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Modification of the physical and chemical properties of *Inpago-Protani* rice flour via fermentation with *Bimo-CF* starter

Nur Aini^{1,2#}, Hadana Sabila Arsyistawa¹, Budi Sustriawan¹, V. Prihananto¹, Santi Dwi Astuti¹, Aisyah Tri Septiana¹, Indarto³, Nageeb Mohammed Suliman⁴

¹Department of Food Technology, Jenderal Soedirman University, Dr. Soeparno Street, Purwokerto, 53123, Indonesia ²Integrated Technology and Management for Halal on Local Resources, Jenderal Soedirman University, Dr. Soeparno Street, Purwokerto, Indonesia

³Department of Agriculture Engineering, Jember University, Jl. Kalimantan Tegalboto No.37, Jember, 68121, Indonesia ⁴Department of Food Science and Technology, Faculty of Agriculture, Omdurman Islamic University, HCFX+4RV Omdurman, Khartoum, Sudan

#Corresponding author; email: <u>nur.aini@unsoed.ac.id</u>

Abstract— *Inpago-Protani* rice is a new rice variety from Jenderal Soedirman University, Indonesia. Producing rice flour as an intermediate product is a practical rice processing method. However, the utilization of rice flour as a wheat flour alternative is limited due to the differences in their gelatinization qualities. Fermentation with *Lactobacillus* sp. changes the gelatinization properties of rice flour by increasing the number of microorganisms and stimulating their metabolism in food materials. This study sought to determine how the physical and chemical qualities of *Inpago-Protani* rice flour are affected by the duration of fermentation, the concentration of Bimo-CF starter, and other factors. Applying 0.6% Bimo-CF starter for 12 hours at 30°C was the most effective method for fermenting *Inpago-Protani* rice flour. The resulting rice flour had a yield of 40.3%, a protein content of 10.3% (db), a carbohydrate content of 77.1%, a fat content of 0.4%, an ash content of 0.5%, a moisture content of 12.2%, and 338.7 kcal of energy. The enhanced characteristics of fermented rice flour may increase its versatility for subsequent processing.

Keywords-Inpago-Protani; fermentation; Bimo-CF starter; application

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I. INTRODUCTION

Rice breeding innovations have been performed by several researchers, including those at Jenderal Soedirman University, Indonesia, who produced a high-protein rice called *Inpago-Protani* [1]. This variety's advantages include a potential yield of 9.06 tons/ha of milled dry grain and a protein content of 9.81%, fluffier texture, and 27 ppm Zn. The high protein content in *Inpago-Protani* rice can be used to overcome stunting when it is processed into porridge [2].

In addition to porridge, there is significant processing potential for *Inpago-Protani* rice. The processing obstacle to producing the right product is that its functional properties are unknown. Among these processing properties, the most important is its gelatinization properties, which will facilitate determining its applications in food products [3].

Producing rice flour as an intermediary product is an extremely viable rice processing technology. For food goods, rice flour can be substituted for wheat flour to create gluten-free options for those with gluten intolerances. However, because rice flour's gelatinization properties differ from those of wheat flour, its usage as a substitute is limited. The flavor of rice flour can be altered via fermentation. Fermentation increases the number of microorganisms and activates their metabolism in dietary materials [4]. The incubation temperature, time, average pH, oxygen content, light level, and agitation level must all be ideal for fermentation to promote maximal development and production.

Nurhayati et al. [5] conducted fermentation research on the production of rice flour using Lactobacillus casei, which results in rice flour with improved digestibility and decreased viscosity. However, the weakness of that study is that it used Lactobacillus casei, so its application is less practical. Cassava can undergo fermentation during flour manufacturing to produce modified cassava flour with improved gelatinization qualities that increase its utility for various culinary products, including noodles, bread goods, and rice analogs [6,7,8,9,10]. Cassava fermentation uses the commercialized Bimo-CF starter, which may be useful for making fermented flour from other ingredients; however, it requires optimization. The character of the modified flour is also influenced by the fermentation time. According to Alfansuri et al. [11], the character of modified cassava flour is influenced by its fermentation time. Oladeji et al. [12] also stated that the character of fermented corn flour is influenced by the fermentation time. Kurniadi et al. [13] and Arifah et al. [14] further support this effect in sorghum flour produced via fermentation as the fermentation time affects the properties of the resulting sorghum flour.

