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The production of corn kernel *miso* based on rice-*koji* fermented by *Aspergillus oryzae* and *Rhizopus oligosporus*

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ABSTRACT

The suitability of corn kernel as raw material to produce *miso* fermented by rice-*koji* containing *Aspergillus oryzae* and *Rhizopus oligosporus* has been investigated. The optimization was conducted on two important factors in *miso* production namely mold composition in rice-*koji* and salt concentration. The mold composition was prepared by inoculating the spores of 2% *A. oryzae*, 2% *R. oligosporus,* and 2% the mixture of both in a ratio of 1:1, 2:1, and 1:2 (v/v) into different rice media. The mold composition was optimized to produce rice-*koji* with high α-amylase and protease activity. Different NaCl concentrations of 10%, 15%, and 20% were subjected to optimization process and added to each mixture after five days of fermentation. The salt concentration was also optimized to produce corn kernel *miso* with high glucose and high dissolved protein concentration. The result showed that rice-*koji* containing *A. oryzae* and *R. oligosporus* in the ratio of 1:1 had the highest α-amylase and protease activity of 0.42 U/mL and 0.45 U/mL respectively. In addition, the presence of 10% NaCl in corn kernel *miso* fermented by *A. oryzae* and *R. oligosporus* in the ratio of 1:1 exhibited the highest glucose and dissolved protein concentration of 0.64 mg/mL and 8.80 mg/mL respectively. The optimized corn kernel *miso* by *A. oryzae* and *R. oligosporus* in the ratio of 1:1 with 10% NaCl was subjected to nutrient content analysis and compared to the result before the corn kernel was fermented. The nutrient content analysis showed nutrient enhancement after corn kernel was fermented and transformed into a *miso*. Glucose, dissolved protein, and fat content increased 6.74, 1.34, 7.63 times respectively. This study concludes corn kernel could be utilized to produce a novel corn kernel *miso* for dietary diversification and for improving nutritional and health status.

1. Introduction

Miso (fermented soybean paste) is a traditional fermented food originally from Japan and has been used worldwide as a basic ingredient to cook soups as well as seasoning for side dishes (Kusumoto and Rai, 2017). *Miso* has several health benefits due to its bioactive compounds such as peptides and isoflavones released during fermentation. This food is capable of reducing cancer cell proliferation and possesses anti-diabetic, anti-hypertensive as well as antioxidant properties (Hirota *et al.,* 2000; Kwon *et al.,* 2010; Zaid and El Shenawy, 2010; Shimizu *et al.,* 2015; Kusumoto and Rai, 2017). *Miso* has sweet, salty, and umami taste, presents in a paste-like half-solid food form with high protein content characteristic. This food is usually made from koji, a solid state fermented grain by *Aspergilus oryzae* served as starter culture in addition to soybean, salt, and water (Bourdichon *et al.,* 2012).

As a starter culture, *koji* has several types which are determined by the substrates (soybean, barley, or rice) in which the *koji* molds grow. The most common type of *koji* used for *miso* production is rice-*koji* fermented by *A. oryzae* which accounts 80% of *miso* production in Japan (Machida *et al.,* 2008). However, *Aspergillus sojae, Aspergillus tamarii, Aspergillus awamori, Aspergillus saitoi, Aspergillus kawachi*, and *Rhizopus oligosporus* have also been used in Japanese food industries for the production of koji (Esaki *et al.,* 1997;

Lin *et al.,* 2006). The molds in *koji* produce hydrolytic enzymes for instance proteases and amylases for degrading biological polymers such as protein and carbohydrate. The monomer produced from various enzymatic reactions happened in the fermentation process gave sweet and umami tastes of *miso*. These enzyme activities are optimized and controlled to yield optimum starter cultures for fermentation by manufacturers (Marui et al., 2013).

Beside *koji*, the type of raw material is important to diversify the production of different *miso* types. In addition to soybean, there are a lot of potential grains that could be utilized to produce *miso*. The use of other grains as raw material substitute for producing *miso* could support dietary diversity to overcome nutrition and health status issues (Fanzo *et al.,* 2013). Dietary diversity directly influences diet quality by guarantying adequate intake of essential nutrients and important non-nutrient factors. Research conducted by Kennedy *et al.,* (2007); Moursi *et al.,* (2008); and Rah *et al.,* (2010) demonstrated dietary diversity has strong association with nutritional status, particularly micronutrient density of the diet. Therefore, substituting soybean with another type of grain could offer another type of *miso* with different nutrient composition and inherent health benefits.

