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Original Paper

Functional Properties of Adlay Flour (*Coix lacryma-jobi* L. var. Ma-yuen) Resulting from Modified Durations of Fermentation Using *Rhizopus oligosporus*

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***Abstract* — Adlay is a nutritious grain that has the potential as an alternative food because it has a high protein and fat content of 14.10% and 7.90%, respectively. The use of Adlay as flour still has a weakness, namely the functional properties of Adlay flour such as low swelling power. One way to improve the characteristics of Adlay flour is by fermentation using *Rhizopus oligosporus*. The fermentation duration is the time that allows changes in the characteristics of the flour due to the fermentation. This study aims to determine the effect of differences in fermentation duration using *Rhizopus oligosporus* on the physical, chemical, and functional characteristics of Adlay flour produced from fermented Adlay seeds. This study used one factor, namely the lengths of fermentation for 0, 12, 24, 30, 36 and 48 hours. The analysis in this study included whiteness, yield, pH, water content, ash content, protein content, fat content, carbohydrate content, swelling power and solubility, oil holding capacity (OHC) and water holding capacity (WHC). The results have shown that the longer the fermentation duration the lower rate of whiteness, pH, yield, water content, fat content, and carbohydrate content will become, while some functional properties of Adlay flour become better with increasing values of ash content, protein content, swelling power, solubility, Oil Holding Capacity (OHC), and Water Holding Capacity (WHC).**

Keywords—Adlay, fermentation duration, characteristics of Adlay flour

I. INTRODUCTION

Adlay is a cereal plant originating from East Asia including Indonesia to East India, which then spreads to China, Egypt, Germany, Haiti, Hawaii, Japan, Indonesia, Panama, Sarawak, Philippines, Thailand, Taiwan, America and Venezuela [1]. Adlay has substantial potential as an alternative food because it has carbohydrates, protein and fat components of 76.40% each; 14.10% and 7.90% [2, 27]. However, local people in Indonesia cultivate Adlay in a simple way, and its use is still very limited.

Efforts to increase the usability of Adlay can be done by diversifying processed Adlay products into Adlay flour. The characteristics of flour determine its use in food products related to the product quality [28]. [3] Adlay flour has a low value of swelling power. This is due to the high fat content surrounding the starch, which can inhibit water hydration into the starch granules thereby reducing or limiting swelling power. One way to improve the characteristics of Adlay flour is by fermentation using *Rhizopus oligosporus* [29].

The fermentation plays a role in the degradation of some complex compounds into simpler compounds. [4] Soy flour starch extract undergoes physical changes due to fermentation using *Rhizopus oligosporus*, which is indicated by an increase in swelling power, solubility, absorption, and water holding capacity as the fermentation duration increases.

Rhizopus oligosporus is one of the species that is widely used to improve the nutritional and functional characteristics of materials [30]. This mold is able to produce lipase enzymes to break down fat, which causes a decrease in fat content. During the fermentation process, *Rhizopus oligosporus* produces amylase, cellulase, and xylanase, which degrade carbohydrates into simple sugars. In addition, during the fermentation there will be proteolytic activity of the mold, which breaks down protein into amino acids, causing an increase in nitrogen [5]. The production of modified Adlay flour by fermentation using *Rhizopus oligosporus* is expected to improve the nutrition and functional characteristics of good flour [31]. The effect of different fermentation duration on Adlay is understudied. To that end, the present study aims to investigate the effect of fermentation using *Rhizopus oligosporus* on the characteristics of Adlay flour within different fermentation duration.

II. MATERIAL AND METHO

A. Materials

The main ingredients used in this study were broken Adlay seeds (*Coix lacryma-jobi L. var. Ma-yuen*) obtained from Tangerang. In addition, the study used several chemicals such as 0.1 N NaOH (KGaA brand, Germany), H2SO4 (Smartlab), n-Hexane (Smartlab), aquades, oil (Bimoli).

