Physicochemical properties and sensory evaluation of gluten-free cookies made from fermented adlay (*Coix lacryma jobi* L.) flour using *Rhizopus sp*.

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Physicochemical properties and sensory evaluation of gluten-free cookies made from fermented adlay (Coix lacryma jobi L.) flour using *Rhizopus sp.*


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**ABSTRACT**

Native adlay flour lacks the characteristics that give it grittiness when applied to food products, which can be improved by flour modifications using the fermentation method. This study aimed to evaluate the characteristics of new gluten-free cookies made from native and modified adlay flours and compare them to those made from wheat flour. Adlay flour was modified by fermentation using various *Rhizopus sp.* inoculum concentrations: 0.5%, 1%, and 1.5% (w/w). The modified adlay flour using 1.5% (w/w) *Rhizopus sp.* inoculum was selected for application in making gluten-free cookies because of its most suitable characteristics, including lower pasting temperature, resistance to retrogradation, good thermal stability, and favorable functional properties such as high swelling volume, solubility, and water and oil absorption capacities. In making cookies, the treatments were the type of flour used, namely native adlay flour cookies (NAFC), modified adlay flour cookies (MAFC), and wheat flour cookies (WFC) as a control. All cookies were made using the same recipe; the only difference was the type of flour used. The results showed that MAFC has higher fat and crude fiber however lower protein content than WFC. The MAFC was not significantly different (p ≥ 0.05) from WFC in hardness and fracturability. The sensory evaluation showed that MAFC did not have significant differences (p ≥ 0.05) in texture, mouthfeel, aftertaste, and overall acceptance compared to WFC. Fermentation modification of adlay flour can eliminate grittiness in gluten-free cookies.

1. Introduction

The gluten content in wheat flour is essential for producing good food products. The gluten content in wheat flour has become the main ingredient in various types of commercial and home-processed foods such as noodles, bread, cakes, and various snacks, and gluten plays a role in the characteristics and stickiness of a dough, responsible for cohesiveness, viscosity, and elasticity, and affects the water absorption capacity of the dough (Ortolan & Steel, 2017). Although it has various uses, wheat flour cannot be consumed by some people because of its gluten content, which is dangerous and causes allergies, especially in individuals with celiac disease (Rakmai et al., 2021). Gluten can also cause other disorders, such as non-celiac gluten sensitivity, dermatitis herpetiformis, gluten ataxia, and wheat allergy (Dale et al., 2019). In addition, the ingredients from whole wheat can only be obtained by imports from Australia, Canada, Brazil, Argentina, and Ukraine because of the physical conditions of the Indonesian environment, which are unsuitable for growing wheat. Indonesia primarily imports wheat rather than cultivating it domestically. Indonesia has some wheat milling industries; however, the country’s climate is not conducive to large-scale wheat cultivation. There have been experiments with growing wheat in Indonesia, mainly for research purposes. Despite these efforts, the challenges of adapting wheat cultivation to Indonesia’s tropical climate, along with factors like land availability and agricultural infrastructure, have led to a reliance on imports to meet the country’s wheat demand. Food diversification is necessary...
to increase food independence. Diversifying food sources, especially using local commodities, is a strategy to reduce dependence on imported ingredients such as wheat.

Adlay (Coix lacyma-Jobi L.) is a cereal plant in the Poaceae family and is widely grown in Indonesia. Adlay originates from Asian countries, such as Indonesia and eastern India, and then spreads to other countries, such as China, Egypt, Germany, Haiti, Hawaii, Japan, Panama, Malaysia, Philippines, Taiwan, America, and Venezuela (Mulyono et al., 2019). The main components of adlay seeds are carbohydrates (65%), protein (15%), water (11%), fat (6%), and several vitamins and minerals, such as calcium, phosphorus, niacin, thiamine, and riboflavin (Mulyono et al., 2019), which makes it possible to process it into flour and other processed food ingredients.

Native adlay flour may lack certain characteristics, such as low thermal stability, swelling volume, and water absorption capacity. According to Masahid et al. (2021), the low swelling volume of native adlay flour was caused by the high non-starch component surrounding the starch, which can inhibit water absorption by starch granules and reduce its swelling volume. It caused a gritty texture in the adlay flour and its products. Modifications can be made to adlay flour to address this issue and improve the characteristics of native flour. Several previous studies have reported that modification treatment could improve the starch/flour properties, such as increasing thermal stability, swelling volume, water absorption capacity, and reducing starch retrogradation (Marta, Cahyana, Djali, et al., 2022; Marta et al., 2019; 2021; 2022; 2023) and increase the nutritional such as slowly digestible starch (Marta et al., 2021; Marta, Cahyana, Djali, et al., 2022).

