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REGULAR ARTICLE

EFFECT OF FOOD-MICROORGANISMS ON GAMMA-AMINOBUTYRIC ACID PRODUCTION BY FERMENTATION

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ABSTRACT

Lactic acid bacteria (LAB) are nice targets in order to study γ-aminobutyric acid (GABA) production that has been reported to be effective in order to reduce blood pressure in experimental animals and human beings. In this study, we aimed to γ-aminobutyric acid (GABA) production in aerobical and anaerobical conditions, using different sources of microorganisms. The highest selectivity of GABA from precursor L-monosodium glutamate (82.22%) has been reported using of microorganisms from banana, and with addition of pyridoxal-5-phosphate (P-5-P). For augmentation of selectivity the application of the further stimulating factors of GABA biosynthesis is needed.

Keywords: γ-aminobutyric acid, fermentation, monosodium glutamate, lactic acid bacteria

INTRODUCTION

Gamma-aminobutyric acid (GABA) is a ubiquitous non-protein amino acid which is produced primarily by the decarboxylation of glutamate catalyzed by the enzyme glutamate decarboxylase (GAD) **(Narayan and Nair, 1990)**. In animals and humans, it is one of the

major inhibitory neurotransmitters in the central nervous system **(Chebib and Johnston, 1999)**. Clinical studies have related increased intake of GABA or analogues to several health benefits, including lowering of blood pressure of mildly hypertensive animals **(Abe et al., 1995; Hayakawa, Kimura, Kamata, 2002)** and humans **(Inoue et al., 2003)**. Furthermore, GABA would also have an inhibitory effect on cancer cell proliferation, stimulate cancer cell apoptosis **(Oh and Oh, 2003)**, and play a role in alcohol associated diseases and schizophrenia **(Caputo et al., 2009; Oh and Oh, 2003)**.

Due to the physiological functions of GABA, development of functional foods containing GABA at high concentration has been actively pursued. GABA enrichment hasbeen achieved in anaerobic-incubated tea (gabaron tea) and in rice germ soaked in water. GABA production by various microorganisms has been reported, including bacteria, fungi, and yeasts **(Komatsuzaki et al., 2005)**.

Recent studies have shown that some lactic acid bacteria (LAB) can produce GABA **(Kim et al., 2009, Siragusa et al., 2007, Sun et al., 2009)**. LAB possess special physiological activities and are generally regarded as safe, and have been extensively utilized in food industries for a long time **(Karahan et al., 2010, Leroy et al., 2006, Yan et al., 2008)**. It is clear that the GABA production by LAB is natural and safe.

In this study, we focused on the GABA production ability of LAB. Several GABA-producing lactic acid bacteria have been reported, including *Lactobacillus brevis* isolated from kimuchi**(Ueno et al., 1997)** and from alcohol distillery lees **(Yokoyama et al., 2002)**, and *Lactococcuslactis* from cheese starters **(Nomura et al., 2000)**.

MATERIAL AND METHODS

Raw materialsas a source of microorganisms were obtain from Encián cheese dough (variants 2 and 5), from Niva cheese dough (variant 1), bacteria obtain from buttermilk (variant 3), bacteria taken from inside a banana in a significant stage of its decomposition (variant 4). Mass of microorganisms was obtained ina sterile environment under the principles of good laboratory work in a microbiological laboratory using a sterile bacterial loop. A 1.0 g of material in serted into the previous lysterilized stop per tube containing 10 ml of MRS broth and suspended. Bacteria strains were pre-cultured in Lactobacilli MRS broth at 33 °C 10 h (variant 1) and 24 h (variants 2-5).

Fermentation process

L-monosodium glutamate (MSG) (1.3mgml^{-1}) was added to the Lactobacilli MRS broth (50 ml), then the pH was adjusted by formic acid to pH 6,5 and subsequently pyridoxal-5-phosphate (P-5-P) was added (0.79 mg/60 ml fermentation mixture), (Table 1). Then pre-cultured microorganisms in 10 ml Lactobacilli MRS broth were added, the inoculum of approximately 10^7-10^8cells according to the source. Fermentation process was performed for 7 days. Samples (1 ml) were taken at regular intervals. pH was regulated periodically during the fermentation implemented under constant temperature conditions. Each of variants was repeated three times. Fermentation was realized in aerobic and anaerobic conditions.

Treatment of sample

The GABA content in the culture broth was measured as follows. A 1 ml of sample was taken and diluted by4.2 mlof 96% ethanol was added into taken sample (water solution). The mixture was allowed tost and for 1 hat room temperature and then sample was vigorous shaked and the supernatant filtered after 2 min of vigorous shaking, and then samples were filtered and evaporated to dryness. The sample was dissolved in 1 ml of Na_2CO_3 (saturated solution). The solution was quantitatively transferred into a 50 ml tube, 4 ml of Na_2CO_3 (saturated solution), and an acetone solution of dansyl chloride were added, and the sample was derivatized at 60 °C for 60 min. Next 1 mlof an L-alanine (100gml^{-1}water) solution was added so dansylation was finished the mixture was left to react for 30 min at 60 °C. Acetone was evaporated from the tube by heating at 40° C. Dansylated GABA was extracted by vigorous shaking for 1 min with 5 ml of diethyl ether and extraction was repeated once more. The mixed extracts were centrifuged for 10 min at 4500g, and the exactamount of diethylether phase was taken after the separation of phases, evaporated to dryness and then dissolved in mobile phase (MP), from which 20 ml was injected into the HPLC apparatus.

