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Optimization of Gamma-Aminobutyric Acid Production in Probiotics Extracted from Local Dairy Products in West Region of Iran using MRS broth and Whey Protein Media

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Abstract

Background and objective: Gamma-aminobutyric acid is a non-protein amino acid produced by lactic acid bacteria in fermented foods and includes unique functions in the human biological system. The aim of this study was optimization of culture media for gamma-aminobutyric acid production in probiotics extracted from local dairy products in west of Iran using two culture media of MRS broth and whey protein.

Material and methods: The potential of gamma-aminobutyric acid production was assessed in *Lactobacillus paracasei*, *Lactobacillus plantarum* and *Pediococos acidilactici*, respectively extracted from doogh, yogurt and cheese using MRS broth and whey protein media and high performance liquid chromatography. To increase gamma-aminobutyric acid production, these media were optimized as pH (4-6), temperature (30-50°C), time (12-72 h) and glutamic acid concentration (25-250 mM).

Results and conclusion: Results have shown that *Lactobacillus plantarum* extracted from doogh includes the highest potential of gamma-aminobutyric acid production (115.24 mg kg^{-1}) under the following conditions of a culture temperature of 37°C, incubation time 60 h at pH 5 in MRS broth containing 50 mM of glutamic acid. After optimization of *Lactobacillus plantarum* media, gamma-aminobutyric acid production increased to 170.492 mg kg^{-1}. The optimum conditions included a glutamic acid concentration of 250 mM, culture temperature at 37.27°C, pH=5.19 and an incubation time of 72 h. Based on the results, use of local isolated dairy products in west region of Iran and optimization of growth conditions increased the ability of gamma-aminobutyric acid production.

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1. Introduction

Probiotics are microorganisms belonged to lactic acid bacteria (LAB) group which improves human and animal health when used in adequate quantities [1]. However, probiotics can be effective only when they reside in gastrointestinal tract [2]. 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 [3]. 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* [4]. 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 [5]. GABA is a non-protein amino acid (AA) with the chemical formula of $C_4H_9NO_2$ 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}) [6]. This agent is produced by the activity of glutamate decarboxylase (GAD) in mitochondria through the irreversible decarboxylation of L-glutamate in the presence of pyridoxal-5'-phosphate coenzyme [7]. 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 [8-10]. Therefore, much attention is paid to GABA as a functional bioactive agent with potential healing properties in foods and pharmaceuticals [11]. Natural GABA was first identified in potatoes and found in small quantities in several agricultural products such as barley, corn, cereals, fruits and vegetables including spinach, broccoli, tomatoes, apples and grapes [12]. 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 [13]. Nowadays, use of functional foods containing GABA is increasing worldwide due to the significant health benefits [5]. Ability of GABA production varies within various strains of LAB [12]. However, a few factors affect GABA production such as carbon source, glutamate concentration, fermentation time, coenzyme pyridoxal 5-phosphate, temperature and pH [14-17]. Of these factors, glutamate concentration, fermentation time, temperature and pH are the most important factors in all bacterial species [18,19]. Use of products with high LAB contents to synthesize GABA has created a new vision for the production of GABA-enriched products [12]. 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.

2. Materials and methods

2.1. Materials

The probiotic strains (*L. plantarum, L. paracasei, P. acidilactici*) for this study isolated from local dairy products from west of Iran in Takgene Zist Laboratories, Tehran, Iran**.** Whey protein, mix peptones (casein peptone, meat peptone and soybean peptone), yeast extract, salt trisodium citrate and MRS broth were purchased from Sigma-Aldrich (USA). Triethylamine, acetonitrile, dihydrogen phosphate, acetonitrile and methanol were purchased from Merck (Germany), and phenyl isothiocyanate, orthophthalic aldehyde (OPA) and GABA standards were supplied by Sigma Aldrich (USA). Whey protein was provided by Kabir Jolgeh industries, Iran.

2.2. Preparation of culture media and probiotic bacterial inoculation

Three LAB of *L. paracasei, L. plantarum* and *P. acidilactici* respectively isolated from yogurt, doogh and cheese with the highest probiotic characteristics [4] were added to MRS broth and whey protein media. Whey protein media was prepared by mixing 20 g of whey protein powder (Kabir Jolgeh industries, Iran) with a mixture of peptones (5 g of each casein peptone, meat peptone and soybean peptone), 3.5 g of yeast extract and 2.2 g of tri-sodium citrate salt (Merck, Germany). A prepared culture media of MRS broth was purchased from Merck (Germany). Furthermore, 50 mM of glutamic acid were added to both media according to Tajabadi et al. Then, culture media was heated at 90 ±3°C for 30 min for pasteurization [19].

