Effect of *Rhizopus oryzae* fermentation on proximate composition, anti-nutrient contents, and functional properties of banana peel flour

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Abstract

The present work aimed to evaluate the effect of fermentation by *Rhizopus oryzae* on the proximate composition, anti-nutrient contents, and functional properties of banana peel flour using the solid-state fermentation method in a tray bioreactor at 30°C for 96 h. Throughout fermentation, samples were obtained at different times (0/NF, 24, 48, 72, and 96 h), and analysed using standard procedures to determine the proximate composition, anti-nutrient contents, and functional properties. Based on the results, there were significant differences observed ($p < 0.05$). Carbohydrate content decreased by 3.35%, while the crude protein, fat, ash, and crude fibre contents increased by 11.12, 2.43, 10.99, and 3.50%, respectively. Hydrogen cyanide, saponin, oxalate, and phytate contents decreased by 42.59, 25, 23.83, and 43.82%, respectively. Water absorption capacity (WAC) and the water solubility index (WSI) increased by 3.94 and 37.14%, respectively, while oil absorption capacity (OAC) decreased by 4.48%. These results showed that the fermentation of banana peel flour by *R. oryzae* has potential benefits for the food industry due to its effect on chemical composition and functional properties.

Introduction

Currently, there is a high availability of agricultural and plantation by-products which can serve as valuable raw materials for developing value-added products. Over 40% of the total mass of fruits is comprised of peels and seeds which are inedible, and therefore regarded as waste (Hasanin and Hashem, 2020). Increasing world population means increasing production and consumption of fruits, as well as the amount of waste produced (Pyar and Peh, 2018). This waste is often disposed of improperly, and can lead to environmental pollution (Ali *et al.,* 2014). Fruit peels have high carbon as well as nitrogen contents, and are therefore suitable substrates for microbial growth in enzymatic processes for producing proteins, organic acids, antioxidants, biological fertilisers, energy, and adsorbents (Pathak *et al.,* 2017).

Banana (*Musa* spp.) is widely grown around Southeast Asia, including Indonesia. Banana tree can grow up to 2 - 8 m, with a leaf length of 3.5 m, and fruits hanging and clustered in bunches, which comprise 3 - 20 levels, and about 20 fruits per level

(Anhwange, 2008). In addition, banana trees bear fruits in and out of season, which can be consumed directly, or processed into chips, cakes, and other products. Banana peel is a waste which accounts for 35 - 40% of fresh banana weight (Al-Sahlany and Al-Musafer, 2020). These peels contain carbohydrates, proteins, minerals, dietary fibres, essential amino acids, polyunsaturated fatty acids, as well as phenolic compounds as antioxidants (Eshak, 2016), and therefore have the potential to be processed into highly nutritious animal feed or alternative food ingredients.

Banana peel flour is used as a substitute for wheat flour with a maximum limit of 10% without reducing the characteristics of the end product (Eshak, 2016; Al-Sahlany and Al-Musafer, 2020). However, the peels are known to contain anti-nutritional compounds such as hydrogen cyanide and saponin which limit the optimal use of banana peel flour in food products (Anhwange, 2008). According to Abu-Bakar *et al.* (2018), the chemical composition and functional properties of banana peel flour influence the characteristics of the food products. Therefore, certain modifications are required to

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improve the physicochemical and functional properties of banana peel flour, and increase the use of banana peel flour in food products. Fermentation is known to increase the protein content of agro-industrial by-products, and improve the functional properties of food products (Sukma *et al.,* 2018). *Rhizopus oryzae* belongs to the Zygomycetes group, and has been widely applied in food fermentation to produce enzymes including cellulase, xylanase, pectinase, and amylase to break down polysaccharides into simple sugars for use in the fermentation process of banana peel flour (Karmakar and Ray, 2010). Previous studies on the effect of fermentation using *R. oryzae* on the proximate composition, anti-nutrient contents, and functional properties of banana peel flour are quite limited. Therefore, fermented banana peel flour is hoped to be utilised optimally in the food processing industry to increase food security of the world population.

Materials and methods

Banana peel flour preparation

Green *kepok* banana (*M. paradisiaca*) peels were obtained from the banana chip processing industry in Sungai Langka village, Gedong Tataan sub-district, Pesawaran district, Lampung province, Indonesia. The peels were then cut into 3×3 cm dimension, and soaked in a solution containing 0.2% sodium metabisulphite ($Na_2S_2O_5$) and 0.2% citric acid ($C_6H_5O_7$) (Merck KGaA, Darmstadt, Germany) for 1 h to prevent enzymatic browning. Subsequently, the peels were oven-dried (Heraeus ST50, Hanau, Germany) at 60°C for 12 h (Abu-Bakar *et al.,* 2018), blended (Phillips HR2115, Batam, Indonesia), sieved using a 35 mesh sieve (Retsch AS200, Haan, Germany), placed in a 3 L polypropylene box, and refrigerated (GEA Expo-480 PH, Jakarta, Indonesia) at 4°C until subsequent use.

