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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

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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

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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