B. Method

This study employed a completely randomized design (CRD) consisting of 1 factor, namely the length of fermentation for 0, 12, 24, 30, 36, and 48 hours. The treatment was repeated 3 times. The fermentation of Adlay seeds refers to the method of making modified soybean cake (*tempeh*) [4]. The crushed Adlay seeds (150 g) were washed to remove dirt and other contaminants and soaked in water (1:4) for 24 hours. Next, the seeds were boiled for 5 minutes and drained. Raprima yeast as much as 1% (initial weight of Adlay seeds) was sprinkled on Adlay seeds. Adlay seeds were packed in porous plastic and incubated at ± 33°C for 0, 12, 24, 30, 36, and 48 hours. At the end of the fermentation, samples were taken and blanched at 90°C for 20 minutes to stop the fermentation. Afterward, the samples were sliced into smaller slices. The slices were drained and dried in an oven at 55°C for 24 hours, cooled, and then ground to make fermented Adlay flour. The resulting flour was then sieved with a sieve size of 80 mesh. The flour was packed in plastic bags, tightly closed, and stored in the freezer. The data obtained were analyzed using Analysis of Variance (ANOVA), with a potential difference tested using DMRT (Duncan Multiple Range Test) aided by SPSS.

III. FINDINGS AND DISCUSSION

A. Whiteness

Whiteness indicates the color level of food material. The whiter the flour is, the higher the level of consumer acceptance of the flour becomes [6, 33]. The results of ANOVA (α 0.05) showed that the fermentation duration posed an effect on the decrease in the whiteness of Adlay flour. The difference in the color of the modified Adlay flour is shown in Figure 1 and the whiteness value of the modified Adlay flour is shown in Figure 2.