Inoculum preparation

Pure culture of *R. oryzae* (FNCC 6157) was obtained from the Food and Nutrition Culture Collection (FNCC) of PAU Food and Nutrition, Gadjah Mada University, Indonesia, in an agar slant tube. The cultures were then transferred onto a Petri dish containing potato dextrose agar (PDA, MerckKGaA, Darmstadt, Germany), and incubated at 30°C for 7 d (Heraeus B6060, Hanau, Germany) (Oliveira *et al.,* 2010). Subsequently, the spores formed were collected, dissolved in 0.2% Tween 80,

and homogenised using a vortex mixer (DLAB MX-S, Beijing, China). The concentration of spores in the suspension was then measured using a haemocytometer, and the suspension was refrigerated (GEA Expo-480 PH, Jakarta, Indonesia) at 4°C until subsequent use.

Fermentation

Fermentation was carried out for 96 h using the solid-state method with a plastic tray bioreactor (unperforated, unagitated, and unmixed) measuring $10 \times 8 \times 4$ cm (Lion Star, Jakarta, Indonesia). A total of 100 g of banana peel flour was added to each tray bioreactor, with a substrate layer thickness of 2 cm. The tray bioreactor containing banana peel flour was then sterilised in an autoclave at 121°C for 30 min (ALP KT-30LDP, Tokyo, Japan), and a sample was collected for analysis before the fermentation. Subsequently, *R. oryzae* spore suspension was added to the banana peel flour substrate with an initial concentration of 4×10^6 spores/g medium (Oliveira *et al.,* 2010), and sterile distilled water was also added until the substrate's water content reached 55% (Sukma *et al,* 2018). The banana peel flour was then fermented in an incubator (Heraeus B6060, Hanau, Germany) at 30°C, and samples were collected for analysis after every 24 h of fermentation.

Proximate analysis

The moisture, ash, and fat contents of the samples were determined using the gravimetric method according to the AOAC (2000). In addition, the crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25, while the carbohydrate content was determined by difference. The crude fibre content was determined through the gravimetrical measurement of the residue obtained by the acid and basic digestion of the samples, based on the neutral detergent fibre (NDF) method.

Hydrogen cyanide content

The hydrogen cyanide content was determined using SNI Standards (2011). For this, 20 g of the sample was transferred to a Kjeldahl digestion flask, and mixed with 200 mL of distilled water for 2 h. The flask was then heated to evaporate the hydrogen cyanide as a distillate which was collected in an Erlenmeyer flask containing 20 mL of 2.5% NaOH solution. This was followed by adding 8 mL of ammonium hydroxide (NH_4OH) solution and 5 mL of

5% potassium iodide (KI) solution as an indicator to the Erlenmeyer flask. The mixture obtained was then titrated against 0.02 N silver nitrate ($AgNO_3$) until turbidity occurred, using blank solutions as control. Subsequently, the hydrogen cyanide content was calculated from the $AgNO_3$ titre volume using Eq. 1:

HCN content (mg/g) =
$(V_1-V_2) \times N \times 27 / (V_3 \times W)$ (Eq. 1)

where, V1, V2, V3, N, and W = titration of blank solution reading, sample titration reading, distillate volume, $AgNO_3$ normality, and sample weight, respectively, and 27 = molecular weight of hydrogen cyanide.

Saponin content

The total saponin content was determined using a slightly modified version of the previous method (Han *et al.*, 2019). For this, 5 g of the sample was extracted with 50 mL of methanol at 80°C under reflux for 1 h, then the mixture was centrifuged (DLAB DM0412, Beijing, China) at 4,500 rpm for 15 min. Subsequently, the supernatant was heated in a rotary evaporator (IKA RV3V, Guangzhou, China) at 45°C until a final volume of 10 mL was obtained. Next, 0.2 mL of saponin extract sample was then collected, heated in a water bath at 70°C to remove any solvent present, chilled, and mixed with 0.2 mL 5% (w/v) vanillin in acetic acid (CH_3COOH) and 0.8 mL 72% perchloric acid ($HClO_4$). The mixture was heated at 70°C for 10 min, and incubated with 5 mL of 17 M acetic acid (CH_3COOH) at room temperature for 10 min. This was followed by measuring the mixture's absorbance at 550 nm using a spectrometer (Shimadzu UV-1800, Kyoto, Japan), with oleanolic acid as the standard. The total saponin content was expressed as mg of oleanolic acid equivalent (OAE) per g dry weight of the sample.

