THE EFFECT OF PROCESS CONDITIONS IN PREPARATION OF *VEGETABLE BROTH* AS SAVORY FLAVOR FROM MUNG BEANS (*Phaseolus radiatus* L.) USING INOCULUM OF *Rhizopus*-C_1

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ABSTRACT

Preparation of vegetable broth from mung beans (Phaseolus radiatus L.) in semi pilot scale is an attempt development to get product of savory flavor in larger scale. The aim of this activity was to find out the effect of process multiplication on composition of vegetable broth from mung beans using inoculum of Rhizopus-C_1 through brine fermentation in mixtures of inoculum, salt, and mung beans of 26, 23, and 51 %. This activity was conducted in both temperature of fermentation (room temperature and 30 °C), various time of fermentation (0, 2, 4, 6, 8, 10, and 12 weeks) and process scales, namely laboratory scale (300 g) and semi pilot scale (± 25 kg), respectively. The result of experiment indicated that fermentation temperature and time and process scales were tend to affect on composition of product. The length of fermentation time would increase concentrations of dissolved protein, N-amino and reducing sugar, decreased concentrations of fat and Volatile Reduction Substance (VRS), while concentrations of total protein, and water were tend to be constant in laboratory and semi pilot scales at the both process temperatures. Multiplication in preparation process of inoculum (7 kg) using starter of Rhizopus-C_1 resulted inoculum with activities of proteolytic of 0.71 U/g, and amilolytic of 17.5 U/g at 56 h of incubation. The whole process in semi pilot scale decreased composition of products. The optimal treatment based on recovery of total protein, and the highest amino acids as N-amino in semi pilot scale was at fermentation temperature of 30 ºC for 10 weeks with concentrations of water of 44.96%, total protein of 11.77% (dry matter), dissolved protein of 8 mg/mL, N-amino of 15.4 mg/mL, reducing sugar of 582.5 mg/mL, fat of 0.26% and, VRS of 90 µeq.reduction/g.

Keywords: *Brine fermentation, vegetable broth, mung beans (Phaseolus radiatus L.), Rhizopus-C_1, semi pilot.*

INTRODUCTION

Preparation process of *vegetable broth* from mung beans (*Phaseolus radiatus* L.) through brine fermentation as savory flavor using inoculum of *Rhizopus* sp., and *Aspergillus* sp. in laboratory scale (± 100 - 300 g, total substrates) result a product with chemical composition equal to miso product, namely *vegetable broth* from soy beans using *Aspergillus oryzae,* and *Aspergillus sojae* as yeast of enzyme source and nutrition for brine fermentation [1].

Mung beans is one of the kinds of endemic beans possesing a potential use as raw material for food industry. Utilization of mung beans in preparation of *vegetable broth* is a development of processed beans for amino acids, and high peptides based functional foods. V*egetable broth* from fermented mung beans is a food ingredient which has potency as taste enhancer, and specific aroma enhancer due to their nutritive contribution, especially protein as amino acids, and high peptides. *Vegetable broth* from mung beans is produced through way of brine fermentation utilizing inoculum of *Rhizopu*s sp. or *Aspergillus* sp. [2]. On this process of brine fermentation occurs converting protein into amino acids, and peptides by activity of protease enzyme, converting fat into fatty acids by activity of lipase enzyme, and converting carbohydrate into sugars, and alcohol by activity of amylase enzyme resulted by yeast during fermentation to form specific flavor [3]. This product is probably potential utilize as source of savory flavor precursor for seasoning agent, and products of meat taste analog (meat like savory flavor).

Use of inoculum of *Rhizopus* sp. in brine fermentation is an alternative way to get savory flavor from inoculum of *Aspergillus* sp. which is mainly used in preparation of brine fermentation based foods, such as ketchup or tauco [4]. Today, inoculum of *Rhizopus* sp is more recognized as inoculum having an important role in preparation of traditional foods from soy beans, namely tempeh. Potency of *Rhizopus* sp. to get savory flavor via brine fermentation utilizing endemic beans (mung beans, kidney beans) is a development of fermentation food products as condiment products (sauces, ketchup) [2]. Use of *Rhizopus*-C_1 as source of enzymes in this preparation *vegetable broth* through brine fermentation is caused by a modification of inoculum which is mainly used in preparation of tempeh. *Rhizopus*-C_1 is a pure inoculum of *Rhizopus oligosporus* isolated from tempeh of Malang [5] possesing a potential use as inoculum in preparation of *vegetable broth* through brine fermentation [2], while an choice on mung beans is caused by still limited use,

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and broad modification of beans for amino acids, and high peptides [6-7].