Fermentation is a method for modifying flour, degrading several complex components into simpler ones. Two types of fermentation can be applied: natural fermentation and fermentation using specific yeasts or microbes such as Rhizopus sp. Rhizopus Sp. is commonly used to improve the nutritional and functional characteristics of fermented materials, such as in making tempeh from soybean. Generally, tempeh fermentation could increase crude protein, crude fiber, ash, vitamin soluble protein, amino acid, and antioxidant contents, while decrease antinutrients and crude lipid content (Ahnan-Winarno et al., 2021). Masahid et al. (2021) reported that adlay fermentation using Rhizopus sp. increased solubility, oil-holding capacity (OHC), and water-holding capacity (WHC). During fermentation, Rhizopus sp. produces proteolytic enzymes that break down proteins into amino acids and increase nitrogen levels (Wikandari et al., 2023); starch granules are broken down, transforming the starch structure from crystalline to amorphous and porous (Dou et al., 2023). This alteration enhances the capacity of starch to retain water, as the porous regions effectively trap water molecules that infiltrate the material. Furthermore, modification increases the water-holding capacity (WHC) by modifying the macromolecules during fermentation. This modification exposes the hydrophilic group of the macromolecule, which exhibits strong attraction to water.

Adlay flour is a staple ingredient in various commercially processed foods such as snack bars, noodles, and cookies. Several studies have reported on gluten-free cookies made from non-wheat flour (Ali et al., 2023; Bala et al., 2015; Kumari et al., 2023; Oladunjoye & Alade, 2024). However, studies on the application of modified adlay flour in cookies are limited. Therefore, in this study, native and modified adlay flours were applied to cookies. This study aimed to evaluate the characteristics of new gluten-free cookies made from native and modified adlay flours and compare them to those made from wheat flour. Additionally, this study aimed to gauge consumer perceptions through sensory evaluations of the various formulations examined.

2. Material and methods

2.1. Materials

The raw materials used for this study were cultivated adlay seeds (Coix lacyma-jobi L. var. ma-yuen cv. Watani Wado) obtained from the laboratory of crop production technology, Faculty of Agriculture, Universitas Padjadjaran, Sumedang, Indonesia, and tempeh starter ‘Raprima’ (LIPI Institute, Bandung, Indonesia) that contains Rhizopus sp. was previously identified by Yarlina et al. (2023) using biochemical analysis and PCR test. The medium wheat flour ‘Segitiga Biru’ (PT Indofood Sukses Makmur TbK, Bogasari Flour Mills, Surabaya, Indonesia) as a raw material for control cookies, margarine, milk, powdered sugar, vanilla, and baking powder as ingredients for cookies.

2.2. Native adlay flour preparation

The native adlay flour preparation method was described by Xu et al. (2023) with a slight modification. The polished adlay seeds were dried in an oven at 50°C for 24h and then ground using a miller
machine (Fomax Miller Machine FGD-500) at high speed for 1 min. The milled flour was sieved through a 100-mesh sieve. Native adlay flour was packed and sealed in a polypropylene plastic bag, and a food-grade silica gel packet was given inside to prevent increased water content in the flour. Native adlay flour was assigned the sample code F1.

2.3. Modified adlay flour preparation

Modified adlay flour was prepared according to the method developed by Masahid et al. (2021). Washing and soaking the adlay seeds softened their cell wall, facilitated the boiling process, and removed dirt and contaminants still attached to the adlay seeds. The adlay soaking process was carried out for 24 h at room temperature with a ratio of adlay seeds to water of 1:4. The adlay seeds were then washed, boiled for 5 min, and cooled at room temperature. Then, 0.5%, 1%, and 1.5% of starter (w/w of the initial weight of the adlay seeds) were mixed with adlay seeds and given the sample code F2, F3, and F4, respectively, and placed in a perforated plastic bag. The adlay seeds were incubated in a dark, dry, and closed environment at 33 ± 2 °C for 12 h. After 12 h, the adlay seeds were blanched in boiling water (90 °C) for 20 min to stop the fermentation. After blanching, the adlay was dried using a cabinet dryer at 50 °C for 24 h. The dried adlay was ground using a Miller machine for 1 min, then sieved using a 100-mesh sieve, packed, sealed in a polypropylene plastic bag, and filled with a food-grade silica gel packet to prevent increased water content in the flour.

2.4. Cookies preparation

Cookies were prepared in three formulations: wheat flour cookies (WFc), native adlay flour cookies (nAFc), and modified adlay flour cookies (MAFc), which was fermented using 1.5% starter (Rhizopus sp inoculum). This flour sample was chosen because it has the lowest breakdown and setback, indicating good thermal stability and lower retrogradation tendency. It also has the highest water and oil absorption capacity and swelling volume. The formulations of wheat flour, native adlay flour, and modified adlay flour cookies are presented in Table 1.

Cookies preparation methods were described by Oladunjoye and Alade (2024) with some modifications. The initial stage of cookies production begins with creaming. The creaming step was performed by mixing margarine and sugar and then kneading at high speed until homogeneous consistency was achieved. Liquid milk was then added and stirred until it blended well. The dry ingredients were then added to the flour. Other dry ingredients, such as vanilla and baking powders, were added and sifted before being added to the mixture. The dough was then stirred until all ingredients were evenly mixed. The molding stage was then performed using a round cookies mold with a diameter of 5 cm. The thickness of the dough when printed is 0.5 cm. The cookies were baked at 180 °C for 25 min in a baking oven. After cooling to room temperature, the cookies were immediately stored in an airtight container to maintain crispiness. The resulted cookies were named based on the type of flour used, such as: wheat flour cookies (WFC), native adlay flour cookies (NAFC), and modified adlay flour cookies (MAFC).

