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Effect of processing and food matrix on calcium and phosphorous bioavailability from milk-based fruit beverages in Caco-2 cells

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ABSTRACT

Calcium and phosphorus bioavailability from different milk-based (whole milk, skimmed milk and soya milk) fruit beverages as affected by thermal treatment (TT) and high-pressure processing (HPP) was determined by means of the paired *in vitro* gastrointestinal digestion (solubility method)/Caco-2 cell model. Ca bioaccessibility was significantly higher in HPP (98.4% ± 1.6%) versus TT (91.3% ± 1.9%), but Ca bioavailability was equal in all different matrixes independently of the processing treatment used. HPP samples improved P bioaccessibility (98.7% ± 2.5% versus 87.3% ± 2.2%) and P bioavailability by Caco-2 cells versus TT samples—soya milk- and whole milk-based beverages being the samples with the highest bioavailability values (56.8% ± 1.3% and 40.1% ± 9.9% versus 15.0% ± 2.1% and 16.8% ± 2.8%, respectively). Therefore, HPP improves Ca and P bioaccessibility, and P bioavailability versus TT samples, and can be used as an alternative to TT in the manufacture of functional foods with improved nutritional value and health benefits.

1. Introduction

Calcium is an essential micronutrient required for biological functions in the body such as nerve conduction, muscle contraction, mitosis, blood coagulation, and structural support of the skeleton (Miller, Jarvis & McBean, 2001). Insufficient calcium uptake results in diseases such as rickets in children and osteoporosis in the elderly (Guénguen & Pointillart, 2000). Phosphorus, in turn, is also an important element of bone and is essential for the growth of organisms, the synthesis of DNA and RNA, and the accumulation and transmission of energy. Deficiency of this mineral is uncommon and is related to muscular weakness and bone status alteration (Pérez, Garaulet, Gil & Zamora, 2005). Due to the close relationship between both minerals in bone formation and maintenance, their intake in 1:1 proportion is recommended (Pérez et al., 2005).

In the current context of functional foods, fruit beverages are often commercially supplemented with milk to provide bioactive components such as vitamin C, carotenoids and phenolic compounds (from the fruit), and to improve nutritional value derived from proteins and minerals such as Ca and P (from the milk). In this sense, for both minerals, and besides their nutritional value, there are Health Claims related to bone and teeth health in fruit drinks with a calcium content of 50 mg/100 g (EFSA, 2009a) and products that contain phosphorus to at least 15% of the recommended dietary allowance (RDA) (EFSA, 2009b). Thus, they can be considered natural functional ingredients within beverages of this kind. In addition, the use of soya milk in the elaboration of beverages of this type can be an alternative to milk for vegetarians and people who are allergic to protein and intolerant to lactose in cow's milk (Bosscher, Van Dyck, Robberecht, Van Caillie-Bertrand & Deelstra, 1998), although it contains much less calcium than cow's milk (20–30 mg/100 mL versus 120 mg/100 mL) (Chaiwanon, Puwastien, Nitithamyong & Sirichakwal, 2000).

Nowadays, functional foods have made use of innovations in food technology. In this sense, pasteurization has been found to imply few losses of minerals in foods (Watzke, 1998). In line with this, no reduction has been reported in Ca solubility due to the pasteurization of skim milk used for the production of either liquid or powder milk infant formulas (Michel, Lavigne & Desrosiers, 1993). On the other hand, non-thermal processing such as high-pressure processing (HPP) recently has been identified as a useful tool for extending the shelf-life and quality as well as for preserving the nutritional and functional characteristics mainly of fruit and vegetable products, evaluating vitamins C, A and E, carotenoids, flavonoids and total antioxidant capacity (Sánchez-Moreno, De Ancos, Plaza, Elez-Martínez & Cano, 2009). However, it would be interesting to determine how this new technology can modulate the bioavailability of minerals. Applying emerging technologies (i.e., high-pressure techniques) as an alternative to traditional heat processing would

Abbreviations: TT, thermal treatment; HPP, high-pressure processing; J, fruit juices; W, whole milk; S, skimmed milk; Sy, soya milk; NT, non-treated; TEER, transepithelial electrical resistance.