One of the most potential grains to substitute raw material of *miso* is corn kernel. The worldwide production of corn is currently higher than soybean. In June 2017, the production of corn was 1031.86 million metric tons whereas the production of soybean was 344.67 million metric tons (USDA, 2017). This higher availability renders corn more reliable for dietary diversification. Besides, the nutrient composition of corn kernel is suitable for *miso* fermentation which consists of 68.40% carbohydrates, 3.62% protein, 1.42% oil (USDA, 2016). This data was based on the analysis of corn kernel for the commodity yellow dent corn on a dry matter basis. Starch and protein contained by corn kernel can be biochemically converted into essential amino acids, simple carbohydrates, and a wide diversity of phenolic compounds that improves flavors, aromas, and textures of fermented food products (Villeger *et al.,* 2017).

Hence, the purpose of this study was to obtain the composition of *A. oryzae* and *R. oligosporus* culture ratio, as well as NaCl concentration which can improve the nutritional status of miso. The fermentation optimization results were evaluated with enzyme activity at the time of fermentation and nutritional status of miso. The optimization of salt concentration was also conducted and evaluated by measuring the concentration of glucose and dissolved protein on each fermented corn kernel. Nutrient content analysis was done to know the amount of glucose, dissolved protein, and fat content in the optimized final product. The result of nutrient content analysis was compared with the analysis conducted before the corn kernel was fermented to know whether the nutrients increased or decreased after fermentation. This study would give an opportunity to introduce a novel corn kernel *miso* for dietary diversification to improve nutritional and health status.

2. Materials and Methods

2.1 Microorganisms and Chemicals

Aspergillus oryzae and *Rhizopus oligosphorus* were obtained from Research Unit for Chemistry, Indonesian Institute of Science. Corn kernel and rice were purchased from local store. (Hayden, 1923). Casein and Trichloroacetic acid (TCA) were purchased from Merck (Darmstadt, Germany) and used for determination of protease activity using Kunitz method (Kunitz, 1950). The analytical grade of Lowry reagent was purchased from Merck (Darmstadt, Germany) and used for determination of dissolved protein concentration using Lowry method (Lowry, 1951). The analytical grade of Nelson reagents and arsenomolybdate were purchased from Merck (Darmstadt, Germany) and used for determination of glucose concentration using Nelson-Somogyi method (Nelson, 1944).

2.2. Methods

2.2.1. Spores Suspension Preparation

A. oryzae and *R. oligosphorus* were inoculated separately into different PDA and incubated at 30^{o}C for seven days. The spores of each mould were diluted in 0.09% NaCl, and the optical density of each pure culture was measured at 600 nm using a spectrophotometer. The optical density was set to 0.7 to obtain a cell concentration around 10^{7} CFU/mL. The total cell number was confirmed by plating them into different PDA. The colony number of each medium was observed and counted after 24 h of incubation at 30^{o}C. One colony formed is assumed coming from one spore.

2.2.2. *Koji* fermentation Process

Rice was cleansed, washed, and mixed with distilled water in a ratio of 1:1 (m/v). This mixture was sterilised at 121^{o}C for 15 minutes and cooled down to room temperature. The spores of 2% *A. oryzae*, 2% *R. oligosphorus,* and 2% the mixture of both in a ratio of 1:1, 2:1, and 1:2 (v/v) were inoculated into different rice media. Each medium was incubated at 30^{o}C for 48 h and dried inside the oven at 50^{o}C for 24 h. Each dried culture was then subjected to a grinding process to obtain rice-*koji* in the form of powder.

2.2.3. Corn Kernel Fermentation

Corn kernel was weight to give a mass of 100 g. Corn kernel was then cleaned, washed, sliced into pieces, soaked, and sterilised at 121°C for 15 minutes. After it was cooled down to room temperature, corn kernel was mixed with different rice-*koji* composition mentioned above. Each rice-*koji* added to corn kernel was 5% (m/m). Fermentation was done at 30°C for five days inside an incubator. During five days-fermentation, the sampling process was conducted.

2.2.4. Enzymatic Activity Analysis

Fermented corn kernel of 10 g was sampled every day and diluted to 100 mL of phosphate buffer. The suspension was shaken at 120 rpm for 90 min and centrifuged at 2500 rpm in the temperature of 4°C for 20 min. The supernatant was collected and subjected to α-amylase and protease enzymatic activity analysis.

