LWT - Food Science and Technology 72 (2016) 90–98

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Enhancing quality attributes of fiber-enriched strawberry juice by application of vanillin or geraniol

L. Cassani [a, c, *], B. Tomadoni [a, b], G. Viacava [a, b], A. Ponce [a, b], M.R. Moreira [a, b]

[a] Grupo de Investigación en Ingeniería en Alimentos, Facultad Ingeniería, UNMdP, J.B. Justo 4302, Mar del Plata 7600, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
19 April 2016
Accepted 21 April 2016
Available online 22 April 2016

Keywords:
Natural preservatives
Quality parameters
Strawberry juice
Dietary fibers

ABSTRACT

In this work, the impact of adding prebiotic fibers (inulin, oligofructose and apple fiber) to strawberry juices on quality properties was investigated. Additionally, the beneficial effects of natural preservatives (vanillin and geraniol) on quality parameters of fiber-enriched juices were studied. Microbiological, physicochemical and sensorial properties were evaluated throughout two weeks of storage at 5 °C. Results showed that the addition of prebiotic fibers significantly increased antioxidant capacity and successfully maintained sensory quality of strawberry juice when compared to fresh control. Vanillin and geraniol treatments had a marked effect in reducing native microflora counts (4–6 log cycles reductions) in comparison to untreated samples. Natural preservatives imparted strong flavor in fiber-enriched strawberry juices, however, it was pleasant for those samples treated with vanillin. Besides, overall visual quality scores in treated samples were maintained in the acceptability level throughout storage. Juices with vanillin showed the highest total phenolic content, indicating high nutritional value of the product. Nevertheless, both preservatives agents exhibited lower antioxidant activity than controls. Thus, prebiotic fibers could enhance nutritional and sensory quality of strawberry juice. Also, geraniol and vanillin could be feasible alternatives to improve microbiological quality with low impact on the organoleptic properties of fiber-enriched strawberry juice.

1. Introduction

Currently, different types of dietary fibers such as pear, apple and citrus fibers, as well as inulin and gums, are regularly incorporated into different foods to improve their nutritional, functional and/or technological properties. In food formulations, inulin and oligofructose may significantly improve organoleptic characteristics of the product (Franck, 2002).

For many years, citrus and apple juices have dominated the fruit juice market, but within the past decade, strawberry juices gained popularity. Strawberry juice is a good source of health related compounds such as anthocyanin and vitamin C, thus having high antioxidant activities (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009). In today's juice industry, pasteurization is one of the dominant preservation methods of eliminating pathogens from juices. Although pasteurization can lead to desirable destruction of microbial pathogens and spoilage organisms, undesirable changes also occur, such as loss of vitamins and minerals and loss of fresh color and flavor of the fruit juices. To facilitate the preservation of thermolabile nutrients many juice processors required more investigation about new alternatives to thermal treatment (Burt, 2004; Duan & Zhao, 2009).

Nowadays, consumers are looking for safer, fresher and healthier food with no chemicals addition and fewer synthetic preservatives. Hence, there is a niche for effective natural antimicrobials and inexpensive non-thermal intervention technologies that can ensure the microbial safety of strawberry juices while fulfilling today's market demand (Duan & Zhao, 2009). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major constituent of vanilla beans and has been reported to act as an antioxidant in complex foods containing polyunsaturated fatty acids. Moreover, some reports have shown that vanillin can be effective in inhibiting yeasts and molds growth when added in fruit purees and fruit based agar systems (Fitzgerald, Stratford, & Narbad, 2003). Geraniol

* Corresponding author. Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, UNMdP, J.B.Justo 4302, Mar del Plata 7600, Argentina. Tel.: +54 223 481 6600.
E-mail address: lcassani@fi.mdp.edu.ar (L. Cassani).

http://dx.doi.org/10.1016/j.lwt.2016.04.037
0023-6438/

is a commercially important terpene alcohol (3,7-dimethylocta-trans-2,6-dien-1-ol) occurring in the essential oils of several aromatic plants. It is considered as Generally Recognized as Safe (GRAS) according to Food Additive Status List (USFDA, 2006) and is used in the food industry as preservative, antioxidant and flavoring agent (Burt, 2004; Chen & Viljoen, 2010).

As far as we know, the addition of vanillin and geraniol to fiber-enriched strawberry juices for maintaining their quality properties has not been studied. Therefore, the aims of this study were: (a) to evaluate the effect of adding prebiotic fibers on quality attributes of strawberry juice; (b) to analyze the impact of applying natural preservatives on microbiological, physicochemical and sensorial properties of fiber-enriched strawberry juices.

2. Materials and methods

2.1. Preparation of strawberry juice and treatments

Strawberries (*Fragaria x ananassa*) at commercial maturity were obtained from a local producer in Mar del Plata, Argentina. The fruits were washed, drained and squeezed by a commercial juice extractor. Strawberry juice was bottled under hygienic conditions in 350 mL PET bottles and sealed with PE caps.

Different prebiotic fibers were incorporated into strawberry juice samples: inulin (Saporiti, Argentina) at 1.50 g/100 mL; oligofructose (Saporiti, Argentina) at 1.50 g/100 mL and apple fiber (Indulleida, Spain) at 0.75 g/100 mL. The amount of fiber was selected following the requirements of The Code of Federal Regulations (Title 21, Part 101.54). To each of these enriched juice samples, vanillin and geraniol were independently added in concentrations previously determined based on the work of Tomadoni, Viacava, Cassani, Moreira, and Ponce (2016) and preliminary experiments carried out in the laboratory (results not shown). For this purpose, vanillin (Firmenich SAICYF, Buenos Aires, Argentina) was applied directly to the enriched juice sample at 0.18 g/100 mL. On the other hand, geraniol (Firmenich SAICYF, Buenos Aires, Argentina) was added at 0.04 mL/100 mL.

Four juice samples were used as control in order to evaluate the effect of adding prebiotic fibers into fresh strawberry juice: (1) untreated strawberry juice; (2) strawberry juice sample enriched with inulin with no treatments application; (3) strawberry juice sample enriched with oligofructose with no applied treatments; (4) strawberry juice enriched with apple fiber without treatments. Along this work, juice samples (2), (3) and (4) will be referred to as "fiber-enriched controls" in order to differentiate them from untreated strawberry juice.

