# POTENCY OF AMINO ACIDS AS SAVORY FRACTION FROM *VEGETABLE BROTH* OF MUNG BEANS (*Phaseolus radiatus* L.) THROUGH BRINE FERMENTATION BY *Rhizopus*-$C_1$

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## ABSTRACT

*Amino acids produced through brine fermentation of mung beans (Phaseolus radiatus sp) by inoculum of Rhizopus-$C_1$ at room temperature for 0, 2, 4, 6, 8, 10, and 12 weeks, respectively had a potential use as savory fraction for seasoning agent. The objective of this experiment was to find out characteristic of produced amino acids and composition of fermentation products relating with proteolitic and amylolitic activities of Rhizopus-$C_1$. The result of experiment showed that the length of fermentation time would increase intensity of savory taste and cloudy color, and increase total protein, soluble protein, and N-amino concentrations, decrease water, while fat concentration was constant. Fermentation of 10 weeks was optimal time to get crude broth with concentrations of total protein of 9.5622%, soluble protein of 8.5 mg/g, N-amino of 5.6 mg/g, fat of 0.2802%, water of 40.7189%, Volatile Reduction Substances (VRS) of 90 µeq/g, and reduction sugar of 672.5 mg/mL. Kinds of dominant non-essential amino acids produced were glutamic acid (1.014%), and aspartic acid (0.507 %), while essential amino acids were lysine (0.474%), and isoleucine (0.644%). The other of amino acids were resulted with concentration of 0.211 – 0.345%, such as leucine, arginine, serine, glycine, histidine, alanine, proline, tyrosine, valine, methionine, cystine, threonine, and phenilalanine. Visually, crude vegetable broth produced through brine fermentation of mung beans by Rhizopus sp-$C_1$ was semi solid, brownish color, rather fatty, salty, and enough strong savory taste.*

***Keywords***: *Amino acids, brine fermentation, mung beans broth, Rhizopus-$C_1$, savory fraction*

## INTRODUCTION

Brine fermentation on mung beans (*Phaseolus radiatus* L.) is a development process in fermentation food industry as *vegetable broth* for seasoning agent**.** The choice of mung bean is based on almost indifferent concentration of protein (24%) to another local legume, such as red bean (29.1%)**.** Use of *Rhizopus* sp. inoculum in brine fermentation is an alternative effort to get savory flavor besides *Aspergillus* sp. inoculum which is mainly used in brine fermentation-based in preparation of foods, such as ketchup, tauco [1]. *Rhizopus* sp. usually is used as inoculum in production of tempeh. Enzymes produced by inoculum for fermentation process (protease, amylase, lipase) will convert and degrade legumes to simpler compounds affecting on characteristic of savory flavor. Proteolytic activity of inoculum and quality of substrate protein has the important role in recovering amino acids as precursor savory flavor [2].

Amino acids are precursor of savory flavor, non-volatile compounds, are reached through synthesize and natural fermentation methods. Amino acids resulted through brine fermentation of legumes had been well-known as precursor of savory flavor, such as ketchup, tauco (Indonesia), Miso and Katsuobushi (Japan), Bagoong and Tao-si (Phillipine), Meju (Korea), and Prahoc (Cambodia) [3]. These amino acids were generally produced by using mixed inoculums of *Aspergillus oryzeae*, *Aspergillus soyae* and less *Rhizopus* sp. as microorganism in resulting enzyme which will convert components in legumes to volatile and non-volatile compounds in forming savory (umami) taste. Utilization of *Rhizopus*-$C_1$ in brine fermentation is caused by a potential exploration as inoculum in preparation of tempeh supported by non-essential amino acids, particularly glutamic acid (7.353 mg/100 gram dry weight) of total non-essential amino acids (44.221 mg/100 gram dry weight of tempeh [4].

The objective of this experiment was to find out the potential use of amino acid and the product composition through brine fermentation at room temperature for 0, 2, 4, 6, 8, 10, and 12 weeks relating with proteolytic and amilolytic activities of *Rhizopus*-$C_1$ using mung beans substrates supporting its important role as savory flavor.

