Journal of Functional Foods 40 (2018) 128–136

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Influence of non-thermal processing and storage conditions on the release of health-related compounds after *in vitro* gastrointestinal digestion of fiber-enriched strawberry juices

Lucía Cassani[a,b,*], Esteban Gerbino[b,c], María del Rosario Moreira[a,b], Andrea Gómez-Zavaglia[b,c]

[a] Research Group of Food Engineering, Faculty of Engineering, National University of Mar del Plata, RA7600 Mar del Plata, Argentina
[b] Argentinean National Research Council (CONICET), Argentina
[c] Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata), RA1900 La Plata, Argentina

ARTICLE INFO

Keywords:
In vitro digestion
Nutritionally relevant compounds
Fruit-based beverage
Natural antimicrobials
Ultrasound
Storage

ABSTRACT

Strawberry juices enriched with inulin and oligofructose were treated using non-thermal processing techniques [ultrasound (7.5 min, 40 kHz, 180 W) combined or not with antimicrobials vanillin (1.25 mg/mL) or geraniol (0.225 μL/mL)] and stored for two weeks at 5 °C. The impact of the non-thermal processing and storage conditions on the release of health-related compounds (phenolic compounds, flavonoids and ascorbic acid), and on the total antioxidant capacity (determined by DDPH and TEAC assays) after *in vitro* gastrointestinal digestion was investigated. After gastric digestion, the release of most of bioactive compounds decreased in comparison with non-processed juices. Conversely, exposing the treated juices to simulated intestinal conditions enhanced the release of phenolic and flavonoid compounds and the total antioxidant capacity (determined by the TEAC assay). Storage conditions led to slight changes in bioactive compounds' content. In conclusion, fiber-enriched strawberry juices preserved with non-thermal processing are an important source of nutritionally relevant compounds.

1. Introduction

Currently, consumers are interested in functional foods and beverages that besides being highly nutritious and healthy, are easy to prepare and ingest. In this context, fruit-based beverages are increasingly popular since they represent an easy and convenient way of consuming fruits, which are important sources of health-promoting compounds (*i.e.,* such as vitamin C, phenolic compounds, among others) (Rodríguez-Roque et al., 2015). Among fruits, strawberries are important sources of dietary fiber and bioactive compounds (micronutrients and phytochemical compounds), having demonstrated many beneficial effects on human health and disease prevention (Duan & Zhao, 2009; Rößle, Brunton, Gormley, Wouters, & Butler, 2011). Furthermore, strawberries are considered "healthy food products", so that, they are frequently consumed by a significant percentage of consumers (Nazzaro, Fratianni, Sada, & Orlando, 2008). To produce functional foodstuffs, strawberry juices can be enriched with prebiotics like inulin and oligofructose, thus improving the nutritional characteristics of the final product (Cassani, Tomadoni, Ponce, Agüero, & Moreira, 2017).

Fruit juices have traditionally been preserved using heat treatments to prevent microbial spoilage and contamination with pathogens. However, such treatments usually lead to undesirable changes, including loss of vitamins and minerals and loss of fresh color and flavor of the product (Cassani, Tomadoni, Moreira, Ponce, & Agüero, 2017). Non-thermal processing technologies, such as ultrasound and natural antimicrobials, have been revealed as useful tools to extend shelf-life and to preserve nutritional and functional characteristics of fruit and vegetable products (Bohn et al., 2015). In this regard, ultrasound combined with vanillin improves quality attributes of fiber-enriched strawberry juices during storage, namely reduction of microbial development, increase of nutritional quality and decrease of the growth of pathogens (Cassani et al., 2017). In turn, geraniol has demonstrated to be highly effective in controlling the native microflora and intentionally inoculated pathogens of fiber-enriched strawberry juices without compromising the nutritional quality of the product (Cassani, Tomadoni, Viacava, Ponce, & Moreira, 2016). However, there are scarce data on the effect of these emerging technologies on bioaccessibility of bioactive compounds (Bohn et al., 2015).

Processing and storage conditions play an important role on the release, transformation and absorption of health-related compounds

* Corresponding author at: Research Group of Food Engineering, Faculty of Engineering, National University of Mar del Plata, Argentina.
E-mail addresses: lcassani@fi.mdp.edu.ar (L. Cassani), estebanq2001@hotmail.com (E. Gerbino), moreira_maria@fi.mdp.edu.ar (M.d.R. Moreira), angoza@qui.uc.pt (A. Gómez-Zavaglia).

https://doi.org/10.1016/j.jff.2017.11.005
Received 10 August 2017; Received in revised form 4 November 2017; Accepted 6 November 2017

during gastrointestinal digestion (Parada & Aguilera, 2007). Applying emerging technologies as an alternative to traditional thermal processing would be more valuable if nutritional quality was considered not only as a stability issue but also as a bioaccessibility concern. In this regard, the concentration of bioactive compounds that is released from the food matrix into the gastrointestinal tract and becomes available for absorption is much more important than that in the corresponding beverage. Furthermore, these concentrations can be even increased with this sort of processing, thus making the final product more nutritious (Cilla et al., 2011a).

In vitro methods have been developed to simulate the physiological conditions and the sequence of events that occur during digestion in the human gastrointestinal tract (Cilla, Bosch, Barberá, & Alegría, 2017). These methods are cost-effective, reproducible and, in general, rapid, thus available for determining the effects of food matrices and processing on bioaccessibility of food bioactive compounds (Cilla et al., 2017). The effects of natural antimicrobials combined or not with ultrasound on the stability of health-related compounds of strawberry juices enriched with inulin and oligofructose have been previously investigated (Cassani et al., 2017). However, the influence of these technologies and of the storage conditions on the release of bioactive compounds from the juice matrix is still missing. Thus, the objective of this work was to evaluate the effect of processing fiber enriched strawberry juices (vanillin combined with ultrasound or geraniol) on the release of total phenolic and flavonoid compounds, ascorbic acid and on their antioxidant capacity when exposed to simulated gastrointestinal conditions. In addition, the impact of storage conditions (two weeks at 5 °C) on the release of the above-mentioned nutritionally relevant compounds after *in vitro* gastrointestinal digestion was evaluated.

2. Material and methods

2.1. Preparation of strawberry juices

Strawberries (*Fragaria x ananassa* Duch.) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentina. Fruits with defects were discarded and fruits with good quality were washed with tap water and the calyx was removed by hand. Then, fruits were squeezed by a commercial juice extractor and the fresh strawberry juice was collected in a glass jar. The juice was homogenized and bottled under hygienic conditions into 100 mL polyethylene terephthalate bottles and sealed with polyethylene caps to be subsequently used in the experiments.

2.2. Application of non-thermal processing

A juice sample was enriched with 1.5 g of inulin/oligofructose mixture (5:3 ratio, Grupo Saporiti, Argentina) and then treated with geraniol (0.225 μL/mL), according Cassani et al. (2016). The second juice sample was also enriched with inulin/oligofructose mixture and then treated with a combined preservation treatment, including vanillin (1.25 mg/mL) and subsequent immersion in a water bath of an ultrasonic chamber (15 × 29 × 15 cm) (TestLab, Argentina) for 7.5 min at 20 °C (monitored using a thermometer). The sonication conditions were: frequency 40 kHz, power 180 W. The inulin/oligofructose ratio, vanillin concentration, and ultrasound conditions were established using response surface methodology, with a Box-Benhken design, as reported in Cassani et al. (2017). In that optimization study, both the interactions and single effects of the three variables (vanillin concentration, ultrasound time and inulin:oligofructose ratio) were evaluated at three levels each. After simultaneous optimization of several response variables, the optimal conditions for the three parameters under study were determined.