After processing, strawberry juice samples were stored at 5 °C. The evolution of microbial populations, total phenolic content, antioxidant capacity, color parameters, total soluble solids, total acidity and sensory evaluation were evaluated at 0, 3, 7, 10 and 14 days of storage.

All assays were carried out by triplicate in two independent experimental runs.

2.2. Extraction of antioxidants

Two mL of strawberry juice from each treatment was homogenized with 10 mL ethanol (80% v/v) and 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 for the determination of total phenolic content (TPC) and antioxidant activity by DPPH method.

2.2.1. Total phenolic content

TPC was determined spectrophotometrically using the Folin–Ciocalteu reagent (FCR) according to the methodology proposed by Viacava and Roura (2015) with modifications. Proper diluted samples 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 were expressed as mg gallic acid equivalents (GAE)/100 mL of juice.

2.2.2. Determination of DPPH radical scavenging activity

The antioxidant capacity was studied by evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the method described by Viacava and Roura (2015). 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 activity was expressed as the inhibition percentage of the DPPH radical.

2.3. Microbiological analysis

The microbial stability of strawberry juices was evaluated through the determination of total aerobic mesophilic bacteria (MES), psychrophilic bacteria (PSY) and yeast and molds (YM) populations. Ten mL of juice from each treatment was sampled at different times of refrigerated storage (0, 3, 7, 10, 14 days). Serial dilutions (1:10) of each sample were made in peptonated water (0.1% w/v) and surface spread by duplicate. The enumeration of the microbial populations was performed according to Ponce, Agüero, Roura, Del Valle, and Moreira (2008) by using the following culture media and culture conditions: MES on Plate Count Agar (PCA) incubated at 35 °C for 48 h; PSY on PCA incubated at 7 °C for 7 d; YM on Yeast-Glucose-Chloramphenicol (YGC) medium incubated at 25 °C for 5 d. All culture mediums were purchased from Britania, Argentina. Microbial counts were expressed as log CFU/mL.

2.4. Total soluble solids (TSS)

Brix (total soluble solid content (g/100 g)) was measured using an Atago refractometer (Abbe 1T74T, Tokio, Japón).

2.5. Titratable acidity (TA)

For determination of titratable acidity, a known volume of each sample was placed in a 250 mL beaker and 50 mL of distilled water were added. Further, this solution was titrated against standardized 0.1 N NaOH to the phenolphthalein end point (pH 8.2 ± 0.1). The volume of NaOH was converted to grams of citric acid per 100 mL of juice (% TA) based on the method of Sadler and Murphy (2010) and the total acidity was calculated using equation (1):

$$\%TA = \frac{V \times 0.1N\ NaOH \times 0.064 \times 100}{V_s} \quad (1)$$

where V is titer volume of NaOH and Vs is the volume of the strawberry juice sample.

2.6. Color evaluation

Color determination was carried out using a LoviBond colorimeter RT500 (Neu-Isenburg, Germany) with an 8 mm diameter measuring area, calibrated with a standard white plate (Y = 93.2, x = 0.3133, y = 0.3192). Color of strawberry juice was measured using the CIE L*, a*, b* coordinates. Hue angle (h°) was calculated according to equation (2).

$$h^{\circ} = arctg\left(\frac{b*}{a*}\right) \tag{2}$$

2.7. Sensory evaluation

Quantitative descriptive analysis was used to evaluate sensory attributes of strawberry juice samples at 0 and 7 days of storage. At each storage time, treated and untreated strawberry juices with and without fibers addition were subjected to a panel of testers to evaluate the sensory quality of the beverages. Ten judges, aged 25–50 years, with sensory evaluation experience were trained in descriptive evaluation of strawberry juices. The attributes evaluated were: color, odor, acid and sweet taste and overall visual quality (OVQ) of the beverages. Evaluations were performed immediately after juices removal from storage conditions. The coded (three-digit) samples were presented one at a time in random order to the panel members, who sat at a round table and made independent evaluations. The intensity of the attributes evaluated was quantified on an unstructured scale from 0 to 5. OVQ was scored from 0 (highly deteriorated aspect) to 5 (fresh aspect). Color was rated from 0 (deteriorated color) to 5 (typical color), odor from 0 (intense off-odors) to 5 (fresh) and sweet and acid taste from 0 (extremely dislike) to 5 (extremely like). The limit of acceptance was 2.5, indicating that score below 2.5 for any of the attributes evaluated was deemed to indicate end of shelf-life (Alvarez, Ponce, & Moreira, 2013).

2.8. Statistical analysis

A completely randomized design was used. Two independent runs were performed. Results reported in this work are mean values accompanied by their standard errors. Analysis of variance ANOVA ($p < 0.05$) was performed and Tukey–Kramer comparison test was used to estimate significant differences between treatments and through storage time ($p < 0.05$).

3. Results and discussion

3.1. Microbiological evaluation in strawberry juice samples

The causal agents of microbiological spoilage in fruits and derivatives can be bacteria, as well as yeasts and molds. The latter are considered the main spoilage agents due to the low pH of most fruits (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2009).

Mesophilic microorganisms give an estimate of total viable populations and are indicative of the endogenous microflora and the contamination undergone by the material (Ponce et al., 2008). Table 1 shows the evolution of mesophilic aerobic bacteria in treated and untreated strawberry juice samples. The addition of prebiotic fibers in the formulations did not show significant differences on the initial MES counts compared to untreated control. Immediately after treatment application (day 0), MES counts on enriched samples containing vanillin did not show significant differences with their respective controls. On the contrary, the application of geraniol reduced initial MES counts (~1 log cycle) in inulin-enriched sample in comparison to control. During the first seven days of storage, enriched samples treated with both natural preservatives decreased their MES counts below detection limit (<2.00 logs), while MES population in untreated sample increased, reaching 6 log cycles. Fiber-enriched controls exhibited significant differences with respect to untreated juice with reductions of 2 logs cycles in their MES loads. By the end of storage period, MES counts of control samples were increased, while enriched samples treated with both natural preservatives had a marked effect in reducing MES counts in approximately 4 log cycles in comparison to controls.