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be more valuable if nutritional quality is considered not only as a stability issue but also as a bioavailability concern, since the bioavailability of nutrients can be increased with this sort of processing, making the final product more nutritious (Fernández-García, Carvajal-Lérida & Pérez-Gálvez, 2009).

In vitro methods for assessing bioavailability constitute good alternatives to *in vivo* procedures and are generally based, in a first step, on the simulation of gastrointestinal digestion followed by the determination of calcium (Roig, Alegría, Barberá, Farré & Lagarda, 1999) or phosphorus (Miquel, Alegría, Barberá & Farré, 2004) bioaccessibility (soluble fraction). However, these methods have been improved by the incorporation of a human colon carcinoma cell line (Caco-2) with many of the functional and morphological properties of mature human enterocytes (Pinto et al., 1983). This paired model combining *in vitro* digestion (solubility method)/Caco-2 cells has been applied to determine calcium bioavailability in infant formulas (Jovaní, Barberá, Farré & Martín de Aguilera, 2001), milk-based formulas and fruit beverages containing milk and cereals (Perales, Barberá, Lagarda & Farré, 2005), and milk with/without calcium fortification (Perales, Barberá, Lagarda & Farré, 2006). However, as far as we are aware, the *in vitro* bioavailability of phosphorus, as well as comparison of the effect of pasteurization and high-pressure processing on calcium and phosphorus bioavailability in Caco-2 cells have not been investigated to date. To our knowledge, this latter topic has only been reported for calcium and phosphorus in ewe's and cow's milk (pasteurization) (De la Fuente, Olano & Juárez, 1998) and in goat's and cow's milk (pasteurization and high-pressure) (De la Fuente, Olano, Casal & Juárez, 1999), but without taking bioavailability into account.

Therefore, the aim of the present study was to determine calcium and phosphorus bioavailability from different milk-based fruit beverages as affected by pasteurization and high-pressure processing, on the basis of *in vitro* gastrointestinal digestion and solubility values and the estimation of mineral uptake and transport by Caco-2 cells.

2. Materials and methods

2.1. Reagents

Pepsin (Porcine, catalog no. P-7000, 975 units/mg protein), pancreatin (Porcine, catalog no. P-1750, activity equivalent to 4×USP specifications), bile extract (Porcine, catalog no. B-8631), magnesium sulfate and glucose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM + GlutaMAX™-I) containing 1 g/L glucose and pyruvate, fetal bovine serum, nonessential amino acids, HEPES, penicillin/streptomycin, fungizone, phosphate buffered solution (PBS) and trypsin-EDTA solution (2.5 g/L trypsin and 0.2 g/L EDTA) were purchased from Gibco (Scotland, UK). Sodium chloride, potassium chloride, standard reference solution of calcium 1000 mg/L ($CaCl_2$ in 6.5% HCl), lanthanum oxide and sulfuric acid (95–97%, sp. gr. 1.84) were purchased from Merck (Darmstadt, Germany). L-(+)-ascorbic acid (99%), ammonium molybdate-4-hydrate (99%), monobasic potassium phosphate (99–101%) and anhydrous citric acid PA-ACS were purchased from Panreac Química (Barcelona, Spain). Distilled deionized water of 18.2 MΩ cm resistivity was from Millipore Iberica S.A. (Barcelona, Spain).