2.5. Pasting properties (RVA analysis) of flours

The pasting properties of the flour samples were determined using a Rapid Visco Analyzer (RVA-SM2; Warriewood, Australia). Flour samples (2.8 g) and 25 mL of distilled water were added to the canister. The flour solution was stirred until homogenized, and the canister was placed into the RVA. At the beginning of the analysis, the RVA temperature was initially held at 50 °C for 1 min, then increased from 50 °C to 95 °C at a rate of 6 °C/min, then held at 95 °C for 5 min, and decreased to 50 °C at a rate of 6 °C/min.

2.6. Swelling volume and solubility measurement of flour

The 0.35 g (dry weight) of flour sample was poured into a centrifuge tube filled with 12.5 mL of distilled water and agitated using a vortex mixer for 30 s. The

<table>
<thead>
<tr>
<th>Table 1. Cookies formulations.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation</strong></td>
</tr>
<tr>
<td>WF (control)</td>
</tr>
<tr>
<td>NAF</td>
</tr>
<tr>
<td>MAF</td>
</tr>
</tbody>
</table>

WF = wheat flour; NAF = native adlay flour; MAF = modified adlay flour.
samples were heated at 90 °C for 20 min in a water-bath and cooled in cold water. The samples were centrifuged at 3500×g using a Beckman Model TJ-6 Centrifuge (Block Scientific, New York, USA) for 30 min at 25 °C. The swelling volume was determined by measuring the volume of the supernatant. The samples were then dried in an oven at 110 °C for 24 h to calculate their solubilities (Marta et al., 2022).

2.7. Water and oil absorption capacity (WAC and OAC) measurement of flour

The WAC and OAC of the flour samples were determined according to Marta et al. (2023). 1 g (db) of flour sample was poured into a centrifuge tube containing 10 mL of the distilled water for WAC measurement, and 10 mL of oil for OAC measurement. The samples were stirred using a vortex mixer for 30 s and conditioned at room temperature (26 ± 2 °C) for 1 h. The samples were then centrifuged using a Beckman Model TJ-6 Centrifuge (Block Scientific, New York, USA) at 3500×g for 30 min. The volume of the supernatant was then measured. The WAC was calculated by dividing the volume of water absorbed by the weighted sample (db). The OAC was calculated by dividing the volume of oil absorbed by the weighted sample (db).

2.8. Freeze-thaw stability measurement of flour

The flour paste was prepared by heating the starch solution (8% w/v) at 95 °C for 30 min in waterbath. Subsequently, the flour paste was cooled to 50 °C. Then, 20 g of flour paste was placed into pre-weighed 50 mL centrifuge tubes. The tubes were then sealed, stored at 4 °C for 24 h, then placed in a freezer at −20 °C for 48 h. The frozen flour paste was thawed at room temperature for 4 h and centrifuged at 3500×g for 15 min. The supernatant was then separated and weighed. The percentage of syneresis was calculated by dividing the weight of the supernatant by that of the flour paste (Liu et al., 2020).

2.9. Chemical composition and calories of cookies

The moisture, ash, total protein, fat, carbohydrate, and total crude fiber contents were determined using standard methods AOAC (2005), while calorie analysis was carried out using proximate analysis results. Moisture content was determined using an oven-drying method (method no. 930.05). Protein content was determined using the Kjeldahl method (method no. 978.04). Lipid content was determined using the Soxhlet extraction (method no. 930.09). Crude fiber content was determined using method no. 978.10. The proximate composition and crude fiber content were expressed in g per 100 g of dry base (db) of flour.

2.10. Color evaluation of flours and cookies

The flour color scale for L*, a*, and b* was measured using a Spectrophotometer CM-5 with the Spectra Magic software (Konica Minolta Co., Osaka, Japan). Calibration was performed using a zero-calibration plate (CM-A124) and a white calibration plate (CM-A120) with a large target mask (CM-A203). The color measurements included L* (lightness, 0 = black/100 = white), a* (+a* = redness/-a* = greenness), b* (+b* = yellowness/-a* = blueness), and hue. The whiteness index, ΔE*, and hue angle of flour and cookies were calculated in Equation (1)–(3), respectively,

\[
\text{Whiteness index} = 100 - \sqrt{(100 - L^*)^2 + a^*^2 + b^*^2} \quad (1)
\]

\[
\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)
\]

\[
h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)
\]

Where the ΔL*, Δa*, and Δb* represent the differentials between the color parameter of the sample and the control: native adlay flour (for adlay flour samples) and wheat flour cookies (for cookie samples).

2.11. Texture evaluation (TPA) of cookies

Texture parameters (hardness and fracturability) were evaluated using a Texture Profile Analyzer and exponent lite express software for data collection and calculation (TA.XTExpress Stable Micro System, Surrey, United Kingdom). The product was pressed using an aluminum cylinder probe P36R with a trigger force of 25 g. Each pre-test, test, and post-test speed was 1.5, 2, and 10 mm/s, respectively.