Psychrophilic bacteria represent an important group of microorganisms in fresh products. Although they may constitute a small percentage of the initial microflora, they could predominate at chill temperatures recommended for the storage of these commodities (Ponce et al. 2008). The growth of psychrophilic microorganisms on strawberry juice samples is shown in Table 2. The prebiotic fibers added to strawberry juice showed no significant impact on the microbial load of the product, with no significant differences to those found in untreated sample. In line with the results reported for MES counts, the initial PSY counts on samples treated with vanillin did not show significant differences with their respective fiber-enriched controls. With regards to the impact of natural preservative agents on PSY, geraniol was found to be more effective than vanillin in reducing initial PSY counts (~1 log cycle) in oligofructose-enriched sample compared to control. At day 3, the addition of both biopreservatives had a marked impact in reducing and maintaining PSY counts of enriched samples below detection limit (<2.00 logs) throughout storage. On the contrary, PSY of control samples raised progressively as storage time increased, reaching counts as high as 8 log cycles at the end of the experiment. According to the Spanish Regulation, 7.00 log CFU/mL is the maximum limit of microorganisms (at expiry date) in minimally processed foods (BOE, 2001). Therefore, from the 10th day of storage, samples without a preservative treatment would not be commercially accepted, while enriched samples treated with both natural preservatives agents would still be safe for consumption, increasing the juices shelf-life for at least 4 days.

Changes in yeast and mold loads on strawberry juice samples throughout refrigerated storage are shown in Table 3. The addition of prebiotic fibers into the formulation did not produce any differences on YM counts in comparison to untreated strawberry juice. No significant differences were observed in the initial YM counts between samples treated with both preservatives agents and their respective fiber-enriched controls. A storage time effect was observed in samples treated with geraniol and vanillin with a significant reduction of approximately 1 log cycle from day 0 to day 7 of storage. Furthermore, at day 7, significant differences (2–3 log cycles) were observed between treated samples and their respective controls. At the end of storage, the effectiveness of both biopreservatives in reducing YM proliferation on fiber-enriched strawberry juice samples was maintained. However, control samples increased their YM counts until 8 log cycles. These values were higher than the maximum limit of microorganisms (at expiry date) in minimally processed foods established by Spanish Regulation. Because of this, only enriched strawberry juices treated with vanillin or geraniol would still be safe for consumption at day 14.

According to Raybaudi-Massilia et al. (2009), the inhibitory activity of natural antimicrobials resides primarily in their ability to negatively affect the integrity of the cytoplasmic membrane, with loss of ion gradients, pH homeostasis, and inhibition of respiratory activity. Microbiological studies have been carried out on related food products such as strawberry puree and melon containing some natural antimicrobials. In agreement with our results,

Table 1
Mesophilic aerobic bacteria evolution in strawberry juice samples during 14 days of storage at 5 °C.

Treatments	Microbial fraction* (log CFU/mL)				
	Storage time (days)				
	0	3	7	10	14
UC	3.76 ± 0.06^{aB}	5.16 ± 0.08^{aAB}	6.61 ± 0.21^{aA}	6.51 ± 0.37^{aA}	6.66 ± 0.14^{aA}
IC	3.62 ± 0.07^{abC}	4.39 ± 0.30^{abBC}	4.20 ± 0.50^{bC}	5.78 ± 0.24^{abAB}	6.65 ± 0.05^{aA}
AC	3.62 ± 0.07^{abC}	4.46 ± 0.49^{abBC}	4.24 ± 0.06^{bBC}	5.85 ± 0.35^{abAB}	6.70 ± 0.07^{aA}
OC	3.56 ± 0.13^{abB}	5.06 ± 0.06^{aAB}	4.05 ± 0.35^{bB}	6.03 ± 0.31^{abA}	6.50 ± 0.10^{aA}
IG	2.70 ± 0.00^{cA}	3.13 ± 0.29^{bcA}	ND	3.70 ± 0.00^{bcA}	3.70 ± 0.00^{bA}
AG	3.00 ± 0.00^{abcA}	3.00 ± 0.17^{bcA}	ND	ND	ND
OG	2.85 ± 0.15^{bcA}	2.70 ± 0.00^{cA}	ND	5.24 ± 0.00^{abcA}	3.15 ± 0.15^{bcA}
IV	3.43 ± 0.13^{abcA}	2.70 ± 0.00^{cA}	ND	3.40 ± 0.00^{cA}	2.94 ± 0.24^{bcA}
AV	3.36 ± 0.20^{abcA}	3.43 ± 0.43^{bcA}	ND	ND	2.70 ± 0.00^{cA}
OV	3.39 ± 0.21^{abcA}	3.36 ± 0.33^{bcA}	ND	ND	ND

*Data is shown as means of 3 determinations ± standard deviation. Values with different letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **ND**: non-detectable level (<2.00 log CFU/mL). **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

Table 2
Psychrophilic bacteria evolution in strawberry juice samples during 14 days of storage at 5 °C.

Treatments	Microbial fraction* (log CFU/mL)				
	Storage time (days)				
	0	3	7	10	14
UC	4.16 ± 0.08^{aB}	4.23 ± 0.29^{aB}	5.84 ± 0.26^{aB}	8.70 ± 0.00^{aA}	8.52 ± 0.18^{aA}
IC	3.98 ± 0.17^{aB}	3.20 ± 0.50^{aB}	4.84 ± 0.16^{aB}	6.98 ± 0.21^{bA}	7.96 ± 0.14^{aA}
AC	3.90 ± 0.10^{abC}	3.35 ± 0.35^{aC}	5.79 ± 0.25^{aB}	6.83 ± 0.21^{bAB}	8.91 ± 0.39^{aA}
OC	3.98 ± 0.18^{abC}	3.20 ± 0.50^{aC}	5.21 ± 0.26^{aBC}	7.00 ± 0.17^{bAB}	7.89 ± 0.19^{aA}
IG	3.40 ± 0.00^{abcA}	ND	ND	ND	ND
AG	3.36 ± 0.20^{abcA}	ND	ND	ND	ND
OG	2.85 ± 0.15^{cA}	ND	ND	ND	ND
IV	3.71 ± 0.14^{abA}	ND	ND	ND	ND
AV	3.36 ± 0.20^{abcA}	2.70 ± 0.00^{aB}	ND	ND	ND
OV	3.39 ± 0.21^{bcA}	ND	ND	ND	ND

*Data is shown as means of 3 determinations ± standard deviation. Values with different letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **ND**: non-detectable level (<2.00 log CFU/mL). **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

Table 3
Yeasts and molds evolution in strawberry juice samples during 14 days of storage at 5 °C.

