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ORIGINAL ARTICLE

Investigating the effect of pH, different concentrations of glutamate acid and salt on production in low-fat probiotic cheese

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ABSTRACT

Background and Objectives: Gamma-aminobutyric acid (GABA) is a non-protein amino acid produced by lactic acid bacteria. Among GABA-producing bacteria, lactic acid bacteria have received more attention due to their probiotic nature and properties such as inhibiting pathogenic bacteria, strengthening the immune system, and so on.

Materials and Methods: Investigation on the effect of three independent variables including pH (4.7, 4.9 and 5.1), glutamic acid (1, 2 and 3 mgg-1) and salt (2, 2.5 and 3%) on GABA production in low fat cheese by probiotic bacteria.

Results: By increasing the amount of glutamic acid and decreasing the pH from 5.1 to 4.7, the amount of GABA production in ultra-filtration cheese significantly increased on the 15th and 30th days of production ($p \leq 0.05$), while by increasing the amount of salt the production GABA decreased on the 15th and 30th days. Simultaneous optimal conditions to achieve maximum GABA production in cheese on the 15th and 30th production day was respectively 167.7917 mg/kg^{-1} and 220.125 mg/kg^{-1} using 3 mg/g glutamic acid, 2% salt at pH 4.7.

Conclusion: The results showed that by identifying probiotic bacteria with the highest potential for GABA production and optimizing the culture medium, more GABA can be produced in food products and a positive step can be taken to produce pragmatic products and promote consumer health.

Keywords: Gamma-aminobutyric acid; Low fat cheese; Probiotic bacteria

INTRODUCTION

Cheese is a general name that refers to a large group of fermented dairy products that are produced in a wide range of shapes and flavors around the world. In different sources, the number of types of cheese has been mentioned differently, and in some sources, up to 1000 types of cheese have been mentioned (1). These days, consumers are paying more attention to the relationship between food and health. For this reason, the consumption of functional food has grown significantly in recent years. Probiotic products are one of the most common functional foods (2). Probiotics are living microorganisms that, when adequately prepared, are beneficial to the health of the host. Probiotic microorganisms are divided into three groups including bacteria, fungi, and yeasts. Lactic acid bacteria and bifidobacteria are the most common types of microbes used as probiotics in dairy products. Probiotics are commonly used as part of microbial fermented foods such as yogurt, soy yogurt, or as supplements (3). On the other hand, probiotics will only be effective if they survive while passing through the stomach and settling in the intestines. Probiotic bacteria are defined as living resources that can improve the microbial balance of the

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gastrointestinal tract. Effects of probiotics is through the production of acid or bacteriocin, compounds with antibiotic effects and strengthen the immune system, and their properties include controlling serum cholesterol levels, preventing intestinal infections and anti-cancer activities. Dairy products are the main carriers of probiotics that are available today. Therefore, the survival of bacteria during storage to consumption is an important issue. In general, different kinds of cheeses are good carriers for probiotics. The high bioavailability of probiotics in cheese can be related to the relatively high pH compared to fermented milk products, the compact and network solid structure, the high tampon capacity due to the high amount of cheese protein. Today, the production of GABA by probiotic bacteria producing lactic acid is one of the new subject that has been drawn the attention of many researchers (4).

Gamma aminobutyric acid (GABA) is a four-carbon amino acid and non-protein that is a major source of free amino acids in most prokaryotes and eukaryotes. GABA has a group of amine on gamma carbon and exists in a non-branched form. GABA structurally has a high solubility in water and a flexible molecule that can show several structures in solution, including a circular structure similar to the amino acid proline (5). Due to its high biological activity, GABA has a wide potential use in functional foods, but the direct addition of chemical GABA to unhealthy and unnatural foods is illegal even in Korea. Therefore, the biosynthesis of GABA should be used for functional food. The production of amines by living organisms can be found in many different foods that deal with microbial fermentation, such as dairy products, beverages, and fermented sausages. Most of these amino compounds, especially histamine and tyramine, are toxic to consumers, but gamma aminobutyric acid is one of the biogenic amino acids that has positive effects on human health (6). The ability to produce GABA varies widely between different strains of lactic acid bacteria and is significantly affected by the conditions of the culture medium and its medium composition. Therefore, optimizing the conditions to increase the amount of GABA is important. To increase the amount of GABA, strains with high activity of glutamic acid decarboxylase should be selected for use in fermentation (7, 8). Key and effective factors in GABA production have been identified, such as culture time, glutamate concentration, culture medium temperature and pH (6). Among them, pH, temperature and glutamate acid concentration are the most common and important factors considered for all species. Therefore, the content of intracellular GABA is very low and its extracting from the cell is very difficult, so it is only necessary to measure the amount of extracellular GABA during optimization (9).