## EXPERIMENTAL SECTION

### Material

Materials used in this experiment were mung beans purchased locally, starter *Rhizopus*-$C_1$ (Research Centre for Chemistry, Indonesian Institute of Sciences), reagent for proximate and dissolved protein [6], and amino acids analyses [7].

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**Instruments**

Equipments utilized in this work were autoclave, incubator, brine fermentation system in laboratory scale, Spectrophotometer UV-1201 and HPLC (Waters 2487, USA).

**Procedure**

***Experimental design and Analyses***

This experiment was employed by using inoculum *Rhizopus*-$C_1$ starter at room temperature for 0, 2, 4, 6, 8, 10, and 12 weeks of fermentation in laboratory scale (100 - 150 g). Analyses were carried out on proteolytic (Walter) and amilolytic (Somogy-Nelson) [5] activities of inoculum of *Rhizopus*-$C_1$. Analyses of composition were employed on raw material of mung beans and product of *vegetable broth* covering water content (Gravimetric), total protein (Kjeldahl), dissolved protein (Lowry), fat (Sohxlet), reducing sugar (Somogyi-Nelson) [6], and N-amino (Cu method) [7]. At the best time of fermentation was reached by analysing and identification on amino acids (HPLC instrument) [8].

***Identification of Amino acids***

Analysis was carried out by weighing 0.1 g crude *broth* of fermented mung beans, diluting to 5 - 10 mL of HCl 6 N and heating at 100 ºC for 24 h. After filtration, 50 mL of sample was added with 50 mL Methanol and 50 mL of derivatitation solvent (Triethylamine), allowed for 20 min, added with acetonitrile of 0.5 - 1 mL, and injected to HPLC (Waters 2487, USA) using Picotas column of amino acid with wavelength of 254 nm at flow rate of 1 mL/min and injection volume of 20 µL. Concentration of amino acids in sample calculated from chromatogram, was expressed in µmol of amino acids (µmol AA), and was determined as percentage of amino acids (weight/dry weight of protein). Chromatogram, prereport from standard concentration and sample concentration of amino acids was presented in Figure 9, 10 and 11. Calculation is expressed in a formula, as follow :

$$\%\ AA = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{Standard Concentration} \times \frac{\text{MW} \times \text{Dilution} \times 100}{\text{Sample weight}/100}$$

***Process Steps***

**Preparation of inoculum of mung beans *broth*.** Starter of *Rhizopus*-$C_1$ was added to rice substrates which had been rinsed overnight, autoclaved at 120 °C for 15 min, cooled and incubated at 35 °C for 72 h with concentration of 0.2% (w/w). This mixture was then dried at 50 °C for ± 24 h in cabinet dryer, powdered by grinder, screened through 80 mesh sieve and ready to be used as inoculum in the process of brine fermentation.

**Preparation of *vegetable broth* of mung beans.** A number of mung beans was washed, rinsed overnight, dehulled, autoclaved at 121 °C for 20 min, and cooled. Sterilized mung bean was then added to salt in ratio 51% and 23%. *Broth* inoculum of *Rhizopus*-$C_1$ of 26% was aseptically added to the mixture and fermented at room temperature up to 12 weeks in closed jar in which each week was investigated and analyzed. Agitating and transferring of jar was aseptically carried out every week.