Treatments	Microbial fraction* (log CFU/mL)				
	Storage time (days)				
	0	3	7	10	14
UC	4.05 ± 0.05^{aC}	3.92 ± 0.07^{aC}	5.21 ± 0.02^{aBC}	7.24 ± 0.36^{aAB}	8.06 ± 0.30^{aA}
IC	3.89 ± 0.04^{abC}	3.70 ± 0.00^{abcC}	4.91 ± 0.02^{aBC}	6.81 ± 0.18^{aAB}	7.55 ± 0.53^{aA}
AC	3.97 ± 0.03^{aC}	3.80 ± 0.10^{abC}	5.41 ± 0.01^{aBC}	7.10 ± 0.31^{aAB}	8.76 ± 0.10^{aA}
OC	3.81 ± 0.03^{abC}	3.54 ± 0.24^{abcdC}	4.19 ± 0.08^{bBC}	7.13 ± 0.28^{aAB}	8.07 ± 0.07^{aA}
IG	3.30 ± 0.30^{bA}	2.85 ± 0.15^{cdA}	2.00 ± 0.00^{cA}	2.78 ± 0.00^{bA}	ND
AG	3.84 ± 0.01^{abA}	2.70 ± 0.00^{dA}	2.30 ± 0.00^{cA}	2.70 ± 0.00^{bA}	2.15 ± 0.15^{bA}
OG	3.57 ± 0.09^{abA}	3.00 ± 0.30^{bcdA}	2.00 ± 0.00^{cA}	2.00 ± 0.00^{bA}	2.00 ± 0.00^{bA}
IV	3.86 ± 0.08^{aA}	2.70 ± 0.00^{dAB}	2.60 ± 0.00^{cAB}	2.30 ± 0.30^{bAB}	2.00 ± 0.00^{bB}
AV	3.86 ± 0.04^{abA}	3.24 ± 0.24^{abcdAB}	ND	2.00 ± 0.00^{bB}	2.00 ± 0.00^{bB}
OV	3.92 ± 0.02^{abA}	3.45 ± 0.15^{abcdA}	2.15 ± 0.15^{cB}	2.00 ± 0.00^{bB}	ND

*Data is shown as means of 3 determinations ± standard deviation. Values with different letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **ND**: non-detectable level (<2.00 log CFU/mL). **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

Rupasinghe, Boulter-Bitzer, Ahn, and Odumeru (2006) demonstrated that the incorporation of vanillin (0.18 g/100 mL) into dipping treatments inhibited microbial growth on “Empire” and “Crispin” apple slices during 19 days of storage at 4 °C in comparison to control. Furthermore, Raybaudi-Massilia, Mosqueda-Melgar, and Martín-Belloso (2008) reported that the addition of geraniol at 0.5% into edible alginate coating of fresh-cut melon (pH 6.06) resulted effectively in reducing native microflora during 21 days of storage. Besides, Cerrutti, Alzamora, and Vidales (1997) evaluated the use of vanillin as a natural antimicrobial for producing shelf-stable strawberry puree (pH 3.1). They prevented the growth of native microflora for more than 60 days of storage at room temperature in pasteurized strawberry puree containing 0.3 g/100 mL vanillin.

3.2. Total phenolic content in strawberry juice samples

Strawberry is an important source of phytochemicals, in particular, the phenolic composition seems to strongly influence the quality of the fruits, contributing to both their sensorial-organoleptic attributes and their nutritional value. Anthocyanins are quantitatively the most important type of polyphenols in strawberry (Tulipani et al., 2008).

Fig. 1 displays the total phenolic content of treated and untreated strawberry juice samples. Regarding the addition of prebiotic fibers into the formulation, no significant differences were observed in TPC in comparison to untreated strawberry juice. At the beginning of storage, TPC of enriched samples treated with vanillin was significantly higher than their respective fiber-enriched controls. This increment was expected given that vanillin is a phenolic compound itself. In contrast, those samples treated with geraniol did not show significant differences in comparison to controls. TPC in fiber-enriched samples treated with both natural preservatives was maintained constant throughout storage, indicating higher nutritional value of the product. In agreement with our results, Tomadoni et al. (2016) evaluated TPC of strawberry juice treated with vanillin and geraniol stored at 5 °C. These authors reported no significant differences between geraniol treated sample and control, and observed a significant increment in TPC of vanillin treated sample and its maintenance throughout refrigerated storage. Besides, Wang, Wang, Yin, Parry, and Yu (2007) investigated the effect of thymol, eugenol and menthol on fruit quality, phenolic content and antioxidant capacity in strawberries stored at 10 °C. They reported that natural essential oils increased or maintained TPC during three weeks of storage.