Oxalate content

The oxalate content was determined using a slightly modified version of the previous method (Oyeyinka and Afolayan, 2019). For this, 1 g of sample was macerated with 75 mL of 3 M sulphuric acid (H_2SO_4) for 3 h, and the mixture was filtered using a Whatman no. 1 filter paper. Subsequently, 25 mL of the filtrate was heated at 75°C, and hot aliquots were titrated against 0.05 M potassium permanganate ($KMnO_4$) until the pink colour persisted for 15 s. The oxalate content was then calculated as 1 mL of 0.05

M potassium permanganate ($KMnO_4$) as an equivalent to 2.2 mg oxalate or (2.2 mg × titre value).

Phytate content

The phytate content was determined using a slightly modified version of the previous method (Oyeyinka and Afolayan, 2019). For this, 2 g of sample was macerated with 2% hydrochloric acid (HCl) in a 250-mL conical flask for 3 h, and filtered using a Whatman no. 1 filter paper. Subsequently, 25 mL of the filtrate was poured into a 250-mL conical flask, and mixed with 5 mL of ammonium thiocyanate 0.03% indicator and 53.5 mL of distilled water. The mixture was then titrated against iron (III) chloride solution (0.00195 g iron per mL) until a brownish yellow colour persisted for up to 5 min, and the % phytic acid was calculated using Eq. 2:

% Phytic acid =
Titre value × 0.00195 × 1.19 × 100   (Eq. 2)

Oil and water absorption capacities

Oil absorption capacity (OAC) was determined using the centrifugation method with slight modifications (Simwaka *et al.*, 2017). For this, 0.5 g of the sample was mixed with 6 mL of palm oil into a pre-weighed centrifuge tube. The mixture was then stirred for 1 min with a thin brass wire to disperse the sample in the oil. After settling for 30 min, the mixture was then centrifuged (DLAB DM0412, Beijing, China) at 3,000 rpm for 25 min. The separated oil was collected with a pipette, and the tube was turned over for 25 min to drain the oil before re-weighing it. Subsequently, the oil absorption capacity was expressed in grams of bound oil per gram of dry sample, using Eq. 3:

OAC (g/g) = $(W_2 - W_1) / W_0$ (Eq. 3)

where, W0, W1, and W2 = weight of the initial sample on a dry basis, the weight of the initial centrifuge tube + weight of the sample, and the weight of the final centrifuge tube, respectively.

The same procedure was used to determine the water absorption capacity, with the oil being replaced with 6 mL of distilled water.

Water solubility index

Water solubility index was determined using the previous method with slight modification (Abu-Bakar *et al.*, 2018). For this, 1 g of sample was added

to 35 mL of distilled water, and homogenised using a vortex mixer (DLAB MX-S, Beijing, China). The sample was then transferred into a pre-weighed centrifuge tube, and incubated at room temperature for 1 h. Subsequently, the mixture was centrifuged (DLAB DM0412, Beijing, China) at 4,500 rpm for 30 min to separate the supernatant. The supernatant was then drained into a pre-weighed Petri dish at 45°C for 10 min, and dried at 105°C for 12 h (Heraeus ST50, Hanau, Germany) until a constant weight was obtained, and the WSI was obtained from the sample residue of BPF (soluble g/dry sample g).

Statistical analysis

Data were obtained from triplicate determinations and the results were presented as mean ± standard deviation (SD). The variation between means was determined using a one-way analysis of variance (ANOVA) with Duncan's multiple range test performed using MSExcel. Significant differences were tested at $p < 0.05$.

Results and discussion

Proximate composition

The proximate composition results are shown in Table 1. A significant ($p < 0.05$) decrease by 3.35% was observed in the carbohydrate content of banana peel flour after 96 h of fermentation. In the early stages of fermentation, the decrease in carbohydrate content was not significant ($p > 0.05$) because these stages involved the adaptation phase of biomass cells to adjust to the substrate conditions. This is in line with previous studies, where fermentation significantly decreased the carbohydrate content of the substrate (Oliveira *et al.,* 2010; Sukma *et al.,* 2018). This condition is due to the metabolic activity of *R. oryzae* growing and developing in the banana peel substrate, as well as producing enzymes to break down polysaccharides such as cellulase, xylanase, pectinase, and amylase into oligosaccharides and glucose. Simple sugars are required for the growth and development of *R. oryzae* biomass cells, resulting in decreased carbohydrate content within the substrate after fermentation (Karmakar and Ray, 2010; Pandey and Negi, 2020). However, the decrease in carbohydrate content is insignificant as compared to previous studies; this could be due to the high C/N ratio in banana peels as evident in the initial crude protein content of banana peels which was only 5.48 g/g dry weight. Nitrogen is also required for the growth and development of biomass cells (Schmidt and Furlong, 2012).