Savory flavor is a flavor product which is umami taste, and is mainly applied in products of meals, soup, dressing, and snack [8]. Savory is described as unique and specific flavor produced usually by Mono Sodium Glutamate (MSG), where Japan is the first invention on this flavor. This flavor is different with sweet, salty, bitter, and sour, and it affect to produce specific flavor. When MSG is combined with Ribonucleotide, such as inosinemonophosphate will yield stronger intensity of umami taste than that single flavor component. Ribonucleotide is a component which is presence in meat, so that savory flavor can be basic taste for products of meat analog [9]. Savory flavor added to soup as MSG increase also consumption of soup at a number of volunters when compared with soup without savory flavor [10].

Products of savory flavor as *vegetable broth* in instant form (powder) produced from fermentation process is a generally natural resulted by enzymatic way, and is natural source of savory (umami) taste precursor. Application of fermentation products as savory flavor had been well-known for long ago. Fermentation process in preparation of soy sauces by *Aspergillus oryzeae*, *Lactobacillus*, *Hansenulla,* and *Saccharomyces* sp. yield the product with specific aroma when it is applied into various foods which is one of potential fermentation products as precursor savory flavor [11].

Larger scale in preparation of v*egetable broth* is an attempt to get more much product, more uniform quality, and consistency of process conditions. Process of brine fermentation in larger scale (semi pilot or pilot) using source of enzyme from inoculum has process condition which is easy to change by various factors. Activities of proteolytic, amilolytic, and lipolytic in inoculum are generally important factors in instability of process. While other important factors are condition of process environment, brine fermentation temperature, and time, humidity, aeration, preparation of inoculum (purity level of inoculum, incubation temperature and time), processes relating with material (beans, rice, salt), treatment of brine fermentation (soaking, washing, sterilizing), and many cases relating with control during fermentation, such as stability of agitating, control on sanitize, and higiene process, control on sampling, and analysis accuracy, and product investigation on aroma, color, and taste. The main case in multiplication step of fermentation product is to produce the best product quality both composition, and organoleptic acceptability. In other words, it will reached the final goal of product, resulted standard process step, yielded product with the similar quality or better at least than that in laboratory scale, known factors affecting multiplication process of product to be efficient, and produced product with beneficial feasibility in techno-economic aspects.

Vegetable broth from mung beans is a natural food ingredients because it is produced via fermentasi in order to increase food nutrition facts, improve food sensory value [12-13], and utilize alternative food products in order to avoid degenerative diseases. *Vegetable broth* is an alternative products as replacing aroma and taste source of animal in which today, aroma, and taste source of animal was well-known as natural *flavour enhancer* added into food syatem to sharpen orinal taste. Substances used are monosodium L-glutamate (MSG), inosine 5'-monophosphate (5'-IMP), and guanosine 5'-monophosphate (5'-GMP) [14].

This experiment was carried out to find out the effect of multiplication condition of process in preparation of *vegetable broth* from mung beans using inoculum of *Rhizopus*-C_1 through treatment of different fermentation temperature, and time in semi pilot scale with laboratory scale as comparison on composition, and physical qualities (color, aroma, taste) to support its role as savory flavor.

EXPERIMENTAL SECTION

Materials and Equipments

The important materials used in this experiment were commercial mung beans purchased from a local market, inoculum of *Rhizopus*-C_1 supplied from Research Centre for Chemistry – Indonesian Institute of Sciences, rice, commercial salt, and chemicals for analyses of chemistry.

The equipments used in this activity were autoclave, incubator, trays, cabinet dryer, laminer air flow hood, fermentation chamber, series of equipments in preparation of broth inoculum in laboratory, and semi pilot scales and Spectrophotometer UV-1201.

Experimental Design and Analysis

The experiments were conducted using starter of *Rhizopus*-C_1 for preparating inoculum of mung bean broth in semi pilot scale (7 kg) with incubation for 56 h. Brine fermentation was carried out at room temperature, and 30 ^oC for 0, 2, 4, 6, 8, and 12 weeks in laboratory scale (300 g), and semi pilot scale (15 kg), respectively. Chemical analyses were carried out on water content (Gravimetric method), fat (Soxtech method), total protein (Kjeldahl method), dissolved protein (Lowry method), reducing sugar (Nelson-Somogyi method) [15], Volatile Reduction Substances (VRS) [16], and N-amino (Cu method) [17], and proteolytic activity (Kunitz method), and amilolytic activity (Nelson- Somogyi method) [18].

