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Changes of probiotic fermented drink obtained from soy and rice byproducts during cold storage

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ABSTRACT

Plant extracts with probiotics have been gaining ground in the functional food market. The present study aimed to evaluate the physicochemical, microbiological and sensory characteristics of probiotic fermented drink made of mixed extract of soy and rice byproducts with added waxy corn starch during cold storage (5 °C for 28 days). Furthermore, the probiotic resistance under conditions similar to the digestive tract was evaluated. Slight physicochemical and colour changes were noted in the beverage during the storage period, but these changes did not affect product quality. The sample remained within acceptable microbiological parameters, and sensory scores were higher than 3.0, within the predefined quality threshold. Fermenting microorganisms presented reduced viability during the storage period. *Lactobacillus acidophilus* and *Bifidobacterium* spp. resisted the tested gastrointestinal conditions. The product showed a 28-day shelf life, but the probiotic effect lasted only14 days. Further studies should evaluate beverage supplementation with components that could improve the viability of probiotic cultures, in order to maintain their probiotic effect for a longer time. Additionally, consumer studies should be carried out in order to assess current products' acceptance by target consumers.

1. Introduction

The search for foods that meet the demands of health-conscious consumers has a direct impact on the food industry, which aims to provide new products with attractive functional features, convenience and adequate sensory quality (Bezerra, Araujo, Santos, & Correia, 2015). Cultures of *Lactobacillus acidophilus* and *Bifidobacterium* spp. have shown health benefits related to decreasing serum cholesterol (Ejtahed et al., 2011), protection against intestinal diseases and disorders related to pathogenic bacteria (Savard et al., 2011), and reducing the risk of diarrhea associated with antibiotics (Beausoleil et al., 2007), among others. Yogurt and fermented milks represent the main category among foods with added probiotics (Cruz et al., 2013).

However, the marked demand for vegetable-based drinks has increased due to the large amount of people with lactose intolerance and allergies to milk proteins, or who by their own choice have opted for vegetarianism. Soy has whole proteins, dietary fiber, low cholesterol, no lactose, and high levels of bioactive phenolic compounds. Isoflavones, classified as flavonoids and phytoestrogens, are characteristic phenolic components of soybean (Zhao & Shah, 2014). Lactic fermentation of soymilk to produce "yogurt" is performed at the industrial level. However, for the majority of western people, soy extract has an unpleasant flavor. Soybeans' characteristic flavor comes from the self-oxidation of polyunsaturated fatty acids or from the enzymatic action of lipoxygenase, which forms volatile compounds responsible for the flavor generally described as stale or beany (Silva, Ascheri, & Pereira, 2007).

Therefore, mixtures of soy and rice extracts may have sensorial advantages, and at appropriate concentrations the mixtures may have a mutual complementary effect among amino acids. According to a study conducted by Valipour (2015a), rice (22%) and soybean (20%) were two of the main crops grown between 2007 and 2011. Due to their nutritional capacity and the use of their byproducts, these crops have great potential for cultivation and dissemination, reducing poverty and hunger in the world, and

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minimizing the impacts related to water and soil scarcity (Valipour, 2015b).

Byproducts result from rice processing (bran and broken rice) have low added value. However, they still preserve appropriate nutritional value that can be applied to the development of new products. At the same time, waxy maize starch has been used to provide consistency to fermented products and to increase consumer satiety. Additionally, waxy maize starchis able to bind to water, thus improving the viscosity characteristics and texture of these products (Costa, Garcia, Ribeiro, Soares Junior, & Caliari, 2016).

A few studies have evaluated the addition of probiotic cultures to fermented drink obtained from rice (Wongkhalaung & Boonyaratanakornkit, 2000; Hassan, Aly, & El-Hadidie, 2012), but none have used the byproducts as raw material or the association with soy. Furthermore, previous studies with probiotic yogurts have usually added prebiotic-based ingredients (Donkor, Henriksson, Vasiljevic, & Shah, 2007; Ozcan & Kurtuldu, 2014; Oh et al., 2016) based on the assumption that food does not have enough nutrients to ensure the survival of probiotic cultures. In this study, it was decided to test the survival and viability of these microorganisms without the addition of ingredients already known for their protective effect, to study how much food maintains probiotics' viability and whether it needs to receive supplementation or not.

Thus, this study aimed to evaluate the physicochemical, microbiological and sensory characteristics of fermented drink based on mixed extract of soy and rice byproducts, at 70:30 ratio, with added waxy maize starch, probiotic cultures of *Lactobacillus acidophilus* and *Bifidobacterium* spp., and flavoring in the form of strawberry syrup and aroma during cold storage. Moreover, cell viability and resistance of probiotic microorganisms to bile and hydrochloric acid were evaluated during 28 days of cold storage.