**RESULT AND DISCUSSION**

**Characteristic of *broth* inoculum from starter of *Rhizopus*-$C_1$**

*Rhizopus* sp. which had been utilized as inoculum in preparation of tempeh has savory taste due to their amino acids and peptides concentrations. In this brine fermentation, starter of *Rhizopus*-$C_1$ which is *Rhizopus oligosporus* is isolated from *laru* is a fermenting agent added to make tempeh [9]. It is source of enzymes (protease, amylase and lipase) generating components formation of savory flavor. Starter inoculum of *Rhizopus*-$C_1$ posses a optimal growth for 56 h of incubation sign by growing dense micellia. Figure 1 (a) and 1 (b) represented the growth of *Rhizopus*-$C_1$ during incubation in rice substrates at optimal time of 56 h (a), and dried inoculum after drying at 50 ºC for 24 h (b). The growth of *Rhizopus*-$C_1$ during 56 h of incubation results dense does not realize initial sporulation. Enough high activities of proteolytic (1.14 Unit/g) and amylolytic (6 Unit/mg) enable to be reached a product with composition and specific volatile components. These high activity of enzymes are caused by incubation time, temperature, humidity, purity of starter, and nutrition. Inoculum composition with concentrations of carbohydrates (71.5%) and total protein (17.19%) are source of nutrition and enzymes of inoculum. Concentrations of dissolved protein (6.95 mg/mL) and N-amino (2.61mg/g) in inoculum give a sufficient high contribution on formulation of *vegetable broth*. In this brine fermentation, inoculum concentration affects directly on the end composition of product because inoculum (26%) is directly used as formulator in final product, while in formulation of ketchup is added palm suiker and spices indicated as condiment [10].

**Composition of mung beans**

Mung beans is sources of carbohydrate, fat and protein of this main product. High concentration of protein (25.3%, dry weight) enables to be yielded amino acids and high dissolved peptides due to protease activity of *Rhizopus*-$C_1$, whereas carbohydrate (62.12%) which is source of starch will be hydrolyzed by α-amylase enzyme into monosaccharide through Maillard reaction which will naturally produce specific taste. The presence of fat (0.47%) will be

(a) (b)

**Figure 1.** The growth of *Rhizopus*-$C_1$ during incubation at rice substrates for optimal time of 56 h (a) and dried inoculum as result of a drying at 50 ºC for 24 h (b)

Composition :
Water : 9.69 % Ash : 2.42 %
Fat : 0.47 % Carbohydrate : 62.12 %
Protein : 25.30 % (dry weight)

**Figure 2.** Mung beans commodity and their compositions as raw material in brine fermentation to prepare *vegetable broth* as savory flavor

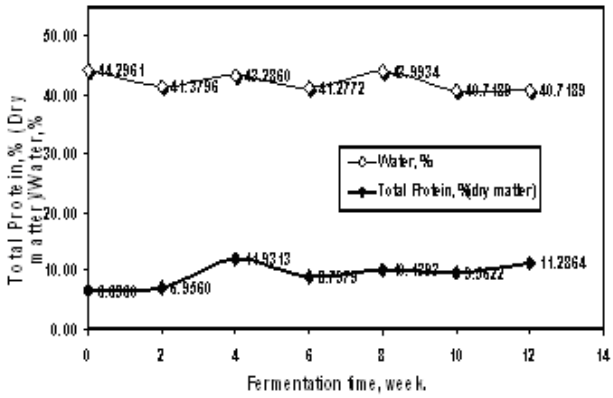


**Figure 3.** Relationship between fermentation time and water content and total protein of *vegetable broth* from mung beans using inoculum *Rhizopus*-$C_1$ at room temperature in laboratory scale


(a) (b) (c)

**Figure 4.** Crude *broth* produced via brine fermentation of mung beans using inoculum *Rhizopus*-$C_1$ at room temperature for 12 weeks (a), substrates as semi solid mass, brownish and rather wet (b), and paste *broth* (c)

converting by lipase enzyme into fatty acids and glycerol contributing specific broth flavor. In this preparation of *vegetable broth*, mung beans used was 51% of all materials and contributed high nutrition on fermentation product. Figure 2 showed mung beans and their composition.

**The effect of brine fermentation on *broth* composition of mung beans**

Brine fermentation generated water content which tends to decrease, but total protein tends to rise, displayed in Figure 3.

During brine fermentation, demand of water to grow and metabolite of inoculum is enough large so it will drop water content of product. Inoculum of *Rhizopus*-$C_1$ will be active at 28 – 35 °C [11]. This range of temperature will be reached because substrates in closed jar can occur an increase of inoculum activity and a demand of water to grow and produce enzymes. In this decrease of water content, flavor intensity will become more and more high due to product thickness.