2.2.5. α-Amylase Assay

The solution containing seven mL of 1% soluble starch in 0.1 M phosphate buffer (pH 7.0) was incubated at 40°C for 5 min. The sample solution was prepared by adding 1 mL of the supernatant as crude enzyme extract to the soluble starch solution and incubated for 30 min at 40°C. Deactivation of the enzymes was done by adding 1.5 mL of 0.67 N sulfuric acid and 0.5 mL of 10% sodium tungstate to the mixture. The blank solution was prepared by adding 1 mL of distilled water to starch solution and was treated the same as sample solution. Each solution was centrifuged at 2500 rpm for 20 min in the temperature of 4°C. Each supernatant was collected and subjected to reducing sugar analysis using Folin Wu method (Folin and Wu, 1919). Folin-Wu method was based on the ability of reducing sugars to reduce alkaline copper tartrate. The product of this reaction was used to oxidise phosphomolybdic acid to yield a blue compound measured using spectrophotometer at 420 nm. The result was compared with enzymatic activity of standard α-amylase obtained through the same method. One unit of α-amylase activity was defined as the amount of enzyme that releases 1 µg of reducing sugar as glucose for 30 min at 40°C and expressed as units per mL supernatant.

2.2.6. Protease Assay

The protease assay was performed based on the method proposed by Kunitz (1971). The solution containing 0.5 mL of 1% casein was incubated at 35°C for 20 min. The sample solution was prepared by adding 0.25 mL of the supernatant as crude enzyme extract and 0.25 mL of phosphate buffer pH 7.6 to casein solution and incubated for 20 min at 35°C. To deactivate the enzyme, 1.5 mL of 5% TCA was added to the mixture and incubated at room temperature for 30 min. The blank solution was prepared by adding 1 mL of distilled water to casein solution and was treated the same as sample solution. Each solution was centrifuged at 2500 rpm for 20 min in the temperature of 4°C. Each supernatant was collected and subjected to absorbance measurement at 280 nm using spectrophotometer. One unit of protease activity was defined as the amount of enzyme that releases 1 µg of amino acid as tyrosine for 20 min at 35°C and expressed as units per mL supernatant.

2.2.7. Optimization of Salt Concentration and Chemical Analysis

Different salt concentrations of 10%, 15%, and 20% (m/m) were added to each fermented corn kernel after five days of fermentation. The mixtures were subjected to further fermentation for eight weeks more. After eight weeks of fermentation, fermented corn kernel of 10 g was sampled and diluted to 100 mL of phosphate buffer. The suspension was shaken at 120 rpm for 90 min and centrifuged at 2500 rpm in the temperature of 4°C for 20 min. The supernatant was collected and subjected to glucose concentration analysis using Nelson-Somogyi method (Somogyi, 1952) and dissolved protein concentration analysis using Lowry method (Lowry e*t al*., 1951).

2.2.8. Nutrient content analysis

The nutrient content analysis was done before the corn kernel was fermented and in the final product of corn kernel *miso* that has been optimized. This was conducted to compare the nutrient before and after fermentation process. The analysis includes glucose concentration analysis using Nelson-Somogyi method (Somogyi, 1952), dissolved protein concentration analysis using Lowry method (Lowry et al., 1951), and fat content analysis using soxhlet extraction method (Manirakiza et al., 2001).

3. Results and Discussions

3.1. Physical properties of *koji* and *Miso*

Koji made from rice that has been steamed and overgrown with mould spores (yeast tempeh) and then incubated for three days. During *koji* fermentation, various enzymes (proteases, amylases and lipases) will be produced by moulds and have an important role in the process of overhauling raw material macromolecules into simpler molecules (Wahyuhapsari *et al*., 2013; Andarti *et al*., 2015).