The physical properties of flour-based products are significantly influenced by the functional properties of the dough [15]. The viscosity of the flour mixture is important for use as a gum substitute. Water holding capacity, flour solubility, and dough viscosity are other important parameters that determine the quality of the carbohydrate source materials used as fat substitutes [16]. The water absorption index and water solubility index are useful in food dough formulation and beverage applications as they describe materials' hydrophobic or hydrophilic nature. Meanwhile, the fat absorption index can indicate the natural interaction between fats and flour components. These functional characteristics need to be studied to facilitate their applications in food products [17].

No studies have yet been performed on using commercial Bimo-CF starter to ferment *Inpago-Protani* rice into flour. This research sought to ascertain the effects of fermentation time and Bimo-CF starter concentration, on the physical and chemical properties *of Inpago-Protani* rice flour.

II. MATERIALS AND METHODS

A. Materials

The main ingredients used in this research were *Inpago-Protani* rice obtained from the Teaching Factory, Jenderal Soedirman University, Purwokerto, Indonesia, and Bimo-CF starter (containing *Lactobacillus* sp.) obtained from an online seller. Other materials and equipment were used for the analyses.

B. Methods

A modified process based on Wang et al. [18] was used to produce *Inpago-Protani* rice flour. First, the rice was sorted and washed clean. The rice was then fermented for 12, 24, and 26 hours using Bimo-CF starter that was dissolved in distilled water (at 0, 0.2, 0.4, and 0.6%) at room temperature. The rice was then rinsed and dried in a cabinet dryer set to 60°C for 6 hours. Then, the rice was processed into rice flour with a disk mill and subjected to further drying in a cabinet dryer at 60°C for 4 hours. Finally, the dried rice flour [19] was sifted through an 80-mesh sieve.

This experiment used a completely randomized factorial design with 2 factors. The first factor was the concentration of Bimo-CF starter, which was 0% (B1), 0.2% (B2), 0.4% (B3), or 0.6% (B4); the second factor was fermentation time, which was 12 hours (T1), 24 hours (T2), or 36 hours (T3). The observed variables included yield, water content [19], protein [19], fat [19], ash [19], crude fiber, carbohydrates, water absorption capacity [20], and oil absorption capacity.

The data were analyzed with an analysis of variance test at a confidence level of 95%. If the treatment had a significant effect, the analysis continued with Duncan's multiple range test (DMRT) at a confidence level of 95%.

III. RESULTS AND DISCUSSION

A. Water contents

The water content of the fermentation-modified rice flour was significantly influenced by the Bimo-CF concentration, the fermentation time, and the combinations of these factors. Certain combinations of Bimo-CF concentration and fermentation time produced rice flour with a water content of 12.2-16.4% (**Table 1**). The highest water content (16.4%) was produced by 12 hours of fermentation with 0.4% Bimo-CF. The lowest water content (12.2%.) was obtained from 12 hours of fermentation with 0.6% Bimo-CF.

TABLE I WATER CONTENT OF INPAGO.PROTANI RICE FLOUR BY STARTER (BIMO-CF) CONCENTRATION AND FERMENTATION TIME

Concentration of Bimo-CF	Fermentation time (hours)		
(%)	12	24	36
0	13.57e±0.03	14.21d±0.05	14.80e±0.26
0.2	14.82e±0.18	15.83f±0.16	14.65e±0.20
0.4	16.43g±0.07	14.95e±0.02	$14.09d{\pm}0.09$
0.6	12.22a±0.13	12.29a±0.11	12.84b±0.05

Different indices indicate values that are significantly different at a confidence level of 95%.

The longer the fermentation time, the lower the water content [21]. This is because fermentation can degrade starches through microorganism activity which can decrease the materials that retain water. The longer the fermentation time, the greater the enzyme activity to degrade starch, liberating more bound water and making the texture of the material soft and porous [22]. This can increase water evaporation during the drying process, decreasing the water content further.

