RESEARCH ARTICLE

Modeling of glutamic acid production by *Lactobacillus plantarum* MNZ

Mohsen Zareian[1] · **Afshin Ebrahimpour**[1] · **Abdul Karim Sabo Mohamed**[1] · **Nazamid Saari**[1] ✉

1 Universiti Putra Malaysia, Faculty of Food Science and Technology, Serdang, Selangor, Malaysia

Abstract

Background: L-glutamic acid, the principal excitatory neurotransmitter in the brain and an important intermediate in metabolism acts as a precursor of γ-amino butyric acid (GABA). In the present study, culture condition for enhanced glutamic acid production by *Lactobacillus plantarum* MNZ was optimized and the influence of such conditions on GABA production was evaluated.
Results: Results indicated that glutamic acid increased up to 3-fold (3.35) under the following condition: pH 4.5, temperature 37ºC, 12% (w/v) glucose and 0.7% (w/v) ammonium nitrate; whilst GABA production was enhanced up to 10-fold under the following condition: pH 4.5, temperature 37ºC, 6% (w/v) glucose and 0.7% (w/v) ammonium nitrate.
Conclusions: This is the first report for dual biosynthesizing activities of a lactic acid bacterium for the production of glutamic acid and GABA. The results of this study can be further used for developing functional foods rich inglutamic acid and subsequently GABA as a bioactive compound.

Keywords: γ-amino butyric acid, functional foods, lactic acid bacteria, medium optimization, response surface methodology.

INTRODUCTION

Amino acids are the building blocks of proteins which play a key role in cellular processes, such as nitrogen metabolism, energy generation, intracellular communication and cell wall synthesis (Stryer et al. 2002). Glutamic acid is the most important amino acid as a flavour enhancer, which is used as a food additive in the form of mono sodium glutamate (MSG). Although this amino acid was primarily used in Asian foods, however; its use became worldwide owing to its unique flavour known as Umami (Jyothi et al. 2005). Apart from its role as a flavour enhancer, L-glutamic acid acts as a neurotransmitter in the brain and possesses a number of physiological functions in general metabolism (Meffert et al. 2003). Glutamic acid plays a major role in therapeutic potential for improving function of the infant gut exhibiting a high rate of epithelial cell turnover (Burrin and Stoll, 2009). Furthermore, possible usefulness of glutamic acid in enhancing nourishment in the elderly and in patients with poor nutrition has already been proven (Yamamoto et al. 2009). When glutamic acid is supplemented to high liquid protein diet, it accelerated gastric emptying to heal post-ingestive gastrointestinal unpleasantness such as heavy stomach (Zai et al. 2009). In addition, glutamic acid is a specific precursor for other amino acids (arginine and proline) as well as for bioactive molecules such as γ-amino butyric acid (GABA) and glutathione (Kravitz et al. 1963; Jinap and Hajeb, 2010). Several well-known physiological functions of GABA has already been reported included anti-hypertension (Inoue et al. 2003) and anti-diabetic (Hagiwara et al. 2004).

Lactic acid bacteria (LAB) with the GRAS status are commercially essential in the processing of food materials and have extensively been used in various sectors of food industry such as dairy, fermented

meats and vegetables (Leroy and De Vuyst, 2004). It is evident that LAB harbour the gene for glutamic acid production (Tanous et al. 2005). The key advantage of the glutamic acid production by LAB is that the amino acid produced in this way is biologically active (L-glutamic acid) and the production process is safe and eco-friendly.

Glutamic acid acts as a precursor for GABA production. GABA is synthesized by decarboxylation of glutamic acid catalyzed by glutamate decarboxylase (GAD) through the following reaction: L-glutamate + H$^+$ $\xrightarrow{\text{GAD}}$ GABA + CO_2. Glutamate decarboxylase has been isolated from a variety of LAB spp and its biochemical properties have been characterized (Li and Cao, 2010). The GABA formation is restricted by the GABA-producing ability of LAB and L-glutamic acid concentration in the medium (Li and Cao, 2010). Using glutamic acid producing LAB strains in food industries can thereby facilitate production of functional foods rich in GABA. The LAB strain reported in this study with dual biosynthesizing activities for glutamic acid and GABA production offers a great advantage to food industry in particular for mass production of GABA enriched foods.

We also studied those parameters which can enhance glutamic acid production and their influence on GABA production. It is important to optimize the medium for enhancing production of glutamic acid during fermentation. Traditional methods of optimization entail changing one independent variable while fixing the others at given levels. This single-dimensional search technique is simple, but often fails to yield optimized conditions because it does not consider possible interactions among factors. Response surface methodology (RSM) is an efficient experimental strategy to seek optimal conditions for multivariable system. The benefit of employing RSM is to decrease the number of experimental trials that are needed to assess numerous parameters and their interactions (Giovanni, 1983). RSM can therefore be far less laborious and time-consuming compared to other methods for running an optimization process. Other advantages of employing RSM is that no complex calculations are required to analyze the resulting data; plus searching for relativity between factors is possible and the most suitable conditions can be found and responses can be forecasted (Berthouex and Brown, 2002). RSM has been successfully employed for optimizing the medium composition (Téllez-Luis et al. 2003). Based on the key factors influencing the glutamic acid production opted with one independent variable, RSM was applied to optimize the factors of medium for enhanced production of glutamic acid and GABA. It is hypothesized that optimizing glutamic acid production can open new horizons for mass production of functional foods rich in GABA.

MATERIALS AND METHODS

Chemicals and media

HPLC-grade solvents were purchased from Sigma-Aldrich (St. Louis, Missouri). HPLC-grade water was prepared using a Sartorius apparatus (Arium 611 UV, Sartorius Stedim Biotech, Germany). MRS medium was purchased from Difco (Detroit, Michigan).

Bacterial strain

The bacterial strain used in this study was *Lactobacillus plantarum* MNZ isolated from indigenous fermented soybean and its ability to produce glutamic acid was previously studied (Zareian et al. 2012).

Glutamic acid and GABA quantification

The contents of extra-cellular glutamic acid accumulated in the culture medium were extracted according to the method of Yang et al. (2008) with minor modifications. First, the culture broth was separated from cells by centrifugation (8000 x g for 15 min at 4ºC) and the supernatant was diluted 50-fold with 7% (v/v) of glacial acetic acid. The diluted sample was centrifuged at 8000 x g for 15 min at 4ºC, and the supernatant was filtered using 0.22 µm-pore-size membranes and then collected for further analysis.

Intra-cellular glutamic acid was determined following the method described by Komatsuzaki et al. (2005) with minor modifications. First, the cells cultured in MRS broth at 30ºC for 7 days, were separated from culture broth by centrifugation (8000 x g for 15 min at 4ºC); the cells were washed with

0.9% NaCl for three times, and re-suspended in 20 ml of phosphate buffer saline (PBS, pH 7.0) which consisted of 8.0 g NaCl, 0.2 g KCl, 0.91 g Na_2HPO_4, 0.12 g KH_2PO_4. The cells were suspended in 1.0 ml of 75% (v/v) ethanol, the homogenate was centrifuged at 8000 x *g* for 15 min at 4ºC, and the supernatant was filtered using Nylon 0.22 µm-pore-size filter. A 100 ml proportion of the filtered supernatant was collected for quantitative analysis of glutamic acid concentration.