Determination of GABA

Method by **Naval et al. (2006)**, **(Hudec et al., 2011)** was used. The results were verified by re-injection of samples into the mass spectrometer Agilent 6410 Triplequat LC-MS/MS.

RESULTS AND DISCUSSION

According to several authorsGABA may be produced at high concentrations by some species of lactic acid bacteria **(Cho et al., 2011, Li et al., 2010)**. We expect their presence in dairy products, which were used in the first three variants and the fifth variant of our experiment (Table 1, Table 2). From these, the largest selectivity of GABA under anaerobic conditions was reached in the fermentation medium of variant 3 (58.57%) where as a source of bacteria was used butter milk. Generally, the greatest selectivity of GABA was achieved in the fermentation medium of variant 4 (82.22%), where as a source of microorganisms was used banana in an advanced stage of decomposition (Table 1). The addition of 50 mMP-5-P to the mixture in an anaerobic environment increased selectivity of GABA 1.5 times. The results of this study confirm the data that GAD activity in creases by addition of P-5-P, and P-5-P can act strict lyas a coenzyme of GAD **(Komatsuzaki et al., 2005)**, what is the contradictory to the views of **Lietal. (2010)**. Change of atmosphere to aerobic negative effected the production of GABA. In the case of Encián cheese (variant 5 in Table 1, and variant 2 in Table 2) aerobic atmosphere decreased the selectivity of GABA by 28.1% relative, which is consistent with the results of **Aokietal. (2003)**. Inaerobic conditions, the highest selectivity of decarboxylation process from MSG was reached in the fermentation medium of variant 4 (62.7%) in the presence of banana microorganisms. Throughout the experiment, pH was adjusted periodically and maintained at 6.5, but the results of **Farrahetal. (2009)** suggests that pH 5.0 is optimal for the biosynthesis of GABA, while the selectivity of GABA decreases closer to pH 6.5. **Farrah et al. (2009)** also suggest that the optimal ratio of MSG:P-5-P (mmol) is10:1, while in our experiment was used the ratio MSG:P-5-P (mM) 10:0.1. Consequently, in the next block of the experiment, it is necessary to verify the impact of increased amounts of P-5-P to the GABA production. GABA biosynthesis in an aerobic environment in the absence of P-5-P is less effective. Sodium glutamate is known flavour enhancer in foods, but its higher intake is associated with human body disorders like stroke, autism, and Alzheimer's disease because of its excitatory neurotransmitter effect in mammalian nervous system. Hence, it is very crucial to minimize the usage of glutamate in food industry for human consumption **(Farrah et al., 2009)** and inour experimentsto achieve the highest selectivity of the GABA from the precursor.

CONCLUSION

In this study we investigated the effect of aerobical and anaerobical conditions, P-5-P addition and the source of microorganisms on biosynthesis of GABA. An anaerobical conditions, microorganisms from banana and P-5-P addition increased the GABA production. Application of the successfully isolated strain into other food matrix has led to a growing interest among food manufactures to food technology to improve nutrition in functional foods for the industry.

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Table 1 Gamma-aminobutyric acid production (µg ml^{-1}) by microorganisms in anaerobic conditions

V.	**Fermentation time** (h)													**Enzyme**	**% max. selectivity**
	12	24	36	48	60	72	84	96	108	120	132	144	168		
1	96.0		177.6		93.3		271.9		139.2		76.2			P-5-P	34.30
2		201.4		150.3		137.9		92.4		232.6		383.5		P-5-P	48.39
3		128.5		136.4		83.8		79.1		228.8		464.2		P-5-P	58.57
4		182.3		110.5		109.1		178.5		83.5		651.7	190.6	P-5-P	82.22
5		232.2		115.8		153.7		79.6		100.7		245.8		———	31.01

Legend: 100% selectivity = 792.6 µg ml^{-1}, V. – variant, V. 1 – microorganisms from Niva cheese, V. 2,5 – microoganisms from Encián cheese, V. 3 – misroorganisms from buttermilk, V. 4 – microorganisms from banana, P-5-P – pyridoxal-5-phosphate.

Table 2 Gamma-aminobutyric acid production (µg ml^{-1}) by microorganisms in aerobic conditions

Variant	**Fermentation time** (h)													**% max. selectivity**
	12	24	36	48	60	72	84	96	108	120	132	144	168	
1	62.1		97.8		113.9		92.1		75.5		88.4			14.37
2		176.7		169.0		180.0		41.6		150.5		131.5		22.29
3		254.6		133.8		111.5		116.7		275.3		152.8		34.73
4		218.5		349.0		152.7		189.9		222.1		170.4	497.0	62.70

Legend: 100% selectivity = 792.6 µg ml^{-1}, V. – variant, V. 1 – microorganisms from Niva cheese, V. 2 – microoganisms from Encián cheese, V. 3 – misroorganisms from buttermilk, V. 4 – microorganisms from banana

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