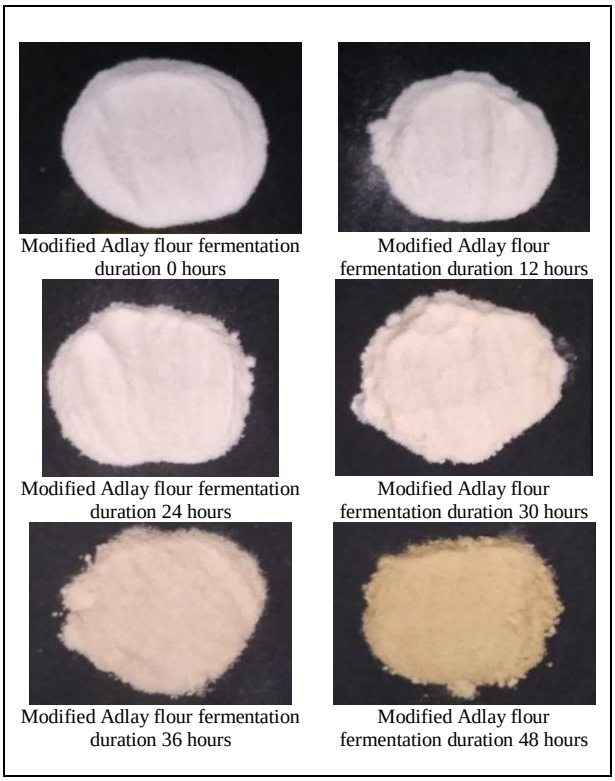

Figure 1. Differences in the color of modified Adlay flour

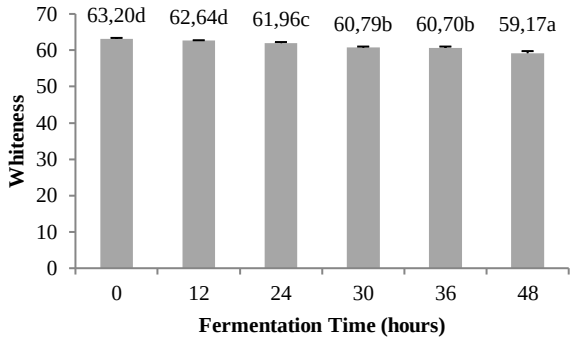

Figure 2. The whiteness of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

The decreased whiteness of the modified Adlay flour was influenced by the mycelia color of the tempeh mushroom which played a role in fermentation. *Rhizopus oligosporus* tends to appear black along with increased fermentation duration [7]. [8] color changes in advanced fermentation are caused by biochemical changes during fermentation, namely: 1) the increasing number of *Rhizopus oligosporus* entering the stationary phase as well as death phase and the activity of spoilage microorganisms; 2) oxidative damage to unsaturated fatty acids (linoleic acid and linolenic acid) resulting from the breakdown of lipids; 3) the presence of vitamin B12 containing cobalt (red).

B. pH

The pH value is one of the quality parameters that affect the flavor and shelf life of the processed product [9]. The results of ANOVA (α 0.05) showed that the difference in fermentation duration posed a significant effect on the pH value of modified Adlay flour. The pH value of modified Adlay flour is shown in Figure 3.

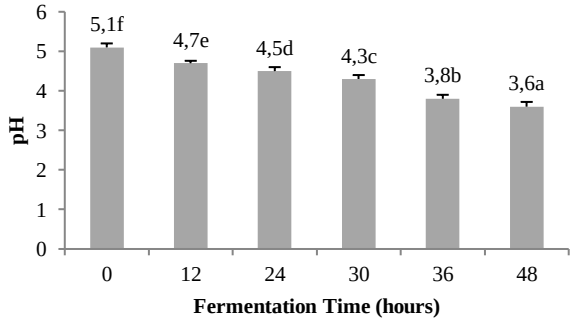

Figure 3. pH of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

Overall, the pH value showed a decrease due to the longer fermentation duration. This is related to the presence of microbial activity that plays a role in the fermentation process. [10] the presence of the fungus *Rhizopus sp* and lactic acid bacteria play a role in tempeh fermentation. Fungi from the genus *Rhizopus sp and* lactic acid bacteria can degrade starch. Simple sugars will be converted into carboxylic acids, especially lactic acid by lactic acid bacteria.

C. Yield

The yield of modified Adlay flour in this study was obtained based on the comparison between the weight of the modified Adlay dry flour produced and the weight of the fresh ingredients. The results of statistical analysis using ANOVA at 95% confidence level (α 0.05) showed that the difference in fermentation duration had a significant effect on the yield value of modified Adlay flour. The yield value of modified Adlay flour is shown in Figure 4.

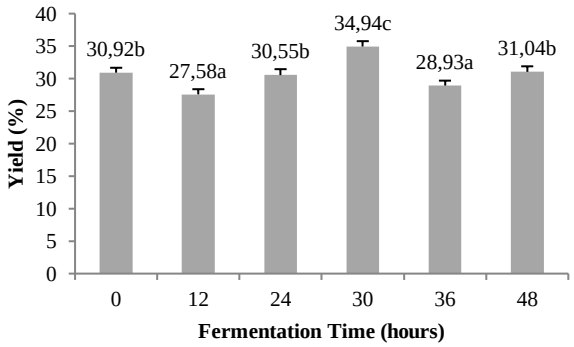

Figure 4. The yield of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

The yield of modified Adlay flour decreased after 36 hours of fermentation. This relates to the increase in the hardness value of Adlay tempeh during the fermentation process caused by the continued reduction in the water content of the material, thereby increasing the compactness of the tempeh constituent particles [11]. The Adlay tempeh becomes more difficult to crush during the grinding process using a blender, so large particles are produced during grinding. The large particles cannot pass through the 80-mesh sieve so that the yield obtained is lower.

D. Water Content

The amount of water content of flour affects the quality and shelf life. The results of statistical analysis using ANOVA at the 95% confidence level (α 0.05) showed that the difference in fermentation duration had a significant effect on the moisture content of modified Adlay flour. The value of the water content of modified Adlay flour is shown in Figure 5.

