Enhancement in Bioaccessibility and Bioavailability of Phenolic Compounds during Black Glutinous Rice *Tape* Fermentation

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ABSTRACT

Black glutinous rice (*Oryza sativa* var. glutinosa) *tape* fermented with various yeast, mold, and bacteria is often rich in phenolics compounds and can contribute positively to health through its antioxidants activity. Despite the potential, these compounds have limited bioavailability value due to their structure, degree of glycosylation or polymerization, and interactions with other components. Therefore, this study aims to determine the effect of fermentation on bioavailability and bioaccessibility of phenolics compounds in black glutinous rice *tape*. During the procedures, cooked black glutinous rice was inoculated with *ragi tape* for 72 hours. Sampling was then performed every 24 hours to analyze bioaccessibility of phenolics compounds, flavonoids, and antioxidants activity. Subsequently, absorption was carried out using an everted gut sac model. The results showed that phenolics compounds were released from the food matrix during gastric and small intestine digestion. Fermentation was shown to increase the content of accessible phenolics compounds from 19.89% to 27.31%, flavonoids from 68.88% to 81.72%, and antioxidants activity from 13.56% to 22.89%. During fermentation, the highest increments were obtained after 72 hours, with 27.31% for total phenolics compounds, 81.72% for flavonoid compounds, and 22.89% for antioxidants activity. The products obtained after 72 hours of fermentation exhibited significantly highest absorption, but no significant differences were observed between the duodenum and ileum segments. The absorption of these compounds in the jejunum from the extract was significantly higher in fermented samples. Therefore, fermentation significantly enhanced bioavailability of phenolics compounds in black glutinous rice *tape*.

Keywords: Antioxidants; bioavailability; black glutinous rice; phenolics; *tape*

INTRODUCTION

Numerous fermented products, such as black glutinous rice tape have been explored for their potential health advantages in Indonesia. Black glutinous rice *tape* is often produced by adding *ragi tape*, which contains various kinds of microorganisms, such as *Pediococcus pentosaceus*, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Endomycopsis burtonii*, *Hansenula anomala*, and *Rhizopus oryzae*, to cooked black glutinous rice (Aryanta, 2000; Marniza et al., 2020). In addition, each group of microorganisms assumes specific roles in fermentation process of the product. For example, the fungal group is adept at simplifying starch into glucose by producing various amylases, while the yeast group is responsible for converting sugars into alcohol and various other organic compounds. In this context, *Acetobacter* can convert alcohol into acetic acid (Novelina et al.,

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2019), while lactic acid bacteria (LAB) have a role in metabolizing sugar present in rice *tape*, leading to lactic acid production (Siebenhandl et al., 2001).

According to previous studies, black glutinous rice *tape* has been found to contain phenolics compounds, including 2,4,6-trihydroxybenzoic acid, sinapic acid, *p*-coumaric acid, ferulic acid, isoferulic acid, vanillic acid, vanillin, protocatechuic aldehyde. The product also has high antioxidants properties, with IC50 of 84.39 µg/g (free), 45.33 µg/g (conjugated free), and 132.95 µg/g (bound) (Azkia et al., 2023). Several studies have reported that phenolics compounds have many health benefits and antioxidants properties. These benefits comprise lowering the risk of diseases associated with oxidative stress, including cancer, diabetes, and cardiovascular (Kumar & Goel, 2019). Phenolics compounds also function as antioxidants because of their redox potential, enhancing their ability to bind on metals, neutralize singlet oxygen, and free radicals, and act as hydrogen donors (Mishra et al., 2020). Understanding bioaccessibility and bioavailability of phenolicss is crucial to providing maximal physiological function in the body. Bioaccessibility is the amount of food substances in the small intestine released from the food matrix following the post-digestive phase, which facilitates potential substance transport over the intestinal barrier. Meanwhile, bioavailability refers to the amounts of substances the body can absorb, digest, and metabolize (McGhie & Walton, 2007). Bioavailability is often influenced by the stability and structure of compounds, their release capability from the matrix (bioaccessible), the ability to traverse the transepithelial pathway, and individual physiological factors (Cosme et al., 2020).