The decarboxylation of glutamate in lactic acid bacterial cells causes the consumption of an intracellular proton and this helps to maintain the neutral pH of the cytoplasm while lowering the pH of the external medium. Due to this pH stability, lactic acid bacteria with high glutamic acid decarboxylase (GAD) activity have the potential to act as probiotics. Siragusa (2007), isolated three strains of *Lactobacillus* that were able to survive under gastric and intestinal conditions and produce GABA. This shows that LAB producing GABA can be established as probiotics in the gastrointestinal tract and produce GABA in its place and consequently, they will promote the health and usefulness of food (10). Lacroix et al. separated GABA-producing strains from different types of cheeses and examined the effect of factors existed in matrix such as sodium chloride, glutamic acid, oxygen and pH and concluded that *Lactococcus lactis* ULAAC-A13 and ULAAC A23 were capable of producing GABA up to 500 mg per 100 ml of fermented milk containing 2% sodium chloride and 367 mg per 100 g of glutamic acid (11). Wu et al. in the study of GABA production efficiency by *Lactobacillus brevis* RK03 observed that at 25 to 45°C of culture medium the highest GABA were produced at 30°C and 1230 and 21936 mgL^{-1} respectively in MRS culture medium along with glutamic acid (12). The use of GABA-producing probiotic strains as starter cultures (inoculant) in fermentation processes can facilitate the production of GABA biosynthesis. In addition to using natural GABA instead of chemical GABA, it also offers new and attractive products to the consumer and reduces production costs by removing excess GABA. Therefore, the aim of this study was to optimize the conditions for the production of the highest amount of GABA in low-fat cheese by probiotic bacteria.

MATERIALS AND METHODS

Skimmed milk powder, milk protein concentrate, skimmed milk ultra-filtrated concentrate from Ca-

seinate Company (Iran), lactic starter from Bioprax Company (France), enzyme rennet from CSk Company (Netherlands), Novagel from Food Chem Company (China), Galactomanan from Veer Company (India), sodium chloride from Golbahar Company (Iran), Butter from Pegah (Tehran) were supplied. The microorganism of *L. brevis* from the Iranian Biological Resource Center (Iran), MRS Broth culture medium, acetic acid and Orthophthalic Aldehyde from Merck Company (Germany), glutamate acid, McFarland solution, Barium chloride, sulfuric acid Methanol, Acetonitrile and Sodium hydroxide from Mina Tajhiz Company (Iran), were supplied.

Inoculation and culture of probiotic bacteria in MRS broth culture medium. The results of the studies of Sharafi et al. showed that *L. brevis* among the 6 probiotic bacteria with the potential to produce GABA (*L. brevis, L. delbrueckii* ssp. *bulgaricus* and *St. thermophilus, Lactococcus lactis* ssp. *lactis, L. rhamnosus* and *L. paracasei*) had the highest potential of GABA production (5960.8 mg/l) (13). Therefore, *L. brevis* was inoculated (10^8 $cfuml^{-1}$) into the samples of low-fat refined cheese studied in this study amount to. The 5% McFarland standard was used to determine the number of inoculated bacteria per milliliter of suspension.

Low fat probiotic cheese production. The optimal formulation of Sharafi et al. was used to produce low-fat Ultra-filtration cheese with desirable textural properties. Initially, skimmed milk Ultra filtrated concentrate, milk protein concentrate and milk powder respectively 70%, 12% and 2% fat were mixed well with water in a pasteurizer at 50°C. Then, butter (82% fat) was used to adjust the final fat content in low-fat Ultra-filtration cheese (9%) and all materials were mixed again at 60°C until the butter was completely melted. Then, in order to improve the textural properties of low-fat Ultra-filtration cheese, Novagel 0.1% and 0.46% Galactomannan were added to the mixture. After that, all the materials were homogenized by Homogenizer model (IKA10, Germany T-Basic) at 75°C with 90 bar pressure and then in pasteurizer (VB-1820J, Faraz Electronic, Iran) at 78°C for 5 minutes. After pasteurization, milk temperature dropped to 34°C and lactic starter amount to 0.3% ww^{-1} along with probiotic bacteria with the highest potential of GABA-producing *(L. brevis)* at a concentration of 10^8 CFU/ ml^{-1} and rennet enzyme amount to 0.5% ww^{-1} was added to the mixture and stirred well and filled into 100 g molds. After clotting formation according to the Table of treatments, salt with 2, 2.5 and 3% ww^{-1} and glutamate amount to (1, 2 and 3 mgg^{-1}) were sprayed to them. Then the final pH was adjusted to (4.7, 4.9 and 5.1) before packing and thermal sealing was done with aluminum coating layered with polypropylene and the samples were kept in an incubator at 8°C for 30 days and then were transferred to refrigerator at 5°C. GABA production test was performed on the 15th and 30th days of storage (14).