2.3. Inoculation of probiotics in culture media

One colony of each *L. paracasei, L. plantarum* and *P. acidilactici* was added to MRS broth and whey protein media and incubated at 37°C for 24-48 h. To calculate number of bacteria inoculated in each milliliter of the suspension, 0.5 of McFarland standard was used. The optical density (OD) was measured at 625 nm with results in a range of 0.08-0.13 [20]. Cultures were stored at 37°C for 60 h (pH 5) [17].

2.4. Measurement of GABA using high performance liquid chromatography (HPLC)

Produced GABA in MRS broth and whey protein media was measured using reverse phase liquid chromatography. Derivation was conducted according to an original protocol by Bartolomeo et al. [21]. After centrifuging of media at 12000 ×g at 25°C for 10 min, 20 µl of the supernatant were poured into a 2-ml vial and then mixed vigorously with 20 µ1 of borate buffer. Then, 10 µl of OPA were added to the mixture stored at room temperature for 1 min. Then, 5 µl of 5% acetic acid were added to the mixture. After derivation, 20 µ1 of each sample were injected to a capillary C18 Column, Rstech Hector-M (150 mm × 4.6 mm × 0.5 µm) at 25°C with UV-Vis detector (Younglin Acme 9000m, YL Instruments Co, South Korea) set at λ = 338 nm, 40 mM of sodium dihydrogen phosphate as mobile phase A (pH 7.8) and acetonitrile:methanol:water (10:45:45) as mobile phase B. A stock solution of GABA (1 mg ml^{-1}) was prepared in water and diluted to 50% v v^{-1} to obtain various concentrations. The analysis was carried out based on the corresponding calibration curves. Concentration of GABA was calculated by the comparison of the peak area with the corresponding GABA standard. The GABA concentration was reported as mg kg^{-1}.

2.5. Optimization of parameters

The optimal fermentation condition for GABA production was modeled using response surface methodology (RSM). The independent variables for the optimization of culture media are shown in Table 1. According to the results by previous researchers [19,22,23], temperature (30-50°C), initial pH (4-6),

incubation time (12-72 h) and initial glutamic acid concentration (25-250 mM) of the culture were selected as independent factors to optimize the rate of GABA production by *L. plantarum*. Each experiment was carried out in triplicate.

Table 1. Independent variables to optimize the culture conditions for gamma-amino butyric acid production

Independents variables	Model parameters	+1	0	-1
Temperature	A	50	40	30
pH	B	6	5	4
Time	C	72	42	12
Glutamic acid	D	250	112.5	25

2.6. Statistical analysis

All experiments were carried out in triplicate. The means comparison was carried out using Duncan's one-way analysis of variance at a 95% confidence level. To optimize the production of GABA, RSM was used (Minitab Software v.16).

3. Results and discussion

3.1. Assessment of GABA production potential in probiotics

Briefly, GABA was produced under the following conditions of temperature at 37°C for 60 h at pH 5 by *L. paracasei*, *L. plantarum* and *P. acidilactici* in MRS broth and whey protein (Table 2). Chromatograms are shown in Figure 1. Of these bacteria, *L. plantarum* produced the highest GABA content of 115.24 mg kg^{-1}. Samples cultured in whey protein showed a significant statistical difference ($P \leq 0.05$).

Table 2. Gamma-amino butyric acid production in MRS broth and whey protein (mg kg^{-1})

Media culture	Bacteria	GABA (mg kg^{-1})
MRS broth	*L. paracasei*	100.09 ± 14.99^{AB}
	L. plantarum	115.24 ± 16.12^{A}
	P. acidilactici	91.09 ± 11.46^{ABC}
Whey protein	*L. paracasei*	59.61 ± 6.36^{BC}
	L. plantarum	51.14 ± 8.63^{C}
	P. acidilactici	55.92 ± 7.35^{BC}

Different small letters in each column show significant differences ($P \leq 0.05$)
L= *Lactobacillus*, *P*= *Pediococcus*, GABA= Gamma-amino butyric acid

Similarly, Shan et al. showed production of GABA by *L. plantarum* NDC75017, a strain that is screened in traditional fermented dairy products in China. The inducing factors for GABA production included L-monosodium glutamate (MSG) at 80 M*m*, pyridoxal-5-phosphate as coenzyme of GAD at 18 µ*M* and a culture temperature of 36°C. Under these conditions, activity of GAD in yogurt resulted in the production of 314.56 mg GABA per 100 g of the product [12]. Tajabadi et al. isolated 24 *Lactobacillus* spp. from honey bees and reported that *L. plantarum* Taj-Apis362 included the highest ability of GABA production (1.15 mM) [19]. Furthermore, Ratanaburee et al. produced a fermented beverage using *L. plantarum* and reported that GABA concentration increased in the presen-ce of MSG [24].