3.3. Antioxidant activity in strawberry juice samples

Strawberry represents one of the most important sources of bioactive compounds with antioxidant activity, together with other berries (Tulipani et al., 2008). Fig. 2 displays the DPPH· radical scavenging antioxidant activity of treated and untreated strawberry juice samples. Regarding the addition of prebiotic fibers in the formulation, it was observed that fiber-enriched controls showed significantly higher antioxidant capacity when compared to untreated strawberry juice during the whole storage period. This indicates that the addition of prebiotic fibers could increase the nutritional value of the product. Immediately after treatments application, no significant differences between treated and untreated samples in antioxidant capacity were found. During refrigerated storage, all samples exhibited fluctuations in the percentage of DPPH inhibition and it gradually decreased. Piljac-Žegarac, Valek, Martinez, and Belščak (2009) also observed some fluctuations in antioxidant capacity of dark fruit juices in refrigerated storage. Similar results were found by Tomadoni et al. (2016) who investigated the effect of vanillin and geraniol on the antioxidant capacity of strawberry juice. They reported that both preservatives agents did not increase the antioxidant activity of the product in comparison to untreated strawberry juice. Besides, they informed that antioxidant capacity of vanillin significantly decreases at low pH and concerning geraniol, in acid medium like strawberry juice, it transforms into α-terpineol, a compound which shows a very low radical scavenging capacity in comparison to conventional antioxidants. Tomadoni et al. (2016) concluded that the weak performance in the DPPH test is probably due to the low number of conjugated bonds capable of trapping free radicals in terpene molecules.

3.4. TSS and TA in strawberry juice samples

Organic acids are found in foods because of biochemical processes, or in the case of fermentations, through the development of certain microorganisms. These acids contribute to the particular flavor and palatability of juices, modifying the degree of sweetness of sugars present and also acting to preserve the juice from spoilage (Rodrigo et al., 2003).

Regarding the incorporation of prebiotic fibers into the formulation, no changes in TSS and TA in comparison to untreated juice were observed, with mean values of 10.25 °Brix and 0.87% citric acid, respectively. These TA and TSS values were consistent with those reported by Kallio, Hakala, Pelkkikangas, and Lapveteläinen (2000). The kind of processing had no effect on TA values in fiber-enriched samples throughout storage. At day 14, a significant increase in TA values of control samples was detected. This change

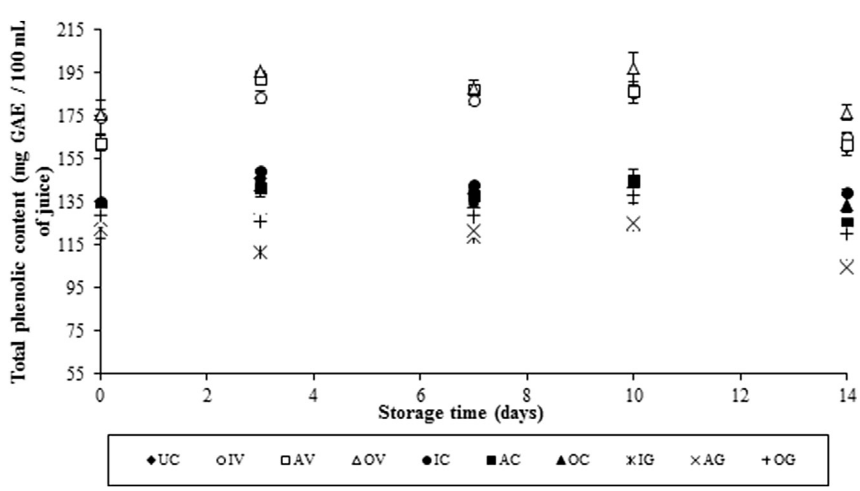

Fig. 1. Total phenolic content evolution in strawberry juices enriched with prebiotic fibers and treated with vanillin and geraniol during 14 days of storage at 5 °C. **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

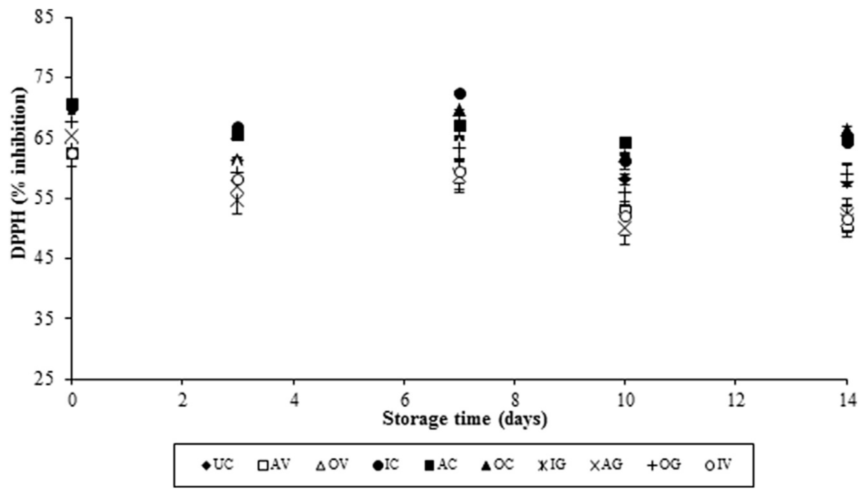

Fig. 2. DPPH radical scavenging activity in strawberry juices enriched with prebiotic fibers and treated with vanillin and geraniol during 14 days of storage at 5 °C. **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

Table 4
Color evolution (parameter L*) in strawberry juice samples during 14 days of storage at 5 °C.

Treatments	L*				
	Storage time (days)				
	0	3	7	10	14
UC	36.11 ± 0.86^{abA}	26.52 ± 1.94 ^{bcAB}	19.92 ± 0.28^{bcdB}	19.54 ± 1.17^{bB}	20.44 ± 1.65^{abcB}
IC	37.98 ± 0.47^{abA}	31.71 ± 1.94^{abcA}	21.07 ± 0.42^{bcB}	20.60 ± 1.15^{abB}	19.29 ± 1.34^{abcB}
AC	37.14 ± 1.08^{abA}	31.71 ± 2.20^{abcAB}	21.64 ± 0.36^{abC}	25.64 ± 1.17^{aBC}	22.38 ± 0.74^{aBC}
OC	39.06 ± 1.51^{abA}	36.93 ± 1.30^{aA}	21.43 ± 0.75^{abB}	20.99 ± 0.63^{abB}	20.17 ± 0.94^{abcB}
IG	24.75 ± 1.06^{cA}	25.29 ± 0.62^{bcA}	21.29 ± 0.41^{abcAB}	22.41 ± 0.98^{abAB}	18.48 ± 0.36^{abcB}
AG	27.19 ± 0.80^{cA}	26.66 ± 0.70^{bcAB}	22.22 ± 0.26^{aC}	23.27 ± 0.13^{abBC}	21.27 ± 0.58^{abC}
OG	25.71 ± 0.51^{cA}	24.63 ± 0.67^{cA}	21.64 ± 0.03^{abAB}	22.65 ± 1.45^{abAB}	18.48 ± 1.01^{abcB}
IV	33.33 ± 0.36^{bA}	31.60 ± 2.78^{abcA}	19.55 ± 0.28^{cdB}	21.61 ± 1.15^{abB}	16.47 ± 0.38^{cB}
AV	38.58 ± 1.46^{abA}	34.42 ± 2.99^{abA}	22.35 ± 0.13^{aB}	21.67 ± 0.47^{abB}	19.02 ± 0.90^{abcB}
OV	39.57 ± 2.25^{aA}	26.47 ± 1.44^{bcB}	19.08 ± 0.04^{dB}	23.55 ± 1.10^{abB}	17.23 ± 0.75^{bcB}