In line with these findings, phenolics are not completely absorbed in the small intestine, with only approximately 12-45% being absorbed (Cañas et al., 2022). Muñoz-González et al. (2013) showed that unabsorbed compounds may be excreted through fecal up to 267 µg/g. Phenolics in food are commonly classified as free, conjugated, and bound (Arruda et al., 2018). In this context, phenolics aglycones are examples of free compounds, which have high absorption through passive diffusion. Conjugated phenolics typically exist as dissolved glycosides, necessitating the activity of lactase phlorizin hydrolase (LPH) to break down glycosidic bonds in the food matrix before absorption (Cosme et al., 2020). Moreover, this conjugated form can be carried by using the active co-transporter 1 Na^+/glucose (SGLT1) to reach epithelial cells, enabling cytosolic *β*-glucosidase to assist in the release of the aglycone (Teng & Chen, 2019). Restricted liberation has been shown by bound phenolics that are covalently bonded to indigestible components, such as structural proteins, polymerized phenolics (like lignin and condensed tannins), and polysaccharides (like pectin, hemicellulose, cellulose, and arabinoxylan) (Shahidi & Ambigaipalan, 2015). These compounds typically have low bioavailability because only a small amount can pass through the intestinal epithelium and reach the bloodstream (Shahidi & Peng, 2018). The release and absorption of phenolics during digestion may be restricted by their chemical structure and interactions with the food matrix, necessitating specific processing methods to overcome the limitations.

Naturally found in food plants, flavonoids belong to phenolics compounds group and have been the subject of numerous studies due to their possible health benefits. However, their impact typically depends on bioaccessibility and ability to be absorbed and metabolized within the body. According to epidemiological studies, consuming a diet high in flavonoids has many health benefits, such as decreasing the risk of chronic diseases, including cancer, type 2 diabetes, and cardiovascular disorders. The macronutrients (proteins, lipids, and carbohydrates) and micronutrients (vitamins, minerals) in the food matrix have a crucial role in modulating the release and bioactivity of flavonoids during digestion (Kamiloglu et al., 2021). Structural composition also plays a pivotal role in the absorption of these compounds within the body. Aglycone-form flavonoids show superior bioavailability compared to their glycoside variants. The molecular weight is a significant determinant affecting the absorption and consequent bioavailability of specific flavonoids. For instance, polymeric proanthocyanidins, which belong to the high molecular weight category, generally exhibit reduced bioavailability compared to oligomeric or monomeric variants (Zhong et al., 2018).

Based on previous studies, fermentation is an effective processing method to improve the release of phenolics compounds. Microorganisms often generate enzymes during fermentation, including xylanase, pectinase, esterase, *β*-glucosidase, *β*-xylosidase, and *β*-galactosidase. These enzymes play a role in releasing bound phenolics compounds into soluble/free forms, thereby increasing their bioactivity (Adebo & Medina-Meza, 2020). A previous study showed that the accessible constituents in fermented rice bran increased after gastric digestion from 347.04 mg GAE/100 g to 567.16 mg GAE/100 g and after small intestine digestion from 395.11 mg GAE/100 g to 649.55 mg GAE/100 g (Chen et al., 2019). Janarny & Gunathilake (2020) also studied the impact of *Rhizopus oryzae* fermentation on enhancing the absorption of rice bran phenolics compounds, with absorption levels increasing from 5.35% to 9.41%. Meanwhile, Khan et al. (2020) studied

the effect of *Saccharomyces cerevisiae* and LAB co-culture fermentation after digestion. According to Azkia et al. (2023), there was a significant increase (49.25%) in extracted free phenolics compounds following fermentation of black glutinous rice *tape*. These products may facilitate the enhancement of bioaccessibility values during digestion and improve their bioavailability. Despite the existing literature, there is no information on their bioaccessibility and bioavailability. Therefore, this study aims to evaluate the effect of fermentation on phenolics compounds' bioaccessibility and bioavailability in black glutinous rice *tape*. The availability of compounds post-digestion and their subsequent absorption using an inverted gut sac model was analyzed. Implementing fermentation technology presents a potential strategy for amplifying the availability and absorption of phenolics compounds, thereby enhancing their potential health benefits for consumers.

METHODS

Materials

The main materials used in this study were black glutinous rice cultivar YR02 from local farmers in Blitar, NKL brand *ragi tape* from Surakarta, and Sprague Dawley rats obtained from the Center for Food and Nutrition Studies at UGM, Indonesia. The chemicals used for analytical purposes included *α*-amylase, pepsin, sodium chloride (NaCl), potassium chloride (KCl), pancreatin, bile salt, sodium bicarbonate ($NaHCO_3$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), and potassium dihydrogen phosphate (KH_2PO_4). Others included sodium hydrogen phosphate (Na_2HPO_4), calcium chloride ($CaCl_2$), methanol, ascorbic acid, quercetin, gallic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), folin-ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitrite ($NaNO_2$), and aluminum chloride ($AlCl_3$) obtained from Sigma Aldrich, USA. Experimental subjects were 3-month-old rats, and all procedures involving these animals were conducted ethically with approval from the Medical and Health Study Ethics Committee of the Faculty of Medicine, Public Health, and Nursing (FK-KMK) at UGM under Permit No. KE/FK/0018/EC/2023.