3.2. Optimization of parameters in culture of *L. plantarum*

L. plantarum extracted from doogh included the highest potential of GABA production (115.24 mg kg^{-1}) in MRS broth containing 50 mM of glutamic acid and under conditions of 37°C for 60 h at pH 5 (Table2). Therefore, to increase the amount of produced GABA, conditions of the culture media were optimized.

A quadratic polynomial model was used to predict the amount of GABA produced by *L. plantarum* as follows.

$$y = 134.277 - 2.052A - 0.887 B + 1.975 C + 34.530 D - 10.409 A^2 - 8.912 B^2 + 2.146 C^2 - 6.092 D^2 - 0.675 AB - 3.302 AC - 0.312 AD - 1.367 BC + 5.553 BD + 2.545 CD$$

In accordance with the above equation the linear impacts of temperature (A), pH (B), glutamic acid (D) and square effects of temperature (A^2), pH (B^2) glutamic acid (D^2) and interactions of pH × glutamic acid (B×D) on GABA production were significant ($P \leq 0.05$). Effects of other factors were not statistically significant. The high R^2 (97.64%) demonstrated the close correlation of effective parameters and response and their significant role in prediction of GABA production. Based on the results, the highest concentration of GABA (179.5 mg kg^{-1}) was produced by increasing the temperature from 30 to 40°C, pH from 4 to 5 and glutamic acid from 25 to 250 mM. Interestingly, Ratanaburee et al. [24] used *L. plantarum* DW12 to produce a fermented beverage and reported that 4000 mg l^{-1} of GABA were produced within 45-60 days under conditions of 1% of MSG, 6% of sucrose and an initial pH of 6. Similarly, Lim et al. [25] reported that 25 mM of GABA could be produced by optimizing culture media for *L. plantarum* B-134 with Makgeolli (traditional rice wine). Park et al. documented the optimized conditions as the cultivate temperature of 37°C, pH 5.7, various concentrations of glutamic acid and a time period of 48 h. They reported that *L. plantarum* K154 produced 154.86 µg ml^{-1}, 170.42 µg ml^{-1} and 201.78 µg ml^{-1} of GABA in MRS broth cultures containing 1%, 2% and 3% of MSG, respectively [26]. In contrast, researchers studied the ability of converting MSG to GABA in 57 LAB isolates from Azorean cheese. Results demonstrated that *L. otakiensis* L3C1R1, three strains of *L. paracasei* and three strains of *L. plantarum* included the highest production ability of GABA (>300 mg l^{-1}). None of Lactococcus, Leuconostoc and Enterococcus were able to produce GABA. Thus, it has been concluded that *L. plantarum* L2A21R1 was the stronger producer of GABA (936.8 mg l^{-1}) [27].

