

Effects of fermentation with lactic acid bacteria from *wikau maombo* on the physicochemical characteristics of sago (*Metroxylon sagu*) flour, and its application in crackers

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Article history

Received: 13 July 2020

Received in revised form:

1 January 2021

Accepted:

7 May 2021

Keywords

fermentation,

LAB,

wikau maombo,

sago,

crackers

Abstract

In the present work, sago was fermented with lactic acid bacteria (LAB) isolated from *wikau maombo*, and used in the production of crackers. The effect of fermentation period, LAB strain, and inoculum concentration on the properties of the flour were investigated. Results showed that the best fermentation treatment was by using LAB UM1.3A with OD 0.75 for 48 h. The fermented flour had a swelling power of 11.39 g/g, water solubility index of 17.58%, pH of 6.32, and distinctive crystallinity and pasting properties as compared to native flour (unfermented; control). Crackers produced from the fermented flour contained higher fat, protein, and crude fibre than those produced from native sago flour. These crackers were salty and crispy. The degree of acceptability of the crackers made from fermented flour was comparable to the crackers made from wheat flour.

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Introduction

The consumption rate of wheat flour-based products such as biscuits, noodles, and breads in Indonesia are increasing over the years (Yanuarti and Afsari, 2016). As a result, Indonesian wheat import values increased from 7.412 million tons in 2015 to 10.1 million tons in 2018 (Statistics Indonesia, 2020). However, wheat flour has a negative health impact on certain population groups due to gluten. Gluten, in excessive amounts, may cause health problems such as bloating, digestive disorders, and irritable bowel syndrome (APA, 2013). Gluten-free food is in demand especially for people suffering from celiac disease (Green and Cellier, 2007).

One of the most abundant sources of carbohydrates and has the potential to replace wheat flour in Indonesia is sago (*Metroxylon sagu*). Sago is also one of the staple foods consumed by people in the Southeast Sulawesi Province. According to the Directorate General of Estate Crops (2019), the area of sago plantation reached 5,105 ha in this province with a production of 6,967 tons. However, native sago starches have several undesirable characteristics such as being too sticky, not resistant to acid treatment, hard-formed, non-translucent paste, and poor in

nutritional content as compared to starch. This causes limited use of sago flour in the food industry. Therefore, the modification of this flour is important to broaden its application as a food ingredient.

One modification that can be done on flour to improve its properties is fermentation with lactic acid bacteria (LAB). Setiarto *et al.* (2018) reported that taro fermented with LAB combined with autoclaving improves the functional properties of flour such as an increase in resistant starch and prebiotic properties. Ogodo *et al.* (2019) reported that sorghum fermented with LAB can increase the nutritional content of flour and digestibility of protein and its starch. LAB is also useful for improving the quality and safety of food ingredients through natural inhibition of pathogenic microorganisms (Ogodo *et al.*, 2019).

LAB can be found naturally in high carbohydrate food products such as cassava. One of the local food products from Buton, Southeast Sulawesi, Indonesia made from cassava is *wikau maombo*. In the production of *wikau maombo*, cassava is soaked in sea water, and naturally fermented at room temperature for three days. LAB isolates are found in *wikau maombo* during fermentation (Herlina, 2016). According to Elvira *et al.* (2016), LAB strains UM1.3A and UM1.4A

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isolated from *wikau maombo* contained amylase that can digest starch cell walls, resulting in the release of starch granules, which is a potential for flour modification.

In the present work, the sago flour was fermented with LAB strains UM1.3A and UM1.4A isolated from *wikau maombo*. The present work aimed to reveal the potential of LAB isolated from Indonesian food product, since this was the first study that used LAB strains from *wikau maombo* to modify sago flour. Extensive analyses have been done to characterise the physicochemical properties of the modified flour and its application in the production of crackers.

Materials and methods

Materials

Sago was purchased from the local market in the form of semi-wet solids. Lactic acid bacterial (LAB) strains UM1.3A and UM1.4A were obtained from the Phytopathology Unit of the Plant Protection Laboratory, Halu Oleo University, Kendari, Indonesia. Rejuvenation of bacterial isolates was done by the spread-plating method. Other chemicals used were standard solution of protein, hexane, bovine serum albumin, Biuret solution, and ethanol.