Procedures

Preparation of broth inoculum

Rice was washed, added tap water with air to rice ratio of 1 and 1, autoclaved at 121 ^{o}C for 15 min, cooled to room temperature, inoculated using starter of *Rhizopus*-C_1 of 0.2%, and incubated at 30 ^{o}C for 0 - 56 h. Control on proteolytic, and amilolytic activities, and the growth of spores were investigated each 12 h. After incubation finished, this broth inoculum was dried using cabinet dryer at 50 ^{o}C for 24 h, powdered, sieved through 80 mesh, and ready to be used.

Preparation of vegetable broth of mung beans

The mung beans were sorted, washed, soaked in water overnight, dehulled, leaked, and autoclaved at 121 ^{o}C for 20 min, cooled to room temperature, and mixed with inoculum of *Rhizopus*-C_1, and salt aseptically in laminer air flow hood in mixtures of mung beans, inoculum, and salt of 51, 26, and 23% in closed container. The capacity of mixing process in semi pilot and laboratory scales were ± 15 kg and ± 300 g, respectively. These product mixtures placed in fermentation chamber were fermented at 30 ºC, and room temperature for 0, 2, 4, 6, 8, 10, and 12 weeks with agitating every week. The yields from this fermentation process at different temperatures, and in laboratory, and semi pilot scales were mung beans broth as crude broth. Finally, the product of crude broth was analysed, including water content, total and, dissolved protein, N-amino, reducing sugar, fat, and Volatile Reduction Substances (VRS).

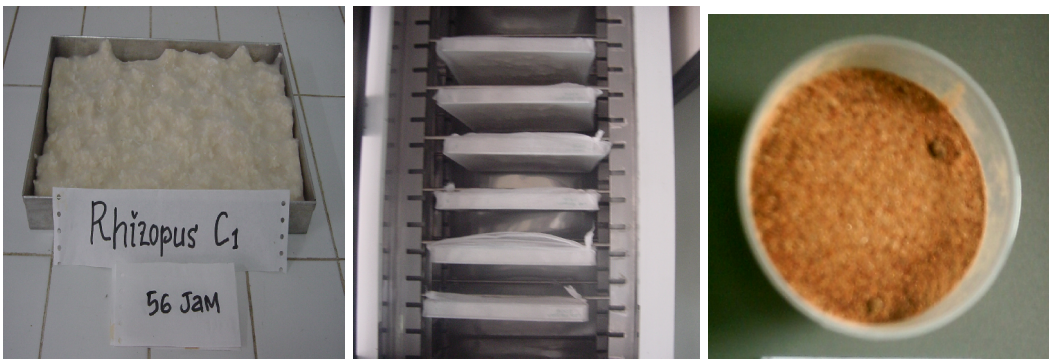
RESULT AND DISCUSSION

Characteristic of mung beans (*Phaseolus radiatus* L.)

Mung beans with water content of 9.69% and ash content of 2.42% in preparation of *vegetable broth* is important source of carbohydrate, protein, and fat in *vegetable broth*. This proximate composition of mung beans indicated by high content of total protein (25.3%, dry matter) was enable obtained amino acids, and dissolved peptides as a result of protease enzyme hydrolysis, while carbohydrate (62.12%) was starch source that was hydrolyzed by α-amilase enzyme into monosakarida, followed acetaldehyde, hydrocarbon,

Table 1. Compotition of vegetable broth from brine fermentation of mung bean using *Rhizopus*-C_1.

