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Original Article

Optimization of culture conditions for gamma-aminobutyric acid production in fermented adzuki bean milk

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ABSTRACT

γ-Aminobutyric acid (GABA), a nonprotein amino acid, is widely distributed in nature and fulfills several physiological functions. In this study, various lactic acid strains commonly used to produce fermented milk products were inoculated into adzuki bean milk for producing GABA. The high GABA producing strain was selected in further experiment to improve the GABA production utilizing culture medium optimization. The results demonstrated that adzuki bean milk inoculated with *Lactobacillus rhamnosus* GG increased GABA content from 0.05 mg/mL to 0.44 mg/mL after 36 hours of fermentation, which showed the greatest elevation in this study. Furthermore, the optimal cultural condition to adzuki bean milk inoculated with *L. rhamnosus* GG to improve the GABA content was performed using response surface methodology. The results showed that GABA content was dependent on the addition of galactose, monosodium glutamate, and pyridoxine with which the increasing ratios of GABA were 23–38%, 24–68%, and 8–36%, respectively. The optimal culture condition for GABA production of adzuki bean milk was found at the content of 1.44% galactose, 2.27% monosodium glutamate, and 0.20% pyridoxine. Under the optimal cultural condition, the amount of GABA produced in the fermented adzuki bean milk was 1.12 mg/mL, which was 22.4-fold higher than that of the unfermented adzuki bean milk (0.05 mg/100 mL). The results suggested that the optimized cultural condition of adzuki bean milk inoculated with *L. rhamnosus* GG can increase GABA content for consumers as a daily supplement as suggested.

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1. Introduction

The adzuki bean (*Vigna angularis*), an annual vine widely grown throughout Southern Taiwan, is used mainly in the production of sweets such as mooncakes, tangyuan, and youkan. It is a good source of a variety of minerals, with 100 g of adzuki beans providing 7.1 mg iron, 162 mg magnesium, 442 g potassium, 3.1 mg zinc, and 94.5 mg calcium. It also contains various amino acids, the most abundant being glutamate (3608 mg/100 g), followed by aspartate, leucine, lysine, and arginine [1]. In addition, the adzuki bean contains proanthocyanidins and polyphenols, which are known to have antioxidant effects, attenuate blood pressure elevation, lower serum triglyceride level, suppress hyperglycemia, and prevent cancer metastasis [2]. Although the adzuki bean is used extensively in the treatment of dropsy and beriberi in traditional Chinese medicine [3], it is seldom the focus of functional food development studies.

Gamma-aminobutyric acid (GABA) is a four-carbon nonprotein amino acid that is known to function as an inhibitory neurotransmitter in the brain and spinal cord of mammals. GABA is produced primarily from the α-decarboxylation of glutamate catalyzed by glutamate decarboxylase (GAD) and is known for relieving menopausal syndrome, enhancing immunity, treating cancer, preventing chronic alcohol-related symptoms, and fighting obesity [4]. Although GABA is available in many fruits and vegetables, its concentration in foods is low in nature, ranging from 0.03 to 2.00 μmol/g fresh weight [5]. Many research studies focus on finding new ways to increase GABA content in natural foods that can benefit human health. Environmental stress, additives, and biotransformation have been found to stimulate the accumulation of GABA [6,7]. Microorganism fermentation is an effective and convenient method to ferment and produce GABA in the food industry. The fermentation of *Escherichia coli*, *Aspergillus oryzae*, and *Rhizopus microspores* var. *oligosporus* can produce high-purity GABA, such as Tempeh, and Chinese fermented soybean product [6,8]. Lactic acid bacteria (LAB) have been applied in GABA production over the past few years. For example, *Lactobacillus brevis*, *Lactobacillus paracasei*, and *Lactococcus lactis* are applied to GABA-rich foods and pharmaceuticals synthesis [9–11]. The GABA-producing LAB are mostly cultivated in synthetic or semisynthetic media; however, the purification of GABA after fermentation is necessary, and this will increase the time and cost of production. Application of natural media such as dairy products, fruits, and vegetables for LAB to obtain GABA-enriched food could avoid the previous problems; however, the literature on this topic is limited.

The adzuki bean contains a high level of glutamate and has the potential via fermentation with LAB for enrichment of the GABA content; further development of fermented milk that is suitable for drinking every day and that assists the consumer to take in enough GABA and calcium to experience the conferred health benefits would then be possible.

It is found that the GABA production ability of LAB is influenced by the cultural compositions suitable for GAD reaction, especially by the type of carbon source, nitrogen source, and other components [12–14]. It is important to optimize the medium for enhancing GABA production during fermentation. Traditional optimizing methods involve changing one independent variable while fixing others at given levels, but the single-dimensional search technique often fails to yield optimized conditions because it does not consider possible interactions among factors. Response surface methodology (RSM) is a combination of statistical and mathematical techniques useful for optimization of bioprocesses. It can be used to evaluate the effect of several factors that influence the responses by varying them simultaneously in a limited number of experiments. Based on the key factors influencing the GABA production opted with one independent variable, RSM was applied to optimize the factors of medium for enhancing the GABA production.

The purposes of this study are aimed to evaluate the effects of various LAB commercially used [15,16] in the production of fermented milk on the GABA yield of fermented adzuki bean milk (ABM) and to find out the optimal culture medium of ABM by RSM. This study provides an alternative to the traditional utilization of cooked adzuki beans, enabling the production of a variety of functional adzuki bean products with beneficial effects to health.

2. Materials and methods

2.1. Materials, reagents, and equipment

Adzuki beans (obtained from Wandan Farmer's Association in Taiwan) were washed and soaked in 5-fold volume of water (w/w) at 37°C for 8 hours. After soaking, the adzuki beans and soaking solution were homogenized to a paste and filtered twice through a cotton cloth. Powdered skim milk (5%) was added to the filtrate, and the mixture was homogenized in a blender for 5 minutes and heated in a water bath at 90°C for 1 hour to prepare for the ABM.

2.2. LAB strains

Bifidobacterium adolescentis (BCRC 14606), *Bifidobacterium longum* (BCRC 14634), *Bifidobacterium bifidum* (BCRC 14615), *Bifidobacterium breve* (BCRC 11846), *Lactobacillus rhamnosus* GG (BCRC 16000), *Lactobacillus plantarum* (BCRC 11697), *Lactobacillus acidophilus* (BCRC 14079), and *Streptococcus salivarius* subsp. *thermophilus* (BCRC 14085) were purchased from the Bioresource and Collection Center of the Food Industry Research and Development Institute (HsinChu, Taiwan). LAB were cultured in MRS (de Man, Rogosa and Sharpe) broth (Difco, Detroit, MI, USA) at 37°C in 5% CO_2 for 48 hours, giving a cells number of about 8 log cfu/mL.

2.3. ABM fermentation

ABM was inoculated with 1% activated probiotic strain and fermentation at 37°C in a 5% CO_2 incubator for 6–60 hours. The LAB count, pH, and GABA content in the ABM were analyzed.

2.4. Cultural conditions for GABA production

2.4.1. Carbon source addition

Glucose, fructose, maltose, and galactose with a concentration of 0.5–2% were added to 100 mL ABM. It was inoculated

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