Sago fermentation

Sago was washed with distilled water, and the sediment was kept in a sterilised glass container. It was inoculated with either LAB UM1.3A or UM1.4A (OD 0.75 or 2.69×10^9 CFU/mL) at 10% v/w. Fermentation was done for 24, 48, and 72 h at 37°C. At the end of fermentation, sago was washed with distilled water, and oven-dried at 60°C for 24 h. After drying, the sago was milled and sieved (80-mesh) to obtain the modified sago flour. The yield of the flour was approximately 68.75%. The optimum fermentation time was determined based on the swelling power and water solubility index (WSI) of the modified sago flour.

After the optimum fermentation period was determined, the experiment was repeated with the variation on the type and concentration of isolates. The types of isolates were U1.3A, UM1.4A, and the combination of both; while the concentrations of inoculum were OD 0.50 (2.25×10^6 CFU/mL), OD 0.75 (2.69×10^9 CFU/mL), and OD 1.00 (3.21×10^{12} CFU/mL).

Characterisation of sago flour

Swelling power, WSI, pH, pasting properties, morphology, and chemical profile of the

sago flour were analysed. Swelling power and WSI were determined by weighing 0.5 g of flour, mixing it with 10 mL of distilled water in a test tube, heating at 85°C for 30 min, and centrifuging at 2,000 rpm for 30 min. Swelling power was the ratio between the weight of sludge left in the tube with the sample dry weight, whereas WSI was the percentage of weight of starch which was soluble in water. pH of flour in water suspension (10% w/v) was determined using a Jenway 3505 pH meter (UK). Morphology was determined using a scanning electron microscope (SEM Philips XL30) with gold coating. Fourier transform infrared spectroscopy (FTIR) was used for chemical profiling of the flour. Sago flour and KBr were made into a pellet (Shimadzu, Tokyo, Japan). The sample spectrum was read with FTIR ABB MB3000 (Clakuaeset Scientific, Northampton, UK) and a DTGS detector in the region of 4000 - 400 cm^{-1} with a resolution of 4 cm^{-1} .

Cracker production

Three types of flour, *i.e.*, modified sago flour, native sago flour, and wheat flour were used to make crackers. The cracker-making process was adopted from Wulandari and Handarsari (2010). Briefly, 40 g of margarine, 40 g of refined sugar, 0.5 g of baking powder, and 0.5 g of baking soda were mixed and stirred using a mixer for 5 min. Next, 20 g of egg yolks were added to the mixture, and stirred for another 10 min. Lastly, 100 g of flour and 0.5 g of salt were added, and stirred until homogeneous. The dough was then rolled and cut into a rectangular shape with uniform dimension, and oven-baked at 130°C for 30 min.

Characterisation of crackers

Crackers were analysed for their sensory properties and nutritional content. Sensory analysis was performed by 30 untrained panellists. The test included hedonic test on colour, aroma, and overall acceptability; and descriptive test included texture, level of saltiness, and level of sweetness. Categorical scales were used, and are described in Table 1. The nutritional content of the crackers was evaluated by measuring moisture content by the oven drying method (method 925.10; AOAC, 2005), ash content by the gravimetry method (method 923.03; AOAC, 2005), protein content by the Biuret method (method 935.11; AOAC, 2005), fat content by the Soxhlet method (method 920.85; AOAC, 2005), crude fibre content (Sudarmaji *et al.*, 1984), and carbohydrate (AOAC, 2005).

Table 1. Categorical scales for sensory analysis.

| Score | Colour | Aroma | Texture | Level of saltiness | Level of sweetness | Overall |
|-------|--------------|--------------|---------------|--------------------|--------------------|--------------|
| 1 | Very dislike | Very dislike | Very tough | Not salty | Not sweet | Very dislike |
| 2 | Dislike | Dislike | Tough | Less salty | Less sweet | Dislike |
| 3 | Rather like | Rather like | Rather crispy | Rather salty | Rather sweet | Rather like |
| 4 | Like | Like | Crispy | Salty | Sweet | Like |
| 5 | Very like | Very like | Very crispy | Very salty | Very sweet | Very like |

Statistical analysis

All experiments were done in triplicate. The obtained data were analysed with one-way analysis of variance (ANOVA) and Tukey’s *post hoc* test ($\alpha = 95\%$) using Minitab Pro 16.2.0.0.

