620, Ciudad Autónoma de Buenos Aires C1428EGA, Argentina

emulsions, such as ice creams and sauces. Moreover, oligofructose is much more soluble than inulin, and its pure form has a sweetness of about 35% in comparison with sucrose (Franck 2002).

Fresh fruit juices have a short shelf life that can be generally attributed to both microbial and enzymatic spoilage. Thus, thermal pasteurization is usually applied to juices in order to inhibit pathogenic microorganisms and extend its shelf life. However, this technology damages nutritional, sensory, and physicochemical properties of foods (Mosqueda-Melgar et al. 2008). For this reason, alternative technologies that offer the advantages of using low processing temperatures, low energy consumption, and retention of nutritional and sensory attributes, while inactivating pathogenic microorganisms to levels that do not cause a public health risk, are being applied. Among these technologies, geraniol, a bioactive compound belonging to the class of monoterpenoids, is an important constituent of essential oils of various aromatic herbs and has applications due to its aroma in food and beverage industries. It has a pleasant aroma characteristic of rose oil and citrus fruits (Prasad and Muralidhara 2017). The antibacterial and antioxidant activity of geraniol against pathogenic and spoilage-forming bacteria were tested in in vitro studies (Zengin and Baysal 2014). In a previous work, geraniol was proved to be highly effective in reducing native microflora counts of fiber-enriched strawberry juices but sensory attributes were affected (Cassani et al. 2016). Thus, an alternative to reduce this negative effect could be to apply this natural antimicrobial together with ultrasound, an environmentally friendly process commonly used to inactivate deteriorative microorganisms and enzymes of foods, in order to achieve the same antimicrobial effect without affecting sensory quality. Besides, it is interesting to investigate the impact of enriching strawberry juices with different proportions of prebiotics (inulin and oligofructose) on sensory attributes and to study the interaction between the mix of prebiotics and the preservation treatments applied. Thus, the objectives of this work were to (1) find the optimal combination of the preservation factors (ultrasound time, concentration of geraniol) and enrichment of strawberry juice (added prebiotics composition) using response surface methodology in order to simultaneously maximize microbial, nutritional, and sensory quality of juices after 2 weeks of storage; (2) evaluate the effect of the optimized treatment on quality parameters (microbiological, antioxidant capacity indicators, and sensory) and safety (simulating an eventual postharvest contamination with *Escherichia coli* O157:H7 and *Listeria innocua*) of strawberry juice during 14 days of storage; and (3) study the performance of the optimized treatment on stability of sugars and prebiotics added to strawberry juices.

Materials and Methods

Juice Obtaining

Strawberries (*Fragaria* x *ananassa* Duch. cv. Aromas) were grown in Sierra de los Padres, Mar del Plata, Argentina. Strawberries intended for the optimization and validation analysis (objective 1) were harvested at commercial maturation in January 2015, those used for quality and safety analysis were collected in January 2016 (objective 2), while those intended for the sugars and prebiotics stability analysis were gathered in February 2016 (objective 3). Fruits with good visual quality were washed with tap water and the calyx was removed by hand. Then, juice was obtained by squeezing the fruits using a commercial extractor and the fresh strawberry juice was collected in a glass jar. The juice was homogenized and bottled under hygienic conditions into 350-mL polyethylene terephthalate bottles and sealed with polyethylene caps to be subsequently used in the experiments.

Enrichment and Treatment Optimization

Experimental Design

A first study was conducted to find the optimum combination of the preservation treatments (ultrasound time, concentration of geraniol) and enrichment of strawberry juice (mixture of inulin and oligofructose) using response surface methodology with a Box-Behnken design, in order to simultaneously minimize microbial counts, maximize nutritional properties, and minimize the impact on sensory quality of juices after 2 weeks of storage. The method of least-squares regression was used to fit data to a quadratic model of the form (for each response variable):

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

where Y_n is the predicted response (Y_1 = mesophilic bacteria counts, Y_2 = yeasts and molds counts, Y_3 = ascorbic acid content, Y_4 = overall visual quality, Y_5 = typical odor, Y_6 = off-odor); β_0 is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, AND β_{ij} is the coefficient for the interaction effect. X_i is a dimensionless coded value of the independent variable, x_i. In this study, x_1 = prebiotics proportion (inulin/oligofructose), x_2 = ultrasound time (min), x_3 = geraniol concentration (μL/mL).

For a 3-level, 3-factor Box-Behnken experimental design with three replicates at the central point, a total of 15 experimental runs are needed, in which each variable was tested at three different coded levels: low (− 1), middle (0), and high (+1) as shown in Table 1. At days 0 and 14, responses were measured by duplicate for each trial. Mean values were

Table 1 Box-Behnken experimental design matrix; initial values of all responses of control samples and mean values of all responses of non-treated and treated samples under different experimental conditions after 14 d of storage at 5 °C

Time of storage (day)	Sample	Independent variables[a]			Response variables					
		x_1 Fiber proportion (inulin/ oligofructose)	x_2 Ultrasound treatment (min)	x_3 Bioactive concentration (μL/mL)	Mesophilic bacteria (log CFU/ mL)	Yeasts and molds (log CFU/mL)	Ascorbic acid (mg ascorbic acid/ 100 mL of juice)	Overall visual quality	Typical odor	Off-odor
0	C1				5.50	5.27	43.94	4.78	4.00	0.61
	C2				5.46	5.22	43.75	4.67	3.16	0.30
	C3				5.49	5.12	44.44	4.67	4.43	0.27
14	1	1:3 (− 1)	0 (− 1)	0.15 (0)	4.47	4.38	23.78	2.77	2.17	1.53
	2	1:3 (− 1)	30 (1)	0.15 (0)	4.79	3.98	24.47	3.24	1.95	2.35
	3	3:1 (1)	0 (− 1)	0.15 (0)	5.01	4.61	26.07	3.33	1.53	2.03
	4	3:1 (1)	30 (1)	0.15 (0)	5.63	5.53	23.20	3.50	1.48	2.47
	5	1:1 (0)	0 (− 1)	0 (− 1)	5.88	5.87	28.20	2.40	0.00	5.00
	6	1:1 (0)	0 (− 1)	0.30 (1)	4.83	4.03	23.92	3.37	1.82	3.23
	7	1:1 (0)	30 (1)	0 (− 1)	7.39	7.32	29.60	3.00	0.00	5.00
	8	1:1 (0)	30 (1)	0.30 (1)	5.06	4.01	23.04	3.33	1.87	3.00
	9	1:3 (− 1)	15 (0)	0 (− 1)	7.49	7.62	24.89	3.10	0.00	5.00
	10	3:1 (1)	15 (0)	0 (− 1)	7.26	7.52	24.05	2.70	0.00	5.00
	11	1:3 (− 1)	15 (0)	0.30 (1)	4.45	3.96	19.94	3.72	2.53	2.23
	12	3:1 (1)	15 (0)	0.30 (1)	4.94	3.90	19.97	3.93	2.67	2.48
	13	1:1 (0)	15 (0)	0.15 (0)	4.45	4.31	22.66	3.78	2.45	2.48
	14	1:1 (0)	15 (0)	0.15 (0)	5.24	4.00	21.45	3.78	2.70	2.20
	15	1:1 (0)	15 (0)	0.15 (0)	4.00	4.48	21.67	3.93	2.60	2.25
	C1				6.84	8.06	29.81	1.50	0.64	5
	C2				6.45	7.66	28.80	1.62	0.52	5
	C3				7.23	7.87	24.05	1.71	0.61	5

[a] *C1* juice sample with an inulin/oligofructose proportion of 1:3 without preservation treatment, *C2* juice sample with an inulin/oligofructose proportion of 3:1 without preservation treatment, *C3* untreated juice sample

Coded independent variables (X_i) are shown between brackets

informed for each response and were considered for fitting the second-order polynomial models (Eq. 1).

The amount of prebiotics (inulin/oligofructose proportion) was selected according to previous studies. In this way, 3 g of fibers in 200 mL of juice were evaluated at three different proportions of inulin/oligofructose (1:3, 1:1, and 3:1). The ultrasound processing times selected for this study were 0, 15, and 30 min, according to Tomadoni et al. (2017), while geraniol was applied at 0, 0.15, and 0.30 μL/mL of juice. These concentrations were selected according to Cassani et al. (2016).

Inulin and oligofructose were added to each strawberry juice bottle and stirred until total dissolution. The ultrasound treatments were performed at 40-kHz frequency (power of the ultrasound waves: 180 W transmitted from bottom to above) using an ultrasonic cleaning bath (TestLab, Argentine) of 15 × 29 × 15 cm in the dark. Temperature in the ultrasonic bath was monitored at 20 ± 1 °C. Geraniol (Firmenich SAICYF, Argentina) was applied directly into the juice samples and stirred until total dissolution.

