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High pressure homogenization processing, thermal treatment and milk matrix affect *in vitro* bioaccessibility of phenolics in apple, grape and orange juice to different extents

Zhiyong He [a,*], Yadan Tao [a], Maomao Zeng [a], Shuang Zhang [a], Guanjun Tao [a], Fang Qin [a], Jie Chen [a,b,*]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China
[b] Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi, Jiangsu 214122, PR China

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ABSTRACT

The effects of high pressure homogenization processing (HPHP), thermal treatment (TT) and milk matrix (soy, skimmed and whole milk) on the phenolic bioaccessibility and the ABTS scavenging activity of apple, grape and orange juice (AJ, GJ and OJ) were investigated. HPHP and soy milk diminished AJ's total phenolic bioaccessibility 29.3%, 26.3%, respectively, whereas TT and bovine milk hardly affected it. HPHP had little effect on GJ's and OJ's total phenolic bioaccessibility, while TT enhanced them 27.3–33.9%, 19.0–29.2%, respectively, and milk matrix increased them 26.6–31.1%, 13.3–43.4%, respectively. Furthermore, TT (80 °C/30 min) and TT (90 °C/30 s) presented the similar influences on GJ's and OJ's phenolic bioaccessibility. Skimmed milk showed a better enhancing effect on OJ's total phenolic bioaccessibility than soy and whole milk, but had a similar effect on GJ's as whole milk. These results contribute to promoting the health benefits of fruit juices by optimizing the processing and formulas in the food industry.

1. Introduction

Fruit juices are consumed by people all over the world and represent an important part of the human diet. The World Health Organization (WHO) recommends having at least 400 g of fruit and vegetables per day (WHO, 2003). Fruit juices retain some nutritional characteristics of the fruits from which they are extracted. Accordingly, fruit juices are not only consumed worldwide for their convenience, but also due to their abundant bioactive compounds such as minerals, vitamins, carotenoids and phenolic compounds, which can increase the antioxidant capacity of plasma, inhibit platelet aggregation, and contribute to positive health outcomes in cases of hormone-dependent diseases such as prostate enlargement, breast, ovarian and endometrial carcinomas, cardiovascular diseases, cancers and stroke (Duffy et al., 2001; Geybels et al., 2013; Linnewiel-Hermoni et al., 2015; Papadopoulou & Frazier, 2004; Scalbert, Morand, Manach, & Rémésy, 2002; Scalbert & Williamson, 2000; Sreekumar, Sithul, Muraleedharan, Azeez, & Sreeharshan, 2014; Thomson & Thompson, 2013; Touvier, Kesse, Volatier, Clavel-Chapelon, & Boutron-Ruault, 2006). Among these bioactive substances, the phenolic compounds may have a major contribution to the health benefits of fruit juices, due to their numerous biological functions such as antioxidant and free radical scavenging activity (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013a). The main phenolics in fruit juices include flavonoids such as quecetin, catechin and anthocyanins, and phenolic acids such as coumaric acid, gallic acid, chlorogenic acid and caffeic acid (Mullen, Marks, & Crozier, 2007).

It is well known that the bioactive polyphenols must be released from the food matrix to exert biological effects on human health. In this respect, the phenolic bioaccessibility is defined as the amount of the ingested polyphenols that are available for absorption in the gut after digestion (Ahmad-Qasem et al., 2014), while the phenolic bioavailability is defined as the fraction of polyphenols secreted into circulation that is available for tissue uptake and metabolism (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2014). The overall bioavailability process includes matrix-release, gastrointestinal digestion (bioaccessibility), absorption, and metabolism. Thus, in the gastrointestinal tract, phenolic compounds may be released from the food matrix and modified under the influence of digestive enzymes and pH changes (Podsędek, Redzynia, Klewicka, & Koziołkiewicz, 2014). Furthermore, it is important to quantify the bioaccessible

* Corresponding authors at: State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China (J. Chen).
E-mail addresses: zyhe@jiangnan.edu.cn (Z. He), chenjie@jiangnan.edu.cn (J. Chen).

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fraction of the ingested bioactive phenolic compounds during gastrointestinal digestion (Wootton-Beard, Moran, & Ryan, 2011). Studies involving the use of animals or human volunteers (*in vivo*) in evaluating the phenolic bioaccessibility are costly and invariably introduce issues of credibility and acceptability due to the ethical consideration. The factors affecting the digestive stability and antioxidant activity of bioactive compounds were assessed by an *in vitro* digestion approach as an alternative method to *in vivo* that was simple, inexpensive, rapid, and reproducible (Stinco et al., 2012).

The bioaccessibility of bioactive substances is affected by different factors such as food processing and the food matrix. Thermal treatment (TT) and high pressure homogenization processing (HPHP) are two commonly used unit operations in the fruit juice industry for extending shelf life and obtaining high-quality products. However, these processes are known to lead to the loss of the health related ingredients and antioxidant capacity of juices (Knockaert et al., 2012). However, previous studies have reported that TT and HPHP can improve the bioaccessibility of carotenoids, lycopene, calcium, and phosphorous by reducing the particle size distribution and inducing a high degree of cell-wall rupture (Bengtsson, Brackmann, Enejder, Alminger, & Svanberg, 2010; Cilla et al., 2011; Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010; Gupta, Kopec, Schwartz, & Balasubramaniam, 2011; Stinco et al., 2012). Furthermore, in response to the growing potential market for fruit juices, new fruit-related products have been designed. Among the new products, fruit juice-milk beverages stand out as products that have enhanced nutritional and organoleptic characteristics compared to fruit juice alone. In these fruit juice-milk beverages, the different matrixes such as whole milk, skimmed milk, and soy milk form distinct environments for bioactive fruit components and play an important role in the release and absorption of the nutrients (tocopherols, carotenoids, and ascorbic acid) (Cilla et al., 2012; Rodríguez-Roque et al., 2013a).

