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Effect of simultaneous consumption of soymilk and coffee on the urinary excretion of isoflavones, chlorogenic acids and metabolites in healthy adults

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ABSTRACT

The effect of the simultaneous ingestion of soymilk and coffee on the urinary excretion of isoflavones (ISO) and chlorogenic acids (CGA) was investigated for the first time in humans. In a randomized crossover study, soy (79.7 μmol ISO), coffee (561.2 μmol CGA) and soy–coffee beverage (79.7 μmol ISO and 561.2 μmol CGA) were ingested by adult subjects (n = 6) after overnight fasting. Urinary excretion of ISO aglycones and metabolites during 48 h after soy and soy–coffee beverages consumption was 22.0 ± 19.1 and 20.3 ± 9.4 μmol, respectively, with no difference between treatments. CGA and metabolite excretion during 24 h after coffee and soy–coffee beverage consumption was 3.4 ± 1.2 and 3.1 ± 0.6 mmol, respectively. However, a 42% decrease in levels of CGA and primary metabolites and not of colonic metabolites was observed in urine after soy–coffee beverage consumption compared to plain coffee, indicating that substances present in soymilk, most likely proteins, bind CGA reversibly, decreasing their absorption in the digestive tract.

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Chemical compounds: Daidzein (PubChem CID: 5281708); Daidzin (PubChem CID: 107971); Genistein (PubChem CID: 5280961); Genistin (PubChem CID: 5281377); Glycitein (PubChem CID: 5317750); Glycitin (PubChem CID: 187808); 5-Caffeoylquinic acid (PubChem CID:1794427); Caffeic acid (PubChem CID: 689043); *p*-Coumaric acid (PubChem CID: 637542); Ferulic acid (PubChem CID: 445858); Isoferulic acid (PubChem CID: 736186); Gallic acid (PubChem CID: 370); Dihydrocaffeic acid (PubChem CID: 348154); Vanillic acid (PubChem CID: 8468); Benzoic acid (PubChem CID: 243); 3,4-Dihydroxyphenylacetic acid (PubChem CID: 547); Trans-3-hydroxicinnamic acid (PubChem CID: 637541); 3-(4-Hydroxyphenyl)propionic acid (PubChem CID: 10394); Syringic acid (PubChem CID: 10742); 2,4-Dihydroxybenzoic acid (PubChem CID: 1491); p-Hydroxybenzoic acid (PubChem CID: 135); Sinapic acid (PubChem CID: 637775); Dihydrodaidzein (PubChem CID: 176907); O-Desmethylangolensin (PubChem CID: 89472); Dihydrogenistein (PubChem CID: 9838356); Equol (PubChem CID: 382975); Hippuric acid (PubChem CID: 464); 3,4-Dicaffeoylquinic acid (PubChem CID: 5459216); 3,5-Dicaffeoylquinic acid (PubChem CID: 6474310); 4,5-Dicaffeoylquinic acid (PubChem CID: 6474309); 5-Feruloylquinic acid (PubChem CID: 9799386).

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1. Introduction

Soy is an excellent dietary source of protein and essential fatty acids and naturally cholesterol-free. It is also relatively cheap in comparison with other food protein sources. Furthermore, over the past 30–40 years, epidemiological studies as well as those using cell cultures, animal models and clinical trials have suggested that soybean consumption is related to various beneficial health effects (Messina, 2014; Nagata et al., 2014; Setchell & Cassidy, 1999). Soybean is the main dietary source of isoflavones (Barnes et al., 2006; Messina, 2014) that may exhibit antiestrogenic or estrogenic effects, depending on the endogenous hormone, quantity and type of estrogen receptor (Thomas et al., 2014; Yuan, Wang, & Liu, 2007). Additionally, isoflavones have shown a variety of non-hormonal effects such as antioxidant, antiproliferative and antiangiogenic effects (Cassidy, Hanley, & Lamuela-Raventos, 2000; Mahmoud, Yang, & Bosland, 2014), that are inherent to polyphenols in general.

Daidzein, genistein and glycitein are the three major isoflavones found in soy and soy products. Each of them may be present in four chemical forms: aglycones (daidzein, genistein and glycitein); β-glycosides (daidzin, genistin and glycitin); acetyl glycosides (6″-O-acetyldaidzin, 6″-O-acetylgenistin, 6″-O-acetylglycitin); and malonyl glycosides (6″-O-malonyldaidzin, 6″-O-malonylgenistin, and 6″-O-malonylglicitin), with the glycosidic conjugates being the most prevalent among them (Kudou et al., 1991; Liu, 2005).

