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REGULAR ARTICLE

CHANGES IN SELECTIVITY OF GAMMA-AMINOBUTYRIC ACID FORMATION EFFECTED BY FERMENTATION CONDITIONS AND MICROORGANISMS RESOURCES

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ABSTRACT

In this study we observe the effect of fermentation conditions and resources of microorganisms for production of γ-aminobutyric acid (GABA). The content of produced GABA depends on various conditions such as the amount of precursor, an addition of salt, enzyme and the effect of pH. The highest selectivity of GABA (74.0 %) from the precursor (L-monosodium glutamate) has been determinate in the follow conditions: in the presence of pre-cultured microorganisms from Encián cheese in amount 1.66 % (w/v) the source of microorganisms/volume of the fermentation mixture, after the addition of 0.028 % (w/v) of $CaCl_2$/volume of the fermentation mixture, 100 μM of pyridoxal-5-phosphate (P-5-P) and the GABA precursor concentration in the fermentation mixture 2.6 mg ml^{-1} in an atmosphere of gas nitrogen. Pure cultures of lactic acid bacteria increased the selectivity of GABA by an average of 20 % compared with bacteria from the path of Encián cheese.

Keywords: GABA, pH, fermentation, microorganisms

INTRODUCTION

γ-Aminobutyric acid (GABA), a four-carbon non-protein amino acid, acts as a major inhibitory neurotransmitter in the central nervous system **(Huang et al., 2007)**. It is synthesized by glutamate decarboxylase (GAD) and pyridoxal-5-phosphate-dependent enzyme that catalyzes irreversible α-decarboxylation of L-glutamate to GABA **(Ueno, 2000)**. GABA mediates pre-synaptic inhibition of primary afferent fibersin the motornervous system. γ-Aminobutyric acid (GABA) serves as a major inhibitory neurotransmitter within the central nervous system, and it is also found in peripheral tissues. It has been shown that GABA plays an important role in the modulation of cardiovascular functions by acting not only within the central nervous system but also in peripheral tissues. Administration of GABA has been shown to reduce blood pressure in both experimental animals and human subjects **(Sakai et al., 2005)**. GABA may reduce inflammation, or, conversely, deficient GABA function may contribute to uncontrolled inflammation. Such deficient function could likely occur at a specific GABA receptor **(Kelly et al., 2008)**. Owing to these physiological functions, the commercial demand for GABA is increasing and GABA-enriched functional foods have been reported as follows: GABA-enriched green tea by anaerobic or cyclic treatments of tea leaves or shoots, GABA-enriched rice germ by soaking in water, GABA-enriched brown rice by high-pressure treatment and germination, tempeh-like fermented soybeans, and dairy products **(Lee et al., 2010)**. GABA is present in small quantities in many plant sources, vegetables, for example spinach, potatoes, cabbage asparagus (broccoli), tomatoes, fruits, for example apples, grapes, in cereals, for example barley, maize **(Ohetal., 2003)**. The increased amount is found mainly in fermented products, especially fermented dairy products **(Hayakawa etal., 2004)**, soy sauce **(Yamakoshietal., 2007)**, cheeses **(Siragusaetal., 2007)** and so on. GABA may be mainly produced by some species of lactic acid bacteria in high concentrations. The most commonly used bacteria are *Lactococcus lactis*, *Lactobacillus brevis*, L. *paracassei*, L. *delbrüeckii susp. Bulgaricus*, but also other microorganisms are used, eg. *Rhyzopus microsporus* **(Watanabe etal., 2007)**.

In this work we determined the impact of the addition of $CaCl_2$ and NaCl, pyridoxal-5-phosphate, the amount of monosodium glutamate on GABA production in anaerobic atmosphere in the presence of microorganisms from the Encián cheese dough. Next, we studied the impact ofpure cultures of microorganisms on the production of GABA (variant 5 *Lactobacillus paracasei subsp. Paracasei*, variant 6 *Lactobacillus brevis*, variant 7 *Lactobacillus casei subsp. Casei*) compared with microorganisms in our source.

The aim of our research is to develop products with high content of GABA and natural ingredients with cardio-, neuro-as well as cancer-protective activity.

MATERIAL AND METHODS

For the fermentation process, bacteria were obtained from Encián cheese dough and also pure cultures of microorganisms were used. The mass of microorganisms was obtained in a sterile environment under the principles of good laboratory work in a microbiological laboratory using a sterile bacterial loop. A 1.0 g of material was inserted 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 for 10 h (versions 1, 2, 3, 4).

