Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 98 (2007) 1675–1679
BIORESOURCE TECHNOLOGY

Short Communication

Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract

Ki-Bum Park [b], Suk-Heung Oh [a,*]

[a] *Department of Medicinal Biotechnology, Woosuk University, Jeonju 565-701, Republic of Korea*
[b] *Department of Life Science and Technology, Graduate School, Woosuk University, Jeonju 565-701, Republic of Korea*

Received 19 September 2005; received in revised form 6 June 2006; accepted 12 June 2006
Available online 18 October 2006

Abstract

Yogurt with high levels of gamma-aminobutyric acid (GABA), free amino acids and isoflavones was developed using lactic acid bacteria (LAB) and germinated soybean extract. Fermented soya milk (GABA soya yogurt) produced with starter and substrate had the GABA concentration of 424.67 μg/g DW, whereas fermented milk produced by a conventional method had GABA less than 1.5 μg/g DW. The GABA soya yogurt also contained significantly high levels of free amino acids and isoflavones compared with other conventional yogurts. The results suggested that the *Lactobacillus brevis* OPY-1 and germinated soybean possessed a prospect to be applied in dairy and other health products with high nutritive values and functional properties.

Keywords: Gamma-aminobutyric acid (GABA); Germinated soybean; Isoflavones; *Lactobacillus brevis*; Yogurt

1. Introduction

Yogurt is a nutrient-rich fermented food made of milk, containing various organic acids, peptones, peptides, other trace activators and lactic acid bacteria. Yogurt has an intestine-cleaning function to promote the proliferation of intestinal lactic acid bacteria (Savaiano et al., 1984; Park et al., 2003). Ingredients such as non-fat dry milk, soya protein, vegetables, sweet potato, pumpkin, plum, etc. are sometimes added into Korean yogurts (Park et al., 2003; Ko, 1989; Joo et al., 2001). Soybean is a very good source of plant protein (Brunsgaard et al., 1994). Glutamic acid (Glu) is one of the most abundant amino acids found in legumes such as soybean, red bean and mung bean (Koh et al., 1997).

Gamma-aminobutyric acid (GABA) is a ubiquitous non-protein amino acid which is produced primarily by the α-decarboxylation of Glu catalyzed by the enzyme glutamate decarboxylase (GAD) (Satya Narayan and Nair, 1990). It is well known that GABA functions in animals as a major inhibitory neurotransmitter (Krogsgaard-Larsen, 1989; Mody et al., 1994). GABA is involved in the regulation of cardiovascular functions, such as blood pressure and heart rate, and plays a role in the sensations of pain and anxiety (Mody et al., 1994). The consumption of GABA-enriched foods such as milk (Hayakawa et al., 2004), soybean (Shizuka et al., 2004), tempeh (Aoki et al., 2003), gabaron tea (Abe et al., 1995), red mold rice (Tsuji et al., 1992), and *Chlorella* (Nakamura et al., 2000) has been reported to depress the elevation of systolic blood pressure in spontaneously hypertensive rats (SHRs).

It has been recently reported that: when chitosan is used in the medium for the germination of brown rice, GAD activity increases. As a consequence, germinated brown rice with increased GABA concentration was produced (Oh and Choi, 2000). GABA and some free amino acids such as alanine in germinated brown rice were further

* Corresponding author. Tel.: +82 63 290 1433; fax: +82 63 290 1429.
E-mail addresses: shoh1573@yahoo.co.kr, shoh@woosuk.co.kr (S.-H. Oh).

0960-8524/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.06.006

increased by joint treatment with chitosan and glutamic acid (Oh and Oh, 2003). By applying such methods, germinated soybean with enhanced levels of GABA and free amino acids was produced, and its extract was used for producing yogurt. The GABA content was also enhanced by applying microorganisms with high GABA producing ability for yogurt. Here we report the methods and materials to produce the yogurt with high levels of GABA, free amino acids and isoflavones.

2. Methods

2.1. Microogranisms and media

Strains used in this study were *Lactobacillus acidophilus* (KCCM 40265), *Lactobacillus plantarum* (KCTC 3105) and *Lactobacillus brevis* OPY-1 (KFCC 11337). The *L. brevis* OPY-1 strain was isolated from *Kimchi* and deposited to Korea Culture Center of Microorganisms. Stock cultures were maintained on agar plates containing 55 g/L of MRS broth and 20 g/L of agar. Seed culture was conducted in MRS broth medium. The initial pH of a medium was adjusted to 6.2 and was not regulated during flask culture. The medium was sterilized in an autoclave at 121 °C and 1.5 psi for 20 min.