Component/Condition process of brine fermentation		Fermentation time (Weeks)						
		0	2	4	6	8	10	12
Water contens, %	Semi pilot scale, room temperature	42.99	45.63	44.12	40.97	44.17	46.38	46.38
	Laboratory scale, room temperature	44.29	41.37	43.28	41.27	43.99	40.71	40.71
	Semi pilot scale, 30°C	42.99	41.52	44.75	39.94	44.19	44.96	55.03
	Laboratory scale, 30°C	43.8	39.26	43.96	40.28	37.78	41.63	58.38
Total Protein, % (dry matter)	Semi pilot scale, room temperature	12.54	12.75	11.98	12.09	10.11	12.15	10.68
	Laboratory scale, room temperature	6.63	6.95	11.93	8.79	10.13	9.56	11.28
	Semi pilot scale, 30°C	12.54	9.76	12.97	11.56	11.72	11.77	10.95
	Laboratory scale, 30°C	17.18	14.25	13.23	13.05	11.26	10.98	11.52
N-amino, mg/mL	Semi pilot scale, room temperature	4.90	3.50	5.60	4.90	1.75	11.20	4.20
	Laboratory scale, room temperature	2.45	2.80	4.20	5.60	2.80	5.60	7.00
	Semi pilot scale, 30°C	4.90	4.20	8.40	5.60	2.80	15.40	7.70
	Laboratory scale, 30°C	2.80	7.00	7.00	5.60	2.80	4.20	9.80
Dissolved Protein, mg/mL	Semi pilot scale, room temperature	2.75	6.95	3.20	9.90	7.60	7.80	7.90
	Laboratory scale, room temperature	2.37	3.35	3.55	10.60	9.60	8.50	8.60
	Semi pilot scale, 30°C	2.75	7.3	3.65	10.20	8.30	8.00	8.30
	Laboratory scale, 30°C	1.30	2.5	3.25	9.00	10.50	8.15	7.70
Reduc-tion Sugar, mg/g	Semi pilot scale, room temperature	36.25	121.25	186.25	571.25	462.50	558.75	502.50
	Laboratory scale, room temperature	52.5	250	142.50	800.00	672.50	672.50	583.75
	Semi pilot scale, 30°C	36.25	136.25	150.00	582.50	547.50	582.50	485.00
	Laboratory scale, 30°C	20.62	198.75	202.50	895.00	772.50	930.00	620.00
Fat, %	Semi pilot scale, room temperature	0.6	0.36	0.43	0.20	0.23	0.20	0.12
	Laboratory scale, room temperature	0.25	0.09	0.47	0.12	0.16	0.28	0.08
	Semi pilot scale, 30°C	0.6	0.82	0.39	0.10	0.14	0.26	0.19
	Laboratory scale, 30°C	0.9	0.11	0.20	0.08	0.06	0.26	0.13
VRS, mikro µeq. red/g	Semi pilot scale, room temperature	55	55	90	80	75	70	20
	Laboratory scale, room temperature	55	105	55	65	95	90	70
	Semi pilot scale, 30°C	55	55	95	80	85	90	80
	Laboratory scale, 30°C	55	100	90	100	95	65	65



(a) (b) (c)

Fig 1. (a).The growth of *Rhizopus*-C_1 in rice substrates at 30 °C for 56 h, (b). incubation of broth inoculum in fermentation room and (c). product of broth inoculum.

ester, and other flavor compounds through yeast metabolism and chemical reactions during fermentation. Fat (0.47%) was hydrolyzed by lipase enzyme into fatty acids, and glycerol. On the whole processes, these interaction and aggregation of components will produce specific taste and flavor. These compositions give the greatest contribution (51%) as substrates in preparation of *vegetable broth* of mung beans through brine fermentation.

Effect of incubation of *Rhizopus*-C_1 inoculum on the growth and proteolytic, and amilolytic activities of *Rhizopus*-C_1 inoculum in semi pilot scale

Use of *Rhizopus*-C_1 inoculum is enables formed specific flavor volatile components. *Rhizopus* sp is mainly utilized as inoculum in preparation of tempeh that produces savory/umami taste due to contents of amino acids, and peptides [19]. In this brine fermentation, *Rhizopus*-C_1 that is pure isolate of *Rhizopus oligospurus* [5] will result enzymes of protease, amilase, and lipase in which they will hydrolyze and convert components in mung beans into taste, and aroma formed compounds in which these compounds are potential use as savory flavor. To prepare mung beans broth in semi pilot scale (± 15 kg) was needed inoculum ± 7 kg that is then utilized in brine fermentation with inoculum-to-mung beans-to-salt mixtures of 26% to 56% to 21%. Fig 1 demonstrated the growth of *Rhizopus*-C_1 inoculum in rice substrates incubated at 30 ºC for 56 h, displayed the growth of *Rhizopus*-C_1 inoculum placed in fermentation room in semi pilot scale at 30 ºC for 56 h, and dried inoculum (ready to be used) as result of drying at 50 ºC for 24 h.

Inoculum of *Rhizopus* sp is good grow at ± 32 ºC, relative humidity of 65 – 85%, minimal water activity for *Rhizopus* sp of > 0.93, and optimal water activity of 0.95 - 0.98%, and broad range of pH (2 – 8.5) [20-21]. In semi pilot scale indicated that the optimal growth of inoculum of *Rhizopus* sp at 56 h is marked by growing micelia at the all substrate surfaces. During incubation in semi pilot scale indicated slowly growth of yeast caused by large volume in which at this initial phase the growth of micelia do not fullfil at all rice substrates surface area yet (0 - 24 h). After 48 h, the growt of spores become more and more uniform and at incubation of 56 h the optimal growth of inoculum will be shown by thick micelia like cotton, white and fullfil at the all rice substrates surfaces. Proteolytic activity of rice substrates at this condition was 0.7 U/g. This proteolytic activity is low when compared with proteolytic activity in laboratory scale (± 300 g, cooked rice), namely 1.20 U/g [22].

