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Antioxidant effect derived from bioaccessible fractions of fruit beverages against H_2O_2-induced oxidative stress in Caco-2 cells

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Abstract

This work evaluates the effect of bioaccessible fractions from fruit beverages against oxidative stress (OS) in Caco-2 cells. A fruit beverage (grape + orange + apricot) (with/without milk and/or iron/zinc) was subjected to *in vitro* gastrointestinal digestion, and bioaccessible fractions were incubated with Caco-2 cell cultures. Following preincubation, OS was induced with 5 mM H_2O_2. Intracellular reactive oxygen species (ROS), mitochondrial potential ($\Delta\psi_m$), mitochondrial metabolism (MTT test), intracellular reduced glutathione (GSH) and superoxide dismutase activity (SOD) were measured. The data evidenced viable cultures with increased mitochondrial metabolism and GSH-Rd activities, without alteration in SOD activity. Accordingly, more preserved mitochondrial integrity was also evidenced, allowing the action of antioxidant systems in preincubated cultures. Based on these data, we can conclude that a cytoprotective effect is derived from bioaccessible fractions of fruit beverages, though this effect failed to prevent intracellular ROS accumulation in Caco-2 cell cultures exposed to 5 mM H_2O_2.

Keywords: Oxidative stress; Caco-2; Fruit beverages; Milk; Minerals

1. Introduction

Fruit beverages are often commercially supplemented with milk, vitamins and/or minerals to improve their nutritional value and to provide bioactive food components with beneficial and healthy effects. It is widely known that fruits (Serafini, Bellocco, Wolk, & Ekström, 2002), and citrus juices (Vinson et al., 2002) possess many components, such as ascorbic acid, carotenoids, α-tocopherol and a wide range of polyphenols and flavonoids, with antioxidant potential. It has been postulated that dietary antioxidants can scavenge reactive oxygen species (ROS) in the body, lowering oxidative stress involved in tissue damage, accelerated aging and chronic degenerative diseases, such as cancer and cardiovascular disorders (Boyle & Langman, 2000; Ferro-Luzzi & Branca, 1995; Serafini et al., 2002; Vinson et al., 2002). Therefore, the consumption of fruit beverages, that are often supplemented with milk as source of functional bioactive peptides and fortified with minerals, could be helpful in complying with the dietary intake recommendations related to oxidative stress. Based on data for daily intakes in Spain (Mercasa, 2006), the annual mean *per capita* consumption of fruit beverages was estimated to be 18.4 l, of which 1.4, 3.9 and 4.7 l corresponded to grape, orange and apricot juices, respectively.

Most recent research on antioxidants, has evaluated the potential total antioxidant capacity (TAC) of pure components or food extracts and dietary supplements in aqueous solutions (Ou, Hampsch-Woodill, & Prior, 2001; Prior et al., 2003; Re et al., 1999). Although, the measured TAC values show possible interactions among different antioxidants and contribute relevant information to better understand the potential protective effects of food components (Manna et al., 2002), these studies lack a biological system with functions that can be impaired by oxidative stress. Furthermore, the protective effects of natural occurring

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antioxidants against oxidative stress using Caco-2 cells (a human colon cancer cell line) have been well documented in several studies. Most of these studies have monitored the antioxidant effects of carotenoids (Bestwick & Milne, 2000), flavonoids (Yokomizo & Moriwaki, 2006), and phenolic apple juice extract (Schaefer, Baum, Eisenbrand, & Janzowski, 2006) upon the accumulation of ROS. These investigations have shown that standard solutions of β-carotene fail to inhibit the ROS accumulation (Bestwick & Milne, 2000), and that phenolic compounds can also exert prooxidant activities (Schaefer et al., 2006; Yokomizo & Moriwaki, 2006). From a metabolic point of view, carotenoids exhibit a cytoprotective effect, increasing antioxidant enzyme activities (Bestwick & Milne, 2000), and both fresh and packaged orange juice increase mitochondrial enzyme activities (Ekmekcioglu, Strauss-Blasche, Leibetseder, & Marktl, 1999). Using only standard solutions, the antioxidant activities of some minerals, such as zinc (Zn), have been described (Zödl et al., 2003). However, the data found in the literature with respect to the pro- (Nuñez, Tapia, Toyokuni, & Okada, 2001) and/or anti-oxidant activity of Fe (Zödl et al., 2004) upon Caco-2 cells are less conclusive and appear contradictory.

