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Processing of soy beverages obtained from the grain, flour and powder extract and fermented by probiotics

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Abstract

Soy-based fermented probiotic drinks are an option in the beverage market. For the food industry to meet the challenge of the demand for new products, the technological simplification of the production process is one factor that facilitates market insertion. The objective of this work was to evaluate the suitability of a process for fermentation, by probiotic bacteria, of liquid soy bases obtained from the grain, flour and powder extract, to have the technology for the industrial production of the beverage from different raw materials. The liquid bases were submitted to the same basic formulation process with sucrose, tricalcium phosphate and potassium sorbate dispersed in water. The probiotic bacteria tested, *Lactobacillus acidophilus* and *Bifidobacterium animalis*, showed fermentative potential in the three liquid bases studied and the final drinks complied with Brazilian legislation, which establishes a minimum concentration of 8 log CFU per serving during at least 45 days of refrigerated storage at 8 °C. Thus, the suitability of the process for the raw materials was proven. Among the drinks prepared, as well as in relation to the microorganism applied, few variations were observed related to the proximate composition, counting of spoilages and monitoring of the acidity from the process of fermentation.

Keywords: soy beverage; soybean extract powder; soy flour; fermentation.

Practical Application: This work demonstrates the feasibility of producing soy beverage fermented by probiotics, from raw material arranged in different ways.

1 Introduction

Foods with functional properties are an excellent alternative to improving quality of life, and well-being and preventing diseases. The food industry has directed resources towards the development of products that offer functional benefits to consumers, who increasingly demand this type of property (Granato et al., 2020).

Internationally, probiotics are defined as live microorganisms, that, when administered in sufficient amounts, confer health benefits on the host. The main bacteria considered beneficial to the intestinal flora and most used by the industry are *Lactobacillus acidophilus* and *Bifidobacterium animalis*. For a clinical effect considered to be probiotic, the minimum viable amount must be in the range of 8 to 9 log CFU/mL in the daily recommendation of the ready-to-eat product (Kumar et al., 2022; Zendeboodi et al., 2020).

The increasingly influential role of the food industry on the diet and lifestyle of the population is accompanied by the challenge of meeting consumer demand for products that are tasty, visually attractive, and that, at the same time, aim at health and well-being. Vegetable drinks are an alternative to dairy products, being among the fastest growing items in the non-alcoholic segment, whether due to adherence to healthy products or lactose intolerance (Pimentel et al., 2021).

Soy extract has been used as a culture medium for the growth of lactic acid bacteria, in addition to the development of fermented products such as tofu and soy yogurt. The challenges in preparing a fermented soy-based probiotic drink are based on the ability of bacteria to grow and reach a minimum population compatible with a probiotic product and on maintaining the viability of microorganisms during the refrigerated storage of the product, as well as its prevalence in the digestive tract of the consumer and have good sensory acceptability (Delgado et al., 2019; Santos et al., 2022).

In this sense, the objective of this study was to evaluate the suitability of a process for fermentation, by probiotic bacteria, of liquid soy bases obtained from the grain, flour, and powder extract, in order to have the technology for industrial production. of the drink with different raw materials.

2 Material and methods

2.1 Preparation of liquid soy bases

The liquid bases obtained by processing the grain into liquid soy extract (ESL), from soy flour (FS) and powdered soy extract (ESP) were used for the development of soy beverages. ESL liquid base was obtained according to the process described

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in Felberg et al. (2009). The soybeans were peeled, cooked and, after grinding, centrifuged using a membrane to separate the okara, a fraction composed of fibers, protein, lipids and other constituents. The FS and ESP liquid bases were prepared by mixing with water. Then, the three liquid soy bases were subjected to processing to produce fermented beverages with probiotics.

Soy flour was obtained by grinding the dehulled soybean without adding water or any other substance and then roasted, without extraction or filtration to remove the fibers from the product. This flour contains both soluble solids and okara from the cotyledon of the grain. The powdered soybean extract used consists of a food ingredient obtained in a technological process that includes a spray-dryer drying step of the liquid extract of the grain. This product does not contain okara, being composed essentially of the soluble solids of the cotyledon, in the same way as the ESL.

2.2 Production of soy beverages fermented with probiotics

Initially, a mixture of 6% (m/m) of sucrose, 6% (m/v) of extract, 6% of soy flour, 0.4% of tricalcium phosphate and 0.1% of potassium sorbate was performed. The drinks were heated and subjected to slow pasteurization until reaching the temperature range of 70 to 75 °C, for 2 min. Cooling was carried out in a water bath at room temperature until the drinks reached a temperature range of 45 to 40 °C. The containers with the cooled drinks were placed in a thermostatic bath without agitation, maintained at a constant temperature of 44 °C.