Three extra juice samples were used as controls: juice sample with an inulin/oligofructose proportion of 1:3 without preservation treatment (C1), juice sample with an inulin/oligofructose proportion of 3:1 without preservation treatment (C2), and untreated juice sample (C3).

Response Variables

The impact of adding functional ingredients in combination with the preservation treatments on microbiological, nutritional, and sensory quality of strawberry juice was simultaneously analyzed. These parameters were assessed at days 0 and 14 of refrigerated storage.

The microbial stability of strawberry juices was evaluated through the enumeration of total aerobic mesophilic bacteria (MES) and yeasts and molds (YM) populations. A 10-mL aliquot of juice from each treatment was sampled and serial dilutions (1:10) were made in peptonated water (1 mg/mL) and surface spread by duplicate. The enumeration of the microbial populations was performed according to Ponce et al. (2008) by using the following culture media and culture conditions: MES on plate count agar (PCA) incubated at 35 °C for 48 h and YM on yeast-glucose-chloramphenicol (YGC) medium incubated at 25 °C for 5 days. All culture mediums were

purchased from Britania, Buenos Aires, Argentina. Microbial counts were expressed as log CFU per milliliter.

Ascorbic acid content (AAC) was determined using the titrimetric assay described by Goyeneche et al. (2015). Briefly, 20 mL of each strawberry juice sample were homogenized with 40 mL of 2% oxalic acid solution (Biopack, Argentina). This mixture was vacuum filtered through glass fiber. Five-milliliter aliquots of the filtrate were titrated with 2,6-dichloroindophenol (Anedra, Argentina). AAC was calculated as milligrams of reduced ascorbic acid/100 mL of juice.

Quantitative descriptive analysis was used to evaluate sensory attributes of strawberry juice samples at days 0 and 14. A panel comprising ten members, aged 25–50 years with sensory evaluation experience was trained and carried out the evaluation of strawberry juices. Samples labeled with three-digit code numbers were randomly provided. The attributes evaluated were overall visual quality (OVQ), typical odor and off-odor. The intensity of the attributes evaluated was quantified on unstructured scale from 0 to 5. OVQ was scored from 0 (highly deteriorated aspect) to 5 (fresh aspect), typical odor from 0 (not detected) to 5 (fresh) and off-odor from 0 (not detected) to 5 (intense) (Cassani et al. 2016).

Simultaneous Optimization

A simultaneous optimization was carried out using the desirability function (D). For this purpose, predicted values obtained from each model (Y_n, Eq. 1) 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 were then combined to obtain the overall desirability D, defined as the geometric average of the individual desirability. An algorithm was then applied to this function in order to determine the set of values that maximizes it (Bezerra et al. 2008).

Validation

In order to test the reliability of the simultaneous optimization, a new set of experiments using optimal operating conditions was performed. For these experiments, a juice sample was prepared according to optimal processing conditions and a control (strawberry juice sample without added fibers and with no treatments) was used. Samples were stored at 5 ± 1 °C for 14 days. The quality parameters, previously described, were assessed at 0 and 14 days of storage.

Evaluation of Quality Parameters and Safety throughout Storage of the Optimized Treatment

Once the optimal combination of the preservation treatments and enrichment of strawberry juice was found, a second study was performed in which changes on several quality parameters (microbiological, nutritional, antioxidant capacity indicators, and sensory analysis) and safety (inoculated *E. coli* O157/H7 and *L. innocua*) of juices were evaluated during 14 days of refrigerated storage. For this study, a new batch of strawberry juice was used and samples were prepared according to optimal conditions of processing and enrichment (treated samples) and were compared with juice samples without fiber addition and without treatments (untreated or control samples). After that, strawberry juice samples were stored at 5 ± 1 °C for 14 days. All assays were carried out by triplicate in two independent experimental runs.

Microbiological Parameters

Native microflora of strawberry juice was assessed during storage. MES and YM were determined as was described above. In addition, the enumeration and differentiation of *Enterobacteriaceae* and total coliforms (ETC) was performed by using a Mac Conkey agar incubated at 35 °C for 24 h and lactic acid bacteria (LAB) was performed by using a Man, Rogosa, and Sharpe (MRS) medium incubated at 35 °C for 24 h. Culture mediums were purchased from Britania, Buenos Aires, Argentina.

Nutritional Quality

Ascorbic acid content was determined using the titrimetric assay described above.

Antioxidant Capacity Indicators

Total phenolic content (TPC), total flavonoids content (TFC), and total antioxidant capacity (TAC) were determined on an extract of antioxidants from juice samples. The extraction of antioxidants was carried out homogenizing 2 mL of strawberry juice from each sample with 10 mL solution of ethanol (80% *v*/*v*) (Merk, Darmstadt, Germany). The homogenate was then centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was collected and filtered using Whatman filter paper #1. The final ethanolic extract was stored at − 20 °C to be used in the determination of antioxidant capacity indicators.

TPC was determined spectrophotometrically using the Folin-Ciocalteu reagent according to the method of Viacava et al. (2015) using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalents (GAE)/100 mL of juice.

TFC was determined based on the method described by Viacava and Roura (2015) and was expressed as milligrams of quercetin equivalents (QE)/100 mL of juice (Sigma-Aldrich, USA).

TAC was studied by evaluation of the free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma-Aldrich, USA), according to the method described by Viacava et al. (2015). The radical scavenging activity was expressed as milligrams of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents/100 mL of juice.

Sensory Parameters

A quantitative descriptive analysis was used to evaluate sensory attributes of strawberry juice samples, as was described above. The evaluated attributes were color, odor, acid and sweet taste, and overall visual quality (OVQ) of the beverages. The intensity of the evaluated attributes was quantified on unstructured scale from 0 to 5. OVQ was scored from 0 (highly deteriorated aspect) to 5 (fresh aspect). Color was rated from 0 (deteriorated color) to 5 (typical color), odor from 0 (intense off-odors) to 5 (fresh) and sweet and acid taste from 0 (not perceived) to 5 (intense).

Performance against *E. coli* O157/H7 and *L. innocua* Contaminations

Another batch of juice was used and samples were inoculated with *E. coli* O157/H7 and *L. innocua*, simulating a contamination with pathogens and then prepared according to optimal conditions of processing and enrichment (treated samples). Another juice sample was inoculated with the corresponding microorganism but not submitted neither to fiber addition nor treatments (control). Then, samples were stored at 5 °C during 14 days. Periodically, the pathogenic microbial counts were assessed.

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

Inoculation of Samples and Sampling Procedure Inoculation was carried out by adding the bacterial suspension to fresh strawberry juice to obtain the final desired concentration of cells (10^5–10^6 CFU/mL approximately). Finally, all inoculated samples were stored at 5 ± 1 °C until analysis.

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

Performance of the Optimized Treatment on the Stability of Sugars and Prebiotics

In a third study, another batch of juice was used and samples were processed at optimal conditions of processing and enrichment (treated samples). Another juice sample was enriched with the optimal proportion of inulin/oligofructose found in the response surface methodology (RSM) study without any preservative treatments (enriched control samples).

In a previous work (Cassani et al. 2018), the composition of commercial inulin and oligofructose was determined by HPLC analysis. From that results, it was concluded that inulin is mainly composed by inulin (73.17%) and short-chain oligosaccharides (fructo-oligosaccharides, FOS) of different degree of polymerization (DP), as DP3 (6.41%), DP5 (5.95%), and DP4 (5.34%). Sucrose (9.13%) was also detected in commercial inulin. On the other hand, oligofructose is composed of a mixture of FOS. DP5 (14.65%), DP4 (17.65%), and DP3 (28.65%), and sucrose (26.42%) were the main compounds present in commercial oligofructose.

To determine sugars and prebiotic fibers of strawberry juices, the analytical technique was based on HPLC with a refractive index detector. The reagents and standards, HPLC equipment (consisting of an HPLC Spectra SYSTEM Isocratic Pump P100 with refractive index detector and a Rheodyne injection valve with a 20-μL-sample loop (Sigma-Aldrich, Missouri, USA)) and chromatographic procedure were kept consistent with our previous study (Cassani et al. 2018). Two separate columns were used for clear and accurate separation of the short-chain carbohydrates and prebiotics of interest. The elution profile of the standards used and the detection and quantification limits have been previously detailed (Cassani et al. 2018).

Statistical Analysis

Data for RSM study were analyzed using the STATISTICA 7.1 (Statsoft Inc. 2004, Tulsa, USA). The statistical analysis was performed using the analysis of variance (ANOVA) including the F ratio, which established the model global significance and the adjusted 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 significant factors affecting each dependent variable were selected according to the Student t test establishing a 95% confidence level (Kuehl 2000).