2.12. Hedonic sensory evaluation of cookies

The sensory characteristics were determined using hedonic testing to determine the panelists’ preference level for the sample being tested. Cookies were served
to the panelists in portions of 2–3 g, and separate intact cookies samples were used as references for the appearance of the cookies. The hedonic test used 20 semi-trained panelists, with analysis points for color, aroma, flavor, texture, mouthfeel, aftertaste, and overall acceptance. The sample assessment was carried out on a six-point scale (1 = extremely dislike, 2 = dislike, 3 = slightly dislike, 4 = slightly like, 5 = like, and 6 = extremely like).

2.13. Statistical analysis

Data were expressed as mean ± SD triplicate and analyzed using one-way ANOVA followed by Duncan’s test for physicochemical properties and Kruskal-Wallis test followed by Mann-Whitney test for the sensory evaluation. The Duncan test was used to compare the sample mean at a significance level of 5% (p ≤ 0.05). The data were analyzed using IBM SPSS Statistical Software version 25 (SPSS Inc, Chicago, USA).

3. Results and discussion

3.1. Pasting properties (RVA analysis) of flour

Pasting properties measurement were performed to show the pasting behavior of flour paste as well as the change in starch viscosity, which plays an important role in the application of flour in the food industry. The viscoamylographs of the flour sample are presented in Figure 1, and the pasting parameters of the flour samples, including pasting temperature (PT), peak viscosity (PV), holding viscosity (HV), breakdown (BD), final viscosity (FV), and setback (SB) are presented in Table 2.

The viscoamylograph profile of wheat flour (WF) was similar to native adlay flour (NAF). The modified adlay flour (MAF) exhibits a different viscoamylograph profile from its native counterpart. The MAF demonstrated a curve that tended to remain constant during testing using a Rapid Visco Analyzer (RVA). The MAF has lower PV, BD, and SB, indicating the paste made from MAF has a higher thermal stability. The viscosity of the MAF paste tends to remain unchanged during the heating and stirring processes. This finding was in line with another study by Zhang et al. (2024), where the fermented proso millet flour by Lactobacillus amylovorus has a lower PV, BD, and SB. In this current study, the higher the starter addition in flour modification, the higher the thermal stability of the MAF paste. In contrast, the WF and NAF showed a high PV, followed by the decrease of viscosity during heating phase and an increase viscosity during cooling phase.

![Figure 1. Viscoamylograph of wheat flour, native adlay flour (F1), modified adlay flour with 0.5% (F2), 1% (F3), and 1.5% (F4) mold inoculum starter.](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pasting temperature (°C)</th>
<th>Peak viscosity (cP)</th>
<th>Hold viscosity (cP)</th>
<th>Breakdown viscosity (cP)</th>
<th>Final viscosity (cP)</th>
<th>Setback viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WF</td>
<td>64.30 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1721 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>943 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>778 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1954 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1011 ± 6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1</td>
<td>66.27 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1675 ± 39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1007 ± 14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>667 ± 27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1868 ± 23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>860 ± 10&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>F2</td>
<td>50.20 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>965 ± 59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>929 ± 50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1511 ± 82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>581 ± 31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F3</td>
<td>50.20 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1053 ± 17&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1031 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22 ± 0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1204 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>173 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F4</td>
<td>50.19 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>920 ± 38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>916 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1026 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (p < 0.05). F1 = native adlay flour, F2 = modified adlay flour with 0.5% Rhizopus sp.; F3 = modified adlay flour with 1% Rhizopus sp.; F4 = modified adlay flour with 1.5% Rhizopus sp.
The pasting properties parameter of WF was not significantly different ($p \geq 0.05$) from that of NAF, except for HV and SB, where the SB of NAF was lower than WF. Generally, the modification treatment significantly decreased ($p \leq 0.05$) all the pasting properties parameters of NAF. Modification treatment decreased the PT and PV of NAF. Compared to the MAF, the different levels of *Rhizopus* sp. starter addition did not significantly affect ($p \geq 0.05$) the PT. A lower PT indicates poor resistance to swelling and breakage (Kumar & Khatak, 2017). This might be caused by amylase produced by *Rhizopus* sp. during fermentation, which may breakdown starch molecules into smaller fragments (Olanipekun et al., 2009). Furthermore, Zhang et al. (2024) reported that fermentation of proso millet flour by lactic acid bacteria for 5 days produces porous starch with lower PT and PV values.

Modification treatment significantly reduced ($p < 0.05$) the BD of NAF from 667.67 ± 4.35 cP to 4–35.67 cP and reduced the SB from 860.00 ± 110 to 581.00 cP. The higher the starter addition, the lower the BD and SB of the MAF. Porous starch allows water to enter more easily and makes gelatinization easier. Flour modification treatment can reduce the BD of native flour by 19–166 times. The low BD indicated the high thermal stability of the MAF. In line with BD, the modification treatment also significantly reduced ($p < 0.05$) the SB of the native flour by 1.5–7.8 times, indicating MAF samples had a lower retrogradation tendency (Pasqualone et al., 2010).

