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In vitro bioaccessibility of health-related compounds as affected by the formulation of fruit juice- and milk-based beverages

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ABSTRACT

The purpose of this research was to evaluate the influence of the beverage formulation on the in vitro digestibility and bioaccessibility of phenolic compounds, vitamin C, and carotenoids, as well as antioxidant activity from milk, a blended fruit juice (BFJ) and a combination of both of them (BFJ–MB). The release of many phenolic substances was improved during gastric digestion of milk and BFJ–MB (around 5 and 75%), but not in BFJ. Vitamin C and carotenoids diminished significantly ($P < 0.05$) in the gastric and intestinal digesta of each beverage. Phenolic acids, flavonoids, vitamin C and hydrophilic constituents with antioxidant activity were more bioaccessible in BFJ (up to 3.4 times) than in milk and BFJ–MB. On the contrary, the bioaccessibility of carotenes, xanthophylls and those compounds with lipophilic antioxidant activity was improved when milk was added to BFJ (up to 1.9 times). Results suggest that the addition of milk improved the bioaccessibility of lipophilic constituents but not that of hydrophilics. Nevertheless, BFJ–MB combines the nutritional ingredients of milk and BFJ. As a result, BFJ–MB could supply a higher diversity of bioaccessible compounds in comparison to that of milk and BFJ alone, promoting health and protecting against several diseases.

1. Introduction

Nowadays, there is a clear trend toward consumption of food that beyond nutritional value, improve health and well-being, reducing the risk of disease. These products are usually known as functional food (Howlett, 2008) and their potential market is currently growing. In fact, new functional food and beverages have been designed in order to satisfy the demand of consumers, standing out the fruit juice- and milk-based beverages.

Fruit juices are considered as the main dietary sources of bioactive substances, such as vitamins, phenolic compounds and carotenoids, which reduce the risk of cardiovascular and neurodegenerative diseases, as well as some cancer types (Aboul-Enein, Berczynski, & Kruk, 2013; Gülçin, 2012). On the other hand, milk contains proteins (essential amino acids), fat (unsaturated fatty acids), vitamins (mainly, A and E), carotenoids (mainly β-carotene), and minerals (Antone, Sterna, & Zagorska, 2012; Claeys et al., 2013). Therefore, both fruit juices and milk possess a high nutritional value and represent a good option to obtain beverages with functional properties.

Nevertheless, the knowledge of the quantity of nutrients contained in the food itself is not enough to attribute functional properties. The most important feature is the proportion of these compounds that is available to exert their biological function. Bioaccessibility corresponds to the fraction of bioactive substance that is released from the food matrix after digestion and solubilized into the gut lumen for uptake in the intestinal mucosa (Ferruzzi, 2010). Bioavailability is defined as the fraction of nutrient secreted into circulation that is available for tissue uptake and metabolism. In this context, in vitro gastrointestinal digestion is usually utilized to assess the bioaccessibility of food constituents and represents an easy and fast approach to in vivo trials (Failla & Chitchumroonchokchai, 2005).

There are some studies assessing the in vitro bioaccessibility of bioactive compounds in simple beverages. For instance, Gil-Izquierdo, Gil, Ferreres, and Tomás-Barberán (2001) analyzed the bioaccessibility of phenolic compounds in orange juice. Pérez-Vicente, Gil-Izquierdo, and García-Viguera (2002) studied the bioaccessibility of pomegranate juice phenolic compounds, anthocyanins and vitamin C. Rodríguez-Roque, Rojas-Graü, Elez-Martínez, and Martín-Belloso (2013b) evaluated the changes in the concentration of vitamin C, phenolic compounds and carotenoids throughout an in vitro gastrointestinal digestion of a blend of orange, pineapple and kiwi juices. However, information on the bioaccessibility of bioactive compounds in heterogeneous food matrices is still very limited. In this sense, Cilla et al. (2012) investigated the bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk- and soy-based fruit beverages. Rodríguez-Roque,

Abbreviations: BFJ, blended fruit juice; BFJ–MB, blended fruit juice–milk beverage; DPPH, 1,1-diphenyl-2-picrylhydrazyl; F–C, Folin–Ciocalteu; HAA, hydrophilic antioxidant activity; HPLC, high-performance liquid chromatography; LAA, lipophilic antioxidant activity; TPC, total phenolic compounds.

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Rojas-Graü, Elez-Martínez, and Martín-Belloso (2014) evaluated the bioaccessibility of vitamin C, phenolic compounds, isoflavones and carotenoids from a blended fruit juice–soymilk beverage. Granado-Lorencio, Herrero-Barbudo, Blanco-Navarro, Pérez-Sacristán, and Olmedilla-Alonso (2009) analyzed the bioaccessibility of carotenoids and α-tocopherol from fruit juices in the presence of absorption modifiers, such as milk. The extent to which these compounds could be available for absorption depends on their stability, interactions with other compounds and the type of food matrix where these substances form part (Rein et al., 2013). Therefore, the aim of this study was to evaluate the influence of the beverage formulation on the in vitro digestibility and bioaccessibility of phenolic compounds, vitamin C, carotenoids, as well as antioxidant activity from milk, a blended fruit juice (BFJ) containing orange, kiwi, pineapple, and mango juices, and a blended fruit juice–milk beverage (BFJ–MB).