Glutamic acid extracted from MRS broth was subjected to derivatization according to the method described by (Rossetti and Lombard, 1996). Quantitative measurement of the glutamic acid was performed by running an HPLC analysis of the glutamic acid according to the method described by Yang et al. (2008). The derivatized samples were dissolved in 200 ml of initial mobile phase, consisting of a mixture of 60% solution A (aqueous solution of 10.254 g sodium acetate, 0.5 ml tri-ethylamine and 0.7 ml acetic acid in 1000 ml, final pH of 5.8), 12% solution B (acetonitrile) and 28% solution C (deionized water). Gradient HPLC separation was performed on a Shimadzu (Kyoto, Japan) LC 20AT apparatus, consisting of a pump system, a CTO-10ASVP model oven with 20 µl injection loop injector, and a model SPD-M20A PDA (Photo Diode Array) Detector, in conjunction with a DELLOptiplex integrator. A Prevail C_{18} column (250 mm x 4.6 mm i.d., Alltech, Illinois,) was used during the analysis. The mobile phase for gradient elution was pumped at 0.6 ml/min flow rate, 27ºC temperature and glutamic acid detection was performed at 254 nm. For the quantification of GABA, the above same procedure was applied. Glutamic acid or GABA were detected by comparison of their retention time (R_t) values and spectra with known standards and determined by peak areas from the HPLC chromatograms.

RSM analysis

Experimental design. To obtain the highest glutamic acid production in the fermentation medium, optimization of the reaction condition was carried out using surface response methodology (RSM). The concentration of growth factors namely glucose, ammonium nitrate, pH, and temperature as independent variables were evaluated. The response variables were modeled as a function of four independent variables to provide information regarding the optimum proportion of growth factors.

The important factors considered in this study included glucose (x_1) and ammonium nitrate (x_2) concentrations, pH (x_3) and temperature (x_4) and the response variables were glutamic acid and GABA production. Independent variable ranges studied were: glucose concentration (0-12% w/v), ammonium nitrate concentration (0.1-1.3% w/v), pH (2.5-6.5) and temperature (27-47ºC). These factors and the level at which the experiments were carried out are shown in Table 1. A total of 30 runs with central points were generated. The central point of the design arrangement used in this study was glucose 6% (w/v), ammonium nitrate 0.7% (w/v), pH 4.5 and temperature 30ºC. A central composite design (CCD) was employed; (1) to study the main and combined effects of the above factors on the level of glutamic acid and GABA produced by the bacterium; (2) to create models between the variables; and (3) to determine the effect of these variables to optimize the production of glutamic acid and GABA in terms of the response variables studied leading to the desirable goals. Table 1 shows the central composite design elements investigated in this study. Experiments were carried out in randomized order to minimize the effects of unexplained variability in the actual responses due to extraneous factors. The medium used was MRS broth in 15 ml universal bottles. All tests were performed in triplicate.

Statistical analyses of RSM. The experimental design matrix, data analysis and optimization procedure were performed using Design Expert version 6.0.6. (Stat-Ease Inc., MN, USA). Regression analysis and analysis of variance (ANOVA) were conducted for determining regression coefficients and statistical significance of model terms and fitting the mathematical models to the experimental data, aiming at an overall optimal region for the response variables. Multiple regression coefficients were determined by employing the least-squares technique to predict linear and quadratic polynomial models for the response variables studied (Myers et al. 2009). The behaviour of the response surface was evaluated for the response function (Y_i) using the regression polynomial equation. The adequacy of the model was determined using model *P*-value; lack-of-fit *P*-value test and coefficient of determination (R^2). Only terms found statistically significant ($P < 0.05$) were included in the reduced models. It should be noted that some independent variable terms were kept in the reduced model despite non-significance ($P > 0.05$). For example, linear terms were kept in the model when a quadratic or interaction term containing this variable was significant ($P < 0.05$).

Verification procedures. The optimal level of four independent variables (x_1, x_2, x_3, x_4) which led to the desirable response goals was determined by graphical and numerical optimization procedures. A three-dimensional (3D) response surface was plotted by keeping one variable constant at the center point and varying the other two variables within the experimental range. The 3D plotting was performed only for the significant ($P < 0.05$) interaction effects. A numerical optimization procedure was also carried out by the response optimizer using Design Expert version 6.0.6. (Stat-Ease Inc., MN, USA) to measure the exact optimum value of individual and simultaneous multiple response optimizations resulting in the desirable response goals. Experimental data was compared with the fitted values predicted by the models in order to verify the adequacy of the regression models.

RESULTS

In the present study, response surface methodology (RSM) was employed for the optimization of glutamic acid production. Application of RSM allowed simultaneously determining main and interaction effects of growth factors on the production of glutamic acid. The variation of each response variable was assessed as a function of linear, quadratic and interaction terms of pH (A), temperature (B), glucose (C) and ammonium nitrate (D). Results showed that the quadratic model was fitted for the response variables (glutamic acid and GABA production) because of its ideal statistic data including the lowest standard deviation (0.22), maximum R^2 (0.944 and 0.963 for glutamic acid and GABA, respectively), maximum adjusted R^2 (0.899 and 0.944 for glutamic acid and GABA, respectively) and the lowest predicted residual sum of squares (3.67 and 6.54 for glutamic acid and GABA, respectively). The predicted R^2 (0.736 and 0.863 for glutamic acid and GABA, respectively) is in reasonable agreement with the adjusted R^2 (0.899 and 0.944 for glutamic acid and GABA, respectively). Analysis of variance for response surface quadratic model of glutamic acid and GABA is presented in Table 2 and Table 3, respectively. The computed model F-value of glutamic acid (20.89) and GABA (22.27) implies that the model is significant and there is only a 0.01% chance that a "model F-value" this large could occur due to noise. Prob > Fvalue less than 0.05, on the other hand, indicates that model terms are significant. Table 2 shows that C, A^2, B^2, C^2, D^2, AB, AC, AD, BD and CD are significant model terms for glutamic acid production where A is pH; B is temperature; C is glucose and D is ammonium nitrate. Table 3 also demonstrates that A, B, A^2, B^2, C^2, D^2, AB and AC are significant model terms for GABA production. Values greater than 0.10 indicate that model terms are not significant. Table 4 illustrates a comparison between experimental and predicted values for glutamic acid and GABA based on the final reduced models.

"Adeqate Precision" is an index that measures the signal to noise ratio. A ratio greater than 4 is desirable. The computed ratio of 24.99 and 22.27 for glutamic acid and GABA, respectively, in this study (data not shown) indicates an adequate signal. This means that this model can be used to navigate the design space. The pure error computed, on the other hand, was very low. This demonstrated a good reproducibility of the data obtained in this study. In addition, with a suitable coefficient of determination ($R^2_{Glu} = 0.944$ and $R^2_{GABA} = 0.963$) and adjusted coefficient of determination ($R^2_{adjustedGlu} = 0.899$ and $R^2_{adjustedGABA} = 0.944$) and very small "model Prob > F" (< 0.0001) from the analysis of ANOVA (Table 2 and Table 3), it can be deduced that the quadratic polynomial model was significant and adequate to represent the actual relationship between the responses (glutamic acid and GABA) and the significant variables. In other words, fitting the data to various models (linear, two factorial, quadratic and cubic) and their subsequent ANOVA revealed that glutamic acid and GABA production were most suitably defined with quadratic polynomial model and the final equation in terms of actual factors were as follows:

Glutamic acid (mM) = - 21.70 + 3.64A + 0.64B+ 0.014C + 9.81D - 0.16A^2 - 0.006B^2 + 0.023C^2 - 1.67D^2 - 0.028AB - 0.059AC - 1.17AD - 0.96BD + 0.191CD

GABA (mM) = - 66 + 4.48A + 2.78B + 2.07C + 9.51D - 0.44A^2 - 0.04B^2 - 0.09C^2 - 6.85D^2 - 0.11AC - 0.01BC

A: pH; B: Temperature (ºC); C: Glucose (%w/v); D: Ammonium nitrate (%w/v)

ANOVA analysis of glutamic acid production (Table 2) revealed that at the first sight, only glucose had significant effect on glutamic acid production and other parameters (pH, growth temperature and ammonium nitrate concentration) were not significant ($P > 0.05$); however, the second order of each parameter was of significant effect on glutamic acid production ($P < 0.05$). In addition, they had

important and significant interactions with each other, thus they have been used to develop the model. On the other hand, among the different interactions, interaction between glucose concentration and growth temperature did not show significant effect on glutamic acid production ($P > 0.05$); however, their interaction was also kept in the final model to have the model less modified.

