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Start this statement with the following words:

This study discover the ---------- that can be beneficial for ……

And the last sentence of this statement could be such as:

This study will help the researcher to uncover the critical areas of ---------- that many researchers were not able to explore. Thus a new theory on ---------- may be arrived at.

A Model Significance Statement:

This study discovers the possible synergistic effect of vitamin E, calcium, and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination, and possibly other combinations, may be arrived at.

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Optimization of Glutamate Production From *Lactobacillus Plantarum* VM Origin Minangkabau Fermented Food As Feed Supplement On Broiler

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ABSTRACT

Background dan obyektif: Glutamate is a non-essential amino acid, as one of the basic tastes for improving umami, serves as a building block of protein and physiological functions of the body. Increased use of glutamate in animal feed causes glutamate to rise global. *Lactobacillus plantarum* VM is a lactic acid bacteria origin Minangkabau fermented foods producing glutamate. Increased of production glutamate from *Lactobacillus plantarum* VM can be done by improving nutrition and the environment. The aim of the study was to obtain the optimization for glutamate producing by *Lactobacillus plantarum* VM. **Materials and Methods:** the research was designed in the form of laboratory experiment descriptive and repeated 3 times. **Results: t**he optimum pH, incubation time, temperature, optimization of carbon and nitrogen source. The results of this study showed that optimum pH 5.5 (161.519 mg/L), 36 h incubation time, 36ºC (350.001 mg/L), 11% glucose (566,535 mg / L) and 0.5% peptone (680.525mg / L).). **Conclusion:** optimization of initial pH of the media, incubation time, temperature, source C and source C can increase glutamate production.

Keywords: Glutamate, Umami, building block, *Lactobacillus plantarum* VM, Minangkabau fermentation food

INTRODUCTION

The use of amino acids as a feed supplement in animal feed was many practiced. One of the amino acids that has been given to animal feed is glutamate. Glutamate is a non-essential amino acid, as one of the basic tastes to enhance umami and building as building blocks of protein[1,2]. Apart from its role as a flavor enhancer and building blocks of protein, glutamate acts as a neurotransmitter in the brain and has a number of physiological functions[3]. Glutamate as a dietary supplement has been widely given to broilers. The addition of glutamate in broiler ration can increase body weight, decrease mortality, crude protein ration and fossil ammonia[4,5,6]. In addition to improving performance, glutamate can also improve carcass quality. According to Berres *et al.*[7]; Fujimura *et al.*[9] giving glutamate can lower abdominal fat, reduce bruising in carcasses and increase the taste umami of meat.

Increased use of glutamate as feed supplement for livestock causes increased glutamate demand globally. According to Sono[9] total world glutamate production with fermentation is estimated 2 million tons/y. Glutamate is an amino acid that dominated demand and was valued over USD 8 billion in 2014[10]. So, it is necessary to innovate to increase glutamate production. Innovations that can be done to increase glutamate production with new organisms, increasing the nutrient content of the media and the growth environment[11,12]. According to Niaz *et al.*[13]; Zareian *at al.*[14] temperature, pH and incubation time affect glutamate production. Glu is excreted by many bacteria in response to different nutritional conditions, and then influenced by the

concentration of C and N in the medium. Nadeem *et al.*15 suggests that the optimization of C and N sources can increase glutamate fermentation using various sources of carbon and nitrogen in the production medium. One of the bacteria that can produce glutamate is *Lactobacillus plantarum* VM.

Lactobacillus plantarum VM is a lactic acid bacteria (LAB) isolated from Minangkabau fermented food. To increase the production of glutamate, it is necessary to optimize the production of *Lactobacillus platarum* VM. The purpose of this study was to obtain optimization of glutamate production by improving the nutrient content of medium and growth environment of *Lactobacillus platarum* VM.

MATERIALS AND METHODS

Microbes and Medium Content

In this study using *Lactobacillus platarum* VM bacteria isolated in Minangkabau fermented foods. *Lactobacillus platarum* VM is a collection of bacteria in previous studies. Lactobacillus platarum VM in culture on MRS broth medium.