A significant ($p < 0.05$) increase by 11.12% was observed in the crude protein content after 96 h of fermentation. In the early stages of fermentation, the crude protein content did not differ significantly ($p > 0.05$); however, a significant increase occurred after 72 h of fermentation. This is consistent with previous research where fermentation increased the substrate's crude protein content (Olukomaiya *et al.,* 2020). Simple sugars produced from the breakdown of polysaccharides serve as an energy source for the production of *R. oryzae* biomass cells protein synthesis. The protein content in filamentous fungal biomass cells is 20 - 30% dry weight, and bound to carbohydrates to form glycoproteins (Garcia-Rubiyo *et al.,* 2020). In addition, the metabolic activity of *R. oryzae* also produces extracellular enzymes, thus increasing the overall crude protein content in fermented banana peels (Adejuyitan *et al.,* 2017).

A significant ($p < 0.05$) decrease by 22.03% was observed in the fat content after 24 h of fermentation. This occurred because *R. oryzae* produced lipase enzymes to convert fatty compounds in banana peel flour to fatty acids for producing chitin

Table 1. Proximate composition (g/100 g) of banana peel flour during fermentation.

Time (h)	Protein	Fat	Ash	Crude fibre	Carbohydrate
0/NF	5.48 ± 0.07[ab]	6.23 ± 0.18[b]	15.33 ± 0.07[a]	14.86 ± 0.28[ab]	72.96 ± 0.12[d]
24	5.44 ± 0.07[a]	4.86 ± 0.20[a]	16.77 ± 0.12[bc]	14.83 ± 0.32[a]	72.92 ± 0.38[d]
48	5.80 ± 0.11[c]	6.32 ± 0.11[bc]	16.61 ± 0.09[b]	15.29 ± 0.18[abc]	71.26 ± 0.21[bc]
72	6.03 ± 0.08[d]	6.34 ± 0.12[bcd]	16.77 ± 0.09[bcd]	15.51 ± 0.24[cd]	70.86 ± 0.06[ab]
96	6.08 ± 0.09[d]	6.38 ± 0.11[bcd]	17.02 ± 0.15[e]	15.38 ± 0.22[cd]	70.51 ± 0.22[a]

Values are mean ± standard deviation of at least three replicates expressed in dry matter basis. Means followed by different lowercase superscripts in a column are significantly different ($p < 0.05$). 0/NF: not fermented.

compounds as a building block for the cell walls of filamentous organisms (Abbate *et al.,* 2010). The fat content increased again after 48 h of fermentation, and did not change significantly ($p > 0.05$) after 96 h of fermentation, due to the growth and development of *R. oryzae* biomass cells (Oboh and Akindahunsi, 2003). A major constituent of the cell wall and plasma membrane of *R. oryzae* is the fat component, in the form of phospholipids and lipoproteins, which increase the fat content of fermented banana peel flour (Shuler and Kargi, 2002). Previous studies also reported an increase in the phospholipid content of *R. oryzae*-fermented rice bran (Oliveira *et al.,* 2011; Massarolo *et al.,* 2016, Sukma *et al.,* 2018).

A significant ($p < 0.05$) increase by 10.99% was observed in the ash content of fermented banana peel flour after 96 h. This is consistent with previous studies where an increase in the ash content of rice bran substrate occurred after fermentation with *R. oryzae* (Feddern *et al.,* 2007; Oliveira *et al.,* 2010). The increase in the ash content was due to the growth of *R. oryzae* biomass cells on the banana peel substrate. Minerals, including phosphorus, potassium, sodium, and sulphur are one of the constituents of cell walls in filamentous fungi (Shuler and Kargi, 2002). Furthermore, filamentous fungi produce calcite biominerals, for instance, calcium carbonate, through the degradation of carbon elements in organic material (Bindschedler *et al.,* 2016).

The crude fibre content significantly increased ($p < 0.05$) by 3.50% after 96 h of fermentation. Previous studies also reported an increase in the crude fibre content of rice bran substrate after 120 h of fermentation using *R. oryzae* (Oliveira *et al.,* 2010). This is due to the increasing number of *R. oryzae*

biomass cells, which are composed mainly of polysaccharides in the form of glucans (50 - 60%) and chitin (10 - 20%) in the dry weight of biomass (Shuler and Kargi, 2002; Garcia-Rubiyo *et al.,* 2020).

Anti-nutrient contents

The anti-nutrient contents are shown in Table 2. The hydrogen cyanide content in banana peel flour significantly decreased ($p < 0.05$) by 32.88% after 72 h of fermentation. However, after 96 h of fermentation, the decrease in hydrogen cyanide content was insignificant ($p > 0.05$). The total decrease in the hydrogen cyanide content of banana peel flour was 42.58%, and this loss occurred partly due to the preparation stage which involved cutting, soaking, heating at 60°C, and sterilising at 121°C. Hydrogen cyanide is easily soluble in water, and has a boiling point of 29°C (Gunawan *et al.,* 2015). The decrease in hydrogen cyanide content was also caused by microbial activity during fermentation. Several bacteria and fungi including *Saccharomyces cerevisiae*, *Lactobacillus bulgaricus*, *Aspergillus fumigatus*, and *Rhizopus stolonifer* have been reported to decrease the hydrogen cyanide content in cassava (Tope, 2014). Previous studies reported that *R. oryzae* was able to decrease the hydrogen cyanide content in cassava by 60.80 - 66.06% (Gunawan *et al.,* 2015). However, there are no known reports on the effect of fermentation with *R. oryzae* on the hydrogen cyanide content in banana peels.