The test measuring the amount of GABA. The amount of GABA produced by probiotic bacteria grown in MRS Broth medium was evaluated by reversed-phase high-performance liquid chromatography. The derivatization method was performed according to the method of Bartolomeo et al. as follows. After centrifugation of culture medium (12000 × g, 10 min and 25°C), about 20 µl of the separated upper phase were poured into a 2 ml vial and mixed thoroughly with 20 µl of borate buffer. Then 10 µl of Ortho-Phthalic Aldehyde (OPA) were added to it and kept in environment temperature for 1 minute. Then 5 µl of 5% of acetic acid was added to the sample. After derivatization, the equivalent of 20 µl of each sample was injected into a C18 (Japan) with the dimensions of 150 mm × 4.6 mm × 0.5 µm at 25°C, with a detector at λ = 338 nm. It was the mobile phase A, 40 mM sodium dihydrogen phosphate, that its pH was adjusted by sodium up to 7.8. The mobile phase B was acetonitrile / methanol / water (10:45: 45, v/v/v). Separation was performed at a flow rate of 1 ml/min with a Gradient program for 35 min. The standard curve was drawn at concentrations of 50, 100, 200, 300, 400 $mgkg^{-1}$ and the GABA concentration was expressed in terms of mg/kg^{-1} according to the storage time and comparison of the area under the curve of the samples with the standard sample (15).

Statistical analysis. In order to design and analyze the results of GABA production in low-fat probiotic cheese, the Box-Behnken Response Surface Methodology in Minitab 16 software was used.

RESULTS

The amount of produced GABA in probiotic ultra-filtration cheese on the 15th and 30th day

of storage. The study of the effect of pH, different concentrations of salt and glutamic acid on GABA production in the 15th and 30th days of low-fat ultra-filtrated cheese production is reported in Table 1. The results showed that by increasing storage time, GABA production gradually increased until the 30th day in cheese samples. Increasing the pH at the mentioned levels caused decrease of GABA, and increasing the concentration of glutamic acid and salt cause increase of GABA production on the 15th and 30th day. On the 15th day, the highest amount of GABA production was observed 158, mg/kg^{-1} in the treatment containing 3 mg/g^{-1} glutamic acid, 2.5% salt and pH equals 4.7. The lowest amount of GABA production was observed 98, mg/kg^{-1} in the treatment containing 1 mg/g^{-1} glutamic acid, 2.5% salt and pH equal to 5.1. On the other hand, on the 30th day, the highest amount of GABA production was observed 211, mg/kg^{-1} in the treatment containing 3 mg/g^{-1} glutamic acid, 2.5% salt and a pH equals to 4.7. The lowest amount of GABA production was observed 142, mg/kg^{-1} in the treatment containing 1 mg/g^{-1} glutamic acid, 2.5% salt and pH equal to 5.1.

Regression model. The results of the regression model for the amount of produced GABA on the 15th and 30th day from the probiotic bacterium *L. brevis* are shown in Table 2. According to Table 2, the results of the predicted produced GABA model on the 15th day showed that the value of R-squared (R^2) was 97.35%, and adjusted R-squared (R^2-adj) was 92.57% indicating a good fit of the model to the experimental data.

In addition, according to Table 2, the results of the predicted produced GABA model on the 30th day showed that the value of R-squared (R^2) of this model was 97.50%, and adjusted R-squared (R^2-adj) was 99.92% indicating a good fit of the model to the experimental data.