A juice sample without any addition or preservation treatment was used as control (untreated). Samples were stored at 5 °C for 0, 7 and 14 days.

2.3. In vitro gastrointestinal digestion

An *in vitro* gastrointestinal digestion procedure was used to evaluate the concentration of health-related compounds that is released from the food matrix into gastrointestinal tract, and becomes available for absorption on both the freshly prepared juices and those stored for 7 and 14 days at 5 °C. The test was divided into two stages, gastric digestion (pepsin, pH 2.5, Sigma Aldrich) and intestinal digestion (pancreatin, bile salts, pH 8, Sigma Aldrich) adapted from Quintana et al. (2017) and Grimoud et al. (2010). Juice samples (10 mL) were mixed with 10 mL of simulated gastric juice (7.30 g/L NaCl, 0.52 g/L KCl, 3.78 g/L $NaHCO_3$, 3 g/L pepsin, at a final pH adjusted to 2.5). The samples were incubated for 1.5 h at 37 °C under continuous shaking (100 rpm, MaxQ 4000, Thermo, Lab-Line, Barnstead, USA). The digests were maintained in ice for 10 min to stop the gastric digestion. Afterwards, 10 mL of simulated intestinal fluid (1.27 g/L NaCl, 0.23 g/L KCl, 0.64 g/L $NaHCO_3$, 1 g/L pancreatin, 1.5 g/L bovine bile salts) was added and the pH was adjusted to 8. The resulting solution was incubated for 3 h at 37 °C, under continuous shaking (100 rpm, MaxQ 4000, Thermo, Lab-Line, Barnstead, USA). To stop intestinal digestion, samples were kept for 10 min in an ice-bath. The test tubes were then centrifuged at 13,500*g* for 20 min. The supernatants were collected and filtered using Whatman filter paper #1. Samples were stored at −20 °C until analysis.

2.4. Health-related compounds determination

Different parameters (total phenolic and total flavonoid contents, antioxidant activity and ascorbic acid content) associated with nutritional quality of strawberry were determined on juices samples before and after *in vitro* gastrointestinal digestion, as well as after 0, 7 and 14 days of storage at 5 °C.

2.4.1. Extraction of phytochemicals from non-digested samples

Total phenolic content, total flavonoid content and total antioxidant capacity were determined on an extract of antioxidants from juice samples prior digestion. The extraction was carried out by homogenizing 2 mL of strawberry juice from each sample with 10 mL solution of ethanol (800 mL/L) (Merck, Darmstadt, Germany). The homogenate was then centrifuged at 13500*g* 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 for determining health-related compounds.

2.4.2. Total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu reagent (Biopack, Argentina) according to the methodology proposed by Viacava, Roura, and Agüero (2015) with modifications. Ethanolic extracts (arising from non-digested or digested samples) properly diluted were added to 150 μL of the Folin–Ciocalteu reagent (diluted 1:10). After 3 min of incubation at room temperature (20 °C), 120 μL of Na_2CO_3 (75 g/L, Merck, Darmstadt, Germany) solution was added and the reaction mixture was incubated for 2 h at the same temperature. The absorbance was read at 765 nm on a microplate reader of 300 μL capacity (Biotek, Synergy HT, Winooski, VT, USA) and the total phenolic content was calculated using gallic acid (Biopack, Argentina) as standard. Results were expressed as mg gallic acid equivalents/100 mL of juice. The standard curve of gallic acid was made up in the range of 0.01–0.14 g/L.

2.4.3. Total flavonoid content

The total flavonoid content was determined according to Viacava and Roura (2015). Ethanolic extracts (arising from non-digested or digested samples) (0.2 mL) were mixed with 1.28 mL of deionized H_2O and 0.06 mL of $NaNO_2$ (50 g/L) (Biopack, Argentina). After 5 min at

20 °C, 60 μL of $AlCl_3$ (100 g/L) (Anedra, Argentina) were added, and 6 min later, 0.4 mL of NaOH (40 g/L, Biopack, Argentina) were incorporated in the same conditions. The mixtures were stirred and the absorbance was read at 510 nm using a microplate reader (BioTek® Synergy HT, USA). The total flavonoid content was expressed as mg of quercetine (Sigma-Aldrich, USA) equivalents/100 mL of juice. The standard curve of quercetine was made up in the range of 0.05–1.2 g/L.

2.4.4. Total antioxidant capacity

2.4.4.1. 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Determination was carried out according to Viacava et al. (2015). An ethanolic DPPH solution (39.43 g/L) was used for determinations. Ethanol (0.1 mL) (Merck, Darmstadt, Germany) was mixed with 3.9 mL of DPPH (39.43 g/L) to determine the initial absorbance of the DPPH solution. Then, 20 μL of ethanolic extracts (arising from non-digested or digested samples) properly diluted was added to 280 μL of DPPH solution (39.43 g/L). The mixture was kept at 20 °C for 60 min in the dark. The decrease in absorbance was read at 517 nm using a microplate reader (BioTek® Synergy HT, USA). The radical scavenging activity was expressed as mg Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents/100 mL of juice. The standard curve of Trolox was made up in the range of 0.005–0.14 g/L.

2.4.4.2. Trolox equivalent antioxidant capacity assay (TEAC). TEAC value was determined according to Viacava and Roura (2015). $ABTS^{\cdot+}$ cation was generated through the interaction of 19.2 mg of ABTS [2,2′-azinobis-(3-ethylbenzotriazoline-6-sulfonic acid)] dissolved in 5 mL of distilled water and 0.088 mL of potassium persulfate ($K_2S_2O_8$; 37.8 g/L). The mixture was incubated in the dark at 20 °C for 16 h to generate the ABTS activated radical ($ABTS^{\cdot+}$). Then, 1 mL of $ABTS^{\cdot+}$ was taken and 88 mL of ethanol were added. The radical was adjusted at an absorbance of 0.70 ± 0.02 at 754 nm. The reaction was initiated by adding 280 μL of $ABTS^{\cdot+}$ and 20 μL of ethanolic extracts (arising from non-digested or digested samples). After 10 min incubation at 20 °C, the absorbance was monitored at 754 nm using a microplate reader (BioTek® Synergy HT, USA). The radical scavenging activity was calculated and the results were expressed as mg of Trolox equivalents/100 mL of juice. The standard curve of Trolox was made up in the range of 0.005–0.14 g/L.

2.4.5. Ascorbic acid content

The content of ascorbic acid was determined by high performance liquid chromatography (HPLC) (Waters, model R-414, Milford, MA, USA) according to Tavera-Quiroz et al. (2015). The method consisted of an isocratic elution procedure with UV–Visible detection at 245 nm. A sample of 1 mL of non-digested or digested strawberry juice was mixed with 1 mL of a solution containing 5 g/L metaphosphoric acid–-acetonitrile (93:7). Then, samples were filtered on 0.45 μm membrane. Separations were carried out on a 5 mm RP C18 column of 150–4.6 mm (WAT 045905, Waters, Dublin, Ireland). A mixture of 5 g/L metaphosporic acid–acetonitrile (93:7) was employed as mobile phase. Ascorbic acid (Sigma Aldrich, USA) was used as standard and its curve was made up in the range of 0.001–0.1 g/L. Results were expressed as mg of ascorbic acid/100 mL of juice.