Each culture was prepared by reconstituting 1.0 g of the lyophilized culture of *Bifidobacterium animalis* and *Lactobacillus acidophilus* in 33 mL of 0.5% saline solution for 20 minutes. Inoculation took place by adding 10 mL of saline solution containing the inoculum, with brief homogenization at 2 and 4 minutes after incubation (Walter et al., 2014).

The beverage fermentation process was systematically monitored by measuring the pH value. The monitoring of the fermentation process continued until the values reached the pH range between 4.90-4.80. pH monitoring was performed using a potentiometer (Mettler Toledo, SevenGo Duo PH/Cond SG23, Schwerzenbach, Switzerland), by direct insertion of the electrode (Mettler Toledo, InLab Expert Pro-ISM-IP67). As the pH value reached the determined range, the jars containing the drinks were immersed in an ice bath and the drinks cooled to a temperature below 10 °C. Storage was carried out in a cold chamber at 8 ± 2 °C.

2.3 Physicochemical analysis

Analyzes of pH (Association of Official Analytical Chemists, 2010), total acidity (Instituto Adolfo Lutz, 2008) and determination of the proximate composition were performed. Gravimetric methods were used for ash and moisture determination (Instituto Adolfo Lutz, 2008), Kjeldahl method for crude protein determination (Association of Official Analytical Chemists, 2010), the enzymatic-gravimetric method for dietary fiber determination (Instituto Adolfo Lutz, 2008), acid hydrolysis method for determination of total lipids (Association of the Official Analytical Chemists, 2005) and the carbohydrate content was determined by difference.

2.4 Microbiological analysis

The viability of probiotic microorganisms was evaluated from the total count of lactic acid bacteria performed at 1, 4 and 6 weeks of storage at 8 °C, in samples of fermented soy beverages ESL, ESP and FS. Quantification was performed in Man Rogosa & Sharpe culture medium supplemented with 100 mg/L of cycloheximide (Oliveira et al., 2022).

Furthermore, the hygienic-sanitary quality of the products was verified, and thermotolerant coliforms, molds and yeasts, *Salmonella* sp, *Bacillus cereus* and *Staphylococcus aureus* were counted (American Public Health Association, 2015).

2.5 Statistical analysis

Analyses were performed in triplicate, following a completely randomized design. The data were submitted to the analysis of variance (ANOVA) and Tukey test ($p < 0.05$), in Statistica (7.0, Statsoft Inc.) software.

3 Results and discussion

3.1 Viability of probiotic bacteria in fermented soy beverages

The viability of *L. acidophilus* and *B. animalis* in ESL, ESP and FS soy fermented beverages during refrigerated storage at 8 °C is shown in Table 1. For the food to be considered probiotic, the minimum viable concentration of microorganisms must be situated in the range of 8 to 9 log CFU/mL in the daily recommendation of the ready-to-eat product (Brasil, 2007). Considering a 100 mL serving of soy beverage, the minimum concentration of probiotics should be on the order of 6 log CFU/mL. During the six weeks of storage, there was a reduction in the concentration of the two probiotic bacteria in the order of 1 logarithmic cycle, regardless of the liquid soy base used in the production of fermented beverages.

In beverages that used liquid and powdered soybean extract (ESL and ESP), *B. animalis* counts were higher than *L. acidophilus* counts when using the same substrates. The behavior of *B. animalis* was also more stable when present in soybean extract.

The ES beverage obtained the highest concentration, being 7 log CFU/mL for *L. acidophilus* and 8 log CFU/mL for *B. animalis*, in addition to presenting greater stability among the three beverages, with a reduction of a logarithmic cycle

Table 1. Viability of probiotic bacteria in fermented soy beverages during refrigerated storage at 8 °C.

Microorganism	Beverage	Week 1	Week 4	Week 6
Lactobacillus acidophilus	ESL	6.27^{a}	6.75^{a}	6.28^{a}
	ESP	7.99^{c}	7.60^{b}	6.00^{a}
	FS	7.00^{b}	6.94^{a}	6.40^{b}
Bifidobacterium animalis	ESL	7.89^{c}	7.94^{b}	6.00^{a}
	ESP	8.26^{c}	8.32^{c}	6.92^{c}
	FS	6.49^{a}	6.66^{a}	6.47^{b}

ESL: liquid soy extract; ESP: soy extract powder; FS: soy flour. Counts are expressed in log CFU/mL. Different letters at the same column indicates significant differences between samples ($p < 0.05$).

at the end of 6 weeks of refrigerated storage. The FS beverage maintained cell viability during the storage period studied, but it was the fermented beverage with the lowest probiotic count result, regardless of the microorganism. Some factors in the extract, such as acidity, acids produced during storage, presence of oxygen, production of antimicrobial compounds and reduction of nutrients in the substrate, are cited as responsible for reducing the viability and, consequently, the probiotic properties of the product (Sanders & Klaenhammer, 2001; Manassi et al., 2022).