Data from quality and safety evaluation during storage of enriched juices treated at optimal conditions and sugars and prebiotics' stability study were analyzed using R, software version 2.12 (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 through storage ($p < 0.05$).

Results and Discussion

Enrichment and Treatment Optimization

Table 1 depicts mean values obtained for samples enriched and treated under conditions established by experimental design, after 14 days of storage. Also, data obtained for control samples (before treatments and after 14 days of storage) were included.

The coefficients of the second-order polynomial equations (Eq. (1)) were calculated from the experimental data. ANOVA showed that each model of each response variable was significant ($p < 0.05$) and adjusted well to experimental data ($R^2 > 0.76$) with non-significant lack of fit ($p > 0.05$) (Table 2). The estimated coefficients, with their standard errors, and t and p values are also shown in Table 2.

The regression analysis shows that mesophilic bacteria and yeasts and molds were significantly affected by geraniol concentration, as linear and quadratic terms associated to this independent variable were significant for these models. This represents a positive effect improving microbiological quality. Ascorbic acid, overall visual quality, and typical odor were significantly affected by ultrasound time (quadratic effect) and geraniol concentration (linear and quadratic effects). Off-odor was significantly affected by geraniol concentration as linear and quadratic terms associated to this independent variable were significant for this model. Both preservation treatments positively affected the sensory attributes studied up to a certain extent. The proportion of inulin/oligofructose added to juices was not significant ($p > 0.05$) in these models, since neither linear and quadratic terms nor interactions with the preservation treatments were significant in all response variables.

Figure 1 shows the results of the simultaneous optimization where the optimal levels for independent variables were found inulin/oligofructose proportion of 5:3, 0.225 μL/mL of geraniol and ultrasound time equal to 0 min, with a desirability value of 0.77. Under these conditions, the predicted optimum responses were 4.30 log CFU/mL for mesophilic bacteria, 3.75 log CFU/mL for yeasts and molds, 24.16 mg ascorbic acid/100 mL of juice for ascorbic acid, 3.47 for overall visual quality, 2.28 for typical odor, and 2.16 for off-odor. This result indicates that, although the positive effects of ultrasound on quality attributes of fruit juices have been widely demonstrated in several works, when combined, this non-thermal technique with geraniol, no ultrasound treatment was necessary to maximize microbial, nutritional, and sensory quality of strawberry juices after 14 days of storage.

Results from validation experiment indicated that the mean experimental values after 2 weeks of storage were 3.59 ± 0.35 CFU/mL for mesophilic bacteria, 3.87 ± 0.36 CFU/mL for yeasts and molds, 34.29 ± 0.77 mg ascorbic acid/100 mL of juice, 2.81 ± 0.31 for overall visual quality, 1.28 ± 0.20 for typical odor, and 2.96 ± 0.32 for off-odor. From these results, it is interesting to compare not only absolute value of response variables (experimental vs. predicted values) but also, the behavior of the optimal sample in comparison to control because in each new elaboration there may be differences associated to the variability of the raw material. In fact, these comparisons were in the typical range, and in almost all cases, were better than those obtained from the predicted values, verifying that optimal conditions improve response variables with respect to control.

Changes in Quality Parameters and Safety throughout Storage of the Optimized Strawberry Juice

Microbiological Quality

Figure 2a–d shows mesophilic bacteria (MES), *Enterobacteriaceae* and total coliform (ETC), lactic acid bacteria (LAB), and yeasts and molds (YM) counts in strawberry juices during storage. Treatment at optimal processing conditions and enrichment was effective in reducing initial MES counts of strawberry juices since significant differences (0.3 log CFU/mL) in comparison to untreated sample were observed (Fig. 2a). Besides, during the whole storage, mesophilic bacteria growth rate was significantly higher in untreated sample than in treated one, indicating that the optimized treatment exerted a significant inhibitory effect on this population. In fact, at the end of storage, MES counts of treated strawberry juice resulted to four log cycles lower than untreated. Regarding ETC counts, no significant differences were observed after treatment application (Fig. 2b). During storage, ETC counts decreased in treated sample up to day 7, but then increased while ETC counts of untreated sample were maintained constant during the whole storage. The

Table 2 Estimated regression coefficients for RSM analysis of response variables

MES[a] (adjR^2 = 0.763; p = 0.031; pLOF = 0.668)					YM[a] (adjR^2 = 0.871; p = 0.008; pLOF = 0.138)				AA[a] (adjR^2 = 0.929; p = 0.002; pLOF = 0.443)			
Term	Coded coefficient	SE coefficient	t value	p value	Coded coefficient	SE coefficient	t value	p value	Coded coefficient	SE coefficient	t value	p value
Intercept	4.56310	0.318036	14.34772	0.000030*	4.26296	0.288217	14.79080	0.000026*	21.92535	0.414067	52.95125	0.000000*
X_1	0.20461	0.194757	1.05060	0.341544	0.20080	0.176496	1.13769	0.306812	0.02734	0.253563	0.10784	0.918318
X_1^2	0.33052	0.286674	1.15296	0.301051	0.40170	0.259795	1.54623	0.182715	− 0.76280	0.373235	− 2.04376	0.096405
X_2	0.33618	0.194757	1.72617	0.144904	0.24437	0.176496	1.38457	0.224788	− 0.20573	0.253563	− 0.81135	0.454038
X_2^2	0.08383	0.286674	0.29242	0.781709	− 0.04078	0.259795	− 0.15698	0.881404	3.21636	0.373235	8.61754	0.000347*
X_3	− 1.09335	0.194757	− 5.61390	0.002481*	− 1.55359	0.176496	− 8.80241	0.000314*	− 2.48438	0.253563	− 9.79786	0.000189*
X_3^2	1.14184	0.286674	3.98307	0.010499*	1.08361	0.259795	4.17103	0.008730*	1.04970	0.373235	2.81243	0.037441*
$X_1 X_2$	0.07404	0.275428	0.26883	0.798800	0.32881	0.249603	1.31734	0.244859	− 0.88932	0.358592	− 2.48004	0.055838
$X_1 X_3$	0.18023	0.275428	0.65437	0.541770	0.01075	0.249603	0.04307	0.967310	0.22005	0.358592	0.61366	0.566283
$X_2 X_3$	− 0.31754	0.275428	− 1.15291	0.301068	− 0.36955	0.249603	− 1.48054	0.198810	− 0.56901	0.358592	− 1.58679	0.173421
OVQ[a] (adjR^2 = 0.834; p = 0.014; pLOF = 0.122)					TO[a] (adjR^2 = 0.907; p = 0.003; pLOF = 0.089)				OO[a] (adjR^2 = 0.921; p = 0.002; pLOF = 0.105)			
Term	Coded coefficient	SE coefficient	t value	p value	Coded coefficient	SE coefficient	t value	p value	Coded coefficient	SE coefficient	t value	p value
Intercept	3.833333	0.110026	34.84040	0.000000*	2.58333	0.186525	13.84978	0.000035*	2.31111	0.204143	11.32104	0.000094*
X_1	0.080729	0.067377	1.19818	0.284539	− 0.12083	0.114223	− 1.05787	0.338521	0.10833	0.125012	0.86659	0.425791
X_1^2	− 0.143229	0.099176	− 1.44420	0.208291	− 0.21042	0.168132	− 1.25150	0.266113	− 0.29722	0.184012	− 1.61523	0.167182
X_2	0.150521	0.067377	2.23402	0.075781	− 0.02708	0.114223	− 0.23711	0.821981	0.12708	0.125012	1.01657	0.355996
X_2^2	− 0.480729	0.099176	− 4.84725	0.004685*	− 0.58958	0.168132	− 3.50668	0.017161*	0.08194	0.184012	0.44532	0.674706
X_3	0.393750	0.067377	5.84402	0.002076*	1.11042	0.114223	9.72149	0.000196*	− 1.13125	0.125012	− 9.04916	0.000275*
X_3^2	− 0.327604	0.099176	− 3.30327	0.021398*	− 1.07292	0.168132	− 6.38141	0.001399*	1.66528	0.184012	9.04983	0.000275*
$X_1 X_2$	− 0.076042	0.095285	− 0.79805	0.461049	0.04167	0.161536	0.25794	0.806733	− 0.09583	0.176793	− 0.54207	0.611048
$X_1 X_3$	0.154167	0.095285	1.61796	0.166596	0.03333	0.161536	0.20635	0.844655	0.06250	0.176793	0.35352	0.738116
$X_2 X_3$	− 0.158333	0.095285	− 1.66168	0.157466	0.01250	0.161536	0.07738	0.941321	− 0.05833	0.176793	−0.32995	0.754811