Table 1. Results of the amount of GABA produced in low fat cheese samples tested on the 15th and 30th day

Treatment	Glutamic acid (mg/g^{-1})	Salt (%)	PH	The amount of GABA (mg/kg^{-1}) on the 15th day	The amount of GABA (mg/kg^{-1}) on the 30th day
T1	2	2.0	5.1	121	162
T2	1	3.0	4.9	111	160
T3	3	2.5	4.7	158	211
T4	3	2.0	4.9	153.	202
T5	2	2.5	4.9	119	173
T6	1	2.5	5.1	98.	142
T7	2	2.0	4.7	142.	199
T8	3	3.0	4.9	135	192
T9	2	3.0	5.1	105	148
T10	2	2.5	4.9	119	174
T11	2	3.0	4.7	128	174
T12	1	2.0	4.9	126	176
T13	3	2.5	5.1	120	164
T14	1	2.5	4.7	132	187
T15	2	2.5	4.9	118	172

Table 2. Regression model of the amount of GABA produced on the 15th and 30th day for independent variables by the response level model

Day	Model	R^2	R^2-adj
Day 15	-118.667 -14.500A -7.875B +12.375C +0.542A^{2*} -4.792B^{2*} -7.792C^{2*} -0.0500AB* -1.000AC* -0.750BC*	97.35	92.57
Day 30	173.000 -19.375 A -8.375 B + 12.750 C -4.625A^{2*}+ 2.375B^{2*} +7.625 C^{2*} + 2.750AB* -0.500AC* + 2.000BC*	97.50	99.92

A= pH, B= Salt, C= Glutamic acid

Analysis of variance of GABA production on the 15th and 30th day. According to Table 3, the linear effects of pH (A), salt (B) and glutamic acid (C) on changes in GABA production on the 15th day were significant ($p \leq 0.05$). However, the square and interaction effects on changes in GABA production on the 15th day were not significant ($p > 0.05$). According to Table 3, the linear effects of pH (A), salt (B) and glutamic acid (C) on changes in GABA production on the 30th day were significant ($p \leq 0.05$), but the square and interactionl effects on the changes of GABA production amount were not significant on the 30th day ($p > 0.05$).

Interaction effects on GABA production on the 15th day. The results of the Interaction effects of pH and different concentrations of glutamic acid and salt on the amount of GABA production on the 15th day are shown in Fig. 1.

Fig. 1-a shows the results of Interaction effects (glutamic acid × salt) on the amount of GABA production on the 15th day at a constant pH and equals to 4.9%. The Interaction effect (glutamic acid × salt) on the amount of GABA production was not significant. As it is clear, under constant pH conditions, an increase in glutamic acid caused increase of GABA, and an increase of salt caused decrease the amount of GABA. GABA production amount to 150 mg/kg^{-1} and higher was observed in salt content of 2 to 2.3% and glutamic acid concentration of 2.9 to 3 mg/g^{-1}.

Fig. 1-b shows the results of the Interaction effects (salt × pH) on the amount of GABA production on the 15th day under constant glutamic acid conditions and equal to 2 mg/g^{-1}. The Interaction effect (salt × pH) on the amount of GABA production was not significant. As it is clear, by increasing salt and pH, the amount of GABA has decreased. So that the amount of GABA production was observed 140 mg/kg^{-1} and higher in the amount of salt less than 2 to 2.19% and pH from 4.70 to 4.78.

Fig. 1-c shows the results of Interaction effects (glutamic acid × pH) on the amount of GABA production on the 15th day under constant salt conditions and equal to 2.5%. The Interaction effect (glutamic acid × pH) on GABA production was not significant. As it is clear, increasing the pH reduces GABA, and by increasing glutamic acid, the amount of GABA production increases. So that, GABA amount to 150 mg/kg^{-1} and higher was observed at glutamic concentrations of 2.4 to 3 mg/g^{-1} and pH of 4.70 to 4.88.

Interaction effects on GABA production on the 30th day. The results of the Interaction effects of pH and different concentrations of glutamic acid and salt on the amount of GABA production on the 30th day are shown in Fig. 2.

Fig. 2-a shows the results of Interaction effects (glutamic acid × salt) on the amount of GABA production on the 30th day at a constant pH and equals to 4.9%. The Interaction effect (glutamic acid × salt)

Table 3. Results of analysis of variance of GABA production on the 15th and 30th day

Source of changes	Day 15		Day 30	
	F-value	P-value	F-value	P-value
Regression	20.37	0.002*	21.62	0.002*
Linear effects	56.21	0.000*	60.17	0.000*
PH (A)	000.0	0.000*	111.43	0.002*
Salt (B)	0.004	0.004*	20.82	0.006*
Glutamic acid (C)	60.70	0.001*	48.26	0.001*
Squares	4.79	0.062ns	4.12	0.081ns
PH × PH (A^2)	0.005	0.826ns	2.93	0.148ns
Salt × Salt (B^2)	40.20	0.096ns	0.77	0.420ns
Glutamic acid × Glutamic acid (C^2)	11.11	0.021ns	7.97	0.037ns
Interaction Effect	0.12	0.945ns	0.58	0.651ns
PH × Salt (A× B)	0.05	0.833ns	1.12	0.338ns
PH × Glutamic acid (A× C)	0.20	0.675ns	0.04	0.855ns
Glutamic acid × Salt (C× B)	0.11	0.752ns	0.059	0.476ns
Residual	-	-	-	-
Total	-	-	-	-