Although the influence of the digestive process on the active substances such as anthocyanins (Liang et al., 2012), isoflavones (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013b) and polyphenols (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007) in fruit juice has been studied, and several reports also have examined the effects of milk on the bioaccessibility of tea polyphenols (Moser, Chegeni, Jones, Liceaga, & Ferruzzi, 2014; van der Burg-Koorevaar, Miret, & Duchateau, 2011; Xie, Kosińska, Xu, & Andlauer, 2013). However, to the best of our knowledge, few studies have evaluated the effects of food processing (TT and HPHP) and the food matrix (whole, skimmed and soy milk beverages) on the *in vitro* bioaccessibility of the bioactive phenolic compounds in fruit juices. Therefore, the aim of this study was to evaluate the total polyphenol content, total antioxidant capacity, and the type and content of individual polyphenols in three familiar fruit (apple, grape and orange) juices subjected to processing (TT and HPHP) and the food matrix (soy, whole and skimmed milk) on the basis of *in vitro* gastrointestinal digestion to determine their bioaccessibility.

2. Materials and methods

2.1. Chemicals

Standards of the phenolic compounds including quercetin, naringenin, naringin, hesperidin, hesperetin, phloridzin, luteolin, proanthocyanidin, caffeic acid, gallic acid, chlorogenic acid, epicatechin and epigallocatechin-3-gallate (EGCG), HPLC-grade acetonitrile, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carbonsaure (Trolox) and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) were purchased from J&K Scientific Co., Ltd. (Beijing, China). Folin–Ciocalteu's phenol reagent, pepsin (porcine gastric mucosa, ⩾400 units/mg protein), pancreatin (porcine pancreas, 4× USP specifications), lipase and bile salt were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample preparation

Apple, grape and orange fruits were purchased at a local farmers market (Wuxi, China) in October 2013. Freshly-squeezed apple juice (AJ), grape juice (GJ) and orange juice (OJ) were obtained using a domestic squeezer (SJ3039, Supor, Zhejiang, China) and filtered through 2 mm filter gauzes. The bovine whole milk (W), skimmed milk (S), and soy milk (Sy) powder products were purchased at a local supermarket (Wuxi, China). The nutritional composition of these commercial milks was as follows: fat, 28% (W), 2% (S), and 1% (Sy); protein, 24% (W), 33% (S), and 80% (Sy); carbohydrates, 37% (W), 54% (S), and 1% (Sy); and calcium, 0.92% (W), 2.4% (S), and 0.12% (Sy).

To study the effect of the milk matrix on the phenolic bioaccessibility of the fruit juices, three formulations of fruit juice-milk beverages were prepared for each fruit juice by mixing 94.8% (w/w) of fruit juice and 4.2% (w/w) of whole milk, 96% (w/w) of fruit juice and 3% (w/w) of skimmed milk, or 97.7% (w/w) of fruit juice and 1.3% (w/w) of soy milk, so the protein content in the beverages was around 1%. Citric acid (1%) was added to the beverages as a preservative. The nine beverage samples were named as follows: AJ-W, AJ-S, AJ-Sy, GJ-W, GJ-S, GJ-Sy, OJ-W, OJ-S, and OJ-Sy. All of the samples were vacuum-packed in aluminum foil bags and kept frozen at −80 °C until analysis.

To evaluate the effect of HPHP on the phenolic bioaccessibility, AJ, GJ and OJ were high-pressure homogenized at 250 MPa for 10 min using a homogenizer (Nano homogenize machine, ATS Engineering Inc., Canada). The processed fruit juice samples, named AJ-HPHP, GJ-HPHP and OJ-HPHP, were collected and quickly cooled in ice water, then stored at −80 °C until analysis.

To investigate the effect of TT on the phenolic bioaccessibility, AJ, GJ and OJ were heated at 80 °C for 30 min (T80) and 90 °C for 30 s (T90) in a water bath (Typ003-2391, Thermo Electron (Karlsruhe) Gmbh, Karlsruhe, Germany), which served as equivalents for the mild and intense thermal pasteurization processes, respectively. The heated samples, named AJ-T80, AJ-T90, GJ-T80, GJ-T90, OJ-T80 and OJ-T90, were then quickly cooled in ice water and kept at −80 °C for further analysis.

All the treatments including HPHP, TT and milk matrix for each fruit juice were repeated three times. The original fruit juices treated without HPHP, TT and the addition of milk were acted as the controls, and named as AJ-C, GJ-C and OJ-C.

2.3. In vitro gastrointestinal digestion

A simulation of the physiological situation of *in vitro* gastrointestinal digestion in the human stomach and intestine was carried out according to a previous publication (Wootton-Beard et al., 2011). For the gastric digestion, 20 ml of thawed fruit juice samples and 3 ml pepsin solution (40 mg/ml) were mixed in a 50 ml tube and adjusted to a total of 30 ml by adding 0.9% NaCl. The mixtures were adjusted to pH 2 with 0.1 M HCl, then flushed with N_2 and incubated at 37 °C for 1 h in a shaking water bath (Shz-82, Xinhang, Jintan, China) at 120 strokes per minute. The gastric digests were maintained in ice for 10 min to stop the pepsin digestion. For the pancreatic digestion, the pH of the gastric digests was adjusted to 5.3 by adding 0.1 M $NaHCO_3$, followed by the addition of 4.5 ml of bile solution (24 mg/ml) and pancreatin-lipase solution