The pure cultures of microorganisms were cultured at 33 °C for 24 has follows: one gelatinous disk with organisms, taken with bacterial loop, was inserted into the previous lysterilized stop per tube containing 10 ml of MRS broth and suspended.

Fermentation process

Sodium glutamate (1.3 $mg.ml^{-1}$) was added to the broth (50 ml), then the pH was adjusted by formic acid to pH 6.5 and subsequently pyridoxal-5-phosphate was added (0.79 mg/60 ml fermentation mixture) (Table 1). Then pre-cultured microorganisms in 10 ml broth were added, the inoculums of approximately 10^7-10^8 cells according to the source. Fermentation process was performed for 7 days. Samples (1 ml) were taken at regular intervals. Periodically pH was regulated during the fermentation implemented under constant temperature conditions. Each of variants was repeated three times.

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 tostand for 1hat room temperatureand thensample was vigorous shaken and the supernatantfiltered and evaporated to dryness. The sample was dissolved in1 ml of Na_2CO_3 (saturatedsolution). The solution was quantitatively transferred into a 50 ml tube, 4 ml of Na_2CO_3 (saturatedsolution), and an acetone solution of dansyl chloride were added, and the sample was derivatized at 60 °C for 60 min. Next 1 ml of an L-alanine solution in water (100g ml^{-1}) was added, and 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 4500 g, and the exact amount of diethylether phase was taken after the separation of phases, evaporated to dryness and then dissolved in mobile phase (MP), from which 20 µl was injected into the HPLC apparatus.

Determination of GABA was performed according to methods by **Naval et al. (2006)** and **(Hudec et al., 2011)**. The results were verified by re-injection of samples into the mass spectrometer Agilent 6410 Triple Quad LC-MS/MS.

Use of lactic acid bacteria for the decarboxylation of glutamic acid and its salts were preferred by several authors **(Ueno etal.2010; Rizelloetal., 2008)** because of their much higher GAD activity.

In the fermentation process 1.66% (w/v) concentration of the source of microorganisms per volume of the fermentation mixture was used in all variants.

RESULTS AND DISCUSSION

Among other things, the amount of GABA also depends on the amount and activity of pyridoxal-5-phosphate, which catalyzes their reversible α-decarboxylation of L-glutamate to GABA **(Ueno, 2009)**. It has been shown also in our results (Table 1, variants 1-3). Microorganisms of Encián cheese dough did not show the sufficient GAD activity, as an increasing of the concentration of the precursor (MSG) did not lead to intensification of the decarboxylation process (variant 1, variant 2). This fact is confirmed by the results of **Bai et al. (2009)**, which showed that the larger amount of precursor may have an inhibitory effect on microorganisms. The addition of double quantity of P-5-P into the increased concentration of L-monosodium glutamate in the fermentation mixture (variant3) activated microorganisms in their decarboxylation activity. In this variant, the selectivity of GABA rapidly increased to 74.0% at a concentration of 2.6 mg of MSG per ml of fermentation mixture and the content of 1.58 mg P-5-P in it. Microorganisms themselves are not capable to provide the sufficient high selectivity of decarboxylation process without the presence of enzyme. The addition of NaCl into the fermentation mixture increased the selectivity of GABA by 20% compared to variant 1, where the addition of $CaCl_2$ was used and all other conditions were identical to the variant 4. It will be necessary to prefer the use of relatively high NaCl concentration in further experiments.

Tab 1 Effect of the fermentation conditions for the production of γ-aminobutyric acid (μg.ml^{-1}) in the presence of bacteria from dough of Encián cheese

V.	Fermentation time (h)							Salt	MSG (mg ml^{-1})	max. sel. (%)	P-5-P (mg)
	48	72	96	120	144	168	192				
1	228.1	340.1	395.7	221.1	241.6	121.3	217.0	$CaCl_2$	1.3	49.9	0.79
2	438.3	343.1	477.4	487.4	168.8	252.8	243.6	$CaCl_2$	2.6	30.7	0.79
3	608.7	539.5	502.4	325.7	1172.6	643.4	265.0	$CaCl_2$	2.6	74.0	1.58
4	306.6	560.1	250.5	250.5	122.9	174.8	329.4	NaCl	1.3	70.7	0.79