MES mesophilic bacteria, *YM* yeasts and molds, *AA* ascorbic acid, *OVQ* overall visual quality, *TO* typical odor, *OO* off-odor

[a] Each response is informed with ANOVA parameters: adjusted R^2 , p value, and p value for lack of fit test

*Coefficients with p value lower than 0.05 were retained in the models

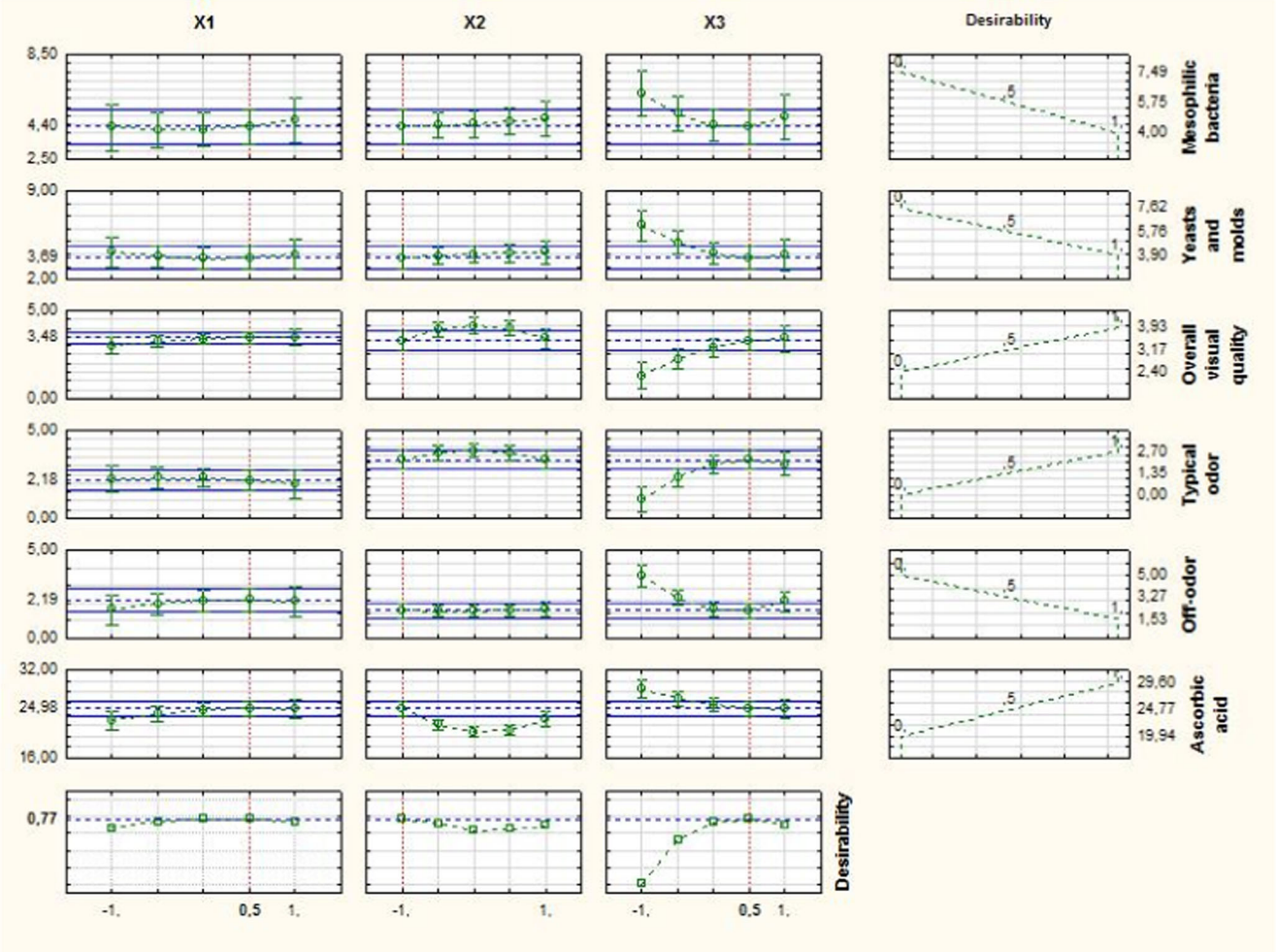

Fig. 1 Profiles for predicted values and desirability function

optimized treatment was not effective in reducing initial LAB counts since no differences with respect to control were observed (Fig. 2c). However, LAB counts of treated sample decreased (0.65 log CFU/mL) up to day 7 of storage and then increased 1 log CFU/mL after 2 weeks of storage. A significant increase was observed in the untreated sample reaching 9 log CFU/mL at the end of the storage. In the case of YM, no statistically significant changes in initial YM counts of treated sample were registered (Fig. 2d). During storage, YM counts of treated sample were significantly lower than those found in the untreated ones. At the end of the storage, noticeable reductions of 4 log CFU/mL in treated samples with respect to control were observed.

According to the Spanish regulation for hygienic processing, distribution, and commerce of prepared meals, the maximum limit of allowed mesophilic total count at expiry is 10^7 CFU/g (BOE 2001). Considering 10^7 CFU/mL as a maximum limit for all the studied populations, microbiological shelf life of untreated sample was 7 days, while the optimized treatment was able to extend its microbiological shelf life for at least seven more days.

Geraniol, as other terpenes, alters cell permeability by penetrating between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing, and changing membrane fluidity (Dalleau et al. 2008). This leads to potassium loss from within the microbial cells (Bard et al. 1988). The antifungal activity can be ascribed to the combined membrane effects such as increase bilayer disorder and ion leakage. These effects disturbed the osmotic balance of the cell through loss of ions, making its membrane-associated proteins inefficient due to increased membrane disorder eventually leading to inhibition of cell growth or death (Chen and Viljoen 2010). The effectiveness of geraniol in reducing native microflora of strawberry juices was studied in our previous researches (Cassani et al. 2016; Tomadoni et al. 2016). In those studies, significant reductions were observed at higher concentrations of geraniol (0.4, 0.6, and 1.2 μL/mL). In the present study, a lower concentration of the natural antimicrobial was also highly effective in reducing native microflora of juices.

Nutritional Quality

Figure 3 shows the evolution of ascorbic acid content of juice samples during storage. Immediately after treatment