Figure 4 (a), (b), and (c) indicated crude *broth* produced via brine fermentation of mung beans using inoculum *Rhizopus*-$C_1$ at room temperature for 12

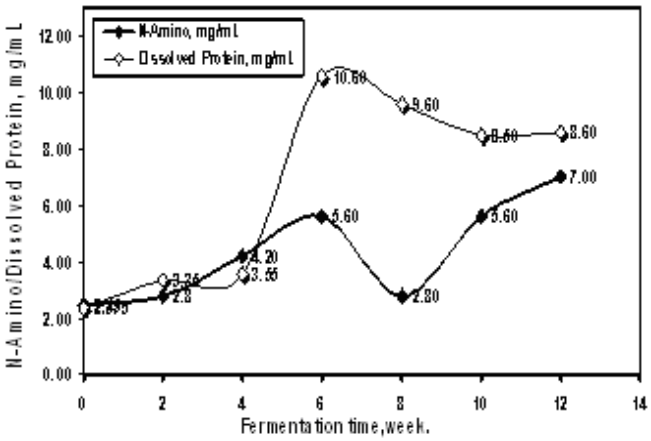


**Figure 5.** Relationship between fermentation time and dissolved protein and N-amino of *vegetable broth* of mung beans using inoculum *Rhizopus* $C_1$ at room temperature in laboratory scale

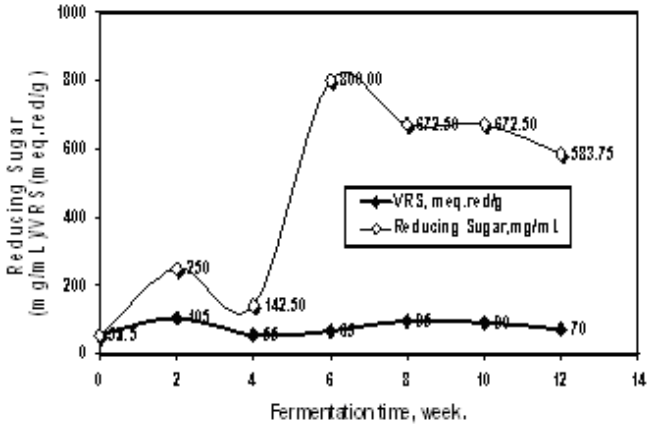


**Figure 6.** Relationship between fermentation time and reducing sugar and VRS of *vegetable broth* of mung beans using inoculum *Rhizopus*-$C_1$ at room temperature in laboratory scale

weeks, substrates as semi solid mass, brownish and rather wet, and paste *broth,* respectively. On increase of total protein concentration, significant raise seemed up to 4 weeks of fermentation, and then tend to 12 weeks of fermentation. This increase is depend on initial concentration of total protein before fermentation, so during fermentation, *Rhizopus*-$C_1$ realize possibility the growth and increase of enzyme production. Increase the inoculum and enzyme give a contribution in raising total protein because inoculum is also a potential source of protein**.** At 4 weeks of fermentation, total protein becomes constant indicating no more difference of total protein in substrates because it occurred equilibrium reaction of fermentation between enzymes production and deamination process into amino acids, peptides, and flavor components.

Fermentation process increase also dissolved protein and N-amino relating with the length time of fermentation, showed in Figure 5.

Sharp increase of dissolved protein seemed at 6 weeks of fermentation (10.69 mg/mL) and then drop to 12 weeks of fermentation (8.6 mg/mL). The same trend was shown in amino acids concentration as N-amino, in which a linear increase seemed to 6 weeks of fermentation (5.6 mg/mL), fluctuated, and became more and more high to the final fermentation (7 mg/mL). Raise of dissolved protein takes place to 6 weeks of fermentation followed by a decline at 12 weeks of fermentation.

