FULL PAPER

Effect of germination period on the antioxidant activities and angiotensin-I converting enzyme inhibitory of Indonesian black rice

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Abstract

Indonesian black rice (*Oryza sativa* L., Ketan Hitam-2) is pigmented rice with high potency as a nutraceutical compound, especially with its high protein content. The effect of the germination period on antioxidant activities and angiotensin I-converting enzyme (ACE-I) inhibitory of Indonesian black rice seed protein was studied to determine its potential use as a nutraceutical ingredient. In this study, the bioactive peptide was produced by protein modification through the germination process for 0, 2, 4, and 6 days. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and amino acid composition were conducted to determine the changes in protein during the germination period. Two antioxidant methods ($ABTS^{\bullet+}$ and $OH^{\bullet}$) were performed to determine the antioxidant activity and the defence against radical-mediated DNA damage by hydroxyl. Meanwhile, the antihypertensive potency was analysed by ACE-I inhibitor activity. The results showed that the antioxidant-protected hydroxyl radical-induced oxidative DNA damage and ACE-I inhibitor activities were increased during the germination period. The IC_{50} value of $ABTS^{\bullet+}$ and hydroxyl radical scavenging on the sixth-day germination were 28.18 μg/mL, and 24.84 μg/mL, respectively, lower than the control (before germinated). Moreover, the IC_{50} value of ACE-I Inhibitory activities during the six-day germination was (9.07 μg/mL). The above results indicated that the germination period could increase the activity of bioactive peptides in Indonesian black rice. It might be used in future nutraceuticals and human health applications.

1. Introduction

Rice is one of the world's essential foods and is classified into pigmented and non-pigmented rice. The pigmented rice usually contains pigments typically in the pericarp, such as red, brown, purple, or black (Samyor *et al*., 2017). Indonesian black rice has been identified as a functional food, which has a double function as a source of nutrition and medicine for various diseases (Goufo and Trindade, 2014). Most researchers have reported that Indonesian black rice contains a variety of bioactive compounds than non-pigmented rice, especially the anthocyanin and bioactive peptides (Kiay *et al*., 2019; Pradipta *et al*., 2020)

Bioactive peptides are short polymers that consist of 2 to 50 amino acids. Bioactive peptides specifically and positively affect human health as antibacterial, antithrombotic, immunomodulatory, antioxidant, and antihypertensive (Murray and Fitzgerald, 2007; Chen *et al*., 2019; Muhialdin *et al*., 2020; Liu *et al*., 2020). Bioactive peptides as antioxidants provide electrons to inhibit an oxidation reaction or prevent the cell destruction process by absorbing the free radicals and reactive molecules. Antioxidants are needed to prevent oxidative stress, which is a condition that occurs when there is an imbalance between the number of free radicals and the number of antioxidants in the body. Based on this, antioxidants can play an essential role in the pathophysiology of various degenerative diseases, one of which is hypertension. Several studies have reported that bioactive peptides from pigmented rice can potentially be a source of antioxidants (Selamassakul *et al*., 2018; Du *et al*., 2020; Liu, Du and Chen, 2020; Bhat *et al*., 2020).

Bioactive peptides as antihypertensive can inhibit the increase of blood pressure by the hormone system that regulates blood pressure and body fluid balance is renin-

angiotensin-aldosterone (RAAS). The plasma renin produced by the kidneys is responsible for converting the angiotensinogen hormone released by the liver into angiotensin I (Nguyen *et al.*, 2002). This compound will be active when catalyzed by the angiotensin-converting enzyme (ACE) into octapeptide angiotensin II. Angiotensin II stimulates the adrenal cortex to produce aldosterone compounds. Thus, sodium salt retention in the blood increases and causes blood pressure to increase. Bioactive peptides that are known to have antihypertensive properties are ACE inhibitor peptides. These peptides can inhibit ACE activity in producing angiotensin II, hence, preventing the increase in blood pressure. ACE inhibitor peptides found in rice with the amino acid composition are Gln-Phe-Tyr-Ala-Val and Ala-Gly-Pro-Val-Leu-Leu (Gu *et al.*, 2012).