The amount of water trapped in starch's hollow structure is indicated by the flour's water content. This measures the large or small amounts of water trapped in the flour as it undergoes the microbial activity that produces fermented flour. According to Sengev et al. [23], the water content of sorghum flour decreased with extended fermentation because the microorganisms degraded starches during fermentation, reducing the material's capacity to retain water. The longer the fermentation process, the more the active enzymes break down starches, releasing a greater volume of bound water. This softens the material and makes it more porous. Therefore, more water will evaporate during the drying process and the water content will further decrease [24].

Over longer fermentation times, more starch is degraded by lactic acid, which is produced by lactic acid bacteria (LAB) in the soaking water [25]. The bound water in the starch will be released as the starch degrades and become free water. The raw material loses free water during the drying process. Therefore, a longer fermentation period permits greater evaporation of free water, resulting in flour with a lower water content than that achieved with other treatments.

B. Ash content

The ash content of rice flour modified using fermentation techniques was influenced by the concentration of Bimo-CF. It was not influenced by fermentation time or the interaction between the two factors. Rice flour fermented with 0.2% Bimo-CF had the lowest ($0.39\pm0.05\%$) ash content, while the highest ash content ($0.59\pm0.08\%$) was obtained from fermentation with 0.6% Bimo-CF, as shown in **Figure 1**.



Fig 1. Average ash content of Inpago-Protani rice flour prepared via 12, 24, or 36 hours of fermentation with different concentrations of starter (Bimo-CF)

Different indices indicate values that are significantly different at a confidence level of 95%.

Rice flour made by fermenting 0.6% Bimo-CF had a higher ash content than rice flour fermented with 0.2% and 0.4% Bimo-CF. The high ash content in rice flour fermented with 0.6% Bimo-CF was inversely proportional to the water content, which was much lower than the water content of fermented rice flour prepared with 0.2% or 0.4% Bimo-CF. Rice flour fermented with Bimo-CF also had a higher ash content than unfermented rice flour (0.53 \pm 0.05%).

This happens due to the water content decreasing under high drying temperatures. Furthermore, the increased amylose content following fermentation accounts for the modified flour's decreased water content [26]. Because amylose has a straight, thick structure that facilitates the efficient absorption and release of water, materials with a high amylose content will more readily release the water they contain during drying, reducing their water content [27]. The fraction of ash content in a substance increases with decreasing water content.

C. Protein

Starter concentration, fermentation time, and their interactions had a significant effect on the protein content of the rice flour. The lowest protein content was found in rice flour produced through fermentation with 0.2% Bimo-CF for 24 hours (**Table 2**). The rice flour with the highest protein content was produced from fermentation with 0.4% Bimo-CF for 36 hours. The increase in the amount of protein reflects the increasing number of microbes that act as single-cell proteins [28].

Protein degradation occurs when the lactic acid bacteria in Bimo-CF create proteinase enzymes during fermentation. These enzymes turn proteins into their component amino acids, which subsequently become lactic acid [29]. Nevertheless, there was an increase in protein after 24-hour fermentation; this is assumed to be the result of an increase in the number of microorganisms. The number of bacteria grows with fermentation time, increasing the quantities of dissolved protein.

TABLE 2 PROTEIN CONTENT OF INPAGO-PROTANI RICE FLOUR BY STARTER (BIMO-CF) CONCENTRATION AND FERMENTATION TIME

Concentration of Bimo-CF (%)	Fermentation time (hours)		
	12	24	36
0	9.13 ^{ef} ±0.02	9.69 ^g ±0.36	8.61 ^{cd} ±0.29
0.2	$8.52^{bc} \pm 0.05$	$8.07^{a}\pm 0.02$	$8.93^{de}{\pm}0.01$
0.4	$8.16^{ab}\pm0.10$	$9.38^{\text{fg}} \pm 0.11$	10.31 ^h ±0.24
0.6	$10.29^{h}\pm 0.01$	$8.48^{bc} \pm 0.04$	10.23 ^h ±0.15

Different indices indicate values that are significantly different at a confidence level of 95%.

The protein content increased with the percentage of starter utilized. This is because the lactic acid bacteria in Bimo-CF produce substantial quantities of protease enzymes during fermentation, which increase the material's protein content. Protease hydrolyzes proteins into simpler peptides. Because more bacteria act as single-cell proteins (SCPs) and proteins are derived from microbes, the quantity of protein increases [30].