From a technological point of view, probiotic microorganisms must allow their production on a large scale, resist processing, maintain stable acidity, present adequate flavors and/or aromas, as well as a pleasant texture after fermentation, in addition to maintaining a viable cell count throughout the product's shelf life (Turkmen et al., 2019).

3.2 Hygienic-sanitary assessment

The three soy beverages fermented with probiotics showed an absence or count below the detection limit for *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus* and thermotolerant coliforms, at the beginning and after six weeks of storage, indicating adequate hygienic-sanitary condition during the process.

As an indicator of deterioration, the count of molds and yeasts during storage was verified. Silva (2007) defines results above 5 log CFU/g as a high count of molds and yeasts. The sample of soy flour (FS) beverage fermented with *L. acidophilus* showed, in the sixth week of storage, a mold and yeast count of 5.18 log CFU/g, a value close to the maximum considered in the literature. Most values were absent or counted below the detection limit.

3.3 Evaluation of the fermentation process

Table 2 shows the pH variation and lactic acid formation in beverages before the fermentation process and during storage. The samples analyzed before fermentation indicate pH values ranging from 6.92 to 7.01 and lactic acid concentration in the order of 0.03 to 0.06 g/100 g. The pH values of the soy extract are compatible with the results expected in the period prior to fermentation.

After fermentation, the pH decreased as expected, with the lowest values observed in beverages fermented by *L. acidophilus*, ranging from 4.59 to 4.85. In beverages fermented by *B. animalis*, pH values remained in the range between 4.94 and 5.07, values considered optimal for the proper maintenance of bacterial viability, according to the process established by Walter et al. (2014). The titratable acidity, expressed as lactic acid, at the end of week 1 of storage was similar for both bacteria used in the fermentation, with values between 0.27-0.42 g/100 g for *B. animalis* and between 0.27-0.43 g/100 g for *L. acidophilus*. These values are similar to the acidity concentration of 0.46 found by Marin et al. (2014), who evaluated a soy-based probiotic drink for the same period.

The behavior of fermented soy beverages during the shelf life was monitored and, in general, there was a slight decrease in pH values in beverages fermented by *L. acidophilus*, from week 1 to week 4, and stability in lactic acid concentrations, for both microorganisms. The pH is one of the main factors that influence the production of lactic acid since the catalytic activity of enzymes and the metabolic activity of microorganisms depend on the extracellular pH (Zhong et al., 2021).

The behavior at week 6 was similar to that at week 4, characterized by a slight change in pH in some beverages fermented by *L. acidophilus* and stability in pH values in those fermented by *B. animalis*. Weak acids, such as lactic acid, inhibit bacterial growth, as with the drop in external pH, the acid is protonated as soon as it is exported out of the bacteria, it diffuses back into the cell and dissociates due to the greater intracellular pH. The cell then needs to use ATP to pump the protons out, resulting in a loss of energy, which causes the growth to stop and the bacteria to die. In addition, autolysis of cells occurs at a high concentration of lactic acid (Hofvendahl & Hahn-Hägerdal, 1997).

From the results achieved for the quantification of lactic acid in soy beverages fermented with probiotics, it is possible to observe an increase in concentration as a function of the fermentation process from 0.03 to 0.49 g/100 g.

3.4 Determination of the centesimal composition

The centesimal composition, calculated on a wet basis, of the different soy beverages is described in Table 3. As shown,

Table 2. pH and acidity values of soy beverages before fermentation and after fermentation with probiotic bacteria, during refrigerated storage at 8 °C.