### 3.2. Functional properties of flour

The functional properties observed in this study include swelling volume (SV), solubility, water absorption capacity (WAC), oil absorption capacity (OAC), and freeze-thaw stability (% syneresis), which are presented in Table 3. The modification treatments affected all functional properties parameters of the NAF, except for syneresis. The SV, solubility, WAC, and OAC of the modified flour were significantly higher ($p < 0.05$) than those of native flour. The increase in SV was in line with WAC, where a higher WAC suggests that the flour can absorb water more easily, leading to an increased swelling capability. It is an important characteristic in various applications, particularly in baking, where flour needs to absorb water to form dough and rise properly.

The WAC of WF ranges from 1.11 to 1.85 g/g (Marta et al., 2023; Nisar et al., 2020; Thongram et al., 2016; Yadav et al., 2014), which was lower than the WAC of NAF and MAF (2.02–2.08 g/g). The higher WAC in adlay flour might be due to the higher crude fiber content of adlay flour. The crude fiber content of wheat flour was 1.2% db (Chauhan et al., 2016), whereas the crude fiber content of adlay flour was 2.37% db (Ahmad et al., 2024). This result was in line with another study, where the higher WAC of flour was associated with its higher fiber content (Oleyinka & Bassey, 2023). The opposite trend to WAC was that the OAC of WF was higher than that of NAF and MAF. OAC of WF was 1.46–150 g/g (Nisar et al., 2020; Yadav et al., 2014), whereas OAC of NAF and MAF was 1.01 and 1.02–1.09, respectively.

The higher the *Rhizopus* sp. starter addition in the adlay seeds fermentation, the higher the SV, solubility, WAC, and OAC of the modified flour. This finding was in agreement with a previous study, where the WAC and OAC of fermented sorghum flour by *Lactobacillus brevis* were higher than those of native flour (Rahayu et al., 2019). The increased WAC and OAC of the modified adlay flour caused by the production of enzymes during the fermentation process, which can break starch granules into simpler molecules (Chelule et al., 2010). The breakdown of starch granules during fermentation changes the starch structure from crystalline to amorphous and porous (Jorge et al., 2023) and could absorb more water and oil (Bankole et al., 2013). The porous structure of starch due to fermentation, when heated with water, binds more water, and the starch expands more easily (Jorge et al., 2023), which was in line with another study by Zhao et al. (2023). Furthermore, Oloyede et al. (2015) reported that the increase WAC of flour due to the modification of macromolecules during fermentation. This modification increased the

### Table 3. Functional properties of native and modified adlay flours.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Swelling volume (ml/g db)</th>
<th>Solubility (% db)</th>
<th>Water absorption capacity (g/g db)</th>
<th>Oil absorption capacity (g/g db)</th>
<th>Syneresis (% db)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>9.35 ± 0.10</td>
<td>5.63 ± 0.00</td>
<td>2.02 ± 0.01</td>
<td>1.01 ± 0.00</td>
<td>64.12 ± 5.77</td>
</tr>
<tr>
<td>F2</td>
<td>10.12 ± 0.19</td>
<td>6.40 ± 0.00</td>
<td>2.05 ± 0.02</td>
<td>1.02 ± 0.00</td>
<td>62.04 ± 2.34</td>
</tr>
<tr>
<td>F3</td>
<td>10.31 ± 0.12*</td>
<td>6.97 ± 0.00</td>
<td>2.12 ± 0.00</td>
<td>1.06 ± 0.00</td>
<td>60.72 ± 3.27</td>
</tr>
<tr>
<td>F4</td>
<td>10.34 ± 0.02*</td>
<td>7.42 ± 0.00</td>
<td>2.28 ± 0.00</td>
<td>1.09 ± 0.00</td>
<td>60.28 ± 1.16</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different ($p < 0.05$). F1 = native adlay flour, F2 = modified adlay flour with 0.5% *Rhizopus* sp.; F3 = modified adlay flour with 1% *Rhizopus* sp.; F4 = modified adlay flour with 1.5% *Rhizopus* sp.
hydrophilic group of the macromolecule, which has a high affinity for water.

Fermentation treatment increases swelling volume, in line with another study on fermented lentil and quinoa grains by *Pleurotus ostreatus* (Badia-Olmos et al., 2024). Whereas the increased solubility of MAF might be due to the breaking of amylopectin bonds to amyllose during fermentation, where amyllose is water-soluble. During fermentation, branched amylopectin chains are broken, thereby increasing the linear chains (amylose) (Yuan et al., 2008). Amylose with shorter chain dissolves easily in water; the higher the amyllose leaching, the higher the solubility. The fermentation treatment did not have a significant effect (*p* ≥ 0.05) on the syneresis of NAF.