Optimizing Glutamate Production

Glutamate production with mineral contained (g/ L): 1 g KH2PO4, 0.4 g MgSO4 _ 7H2O, 0.01 g FeSO4 _ 7H2O and 0.01 g MnSO4 _ 4-5H2O[16]. Optimization of **first** glutamate production, pH of medium. To decrease the pH using 2 M NaOH and raise 2 M HCl, pH values 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7). **Second**, the optimum temperature (30 ° C, 33 ºC, 36 ºC, 39 ºC, 42 ºC, and 45ºC). Third, incubation time (12, 24, 36, 48,60, 72, 96,108) h. **Fourth**, determination of carbon source and dose. Carbon sources are used glucose, sucrose, maltose and lactose. After the best

carbon source, and then determination of optimum concentration of carbon source (1%, 3%, 5%, 7%, 9%, 11%,13%, 15%). **The five** determinations of sources and concentration of nitrogen, nitrogen sources (peptone, yeast extract, skim milk, NH4NO3, and KNO2). After obtaining the best nitrogen source, then dilalukan determination of optimum dosage of nitrogen source. 0,1%, 0,3%, 0.5%, 0.7%, 0.9%, 1 % 1.1%, 1.3%, 1.5%.

Determination of Glutamate Content

Quantitative measurements of glutamate were performed using HPLC analysis by the method described by Yang *et al*.[17]. The mobile phase comprises a mixture of 60% solution A (aqueous solution of 10.254 g of sodium acetate, 0.5 ml tri-ethhylamine and 0.7 ml of acetic acid in 1000 ml, final pH of 5.8), 12% solution B (acetonitrile) and 28% solution C (aquabides). Gradient HPLC separation was performed on a Shimadzu (Kyoto, Japan) LC 20AT apparatus. A Prevail C18 column was used during the analysis. The mobile phase for the gradient elution was pumped at 0.6 mL/min flow rate and 27 °C temperature, and glutamic acid detection was performed at 254 nm.

Experimental design

The research was designed in the form of laboratory experiment descriptive and repeated 3 times.

RESULTS AND DISCUSSION

Optimum pH Medium

Optimizing the production of glutamate by growing *Lactobacillus plantarum* VM isolates at different pH (pH 2 to 7) can be seen in Figure 1.

The results show that at pH 2 to 7 isolates can still grow which is indicated by turbidity formed on the medium and glutamate produced. The optimum pH of growth of *Lactobacillus*

plantarum VM in this study was acid (pH 5). According to Zareian *et al*.[14] acid pH can trigger the gdh gene on LAB to produce higher glutamate. The pH of growth medium of *Lactobacillus plantarum* VM is different compared to other studies. As Zareian *et al*.[18] reported the optimum pH of LAB for glutamate production is pH 4.5. Furthermore, pH 6 is the optimum condition of *Lactobacillus plantarum* to produce glutamate[19].

Medium pH is an important factor of *Lactobacillus* growth in biological processes and glutamate production[20, 21]. Decreased initial pH of the media may inhibit the growth of *Lactobacillus*, thereby causing a redirection of 2-oxoglutarate efflux to glutamate production which increases glutamate excretion[19,22]. In addition to gene and growth factors, glutamate production increases due to *Lactobacillus plantarum* producing ammonia when the acidic environment, which contributes to the pH of homeostasis so as to survive microorganisms through neutralizing pH[23]. So that the ammonia produced can be used to form glutamate.

Incubation Time

The incubation time of *Lactobacillus plantarum* VM can be seen in Figure 2. The best incubation time was 36 hours with production 260,551 mg / L. The incubation time in this study differs from several other studies. Lawal *et al*.[24] reported the optimum incubation time of Bacullus spp is 96 h. Added Zaraien *et al*.[18] the best incubation time *Lactobacillus plantarum* 96 h with glutamate production 1.032 mmol. Furthermore, Ahmad *et al*.[25] 100 hours is the best incubation time in producing glutamate.

