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Development and characterization of an innovative synbiotic fermented beverage based on vegetable soybean

Carolina Battistini[a], Beatriz Gullón[b], Erica Sayuri Ichimura[a], Ana Maria Pereira Gomes[b], Eliana Paula Ribeiro[a], Leo Kunigk[a], José Ubirajara Vieira Moreira[c], Cynthia Jurkiewicz[a,*]

[a] Instituto Mauá de Tecnologia, São Caetano do Sul, SP, Brazil
[b] Universidade Católica Portuguesa, Escola Superior de Biotecnologia, Porto, Portugal
[c] Embrapa Soja, Londrina, PR, Brazil

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ABSTRACT

Soymilk was produced from vegetable soybean and fermented by probiotics (*Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* Bb-12) in co-culture with *Streptococcus thermophilus*. The composition of the fermented beverage and oligosaccharides content were determined. The effect of fructooligosaccharides and inulin on the fermentation time and viability of probiotic microorganisms throughout 28 days of storage at 5 °C were evaluated. The soymilk from vegetable soybeans was fermented in just 3.2 h, when pH reached 4.8. Fermentation reduced the contents of stachyose and raffinose in soymilk. Prebiotics had no effect on acidification rate and on viability of *B. animalis* and *S. thermophilus* in the fermented beverage. The viable counts of *B. animalis* Bb-12 remained above 10^8 CFU mL^{-1} in the fermented soymilk during 28 days of storage at 5 °C while *L. acidophilus* La-5 was decreased by 1 log CFU mL^{-1}. The fermented soymilk from vegetable soybeans showed to be a good food matrix to deliver probiotic bacteria, as well as a soy product with a lower content of non-digestible oligosaccharides.

Introduction

Soymilk has received increasing attention from consumers as an alternative to dairy products due to its protein quality, absence of cholesterol and lactose, and functional properties.[1] However, the consumption of soymilk is limited due to the presence of non-digestible oligosaccharides, such as raffinose and stachyose, which are not hydrolyzed in the small intestine and may cause abdominal cramps, diarrhea and bloating.[2,3]

Lactic acid bacteria and probiotic microorganisms, for example, *Streptococcus thermophilus*, *Lactobacillus acidophilus*,

* *Corresponding author.*
E-mail: cynthia@maua.br (C. Jurkiewicz).
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and *Bifidobacterium*, can grow in soymilk and consume non-digestible oligosaccharides, decreasing or eliminating these anti-nutritional compounds, resulting in a healthier product for consumers.[4–7] A probiotic fermented soymilk combines the beneficial properties of soy with the health benefits of probiotic microorganisms. However, the acidification rate of pure probiotic cultures is usually low, and often off-flavors in the final product are produced. The solution to this problem is to mix the probiotic cultures with yogurt cultures to reduce the fermentation time and improve the sensory characteristics of the product.[8,9] Prebiotic ingredients, such as inulin and fructooligosaccharides (FOS), may also improve the activity and survival of probiotic bacteria in fermented soymilk.[7,10,11] Moreover, the combination of prebiotics and probiotics results in a synbiotic effect on gut microbiota.[12] Non-dairy synbiotic beverage[13,14] is also an alternative to consumer that are lactose intolerant or allergic to milk protein.

Vegetable soybean [*Glycine max* (L.) Merril] is a popular food that is consumed in Asia, the United States and other countries, mainly as a snack, a vegetable for soups or stews, or in salads. It is a soybean that is harvested while the seeds are at approximately 80% of maturity, such that it has a green color and a soft texture.[15,16] These immature seeds have advantages over mature soybean, including improved sensory attributes and nutritional value, such as sweeter flavor and less contents of stachyose and raffinose, resulting in better digestibility.[17,18] Similar to mature soybean, vegetable soybean is rich in good quality protein, has a high mineral content and has the potential to prevent some diseases, including cancer, osteoporosis and menopausal symptoms due to its content in isoflavones.[17]

Numerous studies have been performed on the growth of probiotic cultures in soy beverage[4,8,20]; however, no information is available on the fermentation of soymilk from vegetable soybean. Thus, the aim of this study was to investigate the acidification rate of soymilk produced with vegetable soybeans supplemented with inulin and fructooligosaccharide (FOS), by a mixed culture of *L. acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* Bb-12 and *S. thermophilus*. In addition, the survival of microorganisms during 28 days of storage at 5 °C and the contents of stachyose and raffinose in soymilk and fermented beverages were also evaluated.