2.5. Statistical analysis

The *in vitro* gastrointestinal digestion process was carried out twice. Each studied parameter was analyzed three times in every *in vitro* gastrointestinal digestion. A completely randomized design was used for each experiment. Results reported in this work are mean values accompanied by their standard errors. Experimental data were analyzed using R, software version 2.12 (R Development Core Team, 2011). Analysis of variance ANOVA ($p < .05$) was performed and Tukey-Kramer comparison test was used to estimate significant differences between treatments and through storage time ($p < .05$).

3. Results and discussion

3.1. Changes in total phenolic content

The Folin-Ciocalteu assay is the most commonly used procedure to determine total phenolic compounds of food extracts. The Folin-Ciocalteu assay is a colorimetric method based on electron transfer reactions between the Folin-Ciocalteu reagent and phenolic compounds. However, it is known that other types of compounds that may be present in abundance in strawberries (i.e. ascorbic acid) can also reduce the Folin-Ciocalteu reagent, interfering on the accuracy of the assay. In order to reduce this interference, the ascorbic acid reducing activity should be quantified and subtracted from the total phenolic compounds as determined by Folin-Ciocalteu reaction. Isabelle et al. (2010) proposed the subtraction of ascorbic acid contribution from total phenolic compounds to obtain a more accurate quantification of total phenolic compounds in common vegetables. In that approach, ascorbic acid was first determined by HPLC. In addition, an ascorbic acid standard was tested for total phenolic compounds using the Folin-Ciocalteu assay and it was found that ascorbic acid possesses a reducing activity of 0.872 mg of gallic acid equivalents/g ascorbic acid. To obtain the corrected total phenolic compounds of the juice samples analyzed, the ascorbic acid content of the sample was multiplied by 0.872 and the result was subtracted from the values obtained for total phenolic compounds. ANOVA was applied to analyze differences between the corrected and non-corrected total phenolic compounds values of juice samples, and no significant differences were found (data not shown). Besides that, considering that the content of ascorbic acid was similar for all samples (it will be discussed in Section 3.4), even if it would interfered, such interference would have been similar for all samples. Therefore, it is not relevant for the purpose of comparing the total phenolic compounds between juice samples.

Fig. 1 displays the concentration of total phenolic compounds before (non-digested) and after exposure of stored strawberry juices (0, 7 and 14 days at 5 °C) to simulated gastric and intestinal conditions. Fiber-enriched juices treated with vanillin and ultrasound before digestion and storage (time 0) were those showing the highest concentration of phenolic compounds (180 mg gallic acid equivalent/100 mL of juice). Geraniol treated samples and control showed significantly lower values (123 mg gallic acid equivalent/100 mL of juice and 110 mg gallic acid equivalent/100 mL of juice, respectively). These results are in agreement with previously reported results (Cassani et al., 2017). After gastric digestion, the phenolic content of treated samples diminished between 17 and 28%, while those of the untreated ones increased up to 10%, with regard to the concentration of the corresponding non-digested samples. In this regard, a release of total phenolic compounds from fiber-enriched juice matrices was previously reported for samples treated with vanillin and ultrasound (Cassani et al., 2017). The low pH and the pepsin action of the gastric digestion were expected to release some phenolic compounds bound to carbohydrates, thus leading these bioactive compounds more bioaccessible. A possible explanation of the decrease in the phenolic content can be an incomplete release of phenolic compounds due to the presence of fiber, typically present in the strawberries cell wall (pectin and hemicelluloses) or in the prebiotics exogenously added (inulin and oligofructose), that could interact with these phytochemicals and limit their bioaccessibility. Velderrain-Rodriguez et al. (2016) reported that the interaction between dietary fiber and phenolic compounds is related to non-covalent bonds as electrostatic forces and hydrogen bonds as van der Waals forces. This linkage is formed between the available hydroxyl groups of phenolic compounds and the diverse components of dietary fiber. Since the antioxidant capacity of phenolic compounds is attributed to the number and localization of their hydroxyl groups, their interaction with dietary fiber could not only limit their absorption, but it also prevents these

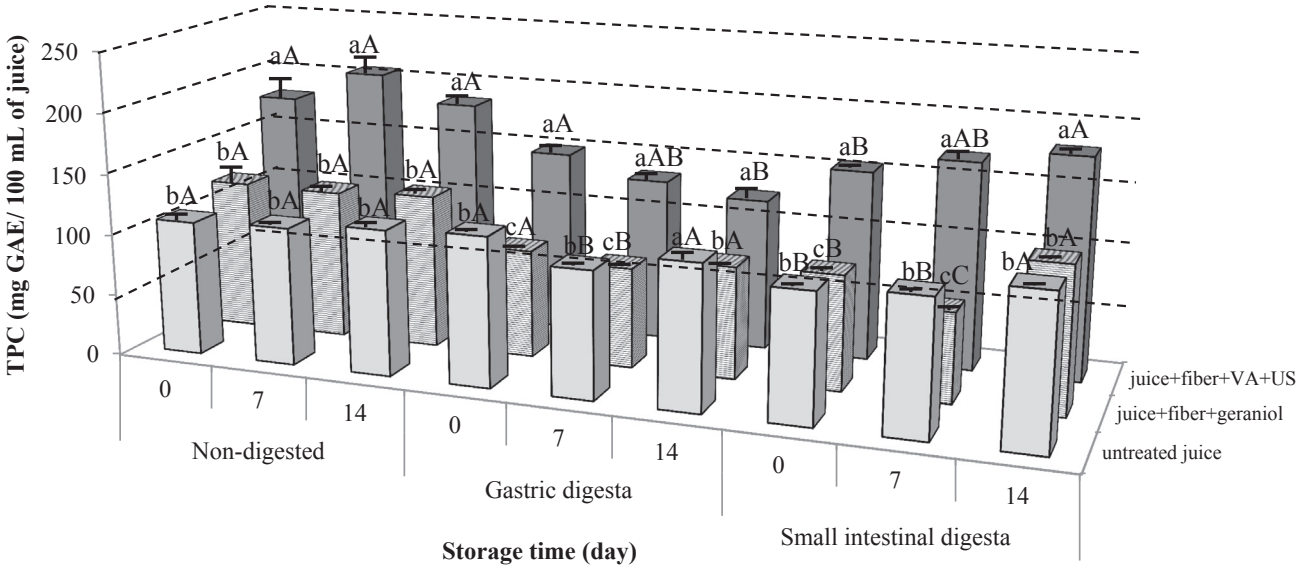

Fig. 1. Total phenolic compounds before (non-digested) and after exposure of stored strawberry juices (0, 7 and 14 days at 5 °C) to simulated gastric and intestinal conditions. Values with different lowercase letters in the same column indicate significant differences ($p < .05$) between treatments at each digestion stage, and values with different capital letters in the same row indicate significant differences ($p < .05$) through storage time. Light gray bars: untreated juices (without any addition or preservation treatment); striped bars: juices enriched with inulin and oligofructose and treated with geraniol (juice + fiber + geraniol); dark gray bars: juices enriched with inulin and oligofructose and treated with vanillin and ultrasound (juice + fiber + VA + US). TPC: total phenolic compounds; GAE: gallic acid equivalent.