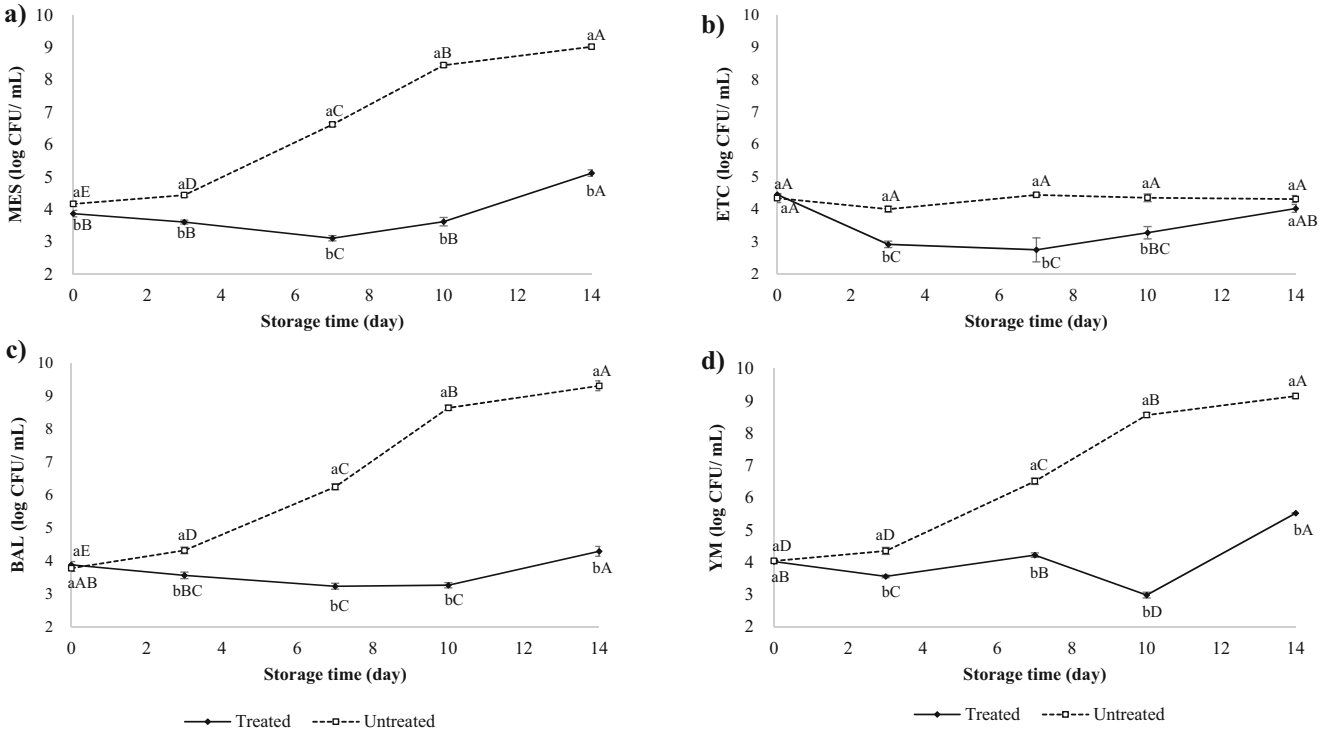

Fig. 2 Effect of optimized treatment on native microflora of strawberry juice during 14 days of storage at 5 °C. **a** Mesophilic bacteria. **b** *Enterobacteriaceae* and total coliform. **c** Lactic acid bacteria. **d** Yeasts and molds. Bars indicate standard error. Values with different lowercase letters (at a constant time) indicate significant differences ($p < 0.05$) between treatments and values with different capital letters (for the same sample) indicate significant differences ($p < 0.05$) through storage. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Untreated = strawberry juice without fiber nor treatments

application, a significant increase in AAC (4.5%, $p < 0.05$) was observed compared to control. Initial AAC was within the range observed in Aaby et al. (2007) and Tiwari et al. (2008), who reported 40.4 and 53.3 mg of AAC/100 g of fresh strawberries, respectively. Storage time had a significant effect in AAC of treated sample, suffering a significant reduction (losses of 12%) at day 3 of storage. Then, AAC was remained constant up to day 10 of storage, and after that, a significant diminution was registered. In contrast, no changes in AAC of control sample, up to day 10 of storage was observed, but from that time, a significant decrease in this parameter was registered, reaching a higher retention than treated sample at day 14 of storage. It is well known that vitamin C is a thermo-labile compound, very susceptible to chemical and enzymatic oxidation during processing and storage. External factors as storage temperature, light, and oxygen can accelerate ascorbic acid oxidative reactions. Possibly, geraniol treatment altered the cell membranes of the strawberry allowing the release of oxidative enzymes (i.e., ascorbic acid oxidase and peroxidase) and favoring contact with ascorbic acid. Therefore, ascorbic acid oxidative reactions could explain the decrease in AAC of the treated sample.

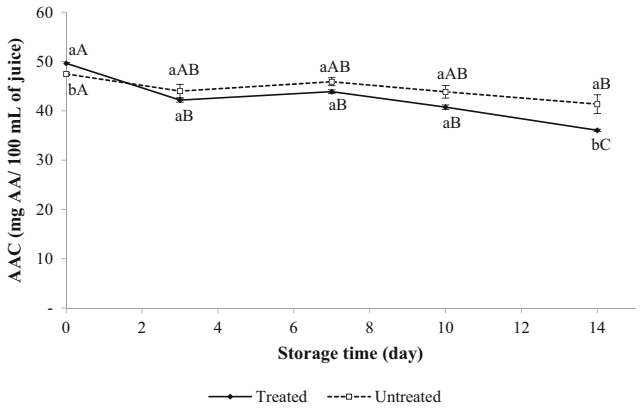

Fig. 3 Effect of optimized treatment on ascorbic acid content of strawberry juice during 14 days of storage at 5 °C. Bars indicate standard error. Values with different lowercase letters (at a constant time) indicate significant differences ($p < 0.05$) between treatments and values with different capital letters (for the same sample) indicate significant differences ($p < 0.05$) through storage. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Untreated = strawberry juice without fiber nor treatments

Antioxidant Capacity Indicators

Figure 4a–c displays total phenolic content, total antioxidant capacity measured by DPPH assay, and total flavonoid content of strawberry juice samples during storage. After treatment application, no effect on TPC of treated sample was observed since no significant differences with respect to control were found (Fig. 4a). These values were in the same range

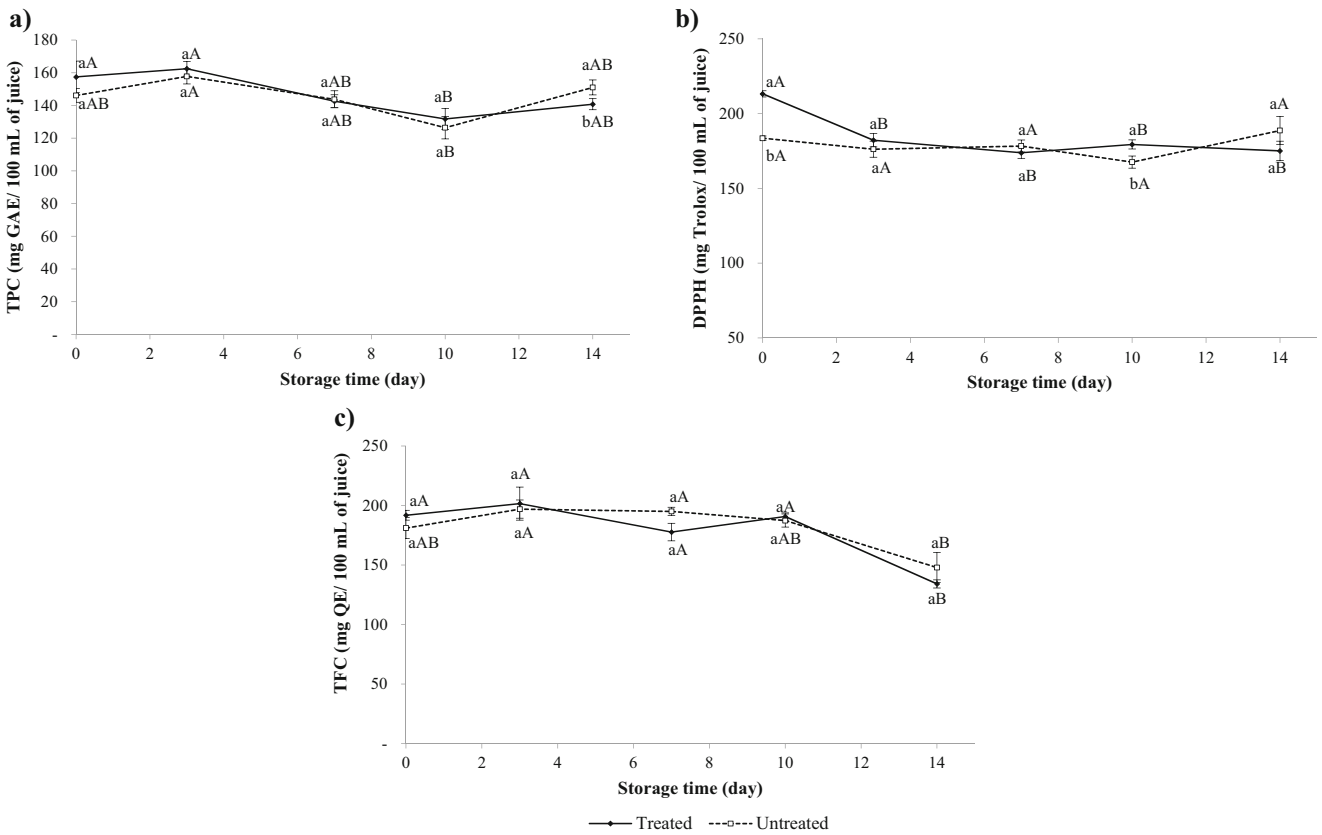

Fig. 4 Effect of optimized treatment on potential antioxidant capacity indicators of strawberry juice during 14 days of storage at 5 °C. **a** Total phenolic content. **b** DPPH radical scavenging activity. **c** Total flavonoid content. Bars indicate standard error. Values with different lowercase letters (at a constant time) indicate significant differences ($p < 0.05$) between treatments and values with different capital letters (for the same sample) indicate significant differences ($p < 0.05$) through storage. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Untreated = strawberry juice without fiber nor treatments

with those reported by Aaby et al. (2007). Storage time had no impact on TPC of treated and control samples since their values did not statistically change along storage. However, at day 14, TPC of treated sample was significantly lower than that observed in the control one.