In case of GABA production, ANOVA analysis (Table 3) showed that only pH and temperature were of significant effect and other parameters included glucose and ammonium nitrate concentration did not significantly ($P > 0.05$) influence GABA production; however, the second order of each parameter was found to be of significant effect ($P < 0.05$). Among the different interactions, only interaction between pH-glucose concentration and temperature-glucose concentration significantly ($P > 0.05$) affect GABA production.

Effect of temperature on glutamic acid and GABA production

The combined effect of temperature-initial pH on glutamic acid production was depicted in Figure 1a demonstrating that glutamic acid production was effectively increased from 32 to 37ºC and decreased thereafter up to 42ºC. Other studies also showed an increased glutamic acid production when the temperature at which bacteria was grown, increased (Delaunay et al. 1999; Lapujade et al. 1999). Such phenomena can be explained by the effect of temperature on glutamic acid production. It is proven that temperature can influence the efflux of glutamic acid from bacteria cells in which the yield and the specific glutamic acid production rate can be increased by a temperature shift-up (Choi et al. 2004). Increasing temperature is primarily responsible for a repression or inhibition of α-ketoglutarate dehydrogenase complex (Uy et al. 2003). This leads to redirecting the 2-oxoglutarate flux towards glutamic acid production and thereby increasing glutamic acid excretion rate (Asakura et al. 2007).

As shown in Figure 2a, b and c, the combined effect of temperature-initial pH, glucose-temperature and ammonium nitrate- temperature on GABA production indicated that GABA production was effectively increased when the temperature increased up to 37ºC and decreased thereafter. Elevation in temperature was previously shown to significantly increase GABA production in *Lactobacillus* (Huang et al. 2007; Kim et al. 2009; Li et al. 2010b). In case of *L. plantarum*, the temperature 34ºC was previously reported as the optimum point for GABA production (Tung et al. 2011) which is quite similar to the temperature value (37ºC) found in our study. Such higher temperature for GABA production in our study (37ºC) can be explained by the higher temperature necessary for GAD activity than the optimum condition for the cell growth of lactic acid bacteria (Yang et al. 2008).

Effect of pH on glutamic acid and GABA production

The combined effect of initial pH-temperature and initial pH-ammonium nitrate on glutamic acid production is shown in Figure 1a, b. According to the plot (Figure 1a), maximum glutamic acid production occurred at an acidic pH (4.5). Further decrease in the pH led to a decrease in glutamic acid production. pH plays an important role in the biological processes and the initial pH of the culture medium is important for glutamic acid production (Eggeling and Bott, 2005). Growth rate of *L. plantarum* is reported the highest at pH 6.5 (Zacharof and Lovitt, 2010), maximum glutamic acid production in this study, however, occurred at lower pH (4.5). Initial pH of the culture medium can influence the growth rate of Lactobacilli and the initial pH value lower than 6.5 decreases the growth rate of *L. plantarum* (Zacharof and Lovitt, 2010). This causes redirection of 2-oxoglutarate efflux towards glutamic acid production which increases glutamic acid excretion rate (Asakura et al. 2007). It is concluded from the result of this study that acidic conditions can improve glutamic acid production by *L. plantarum* MNZ. Previous studies which evaluated the effect of initial pH on glutamic acid yield reported the highest glutamic acid production mostly at a neutral pH range (Momose and Takagi, 1978; Roy and Chatterjee, 1989; Nampoothiri and Pandey, 1995a; Shiratsuchi et al. 1995; Jyothi et al. 2005); nevertheless, acidic fermentation medium in our study induced such elevation in the production of this amino acid. Acidic pH, therefore, could be responsible for triggering the *gdh* gene in lactic acid bacteria resulting in elevated glutamic acid production.

The combined effect of temperature-initial pH, glucose-initial pH and ammonium nitrate-pH on GABA production is also depicted in Figure 2a, d, e, respectively. According to the plots, GABA production was the highest at an acidic pH (4.5). Such similar acidic pH value (4.4) has been shown (Komatsuzaki et al. 2005; Li et al. 2010b) to maximize GABA production in various *Lactobacillus* spp. In microorganisms, GABA can be decomposed through this pathway: GABA transaminase catalyses the

reversible conversion of GABA to succinic semialdehyde using either pyruvate or α-ketoglutarate as amino acceptor and succinic semialdehyde dehydrogenase catalyses the reversible conversion of succinic semialdehyde to succinate (Shelp et al. 1999; Kumar et al. 2000). Since the above mentioned enzymes have the alkaline pH optimum (Shelp et al. 1999; Kumar et al. 2000), therefore, acidic condition can block the enzymes that cause GABA to decompose resulted in enhanced accumulation of GABA in the medium.

Maximum growth rate of *L. plantarum* occurs at moderately acidic initial pH of 6.5 (Zacharof and Lovitt, 2010). High cell density, in general, is also required for efficient GABA biosynthesis (Li and Cao, 2010). On the other hand, acid resistance response in the presence of glutamate was demonstrated to induce the glutamate decarboxylase system (Small and Waterman, 1998; Dilworth and Glenn, 2008). In other words, induction of the GAD gene is one of the acid stress responses in LAB (Small and Waterman, 1998) resulting in GABA production. Based on previous evidence (Hommais et al. 2004), *GadX* and *GadE* are activators of the global bacterial acid response involved in the general adaptation of pH homeostasis maintenance. In addition, pH-regulated genes encoding a GABA transporter was shown to have an acidic pH optimum and preferentially expressed at acidic ambient pH (Espeso and Arst, 2000). Since growth and subsequently expression of the GAD gene requires this type of pH changing therefore, stabilization of pH *e.g.* using buffer system would suppress the gene expression and subsequently GABA production.

Effect of ammonium nitrate on glutamic acid and GABA production

The combine effect of ammonium nitrate-initial pH on glutamic acid production (Figure 1b) showed that glutamic acid production decreased when ammonium nitrate decreased. The highest concentration of glutamic acid was achieved at 0.7% (w/v) ammonium nitrate. Nitrogen as an essential component for amino acid production plays an important role in fermentative cultivation of glutamic acid-producing bacteria. Therefore, nitrogen is typically provided with external nitrogen sources which is taken up by cells, and thereafter assimilated to accomplish their metabolism (Burkovski, 2003a; Burkovski, 2003b). Ammonium is mostly assimilated by glutamate dehydrogenase (Tesch et al. 1999) with high activity in *L. plantarum* (Tanous et al. 2002) to form glutamic acid.