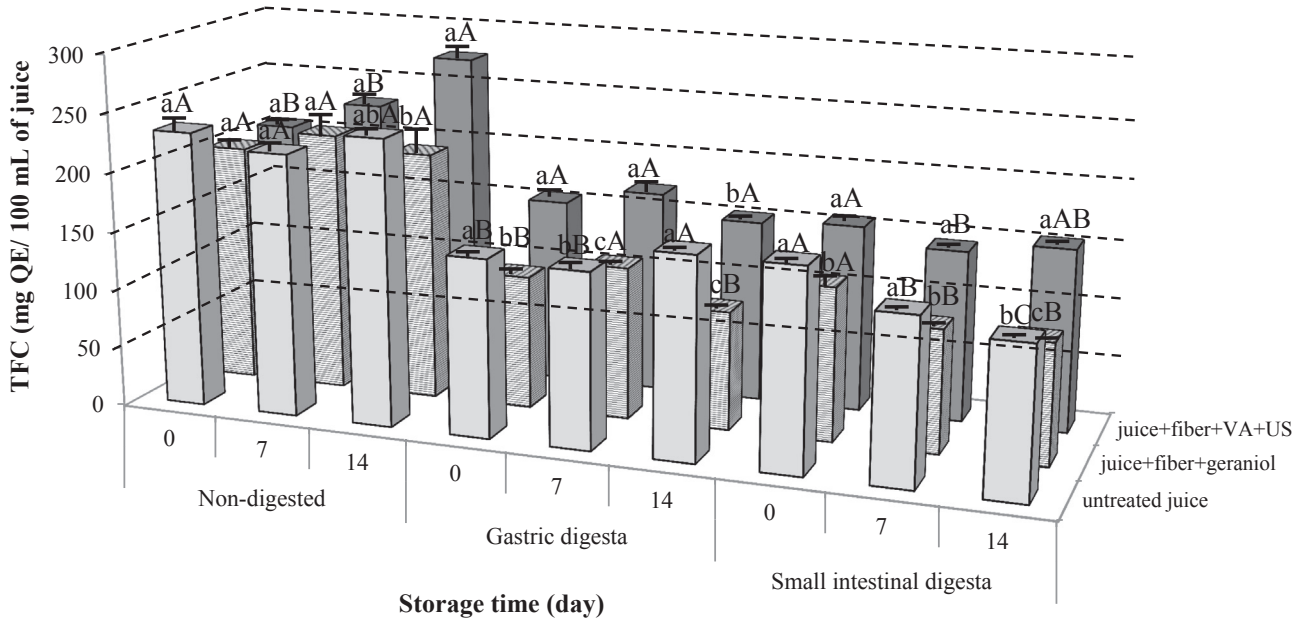

Fig. 2. Effect of storage (0. 7 and 14 days at 5 °C) and simulated gastrointestinal conditions on the release of total flavonoid compounds. Values with different lowercase letters in the same column indicate significant differences ($p < .05$) between treatments at each digestion stage, and values with different capital letters in the same row indicate significant differences ($p < .05$) through storage time. Light gray bars: untreated juices (without any addition or preservation treatment); striped bars: juices enriched with inulin and oligofructose and treated with geraniol (juice + fiber + geraniol); dark gray bars: juices enriched with inulin and oligofructose and treated with vanillin and ultrasound (juice + fiber + VA + US). TFC: total flavonoid compounds; QE: quercetine equivalents.

groups from stabilizing free radicals (Velderrain-Rodriguez et al., 2016).

Simulated intestinal digestion led to interesting results. The phenolic content of juices treated with non-thermal processing techniques slightly increased (between 3 and 5%) with regard to that in the gastric digesta. On the contrary, the phenolic content of the untreated ones decreased up to 14%. The increase in the phenolic content by processing can be explained by the release of ellagic acid (the major phenolic compound present in strawberry) from the ester form, giving ellagitannins upon alkaline treatments (mild saponification in physiological conditions) (Gil-Izquierdo, Zafrilla, & Tomás-Barberán, 2002). Processing conditions probably degrade the cell structures, favoring the release of ellagic acid during the small intestinal digestion of fiber-enriched strawberry juices. These results suggest that the phenolic stability might depend on some factors such as their physicochemical properties and the interaction with dietary or gastric constituents. Similar findings were reported by Bohn et al. (2015) who concluded that many polyphenols may not be detectable in the native matrix following

Table 1
Antioxidant capacity of strawberry juices before (non-digested) and after exposure of stored strawberry juices (0, 7 and 14 days at 5 °C) to simulated gastric and intestinal conditions, as determined by the DPPH assay (mg Trolox/ 100 mL of juice).

	Treatment	Storage time (days)		
		0	7	14
Non-digested	Untreated	120.47 ± 7.80^{aA}	100.91 ± 6.81^{abA}	110.22 ± 2.26^{aA}
	Juice + fiber + geraniol	106.70 ± 5.96^{aA}	115.47 ± 6.36^{aA}	79.00 ± 2.91^{bB}
	Juice + fiber + VA + US	100.89 ± 6.42^{aA}	92.67 ± 5.16^{bA}	82.72 ± 6.03^{bA}
Gastric digesta	Untreated	108.04 ± 4.19^{aB}	75.17 ± 4.69^{aC}	133.99 ± 3.61^{aA}
	Juice + fiber + geraniol	82.38 ± 2.56^{bB}	75.46 ± 4.17^{aB}	98.84 ± 1.99^{bA}
	Juice + fiber + VA + US	73.33 ± 3.85^{bA}	67.49 ± 6.02^{aA}	82.28 ± 2.73^{cA}
Small intestinal digesta	Untreated	93.89 ± 4.64^{aA}	77.62 ± 5.69^{aA}	94.14 ± 4.12^{aA}
	Juice + fiber + geraniol	79.35 ± 6.89^{aA}	45.71 ± 3.33^{bB}	46.18 ± 3.30^{cB}
	Juice + fiber + VA + US	75.30 ± 4.21^{aA}	62.92 ± 3.29^{aB}	77.35 ± 1.49^{bA}

Data are means of 3 determinations ± standard deviation. Values with different lowercase letters in the same column indicate significant differences ($p < .05$) between treatments at each digestion stage, and values with different capital letters in the same row indicate significant differences ($p < .05$) through storage time. Untreated juice: strawberry juices without any addition or treatment; Juice + fiber + geraniol: strawberry juice enriched with inulin and oligofructose and treated with geraniol; Juice + fiber + VA + US: strawberry juices enriched with inulin and oligofructose and treated with vanillin combined with ultrasound.

chemical extraction, but may be released during digestion in the small intestine, such as those bound covalently, though colonic fermentation may further result in the breakdown of non-extractable polyphenols.

