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Utilization of tofu whey concentrate by nanofiltration process aimed at obtaining a functional fermented lactic beverage

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ABSTRACT

Tofu whey was concentrated using the nanofiltration process up to a volume reduction factor of 4.5. The concentrate was used to produce two fermented lactic beverages: (1) with 10% of concentrated tofu whey + 90% of milk; and (2) with 20% of concentrated tofu whey + 80% of milk. Both beverages were analyzed to determine the total lactic acid bacteria count and the physicochemical, rheological and functional properties during storage (30 days). It was observed that the total lactic acid bacteria count was >8 log CFU mL^{-1}. It was observed that the total solids and protein contents of all beverages remained unchanged, but the use of concentrated tofu whey contributed to the post-acidification process. It can be verified that the viscosity was lower for beverage 2. The apparent viscosity of all samples decreased with an increase in the shear rate, indicating shear thinning and thixotropic properties. The Power Law model was successfully applied to describe the rheological behavior. The total isoflavones content was greater for beverage 2 and remained unchanged during storage, while the oligosaccharides content decreased during storage.

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1. Introduction

Functional compounds, such as isoflavones, present mainly in soy products, merit considerable interest since they are strongly associated with the prevention of some diseases, such as breast and prostate cancers, cardiovascular diseases and osteoporosis (Reynolds et al., 2006). Another important component of soybean is the oligosaccharides, which have prebiotic effects and studies have shown that their consumption is related to several health benefits, such as lowering blood cholesterol, reducing blood pressure and preventing some types of cancer (Roberfroid, 2007).

Isoflavones and oligosaccharides have been found to be present in soy products such as tofu as well as its liquid waste known as tofu whey, since these components can remain soluble after coagulation. Currently, the disposal of tofu whey represents an environmental problem due to its negative impact. Thus, a strategy to minimize this impact and allow its utilization in formulated foods needs to be devised at the concentration stage (Sobral and Wagner, 2007).

Nanofiltration is a membrane process which has been successfully employed to concentrate phenolic compounds extracted from natural products (Prudencio et al., 2012). However, the use of concentrated tofu whey to produce fortified products through the addition of exogenous functional compounds is practically non-existent. Thus, dairy products as a natural source of functional or antioxidant compounds appear to be a convenient food format to satisfy consumer interest in health benefits.

The objective of this study was to investigate a potential use of tofu whey concentrated by nanofiltration by obtaining a functional fermented lactic beverage and evaluating its microbiological, physical, chemical, rheological and functional properties during storage time.

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2. Material and methods

2.1. Materials

Tofu whey supplied by Tofutura Indústria de Alimentos Ltda (Campo Largo, Paraná, Brazil) with 0.35 g 100 g^{-1} of protein, 1.00 g 100 g^{-1}of lipids and 0.85 g 100 g^{-1}of sugars; pasteurized cow's milk with 3.5 g 100 g^{-1} of protein, 3 g 100 g^{-1} of lipids and 10 g 100 g^{-1} of sugars; and a thermophilic milk culture (YC-X11 Yo Flex®, Chr. Hansen, Hønsholm, Denmark) composed of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* were used in the production of two fermented lactic beverages. All reagents were of analytical grade.

2.2. Membrane processes

The tofu whey was submitted to the microfiltration process in a pilot filtration unit, using tangential flow and an organic polyimide membrane (PAM Membranas Seletivas, Rio de Janeiro, RJ, Brazil) in a hollow fiber configuration, with an average cross-section diameter of 0.4 μm and a filtration area of 0.7 m^2. The following operating conditions were used in the microfiltration: temperature 22 ± 1 °C; transmembrane pressure 100 kPa; and tangential velocity 1.0 m s^{-1}. The permeate resulting from the microfiltration process was used as the nanofiltration (NF) feed material. The NF was performed according described to Benedetti et al. (2013). However, the operational conditions were as follows: pressure 600 kPa; temperature 22 ± 1 °C; and volume reduction factor (VRF) 4.5, which were applied to obtain the concentrated tofu whey, with 6.12 g 100 g^{-1} of total solids and 1.20 g 100 g^{-1} of proteins. This concentrate was used to produce the fermented lactic beverages. The VRF was calculated as the ratio between the initial volume (L) of tofu whey used in the feed and the final volume (L) of the concentrate after NF. During the NF process the permeate flux (J) (L h^{-1} m^{-2}) was calculated every 3 min, according to Eq. (1):

$$J = \frac{V_p}{t.A_p} \tag{1}$$

where V_p (L) is the amount of permeate collected during the period of time t (h) and A_p (m^2) is the permeation surface area of the membrane.