Results and discussion

Effects of fermentation time on the flour properties

Fermentation times which affected the swelling power, WSI, and pH of modified sago flour are shown in Figure 1. Swelling power and WSI are known to have a positive correlation to the expansion of a starch-based product (Cheow *et al.*, 2004). The swelling power increased slightly when sago flour was fermented for 48 h by both UM13.A and UM14.A isolates, from 8.8 to 10.9 - 11.4 g/g, respectively. However, the swelling power decreased to almost the same value as the control (native flour) when the fermentation continued to 72 h. According to Numfor *et al.* (1995), the proportion of

amylopectin in the amorphous area decreases, and the ratio of short-chain amylose increases. Amylose contents are also known to be negatively related to swelling power and WSI (Numfor *et al.*, 1995).

Fermentation for 48 h also increased the WSI of flour for both isolates (Figure 1). WSI of control was 10.67%, whereas WSI of 48 h fermented flour was 17.20 - 17.58%. Theoretically, during fermentation, LAB would hydrolyse starches into short chain polymers, and a prolonged fermentation would produce more glucose and acids that are highly water soluble. However, results in Figure 1 shows that WSI slightly decreased when fermentation was extended to 72 h. This was probably due to the fact that water-soluble products leached out when the flour was washed after fermentation, thus, they were not counted in WSI analysis. This hypothesis was supported by a uniform pH value between samples, regardless of the difference in the fermentation time. Organic acids, such as acetic acid, formed during fermentation may

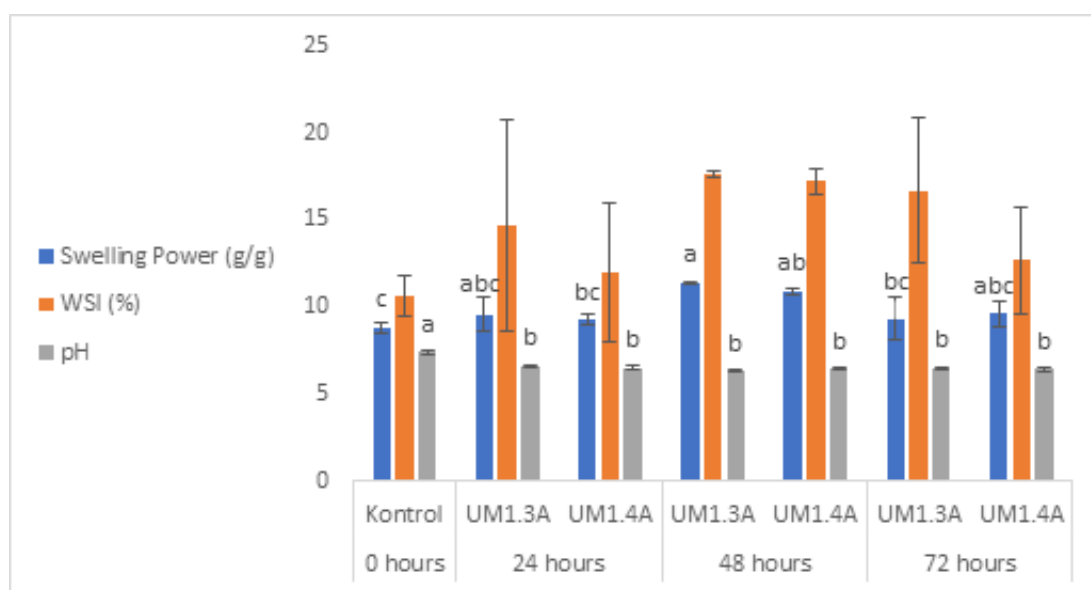


Figure 1. Effects of sago flour fermentation time on flour swelling power, water soluble index (WSI), and pH. Different lowercase letters indicate significant differences at 95% confidence level. WSI values are not statistically different between treatments ($p > 0.05$).

also dissolve in washing water.

In addition to the swelling power and WSI, the functional group of sago flour was also evaluated by FTIR. The FTIR spectrum of sago flour is shown in Figure 2. The length of fermentation did not affect the functional groups of sago flour. All samples contain the same functional groups at the same wave number, namely O-H stretching in the range of 3000 - 3700 cm^{-1} , C=O stretching at 1651.07 cm^{-1} , and C-O-C stretching at 987.55 cm^{-1} (Silfia, 2012) (Figure 2). This indicated that there was no change in the main functional group due to the flour modification.