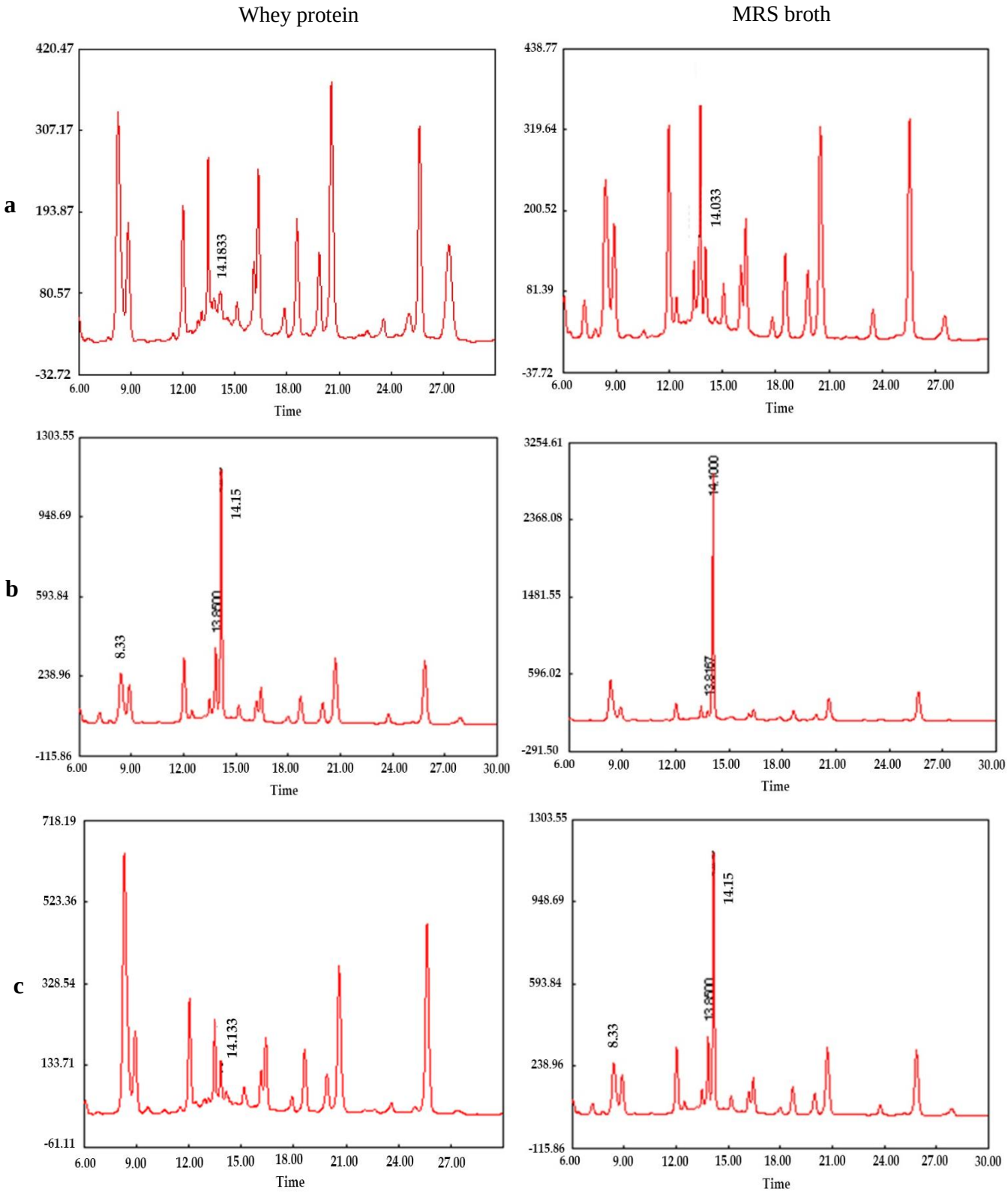

Figure 1. Chromatograms of the gamma-aminobutyric acid (GABA) production by lactic acid bacteria in MRS broth and whey protein media. a) *Lactobacillus paracasei* in whey protein and MRS broth; b) *Pediococos acidilactici* in whey protein and MRS broth; and c) *Lactobacillus plantarum* in whey protein and MRS broth

3.2.1. Effects of temperature on bacterial growth and GABA production

Results from optimization of conditions using RSM showed that temperature rise from 30 to 40°C increased production of GABA. In addition to effect on biocatalyst activity and stability, temperature included an effect on thermodynamic equilibrium of a reaction as well. The highest efficient conversion rate of glutamic acid to GABA occurs when the temperature of the culture media is optimum [28]. Cho et al. similarly described the

optimum temperature for GABA production using *L. buchneri* in MRS broth as 30°C. However, when the values were combined for temperatures, no statistical differences were seen in GABA production ($P>0.05$) in media with initial pH of 3.5, 4, 4.5, 5 and 5.5. The content of GABA in media with initial pH of 6 was significantly lower than that in other media [15]. In the other hand, researcher observed that GABA production in *L. Brevis* NCL912 included positive correlation with cellular density depends on temperature of the culture media. The optimum growth of *L. Brevis* NCL912 was observed at 35°C. This decreased at higher temperatures [28]. Di Cagno et al. reported that the highest rate of GABA production (4.83 mM) was seen for the fermented grapes by *L. plantarum* DSM19463 at temperature 30°C [29].