2.2. Samples

A total of 9 different beverages have been studied, all of them containing a mixture of fruit juices (J) [orange (30% w/v), pineapple (10% w/v), kiwi (25% w/v) and mango (10% w/v)], anhydrous citric acid PA-ACS (1% w/v) and white sugar (7.5% w/v), and differing in the source of commercial pasteurized refrigerated milk (16.5% w/v), freshly prepared [whole milk (W) and skimmed milk (S) (La Priégola, Madrid, Spain)] and soya milk (Sy) (Yosoy, Mercadona, Spain), and the treatment received (non-treated (NT), high-pressure processing (HPP) and thermally treated (TT)): JW-NT, JW-HPP, JW-TT, JS-NT, JS-HPP, JS-TT, JSy-NT, JSy-HPP and JSy-TT. In terms of comparing the HPP and TT treatments, commercial pasteurized refrigerated milks with a mild thermal treatment (72 °C/15 s) have been considered as control products.

Fresh-squeezed juices were obtained from oranges (cv Valencia Late, Valencia, Spain), kiwis (cv Hayward, New Zealand), pineapples (cv MD2 or SuperSweet, Costa Rica) and mangos (cv Keitt, Ecuador) purchased in a local supermarket in December 2009. Orange juices were obtained using a domestic squeezer (Lomi model 4, Madrid, Spain) and filtered through 2 mm steel sieves. Kiwi, mango and pineapple juices were obtained using a domestic liquidizer (Braun MP80 Multipress, Barcelona, Spain). After that, the source of milk [(whole milk and skimmed milk (Priegola) or soya milk (Soja Yosoy))], citric acid and sugar were added to them and mixed. Four aliquots of 250 mL of each fruit beverage were vacuum packed in flexible Doypack plastic bags (polyskin XL, Amcor Flexibles Hispania, Granollers, Spain) and then processed or not. Finally, samples were kept frozen at −20 °C until analysis.

2.3. Sample processing

2.3.1. High-pressure processing (HPP)

Fruit beverages vacuum packed in flexible Doypack bags were introduced in the pressure unit filled with pressure medium (water). High-pressure processing (HPP) at 400 MPa was performed in a hydrostatic pressure unit with 2350 ml capacity, a maximum pressure of 500 MPa and a potential maximum temperature of 95 °C (GEC Alsthom ACB 900 HP, Type ACIP No. 665, Nantes, France). Before pressurization, the pressure chamber was heated/cooled to a desired level by means of a thermostat jacket connected to a water bath. Compression and decompression rates were 2.5 MPa/s. Samples were processed at 36 °C with a holding time of 5 min at 400 MPa. Because of adiabatic compression, the maximum temperature in the vessel was 40 °C at 400 MPa. Pressure, time and temperature were controlled by a computer program, being constantly monitored and recorded during the process. According to preliminary results of the group, these processing conditions were selected due to their effect on enzymatic inactivation (Cano, Hernández & De Ancos, 1997) and microbial reduction (Muñoz, De Ancos, Sánchez-Moreno & Cano, 2007).

2.3.2. Thermal treatment (TT)

Fruit beverages were heated at 90 °C for 30 s in the same packages used for high-pressures processing, quickly achieving uniform heat. Then samples were cooled to room temperature. Treatment was carried out in an autoclave (Autotester-G, Selecta, Barcelona, Spain). TT treatment was selected among different thermal treatments according to results of a previous study (Sánchez-Moreno, Plaza, Elez-Martínez, De Ancos, Martín-Belloso & Cano, 2005).

2.4. In vitro simulated gastrointestinal digestion

An *in vitro* gastrointestinal digestion procedure mimicking the physiological conditions in the upper digestive tract (stomach and small intestine) was used according to Cilla, Perales, Lagarda, Barberá and Farré (2008).

Briefly, 80 g of fruit beverages were adjusted to pH 2.0 with 6 M HCl (GLP 21 pH-meter, Crison, Barcelona, Spain). The pH was checked after 15 min, and if necessary readjusted to 2.0. Then an amount of freshly prepared demineralized pepsin solution sufficient to yield 0.02 g pepsin/g sample was added. The samples were made up to 100 g with cell culture-grade water (Aqua B Braun, Braun Medical, Barcelona, Spain), and incubated in a shaking water-bath at 37 °C/120 strokes per minute for 2 h (SS40-2, Gran Instruments, Cambridge,