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Optimization of fermentation parameters to study the behavior of selected lactic cultures on soy solid state fermentation

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ABSTRACT

The use of solid fermentation substrate (SSF) has been appreciated by the demand for natural and healthy products. Lactic acid bacteria and bifidobacteria play a leading role in the production of novel functional foods and their behavior is practically unknown in these systems. Soy is an excellent substrate for the production of functional foods for their low cost and nutritional value. The aim of this work was to optimize different parameters involved in solid state fermentation (SSF) using selected lactic cultures to improve soybean substrate as a possible strategy for the elaboration of new soy food with enhanced functional and nutritional properties. Soy flour and selected lactic cultures were used under different conditions to optimize the soy SSF. The measured responses were bacterial growth, free amino acids and β-glucosidase activity, which were analyzed by applying response surface methodology. Based on the proposed statistical model, different fermentation conditions were raised by varying the moisture content (50–80%) of the soy substrate and temperature of incubation (31–43 °C). The effect of inoculum amount was also investigated. These studies demonstrated the ability of selected strains (*Lactobacillus paracasei* subsp. *paracasei* and *Bifidobacterium longum*) to grow with strain-dependent behavior on the SSF system. β-Glucosidase activity was evident in both strains and *L. paracasei* subsp. *paracasei* was able to increase the free amino acids at the end of fermentation under assayed conditions. The used statistical model has allowed the optimization of fermentation parameters on soy SSF by selected lactic strains. Besides, the possibility to work with lower initial bacterial amounts to obtain results with significant technological impact was demonstrated.

1. Introduction

Soy is a traditional and central food for Eastern cultures and it has been adopted in the West due to the knowledge and dissemination of its nutritional properties and its potential positive health effects. Argentina ranks third in the world in the production of soybean with an output of 40 million tonnes in 2012 (FAO, 2012) and is the first exporter of soy worldwide, so soy has become an important agricultural commodity in the national economy.

Consumption of soybean has been linked to the prevention of cardiovascular and gastrointestinal diseases, cholesterol reduction, cancer, diabetes and obesity (Gonzalez de Mejia and De Lumen, 2006; Jenkins et al., 2000; Kerckhoffs et al., 2002; Setchell, 1998; Singh et al., 2008; Tikkanen and Adlercreutz, 2000). These health benefits are attributed to the presence of several bioactive compounds which can be separated into protein compounds and non-protein compounds, such as isoflavones. Soy protein has a potential biological value because it is an excellent source of essential amino acids (Kellor, 1974). The hydrolysis of soy protein increases its solubility so the intestinal absorption of protein hydrolysates appears to be more effective than intact proteins (Kong et al., 2008). Different processes have been extensively applied to hydrolyze proteins such as acidic or enzymatic hydrolysis. The latter process can be made with commercial enzymes or enzymes from microorganisms. One of the simplest ways of producing food-grade hydrolyzed proteins is to use lactic acid bacteria (LAB), which are generally recognized as safe and are traditionally used to ferment raw materials of vegetable and animal origin (Aguirre et al., 2008).

On the other hand, isoflavones are phenolic compounds of non-steroid nature with structural similarity to estrogen. Isoflavones responsible for the biological effects are the aglycones which are obtained from the hydrolysis of glycosyl-isoflavones by the enzyme β-glucosidase. In soybean the main isoflavones present are those in glycosyl forms, but little amounts of those in aglycone forms can be present (Wang and Murphy, 1994). Probiotic microorganisms including *Lactobacillus* and *Bifidobacterium* have been known to possess endogenous β-glucosidases which can play an important role in the bioconversion of glycosyl isoflavones during fermentation increasing the amounts of biologically active isoflavones (aglycone form) (Marazza et al., 2009; Otieno et al., 2005).

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LAB play a leading role in the production of novel functional foods. Most of the studies with LAB were developed using submerged fermentation (SmF) and there is a lack of detailed information in the literature about the behavior of LAB on soy solid state fermentation or semi-solid state fermentation (SSF). During this process microorganisms are grown on the surface of solid materials with limited water amounts. Based on the metabolic needs of fermentation microorganisms, the control of water activity, oxygen content, temperature, and pH are the most important SSF parameters (Chen, 2013). SSF is a very interesting technological alternative to obtain new products as it uses waste and/or cheap raw material as substrate. In this sense soy is a great substrate to use for its high nutritional value and low cost.

The aim of this study was to optimize different parameters involved in solid state fermentation (SSF) using selected lactic cultures to improve soybean substrate as a possible strategy for the elaboration of new soy food with enhanced functional and nutritional properties. To optimize soy SSF, we used soy flour and selected lactic cultures under different conditions in order to: a) get the best bacterial growth on solid substrate, b) increase the protein digestibility, and c) obtain higher β-glucosidase activity by application of response surface methodology. The variation of initial bacterial concentration on the behavior of selected strains under previously optimized conditions of soy SSF was also investigated.