2.2. Cultivation of L. brevis OPY-1

L. brevis OPY-1 seed culture was prepared in a 100 mL flask with 10 mL MRS broth incubated at 30 °C and 150 rpm for 24 h. A 4% volume of seed culture was used as its inoculum for the flask culture. In order to investigate the GABA production by *L. brevis* OPY-1, the flask cultures were carried out in a 250 mL flask with 50 mL of MRS broth with 1% (w/v) of monosodium glutamate (MSG) at 30 °C and 150 rpm for 24 h.

2.3. Producing germinated soybean and fermentation substrate

Germinated soybean was produced as described elsewhere (Oh and Oh, 2003). In brief, 50 g of commercial soybean was germinated in an incubator with 100 mL solution to which a chitosan/glutamic acid germination solution (50 ppm chitosan dissolved in 5 mM glutamic acid) was added at 25 °C. The germination solution was exchanged for fresh solution at 12 h intervals until germination was complete at 72 h. After removing the germinated soybean from the solution, it was dried on a filter paper. The germinated soybean was frozen in liquid nitrogen and ground with a mortar and pestle as described (Oh and Oh, 2003). Four volumes of double distilled water were added to the soybean powder, and the mixture was sterilized in an autoclave at 121 °C and 1.5 psi for 20 min. The sterilized sample was filtered and treated with α-amylase (Park and Oh, 2005) to use as a fermentation substrate.

2.4. Producing starter and yogurt

The procedures to produce starter and fermentation were as described by Park and Oh (2005) with minor modifications. The *L. acidophilus*, *L. plantarum* and *L. brevis* OPY-1 strains were inoculated into *Lactobacillus* MRS broth (4% v/v), and the inoculum was activated at three times at 37 °C for 24 h to use as the starter for production of yogurt. Powdered whole milk (18%) and skim milk (2%) were added to the prepared fermentation substrate solution and homogenized in a Warning blender for 5 min. Afterwards, it was sterilized in an autoclave for 20 min at 121 °C. After the sterilized substrate was warmed to 30 °C, the substrate was inoculated with the mixed strain starter (*L. acidophilus* + *L. plantarum* + *L. brevis* OPY-1 strain, 1:1:3 v/v), and was fermented at 30 °C for 24 h.

2.5. GABA assay

Contents of GABA in the cell suspension of *L. Brevis* OPY-1, germinated soybean extract and fermented GABA soya milk were determined by HPLC (Waters, Milford, MA) as described earlier (Oh and Oh, 2003; Park and Oh, 2005). GABA was extracted essentially as described by Baum et al. (1996) with minor modifications (Oh and Oh, 2003). GABA contents were calculated using the Autochro WIN program (Young-Lin, Seoul, Korea).

2.6. Measuring viable count

Sample (1 mL) was collected 4 h after inoculation and diluted 10 fold with sterilized physiological saline. After that 0.1 mL of aliquot was smeared on MRS plate count agar using a micropipette and incubated for 24 h at 37 °C. Visible colonies were then counted and the unit expressed as CFU (colony forming unit)/mL.

2.7. Content of isoflavones in yogurt

The contents of isoflavones in GABA soya milk were determined by HPLC (Waters) as described by Wang et al. (2003) with minor modifications (Kim et al., 2004). Isoflavones were extracted as described by Kim et al. (2004). Isoflavone contents were calculated from standard calibration curves generated by using standard genistein, daidzein and glycitein.

2.8. Sensory evaluation of yogurt

To evaluate the sensory properties of the product, the curd of yogurt, which was incubated at 20 °C for 20 h, was broken and kept in a refrigerator at 4 °C for 5 h. Afterwards, 20 panelists evaluated its overall acceptability, taste, odor, texture, etc., and each item was scored between 1 and 5 points, in which 1 is equal to worst and 5 is equal to best. Differences in preferences between the conventional yogurt and GABA soya yogurt were analyzed with Student's *T*-test