### 3.3. Whiteness index and color difference of flour

The color characteristics of flour were affected by several factors, including flour preparation and the presence of non-starch components, such as fat, protein, ash, and phenolics, etc. Color measurements using a chromameter CM-5 produced L, a*, and b* values to calculate the whiteness index (WI) and color difference (ΔE*) (Table 4). The WI of the MAF was significantly lower (*p* < 0.05) than that of NAF. The higher the starter addition, the lower the WI of the MAF. The WI of the NAF decreased from 54.64 to 52.78–53.48. ΔE* is the difference between two colors (NAF and MAF) in the L*a*b* color space, where the lower the ΔE* value, the more similar the color between two flour samples. The MAF using 1.5% starter had the lowest ΔE* value, indicating a color similar to that of NAF. The modified flour used to make cookies is treated with 1.5% starter. Although its WI was lower than WF and NAF however its ΔE* value was closest to NAF. Additionally, this flour exhibited good heat stability and a lower tendency to retrograde, along with having higher WAC and OAC. The decrease in the WI of the MAF was affected by the color of the fungal mycelia, which played a role in fermentation. *Rhizopus sp.* tended to appear black as the fermentation time increased. According to Muzdalifah et al. (2017), biochemical changes cause a color change during fermentation, with an increasing number of *Rhizopus sp.* entering the stationary phase as well as the death phase, and the activity of spoilage microorganisms, oxidative damage to unsaturated fatty acids (linoleic acid and linolenic acid) due to lipid breakdown, and the presence of vitamin B₁₂, which contains cobalt (red).

### 3.4. Chemical composition and total caloric value of cookies

The chemical composition and total caloric value of the gluten-free cookies were analyzed by testing the moisture, ash, fat, protein, crude fiber, carbohydrate content, and energy values. The chemical compositions and total caloric values of the cookies are listed in Table 5.

The moisture content of NAF had not significantly different (*p* ≥ 0.05) from WFC) and was significantly lower (*p* < 0.05) than MAFC. NAF and MAFC have lower protein and higher fat and crude fiber content than WFC. In comparison, MAFC has higher protein but lower fat and ash content than NAFC.

MAFC had higher moisture content than NAFC, which might be caused by the absorption of water experienced by adlay seeds during the boiling process and post-fermentation blanching during flour modification. However, the moisture content of the NAF had not significantly different (*p* ≥ 0.05) from WFC. All cookie samples had moisture content below 5%. The moisture content of dry biscuits with a good shelf life is less than 5% (Giuberti et al., 2015).

MAFC had higher protein content than NAFC. These results were in line with another study by

### Table 4. Whiteness index and color difference of native and modified adlay flours.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Whiteness Index</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>54.64 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>53.48 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.71 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F3</td>
<td>52.82 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F4</td>
<td>52.78 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (*p* < 0.05). F1 = native adlay flour, F2 = modified adlay flour with 0.5% *Rhizopus sp.*, F3 = modified adlay flour with 1% *Rhizopus sp.*, F4 = modified adlay flour with 1.5% *Rhizopus sp.*

### Table 5. Chemical composition and total caloric value cookies made from wheat, native and modified adlay flours.

<table>
<thead>
<tr>
<th>Cookies</th>
<th>Water (% db)</th>
<th>Protein (% db)</th>
<th>Fat (% db)</th>
<th>Ash (% db)</th>
<th>Crude Fiber (% db)</th>
<th>Energy (kcal/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFC</td>
<td>3.35 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.13 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.67 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.61 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>482.24 ± 3.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NAF</td>
<td>3.13 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.30 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.55 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.95 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.19 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>507.20 ± 4.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MAFC</td>
<td>3.70 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.17 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.32 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.58 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>490.18 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (*p* < 0.05). Cookies made from wheat flour (WFC), native adlay flour (NAF), and modified adlay flour (MAFC).
h. Marta et al., who found an increase in protein content in biscuits due to microbial cell biomass, such as mold derived from yeast used in the fermentation process. Furthermore, Nout and Kiers (2005) reported that the fermentation process triggers nitrogen hydrolysis activity through proteases, thus increasing the amount of dissolved protein and amino acids in sorghum.

MaFc has a lower fat content than NaFc, which might be due to the fermentation process could reduce the fat content of adlay flour, influenced by the activity of lipase, which oxidizes fat, where the fat is broken down into fatty acids and glycerol (Cuevas-Rodríguez et al., 2004). The lower fat content in the MaFc resulted in a lower total calorie than NaFc, however it was not significantly different (p ≥ 0.05) from WFc.

3.5. Color evaluation of cookies

Color characteristics were measured on the top sides of the cookies, including L*(lightness), a* (redness), b* (yellowness), °hue, and ΔE. The visual of cookies sample is presented in Figure 2 and the color parameters of the cookies are presented in Table 6.

Table 6. Color’s parameters of cookies made from wheat, native and modified adlay flours.

<table>
<thead>
<tr>
<th>Cookies</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>°Hue</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFC</td>
<td>74.15 ± 1.26c</td>
<td>5.03 ± 0.30a</td>
<td>36.77 ± 1.48c</td>
<td>82.19 ± 0.74c</td>
<td>0c</td>
</tr>
<tr>
<td>NAFC</td>
<td>67.02 ± 1.20b</td>
<td>7.42 ± 0.38b</td>
<td>28.59 ± 0.42a</td>
<td>76.11 ± 1.15a</td>
<td>2.26 ± 0.08b</td>
</tr>
<tr>
<td>MAFC</td>
<td>63.07 ± 0.98b</td>
<td>6.43 ± 0.36c</td>
<td>31.24 ± 0.62b</td>
<td>78.96 ± 0.96b</td>
<td>1.85 ± 0.21b</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (p < 0.05). Cookies made from wheat flour (WFC), native adlay flour (NAFC), and modified adlay flour (MAFC).