The combine effect of ammonium nitrate-temperature, ammonium nitrate-pH and ammonium nitrate-glucose on the production of GABA showed (Figure 2c, e**,** and f) that GABA production decreased when ammonium nitrate decreased. The highest concentration of GABA was also achieved with 0.7% (w/v) ammonium nitrate.

Effect of glucose concentration on GABA production

The combine effect of glucose-ammonium nitrate and glucose-temperature on GABA production was depicted in Figure 2f and Figure 2b, showing that 6% (w/v) glucose content was the best concentration to yield the highest GABA content. A concentration of 1% glucose was previously reported as the optimum level for GABA production by *Lactobacillus buchneri* (Cho et al. 2007) whilst a 5.5% glucose concentration was found to yield highest GABA in *Lactobacillus brevis* (Li et al. 2010a). GABA is produced through a metabolic pathway called GABA shunt, where the initial step utilizes α-ketoglutarate formed from glucose metabolisms via Krebs cycle (Kumar and Punekar, 1997). Carbohydrates utilized by microorganism are converted into glucose form prior to entering the metabolic pathway of glycolysis (Pommerville, 2009). The glycolytic pathway constitutes a definite sequence of reactions involving intermediate compounds and produced pyruvate. Pyruvate serves as an intermediate compound to enter the TCA cycle before further converting into α-ketoglutarate (Kumar and Punekar, 1997). Instead of being directly converted into succinate, α- ketoglutarate is preferably aminated to form glutamate within the TCA cycle by oxidative decarboxylation process through GABA shunt pathway (Kumar and Punekar, 1997). GABA biosynthesis pathway from carbohydrates has been previously proved (Kumar et al. 2000). It was also shown (Zareian et al. 2012) that among various sugars, glucose effectively enhanced glutamic acid production. Such phenomenon was expected to significantly influence GABA production shown in this study.

Changes in the levels of glutamic acid and GABA before and after RSM optimization

The theoretical values for levels of glutamic acid and GABA were 3.54 and 3.67 mM, respectively, while the actual values were 3.35 and 3.96 mM, respectively. After RSM optimization, the levels of

glutamic acid and GABA were significantly increased 3.3 and 10-fold, respectively, in comparison with the control (non-RSM) (data not shown). Table 5 compares the content of glutamic acid and GABA prior and after RSM optimization. Figure 3 depicts the production profile of GABA *versus* glutamic acid. The correlation between the two compounds shows that GABA is produced during glutamic acid biosynthesis phase. Glutamic acid is a precursor of GABA, hence, increasing glutamic acid content leads to an elevation of GABA production. Enhancement of glutamic acid level has also been proven to increase GABA synthesis in LAB (Komatsuzaki et al. 2005).

Optimal production of glutamic acid and GABA by *L. plantarum* MNZ fermentation

The potency for the production of GABA extensively differs among the strains of lactic acid bacteria and is significantly affected by medium composition and culture conditions. Hence, it is essential to optimize such conditions to enhance GABA production. The optimal conditions for GABA fermentation vary among the different lactic acid bacteria strains, and the main factors affecting glutamic acid and consequently GABA production for *L. plantarum* MNZ have been characterized in this study. The optimal fermentation conditions for glutamic acid production by *L. plantarum* MNZ was found to be 12% glucose, 0.7% ammonium nitrate, pH 4.5 and temperature 37ºC (Figure 1) resulted in 3.35 mM of glutamic. For GABA production, 6% glucose, 0.7% ammonium nitrate, pH 4.5 and temperature 37ºC yielded 3.96 mM GABA.

The effect of various carbon and nitrogen sources on glutamic acid yield has been previously studied and ammonium nitrate (0.53%) and glucose (8%) were reported as the most appropriate nitrogen and carbon sources (Roy and Chatterjee, 1989). In another study, a wide range of different carbon sources including glucose, fructose, sucrose, maltose, lactose, xylose and starch were evaluated for the highest glutamic acid production and a 2% glucose was found to be the best (Nampoothiri and Pandey, 1995b). Assessment of three different nitrogen sources (ammonium nitrate, sodium nitrate and urea) on glutamic acid production showed that ammonium nitrate (1%) was the best than the other nitrogen sources (Jyothi et al. 2005).

Optimization through experimental design is a common method in biotechnology for enhanced production of specific amino acids (Téllez-Luis et al. 2003). In the last decade, various strategies have been employed to optimize glutamic acid production such as cell recycling (Ishizaki et al. 1993), nutrient formulation (Nampoothiri and Pandey, 1995a), culturing the bacteria in solid substrates (Nampoothiri and Pandey, 1996a), and the use of different raw materials (Das et al. 1995; Jyothi et al. 2005). The optimal conditions of the fermentation process for glutamic acid production by different bacterial strains have already been reported (Nampoothiri and Pandey, 1995b; Shiratsuchi et al. 1995; Delaunay et al. 2002; Jyothi et al. 2005). Of these factors, pH, temperature, various carbon and nitrogen sources have been evaluated. For instance, *Brevibacterium lactofermentum* cultured in a medium containing 3.6% glucose, 0.24 g soybean hydrolysate and 0.5% urea as nitrogen sources yielded 1.04 mM glutamic acid (Momose and Takagi, 1978). Cultivation of *Arthrobacter globiformis* with 8% glucose and 0.53% ammonium nitrate yielded the highest glutamic acid (Roy and Chatterjee, 1989). The highest glutamic acid yield by *Brevibacterium* sp. was found to be 0.049 mM using 2% glucose and 0.5% urea (Nampoothiri and Pandey, 1996b). In the presence of 1.0% ammonium nitrate, *Brevibacterium divaricatum* produced the highest glutamic acid yield (26.9 mM) (Jyothi et al. 2005). In our study, with 12% glucose, 0.7% ammonium nitrate, pH 4.5 and temperature 37ºC were shown to be the individual optimum factors leading to the highest glutamic acid yield (3.35 mM).

CONCLUDING REMARKS

A dual production of glutamic acid and GABA by a lactic acid bacterium (*L. plantarum*) was studied and effective parameters were evaluated. Optimization improved production of glutamic acid and GABA up to 3 and 10 folds higher than non-optimized system. Employing such a strain with the potential application as a probiotic together with the proven bioactive compound capacity provides a multi promoting effects for health conscious consumers. Given the broad range of food fermentation industry, such strains can eventually speed up the ever expanding markets of functional foods.

REFERENCES

ASAKURA, Y.; KIMURA, E.; USUDA, Y.; KAWAHARA, Y.; MATSUI, K.; OSUMI, T. and NAKAMATSU, T. (2007). Altered metabolic flux due to deletion of *odh*A causes L-glutamate overproduction in *Corynebacterium glutamicum*. *Applied and Environmental Microbiology*, vol. 73, no. 4, p. 1308-1319. **[CrossRef]**

BERTHOUEX, P.M. and BROWN, L.C. (2002). *Statistics for environmental engineers*. CRC. 489 p. ISBN 978-1566705929.