Saponins are anti-nutrients with the capacity to cause digestive disorders by inhibiting the activity of enzymes such as amylase, glucosidase, trypsin, chymotrypsin, and lipase without being easily hydrolysed by human digestive enzymes (Lee *et al.,* 2015; Ercan and El, 2016). In the present work, the

Table 2. Anti-nutrient contents (mg/g) of banana peel flour during fermentation.

Time (h)	Hydrogen cyanide	Saponin	Oxalate	Phytate
0/NF	1.24 ± 0.13^d	23.87 ± 0.39^e	0.71 ± 0.04^{bcd}	0.30 ± 0.03^d
24	1.18 ± 0.18^{cd}	22.15 ± 0.15^d	0.69 ± 0.05^{bcd}	0.30 ± 0.02^d
48	0.97 ± 0.19^{abc}	20.88 ± 0.15^c	0.68 ± 0.05^{bc}	0.24 ± 0.03^c
72	0.83 ± 0.12^{ab}	18.54 ± 0.23^b	0.63 ± 0.04^b	0.19 ± 0.03^{ab}
96	0.71 ± 0.10^a	17.90 ± 0.24^a	0.54 ± 0.06^a	0.17 ± 0.02^a

Values are mean ± standard deviation of at least three replicates expressed in dry matter basis. Means followed by different lowercase superscripts in a column are significantly different ($p < 0.05$). 0/NF: not fermented.

saponin content significantly decreased ($p < 0.05$) by 25% after 96 h of fermentation, and this could be due to the sterilisation process of raw banana peels using autoclave at 121°C. This is consistent with previous studies where autoclaving and cooking were discovered to decrease the saponin content in bean medium (Maphosa and Jideani, 2017). Similarly, a decrease in saponin content was also reported after fermentation of watermelon peels using *Saccharomyces cerevisiae* (Erukainure *et al.*, 2010). Several strains of bacteria and fungi have the capacity to decrease saponin levels in green peas, using both submerged and solid-state fermentation methods (Oluremi and Okhonlaye, 2020). However, there are no known reports on the effect of fermentation with *R. oryzae* on the saponin content in banana peels.

Oxalate is an anti-nutritional substance in food that can cause kidney disease in cases of excess consumption. This substance also reduces the availability of minerals in the body, especially calcium (Anhwange, 2008). However, heating and natural fermentation processes help to reduce oxalate content in substrates (Tilahun *et al.*, 2013). In the present work, the oxalate content of banana peel flour did not change significantly ($p > 0.05$) after 48 h of fermentation, but significantly ($p < 0.05$) decreased by 23.83% after 96 h of fermentation. The decrease in oxalate content occurred due to the raw material sterilisation at 121°C and fermentation with *R. oryzae*. Previous studies have shown that submerged fermentation or solid-state fermentation with various bacterial and fungal strains including *Bacillus* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Penicillium* sp. could decrease the oxalate content in green peas (Oluremy and Okonlaye, 2020).

Phytate is an antinutritional substance that acts as storage for phosphorus, and has the capacity to bind minerals, for instance, Ca, Fe, K, Mg, Mn, and Zn into insoluble substances, thus leading to mineral deficiency in food. In grains, phytates accumulate within the aleurone layer, and are hydrolysed by phytase enzymes during germination to produce phosphate, inositol, and micronutrients for plant growth (Bohn *et al.*, 2008). In the present work, the phytate content in banana peel flour did not change significantly ($p > 0.05$) after 24 h of fermentation, but decreased significantly ($p < 0.05$) by 43.82% after 96 h of fermentation. This could be due to the metabolic activity of *R. oryzae* which can produce phytase to hydrolyse phytic acid into phosphate, inositol, and other micronutrients (Ramachandran *et al.*, 2005). Previous studies also reported 60% decrease in the phytate content of rice bran after fermentation for 120 h with *R. oryzae* (Oliveira *et al.*, 2010).