Legend: V. – variant, max. sel. – maximal selectivity, V. 1,4 – 100% selectivity = 742.6 mg ml^{-1}, V. 2,3 – 100% selectivity = 1585.2 mg ml^{-1}, $CaCl_2$ – 0.028% w/v in the final volume of the fermentation mixture, NaCl – 2% w/vin the final volume of the fermentation mixture

In fermentation processes conducted in the presence of pure cultures of microorganisms, the highest selectivity of GABA was achieved by using lactic acid bacteria *Lactobacillus casei supsp. casei*. Generally, we have found that using of the pure cultures of lactic acid bacteria in fermentation processes increased the selectivity of the GABA production from precursor by 20% compared to the fermentation process conducted in the presence of microorganisms taken from Encián cheese dough (Figure 1).

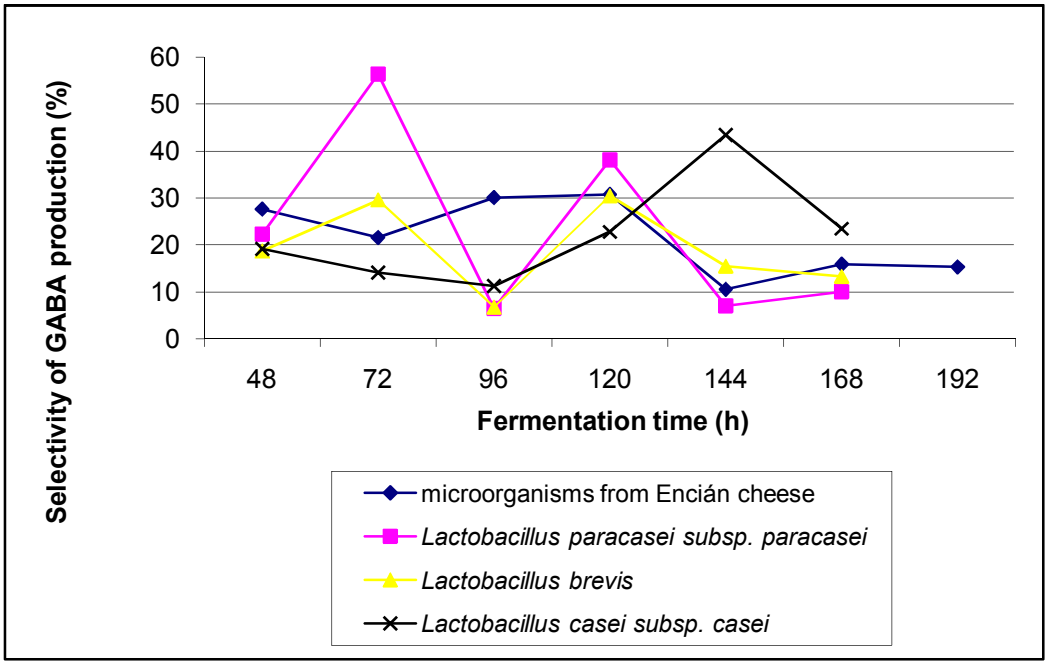

Fig 1 Effect of microorganisms on the selectivity of γ-aminobutyric acid

CONCLUSION

To change the focus of nutritional science from the term "satisfactory nutrition" to "optimalnutrition", new food products that have beneficial effects on mental and physical condition of people and can also reduce the risk tovarious diseases are developed. As far as the effectivity of GABA production in anaerobic conditions, the addition of pyridoxal-5-phosphate, calcium chlorideas well as the optimal amount of the precursor L-monosodium glutamate, which in high doses has an inhibitory effect, is very important for GABA biosynthesis. Using the pure cultures of lactic acid bacteria in fermentation processes increased selectivity of the GABA production from precursor by 20% compared to a fermentation process conducted in the presence of microorganisms taken from Encián cheese dough.

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REFERENCES

BAI, Q. – CHAI, M. – GU, Z. – CAO , X. – LI, Y. – LIU, K. 2009. Effect of components in culture medium on glutamate decarboxylase activity and γ-aminobutyric acid accumulation in foxtail millet (*Setariaitalica*L.) during germination. In *Food Chem.*, vol. 116, 2009, no. 1, p. 152-157.

HAYAKAWA, K. – KIMURA, M. – KASAHA, K. – MATSUMOTO, K. – SANSAWA, H. – YAMORI, Y. 2004. Effect of gamma-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar – Kyoto rats. In *Br. J. Nutr.*, vol. 92, 2004, no. 3, p. 411-417.