The concentration of bioactive compounds that is released from strawberry juices into gastrointestinal tract and becomes available for absorption affected by storage conditions is actually scarce. Therefore, we decided to evaluate the evolution of the phenolic content during two weeks of refrigerated storage. The phenolic content for all non-digested juices remained unchanged for up to 14 days of storage at 5 °C (Fig. 1). Regarding gastric digesta, the phenolic content of samples treated with vanillin and ultrasound significantly decreased during storage (17% with respect to that obtained at day 0), while geraniol treated samples and control exhibited some significant fluctuations. Finally, the phenolic content of all stored samples increased after exposure to simulated intestinal conditions (between 16 and 27% with respect to the phenolic content obtained at day 0). These results indicate that storage conditions (time and temperature) affect the release of phenolic compounds of fiber-enriched strawberry juices.

3.2. Changes in total flavonoid content

Changes in the total flavonoid content due to *in vitro* gastrointestinal digestion and storage of strawberry juices are shown in Fig. 2. Initial total flavonoid content (time equal to 0) of non-digested samples ranged from 203 to 234 mg quercetine/100 mL of juice with no significant differences among samples ($p > .05$). These results were within the range reported in previous works (Cassani et al., 2017). After gastric digestion, the total flavonoid content of all samples significantly decreased, between 23 and 44%, with regard to the content in the non-digested samples ($p < .05$). The subsequent intestinal phase resulted in an increase of *ca.* 10% compared to the amounts released after exposure to the gastric phase.

Flavonoids are present in plants almost exclusively as β-glycosides. Most industrial and domestic food processing procedures do not lead to cleavage of the glycosidic linkage and hence flavonoids in foods are generally present as glycosides (Németh et al., 2003). A possible explanation of the decrease in the total flavonoid content of all samples is that flavonoid glycosides do not undergo acid hydrolysis in the stomach and pass unchanged to the intestine. There, enzymatic hydrolysis and cleavage of the sugar moiety take place (Tarko, Duda-Chodak, Sroka, Satora, & Michalik, 2009). Felgines et al. (2003) demonstrated that prior to absorption into the bloodstream, pelargonidin-3-O-glucoside (the major anthocyanin compound in strawberry) is converted into three monoglucuroconjugates of pelargonidin, one sulfoconjugate of pelargonidin and pelargonidin itself. These conversions may begin with the hydrolysis of the glucoside by the enzymes located in the small intestinal epithelial cells. In the same way, McDougall, Dobson, Smith, Blake, and Stewart (2005) observed that pelargonidin-3-O-glucoside was highly recovered in the dialyzed samples as compared to the other anthocyanins, whereas cyanidin-3-O-glucoside was notably unstable. It is possible that the relative increase in pelargonidin-3-O-glucoside content is caused by the breakdown of other anthocyanins, such as pelargonidin-3-O-rutinoside, pelargonidin-3-O-(2G)-glucosylrutinoside, and/or pelargonidin-3-O-sophoroside. Besides, McDougall, Fyffe, Dobson, and Stewart (2007) observed an increase of non-anthocyanin compounds after small intestinal digestion, resulted from anthocyanin breakdown. Similar to our results, Bouayed, Hoffmann, and Bohn (2011) concluded that the increase in the total flavonoid content after small intestinal digestion could be the result of the additional time of extraction (plus 3 h) and/or the effect of intestinal digestive enzyme (pancreatin) on the complex food matrix, facilitating the release of flavonoids bound to the matrix.

The concentration of flavonoids that is released from strawberry juices into the gastrointestinal tract and becomes available for absorption affected by storage conditions was subsequently studied (Fig. 2). Throughout storage, the total flavonoid content of geraniol treated and control samples was maintained constant and no significant differences between beverages were registered. However, the total flavonoid content of juices treated with vanillin and ultrasound increased at the end of the storage. Different behavior was observed in all samples after gastric digestion along storage. While the total flavonoid content values of samples treated with vanillin and ultrasound remained constant, geraniol treated samples showed some fluctuations and the total flavonoid contents of the untreated juices increased at the end of the storage assay. On the other hand, the total flavonoid contents of geraniol treated samples and control diminished after exposing the stored samples to intestinal fluids (*ca.* 23% with respect to the total flavonoid content obtained at day 0). In turn, the total flavonoid contents of samples preserved with vanillin and ultrasound remained unchanged.

3.3. Changes in total antioxidant capacity

3.3.1. DPPH radical scavenging activity

The influence of gastrointestinal digestion and storage conditions on the total antioxidant capacity of the different strawberry juices were determined using DPPH assay (Table 1). Initial total antioxidant capacity values of non-digested strawberry juices were within 100 and 120 mg Trolox equivalents/100 mL of juice, with no significant differences between treatments ($p > .05$) These results laid within the range

reported in the literature (Piljac-Žegarac, Valek, Martinez, & Belščak, 2009). After gastric digestion, the total antioxidant capacity of all samples significantly decreased with regard to the non-digested ones (*ca.* 24% for treated samples and 10% for the untreated one). Phenolic compounds are among the most important bioactive compounds of strawberry juices with antioxidant activity. As it was mentioned before, these compounds showed low stability under gastric digestion possibly due to the interaction with dietary fiber, which blocks the hydroxyl groups and prevents them from stabilizing free radicals (Velderrain-Rodriguez et al., 2016).

After the intestinal phase, no additional variation in the total antioxidant capacity of samples treated with non-thermal processing was observed (Table 1). Untreated sample exhibited a significant decrease (13%) in these conditions. Interestingly, these results can be also related to those observed for total phenolic compounds, which slightly increased during small intestinal digestion in those samples treated with non-thermal processing (Fig. 1). The increase of phenolic compounds was attributed to the release of ellagic acid. Häkkinen, Kärenlampi, Mykkänen, Heinonen, and Törrönen (2000) observed that this compound had antioxidant activity due to its metal chelating capacity and ability to react with free radicals, which could explain the results obtained in this research. On the other hand, the decrease in total antioxidant capacity of the untreated sample suggests that the bioactive compounds were unstable under intestinal digestion. The alkaline pH, as well as the digestive enzymes action, could transform these bioactive compounds into other substances with different chemical and physical properties.

As well as for total phenolic compounds and flavonoids, information about changes in total antioxidant activity during *in vitro* gastrointestinal digestion and during storage has been addressed hereto. Regarding non-digested beverages, total antioxidant capacity of geraniol treated samples significantly decreased at the end of storage ($p < .05$), while that of samples treated with vanillin and ultrasound and that of the untreated one remained unchanged ($p > .05$). After gastric digestion, geraniol treated samples showed a significant increase in the total antioxidant capacity, while the untreated exhibited some fluctuations and those juices treated with vanillin and ultrasound remained statically unchanged after 14 days of storage. On the other hand, total antioxidant capacity of geraniol treated samples significantly decreased after small intestinal digestion. In turn, total antioxidant capacity of samples treated with vanillin and ultrasound displayed some fluctuations during storage and that of the untreated ones remained constant (Table 1).

Prolonged storage time or processing can promote the progressive enzymatic or chemical oxidation of phenolic compounds, and these reactions proceed at different rates depending on some intrinsic food variables as well as on the processing conditions (aw, pH, time, temperature, oxygen availability, etc.) (Nicoli, Anese, & Parpinel, 1999). Thus, the increases or decreases in the overall antioxidant properties of polyphenol-containing products are consequences of the same oxidation reactions (Nicoli et al., 1999).