The bioactive peptide potential of a plant can be evaluated during the germination period. Bau *et al.* (2020) reported that the protein could be hydrolyzed naturally during the germination process. Seed germination is a stage that experiences many biochemical changes such as protein synthesis and hydrolysis of food reserves due to enzyme activity. One of the mobilized food reserves is protein. Protein mobilization involves the protease enzyme when increased provides a hydrolysis effect (Anna and Karl, 2012). The hydrolysis effect produces low molecular weight proteins or simple peptides and free amino acids. Indonesia black rice seed protein has displayed beneficial properties yet no further information about protein or peptide antioxidant activities and ACE-I inhibitor during germination of black rice seeds were reported. Therefore, this study was designed to evaluate the effect of the germination period on antioxidant activities and angiotensin I-converting enzyme (ACE-I) inhibitory of Indonesian black rice seed proteins.

2. Materials and methods

2.1 Germination process

Indonesian black rice used in this study was *Oryza sativa L.- Ketan Hitam-2*, which was cultivated with conventional plantation at the Center of Excellence on Crop Industrial Biotechnology (PUI-PT BioTIn) in Agrotechno Park Research Area, University of Jember, Jember, East Java, Indonesia. The germination process was performed using germinated paper as the medium. Before germination, 90 seeds of *Ketan Hitam-2* were soaked in 0.5 mL/100 mL liquid solutions of Explore® 250 EC commercial fungicide for 10 mins. The seeds were then rinsed with water and soaked in 100 mL sterile distilled water for 24 hrs to accelerate the seed germination. The seeds were then drained and placed on the media by placing 10 seeds on every paper sheet and

then rolling them with plastic. Then, it was placed in a germinator chamber (Thermo Scientific™, 396 L) at 25±2°C and 90±5% relative humidity in dark conditions. Seed germination periods were 0, 2, 4, and 6 days (ISTA, 1985).

2.2 Morphological observation

The morphological parameter of germinated rice was recorded by the length of plumule, radicule, and germination percentage. The length of plumule and radicule was observed in each germinated rice, followed by calculating the mean data. The following formula to calculate the germination

$$\% \text{ germination} = \frac{\text{total seeds} - \text{nongerminated seed}}{\text{total seeds}} \times 100\%$$

2.3 Sample extraction

Rice seed extraction was done according to Cao *et al.* (2010). The samples of germinated rice were extracted by grinding them with a mortar and then homogenized with phosphate buffer (50 mM, pH 6.8) in a ratio of 1:10 (w:v). The mixture was then centrifuged (10,000 rpm, 4°C, 15 mins). The supernatant was collected and stored (4°C) for further analysis.

2.4 Degree of hydrolysis

The determination of the degree of hydrolysis (DH) values were using 2,4,6-Trinitrobenzenesulfonic acid (TNBS) reaction (Alder-Nissen, 1979), and the measurement was adapted from Noviyanti *et al.* (2020). The 125 µL sample was mixed with phosphate buffer (2 mL, 200 mM, pH 8.2) and TNBS (0.1%, 1 mL), then incubated (50°C, 30 mins) with a water bath. The reaction was stopped by adding Na_2SO_3 (2 mL, 0.1 M), cooled at 26-27°C for 15 mins, and then the absorbance was read at a wavelength of 420 nm. A standard L-leucine curve was used to determine the amino acid concentration.

2.5 Gel electrophoresis

Samples were analysed using sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) according to Kupkanchanakul *et al.* (2018) with slight modifications. Samples were added in 525 µL of sample buffer with or without 75 µL of 5% β-mercaptoethanol (β-ME). Each sample was then heated for 3 mins and then cooled. The dissolved protein was fractionated using SDS-PAGE (using a stacking gel containing 4.5% acrylamide and a solvent gel containing 15% acrylamide) at 20 mA per gel for 210 mins. A series of molecular weight markers are used as a standard. After electrophoresis, the gels were stained simultaneously using 10% Coomassie brilliant blue solution for 24 hrs.

Then, the colour was removed by soaking the gel in a destaining solution (50 mL distilled water, 40 mL methanol, 10 mL glacial acetic acid) until the band was clear.

2.6 Determination of amino acid

Amino acid hydrolysis was carried out by weighing 0.1 g of each of the black rice samples and adding HCl (5 mL, 6N). The hydrolysis process is carried out at $110^{o}C$ for 22 hrs. After hydrolysis, the mixture was cooled at 26-27°C then transferred to a 50 mL measuring flask, added aquabidest up to boundary marker, and then filtered using a 0.45 μm filter. It was then added with alpha aminobutyric acid (0.4 mL, 50 mM) as an internal testing standard. As much as 20 μL of hydrolysate was injected into the UPLC system (Waters 2475, US) using AccQ. Tag Ultra C18 μm (2.1×100 mm) column with Photodiode Array (PDA) as a detector at 260 nm to identify the amino acid compositions.