Data is shown as means of 3 determinations ± standard deviation. Values with different letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

Table 5
Color evolution (parameter °h) in strawberry juice samples during 14 days of storage at 5 °C.

Treatments	°h				
	Storage time (days)				
	0	3	7	10	14
UC	39.10 ± 0.21^{abA}	37.31 ± 1.05^{aA}	39.61 ± 0.22^{aA}	36.63 ± 0.85^{aA}	36.69 ± 1.28^{aA}
IC	38.98 ± 0.18^{abA}	38.67 ± 0.41^{aA}	39.05 ± 0.64^{aA}	36.86 ± 0.99^{aA}	37.10 ± 1.05^{aA}
AC	38.30 ± 0.24^{abcA}	36.42 ± 1.78^{aA}	38.99 ± 0.25^{aA}	38.30 ± 0.91^{aA}	38.52 ± 0.14^{aA}
OC	39.74 ± 0.40^{aA}	38.45 ± 0.14^{aAB}	39.50 ± 0.63^{aA}	39.00 ± 0.45^{aAB}	36.09 ± 0.59^{aB}
IG	36.80 ± 0.28^{bcdA}	38.36 ± 0.78^{aA}	38.56 ± 0.37^{aA}	36.87 ± 0.69^{aA}	37.84 ± 0.48^{aA}
AG	38.19 ± 0.58^{abcAB}	37.91 ± 0.27^{aAB}	39.36 ± 0.17^{aA}	36.04 ± 0.51^{aB}	36.85 ± 0.29^{aAB}
OG	37.22 ± 0.09^{bcdA}	38.75 ± 0.33^{aA}	39.35 ± 0.18^{aA}	35.96 ± 1.24^{aA}	37.89 ± 0.93^{aA}
IV	35.13 ± 0.74^{dA}	35.73 ± 0.71^{aA}	38.25 ± 0.09^{aA}	36.66 ± 1.13^{aA}	37.55 ± 0.41^{aA}
AV	36.19 ± 1.01^{dA}	36.27 ± 1.42^{aA}	38.82 ± 0.13^{aA}	37.33 ± 0.51^{aA}	35.27 ± 0.67^{aA}
OV	37.17 ± 0.36^{bcdA}	37.40 ± 0.42^{aA}	38.20 ± 0.02^{aA}	34.87 ± 0.49^{aA}	36.39 ± 0.93^{aA}

Data is shown as means of 3 determinations ± standard deviation. Values with different letters in the same column indicate significant differences ($p < 0.05$) between treatments and values with different letters in the same row indicate significant differences ($p < 0.05$) trough storage time. **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

may be related to the spoilage of the juice by microorganisms that would contribute to an increase in acidity. As regards to TSS, significant differences were observed in TSS of enriched samples treated with both preservative agents with mean values of 9.40 °Brix, significantly lower than their respective controls. Nevertheless, storage time did not have a significant influence on the