3.3.2. Trolox equivalent antioxidant capacity

Table 2 shows the changes in antioxidant activity during *in vitro* gastrointestinal digestion and storage, as determined by the TEAC assay. Processing significantly affected the initial total antioxidant capacity of non-digested strawberry juices, as significant lower values were observed in treated juices ($p < .05$).

Contrarily to the results obtained with the DPPH assay (Table 1), total antioxidant capacity of all samples significantly increased after gastric digestion (*ca.* 9% for samples treated with vanillin and ultrasound and 14% for geraniol-treated samples and the untreated one, with regard to the corresponding non-digested juices). The subsequent intestinal phase resulted in a significantly higher increase of $ABTS^{\cdot+}$ scavenging capacity with regard to that observed in the gastric digesta (*ca.* 86% for samples treated with vanillin and ultrasound, 14% for geraniol treated samples and 8% for untreated ones). Similar results were found by Tagliazucchi, Verzelloni, Bertolini, and Conte (2010), who observed a significant increase in the antioxidant activity of grapes measured with the TEAC assay, after exposure to the mild alkaline intestinal environment. It is well-known that the radical scavenger activity of polyphenols is strongly pH-dependent, higher pH values noticeably increasing this capacity. This increase in the radical scavenger activity has been attributed to the deprotonation of the hydroxyl moieties present in the aromatic ring. The transition from the stomach to the intestinal environment may induce structural changes in the phenolic molecules, that could be attributed to the ionization of the hydroxyl groups (Tagliazucchi et al., 2010).

Changes in total antioxidant capacity after *in vitro* gastrointestinal digestion of different strawberry juices were also investigated during two weeks of refrigerated storage (Table 2). Throughout storage, total antioxidant capacity of all non-digested samples significantly decreased. After gastric digestion of stored juices, total antioxidant capacity of samples preserved with non-thermal processing remained unchanged, while that of the untreated juices significantly decreased ($p < .05$). Upon intestinal digestion of all stored samples, their total antioxidant capacity significantly increased with regard to that obtained at day 0 (*ca.* 21% for samples treated with vanillin and ultrasound, 14% for geraniol treated samples and 51% for the untreated ones). These results may probably support a more efficient protection of the intestinal cells from oxidative stress by polyphenols, which have a greater scavenger ability at the intestinal pH than at the gastric pH (Tagliazucchi et al., 2010). Similar results were found by Cilla et al. (2011b) who observed a significant increase ($p < .05$) in total antioxidant capacity determined by TEAC assay of the bioaccessible fraction of different fruit beverages stored at 2–4 °C for 135 days.

Some differences were observed between the two radical scavenging assays ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$). On the one hand, $DPPH^{\cdot}$ is a long-lived and stable nitrogen radical with which some antioxidants react more slowly or may be even inert than they would with the peroxyl radical in a biological system due to steric inaccessibility (Huang, Ou, & Prior, 2005). On the other hand, $ABTS^{\cdot+}$ is soluble in both aqueous and organic solvents, and is not affected by ionic strength, thus it can be used in multiple media to determine both hydrophilic and lipophilic antioxidant capacities of extracts (Prior, Wu, & Schaich, 2005). Alterations in the structure of antioxidants following digestion may affect their reactivity with the formation of nitrogen radical, which is biologically less relevant in the DPPH assay, leading to the underestimation of total antioxidant capacity (Wootton-Beard, Moran, & Ryan, 2011).

3.4. Changes in ascorbic acid content

Changes in ascorbic acid content as result of the *in vitro* gastrointestinal digestion and storage conditions are displayed in Fig. 3. The initial ascorbic acid concentrations of all the three non-digested juices were between 42 and 49 mg/100 mL of juice, with no significant differences among them ($p > .05$). According to FAO/WHO, the recommended nutrient intake (RNI) for vitamin C varies regarding the age groups. For children from 1 to 9 years RNI for vitamin C ranges between 30 and 35 mg per day, while for adolescents and adults this value ranges between 40 and 45 mg per day at the moment of being consumed (FAO & WHO, 2001). Thus, the daily requirement of vitamin C can be achieved by drinking 100 mL of strawberry juice. After gastric digestion, the ascorbic acid content decreased 30% in fiber enriched juices preserved with non-thermal techniques, and 10% in the untreated one, with regard to the ascorbic acid content of non-digested beverages. It is well-known that vitamin C is a thermo-labile compound, very susceptible to chemical and enzymatic oxidation during processing (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013). The ascorbic acid and oxidative enzymes (*i.e.* ascorbic acid oxidase and peroxidase) may probably bring into contact when the juice matrix was disrupted upon non-thermal processing. Therefore, oxidative reactions

Table 2
Antioxidant capacity of strawberry juices before and after exposure of stored strawberry juices (0, 7 and 14 days at 5 °C) to simulated gastric and intestinal conditions, as determined by the trolox equivalent antioxidant capacity assay (mg Trolox/100 mL of juice).

	Treatment	Storage time (days)		
		0	7	14
Non-digested	Untreated	152.13 ± 10.08^{aA}	108.90 ± 10.62^{aB}	81.16 ± 5.21^{aB}
	Juice + fiber + geraniol	124.46 ± 3.62^{bA}	102.74 ± 11.25^{aAB}	94.44 ± 3.11^{aB}
	Juice + fiber + VA + US	121.01 ± 4.86^{bA}	83.34 ± 5.06^{aB}	75.89 ± 6.87^{aB}
Gastric digesta	Untreated	173.81 ± 7.73^{aA}	181.11 ± 9.49^{aA}	141.93 ± 7.15^{aB}
	Juice + fiber + geraniol	143.10 ± 6.87^{bA}	126.35 ± 6.14^{bA}	125.23 ± 5.13^{aA}
	Juice + fiber + VA + US	133.13 ± 5.09^{bA}	103.97 ± 7.66^{bB}	124.45 ± 6.87^{aAB}
Small intestinal digesta	Untreated	187.98 ± 3.44^{bC}	236.84 ± 9.78^{bB}	285.20 ± 4.17^{aA}
	Juice + fiber + geraniol	163.20 ± 1.54^{cB}	172.40 ± 5.99^{cAB}	187.14 ± 7.48^{bA}
	Juice + fiber + VA + US	248.18 ± 5.18^{aC}	284.02 ± 4.55^{aB}	301.57 ± 2.41^{aA}

Data are means of 3 determinations ± standard deviation. Values with different lowercase letters in the same column indicate significant differences ($p < .05$) between treatments at each digestion stage, and values with different capital letters in the same row indicate significant differences ($p < .05$) through storage time. Untreated juice: strawberry juices without any addition or treatment; Juice + fiber + geraniol: strawberry juices enriched with inulin and oligofructose and treated with geraniol; Juice + fiber + VA + US: strawberry juices enriched with inulin and oligofructose and treated with vanillin combined with ultrasound.

of ascorbic acid could explain the noticeable decrease in the ascorbic acid content of the preserved samples.