3.2.2. Effects of pH on bacterial growth and GABA production

Results from optimization of conditions using RSM showed that increased pH to 5 increased the production of GABA. Adjustment of GABA biosynthesis in microorganisms is largely affected by pH, which usually includes the greatest effect on the fermentation process [30]. Biochemical properties of GAD vary in various microorganisms; therefore, the effective value of pH for the maximum GABA production depends on the bacterial strain [31]. It has been reported that GABA production can be excellently enhanced by acidizing the cellular pH of the media because GAD needs H^+ ions for the production of GABA [32]. A study on 22 Italian cheeses demonstrated that the highest rate of GABA belonged to Pecorino di Filiano cheese with 391 mg kg^{-1}. They reported that strains of *L. Paracasei* PF6, PF8 and PF13, *L. plantarum* PF14 and *Enterococcus durans* PF15 produced further GABA at pH 5.5 [33]. In another study, Di cango et al. showed that addition of *L. plantarum* DSM19463 to functional grape beverage could synthesize a maximum GABA production rate of 4.83 mM under special conditions of 18.4 mM of glutamic acid and pH 6 at 30°C for 72 h [29]. In a research by Lu et al., they reported that the highest GABA production rate (7.2 g l^{-1}) in *L. lactis* varied at pH 7.1. They reported changes in pH during fermentation process in culture media. Moreover, they showed that initial pH affected the performance of final GABA and pH of the media should be adjusted timely to save the optimum pH [32]. Other bacteria, including pseudomonads as well as higher bacteria, similarly presented the highest activity of GABA transaminase practically at pH 8.5 [34,35]. Succinic semi-aldehyde dehydrogenase isolated from *Saccharomyces* spp. included the optimum pH 8.4 for the highest enzyme activity. Therefore, activities of the two enzymes should be blocked using pH by modifying the pH of the buffer to achieve the maximum production of GABA [36].

3.2.3. Effects of time on bacterial growth and GABA production

Results of optimization of condition using RSM demonstrated that an increased time from 12 to 42 h amplified the GABA production rate. Time factor plays an important role in fermentation and production of GABA. Research have proven that *L. plantarum* dsm19463 and *L. paracasei* NFRI 7415 need 72 and 144 h of fermentation time to achieve the highest GABA production rates of 4.38 and 60 mM, respectively [17,37]. An added time for GABA substrate similarly disturbs the final GABA yield as well as the concentration of the substrate in the media. A significant difference in GABA yield between various adding times of MSG was observed in fermentation of *L. lactis* as the maximum GABA yield was achieved while MSG was added at the starting of fermentation (time 0). Nevertheless, GABA yield dropped when MSG was added during 6 to 96 h of fermentation at a 6-h interval time [32].

3.2.4. Effects of glutamic acid density on bacterial growth and GABA production

Results showed that increased concentration of glutamic acid from 25 to 250 mM increased the rate of GABA production by *L. plantarum*. This can occur due to dicarboxylic acid reaction of glutamate at lab results at oscillometric liberation production of GABA in the final product and proton use. Increased concentration of glutamic acid from 25 to 250 mM increased the concentration of GABA from 80 to 145 mg kg^{-1} in treatments 1 to 6. [37,38]. Furthermore, culture media admixtures such as glutamates and coenzymes (pyridoxal-5-phosphate) are the main factors for the GABA production during fermentation [8,31]. The current results were similar to results by Pimentel et al. who reported that carbon, nitrogen and other elements could affect the production of GABA. Furthermore, concentration of the substrates is important in accessing high GABA functions [38]. Addition of glutamate has been found to increase the concentration of GABA production by *L. Paracasei* and *L. Brevis*. Based on the findings, concentration of GABA after 144 h of fermentation by *L. Paracasei* NFRI 7415 in a culture media containing 500 mM of methyl glutamate increased to 302 mM [17]. In another study, Yang et al. reported that the amount of GABA produced by *S. salivarius* subsp. *thermophilus* Y2 did not increase significantly, when glutamic acid levels increased from 10 to 20 g l^{-1} in the media [18].

3.2.5. Assessment of parameter interaction on GABA production

Figure 2-a demonstrates the effects of glutamic acid and time on GABA production, while pH and incubation temperature were adjusted as 5 and 40°C in culture media, respectively.