2. Material and methods

2.1. Reagents

Pepsin from porcine stomach, pancreatin from porcine pancreas, bovine bile, phenol standards (caffeic, chlorogenic, p-coumaric, ferulic, sinapic and 4-hydroxybenzoic acids; hesperidin, naringenin, quercetin, rutin, and [+]-catechin), carotenoid standards (α-carotene, β-carotene, zeaxanthin, lutein, α-cryptoxanthin and β-cryptoxanthin), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and cellulose dialysis membrane (molecular weight cutoff of 12,000 Da) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid and Folin–Ciocalteu (F–C) reagent were acquired from Scharlau Chemie S.A. (Barcelona, Spain).

2.2. Beverage preparation

Whole cow's milk was purchased at a local supermarket (Lleida, Spain). According to manufacturer, it contained 3.6% of fat, 3.0% of protein and 4.5% carbohydrates. In addition, the pH (Crison Instruments S.A., Alella, Barcelona, Spain) and the soluble solid content (Comecta S.A., Abrera, Barcelona, Spain) were assessed, resulting in 6.75 ± 0.06 and 11.83 ± 0.29 °Brix, respectively.

Orange, kiwi, pineapple and mango fruits were purchased at commercial maturity in a local supermarket (Lleida, Spain). Fruits were washed, peeled and the juice extracted. Each fresh-squeezed juice was filtered through a cheese cloth using a vacuum pump. A blended fruit juice (BFJ) was obtained by mixing 40% of orange, 33% of kiwi, 13.5% of pineapple and 13.5% of mango juices. The pH of BFJ was 3.38 ± 0.04 and the soluble solid content 11.0 ± 0.10 °Brix.

The blended fruit juice–milk beverage (BFJ–MB) was manufactured by mixing 75% of BFJ, 17.5% of milk and 7.5% of sugar. The beverage was filtered through a cheese cloth using a vacuum pump. The pH of BFJ–MB was adjusted to 3.3 ± 0.05 with citric acid if necessary, and the soluble solid content was assessed (17.5 ± 0.03 °Brix). The formulation of the beverage was selected based on a previous study where the combination of these fruit juices with milk displayed a high concentration of vitamin C, as well as antioxidant activity and stability (Salvia-Trujillo, Morales-De La Peña, Rojas-Graü, & Martín-Belloso, 2011).

2.3. In vitro gastrointestinal digestion

The in vitro gastrointestinal digestion was carried out following the methodology described by Rodríguez-Roque et al. (2013b). This procedure consisted of gastric digestion (pH 2, containing pepsin) and small intestinal digestion (pH 7, containing a pancreatin-bile mixture). Small intestinal digestion included dialyzed (bioactive compounds of inside the dialysis membrane) and micellar fractions (centrifugation of small intestinal digesta at 5000 rpm for 20 min), which contained the bioaccessible hydrophilic and lipophilic compounds, respectively.

Aliquots were collected at the end of each digestive phase and immediately placed in a cold water bath during 10 min. All samples were frozen (−45 °C) until analysis.

2.4. Bioactive compound analysis

2.4.1. Phenolic compounds analyzed by HPLC

Extraction, separation, identification and quantification of phenolic compounds were performed using the methodology reported by Rodríguez-Roque, Rojas-Graü, Elez-Martínez, and Martín-Belloso (2013a). Individual phenolic compounds were identified by comparison of their retention time and spectra with those of the standards (caffeic, chlorogenic, ferulic, p-coumaric, sinapic and 4-hydroxybenzoic acids; hesperidin, naringenin, quercetin, rutin, and [+]-catechin). Quantification was carried out by integration of the peak areas and using calibration curves. Results were expressed as mg of phenolic compound/100 mL of sample. Total phenolic compounds were calculated as the sum of individuals (TPC by HPLC).

2.4.2. Total phenolic content analyzed by Folin–Ciocalteu (TPC by F–C) methodology

Total phenolic compounds were determined according to the methodology of Rodríguez-Roque et al. (2013a), using the colorimetric method previously described by Singleton, Orthofer, and Lamuela-Raventós (1998). A calibration curve of gallic acid was used to quantify the concentration of total phenolic compounds in each sample. Results were expressed as mg of gallic acid/100 mL of sample.

2.4.3. Vitamin C

Vitamin C was extracted, separated, identified and quantified (by HPLC) according to the methodology reported by Rodríguez-Roque et al. (2013b). Vitamin C identification was carried out by comparison of its retention time and spectra with the standard (ascorbic acid). Results were expressed as mg of ascorbic acid/100 mL of sample.

2.4.4. Carotenoids

Carotenoids were extracted, separated, identified and quantified (by HPLC) following the methodology reported by Rodríguez-Roque et al. (2013b). Each carotenoid was identified by comparison of its retention time and spectra with the standards (α-carotene, β-carotene, zeaxanthin, lutein, α-cryptoxanthin and β-cryptoxanthin). Quantification was carried out by integration of the peak areas and using calibration curves. Cis-violaxanthin + neoxanthin, cis-antheraxanthin and anteraxanthin were identified according to the retention time and spectra reported in the literature and they were quantified through the calibration curve of zeaxanthin. Results were expressed as μg of carotenoid/100 mL of sample.

2.5. Hydrophilic and lipophilic antioxidant activity

Extraction of hydrophilic and lipophilic fractions of non-digested or digested samples, as well as antioxidant activity, was performed according to the procedure of Rodríguez-Roque et al. (2013a). The determination of the antioxidant activity of extracts was based on a colorimetric method (DPPH radical) reported by Brand-Williams, Cuvelier, and Berset (1995). Results were expressed as percentage of DPPH inhibition.

2.6. Bioaccessibility calculations

Bioaccessibility was determined as the ratio between the concentration of bioactive compound in the dialyzed (for phenolic compounds and vitamin C, as well as hydrophilic compounds with antioxidant activity) or micellar (for carotenoids and lipophilic compounds with