The optimum time difference of bacterial growth in producing glutamate is influenced by the growth of *Lactobacillus* cells. According to Nampoothiri *et al*.[26] the growth of *Lactobacillus* cells increases exponentially between 18 and 72 h of fermentation in MRS broth medium. This stage is recognized as a log phase or exponential phase. Exponential growth phase at a certain

time limit. This is because the nutrients of the medium will decrease, the nutrients used for bacterial growth will affect the production of glutamate produced[18]. In addition, differences in bacterial strains and media nutrition are another factor responsible for the difference in outcomes[14].

Optimum Temperature

To determine the optimum temperature of *Lactobacilus plantarum* VM in producing glutamate in incubation at temperature (30, 33, 36, 39, 42, 43) ^{o}C. The effect of temperature on glutamate production can be seen in Figure 3.

The results showed that the optimum temperature of *Lactobacilus plantarum* VM in producing glutamate was 36^{o}C (366.76 mg / L). There is a difference in the production of glutamate with temperature shifts can be seen in Figure 2. The optimum temperature obtained in this study is different from that reported by other studies. According to Ahmet *et al*.[26] 31^{o}C is the optimum temperature of *Corybacterium glutamicum* in producing glutamate. Furthermore, Zareian *et at*.[14]; Lawal *et al*.[24] states that the optimum temperature of *Lactobacillus plantarum* in producing glutamate is 37^{o}C and 32^{o}C is the optimum temperature of *Bacullus* Spp.

The occurrence of differences in glutamate production of each temperature change caused every microbial has the optimum temperature, maximum and minimum for its growth. If the environment temperature is less than the minimum temperature or greater than the maximum temperature of its growth then the enzyme activity will stop even at too high a temperature will occur enzyme denaturation[27]. According to Lehniger *et al.*[28] low temperatures decrease the fluidity of cell membranes by increasing the fraction of fatty acids containing shorter carbon chains and cis-double bonds. This change in lipid composition increases the fluidity of the lipid double layer in the cell membrane and will increase the transport of metabolites and nutrients to

the same level as normal growth temperatures. So with an increase in membrane fluidity will be achieved and increasing the temperature of cultivation will increase the production of glutamate[11]. The same is conveyed by Uy *et al*.[29] Increasing the temperature of cultivation can inhibit the complex dehydrogenase a-ketoglutarate. Thus it causes the transfer of 2-oxoglutarate flux to glutamate production and thus increases the excretion of glutamate[22].

Carbon Source Optimization

Determination of the best carbon source which comparing some carbon sources to produce glutamate. The carbon sources used in this study were glucose, lactose, sucrose and maltose. Once selected the highest carbon source to produce glutamate, then done the determination of the optimum concentration (1,3,5,7,9,11,13,15)%. The effect of carbon source and optimum concentration can be seen in Figure 4 and Figure 5.

The results showed the best carbon source for glutamate production was glucose 351.14 mg / L. However, it is not much different when compared with sucrose which produced 150 mg/L of glutamate. The difference in glutamate production results due to the ability of microbes to deregulate carbon sources. According to Nadeem *et al*.[16] each microbe has a different metabolism in the degredation of carbon sources which affects the formation of biomass (production of primary or secondary metabolites) produced. Research on the production of glutamate from carbon sources has been reported that glucose is the best source of carbon in producing glutamate [14.24]. high glutamate production because bacteria changed glucose through the Krebs cycle intermediates[30]. Glucose can be changed to pyruvic acid through glycolysis, TCA cycle and electron transport chain [31]. However, different results reported by Kiefer *et al*.[32] fructose and sucrose are the best carbon sources in producing glutamate. Thus, the growth and production activity of glutamate from microbes can be influenced with available carbon sources.

Glucose is the best source of carbon in producing glutamate and and then upgraded to determine optimal glucose concentration. The effect of glucose concentration on glutamate production can be seen in Figure 7. The results showed that glucose optimum concentration was 11% higher than other studies. The best glucose concentrations obtained in this study were lower than that of Zareian *et al*., [18] with a concentration of 12% carbon source. The high production of glutamate at 11% concentration is due to microbial growth itself. This was also stated by Nampoothiri *et al*.[26] the utilization of sugars in the medium by bacteria will continue in accordance with the growth pattern.