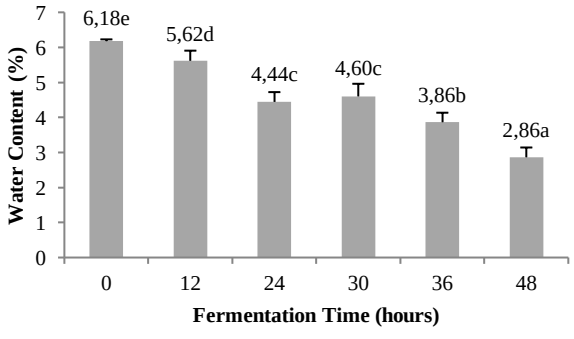

Figure 5. The moisture content of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

The average moisture content of modified Adlay flour ranges from 2-6%, which indicates that modified Adlay flour is a non-perishable food ingredient or food that has a moisture content of less than 13%, namely food that can be stored in a cool, dry and airtight place. [12]. Overall, the water content of modified Adlay flour showed a decrease with the longer fermentation duration. This is caused by the activity of molds that use water for their metabolism. During the fermentation, the complex compounds change into simpler compounds, and the media overhauled by the mold is used as energy for its growth, and some are released into CO2 gas and water vapor (H2O) [13].

E. Ash Content

Ash content is related to the mineral content of a material. The results of statistical analysis using ANOVA at the 95% confidence level (α 0.05) showed that the difference in fermentation duration had a significant effect on increasing the ash content of modified Adlay flour which can be seen in Figure 6.

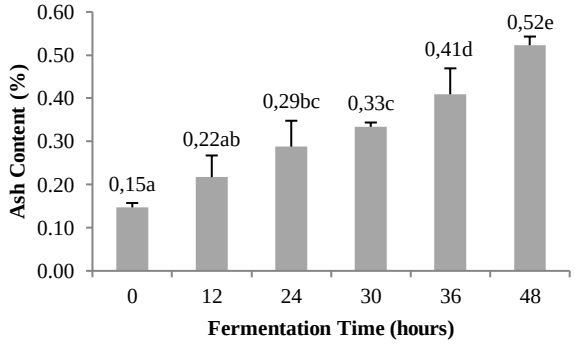

Figure 6. Ash content of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

Rhizopos oligosporus can produce phytase enzymes that can decompose phytic acid (which binds some minerals) into phosphorus and inostol. With the decomposition of phytic acid during fermentation, certain minerals (magnesium, iron, calcium, and zinc) become more available [14].

F. Protein

Protein content is one of the specifications for flour because it is related to water absorption and adhesive strength. The results of ANOVA analysis (α 0.05) showed that the difference in fermentation duration had a significant effect on increasing the protein content of modified Adlay flour. The average value of the modified Adlay flour protein content with the difference in fermentation duration is shown in Figure 7.

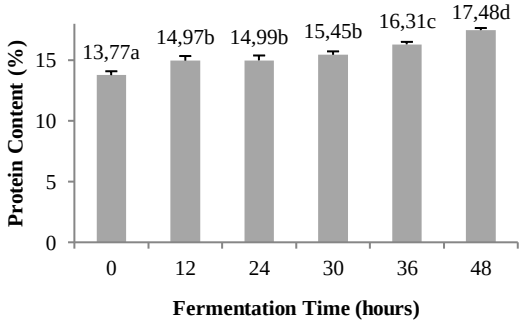

Figure 7. Protein content of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

The increase in protein content can be caused by an increase in the biomass of microbial cells such as fungi from yeast [16]. The protein and N content in yeast were 45% and 57%, respectively [15]. [16] The increase in protein resulted from increased biomass of microbes that grew during fermentation, including mycelium from molds.

G. Fat

The results of ANOVA (α 0.05) showed that the difference in fermentation duration had a significant effect on the fat content of modified Adlay flour as shown in Figure 8.