BURKOVSKI, A. (2003a). Ammonium assimilation and nitrogen control in *Corynebacterium glutamicum* and its relatives: An example for new regulatory mechanisms in actinomycetes. *FEMS Microbiology Reviews*, vol. 27, no. 5, p. 617-628. **[CrossRef]**

BURKOVSKI, A. (2003b). I do it my way: Regulation of ammonium uptake and ammonium assimilation in *Corynebacterium glutamicum*. *Archives of Microbiology*, vol. 179, no. 2, p. 83-88. **[CrossRef]**

BURRIN, D.G. and STOLL, B. (2009). Metabolic fate and function of dietary glutamate in the gut. *The American Journal of Clinical Nutrition*, vol. 90, no. 3, p. 850S-856S. **[CrossRef]**

CHO, Y.R.; CHANG, J.Y. and CHANG, H.C. (2007). Production of gamma-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuronal cells. *Journal of Microbiology and Biotechnology*, vol. 17, no. 1, p. 104-109.

CHOI, S.U.; NIHIRA, T. and YOSHIDA, T. (2004). Enhanced glutamic acid production of *Brevibacterium* sp. with temperature shift-up cultivation. *Journal of Bioscience and Bioengineering*, vol. 98, no. 3, p. 211-213. **[CrossRef]**

DAS, K.; ANIS, M.; AZEMI, B.M.N. and ISMAIL, N. (1995). Fermentation and recovery of glutamic acid from palm waste hydrolysate by Ion exchange resin column. *Biotechnology and Bioengineering*, vol. 48, no. 5, p. 551-555. **[CrossRef]**

DELAUNAY, S.; GOURDON, P.; LAPUJADE, P.; MAILLY, E.; ORIOL, E.; ENGASSER, J.M.; LINDLEY, N.D. and GOERGEN, J.L. (1999). An improved temperature-triggered process for glutamate production with *Corynebacterium glutamicum*. *Enzyme and Microbial Technology*, vol. 25, no. 8-9, p. 762-768. **[CrossRef]**

DELAUNAY, S.; LAPUJADE, P.; ENGASSER, J.M. and GOERGEN, J.L. (2002). Flexibility of the metabolism of *Corynebacterium glutamicum* 2262, a glutamic acid-producing bacterium, in response to temperature upshocks. *Journal of Industrial Microbiology and Biotechnology*, vol. 28, no. 6, p. 333-337.

DILWORTH, M. and GLENN, A. (2008). Problems of adverse pH and bacterial. *Bacterial responses to pH, 4. Novartis Foundation Symposium 221*. John Wiley & Sons. 292 p. ISBN 9780471985990.

EGGELING, L. and BOTT, M. (2005). Handbook of *Corynebacterium glutamicum*. CRC Press. 632 p. ISBN 978-0-8493-1821-4

ESPESO, E.A. and ARST, H.N. (2000). On the mechanism by which alkaline pH prevents expression of an acid-expressed gene. *Molecular and Cellular Biology*, vol. 20, no. 10, p. 3355-3363. **[CrossRef]**

GIOVANNI, M. (1983). Response surface methodology and product optimization. *Food Technology*, vol. 37, no. 11, p. 41-45.

HAGIWARA, H.; SEKI, T. and ARIGA, T. (2004). The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. *Bioscience, Biotechnology and Biochemistry*, vol. 68, no. 2, p. 444-447. **[CrossRef]**

HOMMAIS, F.; KRIN, E.; COPPÉE, J.Y.; LACROIX, C.; YERAMIAN, E.; DANCHIN, A. and BERTIN, P. (2004). GadE (YhiE): A novel activator involved in the response to acid environment in *Escherichia coli. Microbiology*, vol. 150, no. 1, p. 61-72. **[CrossRef]**

HUANG, J.; MEI, L.H.; WU, H. and LIN, D. (2007). Biosynthesis of γ-aminobutyric acid (GABA) using immobilized whole cells of *Lactobacillus brevis*. *World Journal of Microbiology and Biotechnology*, vol. 23, no. 6, p. 865-871. **[CrossRef]**

INOUE, K.; SHIRAI, T.; OCHIAI, H.; KASAO, M.; HAYAKAWA, K.; KIMURA, M. and SANSAWA, H. (2003). Blood pressure lowering effect of a novel fermented milk containing γ amino butyric acid (GABA) in mild hypertensives. *European Journal of Clinical Nutrition*, vol. 57, no. 3, p. 490-495. **[CrossRef]**

ISHIZAKI, A.; TAKASAKI, S. and FURUTA, Y. (1993). Cell-recycled fermentation of glutamate using a novel cross-flow filtration system with constant air supply. *Journal of Fermentation and Bioengineering*, vol. 76, no. 4, p. 316-320. **[CrossRef]**

JINAP, S. and HAJEB, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite*, vol. 55, no. 1, p. 1-10. **[CrossRef]**

JYOTHI, A.N.; SASIKIRAN, K.; NAMBISAN, B. and BALAGOPALAN, C. (2005). Optimization of glutamic acid production from cassava starch factory residues using *Brevibacterium divaricatum*. *Process Biochemistry*, vol. 40, no. 11, p. 3576-3579. **[CrossRef]**

KIM, J.Y.; LEE, M.Y.; JI, G.E.; LEE, Y.S. and HWANG, K.T. (2009). Production of γ-aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis* GABA100. *International Journal of Food Microbiology*, vol. 130, no. 1, p. 12-16. **[CrossRef]**

KOMATSUZAKI, N.; SHIMA, J.; KAWAMOTO, S.; MOMOSE, H. and KIMURA, T. (2005). Production of γ amino butyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiology*, vol. 22, no. 6, p. 497-504. **[CrossRef]**

KRAVITZ, E.A.; KUFFLER, S.W. and POTTER, D.D. (1963). Gamma-aminobutyric acid and other blocking compounds in crustacea: III. Their relative concentrations in separated motor and inhibitory axons. *Journal of Neurophysiology*, vol. 26, p. 739-751.

KUMAR, S. and PUNEKAR, N.S. (1997). The metabolism of 4-aminobutyrate (GABA) in fungi. *Mycological Research*, vol. 101, no. 4, p. 403-409. **[CrossRef]**

KUMAR, S.; PUNEKAR, N.S.; SATYANARAYAN, V. and VENKATESH, K.V. (2000). Metabolic fate of glutamate and evaluation of flux through the 4-aminobutyrate (GABA) shunt in *Aspergillus niger. Biotechnology and Bioengineering*, vol. 67, no. 5, p. 575-584. **[CrossRef]**

LAPUJADE, P.; GOERGEN, J.L. and ENGASSER, J.M. (1999) Glutamate excretion as a major kinetic bottleneck for the thermally triggered production of glutamic acid by *Corynebacterium glutamicum. Metabolic Engineering*, vol. 1, no. 3, p. 255-261. **[CrossRef]**

LEROY, F. and DE VUYST, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, vol. 15, no. 2, p. 67-78. **[CrossRef]**

LI, H. and CAO, Y. (2010). Lactic acid bacterial cell factories for gamma-aminobutyric acid. *Amino Acids,* vol. 39, no. 5, p. 1107-1116. **[CrossRef]**

LI, H.; QIU, T.; GAO, D. and CAO, Y. (2010a). Medium optimization for production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912. *Amino Acids*, vol. 38, no. 5, p. 1439-1445. **[CrossRef]**

LI, H.; QIU, T.; HUANG, G. and CAO, Y. (2010b). Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. *Microbial Cell Factories,* vol. 9, no. 1, p. 85. **[CrossRef]**

MEFFERT, M.K.; CHANG, J.M.; WILTGEN, B.J.; FANSELOW, M.S. and BALTIMORE, D. (2003). NF-κB functions in synaptic signaling and behavior. *Nature Neuroscience*, vol. 6, no. 6, p. 1072-1078. **[CrossRef]**

MOMOSE, H. and TAKAGI, T. (1978). Glutamic acid production in biotin-rich media by temperature sensitive mutants of *Brevibacterium lactofermentum*, a novel fermentation process. *Agricultural and Biological Chemistry,* vol. 42, no. 10, p. 1911-1917. **[CrossRef]**

MYERS, R.H.; MONTGOMERY, D.C. and ANDERSON-COOK, C.M. (2009). *Response surface methodology: Process and product optimization using designed experiments.* John Wiley & Sons. 680 p. ISBN 9780470174463.