Tape Fermentation from Cooked Black Glutinous Rice

The preparation of black glutinous rice *tape* followed the method described in a study by Azkia et al. (2023). Black glutinous rice was soaked overnight in water with a ratio of 1:1.5 (w/v). After draining, rice was steamed for 60 minutes, mixed with 250 mL water per 1kg cooked black glutinous rice (25% v/w) , and then steamed again for another 60 minutes. Subsequently, the steamed black glutinous rice was transferred under clean conditions into vessels and inoculated with 0.3% (w/w) *ragi tape* at a temperature of 30°C, and incubated for 72 hours. *Tape* produced was freeze-dried and kept in airtight, dry containers for further analysis.

Bioaccessibility Test

The *in vitro* digestion processes followed the method of Gawlik-Dziki et al. (2009) with modification. 5 grams of sample was thoroughly mixed with 15 mL saliva solution containing 0.2 U/mL *α*-amylase in a buffer containing 2.38 g/L Na_2HPO_4, 0.19 g/L KH_2PO_4 and 8 g/L NaCl at pH 6.75. The resulting mixture was incubated for 10 minutes at 37°C in a shaking water bath. Subsequently, 5 M HCl was added to adjust the pH to 1.2, and 15 mL of stomach fluid, containing 0.32% pepsin in 0.03 M NaCl. Gastric digestion was stopped by adding 0.1 M $NaHCO_3$ to adjust the pH to 6 after 120 minutes of incubation. 15 mL of intestinal fluid (containing 0.05 g of pancreatin and 0.3 g of bile salts) was added, and the pH was adjusted to 7 with 1 M NaOH to simulate the intestinal phase. Subsequently, 2.5 mL each of 5 mM KCl and 120 mM NaCl were added. The mixture was then incubated for 60 minutes at 37 °C in a shaking water bath. Samples were collected at the end of the gastric and intestinal digestion step and centrifuged at 4 °C and 4000 rpm for 10 min. Supernatants obtained from each digestion stage were used for analysis to determine total phenolics, total flavonoids, and antioxidants activity within the digestive system. The percent bioaccessibility was calculated as the ratio of compounds present in the supernatant after digestion to the total content in the samples.

Bioavailability Test

Phenolics compounds were extracted using ultrasound-assisted extraction, according to Babotă et al. (2022). The powdered sample was dissolved in 50% ethanol solvent (1:10 w/v) and sonicated for 6.5 minutes. The maximum amplitude was set at 35% of the 26 kHz ultrasound wave (Ultrasonic Hielscher UP200St 200W), the solvent was then evaporated using a vacuum rotary evaporator before further testing. In addition, phenolics compounds absorption followed the method of Liu et al. (2022) and Wilson & Wiseman (1954) with modifications. Sprague-Dawley rats were anesthetized with 60 mg/kg body weight of ketamine after fasting, and segments of the duodenum, jejunum, as well as ileum (10 cm) were obtained from the abdominal region. Each segment was thoroughly cleaned with saline solution and everted using a glass rod, while the

end of each section was tied with a thread to form a pouch. Subsequently, the empty pouch was filled with 1 mL Krebs-Ringer buffer (KRB), containing 6.90 g of NaCl, 0.35 g of KCl, 0.29 g of MgSO4·7H2O, 0.16 g of KH2PO4, 2.10 g of NaHCO3, 0.28 g of CaCl2, and 0.2% glucose in 1 L of distilled water, adjusted to pH of 7.3 and immersed in the extract solution. The solution was carefully maintained at 37 °C using a water bath for 2 hours. The absorption percentage was calculated using the Equation 1.

$$\text{Absorption } (\%) = \frac{Cs}{Ci} \times 100 \quad (1)$$

where Cs: Concentration of absorbed phenolics compounds in each intestinal segment (µg/mL) and Ci: Initial concentration of phenolics compounds (µg/mL).

Analysis of Total Phenolics

The total phenolics content in this study was analyzed following the method of Velioglu et al. (1998) with modification. A mixture of 125 µL of sample, 250 µL of water, and 125 µL Folin-Ciocalteu reagent was combined. The mixture was left to settle in the dark for 5 minutes at room temperature. Subsequently, 1.25 mL of 7% w/v sodium carbonate and 1 mL of water were added to the mixture. The homogenized solution was allowed to stand in the dark for 90 minutes. Moreover, the absorbance was measured at 760 nm, and the standard gallic acid curve was used at concentrations 0-250 µg/mL. The total phenolics content of the sample was determined in mg GAE/g.