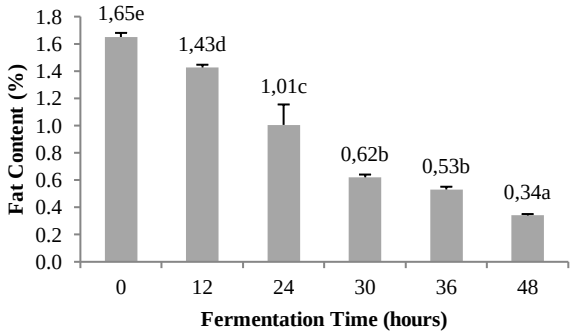

Figure 8. The fat content of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

As the fermentation duration increases, the fat content of Adlay flour decreases. This decrease in fat content is caused by the presence of oxidation and lipase enzyme activity [32]. [16] During the fermentation process, the mold showed high lipase activity and was able to hydrolyze up to 1/3 fat. Fats can be broken down by lipase enzymes through fat catabolism into free fatty acids and glycerol [17]. Furthermore, the glycerol will be converted into glycerol dehyd phosphate and follow the glycolysis pathway to form pyruvate. In contrast, the fatty acids will be broken down into molecules with 2 C atoms and converted into acetyl coenzyme A.

H. Carbohydrate

The results of ANOVA (α 0.05) showed that the difference in fermentation duration had a significant effect on reducing the carbohydrate content of modified Adlay flour, which can be seen in Figure 9.

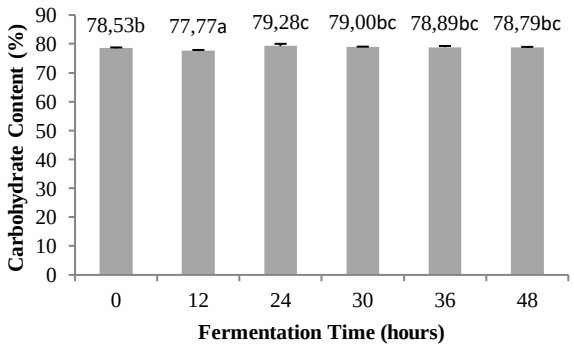

Figure 9. Carbohydrate content of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

The decrease in carbohydrate content in modified Adlay flour could be due to the use of carbohydrates (glucose) as an energy source by microorganisms [18]. [19] during the fermentation process, *Rhizopus oligosporus* produces enzymes amylase, xelulase, xylanase that can degrade carbohydrates, and carbohydrates will be converted into simple sugars. The process of breaking down carbohydrates occurs in an aerobic atmosphere and produces energy (ATP) which is then used for metabolism and fungal growth.

I. Swelling Power

The ANOVA results (α 0.05) showed that the difference in fermentation duration had a significant effect on increasing the swelling power value of modified Adlay flour which can be seen in Figure 10.

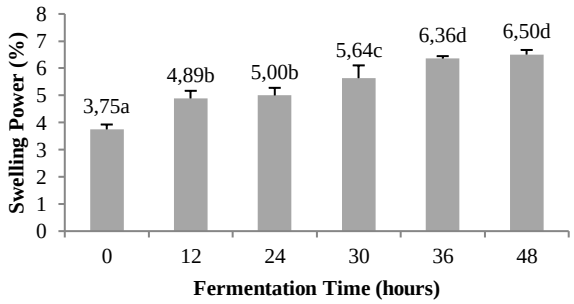

Figure 10. Swelling power of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

This increase in swelling power can be caused by cells released during fermentation which makes amylose and amylopectin come out so that the water-binding part will increase and bind to each other during gelatinization [20]. The porous structure of starch due to the fermentation when heated with water will bind more water and the starch will expand more easily [21]. In addition, there is a relationship between swelling power and fat content which indicates that when the fat content in starch is reduced, swelling will occur more quickly [26].

