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Influence of daily consumption of synbiotic soy-based product supplemented with okara soybean by-product on risk factors for cardiovascular diseases

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ABSTRACT

This study aimed to investigate the effect of a synbiotic fermented soy product supplemented with okara (a by-product from soybean) on cardiovascular disease risk markers in healthy men. In a randomized, double-blinded, placebo controlled trial, thirty-six normocholesterolemic men were assigned to two groups. Subjects consumed daily 100 g of soy-based product fermented with *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* Bb-12, and *Streptococcus thermophilus* (starter culture) (synbiotic group — S) (n = 18) or 100 g of unfermented soy-based product (placebo group — P) (n = 18) for 8 weeks. Fasting blood samples and anthropometric measurements were collected at the baseline (T_0), the end of week 4 (T_4), and the end of week 8 (T_8). Serum lipids, C-reactive protein, fibrinogen, and electronegative LDL were also analysed on T_0, T_4, and T_8. During the period of daily soy-based product consumption (from T_0 to T_8) the LDL-C mean decreased significantly ($p < 0.05$) in group S, resulting in a significant ($p < 0.05$) improvement of the LDL-C/HDL-C ratio. Comparing mean differences (T_8–T_0) between the two groups, the trend of LDL-C and LDL-C/HDL-C ratio reductions was higher in group S (14.1 mg/dL and 0.38 mg/dL, respectively) than group P (4.9 mg/dL and 0.17 mg/dL, respectively) ($p > 0.05$). Our results suggest limited lipid-lowering effects of synbiotic soy-based product supplemented with okara on cardiovascular risk markers in normocholesterolemic men.

1. Introduction

Cardiovascular disease (CVD) is a multifactorial disorder with a high mortality rate and the biggest cause of death worldwide (WHO, 2011). The WHO has predicted that by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.3 million people around the world (WHO, 2013). Dyslipidemia, characterized by the presence of one or more than one abnormal serum lipid concentration (total cholesterol, low density lipoprotein cholesterol, triglycerides, and high density lipoprotein cholesterol), is a major risk factor associated with CVD (Goff et al., 2006).

In addition to dyslipidemia, CVD is typified by low-grade chronic inflammation. Increased levels of inflammatory markers, such as C-reactive protein, have been shown to predict all causes of cardiovascular mortality (Pearson et al., 2003). LDL particles might be oxidatively modified *in vivo* giving rise to different modified particles, including a more electronegative LDL subfraction (Ziouzenkova & Sevanian, 2000). Electronegative LDL [LDL(−)] is a minimally modified form of LDL that is present in plasma and has proinflammatory and proatherogenic properties (Faulin, Sena-Evangelista, Pacheco, Augusto, & Abdalla, 2012). LDL(−) particles have an increased content of hydroperoxides, conjugated dienes, cholesterol oxides, non-esterified fatty acids, lysophosphatidylcholine, and thiobarbituric acid reactive substances, as well as a decreased content of α-tocopherol when compared to the native LDL (Faulin et al., 2008).

Alternative strategies for CVD prevention, such as dietary therapy, have been the subject of attention in the scientific fields. The diet cardioprotective role may be associated to several compounds, including soluble fibres, soy proteins, isoflavones, and probiotic microorganisms (Cavallini, Abdalla, Vendramini, Bedani, Bomdespacho, Pauly-Silveira, Valdez and Rossi, 2009; Cavallini, Bedani, Bomdespacho, Vendramini and Rossi, 2009).

In addition to effects on gastrointestinal disorders, studies have suggested that probiotic bacteria and prebiotic fibres, like inulin, may reduce the risk of CVD (Al-Sheraji et al., 2012; Pereira & Gibson, 2002b). However, although several animal studies show a lipid-lowering effect

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after the administration of probiotics and/or prebiotics (Cavallini, Bedani, Bomdespacho, Vendramini, & Rossi, 2009; Chiu, Lu, Tseng, & Pan, 2006; Xiao et al., 2003), the results in humans are still controversial (Hatakka, Mutanen, Holma, Saxelin, & Korpela, 2008; Ooi & Liong, 2010; Pereira & Gibson, 2002b).

Strains belonging to the *Lactobacillus* and *Bifidobacterium* genera are the most well-known probiotic microorganisms (Saxelin, Tynkkynen, Mattila-Sandholm, & de Vos, 2005). A number of beneficial effects have been attributed to *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12, among them are: prophylactic activity against infectious rotavirus diarrhoea in children (Weichert, Schroten, & Adam, 2012; Weizman, Asli, & Alsheikh, 2005); relief of clinical symptoms of atopic dermatitis in children (Isolauri, Arvola, Sütas, Moilanen, & Salminen, 2000); intestinal microbiota modulation (Savard et al., 2011), and hypocholesterolemic effect (Abd El-Gawad, El-Sayed, Hafez, El-Zeini, & Saleh, 2005).

Even though most probiotic foods are derived from milk, the possibility of using other protein-rich substrates such as soymilk to make these foods has been encouraged. Recently, soy based products like yoghurt have been produced from soymilk fermented with lactic acid bacteria and/or bifidobacteria (Abd El-Gawad et al., 2005; Bedani, Vieira, Rossi, & Saad, 2014).

On the other hand, soy consumption has been limited owing to its undesirable beany flavour and the presence of oligosaccharides that often lead to flatulence and stomach discomfort (Yeo & Liong, 2010). Nevertheless, soymilk fermentation, especially with lactic acid bacteria, may improve the flavour and texture of soy products, as well as enhance its beneficial health properties (Cruz et al., 2009; Donkor, Henriksson, Vasiljevic, & Shah, 2005). Several studies have shown that soy products may be good vehicles for probiotic microorganisms (Bedani, Rossi, & Saad, 2013; Champagne, Green-Johnson, Raymond, Barrette, & Buckley, 2009).