Materials and methods

Production of soymilk

Vegetable soybeans, cultivar BRS-232, were supplied by Embrapa Soybean, Brazil. The plants were harvested mechanically and taken to the laboratory where the pods were removed and immediately bleached in boiling water for 3 min and cooled at 5 °C. Seeds were removed from the pods, packed in plastic bags, frozen at −18 °C and freeze-dried in a lab scale lyophilizer (Enterprise I; TERRONI, São Carlos, SP, Brazil).

Soymilk was produced by soaking fifty grams of freeze-dried vegetable soybeans in 455 g of water at room temperature for 10 min. The mixture was heated at 85 °C and blended for 3 min. The slurry was stirred at 85 °C for 5 min and filtered in a 0.5 mm conical sieve to obtain the soymilk.

Fermentation of soymilk

Soymilk was supplemented with 40 g kg^{-1} FOS (Orafti® P 95, Beneo Latinoamericana), 40 g kg^{-1} inulin (Orafti® GR, Beneo Latinoamericana) or a mixture of 40 g kg^{-1} FOS and 40 g kg^{-1} inulin. Control fermented soymilk was prepared without the addition of the prebiotic ingredients. The four formulations were produced in triplicate. After the addition of the prebiotic ingredients, soymilks were pasteurized at 75 °C for 15 s, cooled at 37 °C, and inoculated with 0.02% of a freeze-dried ABT-4 culture (Christian Hansen, Denmark) containing *L. acidophilus* La-5, *B. animalis* subsp. *lactis* Bb-12 and *S. thermophilus*. Next, the soymilks were distributed in 50 mL sterile bottles and incubated at 37 °C until the pH reached 4.7–4.8. The fermented beverages were stored at 5 °C for 28 days.

Chemical and physicochemical analyses

The chemical composition of lyophilized vegetable soybeans and soymilk without prebiotic ingredients was determined according to AOAC methods.[21] The moisture of the vegetable soybeans was determined by drying the sample in an oven at 105 °C until constant mass, based on AOAC method 925.09B, without vacuum utilization. The total solids content of the soymilks was determined according to AOAC method 990.20, and the moisture was calculated subtracting this value from 100. To determine the ash content, the sample was incinerated in a muffle at 550 °C (method 923.03 and method 945.46 for grains and soymilk, respectively, both from AOAC). The protein content was calculated by the measurement of total nitrogen using the micro Kjeldahl method, and the conversion factor applied was 6.25, based on AOAC method 979.09. The fat content was determined using the Soxhlet, based on AOAC method 920.39, using hexane as solvent (boiling point = 70 °C at 101,325 Pa). The total carbohydrate content was calculated by the difference.

The pH of the soymilk and fermented soymilk were measured using a pH meter (TEC-2; TECNAL, Piracicaba, SP, Brazil), according to AOAC method 981.12 (AOAC[21]).

Extraction of oligosaccharides (stachyose and raffinose) from vegetable soybeans was performed according to Oliveira et al.[22] with adaptations. The freeze-dried vegetable soybeans were ground to pass through a 0.5 mm sieve, and a sample of 2.50 g was mixed with 50 mL of an 80% ethanol solution and stirred for 2 min. The mixture was centrifuged at 5000 rpm for 10 min, and the content of the oligosaccharides was determined in the supernatant.

To analyze the oligosaccharides and organic acids contents in soymilk and fermented beverages, the samples were centrifuged at 5000 rpm for 5 min. The supernatant was centrifuged again under the same conditions, diluted in deionized water at a volumetric ratio of 50%, and neutralized with barium carbonate. Centrifugation was performed again at 5000 rpm for 5 min, and the oligosaccharides content in the supernatant was determined using HPLC.

The HPLC method described by Rivas et al.[23] was used to determine the contents of oligosaccharides. Samples of the liquors were filtered using 0.20 μm cellulose acetate membranes, neutralized with barium carbonate, and assayed

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