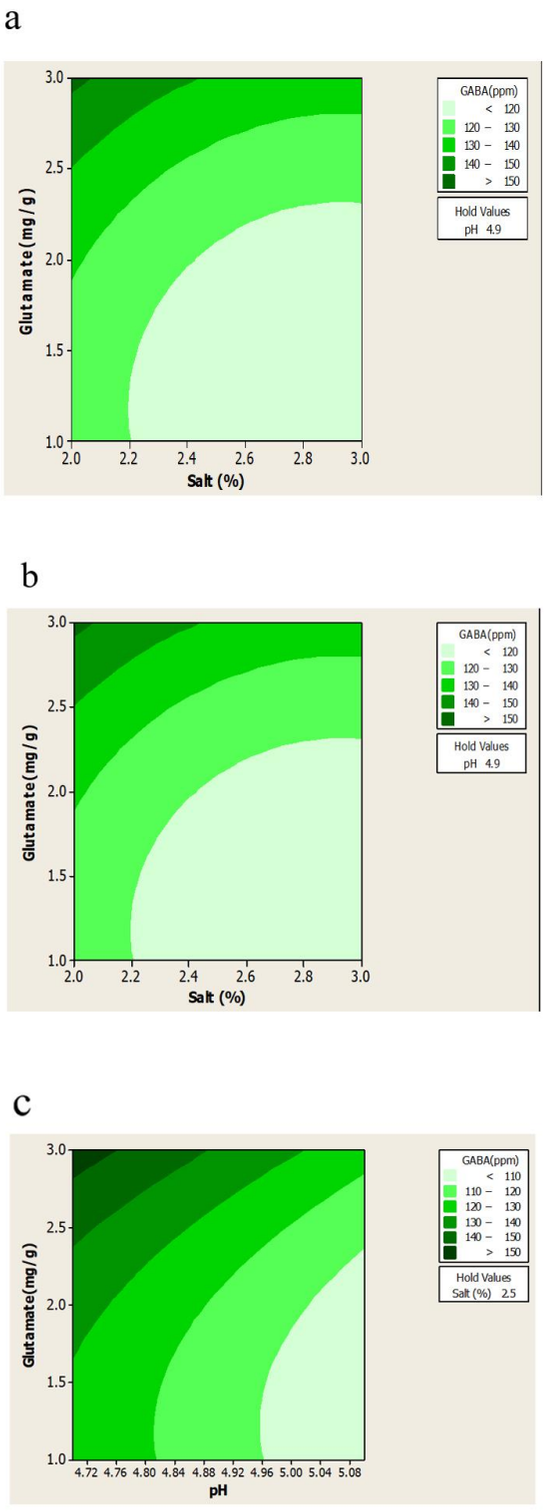

Fig. 1. Interaction effects of independent pH variables, glutamic acid and salt on the amount of GABA production on the 15th day. a) Interaction effect of Glutamic acid × salt b) Interaction effect of Salt × pH c) Interaction l effect of Glutamic acid × pH