NAMPOOTHIRI, K. and PANDEY, A. (1995a). Glutamic acid fermentation using *Brevibacterium* DSM 20411. *Journal of Food Science and Technology (Mysore)*, vol. 32, p. 406-408.

NAMPOOTHIRI, K.M. and PANDEY, A. (1995b). Effect of different carbon sources on growth and glutamic acid fermentation by *Brevibacterium* sp. *Journal of Basic Microbiology*, vol. 35, no. 4, p. 249-254. **[CrossRef]**

NAMPOOTHIRI, K.M. and PANDEY, A. (1996a). Solid state fermentation for L-glutamic acid production using *Brevibacterium* sp. *Biotechnology Letters*, vol. 18, no. 2, p. 199-204. **[CrossRef]**

NAMPOOTHIRI, K.M. and PANDEY, A. (1996b). Urease activity in a glutamate producing *Brevibacterium* sp. *Process Biochemistry*, vol. 31, no. 5, p. 471-475. **[CrossRef]**

POMMERVILLE, J.C. (2009). *Alcamo's Fundamentals of Microbiology: Body Systems.* Jones & Bartlett Learning. 914 p. ISBN 9780763762599.

ROSSETTI, V. and LOMBARD, A. (1996). Determination of glutamic acid decarboxylase by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Applications*, vol. 681, no. 1, p. 63-67.

ROY, D.K. and CHATTERJEE, S.P. (1989). Production of glutamic acid by *Arthrobacter globiformis*: Influence of cultural conditions. *Folia Microbiologica*, vol. 34, no. 1, p. 11-24. **[CrossRef]**

SHELP, B.J.; BOWN, A.W. and MCLEAN, M.D. (1999). Metabolism and functions of gamma-aminobutyric acid. *Trends in Plant Science*, vol. 4, no. 11, p. 446-452. **[CrossRef]**

SHIRATSUCHI, M.; KURONUMA, H.; KAWAHARA, Y.; YOSHIHARA, Y.; MIWA, H. and NAKAMORI, S. (1995). Simultaneous and high fermentative production of L-lysine and L-glutamic acid using a strain of *Brevibacterium lactofermentum. Bioscience, Biotechnology and Biochemistry*, vol. 59, no. 1, p. 83-86. **[CrossRef]**

SMALL, P.L.C. and WATERMAN, S.R. (1998). Acid stress, anaerobiosis and gadCB: Lessons from *Lactococcus lactis* and *Escherichia coli. Trends in Microbiology,* vol. 6, no. 6, p. 214-216. **[CrossRef]**

STRYER, L.; BERG, J. and TYMOCZKO, J. (2002). *Biochemistry.* W.H. Freeman & Co Ltd. 1438 p. ISBN 978-0716746843.

TANOUS, C.; KIERONCZYK, A.; HELINCK, S.; CHAMBELLON, E. and YVON, M. (2002). Glutamic dehydrogenase activity: A major criterion for the selection of flavor producing lactic acid bacteria strains. *Antonie Van Leeuwenhoek*, vol. 82, no. 1-4, p. 271-278. **[CrossRef]**

TANOUS, C.; CHAMBELLON, E.; SEPULCHRE, A.M. and YVON, M. (2005). The gene encoding the glutamate dehydrogenase in *Lactococcus lactis* is part of a remnant Tn3 transposon carried by a large plasmid. *Journal of Bacteriology,* vol. 187, no. 14, p. 5019-5022. **[CrossRef]**

TÉLLEZ-LUIS, S.J.; MOLDES, A.B.; ALONSO, J.L. and VÁZQUEZ, M. (2003). Optimization of lactic acid production by *Lactobacillus delbrueckii* through response surface methodology. *Journal of Food Science*, vol. 68, no. 4, p. 1454-1458. **[CrossRef]**

TESCH, M.; DE GRAAF, A.A. and SAHM, H. (1999). *In vivo* fluxes in the ammonium-assimilatory pathways in *Corynebacterium glutamicum* studied by 15N nuclear magnetic resonance. *Applied and Environmental Microbiology,* vol. 65, no. 3, p. 1099-1109.

TUNG, Y.T.; LEE, B.H.; LIU, C.F. and PAN, T.M. (2011). Optimization of culture condition for ACEI and GABA production by lactic acid bacteria. *Journal of Food Science*, vol. 76, no. 9, p. M585-M591. **[CrossRef]**

UY, D.; DELAUNAY, S.; GERMAIN, P.; ENGASSER, J.M. and GOERGEN, J.L. (2003). Instability of glutamate production by *Corynebacterium glutamicum* 2262 in continuous culture using the temperature-triggered process. *Journal of Biotechnology*, vol. 104, no. 1-3, p. 173-184. **[CrossRef]**

YAMAMOTO, S.; TOMOE, M.; TOYAMA, K.; KAWAI, M. and UNEYAMA, H. (2009). Can dietary supplementation of monosodium glutamate improve the health of the elderly? *The American Journal of Clinical Nutrition*, vol. 90, no. 3, p. 844S-849S. **[CrossRef]**

YANG, S.Y.; LU, F.X.; LU, Z.X.; BIE, X.M.; JIAO, Y.; SUN, L.J. and YU, B. (2008). Production of γ-aminobutyric acid by *Streptococcus salivarius* subsp. *thermophilus* Y2 under submerged fermentation. *Amino Acids*, vol. 34, no. 3, p. 473-478. **[CrossRef]**

ZACHAROF, M.P. and LOVITT, R.W. (2010). Development of an optimised growth strategy for intensive propagation, lactic acid and bacteriocin production of selected strains of *Lactobacilli* genus. *International Journal of Chemical Engineering and Applications*, vol. 1, no. 1, p. 55-63.

ZAI, H.; KUSANO, M.; HOSAKA, H.; SHIMOYAMA, Y.; NAGOSHI, A.; MAEDA, M.; KAWAMURA, O. and MORI, M. (2009). Monosodium L-glutamate added to a high-energy, high-protein liquid diet promotes gastric emptying. *The American Journal of Clinical Nutrition*, vol. 89, no. 1, p. 431-435. **[CrossRef]**

ZAREIAN, M.; EBRAHIMPOUR, A.; BAKAR, F.A.; MOHAMED, A.K.S.; FORGHANI, B.; AB-KADIR, M.S.B. and SAARI, N. (2012). A glutamic acid-producing lactic acid bacteria isolated from Malaysian fermented foods. *International Journal of Molecular Sciences*, vol. 13, no. 5, p. 5482-5497. **[CrossRef]**

Tables

Table 1. Central composite design (CCD).