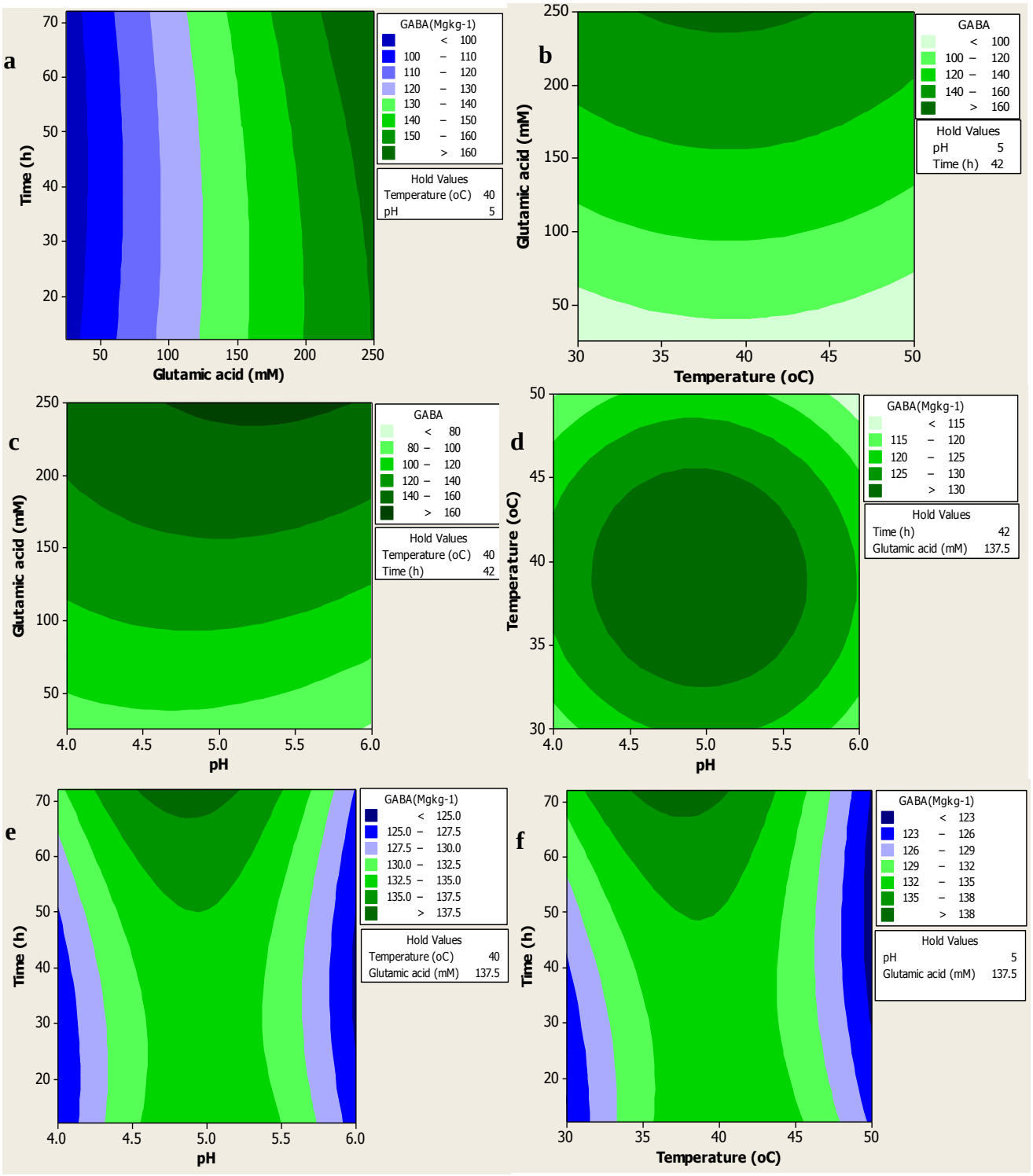

Figure 2. Effects of parameter interaction on gamma-aminobutyric acid (GABA) production a) time × glutamic acid b) temperature × glutamic acid c) pH × glutamic acid
d) Temperature × pH e) time × pH f) time × temperature