HUANG, J. – MEI, L. – WU, H. et al. 2007. Biosynthesis of γ – aminobutyric acid (GABA) using immobilized whole cells of *Lactobacillus brevis.* In *World J. Microbiol. Biotechnol.,* vol. 23, 2007, no. 4, p. 865-871.

HUDEC, J. – MAZUR, R. – KOBIDA, Ľ. TREBICHALSKÝ, P. 2011. Gamma-aminobutyric acid (GABA) production by fermentation in aerobic atmosphere. In *Potravinárstvo*, vol. 5, 2011, special number, p.14-17.

KELLY, J. M. – HUGHES, L. B. – BRIDGES, S. L. Jr. 2008. Does gamma-aminobutyric acid (GABA) influence the development of chronic inflammmation in rheumatoid arthritis? In *J. Neuroinfl.*, vol. 5, 2008, no. 1, p. 32-37.

LEE, B. J. – KIM, J. S. – MI, K. Y. – LIM, J. H. – KIM, Y. M. – LEE, M. S. – JEONG, M. H. – AHN, CH. B. – JE, J. Y. 2010. Antioxidant activity and γ-aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. In *Food Chem.,* vol. 122, 2010, no. 1, p. 271-276.

NAVAL, M. V. – GOMÉZ-SERRANILLOS, M. P. – CARRETERO, M. E. – DE ARCE, C. 2006. Value of high-performance liquid chromatographic analysis of amino acids in the determination of *Panax ginseng* radix extract effect in cultured neurons. In *J. Chromatogr.*, vol. 1121, 2006, no. 2, p. 242 -247.

OH, S.H. – MOON, Y.J. – OH, C.H. 2003. γ-Aminobutyric acid (GABA) content of selected uncooked foods. In *Nutraceuticals Food*, vol. 8, 2003, no. 1, p. 75-78.

RIZZELLO, C. G. – CASSONE, A. – DI CAGINO, R. – GOBETTI, M. 2008. Synthesis of angiotensin I-converting enzyme (ACE)-inhibitory peptides and γ-aminobutyric acid (GABA) during sourdough fermentation by selected lactic acid bacteria. In *J. Agric. Food Chem.,* vol. 56, 2008, no. 16, p. 6936-6943.

SAKAI, T. – OKADA, H. – KISE, M. – KOMATSU, T. – YAMAMOTO, S. 2005. γ-Aminobutyric acid (GABA) supresses antigen-specific imunne responses in ovalbumin γ(OVA)-immunized BALB/c mice. In *Am. J. Immunol.*, vol. 1, 2005, no.3, p. 101 – 105.

SIRAGUSA, S. – DE ANGELIS, M. – DI CAGNO, R. – RIZZELLO, C. G. – CODA, R. – GOBBETI, M. 2007. Synthesis of γ-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. In *Appl. Environ. Microbiol.*, vol. 73, 2007, no. 22, p. 7283-7290.

UENO, H. 2000. Enzymatic and structural aspects on glutamate decarboxylase. In *J. Mol. Catal*., vol.10, 2000, no. 1-3, p. 67-79.

UENO, S. – SHIGEMATSU, T. – WATANABE, T. – NAKAJIMA, K. – MURAKAMI, M. – HAYASHI, M. – FUJII, T. 2010. Generation of free amino acids and γ-aminobutiric acid in water-soked soybean by high-hydrostatic pressure processing. In *J. Agric. Food Chem*., vol. 58, 2010, no. 2, p. 1208-1213.

WATANABE, N. – FUJIMOTO, K. – AOKI, H. 2007. Antioxidant activities of the water-soluble fraction in tampeh-like fermented soybean (GABA-tempeh). In *Int. J. Food Sci. Nutr.*, vol. 58, 2007, no. 8, p. 577-587.

YAMAKOSHI, J. – FUKUDA, S. – SATOH, T. – TSUJI, R. – SAITO, M. – OBATA, A. – MATSUYAMA, A. – KIKUCHI, M. – KAWASAKI, T. 2007. Antihypertensive and nutriuretic effects of less-sodium soy sauce containing gamma-aminobutyric acid in spontaneously hypertensive rats. In *Biosci. Biotechnol. Biochem.*, vol. 71, 2007, no. 1, p. 165-173.