Effects of type and concentration of isolates on the properties of sago flour

Based on the fermentation time analysis results, it can be concluded that fermentation for 48 h was the optimum time at which the flour had maximum swelling power and WSI. Therefore, fermentation for 48 h was used in studying the effect of isolate type and concentration on the properties of sago flour.

The type and concentration of LAB isolates slightly varied with the value of swelling power, WSI, and pH of the flour as shown in Table 2. The ranges of swelling power were between 10.28 - 11.39 g/g, WSI of 16.65 - 17.58%, and pH of 6.32 - 6.70. The difference between the lowest and the highest values was approximately or smaller than one. This shows that during 48 h of fermentation, the activity of UM1.3A and UM1.4A LAB isolates was

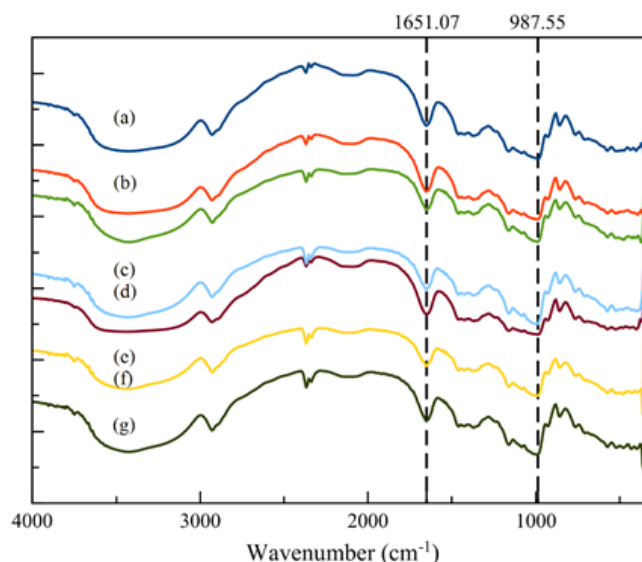


Figure 2. FTIR spectrum of sago flour: (a) Control, (b) UM1.3A_24 h, (c) UM1.3A_48 h, (d) UM1.4A_24 h, (e) UM1.3A_72 h, (f) UM1.4A_48 h, and (g) UM1.4A_72 h.

relatively similar in all concentration levels. However, the variation was statistically different ($p < 0.05$), and there was one treatment that consistently yielded the highest value for swelling power and WSI, which was M1B2, sago flour that was fermented with LAB UM1.3A and OD concentration of 0.75 for 48 h.

Further analysis which consisted of morphological analysis, pasting properties, and crystallinity was carried out specifically to compare M1B2 samples to the control sago flour (Figure 3). M1B2 starch granules were flatter and more

Table 2. Effects of type and concentration of LAB isolates on the swelling power, water solubility index, and pH of modified sago flour.

| Treatment | Swelling power (g/g) | WSI (%) | pH |
|---------------------------------|----------------------------|----------------------------|---------------------------|
| M1B1 (LAB UM1.3A; OD 0.50) | 10.77 ± 0.01 ^c | 17.02 ± 0.08 ^c | 6.70 ± 0.09 ^a |
| M2B1 (LAB UM1.4A; OD 0.50) | 10.36 ± 0.02 ^d | 16.69 ± 0.06 ^{de} | 6.50 ± 0.12 ^c |
| M3B1 (LAB combination; OD 0.50) | 10.34 ± 0.09 ^{de} | 16.65 ± 0.11 ^e | 6.41 ± 0.08 ^c |
| M1B2 (LAB UM1.3A; OD 0.75) | 11.39 ± 0.01 ^a | 17.58 ± 0.04 ^a | 6.32 ± 0.01 ^c |
| M2B2 (LAB UM1.4A; OD 0.75) | 10.89 ± 0.69 ^b | 17.20 ± 0.02 ^b | 6.45 ± 0.04 ^c |
| M3B2 (LAB combination; OD 0.75) | 10.73 ± 0.02 ^c | 17.00 ± 0.05 ^c | 6.46 ± 0.04 ^c |
| M1B3 (LAB UM1.3A; OD 1.00) | 10.76 ± 0.02 ^c | 17.01 ± 0.04 ^c | 6.54 ± 0.10 ^b |
| M2B3 (LAB UM1.4A; OD 1.00) | 10.38 ± 0.02 ^d | 16.78 ± 0.04 ^d | 6.54 ± 0.02 ^{bc} |
| M3B3 (LAB combination; OD 1.00) | 10.28 ± 0.02 ^e | 16.76 ± 0.04 ^d | 6.65 ± 0.03 ^{ab} |