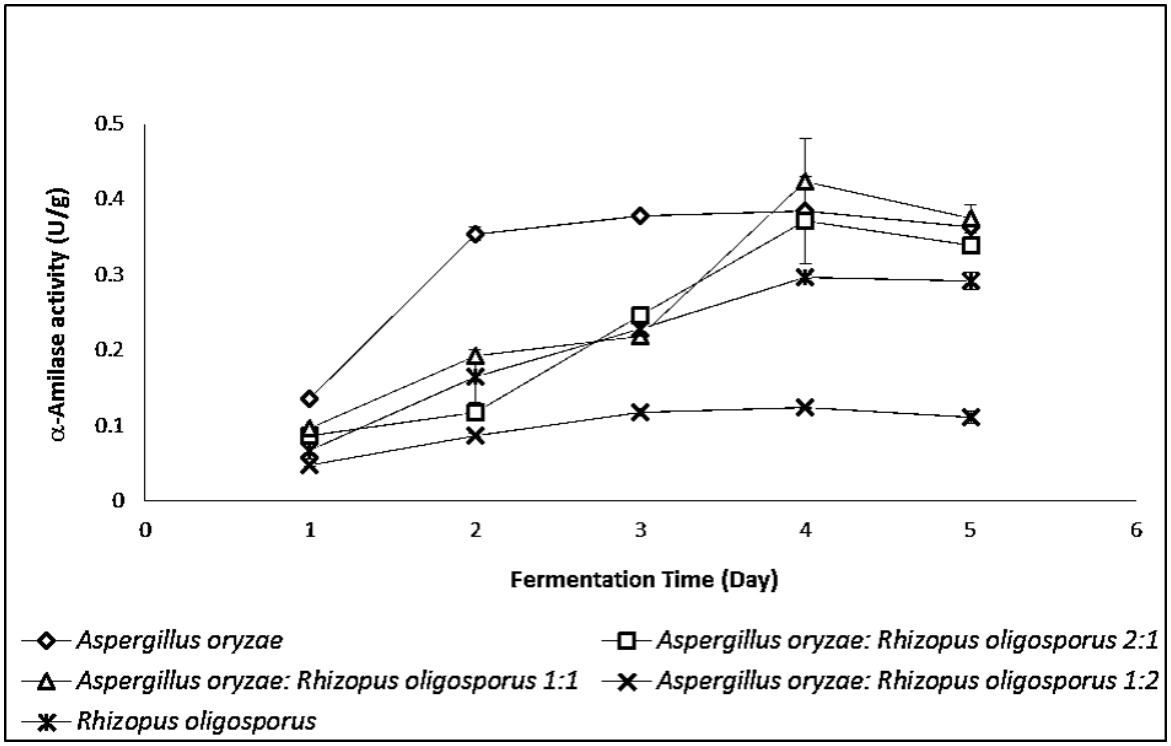

Figure 1. The enzymatic activity of α-amilase in rice-*koji* containing different mold composition.

The second stage of *miso* production is fermentation with a mixture of salt, peanuts and *koji* to form a *miso* flavour. *Miso* is shaped like peanut butter, or coloured peanut butter with a creamy yellowish, light brown, dark brown to blackish with texture is like peanut butter with salty tasted. *Miso* produces a savoury and salty taste unique and commonly used as a spice on a variety of Japanese cuisine for example used as a sauce, soup sauce and soaked spices for pickles.

3.2. The Activity of α-Amylase Enzyme

Regarding α-amylase activity, the result showed each mold composition had different activity to hydrolyze starch from corn kernel (Figure 1). All samples had similar profiles where the activity increased from the first day until the fourth day of fermentation and tend to diminish on the fifth day. This was possibly caused by decreasing nutrient in the media and the stationary phase regulation leading to the reduction of molds' metabolism rate. This restrains the formation of α-amylase enzyme as it has been thought to be a way for microorganisms to save energy (Coronado *et al.,* 2000). Moreover, the excessive amount of maltose as one of α-amylase hydrolysates is able to hedge the production of α-amylase enzyme. The presence of hydrolysates in a certain concentration could inhibit the activity of hydrolase enzyme itself through product inhibition mechanism (Qian *et al.,* 1995). This result was confirmed by Frederick *et al.* (2012) who found increasing maltose concentration could enhance the dissociation constant for starch binding with the enzyme. The binding analysis shows the kinetic action of maltose is entirely competitive. This binding site is large enough to accommodate two molecules of maltose. Hence, when maltose concentration is high and a second molecule may bind at the active site, the affinity of this binding step is approximately 6.5-fold weaker.

The highest α-amylase activity was shown by rice-*koji* containing *A. oryzae* and *R. oligosporus* in the ratio of 1:1. This mixture gave α-amylase activity of 0.423 U/mL (Figure 1). This result is similar to the study conducted by Takefuji *et al.* (2016) who showed rice-*koji* containing *A. oryzae* and *R. oryzae* in the ratio of 1:1 had higher α-amylase activity compared to the other ratios. Figure 1 also showed *A. oryzae* produced α-amylase enzyme more than *R. oligosporus*. This can be attributed to the fact that the growth of *Rhizopus* species in steamed polished rice culture is delayed due to inability of the microbes to utilize heat-denatured proteins contained in rice (Tanaka *et al.,* 1982). *Rhizopus* strains also lack carboxypeptidase activity which in turn makes them incapable of breaking down heat-denatured proteins. In addition, the study conducted by Harayama *et al.* (1989) also reported the α-amylase activity of *R. oryzae* IFO 5418 and *R. oligosporus* NRRL 2710 was extremely weak in rice and soybean-*koji*. Hence, the enzyme activity of *koji* containing *A. oryzae* was generally higher than the ones containing *R. oligosporus* with a higher ratio. The result showed during the first few days of fermentation, the mixed culture exhibited lower α-amylase activity. This was probably because the growth of each mold in the mixed culture was still in the lag phase for adaptation process leading to less α-amylase