Microorganism	Beverage	Before fermentation		Week 1		Week 4		Week 6	
		pH	Acidity (g/100 g)	pH	Acidity (g/100 g)	pH	Acidity (g/100 g)	pH	Acidity (g/100 g)
Lactobacillus acidophilus	FS	6.92^{a}	0.03^{a}	4.80^{a}	0.27^{a}	4.76^{a}	0.31^{a}	4.75^{a}	0.31^{a}
	ESP	6.98^{a}	0.06^{a}	4.67^{a}	0.43^{b}	4.51^{a}	0.50^{b}	4.57^{a}	0.49^{b}
	ESL	7.01^{a}	0.06^{a}	4.69^{a}	0.40^{b}	4.57^{a}	0.45^{b}	4.53^{a}	0.45^{b}
Bifidobacterium animalis	FS	6.93^{a}	0.06^{a}	5.03^{a}	0.27^{a}	5.10^{b}	0.27^{a}	5.07^{b}	0.26^{a}
	ESP	6.97^{a}	0.06^{a}	4.96^{a}	0.42^{b}	5.00^{b}	0.42^{b}	4.99^{b}	0.41^{b}
	ESL	7.01^{a}	0.05^{a}	5.03^{a}	0.35^{b}	5.05^{b}	0.41^{b}	5.07^{b}	0.42^{b}

ESL: liquid soy extract; ESP: soy extract powder; FS: soy flour. Different letters at the same column indicates significant differences between samples ($p < 0.05$).

Table 3. Centesimal composition of soy beverages fermented with probiotics (wet basis, in g/100 g).

Microrganism	Beverage	Moisture	Ash	Protein	Carbohydrate	Lipid
				Week 1		
Lactobacillus acidophilus	FS	86.68[a]	0.71[a]	2.75[a]	8.13[a]	1.73[b]
	ESP	87.37[a]	0.81[a]	2.63[a]	8.63[a]	0.56[a]
	ESL	87.06[a]	0.67[a]	2.50[a]	9.27[a]	0.50[a]
Bifidobacterium animalis	FS	86.68[a]	0.72[a]	2.94[a]	8.03[a]	1.74
	ESP	86.98[a]	0.82[a]	2.85[a]	8.56[a]	0.45[a]
	ESL	86.60[a]	0.75[a]	2.78[a]	9.40[a]	0.48[a]
				Week 6		
Lactobacillus acidophilus	FS	86.54[a]	0.59[a]	2.97[a]	7.46[a]	1.86[b]
	ESP	86.74[a]	0.77[a]	3.06[a]	8.09[a]	1.34[a]
	ESL	86.32[a]	0.65[a]	2.75[a]	8.92[a]	1.36[a]
Bifidobacterium animalis	FS	86.66[a]	0.61[a]	2.76[a]	8.17[a]	1.79[b]
	ESP	86.59[a]	0.76[a]	2.91[a]	8.46[a]	1.29[a]
	ESL	86.90[a]	0.76[a]	2.85[a]	8.22[a]	1.69[b]

ESL: liquid soy extract; ESP: soy extract powder; FS: soy flour. Different letters at the same column indicates significant differences between samples ($p < 0.05$).

Table 4. Fermentation time and pH variation of fermentation processes.

Microrganism	Beverage	Initial pH	Final pH	Variation	Fermentation time
Lactobacillus acidophilus	FS	6.77	4.84	1.93	4 h 18 min
	ESL	8.40	4.64	3.76	4 h 13 min
Bifidobacterium animalis	FS	6.73	5.02	1.71	5 h 7 min
	ESP	6.68	4.96	1.72	3 h 56 min
	ESL	6.73	5.04	1.69	5 h 12 min

ESL: liquid soy extract; ESP: soy extract powder; FS: soy flour.

there were no relevant differences in moisture, ash, protein and carbohydrate contents.

The values found for proteins in the ESP drink ranged between 2.63 and 3.06 g/100 g. Differences between the sample results may be related to the extraction rate and forms, and this directly results in the concentration of compounds contained in the extract. The whole soy flour used to obtain the soy flour drink had higher lipid contents when compared to the drink where the grain was used to prepare the liquid extract and the powder extract.

3.5 Fermentation time

Table 4 shows the pH variation of soy beverages in response to the fermentation time required for the beverage pH to reach pH values between 4.8 and 4.9, as described by Walter et al. (2014). It can be seen that the initial pH values ranged between 6.77 and 8.40, decreasing during fermentation.

The shortest fermentation time of soy beverages found using *L. acidophilus* as fermenting bacteria was 4 h and 13 min. The fermentation time of soy beverages fermented by *B. animalis* ranged between 5 h and 31 min and 03 h 35 min. The process using powdered soybean extract resulted in the process with the shortest duration.

4 Conclusion

The possibility of producing fermented soy-based probiotic drinks formulated from powdered soy extracts and soy flour, raw materials available on the market, has been proven, instead of the traditional process starting from the grain, in a simplified production process, with the maintenance of viable probiotic microorganisms during the shelf life.

Among the prepared beverages, as well as in relation to the applied microorganism, few relevant variations were observed in relation to the proximate composition, deterioration count and monitoring of the acidity of the fermentation process. To carry out a complete validation of the products developed, sensory analysis is recommended.

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