D. Fat content

The fat content of modified rice flour was significantly affected by the concentration of Bimo-CF and the interaction between concentration and fermentation time. Fermentation time alone had no significant influence. Modified *Inpago-Protani* rice flour had a fat percentage of 0.26–0.83% (**Table 3**). The highest fat content was achieved via 12 hours of fermentation with 0.4% Bimo-CF.

TABLE 3 FAT CONTENT OF INPAGO_PROTANI RICE FLOUR BY STARTER (BIMO_ CF) CONCENTRATION AND FERMENTATION TIME

Concentration of Bimo-CF	Fermentation time (hours)		
(%)	12	24	36
0	0.37 ^a ±0.03	$0.26^{a} \pm 0.02$	0.27ª±0.05
0.2	0.28ª±0.01	$0.40^{a} \pm 0.10$	$0.40^{a} \pm 0.07$
0.4	0.83°±0.01	$0.65^{b}\pm 0.13$	0.42ª±0.09
0.6	$0.40^{a} \pm 0.05$	0.35ª±0.07	$0.63^{b} \pm 0.02$

Different indices indicate values that are significantly different at a confidence level of 95%.

The higher the starter concentration, the higher the fat content. This is because the microorganisms contained in the starter, *Lactobacillus* sp., produce microbial oils during fermentation. According to Garrido-Galland [31], microorganisms, like every other living cell system, produce lipids or fats.

E. Fiber content

The fiber content of rice flour modified using fermentation techniques was only significantly influenced by the Bimo-CF concentration and was not influenced by fermentation time or the interaction of these two factors. There was no discernible change in the crude fiber content of Bimo-CF fermented rice flour at concentrations of 0.2–0.6% and 12–36 hours of fermentation (**Figure 2**).



Fig 2. The average crude fiber of Inpago-Protani rice flour prepared via 12, 24, or 36 hours of fermentation with different concentrations of starter (Bimo-CF)

Different indices indicate values that are significantly different at a confidence level of 95%.

However, compared to spontaneously fermented rice flour, which only contained 0.50% crude fiber, fermented rice flour with the addition of Bimo-CF exhibited a substantially larger crude fiber concentration. The crude fiber content in rice flour that was not subjected to spontaneous or targeted fermentation was 0.88%. This indicates that the fermentation process decreased the amount of crude fiber, possibly because lactic acid bacteria naturally broke the fiber down into glucose during the fermentation process, reducing the fiber content as the fermentation time increased. Rice cell walls can be destroyed by pectinolytic and cellulolytic enzymes that are produced by LAB [32]. LAB also produce small amounts of cellulase, which can break down lignocellulose and lignohemicellulose, which are naturally occurring fibers. The breakdown of lignocellulose and lignohemicellulose causes cellulose and hemicellulose to dissolve into simple forms that are easily utilized by bacteria as an energy source, further decreasing the cellulose and hemicellulose content [33].

F. Carbohydrates

Carbohydrates in fermented rice flour are significantly influenced by starter concentration, fermentation time, and the interaction between these factors. The lowest (73.49%) carbohydrate content occurred in rice flour made via fermentation using 0.2% Bimo-CF for 36 hours. Meanwhile, the highest carbohydrate (77.12%) content was obtained by fermenting rice flour with 0.6% Bimo-CF for 12 hours (**Table 4**).

TABLE 4 CARBOHYDRATES OF INPAGO_PROTANI RICE FLOUR BY STARTER (BIMO-CF) CONCENTRATION AND FERMENTATION TIME

Concentration	Fermentation time (hours)		
(%)	12	24	36
0	75.89e±0.0	74.80c±0.18	75.24cd±0.10
0.2	75.18cd±0.29	74.30b±0.27	73.49a±0.01
0.4	73.54a±0.29	73.79a±0.05	75.82e±0.38
0.6	77.12f±0.19	75.62de±0.33	74.86c±0.15

Different indices indicate values that are significantly different at a confidence level of 95%.