enzyme production. Moreover, in the mixed culture, there was competition between both molds to get nutrient from the media. Hence, *A. oryzae* and *R. oligosporus* in the ratio of 2:1 and 1:2 exhibited lesser α-amylase activity compared to balance ratio of 1:1.

3.3. The Activity of Protease Enzyme

Other than α-amylase activity, protease activity of each mold composition in rice-*koji* was also investigated. The result showed all mold composition reached its maximum activity on the fourth day of fermentation (Figure 2). This finding conforms to other studies suggesting that highest protease activity of *R. olisgosporus* and *A. oryzae* was usually obtained at 48 h of incubation and after (de Castro *et al.,* 2014; Han *et al.,* 2003). The protease activity of *R. oligosporus* was higher than *A. oryzae,* however, the highest protease activity was shown by rice-*koji* containing *A. oryzae* and *R. oligosporus* in the ratio of 1:1. This mixture gave protease activity of 0.455 U/mL (Figure 2). The same result was also shown by Starzyńska-Janiszewska *et al.* (2015) who observed the improvement of protein hydrolysis when the dose of *A. oryzae* spores was equal and lower than that of *R. oligosporus* in inoculum. Yigzaw *et al.* (2004) also discovered the improvement of amino acid profile when teff and grass-pea were fermented by *A. oryzae* and *R. oligosporus*. This was probably caused by the ability of *A. oryzae* to eliminate most of protease inhibitors in soya bean increasing the degree of proteolysis when it is mixed with *R. oligosporus* during the fermentation (Hong *et al.,* 2004).

The optimization study of mold composition showed rice-*koji* containing *A. oryzae* and *R. oligosporus* in the ratio of 1:1 exhibited higher α-amylase and protease activities compared to the others. This result suggested controlling the spore numbers of *A. oryzae* and *R. oligosporus* could control the production of α-amylase and protease enzymes. Furthermore, the data shown above also imply that coculturing *A. oryzae* and *R. oligosporus* could raise the production of α-amylase and protease enzymes. In solid-state fermentation, these molds colonized corn kernel as the media through two types of penetrative hyphae. The long ones, also called scouting hyphae are used to help the mold explore the composition of the medium and seek new colonization sites across the medium. These hyphae are formed during the lag and exponential phase where there is still a significant amount of O_2 in the medium. Meanwhile, the short hyphae, also called vegetative hyphae are used to anchor the colony to the solid medium preventing the removal of the entire colony due to physical disturbances like wind and agitation of the fermentation bed. These hyphae are used to improve substrate hydrolysis by secreting large quantities of hydrolytic enzymes into the medium (Sugai-Guérios *et al.,* 2016).

3.4. The Optimization of Salt Concentration

Salt is one of main components to produce *miso* which also needs to be optimized. Different concentrations of salt were added to the mixture in order to know which salt concentration produced higher concentration of glucose and protein as indicators for better fermentation result. The study conducted by Ito *et al.* (2017) revealed an interesting fact