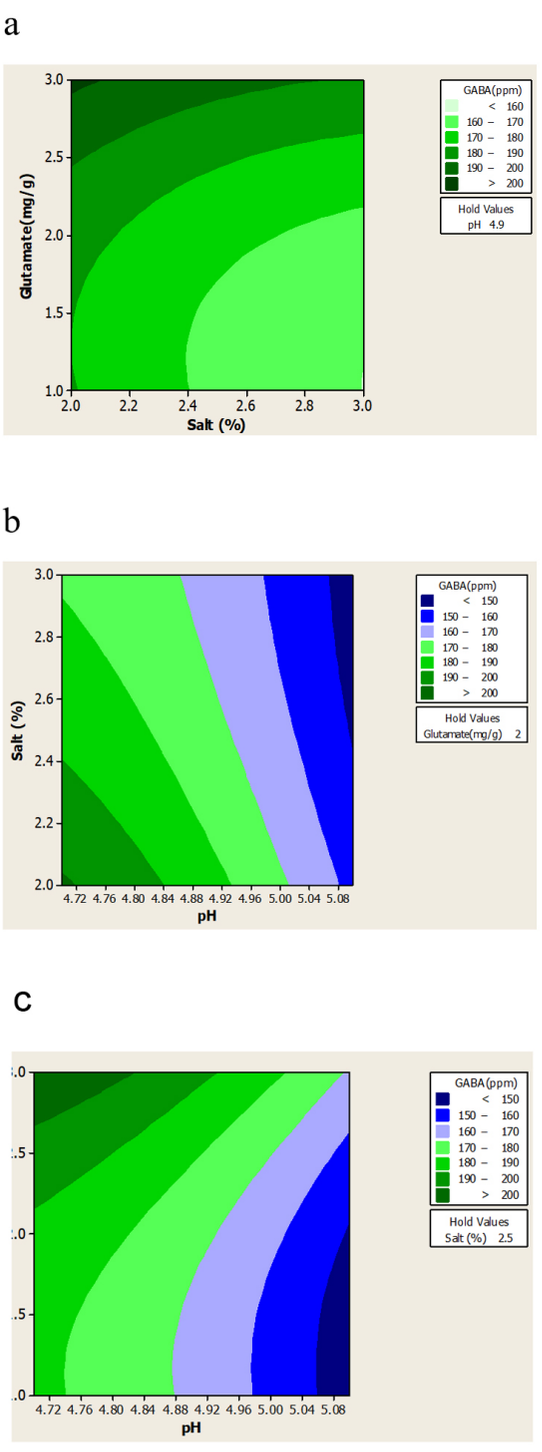

Fig. 2. Interaction effects of independent pH variables, glutamic acid and salt on the amount of GABA production on the 30th day. a) Interaction effect of Glutamic acid × salt, b) Interaction effect of Salt × pH, c) Interaction effect of Glutamic acid × pH

on the amount of GABA production was not significant. As it is clear, under constant pH conditions, an increase in glutamic acid caused increase of GABA, and an increase of salt caused decrease the amount of GABA. So that, GABA production amount to 200 mg/kg^{-1} and higher was observed in salt content of 2 to 2.8% and glutamic acid concentration of 2.5 to 3 mg/g^{-1}.

Fig. 2-b shows the results of the Interaction effects (salt × pH) on the amount of GABA production on the 15th day under constant glutamic acid conditions and equal to 2 mg/g^{-1}. The Interaction effect (salt × pH) on the amount of GABA production was not significant. As it is clear, by increasing salt and pH, the amount of GABA has decreased. So that the amount of GABA production was observed 200 mg/kg^{-1} and higher in the amount of salt less than 2 to 2.1% and pH from 4.70 to 4.72.

Fig. 2-c shows the results of Interaction effects (glutamic acid × pH) on the amount of GABA production on the 15th day under constant salt conditions and equal to 2.5%. The Interaction effect (glutamic acid × pH) on GABA production was not significant. As it is clear, increasing the pH reduces GABA, and by increasing glutamic acid, the amount of GABA production increases. So that, GABA amount to 200 mg/kg^{-1} and higher was observed at glutamic concentrations of 2.6 to 3 mg/g^{-1} and pH of 4.70 to 4.82.

Single optimization of the amount of GABA production in probiotic cheese on the 15th and 30th day of production. According to Fig. 3, the optimal conditions to reach maximum amount of GABA production on the 15th day were predicted. Therefore, the optimal conditions for maximum GABA production amount to 167.7177 mg/kg^{-1} were predicted in glutamic acid conditions of 3 mg/g^{-1}, 2% salt and at pH 4.7 with 100% desirability.

According to Fig. 3, the optimal conditions to reach maximum amount of GABA production on the 30th day were predicted. Therefore, the optimal conditions for maximum production of GABA amount to 220.125 mg/kg^{-1} was determined with glutamic acid 3 mg/g, salt 2% and pH of 4.7.maximum production of GABA amount to 220.125 mg/kg^{-1} was determined with glutamic acid 3 mg/g, salt 2% and pH of 4.7.

Multiple optimization of GABA production on the 15th and 30th days. According to Fig. 4, the optimal conditions to reach maximum amount of GABA production on the 15th and 30th days were predicted. The results of the optimization process showed that the optimal production conditions of GABA were determined 15th and 30th day of production was respectively observed 167.7917 mg/kg^{-1} and 220.1250 mg/kg^{-1} in 3 mgg^{-1} glutamic acid, 2% salt and pH 4.7 Optimal conditions were simultaneously produced experimentally in the laboratory, no significant difference was observed between experimental and predicted GABA amount.