The major class of phenolic compounds in strawberry is represented by the flavonoids (anthocyanins as major components, being pelargonidin-3-glucoside the main anthocyanin found in strawberry, with cyanidin-3-glucoside and pelargonidin-3-rutinoside present as minor components), followed by hydrolyzable tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), with condensed tannins (proanthocyanidins) being the minor constituents (da Silva Pinto et al. 2008, Giampieri et al. 2012). Thus, when the Folin-Ciocalteu assay is used to determine the total phenolic compounds of a strawberry extract, all of those compounds previously mentioned, are measured. In addition, it is worth noting that other types of compounds that may be present in abundance in strawberries (i.e., ascorbic acid) can reduce the Folin-Ciocalteu reagent. Therefore, to explain the behavior of TPC during storage, it important to identify which compound is mainly affecting the measure. In this case, the significant decreased of TPC in treated sample at the end of storage can be attributed to the diminution of ascorbic acid, as it was shown in Fig. 3.

Figure 4b displays DPPH• radical scavenging activity of juice samples. Immediately after treatment application, the antioxidant capacity of treated sample was significantly higher ($p < 0.05$) than control. Ruberto and Baratta (2000) found that geraniol showed an appreciable antioxidant capacity due to the presence of allylic alcohols. Choi et al. (2000) also demonstrated that geraniol showed marked scavenging activities in DPPH assay (235.9 mg of Trolox equiv./mL). However, a significant decrease in this parameter of treated samples at day 3 of storage was observed; but from that day, the antioxidant capacity of treated sample was maintained constant. In contrast, storage time did not statistically affect the DPPH values of untreated samples.

The individual contribution of different phytochemical compounds in fruit is an important factor in determining their total antioxidant capacity (Giampieri et al. 2012). Tulipani et al. (2008) investigated the individual contribution of phytochemical compounds in different strawberry cultivars. These authors found that vitamin C was one of the most important components, responsible for more than 30% of the total antioxidant capacity of strawberry extracts, followed by

anthocyanin contributing 25 to 40%, and the rest was composed mainly of ellagic acid derivatives and flavonols. In the present study, a clear relationship can be observed between TAC measured by DPPH and ascorbic acid content (Fig. 3), indicating that ascorbic acid would represent the major contribution of TAC of strawberry juices treated at optimal conditions. On the other hand, a relationship was also detected between TAC and TPC (Fig. 4a), demonstrating that total phenolic compounds had a contribution of TAC.

Another possible explanation of reductions on DPPH values of treated samples during storage could be the transformation of geraniol into α-terpineol, which occurs in acid medium, showing a lower antioxidant capacity than others oxygenated monoterpenes (Choi et al. 2000).

Figure 4c displays TFC of strawberry juices during storage. The application of the optimized treatment was not detrimental for this parameter, since initial TFC of treated sample was similar to that registered in control. Up to day 10 of storage, no relevant changes in TFC of both treated and untreated samples were found. However, at the end of the evaluation period, a significant decrease in treated sample was registered. The decrease in this parameter could be attributed to the anthocyanin degradation. In general, several factors are believed to affect the stability of anthocyanin in fruits and their products during preparation, processing, and storage, which include pH, temperature, light, oxygen, metal ions, enzymes, and sugars (Patras et al. 2010). Thus, the degradation of anthocyanin in treated sample at day 14 of storage, could be related to the degradation of sugars and ascorbic acid (Fig. 3), which leads to the condensation of anthocyanin with the formation of brownish polymers (Patras et al. 2010). The reactivity of sugar degradation products with anthocyanin is higher due to the pH effect on stability of the flavylium form. Sugars such as fructose, which are relatively unstable, give a higher degradation rate with pelargonidin-3-glucoside than more stable disaccharides, such as maltose and sorbitol (Krifi and Metche 2000).

Sensory Quality

Figure 5 displays changes in sensory parameters (color, OVQ, odor, and acid and sweet taste) in treated and untreated samples at days 0, 7, and 14 of storage. The application of geraniol treatment significantly affected initial odor and sweet taste scores of fiber-enriched strawberry juices. Despite having oligofructose (a natural sweetener) in its composition, the low concentration of geraniol masked the sweet taste of juice samples. Panelists perceived an intense citric odor in those samples treated with the natural antimicrobial, which did not decrease along storage.

Immediately after treatment, overall visual quality scores of both treated and untreated samples were similar. During storage, OVQ scores of untreated samples significantly decreased, reaching the lowest values at the end of the experimental period. This decrease is attributed to the visible signs of fermentation

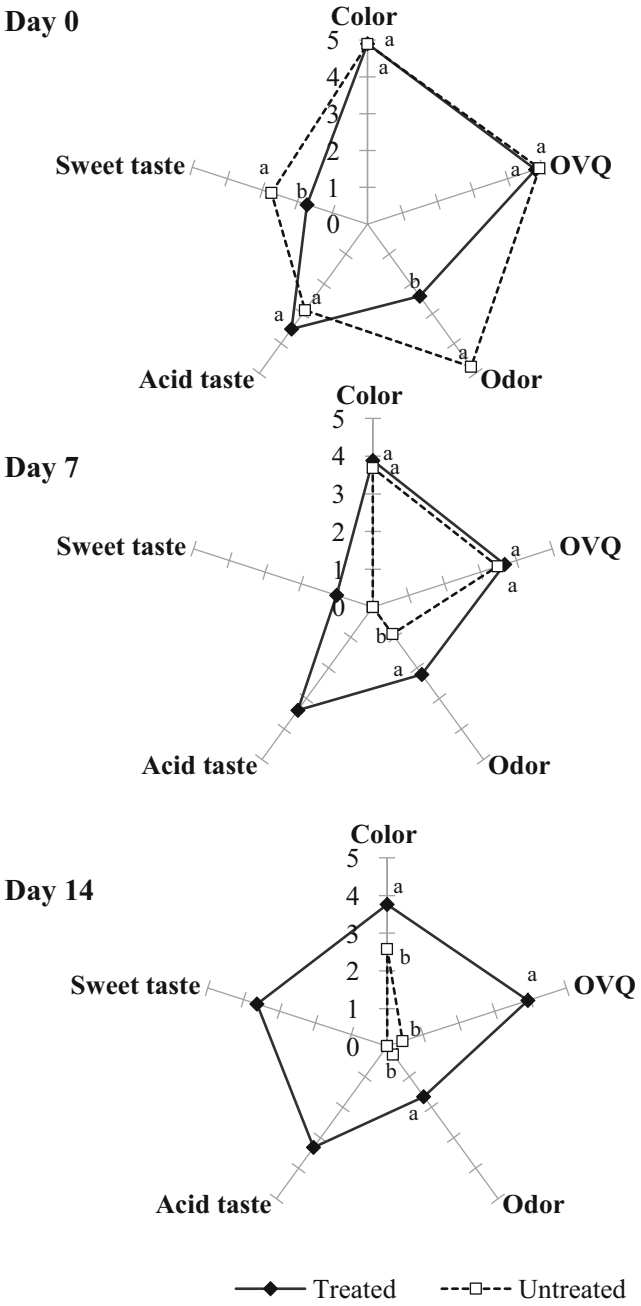

Fig. 5 Effect of optimized treatment on sensory attributes of strawberry juice at 0, 7, and 14 days of storage at 5 °C. Values with different lowercase letters (at a constant time) indicate significant differences ($p < 0.05$) between treatments. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Untreated = strawberry juice without fiber nor treatments

and microbial growth. On the other hand, a diminution in OVQ scores of treated samples was registered, but at a lower rate than the untreated ones, being above the acceptability limit (2.5) at day 14 of storage. In this case, no visible signs of fermentation or microbial growth were observed, which is consistent with the lower counts of yeasts and molds in these samples. However, a visible loss of viscosity in treated samples could be attributed to

the action of geraniol on pectins, possibly activating the action of pectin methylesterase and polygalacturonase enzymes, causing changes in the viscosity of juices. With respect to color, its decrease in treated and untreated samples during storage could be associated with the formation of dark color compounds in the juices due to the non-enzymatic browning reactions.

The optimized treatment significantly affected the initial odor scores of strawberry juices, since a noticeable diminution with respect to control was observed. This result was expected, as geraniol has characteristic rose-like odor and taste (at 10 ppm) which is described as sweet floral rose-like, citrus with fruity, waxy nuances (Chen and Viljoen 2010). During storage, odor scores decreased in treated sample at a lower rate than control. The marked decline in the untreated samples is attributed to the fermentation and microbial growth.

Regarding taste attributes, the optimized treatment significantly decreased sweet taste and increased acid taste of juices in comparison to control. Throughout storage, sweet taste scores gradually diminished in treated samples while acid taste was maintained constant. Taste attributes at 7 and 14 days of storage, in untreated samples, were not evaluated due to their high microbial load.

