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Biotechnological process for producing black bean slurry without stachyose

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ABSTRACT

Beans are important sources of proteins and other nutrients. However, stachyose and other oligosaccharides (RFOs) are present in this legume causing flatulence (H_2, CO_2 and CH_4), abdominal pain, and diarrhea. The problematic digestibility of these sugars in the small intestine is attributed to a lack of α-galactosidase, which is essential for the hydrolysis of α-1,6 linkages. The aim of the present work was to reduce the stachyose of black bean slurry by lactic acid fermentation using a selected *Lactobacillus* LPB56, an α-galactosidase producer. The bean slurry (6L) was fermented in a bioreactor with 1.3% (w/v) of $CaCO_3$, at 37 °C and 160 rpm. Bacterial cells increased from 2.4×10^7 to 7.0×10^8 CFU/mL, and the stachyose and other sugars were totally consumed after 18 h of fermentation. The maximum activity of α-galactosidase was 0.162 U/mL after 6 h. The fermentative process did not cause significant changes on the composition of the bean product.

1. Introduction

Black beans and other types of leguminous beans are important sources of proteins and other nutritive compounds. Brazil is the largest producer and consumer of beans in the world. Among the bean producing countries are Brazil, India, China, Mexico, and others (FAO, 2005).

Beans are considered the main source of protein for Brazilians, followed by bovine meat and rice (Lajolo, Genovese, & Menezes, 1996). They are excellent food since they provide essential nutrients for human nutrition, like proteins, carbohydrates, iron, calcium, zinc, magnesium, vitamins (including complex B), and fibers (Soares, 1996).

Bean grains are constituted of 20–25% protein, 1–3% lipids, 60–65% carbohydrates, and 1–20% dietary fiber (Geil & Anderson, 1994), depending on cultivation and culture conditions. Common beans have variable composition regarding lipids, with a considerable quantity of unsaturated fatty acids (Reyes-Moreno & Paredez-López, 1993). Besides, they have a high content of essential minerals and a low content of sodium (Sgarbieri, 1989).

The chemical composition of beans is shown in Table 1.

It has been suggested that many micronutrients present in beans – such as anthocyanins, lecithin, and trypsin inhibitors – have protective and therapeutic effects against cancer and, thus, may be used in dietary chemopreventive strategies (Wang & Murphy, 1994). According to Madhujith and Shadidi (2005), the antioxidants present in beans remove the free radicals and chemical species that react with oxygen, preventing DNA oxidative damages and cellular transformations that may lead to degenerative diseases. Besides, the fibers present in beans are associated to prevention of cardiovascular diseases, diabetes, and colon cancer (Vanderhoof, 1998).

In spite of its nutritional properties, the consumption of beans is somewhat limited in part of the Brazilian population due to some digestion problems it provokes, which are associated to undesirable components like tannins, phytates, protease and amylase inhibitors, lectins, tannins, phytates, and to the presence of the raffinose family of oligosaccharides (RFOs), like raffinose, stachyose, and verbascose. These α-galactosides oligosaccharides are not hydrolyzed in the small intestine due to a lack of the specific hydrolase enzyme; while, in the low intestinal tract, bacterial colonies are able to ferment these sugars producing CO_2, H_2 and CH_4, often with flatulence (Iyer, Salunkhe, Sathe, & Rockland, 1980; Vidal-Valverde, Frias, & Valverde, 1993). Other symptoms that can appear are nausea, cramps, abdominal pain (Cristofaro, Mattu, & Wuhrmann, 1974), headache, mental disturbance, and a decrease in concentration (Sanni, Onilude, & Ogundoye, 1997).

The raffinose family of oligosaccharides (RFOs) is present in soybean and other legumes in relatively small quantities as components of the carbohydrate fraction (Bach Knudsen & Li, 1991). In general, the predominant RFO is stachyose, followed by raffinose and verbascose, depending on the type of grain (Petterson & Mackintosh, 1994). On the oligosaccharide structure, sucrose may contain one or more α-D-galactopyranosyl (α-D-Gal) groups, in which the α-galactosyl units are always to the left of sucrose, connected

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Table 1
Chemical composition of common beans (*Phaseolus vulgaris* L.).

Compounds	Quantity (g/100 g)
Moisture	9.4
Proteins	18.4
Total lipids	2.3
Ash	0.6
Fibers	4.6
Total carbohydrates	64.7
Stachyose[a]	*3.23*
Raffinose[a]	*0.4*
Verbascose[a]	*0.12*

Adapted from de Oliveira et al. (2001).
[a] Raffinose family of oligosaccharides is included in the fraction of total carbohydrates.

to the molecule of glucose. (Silva, Braga, Bianchi, & Lopes, 1992). The structural relationship of these sugars is shown in Fig. 1.

The RFOs are considered the cause of flatulence (Dey & Pridham, 1972). The absence of the α-galactosidase enzyme in the low gastrointestinal tract (responsible for the hydrolysis of α-1,6 galactosides linkages) and its accumulation in the large intestine, result in the fermentation by anaerobic bacteria (Fleming, 1981).

Tomlin, Lowis, and Read (1991) evaluated the influence of adding 200 g of baked beans in the diet of ten healthy patients. They observed that the volume of gases increased from 476 mL to 1491 mL per day (average = 705 mL/24 h).