DISCUSSION

Gamma aminobutyric acid (GABA) is a four-carbon amino acid, non-protein produced by various microorganisms, especially lactic acid bacteria (LAB) (16). To increase the amount of GABA, strains with high activity of glutamic acid decarboxylase should be selected to use in fermentation (7, 8). In addition, the concentration of glutamic acid in the food matrix as a precursor of GABA production should be sufficiently high. Optimization of conditions is important to increase GABA production. These conditions for GABA fermentation vary among different strains of lactic acid bacteria and key and effective factors including culture time, glutamate concentration, culture medium temperature and pH in GABA production have been identified (6).

By increasing glutamic acid, the production of GABA in cheese increased linearly, which could be due to the appropriate amount of glutamate as a precursor of GABA production by *L. brevis* (17, 18). In this regard, Komatsuzaki et al. reported in their research that the concentration of GABA reached to 161 mM after 144 hours of culture of *L. casei* NFRI 7415 in a culture medium containing 500 mM glutamic acid (9). Li et al. also reported that by increasing glutamate concentration, GABA production increased by *L. brevis* NCL912 and *L. brevis* (8). To increase the amount of GABA, strains with high activity of glutamic acid decarboxylase should be selected for use in fermentation (7, 8). In addition, the concentration of glutamic acid in the food matrix as a precursor to GABA production should be sufficiently high. GABA biosynthesis in microorganisms is mainly adjusted by pH, which usually has the greatest effect on the fermentation process (19). The biochemical properties of glutamate carboxylase vary among different microorganisms, so the optimal pH for maximal