Optimization of Nitrogen Source

Determination of the best nitrogen source which comparing the sources of nitrogen (pepton, yeast extract, skim milk, NH4NO3 and KNO3). After obtaining the best nitrogen source then determined the optimum concentration of nitrogen. The nitrogen source and the optimum concentration can be seen in Figures 6 and 7.

The results showed that the best source of nitrogen in producing glutamate was peptone with production 415.23 mg / L. In this study, the best results obtained differ from those reported by Li *et al*.[33]; Nadeem *et al*.[15] ammonium sulfate is the best source of nitrogen to produce glutamate. According to Savijoki *et al.* [34] the determination of suitable nitrogen sources is essential for the needs of amino acids and microbial peptides in producing glutamate. The ability of microbes in synthesizing nitrogen sources has an effect on the production of glutamate in the expression[34]. Furthermore, each LAB strain has a different need for each nitrogen source, nitrogen is an essential growth factor or a stimulating factor[35,36].

After obtaining pepton as the best source of nitrogen, then determination of pepton optimum concentration can be seen in Figure 10. The results showed the best pepton

concentration was 0.9%. Nitrogen function an important role in the fermentation of glutamate-producing bacteria. Nitrogen is taken up to bacterial cells, then assimilated to achieve metabolism[37]. Utilization of nitrogen sources into bacterial cells through passive diffusion (ammonium) or active transport[38]. The ammonia concentration is low so that the diffusion into the cells becomes limited, the ammonium transporter (AmtB) activates to overcome the nitrogen deficiency, and the nitrogen is assimilated with glutamine syntetase[39]. Conversely, if high nitrogen concentrations, nitrogen diffusion (NH3) occur through cytoplasmic membranes. This will encourage the growth of bacterial cells and than nitrogen is assimilated with glutamate dehydrogenase to form glutamate. Thereby also De Angelis et al.40 shows that most of the nitrogen is assimilated by glutamate dehydrogenase to form glutamate that has been shown to exhibit high activity in *Lactobacillus plantarum*.

CONCLUSION

The results show an increase in glutamate production by the optimized *Lactobacillus plantarum* VM. Optimization, adjustment of nutrients and environmental conditions affect glutamate fermentation resulting in increased production of glutamate. The effect of medium pH, incubation time, temperature, source C and source N on glutamate production.