Despite the beneficial nutritional and functional implications of soy consumption, the acceptance of soy food products in the Western countries is still low. While Eastern countries populations consume 20–40 g of soy foods daily (Song et al., 2007), the consumption in Western countries is lower than 3 g daily. Soy beverages are an easy, practical and palatable way to improve soy intake in the Western countries, and their acceptability increases when fruit concentrates (Rodrigues & Moretti, 2008), nuts (Felberg et al., 2004) and other flavours are added.

Coffee is one of the most consumed beverages in the world due to its pleasant aroma and taste. Additionally, it has a potential health value due to the high content of phenolic compounds, particularly, the chlorogenic acids. The most abundant groups of chlorogenic acid isomers in coffee are the caffeoylquinic acids, dicaffeoylquinic acids and feruloylquinic acids. The consumption of these compounds has been associated with reduction of the relative risk of cardiovascular disease, diabetes type 2, Alzheimer's disease and with antibacterial and anti-inflammatory activities (Farah, 2009). In addition to chlorogenic acids, coffee also contains caffeine which increases alertness and reduces fatigue when ingested moderately, as well as other bioactive compounds, such as trigonelline and potentially prebiotic polysaccharides (Farah, 2009; Farah & Donangelo, 2006).

Considering the aforementioned aspects, the consumption of a beverage combining the positive characteristics of soy and coffee food products would be of interest. Furthermore, the use of coffee in a soy beverage would dispense colouring, flavouring and artificial aroma, thus resulting in a natural and healthy beverage alternative. Consequently, a soy–coffee instant beverage was formulated and sensory tested with good acceptance by habitual coffee consumers (Felberg, Deliza, Farah, Calado, & Donangelo, 2010). However, we hypothesized that combining soymilk and coffee could affect the bioavailability of their specific phenolic compounds because previous studies have shown that the bioavailability of polyphenols may be affected by the interaction with other dietary constituents, especially proteins (Jakobek, 2015; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Ozdal, Capanoglu, & Altay, 2013). For example, *in vitro* studies have shown interactions between chlorogenic acids and milk proteins (Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006). Duarte and Farah (2011) also observed that the addition of cow's milk to coffee reduced the excretion of chlorogenic acids and metabolites in humans, compared to plain coffee. Regarding soy matrix, it has been reported in *in vitro* studies that different types of soy proteins can interact with chlorogenic and caffeic acids as well (Budryn et al., 2015; Rawel, Czajka, Rohn, & Kroll, 2002). Considering that no *in vivo* studies in humans have investigated this interaction to date, the aim of the present study was to evaluate the effect of simultaneous ingestions of coffee and soymilk on the urinary excretion of soy isoflavones, coffee chlorogenic acids and metabolites in humans.

2. Materials and methods

2.1. Subjects

Six nonsmoking subjects (four females and two males), 24–42 years of age, were recruited at the Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil. They were healthy as judged by a medical questionnaire, with normal blood values for haemoglobin and haematocrit, and were not taking any medication or nutritional supplements at the time of the study. Subjects had, on average, an adequate body mass index (20.6 ± 3.3 kg/m^2), and according to the subjects' average body mass index during treatments, five subjects were characterized as euthrophic and one with BMI of 28 kg/m^2 as overweight (WHO, 1995). The study protocol was approved by the Ethics Committee of Veiga de Almeida University (Rio de Janeiro, Brazil) and fully explained to the subjects who gave their written informed consent prior to participation.

2.2. Soy, coffee and soy–coffee beverages

The soymilk beverage was prepared by mixing 20 g of soymilk (79.7 μmol of isoflavones) powder provided by Olvebra Industrial S/A (Eldorado do Sul, Brazil), with 200 mL of filtered hot water (60–70 °C). The coffee beverage was prepared by addition of 4 g of commercial medium roast instant coffee powder (*Coffea canephora* cv. Conillon) (561.2 μmol of chlorogenic acids) provided by COCAM Co. (São Paulo, Brazil) to 200 mL of filtered hot water (60–70 °C). The soy–coffee beverage was prepared by adding 20 g of soymilk powder and 4 g of instant coffee to 200 mL of filtered hot water (60–70 °C). Beverages were prepared on each day of the study, immediately before consumption and sweetened with standard amount of a commercial sugar-free sweetener (aspartame, Finn®, São Paulo, Brazil).