This condition was possibility causedby the optimal proteolytic activity of *Rhizopus*-$C_1$ at 6 weeks of fermentation so protein and protease enzymes stock in substrates became more and more low decresing dissolved protein. Increase of N-amino is still continuing to 12 weeks of fermentation, which is an indication of their formations of taste and specific aroma by contributing amino acids, such as glutamic acid. *Rhizopus*-$C_1$ produced acid, alkaline, and neutral proteases with metabolic activity at the optimal temperature of 30 – 37 °C, and the different range of substrates pH. Protease enzyme will hydrolyze chain of protein peptides into amino acids and simpler peptides so it might change orientation of all protein molecules, namely side chain of hydrophobic, non-polar is arranged to inner surface, and side chain of polar hydrophylic is outer section to increase their solubility in water as polar solvent [12].

The presence of salt in this fermentation can increase production and protease activity by increasing free amino acids concentration [13]. Inoculum of *Rhizopus*-$C_1$ is a single isolate of *Rhizopus oligosporus* [9] having high activity of proteolytic in preparation of tempeh and results tempeh's aroma which is not strong with longer life time [14]. Its application on brine fermentation enables to be produced specific flavor due to lower interaction among species so flavor resulted is not uniform. In other words, flavor resulted is affected by reactions of other microbes, as well.

The length time of fermentation will increase reducing sugar concentration, while volatile components formed tend to be constant, shown in Figure 6.

Sharp increase of reducing sugar takes place at 6 weeks of fermentation (809 mg/mL), and then drop to the final fermentation (583.75 mg/mL). This matter is relating with amilolytic activity of inoculum in hydrolyzing glycoside binding from a carbohydrate chain at inoculum of rice substrates (26%) and mung beans (51%) into monosaccharide having reducing sugar group, such as glucose. In fermentation range of 4 to 6 weeks, amilase activity is possibility very reactive, and then decrease because all their activities used have drop reducing sugar.

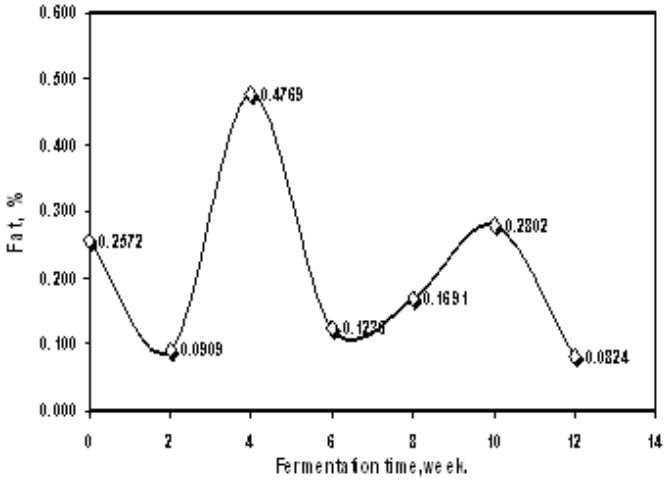


**Figure 7.** Relationship between fermentation time and fat of *vegetable broth* of mung beans using inoculum *Rhizopus*-$C_1$ at room temperature in laboratory scale

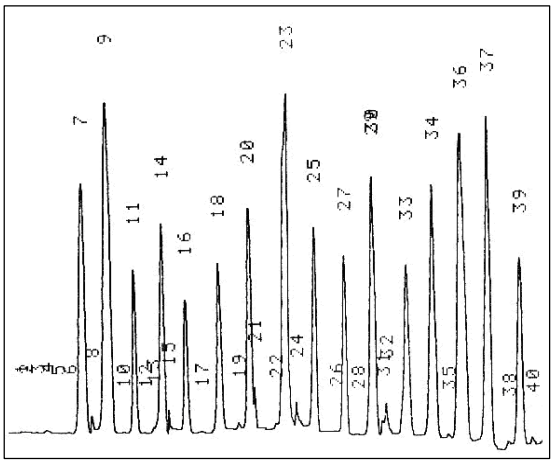

**Figure 8.** Relationship between time of analyses amino acids and member of peak Chromatogram of amino acids using HPLC from *vegetable broth (crude)* of mung beans (crude) using inoculum of *Rhizopus*-$C_1$