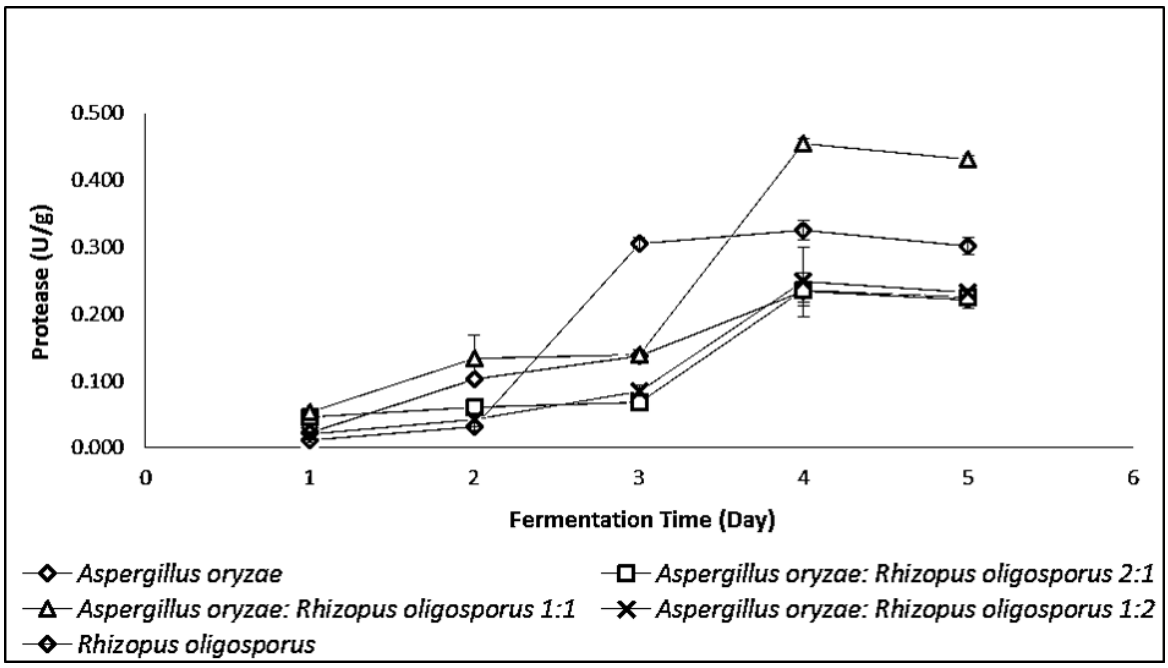

Figure 2. The protease activity in rice-*koji* containing different mold composition

that the salt contained in *miso* did not increase blood pressure and was not associated with hypertension in humans. However, the reason remains unclear and the study still needs to be conducted further. The result showed when 10% of NaCl was added to corn kernel, the one fermented by *A. oryzae: R. oligosporus* in the ratio of 1:1 produced the highest glucose concentration of 0.64 mg/mL compared to the others (see Figure 3). This mold composition had the highest α-amylase activity as described above leading to the highest glucose concentration. However, the concentration of glucose in corn kernel *miso* fermented by those molds decreased as salt concentration increased. The result also showed the optimum salt concentration for each mold composition was different. When corn kernel was fermented by *R. oligosporus* the sample reached its maximum glucose concentration in the presence of 15% NaCl. A similar result was shown when *A. oryzae* and *R. oligosporus* in the ratio of 1:2 and 2:1 was used to ferment corn kernel. The maximum glucose concentration was also reached when 15% of NaCl was added to the mixture.

The result also showed all corn kernel *miso* had the lowest concentration of glucose in the presence of 20% NaCl except for *R. oligosporus* which had the lowest glucose concentration when 10% NaCl was added to the mixture. In the case of *R. oligosporus*, this was probably caused by the low concentration of chloride ion in the 10% of NaCl which is one of the allosteric activators for amylase enzyme (Kumar and Khare, 2015; Mohapatra *et al.,* 1998; Obo and Ajele, 1997). Chloride ions could bind and interact with catalytic residues and ultimately turn on -amylase activity (Maurus *et al.,* 2008; Williams *et al.,* 2006). The amylase enzyme possessed by *R. oligosporus* probably requires a higher concentration of chloride ions to be active. Hence, the production of glucose as one of the hydrolysis products was reduced when 10% of NaCl was applied in corn kernel *miso* fermented by *R. oligosporus*. However, the glucose concentration decreased when 20% of NaCl was added. High concentration of salt could also inhibit the growth of the molds especially if both molds are not halophile microbes. Therefore, when 20% NaCl was added to the mixture, the production of α-amylase in each mold composition was limited leading to lower production of glucose. This finding was in line with some scientific reports (Almansouri *et al.,* 2001; Dutta *et al.,* 2006; Fentahun and Kumari, 2017).