Glucose (%w/v)	Ammonium nitrate (%w/v)	pH	Temperature (°C)
3	1	5.5	42
9	1	5.5	32
6	0.7	4.5	47
9	0.4	3.5	42
6	0.7	6.5	37
9	1	3.5	42
6	0.7	4.5	27
3	1	3.5	42
6	**0.7**	**4.5**	**37**
3	0.4	3.5	32
6	1.3	4.5	37
9	1	3.5	32
6	0.1	4.5	37
3	1	5.5	32
9	0.4	5.5	42
9	0.4	5.5	32
6	**0.7**	**4.5**	**37**
12	0.7	4.5	37
6	**0.7**	**4.5**	**37**
9	1	5.5	42
3	0.4	5.5	42
6	**0.7**	**4.5**	**37**
6	**0.7**	**4.5**	**37**
6	**0.7**	**4.5**	**37**
3	0.4	5.5	32
3	0.4	3.5	42
9	0.4	3.5	32
0	0.7	4.5	37
3	1	3.5	32
6	0.7	2.5	37

Bold numbers indicate center points.

Table 2. Analysis of variance for response surface quadratic model of glutamic acid production by *L. plantarum* MNZ.

Source	Sum of squares	DF	Mean square	*F*-value	Prob > F	
Model	13.181	13	1.014	20.898	< 0.0001	significant
A	0.088	1	0.088	1.814	0.1968	
B	0.200	1	0.200	4.131	0.0590	
C	5.575	1	5.575	114.904	< 0.0001	
D	0.137	1	0.137	2.824	0.1123	
A^2	0.719	1	0.719	14.817	0.0014	
B^2	0.664	1	0.664	13.682	0.0019	
C^2	1.207	1	1.207	24.886	0.0001	
D^2	0.623	1	0.623	12.838	0.0025	
AB	0.334	1	0.334	6.893	0.0184	
AC	0.510	1	0.510	10.517	0.0051	
AD	1.987	1	1.987	40.951	< 0.0001	
BD	0.338	1	0.338	6.960	0.0179	
CD	0.476	1	0.476	9.817	0.0064	
Residual	0.776	16	0.049			
Lack of Fit	0.701	11	0.064	4.205	0.0625	not significant
Pure Error	0.076	5	0.015			
Core Total	13.958	29				

A: pH; B: Temperature (ºC); C: Glucose (%w/v); D: Ammonium nitrate (%w/v).

Table 3. Analysis of variance for response surface quadratic model of GABA production by *L. plantarum* MNZ.

Source	Sum of squares	DF	Mean square	*F*-value	Prob > F	
Model	45.99	10	4.60	50.16	< 0.0001	significant
A	0.69	1	0.69	7.53	0.0129	
B	0.86	1	0.86	9.39	0.0064	
C	0.26	1	0.26	2.84	0.1080	
D	0.01	1	0.01	0.15	0.7022	
A^2	5.33	1	5.33	58.18	< 0.0001	
B^2	23.53	1	23.53	256.63	< 0.0001	
C^2	17.87	1	17.87	194.91	< 0.0001	
D^2	10.43	1	10.43	113.74	< 0.0001	
AC	1.88	1	1.88	20.48	0.0002	
BC	0.52	1	0.52	5.69	0.0276	
Residual	1.74	19	0.09			
Lack of Fit	1.57	14	0.11	3.27	0.0985	not significant
Pure Error	0.17	5	0.03			
Cor Total	47.73	29				

A: pH; B: Temperature (°C); C: Glucose (%w/v); D: Ammonium nitrate (%w/v).

Table 4. Comparison between experimental and predicted values for glutamic acid and GABA production by *L. plantarum* MNZ based on the final reduced model.

Experimental value		Predicted value		Absolute deviation	
Glu	GABA	Glu	GABA	Glu	GABA
0.903	0.860	0.576	0.635	0.327	0.225
1.762	0.929	1.806	0.981	-0.044	-0.052
0.899	0.593	0.973	0.617	-0.074	-0.024
1.703	0.821	1.625	0.963	0.079	-0.143
1.706	2.102	1.552	1.890	0.154	0.212
2.211	0.654	2.068	0.865	0.143	-0.211
2.003	0.810	1.949	1.150	0.054	-0.340
1.890	0.007	1.886	0.125	0.004	-0.118
1.258	0.563	1.075	0.587	0.183	-0.024
1.119	1.361	0.895	0.933	0.224	0.428
0.868	0.372	0.891	0.569	-0.023	-0.197
0.009	0.948	0.133	0.915	-0.124	0.033
2.783	1.774	2.741	1.842	0.042	-0.068
1.951	0.776	1.847	0.817	0.104	-0.041
2.788	0.592	2.557	1.102	0.231	-0.510
1.035	0.028	1.085	0.077	-0.050	-0.049
0.917	2.498	1.211	2.246	-0.294	0.253
0.954	1.534	0.968	1.567	-0.014	-0.033
0.885	0.000	1.298	0.344	-0.413	-0.344
1.037	0.151	0.932	-0.413	0.105	0.564
1.492	0.000	1.612	0.233	-0.120	-0.233
3.353	1.103	3.540	0.650	-0.187	0.453
1.117	1.367	1.285	1.252	-0.168	0.115
0.844	1.260	0.983	1.156	-0.139	0.104
1.659	3.568	1.737	3.670	-0.078	-0.102
1.850	3.650	1.737	3.670	0.113	-0.020
1.705	3.403	1.737	3.670	-0.032	-0.267
1.736	3.960	1.737	3.670	-0.001	0.290
1.569	3.710	1.737	3.670	-0.168	0.040
1.903	3.730	1.737	3.670	0.166	0.060

Table 5. Effect of optimization on the final concentration (µmol/L) of glutamic acid and GABA produced by *L. plantarum* MNZ.

Before optimization		After optimization		Percentage increased	
Glutamic acid	GABA	Glutamic acid	GABA	Glutamic acid	GABA
489	128	3353	3960	685	3093

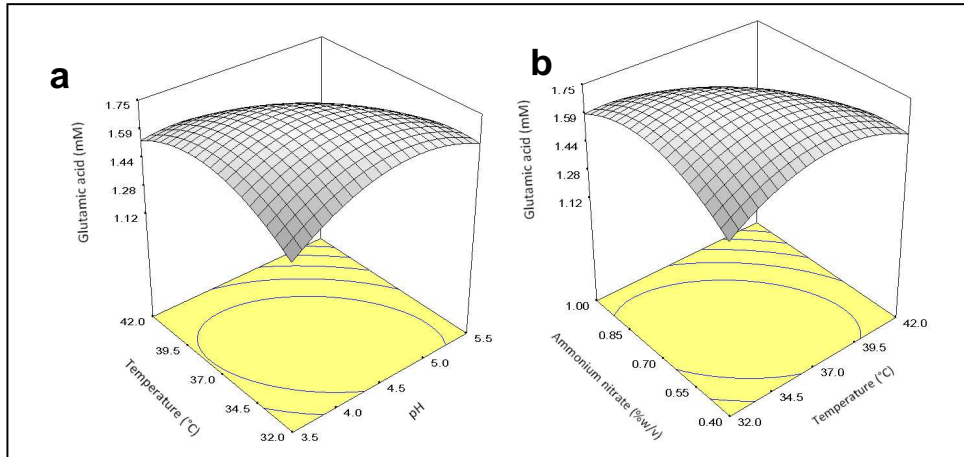Concentration of the compounds reported in µM.