Analysis of Total Flavonoid

The analysis of total flavonoids followed the method of Zhishen et al. (1999) with modification. A total of 0.2 mL of the sample was combined with 0.8 mL of water and then let to stand for 6 minutes, before adding 50 µL of sodium nitrite (5% w/v). Subsequently, 100 µL of aluminum chloride (10% w/v) was added after 5 minutes. The final amount of 2.5 mL was reached by adding 0.5 mL of 1 M sodium hydroxide and diluting it with 0.85 mL of water. The resulting blend was thoroughly mixed, and absorbance measurements were taken at a wavelength of 510 nm. The results were presented as mg QE/g based on the initial dry weight.

Antioxidants Activity

The DPPH radical scavenging activity (RSA) method determined antioxidants activity value according to Brand-Williams et al. (1995) with modification. 200 µL of the sample was thoroughly combined with 1 mL of 0.1 mM DPPH reagent. The mixture was left in darkness for 60 minutes following homogenization, while the measurements of absorbance were taken at a wavelength of 515 nm. Furthermore, control absorbance was determined using an identical process, however the sample was replaced with water. Ascorbic acid served as the standard, and antioxidants activity was showed in mg AAE/g based on the initial dry matter.

Data Analysis

Statistical analysis was carried out using One-way analysis of variance (ANOVA) using IBM SPSS Statistics Version 25.0 (SPSS Inc., Chicago, IL, USA). Mean values by triplicated data along with their corresponding standard deviations were reported for all tests. Duncan's Multiple Range Test (DMRT) was used to identify noteworthy distinctions between variables at a 5% significance level.

RESULTS AND DISCUSSION

Bioaccessibility of Phenolics after *In Vitro* Digestion

This study showed that phenolic substances were crucial to human health. Understanding bioaccessibility or the availability of these compounds in an effectively accessible was essential to achieve the biological activity of phenolic compounds that could be liberated from the food matrix after digestion. This study used models of gastrointestinal digestion to model the in vivo physiological environment of the digestive tract. Figure 1 presented bioaccessibility of phenolic compounds in the gastric phases of fermented black glutinous rice *tape* was 11.18%, 17.38%, 18.58%, and 19.58% at fermentation times of 0, 24, 48, and 72 hours, respectively. Fermentation process showed a significant increase in bioaccessibility phenolic value ($p < 0.05$) observed in both the gastric and gastrointestinal phases. The effectively accessed phenolics content in these phases of fermented black glutinous rice *tape* was measured as 19.89%, 23.00%, 26.19%, and 27.31% at fermentation times of 0, 24, 48, and 72 hours, respectively. Khan et al. (2020) studied the enhanced bioaccessibility of phenolic compounds in extruded brown rice following co-culture fermentation. These compounds increased by approximately 13% after gastrointestinal digestion. The increased phenolic compounds following gastric and small intestinal digestion could be attributed to the breakdown of complex food matrices by digestive enzymes, leading to the release of bound phenolics during digestion (Cuvas-Limon et al., 2022).

Phenolic compounds were classified into 2 forms namely soluble and insoluble forms. Soluble phenolic compounds underwent synthesis within the endoplasmic reticulum intracellular of plants and were

stored within the cell vacuole in free or conjugated form. Meanwhile, insoluble phenolic compounds were bound to the structural proteins, cellulose, and arabinoxylan components of cell walls (Zhang et al., 2020). The availability of compounds to be absorbed in the bloodstream was influenced by their structure. Free and conjugated phenolic compounds were available for absorption through the intestinal brush border, either through passive or facilitated diffusion (Cosme et al., 2020). In contrast, bound phenolic compounds had limited release ability because the cell wall matrix was bound. During digestion, specifically in the stomach and small intestine, the release of these bound compounds could increase due to the activity of protease and lipase enzymes, which played an important role in hydrolyzing the cell wall matrix (Cuvas-Limon et al., 2022). Proteases such as pepsin, trypsin, and chymotrypsin in the digestive tract could hydrolyze proteins into peptides with lower molecular weights, enhancing the release of phenolic substances that interacted with structural proteins (Yilmaz et al., 2022). Meanwhile, pancreatic lipase contributed to heightened lipid hydrolysis, releasing more fatty acids, thereby potentially increasing the liberation of phenolic compounds conjugated with fatty acids (Berton et al., 2012). After digestion, the total of free and conjugated phenolics was more accessible for absorption.