Functional properties

The functional properties of food consist of the structure, quality, texture, nutritional value, and acceptability, which are determined by the organoleptic, physical, and chemical properties of the foodstuff. These properties are used to determine the characteristics of food during processing, production, consumption, as well as storage, and also serve as a reference for manufacturing suitable types of food products (Abu-Bakar *et al.*, 2018). Water absorption capacity (WAC) refers to the amount of water absorbed by a material to form the desired consistency (Awuchi *et al.*, 2019). The WAC result is shown is Table 3. There was no significant change in the WAC ($p > 0.05$) of banana peel flour after 48 h of fermentation, however, the changes were significant ($p < 0.05$) after 72 h, with 3.94% increase in the WAC observed after 96 h. This is consistent with previous studies, where an increase in WAC of mahogany

Table 3. Functional properties (g/g) of banana peel flour during fermentation

Time (h)	Water absorption capacity (WAC)	Oil absorption capacity (OAC)	Water solubility index (WSI)
0/NF	7.36 ± 0.14[a]	1.93 ± 0.03[cd]	0.12 ± 0.03[ab]
24	7.41 ± 0.10[ab]	1.93 ± 0.04[cd]	0.11 ± 0.02[a]
48	7.59 ± 0.09[bcd]	1.87 ± 0.04[abc]	0.13 ± 0.02[abc]
72	7.54 ± 0.08[abc]	1.84 ± 0.03[a]	0.15 ± 0.01[cd]
96	7.65 ± 0.11[cd]	1.85 ± 0.04[ab]	0.16 ± 0.02[cd]

Values are mean ± standard deviation of at least three replicates expressed in dry matter basis. Means followed by different lowercase superscripts in a column are significantly different ($p < 0.05$). 0/NF: not fermented.

beans was observed after fermentation for 72 h (Igbabul *et al.*, 2014). Similarly, the fermentation of canola with *Aspergillus* for 7 d also increased the WAC by 10 - 24% (Olukomaiya *et al.*, 2020). The increase in WAC after fermentation is believed to be due to the formation of amino acids with polar groups, which increases the hydrophilicity of proteins (Ghumman *et al.*, 2016). The increase in WAC is also thought to be caused by the increase in the crude fibre content of fermented banana peel flour. An increase in fibre/polysaccharide content of a material is bound to increase the WAC (Chandra and Samsher, 2013). However, there are no known studies regarding the effect of fermentation with *R. oryzae* on WAC of banana peels.

Oil absorption capacity (OAC) is a functional property of food ingredients that affects the texture in the mouth, as well as the taste (Awuchi *et al.*, 2019). The oil absorption mechanism involves capillary interactions influenced by hydrophobic proteins and the non-polar amino acid side chains, which play a major role in oil absorption (Du *et al.*, 2014). In the present work, the OAC of fermented banana peel flour significantly decreased ($p < 0.05$) by 4.48%. This is consistent with previous studies, where natural fermentation for 36 h was reported to decrease the OAC of various types of grains (Simwaka *et al.*, 2017). A separate study also reported a 60% decrease in the OAC of *Mucuna* bean protein isolates after natural fermentation for 72 h (Udensi and Okoronkwo, 2006). The decrease in OAC is believed to be caused by the increase in protein content with the polar amino acid composition in banana peel flour fermented by *R. oryzae*. Hydrophilic polar amino acids have the capacity to decrease OAC (Ghumman *et al.*, 2016). However, there are no studies regarding the effect of fermentation with *R. oryzae* on OAC of banana peels.

Solubility in food is a chemical and functional property referring to the ability of a particular food substance to dissolve in a solvent, which is an indicator for the ease of digestion (Awuchi *et al.*, 2019). In the present work, the water solubility index (WSI) of fermented banana peel flour did not change significantly ($p > 0.05$) after 48 h of fermentation, but significantly increased ($p < 0.05$) by 37.14% after 96 h. These results are in line with previous studies, where an increase in the WSI values of millet,

sorghum, pumpkin, and amaranth seed was observed after natural fermentation. The increase in WSI in the present work indicated that the fermented banana peel flour particles were significantly degraded by the fermentation process and made more readily available for digestion (Simwaka *et al.*, 2017). A previous study also reported an increase in amino acid digestibility of rice bran after fermentation with *R. oryzae* for 120 h (Oliveira *et al.*, 2010). The increase in WSI was also caused by an increase in fermented proteins composed of amino acids with polar groups, and this condition increased protein solubility in water (Ghumman *et al.*, 2016).

Conclusion

Based on the results, solid-state fermentation with *R. oryzae* changed the proximate composition of the banana peel flour. The carbohydrate content of the banana peel flour significantly decreased by 3.35%, while the crude protein, fat, ash, and crude fibre contents significantly increased by 11.12, 2.43, 10.99, and 3.50%, respectively. In addition, there was a significant decrease in the levels of anti-nutritional substances including hydrogen cyanide, saponin, oxalate, and phytate contents by 42.59, 25, 23.83, and 43.82%, respectively. Several significant changes in the functional properties of the banana peel flour were also observed. Water absorption capacity (WAC) and water solubility index (WSI) significantly increased by 3.94 and 37.14%, respectively, while oil absorption capacity (OAC) decreased by 4.48%. These findings showed that fermented banana peel flour has the potential to be applied in the food industry, based on the desired chemical composition and functional properties. However, further studies using various kinds of bacteria and fungi, as well as various fermentation parameters are encouraged to improve the chemical composition and functional properties of banana peel flour.