Kuriyama and Mendel (1917) related the ingestion of galactooligosaccharides (GOS) to an increase in the incidence of diarrhea in mice. This fact was confirmed by Li et al. (2003), when a diet supplemented with 2% purified stachyose increased the occurrence of diarrhea in pigs when compared to control.

Soluble saccharides present in black bean composition (e.g. sucrose) are fermentable by most lactic acid bacteria; while, raffinose and stachyose are not fermented by most lactic acid bacteria (Silver, 2001). However, several works reported the production of different levels of the α-galactosidase enzyme by lactic acid bacteria – like *Bifidobacterium* – which metabolize these α-galactosides present in soybean milk (Scalabrini, Rossi, Spetolli, & Matteuzzi, 1998). Results from Donkor, Henriksson, Vasiljevic, and Shah (2007) showed that *Lactobacillus acidophilus* (La4962 and L10), *Bifidobacterium lactis* B94 and *Bifidobacterium longum* Bl536, *Lactobacillus casei* Lc279 and *Lactobacillus casei* L26, *Streptococcus thermophilus* St1342 and *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 presented variable enzymatic activity of α-galactosidase.

The aim of the present work was to use a selected strain of *Lactobacillus*, with a high α-galactosidase activity, to reduce the stachyose and other galactosides present in black bean (*Phaseolus vulgaris* L.) slurry, and to compare the physical–chemical characteristics of the fermented slurry with the unfermented slurry (control).

Sucrose
α-D-Glu-(1→2)-β-D-Fru

Raffinose
*α-D-Gal-(1→6)-*α-**D-Glu-(1→2)-β-D-Fru**

Stachyose
*α-D-Gal-(1→6)-α-D-Gal-(1→6)-*α-**D-Glu-(1→2)-β-D-Fru**

Verbascose
*D-Gal-(1→6)-α-D-Gal-(1→6)-α-D-Gal-(1→6)-*α-**D-Glu-(1→2)-β-D-Fru**

Fig. 1. Structural relationship of the RFOs (Silva et al., 1992).

2. Material and methods

2.1. Bean slurry preparation

Black beans (*Phaseolus vulgaris* L.) were washed and soaked for about 16 h (de Oliveira, Queiroz, Helbig, Reis, & Carraro, 2001) at 20 °C. To prepare the slurry, 1000 g beans and 5000 mL distilled water (w/v) were used. Cooking was performed at 121 °C/15 psi for 30 min (Sat & Keles, 2002). The cooked beans and the soaking water were treated in a blender for around 3–5 min until producing a homogeneous slurry.

2.2. Standardization of the medium

Calcium carbonate (1.3% w/v) was added to the slurry and pH was set to 6.5 with NaOH 2.5 N. The medium was sterilized at 121 °C/15 psi for 15 min.

2.3. Inoculum preparation

Lactobacillus LPB56 stock (−20 °C) was reactivated in MRS broth (Merck) and plated on MRS agar. Isolated colonies were inoculated in 20 mL of MRS broth and incubated at 37 °C for 16 h. The inoculum was prepared using a culture previously grown in MRS broth and inoculated at 10% (v/v) in sterile bean slurry (10 g beans in 100 mL distilled water, pH 6.5), cultivated without agitation at 37 °C for 24 h.

2.4. Biotechnological process

The lactic acid fermentation was performed using 6 L sterile black bean slurry in a bioreactor MDL B. E. Marubishi, and inoculating with 10% inoculum (*Lactobacillus* LPB56) adapted to bean slurry. The incubation was at 37 °C for 24 h with slow agitation (160–200 rpm), to obtain a uniform distribution of bacterial cells and calcium carbonate. The growth of lactic bacteria was evaluated by periodic viable cell counting on MRS agar plates, according to Hoben and Somasegaran (1982), and incubated at 37 °C for 24–36 h.

2.5. Determination of sugars and lactic acid

The quantification of sugars as stachyose, raffinose, glucose, fructose, and galactose, as well as that of the lactic acid (g/L), was performed by HPLC (High Performance Liquid Chromatography) using Chromatograph Shimadzu, Aminex HPX-87 H column and refraction index detector. The mobile phase was H_2SO_4 5 mM, flow 0.6 mL/min, 60 °C. The samples were extracted according to optimal conditions determined by Xiaoli et al. (2008). Triplicate samples (1 g) were extracted three times with 10 mL of a 50% ethanol–water solution in water bath at 50 °C for 30 min. After each extraction, the samples were centrifuged at 10,000 rpm for 20 min. Supernatants from three cycles of extraction were combined and concentrated in vacuum oven (Vacucell 22,55 1 M) at 40 °C. The samples were dissolved in 5 mL of the mobile phase of HPLC and filtered through 0.22 μm membranes (Millipore).

Standards for HPLC analysis were Stachyose tetrahydrate, D(+)-raffinose pentahydrate, D(+)-glucose anhydrous, and D(+)-galactose (Acros Organics, Belgium); Sucrose and L-lactic acid 85.0% P. A. (Synth, Brazil); D(+)-Fructose anhydrous (Vetec, Brazil). All reagents were of analytical-reagent grade.