J. Solubility

In this study, solubility is the ability of flour to dissolve in water. The results of ANOVA (α 0.05) showed that the difference in fermentation duration had a significant effect on increasing the solubility value of modified Adlay flour, which can be seen in Figure 11.

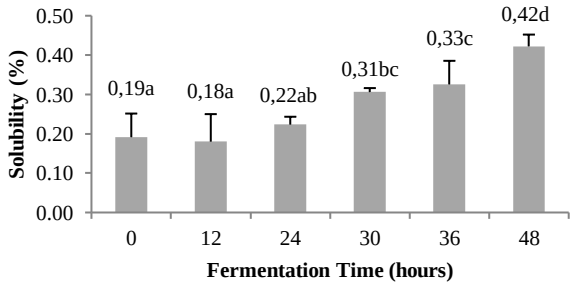

Figure 11. Solubility of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

The increased solubility of modified Adlay flour can be caused by cells released during fermentation, which will cut the starch bonds, so that its structure becomes simpler and also changes to its basic structure, namely glucose [22]. Starch is a polysaccharide insoluble in water, while glucose is easily soluble in water.

K. Oil Holding Capacity (OHC)

The results of ANOVA (α 0.05) showed that the difference in fermentation duration had a significant effect on the OHC value of modified Adlay flour. The average OHC value of modified Adlay flour with differences in fermentation duration ranges from 1.14% to 3.29%, which can be seen in Figure 12.

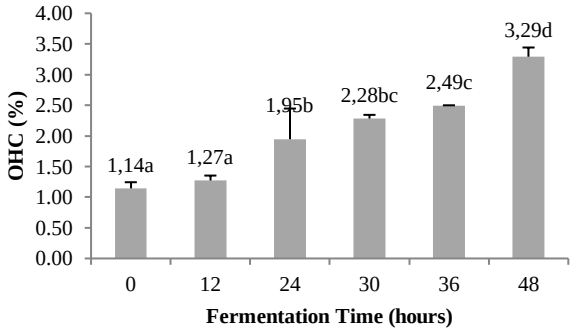

Figure 12. Oil Holding Capacity (OHC) of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

As the fermentation duration increases, the OHC value increases. This can be caused by the liberation of the granules, which can change the starch structure so that its characteristics also change [23]. The rupture of the starch granules will create a hydrophobic structure in the granule, which was originally on the inside to open outward. This causes the starch to have the ability to trap oil [24].

L. Water Holding Capacity (WHC)

The results of ANOVA (α 0.05) showed that the difference in fermentation duration had a significant effect on the increase in the WHC value of modified Adlay flour which can be seen in Table 3. During fermentation, starch granules were broken down, which would change the starch structure from crystalline to amorphous and porous. This increases the ability of starch to trap water because water that enters the material will be trapped in the porous parts [25]. The increase in WHC can also be caused by the modification of macromolecules during the fermentation. This modification exposes the hydrophilic domain of the macromolecule, which has a high affinity for water [24].

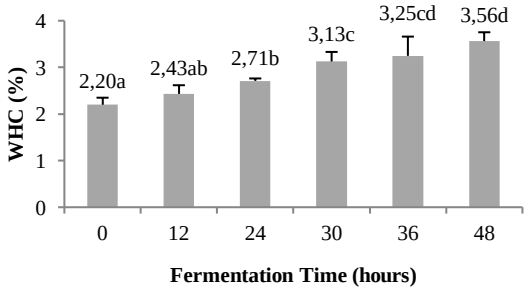

Figure 13. Water Holding Capacity (WHC) of modified Adlay flour with different fermentation durations using *Rhizopus oligosporus*

IV. CONCLUSION

Based on the research results, the present study has concluded that the duration of fermentation using *Rhizopus oligosporus* poses a significant effect on decreasing the value of whiteness, pH, yield, water content, fat, and carbohydrates, but simultaneously increases the ash content, protein content, swelling power, solubility, Oil Holding Capacity, (OHC), and Water Holding Capacity (WHC).

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