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Figure

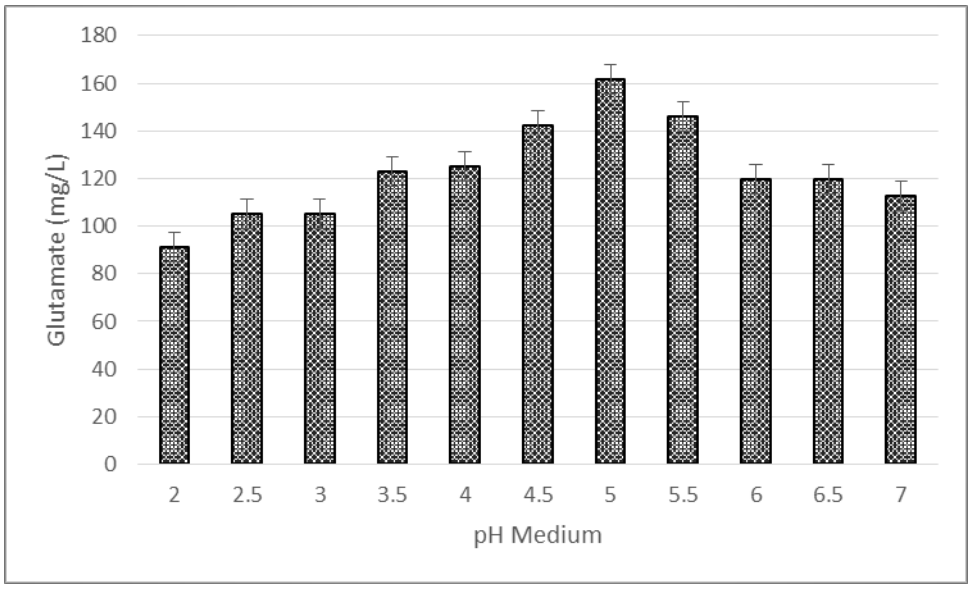

Figure 1. Effect of pH of medium on glutamate production

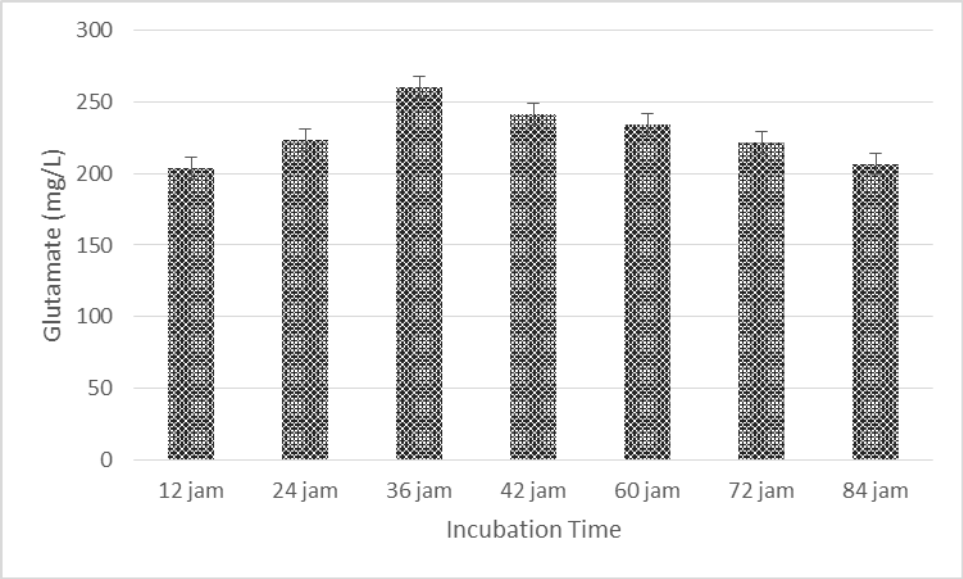

Figure 2. Effect of incubation time on glutamate production