Rice flour fermented with 0.2% Bimo-CF for 36 hours contains fewer carbohydrates than unfermented rice flour. This is consistent with the study by Ogodo [34], which demonstrated that the carbohydrate content of sorghum flour can be lowered through fermentation with *Lactobacillus plantarum*, *L. rhamnosus*, *L. nantensis*, and *L. fermentum*. Furthermore, the amount of carbohydrates in fermented sorghum flour decreased with increased starter concentrations and longer fermentation times. During fermentation, the amount of carbohydrates decreases, suggesting that a process of carbohydrate breakdown occurs.

G. Energy

The rice flour yield was significantly affected by the starter concentration and its interaction with the fermentation time; however, the output was not significantly affected by the fermentation time alone. The rice flour fermented with 0.2% Bimo-CF for 24 hours had the lowest energy content (324.51 kcal). Conversely, the combination of 0.6% Bimo-CF and a 12-hour fermentation period resulted in the highest energy content (338.71 kcal; see **Table 5**). Shorter fermentation times also resulted in higher carbohydrate content. Carbohydrates are the macronutrient component that delivers the most energy to consumers; therefore, high carbohydrate content affects how much energy the material contains.

TABLE 5 TOTAL ENERGY OF INPAGO_PROTANI RICE FLOUR BY STARTER (BIMO_ CF) CONCENTRATION AND FERMENTATION TIME

Concentration of Bimo-CF	Fermentation time (hours)		
	12	24	36
0	334.5g±2.1	332.7f±2.0	328.8bc±8.1
0.2	328.4b±1.6	324.5a±6.0	330.5de±9.01
0.4	325.1a±9.2	330.0cd±5.4	331.9ef±8.3
0.6	338.7h±9.1	338.3h±4.9	337.5h±5.1

Different indices indicate values that are significantly different at a confidence level of 95%.

The energy content of the fermented flour was equal to or less than that of the control (unfermented) flour, which had an energy value of 346.25 kcal. This was also consistent with the findings regarding carbohydrate levels, which indicated a decline during the fermentation process. Omoba and Isah [35] stated that more carbohydrates will be broken down and more energy will be lost during fermentation at a higher rate over a longer period.

H. Yields

Bimo-CF concentration, fermentation period, and the combination of these factors did not significantly affect the production of fermented rice flour. The relatively similar average yields (21.50–40.30%) likely reflect the slight concentration difference between the treatments. The yield of fermented rice flour was higher than that of rice flour made without fermentation (22.34%).

G. Water absorption capacity

Bimo-CF concentration, fermentation time, and the interaction between these factors did not have a significant effect on water absorption capacity. The water absorption capacity of the fermented rice flour ranged from 53-127%. These results are also not significantly different from those of unfermented rice flour, which has a water absorption capacity of 107%. Similar results were also obtained by Aini et al. [36], who affirmed that spontaneous fermentation with yeast, *L. bulgaricus*, and *L. casei* for 20–80 hours did not significantly affect water absorption. However, the longer the fermentation time, the higher the flour's water absorption capacity. This was caused by macromolecules that had been relatively compact becoming more porous. As the molecules broke down into simpler and smaller masses, they became looser and able to absorb water more easily.

I. Oil absorption capacity

The amount of applied Bimo-CF, the fermentation time, and the combinations of these factors had no discernible effect on the fermented rice flour's ability to absorb oil. Fermented rice flour had an oil absorption capacity of 83–140% compared to the control oil absorption capacity of 82%. This limited difference was caused by a lack of fermentation time. Research conducted by Aini et al. [36] resulted in significant differences in oil absorption in corn flour fermented for 20–80 hours, whereas in this study, the fermentation time was only 12–36 hours. The longer the fermentation time, the greater the degradation of macromolecules into simpler molecules [37]. The starch granules in flour that expand due to water absorption during soaking also improve oil absorption due to the breakdown of complex molecules into simpler molecules [36].

IV. CONCLUSION

In this study, the best way to prepare modified *Inpago-Protani* rice flour was through fermentation with 0.6% Bimo-CF starter (mainly consisting of *Lactobacillus sp*). for 12 hours. This resulted in rice flour with 40.3% yield, 10.3% db protein content, 77.1% carbohydrate content, 0.4% fat content, 0.5% db ash content, 12.2% moisture content, and 338.7 kcal of energy. Improving the characteristics of fermented rice flour may make it more versatile and support wider applications.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

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