The highest concentration of GABA was produced within 25-70 h and a glutamic acid concentration of higher than 250 mM. Effects of culture temperature and glutamic acid on GABA production are shown in Figure 2-b; in which, the primitive time and pH at the central point were held as 42 h and 5 constantly; thus, the highest GABA production was observed at a temperature between 30 and 45°C and a glutamic acid content of greater than 250 mM. Figure 2-c shows effects of pH and glutamic acid on GABA production; in which, the primitive time and temperature at the central point were held at 42 h and 40°C constantly. Therefore, the highest GABA concentration was produced at pH between 4.3 and 5.7 and a glutamic acid concentration of higher than 250 mM. Figure 2-d shows effects of pH and temperature on GABA production; in which, the primitive concentration of glutamic acid and time at central point were held at 137.5 mM and 42 h constantly. Consequently, the highest GABA concentration was produced at pH between 4.7 and 5.7 and a temperature between 25 and 45°C. Figure 2-e demonstrate effects of pH and time on GABA production; in which, the primitive concentration of glutamic acid and temperature at central point were held at 137.5 mM and 40°C constantly. Therefore, the highest GABA concentration was produced at pH between 4.5 and 5.3 and a time period of longer than 65 h. Figure 2-f illustrates effects of temperature and time on GABA production; in which, the primitive concentration of glutamic acid and pH at the central point were held at 137.5 mM and 5 constantly. Therefore, the highest GABA concentration was produced at a temperature between 35 and 45°C and a time period of longer than 65 h.

3.2.6. Optimization of conditions for GABA production

Figure 3 demonstrates optimization of the culture conditions using MRS broth to reach the highest GABA production by *L. plantarum*. Based on the results from the current study, the highest GABA production under optimal conditions of temperature of 37.27°C, time of 72 h, pH 5.19 and glutamic acid concentration of 250 mM included 170.492 mg kg^{-1} with 91% desirability. According to Shan et al., GABA production in dairy products by "*L. plantarum* NDC 75017" was associated with the activity of GAD [12]. Glutamic acid and its derivatives are key parameters in GAD activity and GABA production. Relatively, several studies have been carried out on the addition of glutamic acid-based substrates to foods. Park et al. reported that *L. plantarum* K154 isolated from kimchi produced 154.86 µg ml^{-1} of GABA in MRS broth containing 1% of MSG [26]. Guo et al. described that *Pichia anomala* MR-1 produced high levels of GABA (350 mg per 100 ml of media) with the supplementation of 5% of glucose and 1% of MSG. However, inadequate temperature profile and reaction mixture decreased the concentration (120 mg per 100 ml of media) [39]. In a study carried out on *L. Brevis* HYE1 isolated from Kimchi in South Korea, optimization of the culture media conditions using RSM increased GABA from 18.97 to 21.44 mM. Results by Lim et al. [25] demonstrated that changing the MSG concentration and pH from 1% w v^{-1} and 5 to 2.38% w v^{-1} and 4.74 respectively optimized the bacterial GABA production. They concluded that pH was the most effective variable in optimizing the conditions. Furthermore, they studied the culture composition and reported no effects for various temperatures. Results from the current research showed that various bacterial species produced various levels of GABA. Identification of native *Lactobacillus* spp. with higher capabilities of GABA production may favor the formulation of novel health promoting foods [19].

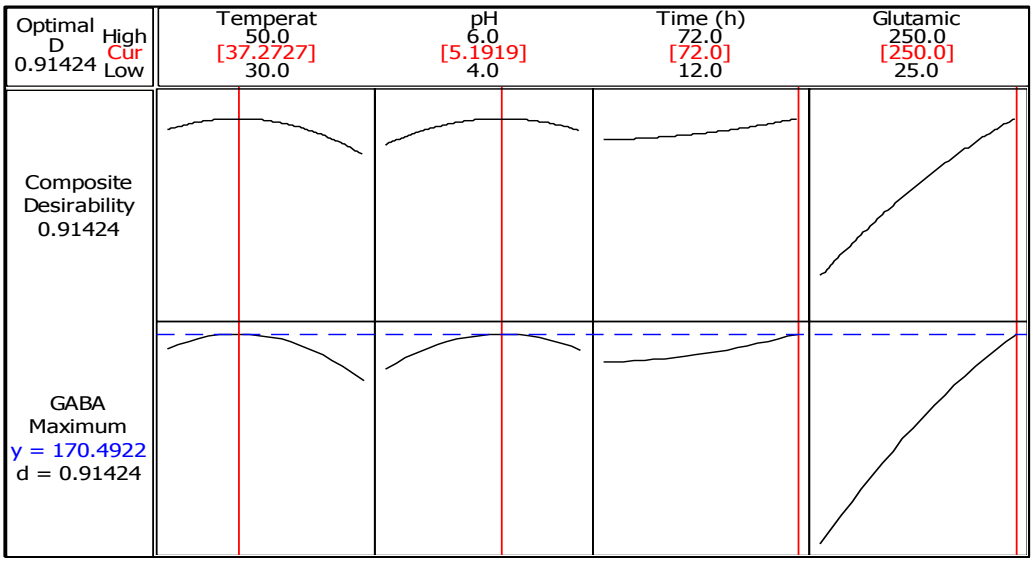

Figure 3. Optimization of the extracted gamma-aminobutyric acid in MRS broth produced by *Lactobacillus plantarum*