Acknowledgement

References

Abbate, V., Bassindale, A. R., Brandstadt, K. F., Lawson, R. and Taylor, P. G. 2010. Enzyme mediated silicon–oxygen bond formation; the use of *Rhizopus oryzae* lipase, lysozyme and phytase under mild conditions. Dalton Transactions 39: 9361-9368.

Abu-Bakar, S. K. S., Ahmad, N. and Jailani, F. 2018. Chemical and functional properties of local banana peel flour. Journal of Food and Nutrition Research 6(8): 492-496.

Adejuyitan, J. A., Otunola, E. T., Bolarinwa, I. F. and Olanipekun, B. F. 2017. Changes in the protein and cyanide contents of pupuru as affected by duration of fermentation of cassava with species of *Rhizopus*. Journal of Food Chemistry and Nanotechnology 3(1): 19-23.

Ali, S. M., Pervaiz, A., Afzal, B., Hamid, N. and Yasmin, A. 2014. Open dumping of municipal solid waste and its hazardous impacts on soil and vegetation diversity at waste dumping sites of Islamabad city. Journal of King Saud University - Science 26: 59-65.

Al-Sahlany, S. T. G. and Al-Musafer, A. M. S. 2020. Effect of substitution percentage of banana peels flour in chemical composition, rheological characteristics of wheat flour and the viability of yeast during dough time. Journal of the Saudi Society of Agricultural Sciences 19: 87-91.

Anhwange, B. A. 2008. Chemical composition of *Musa sapientum* (banana) peels. Journal of Food Technology 6(6): 263-266.

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis of AOAC International. 17th ed. United States: AOAC.

Awuchi, C. G., Igwe, V. S. and Echeta, C. K. 2019. The functional properties of foods and flours. International Journal of Advanced Academic Research 5(11): 139-160.

Bindschedler, S., Cailleau, G. and Verrecchia, E. 2016. Role of fungi in the biomineralization of calcite. Minerals 6: 41-60.

Bohn, L., Meyer, A. S. and Rasmussen, S. K. 2008. Phytate: Impact on environment and human nutrition. A challenge for molecular breeding. Journal of Zhejiang University Science B 9(3): 165-191.

Chandra, S. and Samsher. 2013. Assessment of functional properties of different flours.

African Journal of Agricultural Research 8(38): 4849-4852.

Du, S., Jiang, H., Yu, X. and Jane, J. 2014. Physicochemical and functional properties of whole legume flour. LWT - Food Science and Technology 55(1): 308-313.

Ercan, P. and El, S. N. 2016. Inhibitory effects of chickpea and *Tribulus terrestris* on lipase, α-amylase and α-glucosidase. Food Chemistry 205: 163-169.

Erukainure, O., Oke, O. O., Daramola, A. O., Adenekan, S. O. and Umanhonlen, E. E. 2010. Improvement of the biochemical properties of watermelon rinds subjected to *Saccharomyces cerevisiae* solid substrate fermentation. Pakistan Journal of Nutrition 9(8): 806-8092.

Eshak, N. S. 2016. Sensory evaluation and nutritional value of balady flat bread supplemented with banana peels as a natural source of dietary fiber. Annals of Agricultural Science 61(2): 229-235.

Feddern, V., Furlong, E. B. and De Souza Soares, L. A. 2007. Effects of fermentation on the physicochemical and nutritional properties of rice bran. Ciência Tecnologia de Alimentos 27(4): 800-804.

Garcia-Rubiyo, R., de Oliveira, H. C., Rivera, J. and Trevijano-Contador, N. 2020. The fungal cell wall: *Candida, Cryptococcus*, and *Aspergillus* species. Frontiers in Microbiology 10: 2993.

Ghumman, A., Kaur, A. and Singh, N. 2016. Impact of germination on flour, protein and starch characteristics of lentil (*Lens culinari*) and horsegram (*Macrotyloma uniflorum L.*) lines. LWT - Food Science and Technology 65: 137-144.

Gunawan, S., Widjaja, T., Zullaikah S., Ernawati, L., Istinah, N., Aparamarta, H. W. and Prasetyoko, D. 2015. Effect of fermenting cassava with *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Rhizopus oryzae* on the chemical composition of their flour. International Food Research Journal 22(3): 1280-1287.

Han, Y., Chi, J., Zhang, M., Zhang, R., Fan, S., Dong, L., ... and Liu, L. 2019. Changes in saponins, phenolics and antioxidant activity of quinoa (*Chenopodium quinoa* willd) during milling process. LWT - Food Science and Technology 114: 108381.

Hasanin, M. S. and Hashem, A. H. 2020. Eco-friendly, economic fungal universal medium from watermelon peel waste. Journal of Microbiological Methods 168: 105802.