Means in the same row followed by different lowercase superscripts are significantly different at 95% confidence level.

rectangular than those of the control flour which were elliptical. This suggested that the hydrolysis of starch during fermentation mainly occurred on the surface of the granules.

The hydrolysis of starch by LAB UM1.3A during fermentation also affected the pasting properties of sago flour (Figure 3). Maximum viscosity, breakdown, final viscosity, and setback viscosity decreased after fermentation. The decrease in maximum viscosity and breakdown indicated the reduction in the starch granule development, and an increase in the starch stability during heating (Pukkahuta and Varavinit, 2007). The level of

retrogradation of sago starch also decreased due to fermentation which was marked by the reduction of the setback viscosity. Similar results were also found in sago flour treated with acid, and rice flour fermented with LAB (Yang and Tao, 2008; Fouladi and Nafchi, 2014).

The decrease in the values of pasting properties may be related to the damage of starch granules due to the LAB activities. This is shown by the decrease in the degree of crystallinity of fermented sago flour (M1B2) as compared to the control flour (Figure 3). LAB, which hydrolyses starch, seems to not only reduce the crystal region but

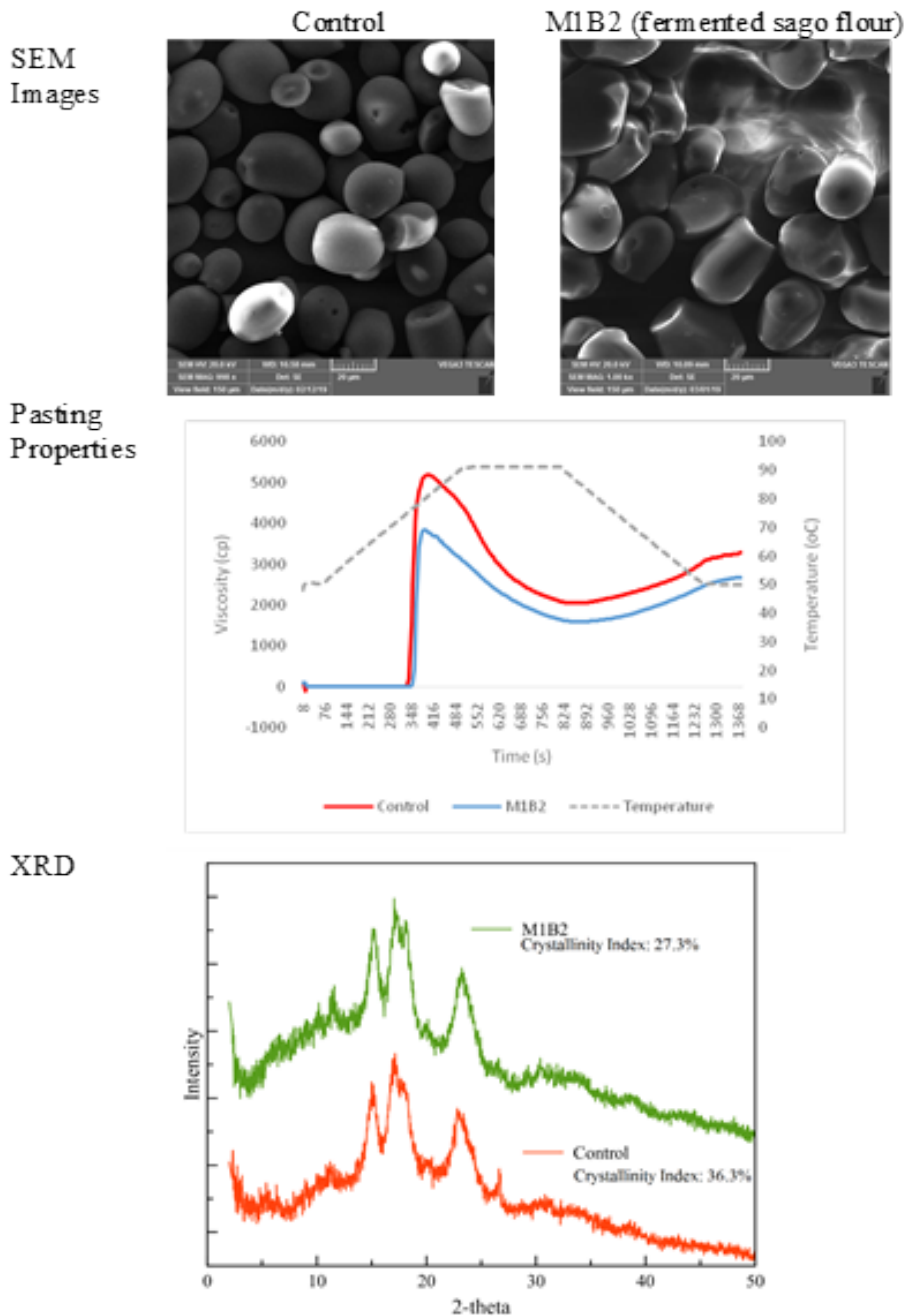


Figure 3. SEM images, pasting properties, and XRD spectra of control (unfermented) and M1B2 (fermented) sago flour samples.

also modify the type of granules. According to Ahmad *et al.* (1999), sago starch is a combination of type A and type B crystalline starches, or commonly referred to as crystalline type C. Sago type B is characterised by a peak at 2θ 5.5° , while peaks at 19.7° , 20.8° , and 26.6° indicate the presence of crystalline type A (Ahmad *et al.*, 1999; Wang *et al.*, 2008). As shown in Figure 3, the control flour had a peak at 26.7° . However, this peak was absent in fermented flour. The peak at 5.5° was also not apparent in the M1B2 sample, and it had a wider amorphous area as compared to the control flour.