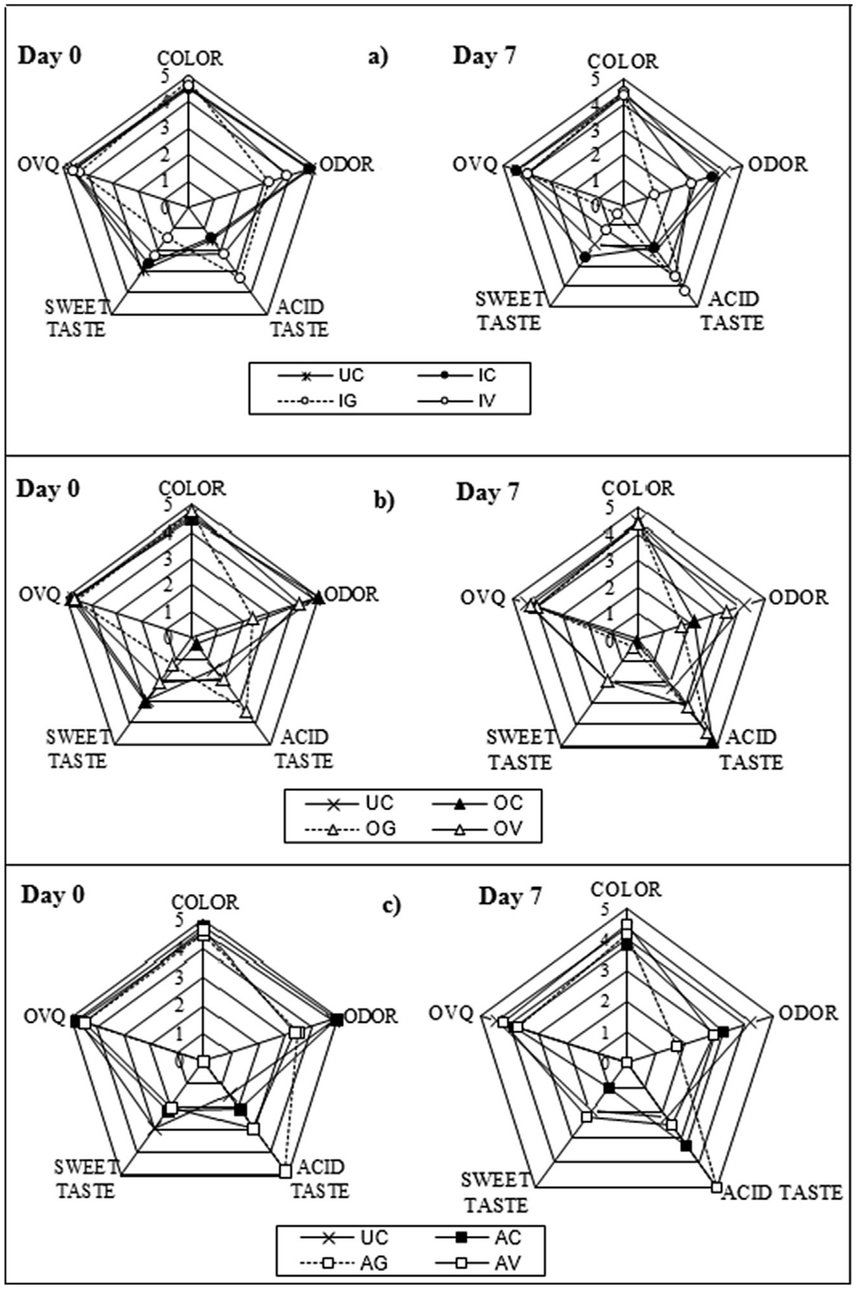

Fig. 3. Natural antimicrobials effect on sensory attributes in strawberry juices enriched with: (a) inulin, (b) oligofructose, (c) apple fiber at 0 and 7 days of storage at 5 °C. **UC**: untreated control; **IC**: inulin-enriched control; **AC**: apple fiber-enriched control; **OC**: oligofructose-enriched control; **IG**, **AG**, **OG**: inulin, apple fiber and oligofructose-enriched sample treated with geraniol, respectively; **IV**, **AV**, **OV**: inulin, apple fiber and oligofructose-enriched sample treated with vanillin, respectively.

evolution of °Brix.

3.5. Color in strawberry juice samples

Color is one of the most important sensory attributes of food and beverages. It is the first thing that consumers notice on the shelves and it may influence the product acceptability (Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martin-Belloso, 2013). Tables 4 and 5 show the evolution of L* and h° of the strawberry juice samples through refrigerated storage, respectively. The incorporation of prebiotic fibers into the formulation did not produce any significant difference with respect to untreated juice, indicating that added fibers themselves did not exert any effect on color of strawberry juice. Immediately after treatment application, enriched samples treated with vanillin did not show significant differences in L* values compared to their respective controls. However, those fiber-enriched samples containing geraniol exhibited the lowest L* values. During storage, L* values of juice samples treated with both preservatives agents decreased, but no significant differences were detected in comparison to the untreated ones. Raybaudi-Massilia et al. (2008) reported that the incorporation of geraniol at 0.5% into edible alginate coating of fresh-cut melon caused a higher diminution of whiteness index and this decrease was gradual along storage time. Furthermore, Cao et al. (2011) reported that a key role in color degradation is attributed to enzymatic browning of phenolic compounds, degradation of anthocyanins and products of the Maillard reaction during or after processing.

The h° value is effective for visualizing true color appearance of food products (Morales-de La Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2010). Immediately after treatment application, enriched samples containing vanillin exhibited lower h° values than their respective controls. However, those samples with geraniol did not present any difference with untreated samples (Table 5). During storage, h° values were maintained constant and no significant differences between treated and untreated samples were registered through time.

3.6. Sensory evaluation in strawberry juice samples

Many nutraceutical compounds have natural bitter, astringent or other off-flavors that could lead to rejection of the product by consumers (Grigelmo-Miguel & Martın-Belloso, 1999). As a result of this, the incorporation of dietary fiber and natural preservatives into strawberry juice could change the original sensory attributes of the product (Sun-Waterhouse et al., 2010).

Fig. 3 displays a sensory evaluation of strawberry juice samples at 0 and 7 days of refrigerated storage. Color, OVQ, odor and acid and sweet taste scores for different samples are shown in Fig. 3. It can be observed that, initially, samples enriched with inulin did not modify the sensory attributes of untreated juice (Fig. 3a). However, those samples enriched with oligofructose presented lower sensory score for acid taste with respect to control (Fig. 3b). Although sweet taste of strawberry juice with oligofructose did not show any difference in comparison to control, preliminary experiments showed that enriched samples were perceived sweeter. This is because, the oligofructose sweetness is 35% higher than sucrose and, as a result, this prebiotic fiber is used as a sugar substitute in many foods (Franck, 2002). In contrast, juices with apple fiber showed lower sweet taste and higher acid taste values in comparison with fresh control (Fig. 3c). The overall preference of strawberry juices enriched with prebiotic fibers received higher scores by panelists. On the other hand, the main sensory characteristics damaged by the use of vanillin and geraniol were acid and sweet taste and odor, but the odor of those samples treated with vanillin was not considered unpleasant. The bioactive compounds imparted a strong flavor to the strawberry juices which did not abate over the course of the experiment.

After the first week of storage, the scores of sensory attributes of samples containing vanillin were remained constant, in contrast with the scores showed by samples containing geraniol which were strongly affected by the incorporation of this natural compound. Some consumers detected a residual aromatic citric taste which increased the acid taste and decreased the sweet taste values of these samples. With respect to color, the incorporation of bioactive compounds in the juices formulation did not affect the typical color of the samples, maintaining its color scores during the entire period of evaluation (Tables 4 and 5).

Up to 14 days of refrigerated storage (data not shown), no visible deleterious effects or color changes were detected on samples treated with both natural preservatives. Besides, treated samples showed OVQ scores higher than the acceptability level (2.50) and presented good appearance. Control samples (without natural preservatives), at day 14 of storage, were only evaluated by consumers in terms of OVQ, odor and color characteristics (obtaining low scores), since microbiological counts were higher than Spanish Regulation establishes as the maximum limit of microorganisms (at expiry date) in minimally processed foods.