This study showed that black glutinous rice *tape* fermentation could significantly improve the bioaccessible value of phenolic compounds. Enzyme activity during fermentation, such as protease, *β*-glucosidase, xylanase, cellulase, and esterase, improved the liberation of bound phenolic compounds and enhanced the release of phenolic compounds into more superficial structures (Maia et al., 2020). The release capability of bound phenolic compounds was limited due to their binding

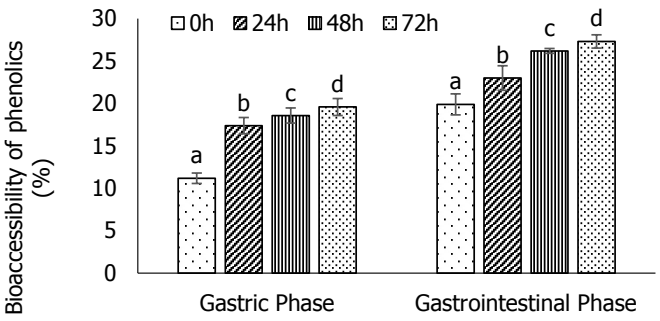

Figure 1. Phenolics compounds bioaccessibility (%) of black glutinous rice *tape* after different times of fermentation. For any specific digestion phase, identical letters showed no statistically significant differences, $p < 0.05$.

to the structural matrix of the cell wall. Consequently, the digestive enzymes produced by the body could not comprehensively break down this matrix. Phenolic compounds bound to cellulose components were not released in the digestive system due to the absence of the cellulase enzyme. Furthermore, fermentation process allowed cellulose-bound phenolics to be liberated by cellulase enzymes produced during *tape* fermentation (Abduh et al., 2022; Xue et al., 2017). Schmidt et al. (2014) showed an increase in soluble phenolic acids following fermentation duration of 24 to 120 hours. There was an increase specifically in gallic acid, *p*-hydroxybenzoic acid, caffeic acid, syringic acid, vanillin, *p*-coumaric acid, protocatechuic acid, chlorogenic acid, and ferulic acid. Azkia et al. (2023) observed an increase in soluble phenolics compounds in free and conjugated forms after fermentation of black glutinous rice *tape*. Several phenolics found in black glutinous rice *tape* included 2,4,6-trihydroxy benzoic acid (45.47 ppm), protocatechuic acid (143.56 ppm), protocatechuic aldehyde (5.29 ppm), caffeic acid (1.73 ppm), vanillic acid (91.18 ppm), vanillin (7.56 ppm), *p*-coumaric acid (22.87 ppm), ferulic acid (89.40 ppm), sinapic acid (5.78 ppm), and isoferulic acid (3.56 ppm) (Azkia et al., 2023).

Bioaccessibility of Flavonoids after *In Vitro* Digestion

Figure 2 showed the bioaccessible flavonoid values in black glutinous rice *tape* fermentation in this study. During the gastric digestion, bioaccessibility of flavonoid values was between 41.66% - 51.08% over the 0-72 hour fermentation period. Meanwhile, during the gastrointestinal digestion phase, bioaccessibility of flavonoid values ranged from 68.88% - 81.72% over the 0-72 hour fermentation period. Some of the studied flavonoid compounds in black rice included catechin, epicatechin, cyanidin 3-O-rutinoside, hesperidin, isorhamnetin, isorhamnetin-3-O-glucoside, luteolin, myricetin-3-galactoside, naringenin, pelargonidin-3-glucoside, procyanidin, quercetin, quercetin-3-galactoside, rutin, cyanidin-3-O-glucoside, and peonidin-3-glucoside (Liu et al., 2023; Xiong et al., 2023). Flavonoids could interact with various food constituents, such as proteins and polysaccharides, affecting their individual properties and functions, which were recognized for their ability to bind with proteins, and this interaction could potentially reduce the system's antioxidants capacity (Arts et al., 2002). Moreover, the high availability of flavonoid compounds after gastric and gastrointestinal digestion could be due to enzymatic activity in gastric and intestinal fluids. Qin et al. (2022) studied post *in vitro* digestion using pepsin

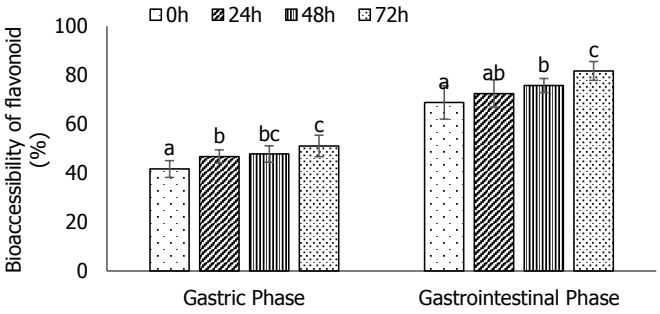

Figure 2. Flavonoid compounds bioaccessibility (%) of black glutinous rice *tape* after different times of fermentation. For any specific digestion phase, identical letters showed no statistically significant differences, $p < 0.05$

and pancreatin enzymes, which showed an increase in flavonoid release compared to non-enzymatic digestion. Enzymatic reactions could disrupt chemical bonds between phenolic compounds and proteins, carbohydrates, as well as lipids, thereby increasing the solubility and release of phenolic compounds. Balakrishnan & Schneider (2020) further studied increased soluble flavonoids after gastric and small intestinal digestion phases.