2. Material and methods

2.1. Material

Soybeans from theW711RR cultivar were donated by the Instituto Federal Goiano (IFG), Rio Verde – GO (Brazil), rice byproducts (broken rice and rice bran) by Arroz Cristal, Aparecida de Goiânia – GO, waxy starch by Febela, Bela Vista de Goiás – GO, Guar gum by Fego, Goiânia – GO, and artificial strawberry flavor (Gemacom 201.110 R) by Leite& Cia, Goiânia– GO. Rich® lactic culture composed by *Streptococcus thermophilus* (concentration not specified by manufacturer), *Bifidobacterium* spp BB-12 (10^6 CFU g^{-1}), and *Lactobacillus acidophilus* La-5 (10^6 CFU g^{-1}), crystallized saccharose (Cristal®) and fresh strawberries were purchased in local shops in Goiânia.

2.2. Preparation of soy and rice byproducts extracts and flavored fermented beverage

The soybeans were heat-treated (100 °C for 5 min) in aqueous solution (1:1 ratio) to inactivate the enzymes that provide soy-based products' beany flavor and were then washed with cold running drinking water. The grains were cooked in water (1:5 ratio) at boiling temperature for 25 min in order to optimize extraction. After reaching the appropriate soybean cooking point, the grains were disintegrated in an industrial blender and centrifuged to separate water-soluble extract from solid residue.

The rice bran was heated for 3 min in a 900 W microwave oven. This was sufficient time to inactivate the enzymes and to avoid acidification (Abdul-Hamid, Sulaiman, Osman, & Saari, 2007). Soon after, the bran was toasted in batches of 500 g in a stainless steel container on direct fire at a temperature of about 110 °C for 10 min, and then it was homogenized manually. Next, the product was sieved, packed in a laminated vacuum packing bag, and stored at −18 °C until processing. The broken rice and toasted rice bran (92:8 ratio) were mixed. This ratio was selected because it is similar to the composition of brown rice. The byproducts were cooked in water (1:3 ratio) in order to obtain a final product with an average yield of 300%. Then, for each batch, disintegration of the drained cooked product was carried out by using 750 mL of product in 750 mL of water for 3 min in an industrial blender until an homogeneous mixture was obtained. The homogenized mixture, previously sterilized in an autoclave at 121 °C for 30 min, was immediately sieved in cotton fabric and a finemesh sieve with a 2 mm opening, which had previously been sanitized with sodium hypochlorite solution (200 mg L^{-1}). The opaque and whitish liquid permeate was defined as water-soluble extract.

For the processing of the flavored fermented beverage (FFB), previously obtained water-soluble extracts of soy and rice byproducts were used at 70:30 ratio, based on the chemical scores of soy amino acids and rice flour available in scientific literature. Saccharose (10 g 100 g^{-1}) and guar gum (0.5 g 100 g^{-1}) were added to the mixed extract. The mixture was homogenized in a mechanical shaker at 700 rpm for 5 min; then it was pasteurized at 85 °C for 30 min in a water bath, cooled to a temperature of 45 °C, combined with 50 g L^{-1} of waxy maize starch, and again homogenized. The temperature was raised to approximately 73 °C and maintained for 10 min under continuous manual agitation. Then, the mixture was cooled to 45 °C, combined with lactic culture (400 mg L^{-1}), as recommended by the manufacturer, and incubated in a BOD incubator at 42 °C until it reached pH 4.5. After this process, the beverage was cooled for 12 h at 5 ± 1 °C, and artificial strawberry flavor (0.08 g 100 g^{-1}) and strawberry syrup (300 g L^{-1}) were added, as described by Miranda, Lafetá, Dessimoni-Pinto, &Vieira (2012), resulting in FFB.

For the syrup processing, granulated saccharose and water were mixed (100 g L^{-1}) in a stainless steel container for approximately 10 min until dissolution was complete, in order to obtain a concentration of 60°Brix. The strawberries were chopped and boiled into solution for 30 min, and then they were packaged in a glass container with a metal lid previously sterilized in boiling water (100 ± 5 °C) for 15 min. Soon after, the containers with syrup were pasteurized in boiling water for 30 min and then cooled. After homogenization, the FFB was bottled in glass jars (25 mL) between two burners, with a screw cap previously autoclaved at 121 °C for 30 min, and then the jars were stored at refrigeration temperature (5 ± 1 °C) up to the time of analysis.

2.3. Changes during cold storage

Differences in the instrumental colour, physicochemical characteristics, microbiological risk, sensory analysis, cell viability of starter culture and probiotics, and resistance of probiotics to hydrochloric acid and bile salts were determined at 7-day intervals, from the first to the 28th day of storage. A completely randomized design (CRD) was used, with five treatments (0, 7, 14, 21, and 28 days of cold storage) in two independent repetitions, totalizing 10 experimental units. For sensory analysis, a randomized block design with 5 treatments and 8 blocks was used, considering each judge a block.

2.4. Physicochemical characteristics and difference of colour

The pH was measured using a potentiometer, total acidity was determined by titration with 0.1 N NaOH, using phenolphthalein as an indicator, and the total soluble solids content at 20 °C was