Sensory studies have been carried out on related food products such as fruit puree containing some natural antimicrobials. Cerrutti et al. (1997) evaluated the sensory properties of fruit purees containing different concentrations of vanillin and found that they had a pleasant vanillin flavor which maintained the typical taste of the fruit. Also, Yousaf, Yusof, Manap, Bin, and Abd-Aziz (2010) informed that clarified banana juice fortified with inulin and oligofructose remained acceptable during the 8 weeks of storage at 4 °C and were rated the best for all the sensory parameters.

4. Conclusions

The results achieved demonstrated the feasibility of the prebiotic fibers addition into strawberry juice for increasing the nutritional value without compromising their fresh-like sensory acceptability. On the one hand, the application of vanillin and geraniol was effective in reducing microorganism proliferation. A significant reduction of 4–6 log cycles was achieved, depending on population analyzed, in comparison to untreated samples. Furthermore, samples treated with vanillin showed the highest total phenolic content during refrigerated storage. In contrast, both bioactives compounds exhibited lower antioxidant activity in a DPPH radical scavenging assay in comparison to controls. As regard to color parameters, no detectable differences between treated and untreated samples in L* and h° values were presented along storage period. Concerning physical properties, total acidity content of enriched strawberry juices was not affected but total soluble solid was decreased by treatments. On the other hand, natural preservatives, imparted strong flavor in the different beverages. Therefore, based on the concept of hurdle technologies, the use of vanillin and geraniol in combination with other barriers may be a good alternative to improve quality attributes of fruit juices, to control microbial growth and to minimize undesirable changes in organoleptic properties.

Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas under project PIP 0007 and Agencia Nacional de Promoción Científica y Tecnológica under project PICT-2012-1121.

References

Alvarez, M. V., Ponce, A. G., & Moreira, M. d. R. (2013). Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. *LWT-Food Science and Technology, 50*(1), 78–87.

BOE, B. O. d. E. (2001). Madrid, Spain, Real Decreto. *Normas de higiene para la elaboración, distribución y comercio de comidas preparadas, 3484(2000)* pp. 1435–1441).

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223–253.

Cao, X., Zhang, Y., Zhang, F., Wang, Y., Yi, J., & Liao, X. (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*(5), 877–885.

Cerrutti, P., Alzamora, S. M., & Vidales, S. L. (1997). Vanillin as an antimicrobial for producing shelf-stable strawberry puree. *Journal of Food Science, 62*(3), 608–610.

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

Duan, J., & Zhao, Y. (2009). Antimicrobial efficiency of essential oil and freeze–thaw treatments against Escherichia coli O157: H7 and Salmonella enterica Ser. Enteritidis in strawberry juice. *Journal of Food Science, 74*(3), M131–M137.

Fitzgerald, D. J., Stratford, M., & Narbad, A. (2003). Analysis of the inhibition of food spoilage yeasts by vanillin. *International Journal of Food Microbiology, 86*(1), 113–122.

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

Grigelmo-Miguel, N., & Martín-Belloso, O. (1999). Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *LWT-Food Science and Technology, 32*(8), 503–508.

Kallio, H., Hakala, M., Pelkkikangas, A.-M., & Lapveteläinen, A. (2000). Sugars and acids of strawberry varieties. *European Food Research and Technology, 212*(1),

81–85.
Morales-de La Peña, M., Salvia-Trujillo, L., Rojas-Graü, M., & Martín-Belloso, O. (2010). Impact of high intensity pulsed electric field on antioxidant properties and quality parameters of a fruit juice–soymilk beverage in chilled storage. *LWT-Food Science and Technology, 43*(6), 872–881.
Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT-Food Science and Technology, 42*(1), 93–100.
Piljac-Žegarac, J., Valek, L., Martinez, S., & Belščak, A. (2009). Fluctuations in the phenolic content and antioxidant capacity of dark fruit juices in refrigerated storage. *Food Chemistry, 113*(2), 394–400.
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.
Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Martín-Belloso, O. (2008). Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. *International Journal of Food Microbiology, 121*(3), 313–327.
Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit Juices by traditional and alternative natural antimicrobials. *Comprehensive Reviews in Food Science and Food Safety, 8*(3), 157–180.
Robles-Sánchez, R. M., Rojas-Graü, M. A., Odriozola-Serrano, I., González-Aguilar, G., & Martin-Belloso, O. (2013). Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. *LWT-Food Science and Technology, 50*(1), 240–246.
Rodrigo, D., Arranz, J., Koch, S., Frígola, A., Rodrigo, M., Esteve, M., … Rodrigo, M. (2003). Physicochemical characteristics and quality of refrigerated Spanish orange-carrot juices and influence of storage conditions. *Journal of Food Science, 68*(6), 2111–2116.
Rupasinghe, H. V., Boulter-Bitzer, J., Ahn, T., & Odumeru, J. A. (2006). Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples. *Food Research International, 39*(5), 575–580.
Sadler, G. D., & Murphy, P. A. (2010). pH and titratable acidity. In *Food analysis* (pp. 219–238). Springer.
Sun-Waterhouse, D., Nair, S., Wibisono, R., Wadhwa, S. S., Massarotto, C., Hedderley, D. I., … Corrigan, V. (2010). Insights into smoothies with high levels of fibre and polyphenols: factors influencing chemical, rheological and sensory properties. *World Academy of Science, Engineering and Technology, 65*, 276–285.
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., … Battino, M. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry, 56*(3), 696–704.
U. S Food and Drug Administration. Food Additive Status List. (2006). http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm091048.htm#ftnG.
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.
Wang, C. Y., Wang, S. Y., Yin, J.-J., Parry, J., & Yu, L. L. (2007). Enhancing antioxidant, antiproliferation, and free radical scavenging activities in strawberries with essential oils. *Journal of Agricultural and Food Chemistry, 55*(16), 6527–6532.
Yousaf, M. S., Yusof, S., Manap, A., Bin, M. Y., & Abd-Aziz, S. (2010). Storage stability of clarified banana juice fortified with inulin and oligofructose. *Journal of Food Processing and Preservation, 34*(s2), 599–610.