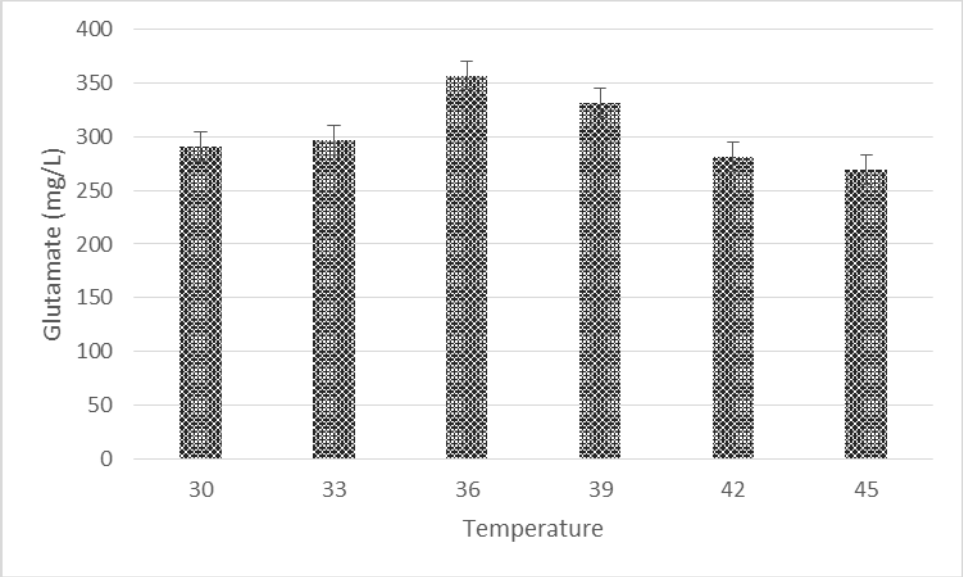

Figure 3. Effect of temperature on glutamate production

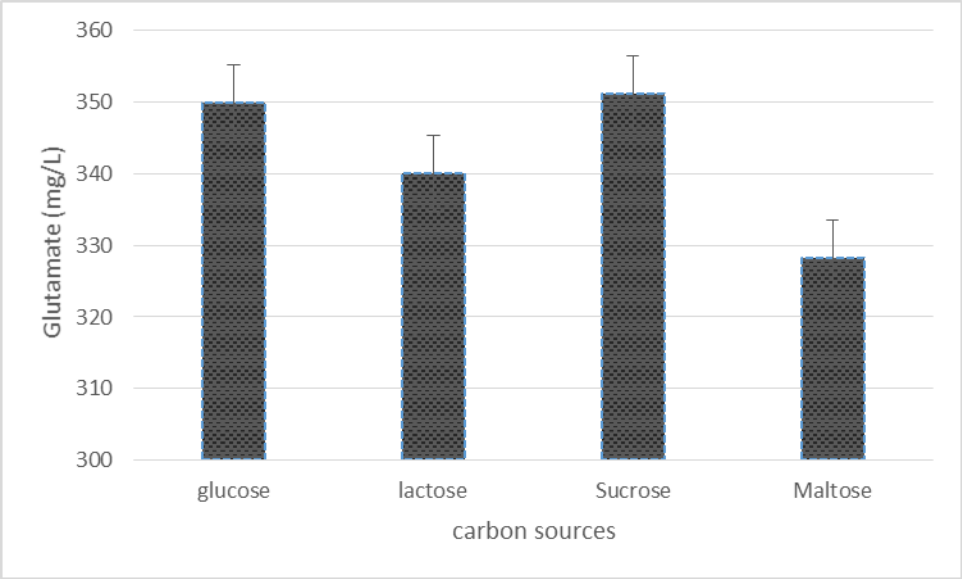

Figure 4. Effect of carbon source on glutamate production

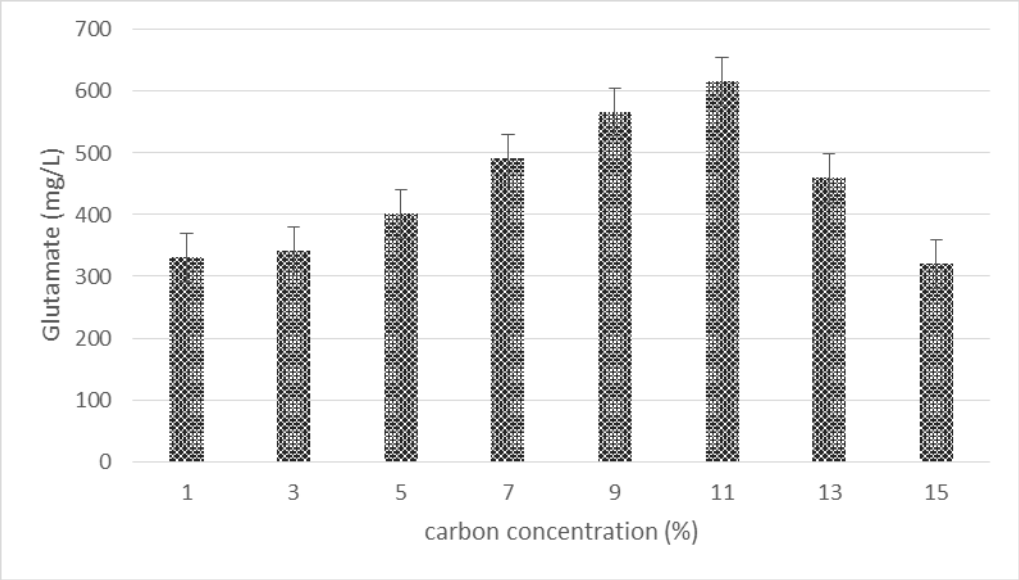