2.3. Production of fermented lactic beverages

Two fermented lactic beverages were produced according to the procedures described by Najgebauer-Lejko et al. (2011), with modifications. The proportions used for the two beverages were as follows: 10% of concentrated tofu whey and 90% of milk (beverage 1); and 20% of concentrated tofu whey and 80% of milk (beverage 2). The milk and concentrated tofu whey were heated to 42 ± 1 °C and mixed in the proportions mentioned above. The lactic culture (5 g 100 g^{-1} of inoculum) was added before incubation (42 ± 1 °C) until pH 4.6 ± 0.2 was reached. The lactic beverages were then cooled to 10 ± 1 °C, being gently stirred and stored at 5 ± 1 °C until the analysis. Following the same procedure, a control sample was produced using only milk (100%).

2.4. Microbiological analysis

A lactic acid bacteria (LAB) count was carried out for all samples. The counts were obtained in triplicate according to the methodology described in APHA (2001) and the results were expressed as the logarithm of the colony count per mL of beverage (log CFU mL^{-1}).

2.5. Physicochemical analysis

All samples were analyzed to determine the total solids content (g 100 g^{-1}), through the drying of the samples until constant weight, and the protein content (g 100 g^{-1}), by the Kjeldahl method (N × 6.38) (AOAC, 2005). The acidity (g 100 g^{-1} of lactic acid) was determined according to the methodology described by the Analytical Norms of the Adolfo Lutz Institute (IAL, 2008). The pH measurements were obtained with a pH meter (MP220, Metler-Toledo, Greinfensee, Switzerland).

2.6. Rheological analysis

The rheological analysis of the samples was carried out using a Thermo Haake DC 10 rotational viscosimeter (model VT 550, Thermo Haake, Karlsruhe, Germany), with concentric cylinders (NV ST 807-0713 CE and NV 807-0702), and data was collected using the software program Pro Rheowin® (version 2.93, Haake). The flow curves were generated from the linear shear rate increase from 0.02 s^{-1} to 200 s^{-1} during the first 5 min (upward curve) and the return to 0.02 s^{-1} over the following 20 min (downward curve), under controlled temperature 5.0 ± 0.1 °C through water circulation in a temperature controlled bath (Phoenix P1, Thermo Haake, Karlsruhe, German) coupled to the equipment. The rotational speed was increased from 2 rpm to 41 rpm at 2 rpm per minute.

The flow behavior was described through the Power Law model according to Eq. (2):

$$\sigma = K(\gamma)^n \tag{2}$$

where σ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index (Pa s^{-1}), n is the flow behavior index. Viscosity values on the downward (viscosity/shear rate) curves at a rate of 50 s^{-1} were considered as the apparent viscosity (g) for beverages 1 and 2 and the control sample since, according to Bourne (2002), these values represent the approximate viscosity perceived on the palate. The thixotropic behavior was calculated from the hysteresis loop area between the upward and downward flow curves.

2.7. Extraction and determination of isoflavones

The extraction of isoflavones and the determination of their components were carried out with lyophilized samples (beverages 1 and 2), in accordance with the methodology proposed by Carrão-Panizzi et al. (2002) and Benedetti et al. (2013). The separation and quantification of the isoflavones were performed using HPLC, as proposed by Berhow (2002), with a photodiode array detector (Model 996) and an automatic sample injector (Model 717 Plus), both produced by WATERS® (Milford, USA). In this stage, a reverse phase column (YMC-Pack ODS-AM, C18, S-5 lm, diameter of 250.0 × 4.6 mm) was used. For the separation of the isoflavones, the binary linear gradient system was used and the mobile phases were: (a) methanol containing 0.025% trifluoroacetic acid (TFA) (Phase A); and (b) ultrapure water (Millipore®, Billerica, MA, USA) containing 0.025% TFA (Phase B). The initial condition of the gradient was 20% Phase A, reaching 90% in 35 min, followed by cleaning of the column with 100% of Phase A for 5 min and subsequently a return to 20%, retaining these conditions for up to 60 min. The mobile phase flow was 1 mL min^{-1} and the temperature during the analysis was 25 °C. For the isoflavone detection, the wavelength of the detector was adjusted to 254 nm. The software used to control the equipment and the data acquisition was Millennium 32 (version 3.05.01) (GCLC®, Toronto, Pickering, ON, Canada). For the identification and quantification of the peaks corresponding to each of the isoflavones, calibration curves with

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