Cuevas-Rodríguez et al. (2004), who found an increase in protein content in biscuits due to microbial cell biomass, such as mold derived from yeast used in the fermentation process. Furthermore, Nout and Kiers (2005) reported that the fermentation process triggers nitrogen hydrolysis activity through proteases, thus increasing the amount of dissolved protein and amino acids in sorghum.

MAFc has a lower fat content than NaFc, which might be due to the fermentation process could reduce the fat content of adlay flour, influenced by the activity of lipase, which oxidizes fat, where the fat is broken down into fatty acids and glycerol (Cuevas-Rodríguez et al., 2004). The lower fat content in the MAFc resulted in a lower total calorie than NaFc, however it was not significantly different (p ≥ 0.05) from WFc.

3.6. Texture evaluation of cookies

The texture properties parameters of cookies, including the hardness and fracturability are presented in Table 7. Hardness and fracturability are textural properties of bakery products that are closely related to consumers’ perceptions of product freshness (Kuchtová et al., 2018).

The results showed that the hardness and fracturability of MAFC were not significantly different (p ≥ 0.05) from those of WFC. NAFC has lower hardness and fracturability than WFC and MAFC. The MAF had a lower SB than the NAFC. It is desirable to make bakery products, where bakery products, in these case cookies, do not easily undergo retrogradation during storage to maintain the stability of the texture of the cookies. The lower SB of modified adlay flour showed a lower retrogradation tendency, affecting the final product’s firmness (Pasqualone et al., 2010). In this study, the SB was not positively correlated to the hardness of cookie samples. MAF had the lower SB than WF and NAFC; however, MAFC had a higher hardness than NAFC and was not significantly different (p ≥ 0.05) from WFC. This might be due to non-starch components such as protein and crude fiber content on MAF. The hardness of the food product could be affect by the protein content (Hussein et al., 2021; Min et al., 2010) and crude fiber

Table 7. Texture’s parameters of cookies made from wheat, native and modified adlay flours.

<table>
<thead>
<tr>
<th>Cookies</th>
<th>Hardness (gF)</th>
<th>Fracturability (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFC</td>
<td>1566.51 ± 32.30b</td>
<td>6.17 ± 0.21b</td>
</tr>
<tr>
<td>NAFC</td>
<td>1468.28 ± 24.73a</td>
<td>4.55 ± 0.45a</td>
</tr>
<tr>
<td>MAFC</td>
<td>1564.34 ± 14.39b</td>
<td>5.70 ± 0.58b</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (p < 0.05). Cookies made from wheat flour (WFC), native adlay flour (NAFC), and modified adlay flour (MAFC).
content (Singh et al., 2013). These results were in line with the protein and crude fiber content of cookies (Table 5). Furthermore, Aidoo et al. (2022) have reported that flours with a lower tendency for retrogradation typically have better moisture retention and produce baked goods with a good texture and improved shelf life. It maintained moisture better, resulting in softer, and fresher baked goods for a more extended storage period.

When subjected to force, cookies fracturability refers to the ease of breaking or fracturing cookies. The fracturability of MAFC was more similar to that of WFC, where the fracturability of both cookies was not significantly different (p ≥ 0.05). Fracturability is a textural attribute often evaluated in sensory analysis and is an important aspect of overall texture and mouthfeel.

### 3.7. Hedonic sensory test of cookies

Hedonic evaluation involves assessing attributes like color, aroma, texture, flavor, mouthfeel (gritty taste), aftertaste, and overall acceptance. The panelists expressed their preferences for cookie qualities using a 6-point hedonic scale. The results of the panelists’ preferences for cookies are presented in Table 8, and the spider web obtained from the sensory test represents six attributes, as shown in Figure 3.

<table>
<thead>
<tr>
<th>Cookies</th>
<th>Color</th>
<th>Aroma</th>
<th>Texture</th>
<th>Flavor</th>
<th>Mouthfeel</th>
<th>Aftertaste</th>
<th>Overall acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFC</td>
<td>4.53 ± 1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.29 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.29 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.41 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.77 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.39 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.29 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NAFC</td>
<td>3.59 ± 0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.82 ± 0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.18 ± 0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.24 ± 0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.59 ± 1.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.18 ± 0.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MAFC</td>
<td>3.95 ± 1.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.83 ± 1.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.75 ± 1.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.41 ± 0.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.59 ± 0.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.42 ± 0.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.78 ± 0.95&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts are significantly different (p < 0.05). Cookies made from wheat flour (WFC), native adlay flour (NAFC), and modified adlay flour (MAFC).

The color and aroma preferences of cookies made from both NAF and MAF were significantly lower (p < 0.05) than those of WFC (control). Meanwhile, the texture, mouthfeel, aftertaste, and overall acceptance of MAFC were not significantly different (p ≥ 0.05) from those of WFC. The texture preference of the MAFC and WFC ranged from 4.75 to 5.29, which indicated that the panelist liked the sample textures. Like texture preference, panelists also like mouthfeel, aftertaste, and overall acceptance of MAFC and WFC. The NAFC had a lower liking value than the other cookies, especially for color, flavor, mouthfeel, aftertaste, and overall acceptance. It suggests that fermenting adlay flour using *Rhizopus* sp. mold enhances its functional qualities and improves its taste and texture, making it more similar to traditional WFC. Furthermore, Rizzello et al. (2017) reported a similar trend, in which fermenting faba bean flour using *Lactobacillus plantarum* at 30 °C for 24 h offers notable advantages over unfermented faba bean flour, as fermentation improves the nutritional content and sensory characteristics of pasta.

