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Production of γ-aminobutyric acid in whey protein juice during fermentation by *Lactobacillus plantarum*

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ABSTRACT

Probiotics are microorganisms belonged to lactic acid bacteria (LAB) group which improves human and animal health when used in adequate quantities. However, probiotics can be effective only when they reside in gastrointestinal tract. Identification and isolation of microorganisms from natural resources is an effective approach to find bacterial species with further functional activities than commonly used species. From one hand, due to Iran wide genetic diversity and, on the other hand, the importation of probiotics to the country, native probiotic strains and their industrial applications should be investigated. In Iran, LAB have previously been extracted from dairy products in west of Iran; mostly belonged to *Lactobacillus* (*L.) paracasei*, *L. plantarum* and *Pediococcus* (*P.*) *acidilactici*. In general, LAB with probiotic properties are mainly used in various fermented foods, particularly dairy products. Based on their ability to synthesize gamma-aminobutyric acid (GABA), screening of LAB could open new horizons to GABA enriched dairy products. GABA is a non-protein amino acid (AA) with the chemical formula of C4H9NO2 and a molecular weight of 103.12 g mol-1. In 1950, it was shown that a large quantity of GABA was released by the central nervous system (CNS) of mammals (nearly 1 mg ml-1). This agent is produced by the activity of glutamate decarboxy-lase (GAD) in mitochondria through the irreversible decar-boxylation of L-glutamate in the presence of pyridoxal-5'-phosphate coenzyme. The biological functions of GABA include lowering of blood pressure in humans, diuretic effects, sleep regulation, insomnia and depression mitigation, auto-immune response suppression, treatment of chronic alcohol-related illnesses, reduction of stress and stimulation of immune cells. Therefore, much attention is paid to GABA as a functional bioactive agent with potential healing properties in foods and pharma-ceuticals. Natural GABA was first identified in potatoes and found in small quantities in several

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agricultural products such as barley, corn, cereals, fruits and vegetables including spinach, broccoli, tomatoes, apples and grapes. In developed countries, GABA is used as a health AA. Furthermore, it is popular as an extra supplement in various foods and nonprescription drugs used for many symptoms such as sleep disorders and stress. Relatively, studies on use of GABA supplementation in healthy individuals for up to 18 g for 4 days or 120 mg for 12 months have shown positive results. Nowadays, use of functional foods containing GABA is increasing worldwide due to the significant health benefits. Ability of GABA production varies within various strains of LAB. However, a few factors affect GABA production such as carbon source, glutamate concentration, fermentation time, coenzyme pyridoxal 5-phosphate, temperature and pH. Of these factors, glutamate concentration, fermentation time, temperature and pH are the most important factors in all bacterial species. Use of products with high LAB contents to synthesize GABA has created a new vision for the production of GABA-enriched products. To the best of the authors' knowledge, no studies have been carried out on development of functional products using probiotics extracted from local dairy products in west of Iran. Therefore, the main objectives of this study were to optimize culture media and investigate the potential of bacterial GABA production in probiotics extracted from Iranian dairy products using two culture media of De Man, Rogosa and Sharpe (MRS) broth and whey protein.

Materials & Methods: Lactobacillus plantarum was injected with 108 to whey protein juice containing concentrate of banana and strawberry adding 250 mM glutamic acid. Viability, pH, GABA production and sensory evaluation of the treatments were evaluated for 30 days at 4 and 25° C.

Findings: Lactobacillus plantarum has the potential for producing GABA in all treatment beverages. The highest amount of GABA production (195.5 ppm), the probiotic bacterial viability (8.01 log10 cfu/ml) and pH (3.81) after 30 days of storage in whey drink containing banana concentrate kept at 25° C. It turned out sensory evaluation results showed that the overall acceptance scores of all treatments were not significantly different ($p>0.05$).

Conclusion & Significance: In summary, the aims of the current study were assessment of potential GABA production by three probiotic bacteria extracted from local dairy products of western Iran in two culture media of whey protein and MRS broth. Of the three studied strains, *L. plantarum* showed the highest potential of the GABA production (115.24 mg kg-1) in MRS broth. To increase the amount of GABA produced by *L.*

plantarum, the conditions of culture medium including pH (4-6), temperature (30-50°C), time (12-72 h) and glutamic acid concentration (25-250 mM) were optimized. *L. plantarum* can have a viable function and produce GABA in a whey drink containing concentrate of banana and strawberries, without undesirable effects on the sensory properties of the beverage. Probiotic-based GABA-in beverages can be taken as a positive step in the development of functional products and the promotion of consumer health.