Besides, it is enabled to convert glucose to energy through glycolysis reaction for growing inoculum or it takes place a conversion of glucose into alcohol and organic acids by enzyme activity of inoculum to result flavor. A number of monosaccharide react also with amino acids through Maillard reaction in order to form melanoidin pigment and a number of intermediate components (furan, pyrazine, etc.) contributing on taste and specific aroma [15], displayed in Figure 4 (b). During fermentation, enzymes (protease, amilase, and lipase) hydrolyze carbohydrate, protein, and fat contained in substrates to form volatile components accumulating to yield specific flavor. Volatile Reduction Substances (VRS) is a volatile compound which can be oxydized, such as alcohol, aldehyde, ester, hydrocarbon and other organic compounds. The prolong time of fermentation can cause an increase or decrease of VRS concentration. The highest concentration of VRS takes place at 2 weeks of fermentation, namely 105 µeq/g.

On total fat concentration, longer time of fermentation tends to produce *broth* with fluctuation concentration of fat, and decrease to 12 weeks of fermentation, displayed in Figure 7. Fat content of this fermentation product is relating with fat of mung beans (0.47%) and *broth* inoculum (1.8%) in which for 0 - 12 weeks of fermentation, fat content changes to 0.2572 – 0.0824%. Change in fat concentration is affected not only by lipolytic activity, but also by water content in substrates, in which more and more high water content will increase lipase activity. It had been indicated that lipase enzyme has 5 folds of activity at water content of 15% than water content of 8.8% [16] so water content in substrates ranging of 40.7189 – 44.2961% will cause sufficient high activity of lipolytic to convert fat content of product to fatty acids and glycerol. *Rhizopus* sp. has high activity of lipolytic indicated in production of tempeh in which hydrolysis of lipase in degrading fat to unsaturated fatty acids can reach 20 – 22% [17] and has a potency in forming flavor**.**

**The effect of brine fermentation on recovery of amino acids**

Analyses of amino acids are carried out at 10 weeks of fermentation according to sensory aspect. From kinds of dominant amino acids, essential amino acid (histidine, arginine, isoleucine, leucine, lysine, methionine, phenilalanine, threonine and valine), particularly isoleucine (0.644%), leucine (0.437%) and lysine (0.474%) are kinds of amino acids with the highest concentration. Figure 8 represented a chromatogram profile of amino acids in crude *broth* produced through brine fermentation for 10 weeks with a chromatogram sequential consisting of aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cystine, isoleucine, leucine, phenilalanine and lysine as shown in peak no. 7, 9, 11, 14, 16, 18, 20, 23, 25, 27, 29, 33, 34, 36, 37 and 39. Essential amino acids (histidine, arginine, isoleucine, leucine, lysine, methionine, phenilalanine, threonine and valine) are obtained in range of 0.252 – 0.,644% (protein, dry weight), while non-essential amino acids (glutamic acid, glycine, proline, tyrosine, cystine, aspartic acid, alanine, and serine) range 0.211 – 1.014% (protein, dry weight) in concentration dominated by glutamic acid (1.014%), aspartic acid (0.507%) and cystein (0.4%) (see Table 1). These concentrations of acids are affected by initial material and result of degrading of

**Table 1.** The composition and concentration of free amino acids in fermented mung bean *broth* by *Rhizopus*-C1 on fermentation on 10 week.

| Peak number | Kinds of amino acids | Concentration (%, w/v dry protein) | Peak number | Kinds of amino acids | Concentration (%, w/v dry protein) |
|---|---|---|---|---|---|
| | Non-Essential | | | Essential | |
| 9 | Glutamic acid | 1.014 | 16 | Histidine | 0.252 |
| 11 | Serine | 0.212 | 18 | Arginine | 0.307 |
| 14 | Glycine | 0.244 | 34 | Isoleucine | 0.644 |
| 23 | Alanine | 0.345 | 36 | Leucine | 0.437 |
| 25 | Proline | 0.211 | 39 | Lysine | 0.474 |
| 27 | Tyrosine | 0.312 | 30 | Methionine | 0.300 |
| 33 | Cystine | 0.400 | 37 | Phenilalanine | 0.332 |
| 7 | Aspartic acid | 0.507 | 20 | Threonine | 0.314 |
| | | | 29 | Valine | 0.242 |