From a physiological perspective, food (after consumption) is subjected to a gastrointestinal digestion process before reaching the proximal intestine, which is considered to be the main site of nutrient and mineral absorption (Ganong, 1990). It is also notable that, during the gastrointestinal digestive process, antioxidant and other functional components could be metabolized or not released from foods, thus affecting the native antioxidant potential of each of these components.

The aim of this study was to evaluate the cytoprotective effect against H_2O_2-induced oxidative stress in Caco-2 cells, derived from the bioaccessible fraction of fruit beverages with/without milk and minerals, after being subjected to a simulated gastrointestinal digestion procedure. In this sense, the present study could improve knowledge of the cytoprotective effects of fruit beverages, based on an intestinal epithelial model which more precisely could simulates the *in vivo* situation.

2. Materials and methods

2.1. Samples

A fruit beverage (Fb) (grape + orange + apricot) with/without iron (Fe) and/or zinc (Zn) and with/without skimmed milk (M), was used in this work, with the following references: Fb, FbFe, FbZn, FbFeZn, FbM, FbMFe, FbMZn, FbMFeZn. The compositions of the aforementioned samples are shown in Table 1.

Table 1
Compositions of the fruit beverages assayed

Component (g/100 g)	Sample	
	Fb	FbM
Osmosis water	58.7	57.7
Apricot puree	24.5	24.5
Grape concentrate	7.2	7.2
Orange concentrate	4.2	4.2
Sugar	5.1	5.1
Skimmed milk powder	–	1.0
Classic pectin	0.4	0.4
Vitamin C (L-ascorbic acid)	0.054	0.054

Fb, fruit beverages with or without mineral supplementation; Fe (sulphate), 3 mg Fe/100 ml fruit juice and/or Zn (sulphate), 1.6 mg Zn/100 ml fruit juice; FbM, fruit juices with milk (11% v/v) and supplemented or not with mineral.

2.2. In vitro digestion

To simulate the human gastrointestinal digestive process, samples of fruit juices (80 g) were subjected to an *in vitro* procedure, as previously described (Perales, Barbera, Lagarda, & Farre, 2005). After gastric (pepsin/pH 2) and intestinal (pancreatin and bile extract/pH 6.5) steps, and prior to the assays with Caco-2 cells, the digests were heated for 4 min. at 100 °C to inhibit sample proteases, and were then cooled by immersion in an ice bath. Twenty-gramme aliquots of the inactivated digests were transferred to polypropylene centrifuge tubes and centrifuged at 3890*g* for 60 min at 4 °C to separate the soluble fraction (bioaccessible fraction), which was pooled.

2.3. Caco-2 cell culture

The Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC 86010202, Salisbury, UK). Cultures were maintained and grown as previously described (Laparra, Vélez, Montoro, Barberá, & Farré, 2005).

For the assays, Caco-2 cells were seeded onto 24-well plates (Costar Corp., USA), at a density of 5×10^4 cells cm^{-2}, with 1 ml of MEM (Minimum Essential Medium, MEM; Gibco BRL Life Technologies, Scotland), and culture media was changed every two days. Fifteen to eighteen days after initial seeding, the culture medium was aspirated from wells, and cell monolayers were washed with PBS warmed to 37 °C. Cell cultures were preincubated (37 °C/5% CO_2/95% relative humidity) for 24 h with bioaccessible fractions of digested samples, added in 1:1 proportion (v/v) with culture media to preserve cell viability. Afterwards, culture medium was removed and the cells were washed with PBS at 37 °C; the induction of oxidative stress was carried out by exposure to a 5 mM H_2O_2 solution in MEM for 1 h (37 °C/5% CO_2/95% relative humidity).

2.4. Intracellular accumulation of ROS and mitochondrial membrane potential changes

The intracellular accumulation of ROS in Caco-2 cells was evaluated using a 2 mM dihydrorhodamine (DHR,