The data showed that fermentation treatment could increase bioaccessibility flavonoid value by 12.84% after gastrointestinal digestion. Zieliński et al. (2022) also studied several individual flavonoid compounds released after digestion. This caused an increase in LAB fermented wheat biscuit samples compared to non-fermented samples, rising to 37.28 µg/g for epicatechin, 14.07 µg/g for vitexin, 5.97 µg/g for orientin, 3.55 µg/g for apigenin, and 0.20 µg/g for luteolin. The effect of black glutinous rice tape fermentation for up to 72 hours was observed to effectively increase bioaccessibility flavonoid values, with an increase of 9.42% in the gastric phase and 12.84% in the gastrointestinal phase. In addition, enzymes produced during fermentation could break ester bonds and hydrolyze *β*-glucosidic bonds, releasing flavonoid compounds (Adebo & Medina-Meza, 2020). Guo et al. (2020) investigated fermentation with *Monascus anka*, effectively increasing quercetin and kaempferol aglycones from mulberry leaves, increasing up to day 10 of fermentation. The observation of mulberry leaf morphology also showed cell wall structure damage due to fermentation process, causing dissociation and glycoside flavonoid conversion into quercetin and kaempferol aglycones. Breaking down cell walls was facilitated by several carbohydrate-hydrolyzing enzymes, including *β*-glucosidase, xylanase, and cellulase. This process resulted in phenolics dissociation and glycoside deglycosylation into aglycones. Other studies had further investigated various enzymes involved in the hydrolysis of polymeric flavonoid compounds, such as tannase, esterase, phenolics decarboxylase, and glucosidase. Tannase, also referred to as tannin acyl hydrolase, was an enzyme capable of catalyzing the breakdown of hydrolyzable tannins. For instance, it facilitated the hydrolysis of epigallocatechin gallate (EGCG) into monomeric flavonoids, including epicatechin gallate (ECG), epigallocatechin (ECG), and gallic acid (GA) (Yang et al., 2023).

Bioaccessibility of Antioxidants Activity after *In Vitro* Digestion

Bioaccessibility of antioxidants activity referred to compounds with antioxidants activity from food sources available for absorption by the body after passing through the digestive system. Antioxidants had been exhibited to be effectively released through the application of fermentation processes, and digestion could influence how bioaccessible these compounds were. Gastric digestion showed bioaccessibility of antioxidants activity at 6.88%, 7.99%, 12.22%, and 20.21% at 0, 24, 48, and 72 hours of fermentation. Meanwhile, after intestinal digestion, bioaccessibility of antioxidants activity values increased to 13.56%, 16.12%, 17.79%, and 22.89% at 0, 24, 48, and 72 hours of fermentation. The study results showed that bioaccessible antioxidants activity values increased following the small intestinal and gastric digesting phase. Janarny & Gunathilake (2020) found a similar occurrence, showing that following small intestine digestion, the bioaccessible antioxidants value of Bw367 rice bran increased from 2.85 mg AAE/g to 3.12 mg AAE/g (9.47%). In addition, Seraglio et al. (2017) also observed an increase in bioaccessibility DPPH antioxidants activity after *in vitro* digestion of

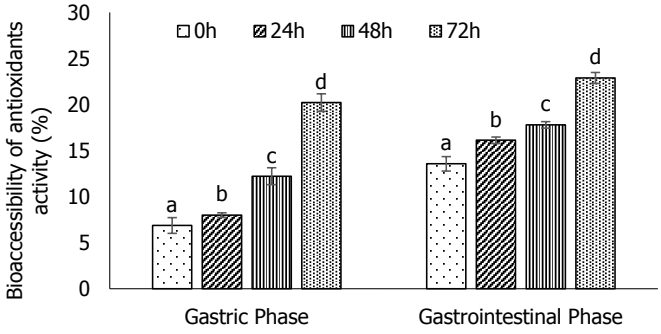

Figure 3. Bioaccessibility of antioxidants activity (%) of black glutinous rice *tape* after different times of fermentation. For any specific digestion phase, identical letters showed no statistically significant differences, $p < 0.05$

Mimosa scabrella Benth., approximately 79.84% for urupema type, 59.91% for urubici type, and 56.77% for large type.