An additional decrease of *ca.* 65% in the ascorbic acid content was observed upon exposure to intestinal conditions, with regard to gastric digesta. No significant differences between treatments were registered ($p > .05$). These results clearly showed the instability of ascorbic acid in the intestinal conditions. The alkaline pH, complexation with other constituents and some factors inherent to *in vitro* gastrointestinal digestion (*i.e.,* temperature, oxygen, light, enzyme activity) could enhance the ascorbic acid oxidation (Cilla et al., 2011b; Rodríguez-Roque et al., 2015). Similar results were found by Pérez-Vicente, Gil-Izquierdo, and García-Viguera (2002), who have reported ascorbic acid losses of 29% after the pepsin digestion of pomegranate juice, and additional 51% after intestinal digestion, leading to losses greater than 80% in the whole digestion process. Cilla et al. (2011b) reported that ascorbic acid content significantly decreased after *in vitro* digestion (16.3–56%) in the bioaccessible fraction of different fruit beverages.

As far as we know, this is the first study that investigates whether the concentration of ascorbic acid that is released from strawberry juices in the gastrointestinal tract and becomes available for absorption is affected by storage conditions. The ascorbic acid content of non-digested samples significantly decreased (30%) throughout storage, with no statistical differences between treatments (Fig. 3). As it was mentioned above, ascorbic acid is very susceptible to chemical and enzymatic oxidation. Therefore, it could be possible that ascorbic acid suffered oxidative reactions during two weeks of storage at 5 °C. Regarding gastric digesta, ascorbic acid content of all samples significantly decreased during storage (57% with respect to the ascorbic acid content obtained at day 0) and no statistical differences between treatments were found. On the other hand, the ascorbic acid content of all stored samples further decreased when exposed to simulated intestinal fluids (30% with respect to the ascorbic acid content obtained at day 0). Possibly, the storage of fiber-enriched strawberry juices favors the precipitation of the phenolic compounds to the cloud fraction (solids in

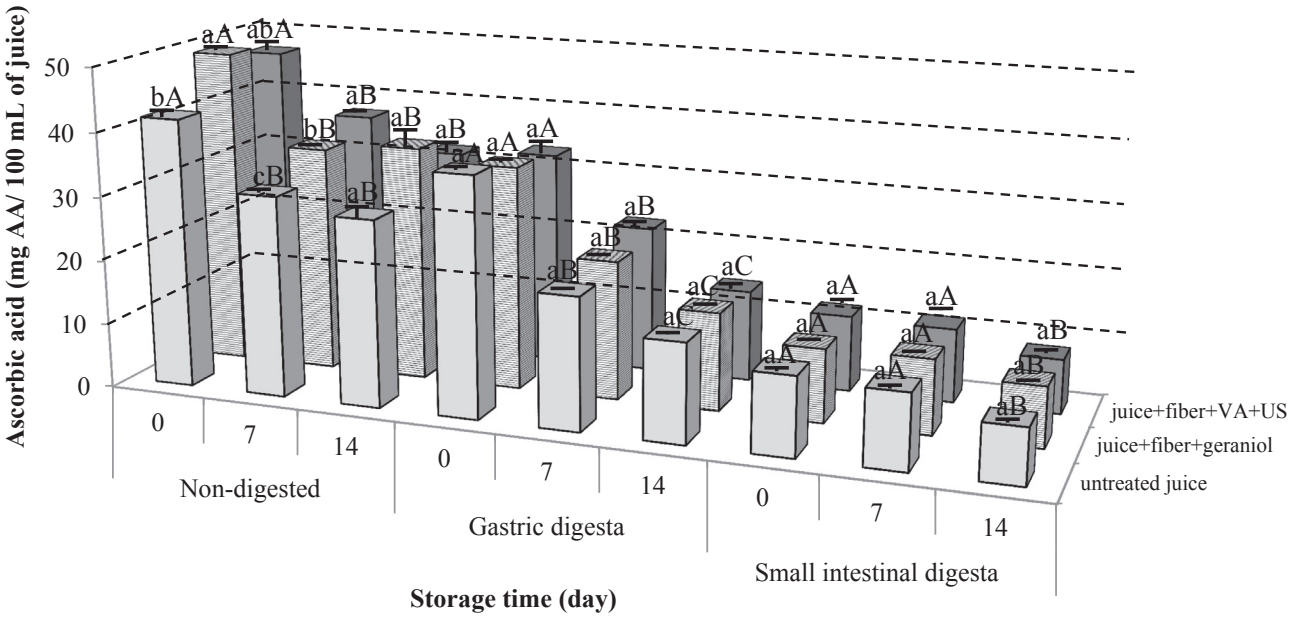

Fig. 3. Ascorbic acid content before (non-digested) and after exposure of stored strawberry juices (0, 7 and 14 days at 5 °C) to simulated gastric and intestinal conditions. Values with different lowercase letters in the same column indicate significant differences ($p < .05$) between treatments at each digestion stage, and values with different capital letters in the same row indicate significant differences ($p < .05$) through storage time. Light gray bars: untreated juices (without any addition or preservation treatment); striped bars: juices enriched with inulin and oligofructose and treated with geraniol (juice + fiber + geraniol); dark gray bars: juices enriched with inulin and oligofructose and treated with vanillin and ultrasound (juice + fiber + VA + US). AA: ascorbic acid.

suspension as a result of squeezing the strawberries without a further centrifugation) leading to an increase in viscosity and making the ascorbic acid molecules less accessible in the *in vitro* gastrointestinal model (Cilla et al., 2012). These results indicate that although the consumption of about 100 mL of fiber-enriched strawberry juices fulfills the vitamin C requirements at the moment of being consumed (FAO & WHO, 2001), only about 25% safely arrive to the gut. This underlines the importance of determining the concentration of health-related compounds that is released from strawberry juices in the gastrointestinal tract and becomes available for absorption.

4. Conclusion

The present work has shown that strawberry juices enriched with inulin and oligofructose and preserved with non-thermal techniques are a valuable source of antioxidant compounds. The *in vitro* digestion procedure provided a simple and rapid method to assess the stability of phytochemicals from fresh and stored fiber-enriched strawberry juices. Some loss of phytochemicals occurred during gastric digestion rather than in intestinal fluids. On the contrary, significant increases in the content of bioactive compounds were registered during small intestinal digestion. The antioxidant capacity after *in vitro* gastrointestinal digestion was significantly higher as determined using the TEAC assay than using the DPPH one. It is worth noting that as only small changes in the concentration of health-related compounds throughout storage were registered, phytochemicals are still bioaccessible at the end of the storage period. These results reveal that fiber-enriched strawberry juices preserved with non-thermal processing are an important source of bioaccessible health-related compounds. In this way, the applied preservation techniques demonstrated to be adequate alternatives to the traditional thermal treatments, not only because they are clean, and hence, environment friendly, but also because as they do not involve any thermal treatment, health-related compounds included in the matrices become more bioaccessible. Moreover, enriching strawberry juices with inulin and oligofructose was a good strategy to add value to the product, since no negative impact on the release of phytochemicals after the simulated gastrointestinal process was observed.

Acknowledgments

This work was supported by the Argentinean Agency for the Scientific and Technological Promotion (ANPCyT) (Projects PICT/2014/0912), Argentinean Research Council (CONICET) and National University of Mar del Plata. The authors acknowledge Mr. Claudio Reyes for technical assistance.

References

Bohn, T., Mc Gougall, G. J., Alegría, A., Alminger, M., Arrigoni, E., Aura, A.-M., ... Santos, C. N. (2015). Mind the gap-deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites—A position paper focusing on carotenoids and polyphenols. *Molecular Nutrition & Food Research, 59*, 1307–1323.