Applications of modified sago flour in crackers production

The modified sago flour chosen as the raw material for making crackers was M1B2 flour (LAB UM1.3A, OD 0.75, 48 h). As a comparison, control sago flour (without fermentation) and wheat flour were also used in making crackers. Table 3 outlines the nutritional values of both flour samples as the raw ingredients, and crackers as the final product.

Naturally, the carbohydrate content of sago is higher than the wheat flour (Table 3). However, this also means that sago flour is poor in nutrients other than carbohydrates. For example, control sago flour contained 98.82% total carbohydrate but only

had 0.38% fat and 0.38% protein. On the other hand, wheat contained 11% protein and 1.5% fat thus the total carbohydrate was less than 90%.

Modification of sago by fermentation increased the proportion of the non-carbohydrate content of sago flour. As compared to the control flour, the fat and protein contents of modified sago flour increased by more than 100 and 600%, respectively. The increase in the level of fat and protein of modified flour could be caused by the decrease in the carbohydrate content due to fermentation. The decrease in the amount of carbohydrates automatically increases the amount of other nutrients in a material.

The increase in the protein level in modified sago flour could also be due to the synthesis of new proteins from amino acids or the joining of short chain peptides produced by LAB during fermentation. This is confirmed by Chavan *et al.* (1989) who observed that during fermentation with LAB, there was a slight increase in total protein. Moreover, the functional properties of proteins also changed, including the level of solubility, where the amount of soluble protein increased dramatically (Chavan *et al.*, 1989). Given that the protein analysis in the present work was the Biuret method, which depends on the level of water-soluble protein, the

Table 3. Nutritional compositions and organoleptic properties of flours and crackers.