Performance of the Optimized Treatment against Pathogens Inoculated

Table 3 depicts the survival of *E. coli* O157H7 and *L. innocua* in inoculated strawberry juice samples during storage. Presence of endogenous *L. innocua* and *E. coli* in non-inoculated juices was studied during the whole storage and no colony of either pathogen was detected. Treatment at optimal conditions of processing and enrichment did not affect initial counts of the inoculated pathogens since no statistically significant differences with respect to control were registered. *E. coli* and *L. innocua* counts of untreated samples decreased along storage probably due to low storage temperature (5 °C), low pH (3.25), and competitive native microflora that, when combined, affected *E. coli* and *L. innocua* growth.

It is worth noting that reductions on these pathogens counts in treated samples were even greater than in control, reaching non-detectable levels (< 2 log CFU/mL) from day 7 to the end of the evaluation period. Thus, geraniol treatment was effective in controlling the growth of inoculated pathogens in strawberry juices.

Few researches have studied the impact of geraniol on pathogens inoculated in fruit juices. Raybaudi-Massilia et al. (2006) observed that the application of geraniol (at higher concentration 2 μL/mL) was effective in inhibiting the growth of *Salmonella enteritidis*, *E. coli*, and *L. innocua* in inoculated pear and apple juices. Tomadoni et al. (2016) found that high concentrations of geraniol (0.6 and 1.2 μL/mL) were sufficient to immediately reduce *E. coli* counts (ca. 3 log CFU/mL) with respect to control. In the present study, the concentration of geraniol was lower (0.225 μL/mL), thus it could not immediately inhibit *E. coli* or *L. innocua* growth, but probably exerted a deleterious effect on these microorganisms, leaving these populations more susceptible to other barriers such as low temperatures or low pH.

The efficiency of geraniol to inhibit *E. coli* O157:H7 growth can be attributed to permeability alteration of the outer membrane and alteration of cell membrane function and leakage of intracellular materials of pathogenic bacteria. In fact, Zengin and Baysal (2014) evaluated the antibacterial activity of essential oil terpenes against pathogenic bacteria by scanning electron microscope and found that treatment with terpenes caused pores on the outer membrane of *E. coli* O157:H7 cells, which enabled the cell constituents to pass easily through these and also caused collapsing of the cells.

Table 3 Effect of the optimized treatment on *E. coli* O157:H7 and *L. innocua* survival (log CFU/mL) of strawberry juices during 2 weeks of refrigerated storage

Microorganism	Sample	Time of storage (day)				
		0	3	7	10	14
E. coli O157:H7						
	Treated	5.24 ± 0.03aA	3.78 ± 0.13bB	ND	ND	ND
	Untreated	5.20 ± 0.07aA	5.04 ± 0.01aAB	4.85 ± 0.01aB	4.36 ± 0.06aC	4.12 ± 0.04aC
L. innocua						
	Treated	4.87 ± 0.07aA	3.02 ± 0.16bB	ND	ND	ND
	Untreated	4.94 ± 0.07aA	3.88 ± 0.10aB	2.60 ± 0.16aC	2.58 ± 0.11aC	2.50 ± 0.00aC

ND non-detectable level (< 2 log CFU/mL)

Data are shown as means values ± standard error. Values with different lowercase letter 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. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Untreated = strawberry juice without fiber nor treatments

Performance of the Optimized Treatment on Stability of Sugars and Prebiotics

The main carbohydrates present in strawberry juices are glucose, fructose, and sucrose (Table 4). Initial fructose and glucose content of treated sample were in the range of those observed in control. In turn, initial sucrose concentration was almost twofold higher in treated sample than in the untreated ones. The higher concentration of sucrose observed in treated sample can be ascribed to the higher concentration of sucrose in oligofructose and inulin (Cassani et al. 2018). During storage, the concentration of sucrose significantly decreased in both treated and untreated samples. However, in untreated sample, the diminution was more pronounced (88% in comparison to day 0) and the sucrose concentration decreased up to 4 mg/g dry matter. Along storage, the content of fructose and glucose were significantly higher in control samples than in the treated one, indicating that untreated juices suffered a marked hydrolysis of sucrose to form the monosaccharides. At the end of storage, a sharp decreased in fructose and glucose content of the control samples was observed but sucrose content remained constant. This can be interpreted that glucose and fructose were consumed in the yeasts and molds growth, taking into account that at that moment, the yeasts and molds counts were high (9 log CFU/mL) and high amounts of simple sugars were consumed to produce metabolites, such as ethanol, lactic, or acetic acids, as reported in Ragaert et al. (2006).

Table 5 displays the effect of the optimized treatment on the stability of oligofructose and inulin of juice samples during storage. A juice sample enriched with oligofructose and inulin without preservative treatment (enriched control) was employed in order to evaluate the impact of the preservative treatment on the stability of added prebiotics. The optimized treatment did not affect the initial prebiotic compounds' concentration of strawberry juices since no significant differences were observed in comparison to enriched control. During storage, the concentration of DP5 and inulin was significantly decreased in the enriched control, probably due to the consumption of carbohydrates during the growth of the main spoilage microflora (BAL and YM). On the contrary, the concentration of FOS and inulin of treated sample did not show significant changes up to 14 days of storage.

The chemical hydrolysis of FOS and inulin can easily occur at low pH matrices, such us fruit juices (pH < 5). Therefore, to be classified as functional food ingredients, these fibers must be chemically stable to food processing treatments, such as heat, low pH, and Maillard reaction conditions (Cassani et al. 2018). In this work, monitoring changes in prebiotics concentration during storage was an adequate strategy to determine if any partial hydrolysis occurred. It is worth mentioning that FOS and inulin added to those juices treated at optimal conditions were not degraded either during the storage or at the low pH of strawberry. Thus, these results demonstrated that the application of geraniol treatment ensured the stability of inulin and oligofructose during storage. These findings are consistent with Cassani et al. (2018) who reported no significant hydrolysis during storage of strawberry juices enriched with oligofructose and inulin and treated with vanillin and ultrasound.

In the literature, little information is available about changes in sugars composition or prebiotics stability of fruit juices due to preservation treatments and storage. In general, when a preservation technique is applied to a fruit juice, only microbiological, nutritional, and sensory parameters are evaluated. Here, in this study, we also investigated how the optimized treatment

Table 4 Effect of the optimized treatment on the carbohydrates composition (mg/g dry matter) of strawberry juices during 2 weeks of storage at 5 °C

Carbohydrate	Sample	Time of storage (day)				
		0	3	7	10	14
Sucrose						
	Treated	60.03 ± 3.62aA	19.60 ± 2.54aB	16.58 ± 2.60aB	21.34 ± 1.79aB	17.87 ± 2.66aB
	Untreated	35.31 ± 0.36bA	4.29 ± 0.00bB	4.09 ± 0.01bB	4.27 ± 0.20bB	4.08 ± 0.07bB
Glucose						
	Treated	188.37 ± 4.26aAB	200.18 ± 0.51bA	194.10 ± 3.82bA	152.17 ± 0.54bC	170.84 ± 4.44aB
	Untreated	184.99 ± 1.61aB	235.62 ± 0.86aA	250.27 ± 5.10aA	248.44 ± 1.05aA	55.09 ± .34bC
Fructose						
	Treated	200.36 ± 0.12aB	220.50 ± 1.97bA	214.68 ± 0.33bA	168.25 ± 0.21bD	191.82 ± 2.36aC
	Untreated	192.43 ± 1.16bC	253.20 ± 1.70aAB	251.53 ± 0.02aB	263.15 ± 0.02aA	66.99 ± 2.45bD

Data are shown as means values ± standard error. Values with different lowercase 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. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Untreated = strawberry juice without fiber nor treatments

Table 5 Effect of the optimized treatment on the inulin and fructo-oligosaccharides concentrations (mg/g dry matter) of strawberry juices during 2 weeks of storage at 5 °C

Prebiotic compound	Sample	Time of storage (day)				
		0	3	7	10	14
DP3						
	Treated	4.30 ± 0.00aA	3.45 ± 0.31aAB	3.37 ± 0.30aAB	3.02 ± 0.09aB	3.32 ± 0.07aAB
	Enriched control	4.27 ± 0.07aA	4.09 ± 0.33aA	2.97 ± 0.14aA	2.98 ± 0.35aA	3.59 ± 0.17aA
DP4						
	Treated	3.43 ± 0.24aA	4.01 ± 0.71aA	3.64 ± 0.59aA	2.42 ± 0.18aA	3.34 ± 0.64aA
	Enriched control	3.48 ± 0.29aA	3.00 ± 0.05aAB	1.97 ± 0.04bB	3.04 ± 0.34aAB	3.26 ± 0.16aA
DP5						
	Treated	3.17 ± 1.13aA	4.18 ± 0.35aA	4.20 ± 0.59aA	4.26 ± 0.37aA	4.80 ± 0.08aA
	Enriched control	2.53 ± 0.29aB	3.79 ± 0.19aB	5.49 ± 0.30aA	2.29 ± 0.14bB	2.20 ± 0.48bB
Inulin						
	Treated	61.25 ± 12.97aA	ND	62.50 ± 7.50aA	ND	50.00 ± 2.50aA
	Enriched control	47.50 ± 2.50aA	ND	46.66 ± 3.33aA	ND	25.41 ± 3.14bB