Igbabul, B., Hiikyaa, O. and Amove, J. 2014. Effect of fermentation on the proximate composition and functional properties of mahogany bean (*Afzelia africana*) flour. Current Research in Nutrition and Food Science 2(2): 1-7.

Karmakar, M. and Ray, R. R. 2010. Extra cellular endoglucanase production by *Rhizopus oryzae* in solid and liquid state fermentation of agro waste. Asian Journal of Biotechnology 2(1): 27-36.

Lee, S. S., Esa, N. M. and Loh, S. P. 2015. *In vitro* inhibitory activity of selected legumes against pancreatic lipase. Journal of Food Biochemistry 39(4): 485-490.

Maphosa, Y. and Jideani, V. A. 2017. The role of legumes in human nutrition. In Chavarri, M. (ed). Functional Food-Improve Health through Adequate Food, p. 103-109. United Kingdom: IntechOpen.

Massarolo, K. C., De Souza, T. D., Ribeiro, A. C., Furlong, E. B. and De Souza Soares, L. A. 2016. Influence of cultivation *Rhizopus oryzae* on rice bran on lipid fraction: Fatty acids and phospholipids. Biocatalysis and Agricultural Biotechnology 8: 204-208.

Oboh, G. and Akindahunsi, A. A. 2003. Biochemical changes in cassava products (flour & gari) subjected to *Saccharomyces cerevisiae* solid media fermentation. Food Chemistry 82(4): 599-602.

Oliveira, M. D., Feddern, V., Kupski, L., Cipolatti, E. P., Badiale-Furlong, E. and de Souza-Soares, L. A. 2010. Physico-chemical characterization of fermented rice bran biomass. CyTA - Journal of Food 8(3): 229-236.

Olukomaiya, O. O., Fernando, W. C., Mereddy, R., Li, X. and Sultanbawa, Y. 2020. Solid-state fermentation of canola meal with *Aspergillus sojae, Aspergillus ficuum* and their co-cultures: Effects on physicochemical, microbiological and functional properties. LWT - Food Science and Technology 127: 109362.

Oluremi, A. E. and Okhonlaye, O. O. 2020. Effects of fermentation on the antioxidant and antinutritional compositions of green pea. South Asian Journal of Research in Microbiology 7(3): 1-14.

Oyeyinka, B. O. and Afolayan, A. J. 2019. Comparative evaluation of the nutritive, mineral, and antinutritive composition of *Musa sinensis* L. (banana) and *Musa paradisiaca* L. (plantain) fruit compartments. Plants 8: 598.

Pandey, A. K. and Negi, S. 2020. Enhanced cellulase recovery in SSF from *Rhizopus oryzae* SN5 and immobilization for multi-batch saccharification of carboxymethylcellulose. Biocatalysis and Agricultural Biotechnology 26: 101656.

Pathak, P. D., Mandavgane, S. A. and Kulkarni, B. D. 2017. Fruit peel waste: Characterization and its potential uses. Current Science 113(3): 444-454.

Pyar, H. and Peh, K. K. 2018. Chemical compositions of banana peels (*Musa sapientum*) fruits cultivated in Malaysia using proximate analysis. Research Journal of Chemistry and Environment 22: 108-113.

Ramachandran, S., Roopesh, K., Nampoothiri, K. M., Szakacs, G. and Pandey, A. 2005. Mixed substrate fermentation for the production of phytase by *Rhizopus* spp. using oilcakes as substrates. Process Biochemistry 40: 1749-1754.

Schmidt, C. G. and Furlong, E. B. 2012. Effect of particle size and ammonium sulfate concentration on rice bran fermentation with the fungus *Rhizopus oryzae*. Bioresource Technology 123: 36-41.

Shuler, M. L. and Kargi, F. 2002. Bioprocess engineering basic concepts. New Jersey: Prentice-Hall International Inc.

Simwaka, J. E., Chamba, M. V. M., Huiming, Z., Masamba, K. G. and Luo, Y. 2017. Effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours. International Food Research Journal 24(5): 1869-1879.

SNI Standard. 2011. SNI 7622 - modified cassava flour. Jakarta: National Standardization Agency of Indonesia.

Sukma, A., Jos, B. and Sumardiono, S. 2018. Kinetic of biomass growth and protein formation on rice bran fermentation using *Rhizopus oryzae*. MATEC Web of Conferences 156: 01023.

Tilahun, A. T., Shimelis, A. E. and Gulelat, D. H. 2013. Effect of processing on physicochemical composition and anti-nutritional factors of

cassava (*Manihot esculenta* Crantz) grown in Ethiopia. International Journal of Science Innovations and Discoveries 3(2): 212-222.

Tope, A. K. 2014. Effect of fermentation on nutrient composition and anti-nutrient contents of ground Lima bean seeds fermented with *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*. International Journal of Advanced Research 2(7): 1208-1215.

Udensi, E. A. and Okoronkwo, K. A. 2006. Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate. African Journal of Biotechnology 5(10): 896-900.