In addition to glucose, the protein concentration of each corn kernel *miso* in different salt concentration was also measured. The result showed corn kernel *miso* fermented by *A. oryzae: R. oligosporus* in the ratio of 1:1 produced the highest dissolved protein concentration of 8.8 mg/mL when 10% of NaCl was added to the mixture (Figure 4). This was because according to the previous result, this composition had the highest protease activity. Moreover, all mold compositions in this study reached maximum dissolved protein concentration in the presence of 10% NaCl. The more NaCl added to the mixture, the lower the protein concentration detected due to salting out and protease denaturation (Su *et al.,* 2005). High salt concentrations also inhibit the growth of many microorganisms, which in turn greatly decreases the likelihood of the protease enzymes production. These are responsible for protein hydrolysis to form amino acids. Therefore, the result showed when salt concentration increased to 15% and 20%, dissolved protein in

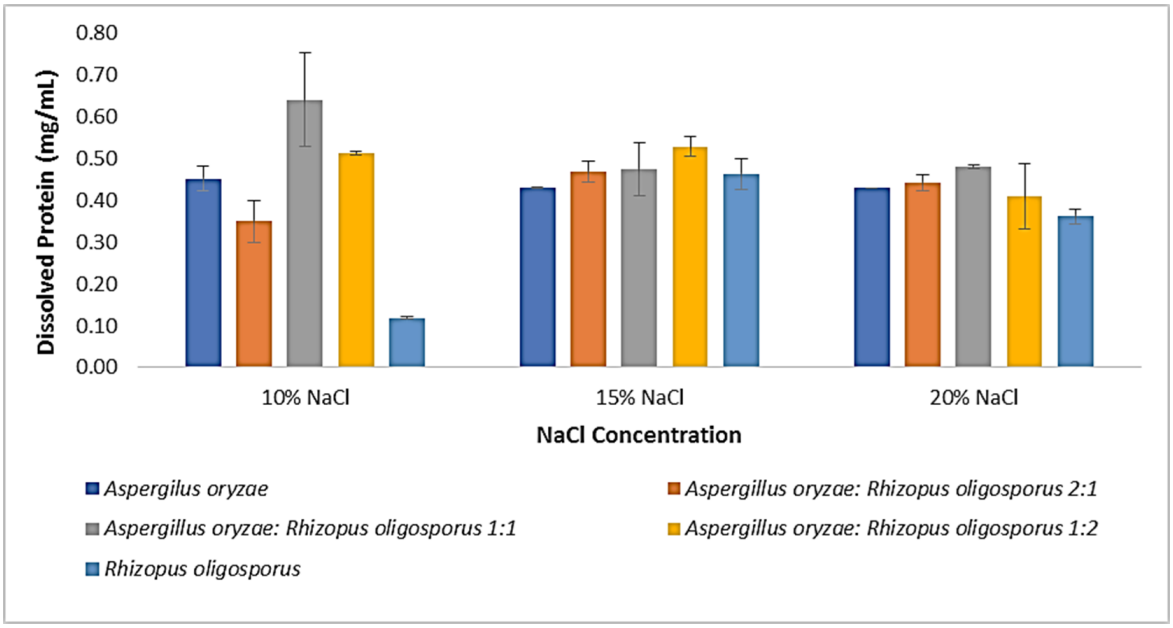

Figure 3. The effect of various salt concentration with ratio comparison of *A oryzae* dan *R. oligosporus* on glucose concentration in corn kernel *Miso*