| Component | Flour | | | Cracker | | |
|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | Native sago flour | Sago flour M1B2* | Wheat flour | Native sago cracker | M1B2 cracker | Wheat cracker |
| Moisture (% wb) | 12.6 ± 0.49 ^b | 11.60 ± 0.15 ^c | 14.50 ± 0.51 ^a | 4.64 ± 0.23 ^a | 4.60 ± 0.13 ^{ab} | 3.83 ± 0.29 ^b |
| Ashes (% db) | 0.32 ± 0.10 ^b | 0.30 ± 0.05 ^c | 0.60 ± 0.09 ^a | 1.17 ± 0.29 ^a | 1.15 ± 0.15 ^{ab} | 1.02 ± 0.88 ^b |
| Fat (% db) | 0.38 ± 0.16 ^c | 0.77 ± 0.23 ^b | 1.50 ± 0.36 ^a | 7.05 ± 0.74 ^c | 9.68 ± 0.55 ^b | 14.65 ± 0.64 ^a |
| Protein (% db) | 0.38 ± 0.04 ^c | 2.98 ± 0.11 ^b | 11.00 ± 0.36 ^a | 7.70 ± 0.13 ^c | 9.03 ± 0.52 ^b | 10.90 ± 1.18 ^a |
| Crude fibre (% db) | 2.29 ± 0.15 ^c | 7.52 ± 0.21 ^a | 2.50 ± 0.02 ^b | 2.36 ± 0.25 ^c | 7.88 ± 0.33 ^a | 2.53 ± 0.12 ^a |
| Carbohydrate (% db) | 98.82 ± 0.47 ^a | 95.95 ± 0.34 ^b | 88.50 ± 0.07 ^c | 84.07 ± 0.70 ^a | 80.12 ± 0.40 ^b | 73.43 ± 1.75 ^c |
| Property | | | | | | |
| Colour | | | | 3.7 ± 0.64 ^a | 4.0 ± 0.64 ^a | 4.2 ± 0.59 ^a |
| Aroma | | | | 3.5 ± 0.73 ^a | 4.1 ± 0.45 ^a | 4.0 ± 0.72 ^a |
| Texture | | | | 3.5 ± 0.63 ^a | 3.9 ± 0.37 ^a | 4.5 ± 0.51 ^a |
| Saltiness | | | | 3.5 ± 0.63 ^a | 3.8 ± 0.67 ^a | 3.6 ± 0.67 ^a |
| Sweetness | | | | 3.3 ± 0.48 ^a | 3.4 ± 0.50 ^a | 3.6 ± 0.76 ^a |
| Overall | | | | 3.4 ± 0.50 ^a | 3.8 ± 0.70 ^a | 4.1 ± 0.74 ^a |

Means in the same row followed by different lowercase superscripts are significantly different at 95% confidence level. wb: weight basis; db: dry basis. *Sago flour fermented with LAB UM1.3A at OD concentration of 0.75 for 48 h.

increase in the amount of soluble protein can directly affect the concentration of the detected protein.

Besides fat and protein, crude fibre of modified sago flour also increased significantly ($p < 0.05$) as compared to the control sago flour. The increase in the proportion of crude fibre could simply be due to the reduction of starch content during fermentation or due to the formation of oligosaccharides that behave like a food fibre (Leroy and De Vuyst, 2004). Oligosaccharides are intermediate products before starch is converted to glucose and lactic acid (Leroy and De Vuyst, 2004).

The improvement in the nutritional content of modified sago flour was also reflected in the results of the proximate analysis of crackers (Table 3). As compared to crackers made of control sago flour, crackers made of modified sago flour contained higher fat, protein, and crude fibre. However, since the fat and protein contents of the modified sago flour were lower than the wheat flour, the concentration of these nutrients in M1B2 crackers was also lower than the wheat crackers.

In contrast to the nutritional content, the fermentation treatment had no significant effect ($p > 0.05$) on the organoleptic properties of the crackers as shown in Table 3. The average score for hedonic level of colour and aroma fell into the same category for all cracker samples. Even though the overall hedonic categories were not the same for all samples, the average scores were not significantly different ($p > 0.05$). Similar results were also observed for texture and sweetness, where the average score was not statistically different ($p > 0.05$). This indicated that the sago crackers were comparable to the wheat crackers in terms of the sensory properties.

Conclusions

The properties of the fermented sago flour were affected by the fermentation time as well as the type and concentration of LAB isolates. The most favourable fermentation condition that produced flour with the highest swelling power and water solubility was fermentation with UM1.3A at OD concentration of 0.75 for 48 h. The fermentation treatment significantly increased the fat, protein, and crude fibre of sago flour ($p < 0.05$). Overall, crackers from modified sago flour were preferred by panellists, and had a crispy texture, although statistically, the sensory properties of crackers were not significantly different ($p > 0.05$) from the crackers made from control (unfermented) sago flour and wheat flour.

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