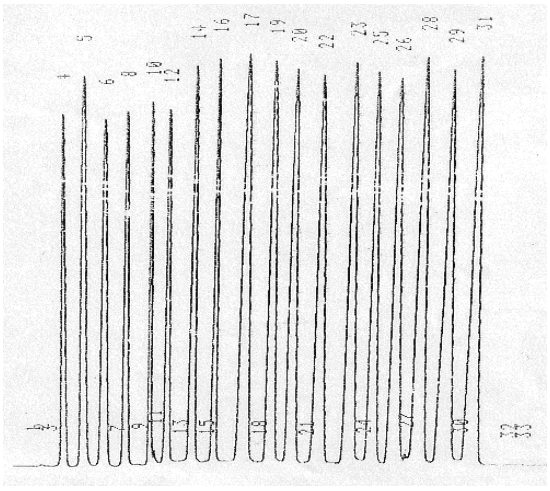

**Figure 9.** Chromatogram from standard concentration of amino acids

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METHOD: TAG: 12 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

| NO. | RT | AREA | CONC | BC |
|---|---|---|---|---|
| 4 | 1.82 | 387347 | 3.755 | VB |
| V5 | 2.79 | 511086 | 4.954 | BV |
| 6 | 3.76 | 439480 | 4.260 | VB |
| 8 | 4.77 | 399240 | 3.870 | BV |
| 10 | 5.91 | 391777 | 3.798 | VV |
| 12 | 6.85 | 440136 | 4.267 | VV |
| 14 | 8.10 | 593938 | 5.757 | VB |
| 16 | 9.30 | 538434 | 5.219 | VB |
| 17 | 10.71 | 719638 | 6.976 | BB |
| 19 | 12.00 | 685463 | 6.645 | VV |
| 20 | 13.14 | 592109 | 5.740 | VB |
| 22 | 14.35 | 669977 | 6.495 | VB |
| 23 | 15.72 | 658117 | 6.380 | BV |
| 25 | 16.79 | 591298 | 5.732 | VV |
| 26 | 17.88 | 652641 | 6.327 | VV |
| 28 | 19.06 | 706813 | 6.852 | VV |
| 29 | 20.23 | 681110 | 6.603 | VV |
| 31 | 21.43 | 657310 | 6.372 | VB |
| TOTAL | | | 100.000 | |

PEAK REJ : 5000

**Figure 10.** Prereport of standard concentration of amino acids-HPLC

proteolytic enzyme by *Rhizopus*-$C_1$. Glutamic acid obtained with the highest concentration (1.014%, w/w dry protein) is total glutamic acid covering free glutamic acid and peptides. Glutamic acid concentration is generated higher than other amino acids. This matter is caused by the presence of 2 kinds of L-glutamic dehydrogenase enzyme in inoculum having specific

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METHOD: TAG: 11 CH: 1

FILE: 0 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

| NO. | RT | AREA | CONC | BC |
|---|---|---|---|---|
| 7 | 2.82 | 155932 | 6.509 | BV |
| 9 | 3.82 | 242396 | 10.118 | VV |
| 11 | 4.87 | 64753 | 2.703 | VV |
| 14 | 6.01 | 102599 | 4.283 | VB |
| 16 | 6.92 | 57415 | 2.397 | VB |
| 18 | 8.20 | 83949 | 3.504 | BV |
| 20 | 9.41 | 113815 | 4.751 | VV |
| 23 | 11.00 | 223892 | 9.345 | VV |
| 25 | 12.10 | 100778 | 4.207 | VB |
| 27 | 13.31 | 81837 | 3.416 | VB |
| 29 | 14.44 | 79084 | 3.301 | VV |
| 30 | 14.48 | 98295 | 4.103 | VV |
| 33 | 15.86 | 106100 | 4.429 | VV |
| 34 | 16.88 | 157620 | 6.579 | VV |
| 36 | 18.02 | 256875 | 10.722 | VV |
| 37 | 19.08 | 189622 | 7.915 | VB |
| 39 | 20.35 | 109824 | 4.584 | VV |
| 41 | 21.55 | 170940 | 7.135 | VV |
| TOTAL | | 2395726 | 100.000 | |