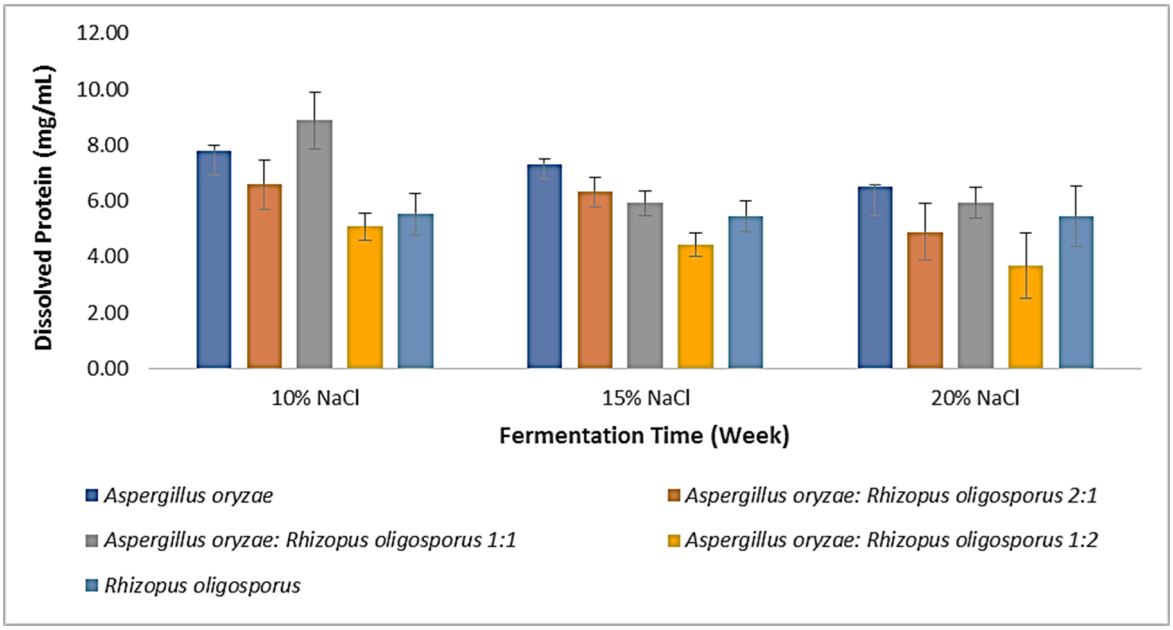

Figure 4. The Interaction between various of salt concentration and composition *A. oryzae* and *R. oligosporus* on protein concentration in corn kernel *Miso*

corn kernel *miso* decreased. This finding was similar to some reported articles saying proteolytic activity decreased as salt concentration increased (Dodia *et al.,* 2006; Gardini *et al.,* 2001; Patel *et al.,* 2005; Kurniawan et al 2008). Based on the optimization results above, in the presence of 10% NaCl, corn kernel *miso* fermented by *A. oryzae* and *R. oligosporus* in the ratio of 1:1 produced highest glucose and dissolved protein concentration. This mold composition has also been proven to reduce 80% of the neurotoxin β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP) in fermented grass pea (Yigzaw *et al.,* 2004).

3.5. Nutrient Content Analysis

Once mold composition and salt concentration have been optimized, the chosen corn kernel *miso* fermented by *A. oryzae* and *R. oligosporus* in the ratio of 1:1 with 10% NaCl was subjected to nutrient content analysis and compared to corn kernel before it was fermented. There were three measured indicators including dissolved protein concentration, glucose concentration, and fat content. This was done to determine whether the nutrient content increased or decreased after fermentation. The result showed that all measured indicators increased after fermentation. The concentration of dissolved protein in corn kernel increased 6.74 times after fermentation from 2.38 mg/mL to 16.03 mg/mL (Table 1). Dissolved protein of corn kernel *miso* was concentrated due to water loss in the drying process and increased due to protease activity produced by *A. oryzae* and *R. oligosporus*. Meanwhile the glucose concentration increased 1.34 times from 0.37 mg/mL to 0.49 mg/mL due to amylase activity by those molds during the fermentation process. The fat content of corn kernel *miso* also increased 7.63 times from 1.94% to 14.80% due to lipase activity produced by *R. oligosporus* and *A. oryzae* which aids the hydrolysis of triglyceride to form fatty acids and glycerol. Fat content is an important factor to determine the aroma of miso since fatty acids are prone to oxidation which in turn form volatile compounds for specific aromas (Peinado *et al.,* 2016).

Table 1. Nutritional content Analysis of corn kernel Miso fermented by *A. oryzae*: *R. oligosporus* in the ratio of 1:1

Indicator	Samples	
	Corn kernel before fermentation	Optimized corn kernel Miso
Dissolved protein (mg/mL)	2.34 ± 0.07	16.03 ± 0.01
Glucose (mg/mL)	0.36 ± 0.01	0.50 ± 0.04
Fat Content (%)	1.94 ± 0.02	14.85 ± 0.02

4. Conclusion

The corn kernel employed in this study was suitable to be used as a raw material for *miso* production. The combination between *A. oryzae* and *R. oligosporus* in the ratio of 1:1 inside the rice-*koji* could yield highest α-amylase and protease activity of 0.42 U/mL and 0.45 U/mL respectively. The addition of 10% NaCl influenced better fermentation results as the glucose and dissolved protein concentrations were the highest measured in the *miso* fermented by the optimised rice-*koji*. The glucose and dissolved protein concentration measured in the optimised product was 0.64 mg/mL and 8.80 mg/mL respectively. These data imply increased concentration of glucose, protein, α-

amylase activity, and protease activity could be obtained by coculturing mould composition and controlling salt concentration. The nutrient content enhanced as shown by glucose, dissolved protein, and fat content in corn kernel *miso* was higher than corn kernel before it was fermented. To the best of our knowledge, this is the first report of *miso* production from corn kernel. Further research should be focused on the organoleptic study and bioactivity studies of the corn kernel *miso* for the exploration of health benefits.

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