2. Materials and methods

2.1. Microorganisms and growth conditions

Lactobacillus (*L.*) *paracasei* subsp. *paracasei* CRL 207 and *Bifidobacterium* (*B.*) *longum* CRL 849 were obtained from the culture collection (CRL) of the Centro de Referencia para Lactobacilos (CERELA). These organisms were selected by their ability to grow on soy substrate using its available carbohydrates and produce β-glucosidase enzyme or hydrolyzed proteins. Before experimental use, cultures were propagated (2%, v/v) twice in MRS medium (De Man et al., 1960) for *Lactobacillus* and incubated at 37 °C for 18 h without agitation. *Bifidobacterium* was grown in MRS supplemented with 1% sucrose, 0.00005% vitamin K and 0.0005% hemin, and incubated at 37 °C for 18 h in microaerophilic conditions without agitation. All solutions were sterilized separately (0.22 μm filtration), and then added to the MRS base. In order to obtain the inoculums for the fermentation process, cells at the end of the exponential phase of growth (5.0 mL initial volume) were collected by centrifugation (10,000 g, 10 min, 4 °C), washed twice with sterile physiological solution and resuspended in 2.5 mL of the same solution. This concentration of the starter cultures was important to avoid the change in the initial moisture of the soy pastes.

2.2. Factorial design and solid state fermentation

Response surface methodology was applied to analyze the solid state fermentation parameters with *L. paracasei* subsp. *paracasei* CRL 207 and *B. longum* CRL 849 in terms of growth (*y1*), free amino acids (*y2*) and β-glucosidase activity (*y3*). The response function *y1* was expressed as the pH difference between fermented soy paste at 24 h and uninoculated soy paste treated in the same way (control), or as the difference between final and initial count cells of fermentation for each condition. The response function *y2* was defined as the difference between the amino acid amounts from fermented soy paste at 24 h and control, and *y3* as the difference in the β-glucosidase activity between the fermented paste at 24 h and control. The coded independent variables and uncoded variables are shown in Table 1 with their variation levels.

In order to evaluate the effects of temperature (T) and moisture (M) and their possible interactions on the three responses, the levels of the independent variables were defined according to 2^2 full-factorial Central Composite Design (CCD), comprising 11 experimental runs in 2 blocks: block 1 was conducted with 6 random assays (4 factorial points and 2 central points), and block 2 with 5 random assays (4 axial points and 1 central point) (Tables 2 and 3).

Table 1
Independent variables and levels of variation in Central Composite Design (CCD).

Independent variables	Levels of variation				
	−1.5	−1	0	+1	+1.5
x1: moisture (%)	50	55	65	75	80
x2: temperature incubation (°C)	31	33	37	41	43

The observations on the CCD were fitted to the second-order polynomial model as follows:

$$Y = \beta_0 + \beta_j + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{12} X_1 X_2 + e$$

where Y is the predicted response of the dependent variable, β_0 is the mean of the total observations (constant), β_j is the estimated coefficient of the block for the response surface model, X_1 and X_2 are coded variables and β is the estimated coefficient for each term of the response surface model.

For each assay, SSF employed 150 g of soy paste (wet weight) which was prepared from commercial soy flour and distilled water to achieve the different moisture contents (*x1*), into 250 mL Erlenmeyer. After water was added, the pastes were homogenized and sterilized by autoclaving at 118 °C for 20 min. When the pastes reached room temperature, they were inoculated with 4% (v/w) of each culture, homogenized and uniformly distributed into Petri plates and incubated at the established temperatures for the design (*x2*) during 24 h. Uninoculated soy paste treated in the same way was used as a control. Samples at different times (0, 4, 8, 12, 16 and 24 h) were taken and linear effects such as the quadratic of the proposed variables (M and T) on three responses (*y1*, *y2* and *y3*) were analyzed. The response functions (*y1*, *y2* and *y3*) were used to perform regression analyses and analyses of variance (ANOVA) for the regression. The experiment results were analyzed using the minitab-15 statistical package (MINITAB Inc., PA, USA), and response surface curves were drawn.

2.3. Moisture determination

The initial moisture of the soy pastes (after sterilization) was expressed as wet basis moisture content, experimentally determined by the method 950.46.B AOAC (1995) and calculated according to the

Table 2
Experimental conditions and results of the statistical experimental design for *B. longum* CRL 849.

Assays	Independent variables coded and uncoded		Response functions		
	x1	*x2*	*y1*	*y2*	*y3*
1	75 (+1)	41 (+1)	−1.33	−0.89	16.210
2	65 (0)	37 (0)	−1.62	−4.64	19.182
3	55 (−1)	33 (−1)	−0.81	0.46	27.356
4	75 (+1)	33 (−1)	−1.42	−0.63	21.256
5	65 (0)	37 (0)	−1.62	−4.31	20.195
6	55 (−1)	41 (+1)	−0.63	−5.25	18.677
7	65 (0)	31 (−1.5)	−0.80	0.61	25.910
8	65 (0)	43 (+1.5)	−0.69	−0.13	27.976
9	65 (0)	37 (0)	−1.39	−4.58	20.276
10	50 (−1.5)	37 (0)	−0.95	−1.34	28.569
11	80 (+1.5)	37 (0)	−1.64	−2.72	16.887

Note: *x1*: moisture (%), *x2*: temperature (°C). Response functions *y1*: ΔpH, *y2*: ΔOPA and *y3*: β-glucosidase activity.