This probably was caused by high humidity in which dry air is not proportional with yeast amount. The occurence in heat accumulation causing the growth of *Rhizopus*-C_1 is not optimal. In this case enables the occurence in decrease of proteolytic activity. *Rhizopus*-C_1 produced acidic protease enzyme in which this activity is influenced by the presence of two carboxyl group at its activated side so that proteolytic activity in rice substrates is higher because acidic protease enzyme is independent by the presence of metal in rice substrates. *Rhizopus*-C_1 is *Rhizopus oligosporus* possesing the optimal range of growth in pH, 3 - 4 and spesific property for hydrophobic residue of amino acids or aromatic at both hydrolyzed carboxyl sides [1]. On production of amylase enzyme, inoculum of *Rhizopus*-C_1 in semi pilot scale indicated that amilolytic activity in incubation for 56 h is 17.5 U/g. This activity was enough high because at incubation 56 h really start to indicate a decrease, and is not found in incubation 25 - 30 h, but in this case is depend on pH of incubation from substrates produced enzyme. *Rhizopus*-C_1 resulted less amount of amylase enzyme so that its amilolytic activity is weak but combination with their proteolytic, and lipolytic activities will result a better fermentation product, namely in tempe production [22].

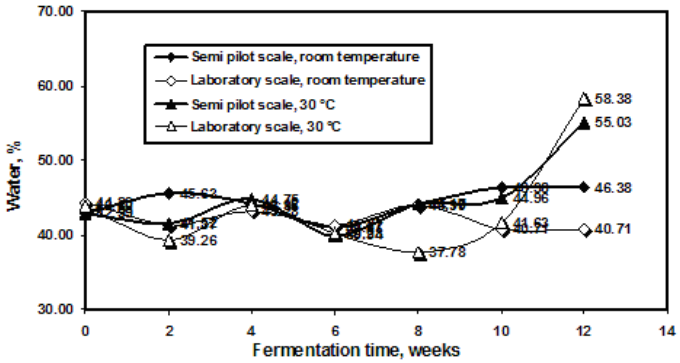


Fig 2. Effect of fermentation time on water concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

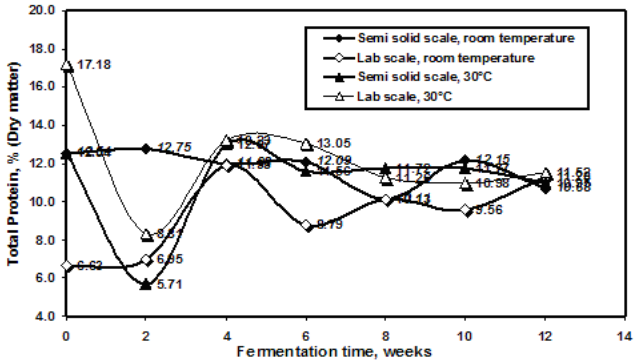
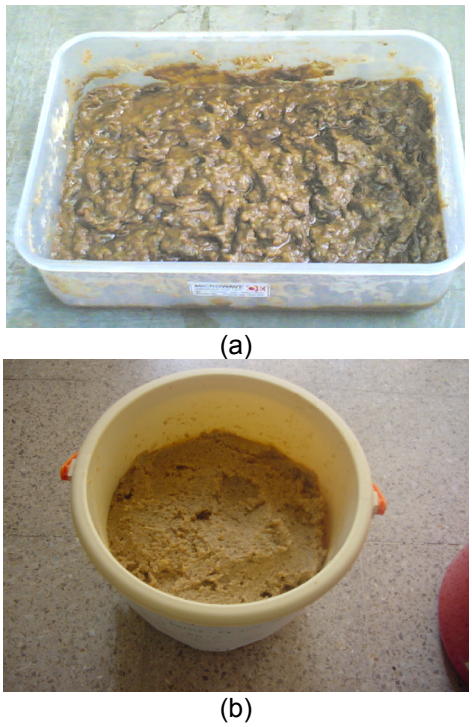


Fig 3. Effect of fermentation time on total protein concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

Effect of process multiplication conditions in brine fermentation on *vegetable broth* composition of mung beans.

Treatment interaction of their fermentation temperature, and time was tend to influence on their composition, and organoleptic quality of product. Fig 2, and 3 indicated an effect of process condition on contents of water, and total protein of fermentation product. Water content of product in semi pilot, and laboratory scales at room temperature were constant to 12 weeks of fermentation. While, water content of product in semi pilot, and laboratory scales at 30 ºC for above 8 weeks, and optimal 12 weeks were 58.36, and 55.03%, respectively, as shown in Fig 2. Thus, treatment at 30 ºC resulted higher water content in product than that in storage at room temperature**.** In this case is probably caused by the presence of stable heat during fermentation (30 ºC, in closed container) causing enzyme activity runs stronger so that it produce water mass as much more metabolism result. Between 8, and 12 weeks of fermentation showed a significat increase, while water content in products resulted in laboratory, and semi pilot scales at room temperature were constant.