4. Conclusion

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. By optimizing the culture media conditions, the concentration of GABA produced by *L. plantarum* in MRS broth increased to 170.492 mg kg^{-1}. The optimum conditions for *L. plantarum* growth in MRS broth included temperature of 37.27°C, glutamic acid concentration of 250 mM, pH 5.19 and time period of 72 h. Results of this study revealed that *L. plantarum* isolated from the local dairy products in west of Iran produced GABA appropriately. On the other hand, by optimizing the conditions of culture media for *L. plantarum*, GABA production capability increased significantly. Optimization of culture media could increase the concentration of GABA. Furthermore, *L. plantarum* extracted from doogh in west of Iran showed the highest probiotic properties and GABA production. Use of this bacterial strain in food products will introduce new opportunities in formulation of functional foods and enhancing general health condition of the consumers.

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6. Conflict of interest

The authors declare no conflict of interest.

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بهینه‌سازی تولید گاما آمینوبوتیریک اسید در زیست‌یارهای جداشده از محصولات لبنی بومی غرب ایران با استفاده از محیط کشت‌های MRS broth و پروتئین آب پنیر

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چکیده

سابقه و هدف: گاما-آمینوبوتیریک اسید آمینواسیدی غیرپروتئینی تولید شده توسط باکتری‌های لاکتیک اسید در مواد غذایی تخمیر شده می‌باشد و عملکرد منحصر به فردی در سامانه زیستی انسان دارد. هدف این مطالعه بهینه‌سازی محیط کشت برای تولید گاما-آمینوبوتیریک اسید در زیست‌یارهای جدا شده از فرآورده‌های شیری بومی غرب ایران با استفاده از دو محیط کشت MRS broth و پروتئین آب پنیر بوده است.

مواد و روش‌ها: تولید بالقوه گاما-آمینوبوتیریک اسید توسط *لاکتوباسیل پاراکازئی*، *لاکتوباسیلوس پلانتاروم* و *پدیوکوکوس اسیدی لاکتیسی* که به ترتیب از دوغ، ماست و پنیر جدا شده بودند با استفاده از محیط‌های کشت MRS broth و پروتئین آب پنیر و کروماتوگرافی مایع با کارایی بالا ارزیابی شد. به منظور افزایش تولید گاما-آمینوبوتیریک اسید، محیط‌های کشت در pH (۶-۴)، درجه حرارت (۵۰-۳۰°C)، مدت زمان (۷۲-۱۲ ساعت) و غلظت گلوتامیک اسید (۲۵۰-۲۵ میلی مول) بهینه شدند.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد که در شرایط درجه حرارت کشت ۳۷°C، مدت زمان گرمخانه‌گذاری ۶۰ ساعت، pH برابر با ۵ در MRS broth حاوی ۵۰ میلی مول گلوتامیک اسید، *لاکتوباسیلوس پلانتاروم* جدا شده از دوغ بیشترین تولید گاما -آمینوبوتیریک اسید (۱۱۵/۲۴ میلی‌گرم در کیلوگرم) را داشت. پس از بهینه‌سازی محیط کشت *لاکتوباسیلوس پلانتاروم* تولید گاما-آمینوبوتیریک اسید تا ۱۷۰/۴۹۲ افزایش یافت. شرایط بهینه شامل غلظت گلوتامیک اسید ۲۵۰ میلی مول، درجه حرارت ۳۷/۲۷°C، ۵/۱۹ pH و مدت زمان گرمخانه‌گذاری ۷۲ ساعت بود. بر اساس نتایج به دست آمده، کاربرد جدایه‌های فرآورده‌های شیری بومی غرب ایران و بهینه‌سازی شرایط رشد توانایی تولید گاما-آمینوبوتیریک اسید را افزایش داد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی

- گاما-آمینوبوتیریک اسید
- *لاکتوباسیلوس پلانتاروم*
- MRS broth
- روش سطح پاسخ
- پروتئین آب پنیر

***نویسنده مسئول**

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