Figures

Fig. 1 Three dimensional plot showing the effect of: (a) temperature, pH; (b) ammonium nitrate, temperature and their mutual effects on glutamic acid production by *L. plantarum* MNZ.
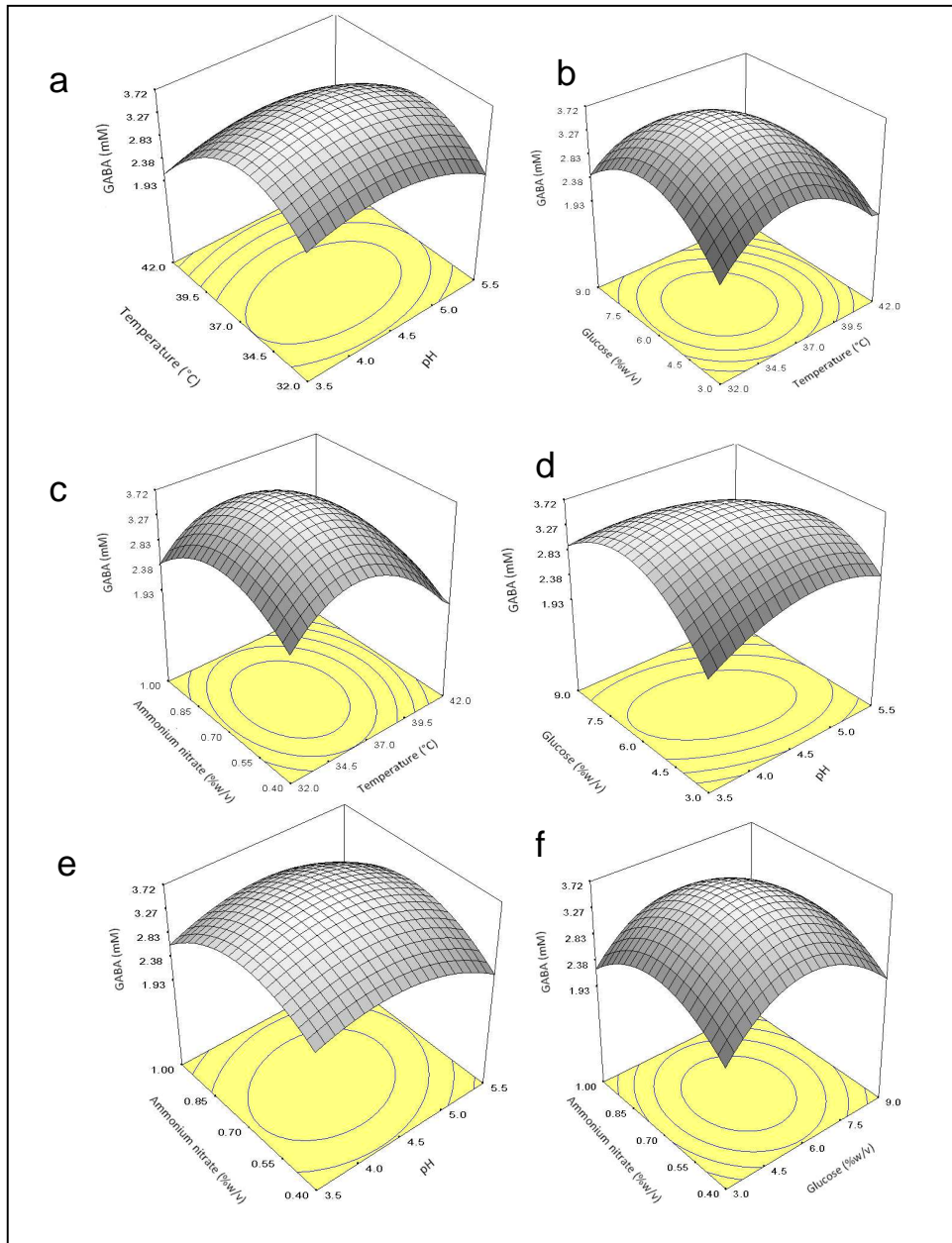
Fig. 2 Three dimensional plot showing the effect of: (a) temperature, pH; (b) glucose, pH; (c) ammonium nitrate, pH; (d) ammonium nitrate, temperature; (e) glucose, temperature; (f) ammonium nitrate, glucose and their mutual effects on GABA production.
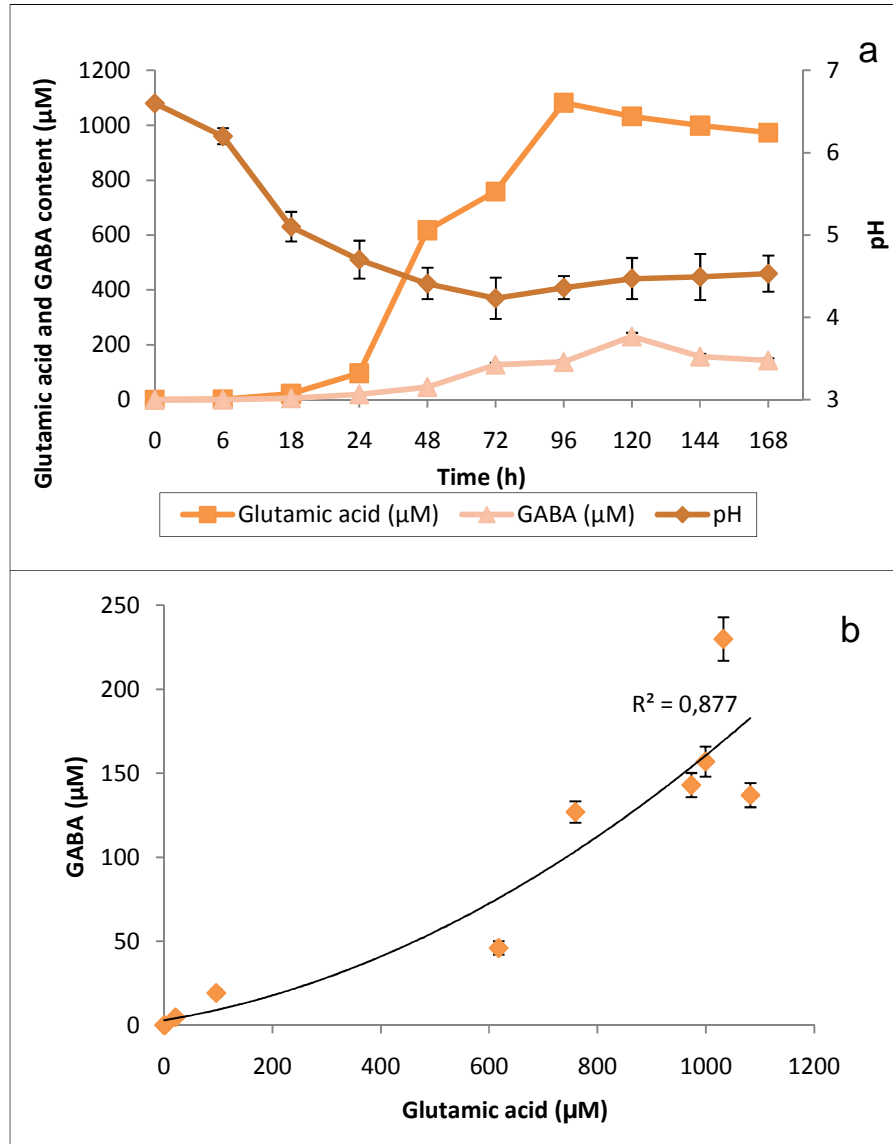
Fig. 3 Glutamic acid and GABA production by *L. plantarum* MNZ (a) Production profile of glutamic acid and GABA and the pH change during the fermentation process; (b) Correlation between glutamic acid and GABA production. Error bars indicate the mean ± standard deviation of three independent experiments.