Content of total protein of fermentation product at 30 ºC for 0 – 2 weeks underwent a decrease in laboratory, and semi pilot scales, but content of total protein for 12 weeks of fermentation in semi pilot scale increase, while content of total protein in laboratory scale fluctuate, as demonstrated in Fig 3. On the whole fermentation processes, total protein content at room temperature, and 30 ºC in semi pilot scale underwent a decrease. The best condition of fermentation in semi pilot scale were at room temperature for 10 weeks with total protein content 12.15% (dry weight basis), and at 30 ºC for 4 weeks with total protein content 12.97% (dry matter)**.** This total protein content was proportional to commercial *vegetable broth* product of soy bean fermented by *Aspergillus soyae* and *Aspergillus oryzeae*



(a)

(b)

Fig 4. Brine fermentation of mung beans using inoculum of *Rhizopus*-C_1 in (a) laboratory scale at 30 ºC and (b) semi pilot scale at room temperature.

(miso) for 3 - 12 months, namely total protein 11 – 13.5 % [1]. This total protein of *vegetable broth* from mung beans was also suitable to quality standard of broth, and consome in Indonesian National Standard (SNI) 01-4218-1996, in which protein as total nitrogen is minimal, namely 100 - 350 mg/L for animal broth (beef/chicken and so on). *Vegetable broth* is an animal broth analog in which quality standard of v*egetable broth* had been yet available in Indonesia today [23].

Fig 4(a) and 4(b) showed that 12 weeks brine fermentation of mung beans in laboratory scale at 30 ºC, and semi pilot at room temperature were seemed

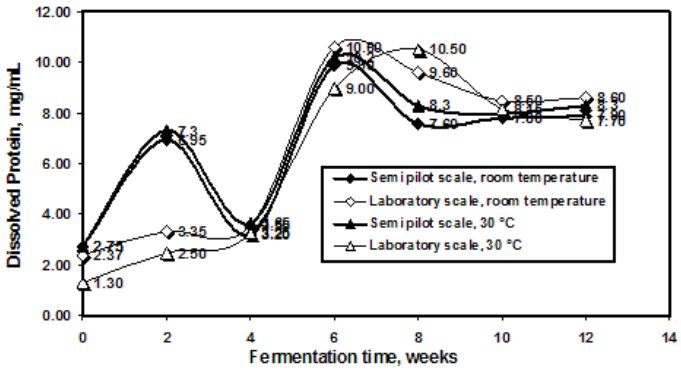


Fig 5. Effect of fermentation time on dissolved protein concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

(a) (b)

Fig 6. Brine fermentation of *vegetable broth* (a) at room temperature in semi pilot scale and (b) at 30 ºC in semi pilot scale in fermentation room.

wet condition, and more compact, and not syneresis, respectively.

These process conditions were tend to affect on contents of dissolved protein of product as presented in Fig 5. On content of dissolved product indicated that treatments in semi pilot scale at room temperature, and 30 ºC fluctuated to 4 weeks and then was optimal to 6 weeks of fermentation with content of dissolved protein 9.9, and 10.6 mg/mL, respectively, while treatment in semi pilot scale at 30 ºC gave dissolved protein content of 10.2 mg/mL. In this case demonstrated that at high temperature of fermentation, proteolytic activity became more and more increase in hydrolysing substrates protein so that to result higher content of dissolved protein was needed shorter time of fermentation. In this difference of process volume caused more focus in reaction due to smaller container surface so that at laboratory scale yielded higher content of dissolved protein (10.6 mg/mL) than in semi pilot scale at 30 ºC, as displayed in Fig 5.

This decrease of dissolved protein content started to occur for fermentation 8 weeks at treatment of semi pilot scale for both storage times. In these cases were probably caused by decrease of activity of inoculum proteolytic or extrinsic factors (distribution of oxygen, container surface, humidity, and mechanic agitating). Figs 6a and 6b presented fermentation process at room

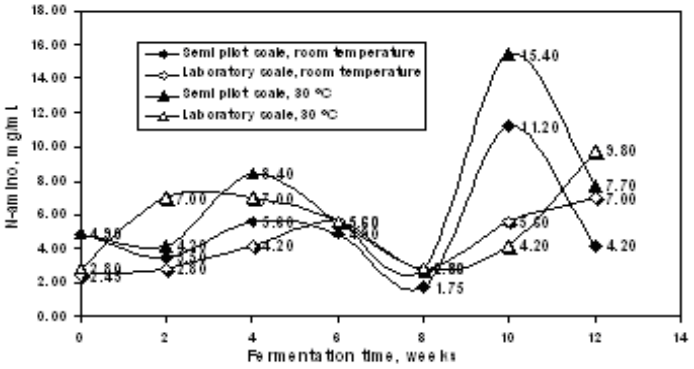


Fig 7. Effect of fermentation time on N-amino concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

temperature in semi pilot scale, and at 30 ºC in semi pilot scale in fermentation room, respectively.