The release of phenolics and flavonoid compounds over digestion was related to the available antioxidants following the *in vitro* digestion process. Phenolics compounds possessed antioxidants qualities through multiple mechanisms, such as transition metal chelation, electron transfer, and hydrogen atom transfer. Phenolics compounds could act as hydrogen donors to free radical substrates, generating non-radical substrate species (RH, ROH, or ROOH). Phenolics compounds' structure, specifically the benzene ring and the number and location of -OH groups, impacted their antioxidants capacity. The stabilization of antioxidants molecules during their interaction with free radicals was attributed to the benzene ring (Zeb, 2020). Figure 3 showed an increase in bioaccessibility of antioxidants activity values after 24, 48, and 72 hours of fermentation, during the gastric digestion phase and the gastrointestinal digestion phase. This showed that as fermentation time increased, the increment in values was greater, reaching 72 hours of fermentation. Leksono et al. (2022) investigated fermentation process of LAB in black soybean, resulting in the production of *β*-glucosidase enzymes which could hydrolyze isoflavone glucosides into glucose and isoflavone aglycones daidzein and genistein. Consequently, this increased its antioxidants activity by DPPH radical scavenging and its phenolics content.

Bioavailability of Phenolics Compounds

Figure 4 depicted the percentage absorption values of phenolics compounds, flavonoids, and antioxidants in black glutinous rice *tape* through the inverted gut sac method. The absorption of phenolics compounds, flavonoids, and antioxidants ranged from 27.60% to

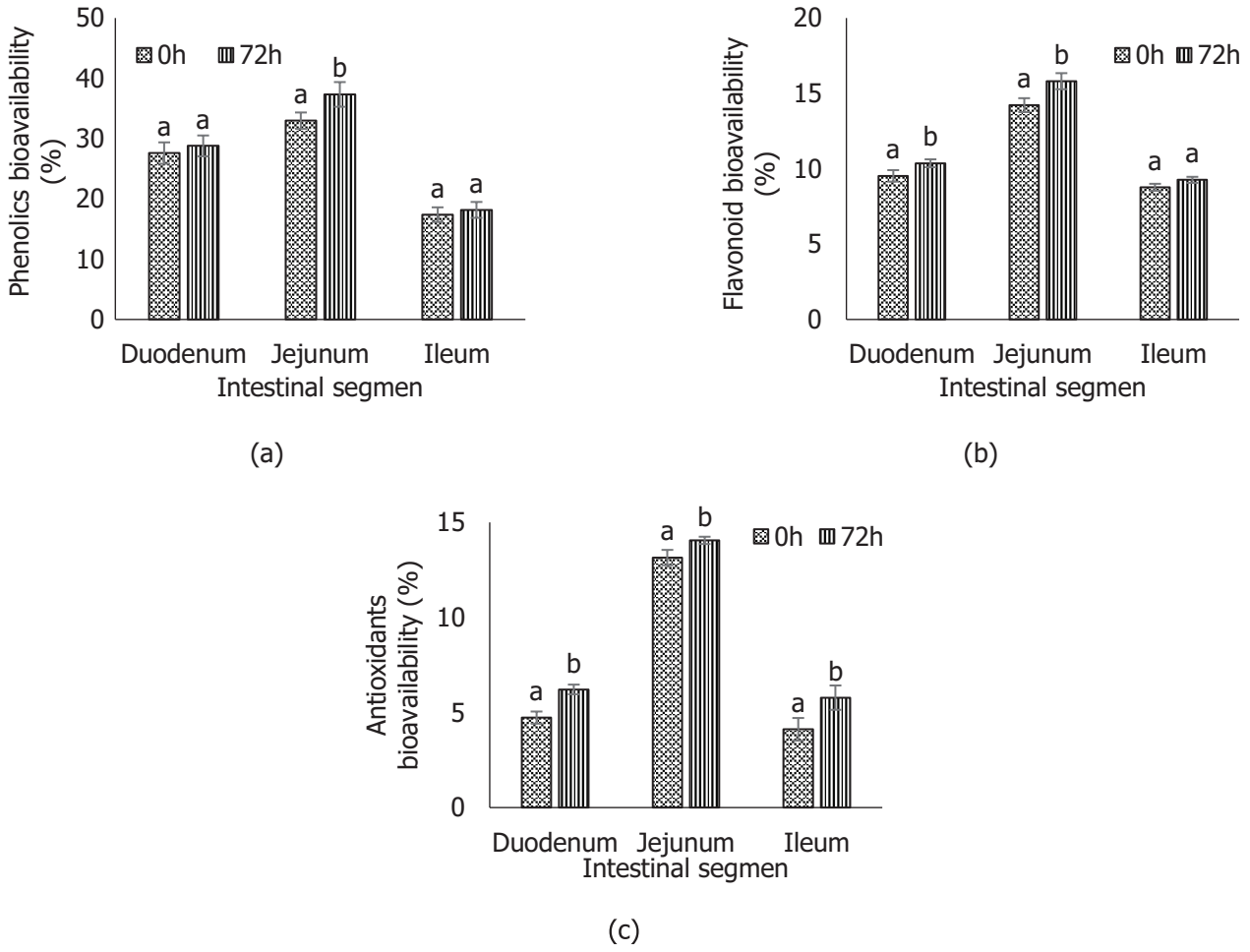

Figure 4. Bioavailability values of phenolic compounds (%) of black glutinous rice *tape*; a) phenolic bioavailability (%), b) flavonoid bioavailability (%), and c) antioxidants bioavailability (%). The sample codes corresponded to various fermentation durations: 0H for 0 hours and 72h for 72 hours. In each specific segment of the small intestine, identical letters showed no statistically significant differences, $p < 0.05$.