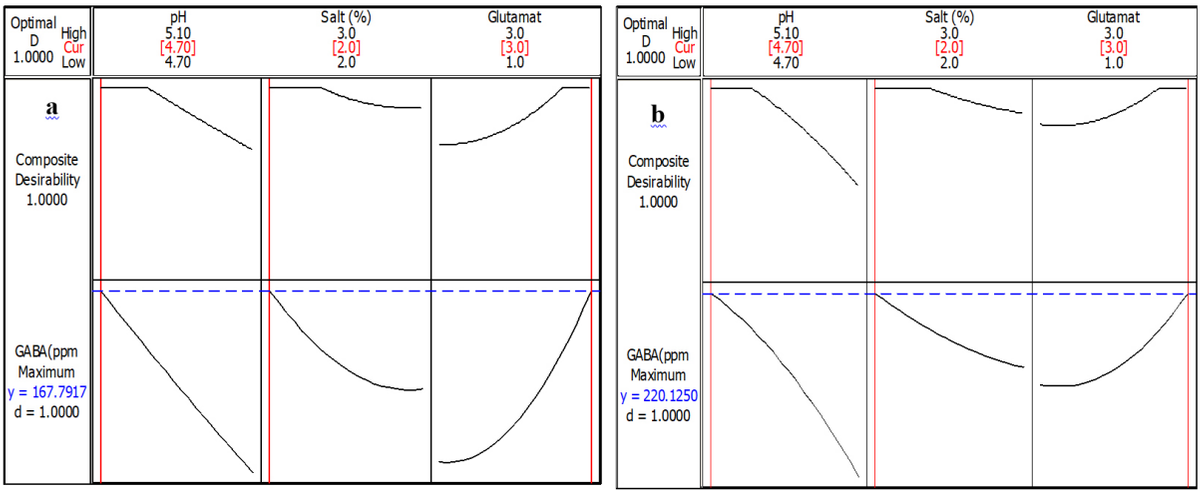

Fig. 3. Single optimization of GABA production on the a)15th and b) 30th day

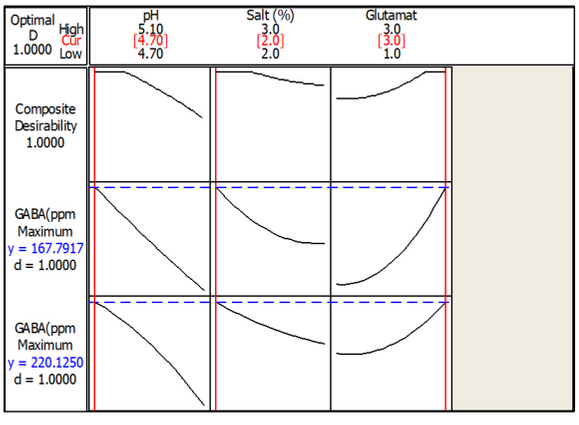

Fig. 4. Multiple optimization of GABA production amount on the 15th and 30th day

GABA production depends on species (8, 19). According to the results of this study, it is determined that by decreasing pH from 5.1 to 4.7, the amount of GABA production increased on the 15th and 30th day, which could be due to providing the suitable pH for the activity of lactic acid bacteria, including *L. brevis.* Consequently, this caused increase in GABA production (17, 18). Regarding the cause of increasing GABA by decreasing the amount of salt, also, it can be said that it is related to the inhibitory effect of salt on the growth and activity of probiotic bacteria. Lacroix et al. separated GABA-producing strains from different kind of cheeses and examined the effect of factors in its matrix such as sodium chloride, glutamic acid, oxygen and pH. They concluded that *Lactococcus lactis* ULAAC-A13 and ULAAC-A23 are able to produce GABA up to 500 mg per 100 ml of fermented milk containing 2% sodium chloride and 367 mg per 100 g of glutamic acid (11).

Di Cagno et al. reported that the maximum GABA production (59 mg/kg^{-1}) by *L. plantarum* DSM19463 at pH was 6. In another study (20), Siragusa et al. concluded that lactobacilli (*L. paracasei* PF6, *L. plantarum* PF14 and *L.* sp. strain PF7) in cheese produced large amounts of GABA (289 mg/kg^{-1} - 391 mg/kg^{-1}) in the range of 4.68-5.70 pH (10). Lu et al. stated in their research that *L. lactis* produced the highest amount of GABA (7.2 gl^{-1}) at pH from 7.5 to 8 that the alkaline pH is weak (18).

So far, several species and subtypes of lactic acid bacteria being able to produce GABA have been reported. The study of researchers also showed that *L. brevis* produced the highest amount of GABA, and *Lactobacillus* had the highest production among GABA-producing strains. Almost all strains were separated from traditional fermented foods with acidic pH, such as cheese, tea, sourdough, kimchi and paocai, etc. (21), except *L. brevis* CGMC 1306 separated from fresh and unpasteurized milk. The cause of GABA production at acidic pHs may be related to the maintenance of glutamate decarboxylase activity in the pH range of 4 to 5 (13).

In connection to *L. brevis*, having the highest rate of GABA production, it can be stated that this organism has the ability to produce glutamic acid decarboxylase, which this intracellular enzyme is responsible for the irreversible catalysis of L-glutamate decarboxylation for the production of GABA (8). Although the decarboxylation reaction for glutamate

decarboxylase is similar for lactic acid bacteria, but the primary structure is significantly different in the N-terminal and C-terminal regions. Differences in this basic structure of the enzyme can affect its ability to produce GABA (9). In addition, in most lactic acid bacteria, the active form of glutamate decarboxylase is dimer, but the active form of this enzyme in *L. brevis* IFO12005 is tetramer (22). In the strains of *L. paracasei* NFRI7415 (9) having the highest GABA production, the activity of glutamate decarboxylase enzyme at pH is about 4 to 5.5. At pH, 4 the amount of enzyme activity is very low and at pH above 5.5, this enzyme lacks activity in some lactic acid bacteria such as *Lactococcus lactis* (23).

The results showed that by decreasing pH during cheese production process, the amount of gamma aminobutyric acid increased, which was similar to the results of the present study. The results of the present study and other researchers showed that the pH range is very effective on enzyme activity and GABA production (13).

CONCLUSION

Due to the physiological effects of GABA and given that received GABA can be effective in treating various neurological disorders including, epilepsy, parkinson, schizophrenia, etc., GABA can also regulate the cardiovascular system such as blood pressure, heart beat pain and fatigue. Therefore, this substance can be used as a bioactive in foods and medicines. Whereas adding chemical GABA to foods is unsafe and can cause problems, by adding bacteria that have the ability to produce GABA, food drugs can be produced that in addition to having probiotic properties, also have sedative effects.

Therefore, in this research, GABA production was performed in low-fat probiotic cheese under different conditions of pH, glutamic acid and salt. Optimal conditions to achieve maximum amount of GABA production in cheese on the 15th and 30th day of production was respectively observed 167.7917 mg/kg^{-1} and 220.1250 mg/kg^{-1} under the condition of pH 4.7, glutamic acid 3 mg/g and salt 2%. The results of this study successfully showed that by optimizing the growth conditions of probiotic bacteria, low-fat ultra-filtered cheese can be produced with desirable nutritional properties, and in terms of qualitative properties close to the high-fat sample.

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