Formation of amino acids as N-amino during fermentation indicated that an increase of N-amino content for fermentation 10 weeks in semi pilot scale at both storage temperature were optimal, while increase of N-amino content in laboratory scale was optimal for 12 weeks fermentation, as showed in Fig 7. Change in N-amino content for all treatments indicated that fermentation at 30 ºC gave higher content of N-amino. In this case was showed in semi pilot scale at 30 ºC (15.4 mg/mL), and at room temperature (11.2 mg/mL) for fermentation 10 weeks. In similar case, it would decrease for fermentation 12 weeks giving contents of N-amino of 7.7 and 4.2 mg/mL, respectively. This case identified that glutamic acids content as N-amino in fermentation of 10 weeks which has important role in non-volatile savory flavor was in the best condition on production of glutaminase enzyme by the optimal concentration of *Rhizopus*-C_1. Glutaminase enzyme has important role in hydrolysis of gamma-amino to form L-glutamat as source of savory flavor [26].

Treatment combination in laboratory scale showed still an increase N-amino content to 12 weeks fermentation and demonstrated fluctuated change during fermentation. This difference occured was caused by process factors (distribution of oxygen, container surface area, humidity, mechanic agitation), and activity of inoculum proteolytic in hydrolising substrates protein into amino acids as source savory flavor relating with amount of substrate, and initial composition of material.

Formation of reducing sugar in product is amilolytic activity of *Rhizopus*-C_1 inoculum in which sharply increase of reducing sugar content take placed at fermentation 6 weeks for all treatments, as presented in Fig 8. After 6 weeks fermentation, reducing sugar content seemed fluctuate to 12 weeks

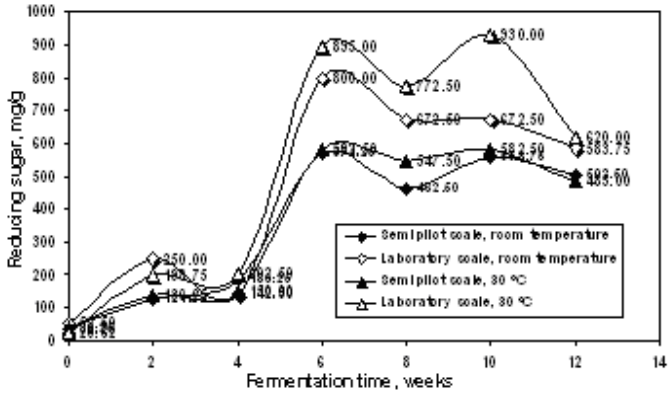


Fig 8. Effect of fermentation time on reducing sugar concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

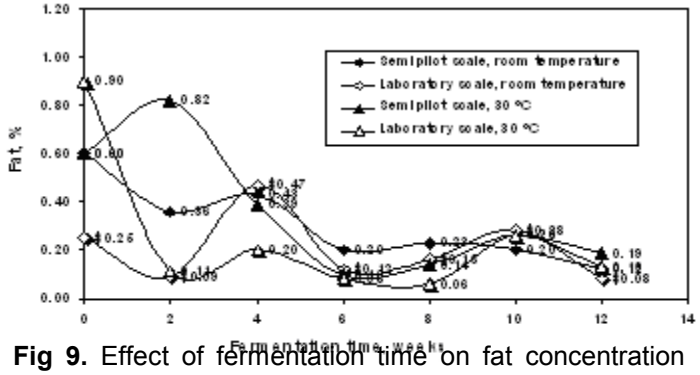


Fig 9. Effect of fermentation time on fat concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

fermentation. Influences on temperature, and process scale showed a significant difference in which in semi pilot scale, and 30 ºC, the highest content of reducing sugar was achieved at 10 weeks fermentation (582,5 mg/g), while in semi pilot scale, and room temperature, the highest content of reducing sugar was achieved at 6 weeks fermentation, namely 571.25 mg/g.