28.79%, 14.22% to 15.81%, and 13.15% to 14.06%, respectively, within the duodenum segment. Absorption in the jejunum segment was higher compared to the duodenum and ileum segments. In this analysis, phenolics compounds ranged from 32.97% to 37.33%, flavonoids from 14.22% to 15.81%, and antioxidants from 13.15% to 14.06%. Casteleyn et al. (2010) outlined how the surface area of the absorption side epithelium affected intestinal absorption. The jejunum had a larger intestinal diameter and a greater microvilli surface area, which facilitated maximal absorption. The study by Cervantes et al. (2020) examined the capacity for intestinal absorption of phenolic compounds in blueberries (35.45%), raspberries (18.18%), and strawberries (34.12%). Meanwhile, the intestinal absorption of flavonoids in strawberries, raspberries, and blueberries was 34.62%, 54%, and 44.83% respectively. This study also observed the percentage absorption of antioxidants in these 3 commodities, ranging from 18.92% to 39.47%. Olthof et al. (2001) investigated the absorption of phenolic substances, including chlorogenic acid at 33% and caffeic acid at 95%. Furthermore, protocatechuic acid was detected at 23.79%, 12.17%, and 12.79%, respectively, from the dose ingested in blood circulation, urine, and feces (Zheng et al., 2019). The absorption rate of flavonoid compounds was 0.21-0.27 $\mu g \cdot min^{-1} \cdot cm^{-2}$ (Liu et al., 2022).

The breakdown of bonds and the release of aglycones from food matrices occurred due to enzymatic hydrolysis in the small intestine. Lactase phlorizin hydrolase was an enzyme that broke down glycoside bonds in food matrices before being absorbed, appearing at the small intestine's brush border. LPH could produce aglycones that passively diffuse into epithelial cells, becoming more accessible to the membrane (Cosme et al., 2020). Meanwhile, the cytosolic *β*-glucosidase in epithelial cells could break down certain phenolic glycosides after passing through the epithelial cell wall. With the help of the active co-transporter, were carried into epithelial cells during the deglycosylation process by this enzyme (Teng & Chen, 2019).

Figure 4 showed that in the jejunum segment, the 72-hour fermented black glutinous rice sample exhibited significantly higher absorption values of phenolic compounds compared to the 0-hour fermentation sample ($p<0.05$). Enzymes generated by microorganisms from fermentation catalyze ester and *β*-glucosidic bond cleavage to liberate free phenolics and create low molecular weight phenolic metabolites, improving absorption in the small intestine's border. In addition, bound phenolics were released from the food matrix, increasing their bioactivity and bioavailability (Yang et al., 2023). Janarny & Gunathilake (2020) investigated fermented Bg406 rice bran with *Rhizopus oryzae*, showing improved bioactivity such as antioxidants, anti-inflammatory, and antidiabetic properties compared to non-fermented rice bran. In contrast, no significant difference was observed in the absorption of phenolic compounds between the 72-hour and 0-hour fermentation samples in the duodenum and ileum segments. This phenomenon could increase from the low activity of the enzyme lactase-phlorizin hydrolase (LPH) in the duodenum and ileum segments of the gastrointestinal tract, to unoptimal absorption. Lactase-phlorizin hydrolase (LPH) catalyzed the hydrolysis of phenolic conjugates, releasing aglycones that could be readily taken up by enterocytes in the small intestine (Day et al., 2000; Tanaka et al., 1997).

CONCLUSION

In conclusion, this study used the everted gut sac method to analyze absorption and observed bioavailability of phenolics compounds found in black glutinous rice *tape* after gastric and small intestinal digestion *in vitro*. The results showed a significant release of antioxidants and phenolic compounds after digestion, which was explained by the presence of certain enzymes and conditions during digestion that broke down the food matrix and released more bioactive chemicals. In addition, fermentation processes elevated the absorption of phenolics, potentially linked to the liberation of aglycone compounds during fermentation. These results provided insight into how digestion and fermentation could increase a beneficial compound's bioavailability, showing the potential of black glutinous rice *tape* as a source of readily absorbable phenolics.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest to declare.

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