Bouayed, J., Hoffmann, L., & Bohn, T. (2011). Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chemistry, 128*(1), 14–21.

Cassani, L., Tomadoni, B., Moreira, M., Ponce, A., & Agüero, M. (2017). Optimization of inulin: Oligofructose proportion and non-thermal processing to enhance microbiological and sensory properties of fiber-enriched strawberry juice. *LWT-Food Science and Technology, 80*, 446–455.

Cassani, L., Tomadoni, B., Ponce, A., Agüero, M., & Moreira, M. (2017). Combined use of ultrasound and vanillin to improve quality parameters and safety of strawberry juice enriched with prebiotic fibers. *Food and Bioprocess Technology, 10*, 1–12.

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.

Cilla, A., Alegría, A., de Ancos, B. A., Sánchez-Moreno, C. N., Cano, M. P., Plaza, L., ... Barberá, R. (2012). Bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk-and soy-based fruit beverages: Influence of food matrix and processing. *Journal of Agricultural and Food Chemistry, 60*(29), 7282–7290.

Cilla, A., Bosch, L., Barberá, R., & Alegría, A. (2017). Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols. *Journal of Food Composition and Analysis*. http://dx.doi.org/10.1016/j.jfca.2017.01.009.

Cilla, A., Lagarda, M. J., Alegría, A., de Ancos, B., Cano, M. P., Sanchez- Moreno, C., ... Barberá, R. (2011a). Effect of processing and food matrix on calcium and phosphorous bioavailability from milk-based fruit beverages in Caco-2 cells. *Food Research International, 44*, 3030–3038.

Cilla, A., Perales, S., Lagarda, M. J., Barberá, R., Clemente, G., & Farré, R. (2011b). Influence of storage and in vitro gastrointestinal digestion on total antioxidant capacity of fruit beverages. *Journal of Food Composition and Analysis, 24*, 87–94.

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), 131–137.

FAO, & WHO (2001). *Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation.* Rome: Bangkok, Thailand Food and Nutrition Division, FAO.

Felgines, C., Talavéra, S., Gonthier, M.-P., Texier, O., Scalbert, A., Lamaison, J.-L., & Rémésy, C. (2003). Strawberry anthocyanins are recovered in urine as glucuro-and sulfoconjugates in humans. *The Journal of Nutrition, 133*(5), 1296–1301.

Gil-Izquierdo, A., Zafrilla, P., & Tomás-Barberán, F. A. (2002). An *in vitro* method to simulate phenolic compound release from the food matrix in the gastrointestinal tract. *European Food Research and Technology, 214*(2), 155–159.

Grimoud, J., Durand, H., Courtin, C., Monsan, P., Ouarné, F., Theodorou, V., & Roques, C. (2010). *In vitro* screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics. *Anaerobe, 16*(5), 493–500.

Häkkinen, S. H., Kärenlampi, S. O., Mykkänen, H. M., Heinonen, I. M., & Törrönen, A. R. (2000). Ellagic acid content in berries: Influence of domestic processing and storage. *European Food Research and Technology, 212*(1), 75–80.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W. P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common vegetables in Singapore. *Food Chemistry, 120*(4), 993–1003.

McDougall, G. J., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Assessing potential bioavailability of raspberry anthocyanins using an *in vitro* digestion system. *Journal of Agricultural and Food Chemistry, 53*(15), 5896–5904.

McDougall, G. J., Fyffe, S., Dobson, P., & Stewart, D. (2007). Anthocyanins from red cabbage–stability to simulated gastrointestinal digestion. *Phytochemistry, 68*(9), 1285–1294.

Nazzaro, F., Fratianni, F., Sada, A., & Orlando, P. (2008). Synbiotic potential of carrot juice supplemented with *Lactobacillus* spp. and inulin or fructooligosaccharides. *Journal of the Science of Food and Agriculture, 88*(13), 2271–2276.

Németh, K., Plumb, G. W., Berrin, J.-G., Juge, N., Jacob, R., Naim, H. Y., ... Kroon, P. A. (2003). Deglycosylation by small intestinal epithelial cell β-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition, 42*(1), 29–42.

Nicoli, M., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science & Technology, 10*(3), 94–100.

Parada, J., & Aguilera, J. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science, 72*(2), 21–32.

Pérez-Vicente, A., Gil-Izquierdo, A., & García-Viguera, C. (2002). *In vitro* gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. *Journal of Agricultural and Food Chemistry, 50*(8), 2308–2312.

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.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290–4302.

Quintana, G., Simões, M. G., Hugo, A., Alves, P., Ferreira, P., Gerbino, E., ... Gómez-Zavaglia, A. (2017). Layer-by-layer encapsulation of Lactobacillus delbrueckii subsp. Bulgaricus using block-copolymers of poly (acrylic acid) and pluronic for safe release in gastro-intestinal conditions. *Journal of Functional Foods, 35*, 408–417.

Rodríguez-Roque, M. J., de Ancos, B., Sánchez-Moreno, C., Cano, M. P., Elez-Martínez, P., & Martín-Belloso, O. (2015). Impact of food matrix and processing on the *in vitro* bioaccessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods, 14*, 33–43.

Rodríguez-Roque, M. J., Rojas-Graü, M. A. A., Elez-Martínez, P., & Martín-Belloso, O. (2013). Changes in vitamin C, phenolic, and carotenoid profiles throughout *in vitro* gastrointestinal digestion of a blended fruit juice. *Journal of Agricultural and Food Chemistry, 61*(8), 1859–1867.

Rößle, C., Brunton, N., Gormley, R. T., Wouters, R., & Butler, F. (2011). Alginate coating as carrier of oligofructose and inulin and to maintain the quality of fresh-cut apples. *Journal of Food Science, 76*(1), 19–29.

Tagliazucchi, D., Verzelloni, E., Bertolini, D., & Conte, A. (2010). *In vitro* bio-accessibility and antioxidant activity of grape polyphenols. *Food Chemistry, 120*(2), 599–606.

Tarko, T., Duda-Chodak, A., Sroka, P., Satora, P., & Michalik, J. (2009). Transformations of phenolic compounds in an *in vitro* model simulating the human alimentary tract. *Food Technology and Biotechnology, 31*(4), 456–463.

Tavera-Quiroz, M. J., Romano, N., Mobili, P., Pinotti, A., Gómez-Zavaglia, A., & Bertola, N. (2015). Green apple baked snacks functionalized with edible coatings of methylcellulose containing *Lactobacillus plantarum*. *Journal of Functional Foods, 16*, 164–173.

Velderrain-Rodriguez, G., Quiros-Sauceda, A., Mercado-Mercado, G., Ayala-Zavala, J. F., Astiazaran-Garcia, H., Robles-Sánchez, R. M., ... González-Aguilar, G. A. (2016). Effect of dietary fiber on the bioaccessibility of phenolic compounds of mango, papaya and pineapple fruits by an *in vitro* digestion model. *Food Science and Technology (Campinas), 36*(2), 188–194.

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.
Wootton-Beard, P. C., Moran, A., & Ryan, L. (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. *Food Research International, 44*(1), 217–224.