This trend was also showed at comparison product in which in laboratory scale and 30 ºC gave the highest content of reducing sugar for 10 weeks fermentation (930 mg/mg), while in semi pilot scale, and 30 ºC was 582.5 mg/g for same time of fermentation. In this case indicated that large volume of substrates needed higher temperature, and longer time of fermentation in order to obtain the optimal content of reducing sugar. This content of reducing sugar affected on product taste relating with formation of savory flavor. Savory flavor is savory/umami compounds produced by reaction between monosaccharide, and amino acids through Maillard reaction and formation of brown pigment (melanoidin), namely specific flavor compounds as result of fermentation products in Thermal Reaction, such as piron, furan [25].

The highest content of reducing sugar obtained in semi pilot scale, and 30 ºC (582.50 mg/g) for 10 weeks fermentation room temperature (571.25 mg/g) for 6 weeks fermentation were equals to characteristic of reducing sugar content as total carbohydrate of *vegetable broth* product from soy bean fermentation by *Aspergillus soyae* and *Aspergillus oryzeae* (miso) using rice substrates in which sweet miso products fermented for 3 - 6 months contain reducing sugar 11 – 13% with brownish yellow to brownish red color [3] like appearance of *vegetable broth* product from mung beans in Fig 4b above mentioned.

Length of fermentation time would decrease fat content of product, as demonstrated in Fig 9. This decrease of fat content was relating with lipase activity in hydrolysing fat into fatty acids and glycerol contributing on aroma, and taste of product, and forming flavor compounds as result of their degradation with other compounds [12]. Treatment interaction between temperature, and fermentation time, and substrates volume showed an influence on decrease of fat content of product in which at room temperature accelerated in hydrolysing fat both semi pilot, and laboratory scale. In this case was showed fat content resulted from 12 weeks fermentation in semi pilot scale – 30 ºC, and laboratory scale – room temperature, namely 0.72 and 0.08%, that are lower than that semi pilot scale – 30 ºC, and semi pilot – room temperature, namely 0.19 and 0.13%.

Volatile Reduction Substance (VRS) is volatile compounds produced during fermentasi which can be reduced by oxydizing compound. VRS is an qualitative identification, and parameter of the presence of volatile compounds, such as alcohol, aldehyd, carbonil [26], and give a contribution on savory flavor through Maillard reaction due to presence amino acids, and sugar. Length of fermentation time, VRS become more and more low for all treatments, as presented in Fig 10. At laboratory scale, VRS yielded for fermentation 12 weeks showed high concentration at room temperature (105 µeq.red./g), and at 30 ºC (100 µeq.red./g), while VRS yielded for fermentation 4 weeks at semi pilot scale – room temperature, and semi pilot scale – 30 ºC were 90 µeq.red./g, and 95 µeq.red.g, respectively. In this case demonstrated that formation of VRS were effected by fermentation temperature, and substrates volume.

Low temperature of fermentation (room temperature, ± 25 ºC) would needed longer time of fermentation to result the optimal VRS. In smaller volume of substrates (laboratory, ± 300 g), and higher temperature of fermentation (30 ºC) would be produced more optimal concentration of VRS. In this case was relating with their growth, and metabolisms of inoculum in degrading or converting components in mung beans into reductive flavor.

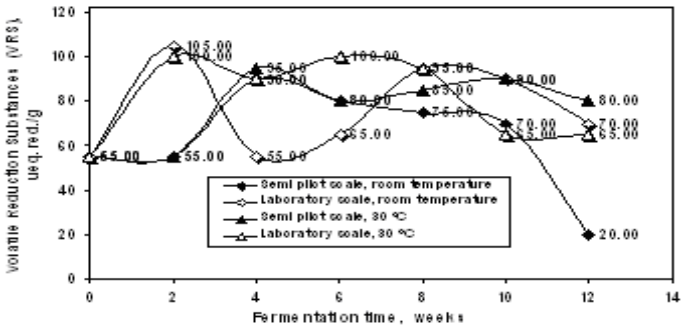


Fig 10. Effect of fermentation time on volatile reduction substances (VRS) concentration from *vegetable broth* of mung beans by inoculum of *Rhizopus*-C_1 at 30 °C, and room temperature in laboratory, and semi pilot scales.

CONCLUSION

Conditions of process multiplication were tend to effect on composition, and organoleptic quality of product. Length of fermentation time at room temperature, and 30 ^oC would increase concentrations of dissolved protein, N-amino and reducing sugar, and organoleptic quality, such as dark color, taste and aroma of product, decrease fat content, and volatile reduction substances (VRS), and be constant on contents of total protein and water for both laboratory, and semi pilot scales;

Process multiplication resulted product composition in semi pilot scale that was lower than that in laboratory scale at both room temperature, and 30 ºC;

Concentrations of total protein and the highest amino acids as N-amino based the optimal process treatment in semi pilot scale was at both room temperature, and 30 ºC for 10 weeks of fermentation.

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