Figure 5. Effect of carbon concentration on glutamate production

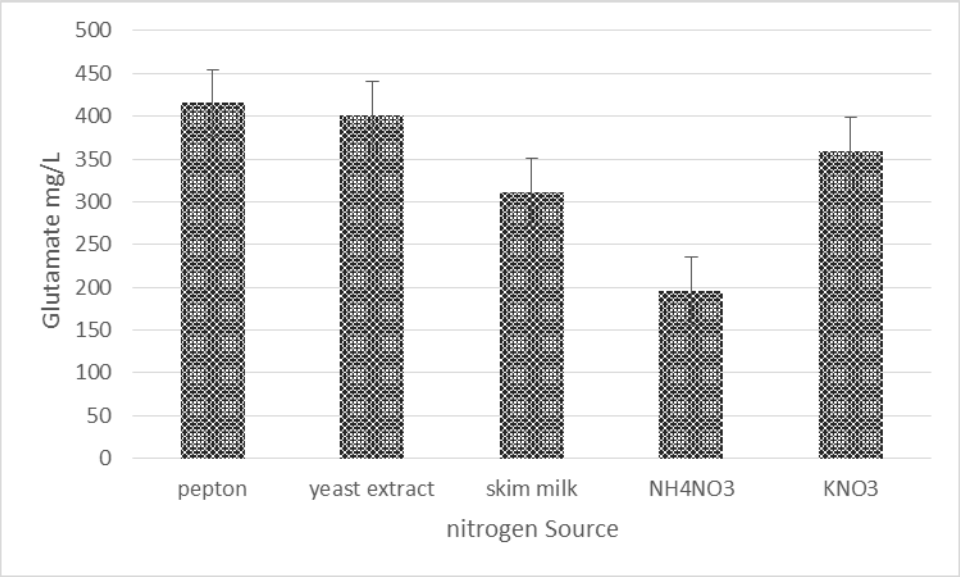

Figure 6. Effect of nitrogen source on glutamate production

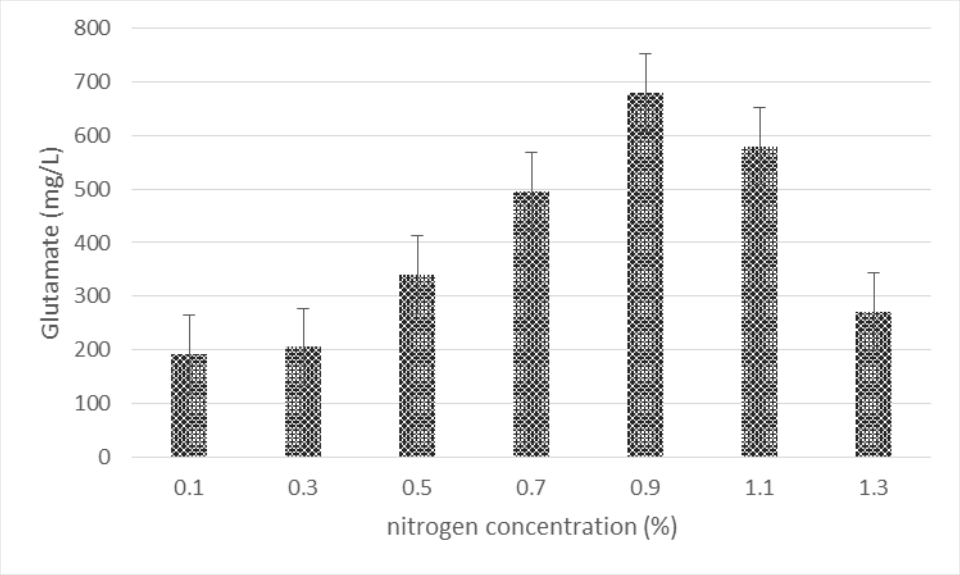

Figure 7. Effect of nitrogen concentration on glutamate production