DP degree of polymerization, *DP3* 1-kestose, *DP4* nystose, *DP5* 1^F-fructofuranosyl nystose. These prebiotic compounds were not evaluated in those juices samples without fiber addition and preservative treatments. *ND* not determined

Data are shown as means values ± standard error. Values with different lowercase 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. Treated = strawberry juice sample treated according to optimal conditions of processing and enrichment found in RSM study. Enriched control = strawberry juice enriched with the optimal proportion of inulin/oligofructose without any preservative treatment

together with storage time affected the sugars composition and prebiotics stability of strawberry juice. This studied provided valuable information, which can be related to the observed changes in taste attributes of treated strawberry juice. On the other hand, it is of paramount importance to investigate the stability of prebiotics added to fruit juice during storage to ensure their functional properties over the storage.

Conclusion

Combining preservation treatment (geraniol) with prebiotics (inulin and oligofructose) using a response surface methodology showed to be an efficient strategy to control the native microflora, as well as, to inhibit inoculated pathogens in strawberry juice during 2 weeks of refrigerated storage, extending its shelf life from a microbiological point of view. Furthermore, the optimized treatment did not induce any negative effect either on antioxidant capacity indicators or ascorbic acid content along storage, improving their nutritional quality. In addition, the optimized treatment proved to ensure the stability of those added prebiotics to juices, ensuring their functional properties over the storage. The optimized treatment induced some changes in sensory parameters, mainly in odor and taste attributes, which were maintained almost constant throughout storage. In conclusion, on the one hand, enriching strawberry juices with prebiotics offers a broad range to increase the nutritional properties of foods to achieve a new range of functional and innovative food products, and on the other hand, preserving with natural antimicrobials offers a new alternative to conventional preservative treatment currently used in fruit juice industry.

Funding Information This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and Universidad Nacional de Mar del Plata (UNMDP).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

References

Aaby, K., Wrolstad, R. E., Ekeberg, D., & Skrede, G. (2007). Polyphenol composition and antioxidant activity in strawberry purees; impact of achene level and storage. *Journal of Agricultural and Food Chemistry, 55*(13), 5156–5166.

Aday, M. S., Temizkan, R., Büyükcan, M. B., & Caner, C. (2013). An innovative technique for extending shelf life of strawberry: ultrasound. *LWT-Food Science and Technology, 52*(2), 93–101.

Bard, M., Albrecht, M. R., Gupta, N., Guynn, C. J., & Stillwell, W. (1988). Geraniol interferes with membrane functions in strains of *Candida* and *Saccharomyces*. *Lipids, 23*(6), 534–538.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76*(5), 965–977.

Boletin Oficial del Estado, BOE. (2001). Normas de higiene para la elaboraciòn, distribución y comercio de comidas preparadas, Madrid, Spain. *Real Decreto, 3484*(/2000), 1435–1441.

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

Cassani, L., Quintana, G., Moreira, M. R., & Gómez-Zavaglia, A. (2018). Relationship between carbohydrate composition and fungal deterioration of functional strawberry juices preserved using non-thermal treatments. *Journal of the Science of Food and Agriculture., 98*(9), 3271–3279.

Chen, W., & Viljoen, A. (2010). Geraniol—a review of a commercially important fragrance material. *South African Journal of Botany, 76*(4), 643–651.

Choi, H.-S., Song, H. S., Ukeda, H., & Sawamura, M. (2000). Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *Journal of Agricultural and Food Chemistry, 48*(9), 4156–4161.

Dalleau, S., Cateau, E., Bergès, T., Berjeaud, J.-M., & Imbert, C. (2008). *In vitro* activity of terpenes against *Candida* biofilms. *International Journal of Antimicrobial Agents, 31*(6), 572–576.

da Silva Pinto, M., Lajolo, F. M., & Genovese, M. I. (2008). Bioactive compounds and quantification of total ellagic acid in strawberries (*Fragaria* x *ananassa* Duch.). *Food Chemistry., 107*(4), 1629–1635.

Franck, A. (2002). Technological functionality of inulin and oligofructose. *British Journal of Nutrition, 87*(S2), S287–S291.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., & Battino, M. (2012). The strawberry: composition, nutritional quality, and impact on human health. *Nutrition, 28*(1), 9–19.

Goyeneche, R., Roura, S., Ponce, A., Vega-Gálvez, A., Quispe-Fuentes, I., Uribe, E., & Di Scala, K. (2015). Chemical characterization and antioxidant capacity of red radish (*Raphanus sativus L.*) leaves and roots. *Journal of Functional Foods, 16*, 256–264.

Keenan, D. F., Brunton, N., Butler, F., Wouters, R., & Gormley, R. (2011). Evaluation of thermal and high hydrostatic pressure processed apple purees enriched with prebiotic inclusions. *Innovative Food Science & Emerging Technologies, 12*(3), 261–268.

Krifi, B., & Metche, M. (2000). Degradation of anthocyanins from blood orange juices. *International Journal of Food Science & Technology, 35*(3), 275–283.

Kuehl, R. O. (2000). *Designs of experiments: Statistical principles of research design and analysis*. Duxbury Press.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2008). Non-thermal pasteurization of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. *Innovative Food Science & Emerging Technologies, 9*(3), 328–340.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2012). Microbiological shelf life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. *Food and Bioproducts Processing, 90*(2), 205–214.

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology, 21*(1), 3–11.

Ponce, A., Agüero, M., Roura, S., Del Valle, C., & Moreira, M. (2008). Dynamics of indigenous microbial populations of butter head lettuce grown in mulch and on bare soil. *Journal of Food Science, 73*(6), M257–M263.

Prasad, S. N., & Muralidhara, M. (2017). Analysis of the antioxidant activity of geraniol employing various in vitro models: relevance to neurodegeneration in diabetic neuropathy. *Asian Journal of Pharmaceutical and Clinical Research, 10*(7), 101. 10.22159/ajpcr.2017.v10i7.18564.

R Development Core Team. (2011). R: A language and environment for statistical computing. Vienna: the R Foundation for Statistical Computing.

Ragaert, P., Devlieghere, F., Loos, S., Dewulf, J., Van Langenhove, H., & Debevere, J. (2006). Metabolite production of yeasts on a strawberry-agar during storage at 7 C in air and low oxygen atmosphere. *Food Microbiology, 23*(2), 154–161.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Martin-Belloso, O. (2006). Antimicrobial activity of essential oils on *Salmonella enteritidis*, *Escherichia coli*, and *Listeria innocua* in fruit juices. *Journal of Food Protection, 69*(7), 1579–1586.

Ruberto, G., & Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry, 69*(2), 167–174.

Tiwari, B. K., O Donnell, C. P., Patras, A., & Cullen, P. J. (2008). Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *Journal of Agricultural and Food Chemistry, 56*(21), 10071–10077.

Tomadoni, B., Cassani, L., Viacava, G., Moreira, M. D. R., & Ponce, A. (2017). Effect of ultrasound and storage time on quality attributes of strawberry juice. *Journal of Food Process Engineering, 40*(5).

Tomadoni, B., Viacava, G., Cassani, L., Moreira, M., & Ponce, A. (2016). Novel biopreservatives to enhance the safety and quality of strawberry juice. *Journal of Food Science and Technology, 53*(1), 281–292.

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., De Vos, C. R., et al. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry, 56*(3), 696–704.

Viacava, G. E., & Roura, S. I. (2015). Principal component and hierarchical cluster analysis to select natural elicitors for enhancing phytochemical content and antioxidant activity of lettuce sprouts. *Scientia Horticulturae, 193*, 13–21.

Viacava, G. E., Roura, S. I., & Agüero, M. V. (2015). Optimization of critical parameters during antioxidants extraction from butterhead lettuce to simultaneously enhance polyphenols and antioxidant activity. *Chemometrics and Intelligent Laboratory Systems, 146*, 47–54.

Zengin, H., & Baysal, A. H. (2014). Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules, 19*(11), 17773–17798.

Zuleta, A., & Sambucetti, M. E. (2001). Inulin determination for food labeling. *Journal of Agricultural and Food Chemistry, 49*(10), 4570–4572.