The potential role of dietary soy in the reduction of risk for chronic disease, in particular heart disease, has been recognized for a long time (Potter, 1998). Some soy compounds, such as protein, isoflavones, and dietary fibres have been reported to affect cholesterol metabolism (Clarkson, 2002; Villanueva, Yokoyama, Hong, Barttley, & Rupérez, 2011). Additionally, studies have suggested that okara, a soybean waste generated from soymilk and tofu (bean curd) production, might be used in the food industry to confer increased nutritional and functional properties to products (Jiménez-Escrig, Tenorio, Espinosa-Martos, & Rupérez, 2008; Villanueva-Suárez, Pérez-Cózar, & Redondo-Cuenca, 2013). Dry okara contains protein, lipid, dietary fibre, and minerals, along with unspecified monosaccharides, and oligosaccharides. It also contains isoflavones (Jiménez-Escrig et al., 2008; Mateos-Aparicio, Mateos-Peinado, Jiménez-Escrig, & Rupérez, 2010). Huge quantities of okara are produced worldwide with the increase in soybean consumption, representing a significant disposal problem (Lu, Liu, & Li, 2013). For each kilogramme of soybean processed into soymilk, an equal weight of okara is produced or even more (Lu et al., 2013; Villanueva et al., 2011).

Although okara is frequently treated as an industrial waste, studies revealed that this by-product is a potential source of antioxidant components (Mateos-Aparicio et al., 2010). It might be useful as a weight-loss dietary supplement (Préstamo, Rupérez, Espinosa-Martos, Villanueva, & Lasunción, 2007) and also protect the gut environment due to its antioxidant status and prebiotic effects (Jiménez-Escrig et al., 2008; Mateos-Aparicio et al., 2010; Villanueva-Suárez et al., 2013). Studies have also suggested that okara consumption could lead to a beneficial effect on plasma lipid levels (Villanueva et al., 2011).

To our knowledge, no study is available in the scientific literature regarding the impact of a synbiotic soy-based product with okara on CVD risk factors in humans. Therefore, the present study aimed to investigate the effect of a synbiotic soy product, fermented with an ABT culture (*L. acidophilus* La-5, *B. animalis* Bb-12, and *Streptococcus thermophilus*), supplemented with okara, on the risk markers for CVD in normocholesterolemic men.

2. Material and methods

2.1. Production of okara flour

Okara was supplied by the Development and Production Unit for Soybean Derivatives (UNISOJA) located at the School of Pharmaceutical Sciences of the São Paulo State University (Araraquara, Brazil) and was obtained according to Bedani et al. (2013).

2.2. Production of the synbiotic soy-based product and of the placebo product

The soy-based products were processed at UNISOJA according to the method described by Bedani et al. (2013). The ingredients employed in the production of the synbiotic soy product and of the placebo product are shown in Table 1.

Both the placebo product and the synbiotic product contained inulin. The synbiotic soy product was fermented with an ABT-4 culture, containing the probiotic strains *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* Bb-12, and the starter *S. thermophilus* (Christian Hansen, Hørshoolm, Denmark). Microbiological analyses of the synbiotic product showed that the average population of La-5 and Bb-12 ranged from 8 up to 9 log CFU/g during the experimental period. The placebo product (unfermented soy product) had the same ingredients as the synbiotic soy product, but without the bacterial culture. As this product did not undergo fermentation, it was acidified to the same pH as the fermented soy product (4.5) by adding food-grade lactic acid (Purac, São Paulo, Brazil). Portions of 100 g of soy-based products were packaged in appropriate polypropylene plastic pots for food products (68 mm diameter, 71 mm high, 115 mL total volume, Tries Aditivos Plásticos, São Paulo, Brazil) and sealed with metallic covers with varnish in a sealer (Delgo Metalúrgica, Cotia, Brazil). The products were stored under refrigeration (4 ± 1 °C) until consumption. The products were freshly produced every week and delivered refrigerated to each volunteer in plastic vials labelled with the date of manufacture and shelf-life.

2.3. Participants

Thirty-six men with mean ages of 45.36 ± 9.67 y took part in the study. Inclusion criteria were LDL-C < 160 mg/dL, not having hypertension, diabetes, gastrointestinal disorders, liver, kidney or cardiovascular diseases, and not taking lipid-regulating medications. Participants were recruited from the School of Pharmaceutical Sciences of the São Paulo State University, including teachers, students, and staff. The study was approved by the Ethical Committees of the School of Pharmaceutical Sciences of the São Paulo State University and of the School of Pharmaceutical Sciences the University of São Paulo. All subjects provided written consent form before participating in the study.

Table 1
Ingredients employed in the production of soy-based products with okara.

Ingredients (g/100 mL of soymilk)	Soy products	
	Synbiotic	Placebo
Sugar	8.00	8.00
Okara flour	5.00	5.00
Inulin	3.00	3.00
Skimmed milk powder	2.50	2.50
Lactose	1.00	1.00
Soybean oil	0.80	0.80
Gelatin	0.30	0.30
Probiotic culture[a]	0.50	0.00

[a] ABT-4 culture (containing the probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12, and the starter *Streptococcus thermophilus*; Christian Hansen, Hørsholm, Denmark) with around 10^{10} cfu/g of La-5 and 10^{11} cfu/g of Bb-12.