The spider web diagram enables visual representation of complex organoleptic profiles. Each axis within the web represents a specific sensory attribute, and the values obtained for these attributes facilitate an easy direct comparison between different products or formulations (Elmacı et al., 2007).

As shown in Figure 3, the aroma of the NAFC had a lower flavor preference level than the MAFC. The savory taste might cause a higher preference level for MAFC due to protein hydrolysis during fermentation. MAFC and NAFC were influenced by the unpleasant aroma of phenol in adlay seeds, where adlay contained aromatic compounds in the form of phenols, which could give adlay a distinctive aroma. The unpleasant aroma of adlay can also be produced through the activity of lipoxygenase, which reacts with fat during the grinding process of adlay seeds.

Based on texture, NAFC showed grittiness when consumed, where the panelists did not like. It was due to the low SV of NAF. According to Dechkunchorn and Thongngam (2016), the protein and fat in adlay flour have the potential to inhibit granule solubility and reduce flour viscosity. The inhibition of solubility of starch granules will result in a rough and
MAF decrease their gritty taste because the modification affects their starch properties. These data were in line with SV and WAC, where the SV and WAC of the MAF were higher than those of the NAF. The mouthfeel preference level of MAFC was higher than that of NAFC, which was caused by reduced protein content and can increase swelling power, degree of starch hydrolysis, and enthalpy of gelatinization in modified adlay flour (Ding et al., 2021).

Based on overall acceptance, MAFC through fermentation did not show a significant difference (p ≥ 0.05) in acceptance compared to WF. Fermentation can enhance the organoleptic properties of adlay flour, including its aroma, taste, and texture, thereby making the final product either more preferred or at least as well accepted as those made from NAF. Fermentation of cassava flour using lactic acid bacteria frequently yields a more complex flavor and more pleasing texture, which can affect consumer acceptance (Kresnowati et al., 2019).

The texture characteristics analyzed using instruments show similar trends to texture preferences analyzed through hedonic testing. The hardness and fracturability of WF and MAF were not significantly different (p ≥ 0.05), nor were the texture preference. However, differences arise when comparing NAF with MAF. In instrument-based texture analysis, the hardness and fracturability of NAF and MAF were significantly different (p < 0.05), whereas, in texture preference testing, NAF and MAF did not exhibit significant differences (p ≥ 0.05). Instrument tests are often more sensitive than organoleptic tests in many instances. Organoleptic assessments rely on human senses such as taste, smell, touch, and sight to evaluate qualities like flavor, texture, color, and aroma. Although these evaluations can yield valuable insights, they are subjective and susceptible to influences such as individual sensitivity and bias.

4. Conclusions

MAF using fermentation by Rhizopus sp. mould can improve its pasting properties, such as increasing thermal stability, reducing PT, and inhibiting starch retrogradation, with the level of improvement corresponding to the amount of inoculum starter used. The pasting properties of NAF were more similar to WF, where all pasting parameters of NAF were not significantly different (p ≥ 0.05) from WF, except for SB. MAF exhibited higher SV, SOL, WAC, and OAC than its native counterpart, making it advantageous for cookie production. MAF with 1.5% inoculum starter was chosen for application in cookies because it had the most suitable characteristics, including lower PT, resistance to retrogradation, good thermal stability, and favorable functional properties (high SV, solubility, WAC, and OAC) compared to other MAFs. MAF has higher fat and crude fiber but lower protein content than WFC. The hardness and fracturability of the MAFC were not significantly different (p ≥ 0.05) from those of the WFC. According to sensory evaluation, MAFC showed no significant differences (p ≥ 0.05) in texture, mouthfeel, aftertaste, and overall acceptance compared to WFC. In other words, applying modified flour in cookie production can eliminate the grittiness in NAFC.

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Herlina Marta: Conceptualization, Funding acquisition, writing—review and editing, writing—original draft, Visualization, Supervision, Methodology, Formal analysis, and data curation. Ilman Noor Firmansyah: Investigation, Formal analysis, Visualization, Project administration. Christine Febiola: Writing—original draft, formal analysis, visualization. Putri Reina Artata: Formal analysis, data curation, writing—review, and editing. Tri Yulliana: Writing—original draft, Validation, Data curation, and supervision. Yana Cahyana: Methodology, Investigation, Writing—Original Draft, Supervision. Vira Putri Yarlina: Software, Validation, Writing—review and editing, supervision. Mohamad Djali: Methodology, Conceptualization, Supervision, Data curation. Tati Nurmala: Conceptualization, Validation, Supervision, Methodology, Funding acquisition.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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**Data availability statement**

The data that support the findings of this study are available from the corresponding author [H.M.] upon reasonable request.

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