PEAK REJ : 20000

**Figure 11.** Prereport of amino acids concentration (sample) from *vegetable broth (crude)* of mung beans (crude) using inoculum of *Rhizopus*-$C_1$-HPLC

for $NAD^+$ and $NADP^+$, while bacteria contain only dehydrogenase enzyme which need $NAD^+$ and can synthesize glutamic through different reaction [18]. In its function as savory flavor, amino acids acting as basic precursor are glutamic acid, valine, glycine, cysteine, and cystine. From recovery of amino acids with various concentrations can be predicted taste intensity supporting its role as savory flavor. The dominant concentrations of amino acids are glutamic acid (1.014 %), lysine (0.474 %), leucine (0.437%), aspartic acid (0.507%) and isoleucine (0.644%). This matter showed that this fermentation product has mixture intensity of savory (umami) taste, sour, salty, and sweet interacting among amino acid, sucrose, anorganic salts, and certain peptides. The existence of savory taste is caused by the presence of acid-based-*amino acid*, namely glutamic acid (1.014%) and aspartic acid (0.507%), and the presence of another hydrophobic amino acids, such as phenylalanine (0.332%), tyrosine (0.312%), leucine (0.437%), isoleucine (0.644%) and valine (0.242%). This sour taste is possibility caused by the presence of glutamic acid (1.014%) and aspartic acid (0.507%), histidine (0.252%), and dipeptides, namely peptide consisting of

two residues, acid amino acid, neutral, and alkaline, and aromatic amino acid. Bitter taste is forecasted by its high concentration of arginine (0.307%), lysine (0.474%) and proline (0.211%) or by the existence of neutral amino acid, and has large alkyl groups, amino acid with large and small alkyl groups or aromatic amino acid. This bitter taste is covered by the presence of savory taste from high free glutamic acid. Whereas, the presence of NaCl from brine fermentation and and anorganic salts, glutamic acid (1.014%) and aspartic acid (0.507%) generated salty taste. Sweet taste is caused by the existence of glycine, serine, and alanine hydroxiproline, praline, alanine, lysine, valine, alanine, threonine, serine, glycine and glutamic acid [19], and glucose, fructose, ribose generated from the initial material as a result of activity of amylase enzyme in degrading carbohydrate to monosaccharides.

## CONCLUSION

*Rhizopus*-$C_1$ contained in rice substrates had the potential application as inoculum to produce *vegetable broth* from mung beans as savory flavor for seasoning agent. Proteolytic (1.14 Unit/g) and amilolytic (6 Unit/mg) activities increased concentrations of total protein, dissolved protein, N-amino, reducing sugar, and VRS, but decreased water and fat contents relating with the prolong time of fermentation. Based on organoleptic aspect, 10 weeks of fermentation was the best time for recovering crude *vegetable* with concentrations of total protein of 9.5622%, dissolved protein of 8.5 mg/g, N-amino of 5.6 mg/g, fat of 0.2802%, water of 40.7189%, VRS of 90 µ/g reduction, and reducing sugar of 672.5 mg/mL. Kinds of non-essential dominant amino acids generated for 10 weeks of fermentation were glutamic acid of (1.014%) and aspartic acid of (0.507%), while essential amino acids were isoleucine (0.644%), leucine (0.437%) and Lysine (0.474%). Glutamic acid was amino acid contributing the highest concentration (1.014%) to give intensity of savory taste.

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