2.7 2,2'-azino-bis (3-ethyl benzothiazole-6-sulfonic acid) (ABTS) radical scavenging

The ABTS method was carried out according to the procedure of Re *et al.* (1999). The ABTS reagent was prepared by mixing an equal amount of 7 mM ABTS and 2.45 mM potassium persulfate, then incubated for 12-16 hrs in the dark at room temperature. Before starting the test, the ABTS reagent was diluted with PBS (phosphate buffer saline) (0.2 M, pH 7.4) to an absorbance of 0.70±0.02 at a wavelength of 734 nm. Blank controls were made in the same way without adding samples.

2.8 Hydroxyl radical scavenging

The antioxidant activity test with hydroxyl was carried out based on Kumar *et al.* (2013). Sample solutions were prepared with 20, 40, 60, 80, and 100 μg/mL sample concentrations. Tests were carried out by adding each sample with a solution containing $FeCl_3$ (100 μL, 10 mM), EDTA (100 μL, 1 mM), ascorbic acid (50 μL, 1 mM), H_2O_2 (50 μL, 1 mM), phosphate buffer (150 μL, 28 mM, pH 7.4), and deoxyribose (50 μL, 28 mM), then incubated (37°C, 1 hr). After incubation, TBA (400 μL, 1%) and TCA (400 μL, 2.8%) were added to reveal a pink chromogen colour. The test tube containing the sample was then heated at $80^{o}C$ for 30 mins. Then cooled and absorbance was read at a wavelength of 532 nm. As a comparison, positive control was used, namely the addition of glutathione (GSH). Antioxidant activity through reduction of hydroxyl radicals (OH•) was expressed as per cent (%) of inhibition against hydroxyl radicals and IC_{50} (μg/μL). The calculation of hydroxyl radical reduction is determined by the equation of hydroxyl radical reduction (%) = $(A_{control}-A_{sample})/A_{control} \times 100\%$. $A_{control}$ is the absorbance value without the addition of the sample, and the A_{sample} is the absorbance value with the addition of the sample.

2.9 Protective DNA Assay

Protective DNA was analysed using the method described by Siswoyo *et al.* (2011). The *p*BT7 plasmid from the collection of the Center of Excellence on Crop Industrial Biotechnology (PUI-PT BioTIn), University of Jember, Jember, East Java, Indonesia, was used. The DNA plasmid (2.5 μg) was treated with a Fenton's reagent (80 mM $FeCl_3$, 30 mM H_2O_2, and 50 mM ascorbic acid) until the final volume of 1 mL was incubated at 37°C for 15 mins. The protein samples were then added and made the final volume up to 20 μL with double-distilled H_2O. The mixture was then incubated at 37°C for 15 mins. The mixture was then run through a 1.5% gel electrophoresis and was visualized through the gel documentation system (Major Science, USA).

2.10 Determination of ACE-I inhibitory activity

The ACE inhibitor activity was carried out based on Arihara *et al.* (2001). The test was carried out by adding 50 μL of the sample (15 mg/mL) to 125 μL of buffer substrate (7.6 mM HHL and 608 mM NaCl in 10 mL borate buffer pH 8.3). The mixture was incubated at $37^{o}C$ for 15 mins. Then 50 μL of ACE enzyme 50 mU/mL was added to the mix and then incubated for 30 mins. The reaction was ended by adding 200 μL of 1 N HCl. The mixture was vortexed and added with 1140 μL of ethyl acetate, then centrifuged at 10,000 rpm for 10 mins. Finally, 1000 μL of supernatant was taken and dried at $95^{o}C$ for 90 mins. The hippuric acid formed was dissolved in 1 mL of aquabidest. The absorbance was measured at a wavelength of 228 nm using a UV-VIS spectrophotometer, and a positive control used captopril.

2.11 Statistical analysis

Data were analysed using the analysis of variance (ANOVA) and Duncan's Multiple Range Test with a significance level (p<0.005).

3. Results

3.1 Germinated black rice morphological

The seeds of *Ketan Hitam-2* were germinated for 0, 2, 4, and 6 days (Figure 1). The morphology of each germinated rice was then recorded (Table 1). The data showed that the radicules formed on the 2nd day of germination, while plumules formed on the 4th day of germination. The germination percentage was 86.67% on the 6th day of germination, and it was indicated that the seeds of Indonesian black rice used in this study were in good viability condition.
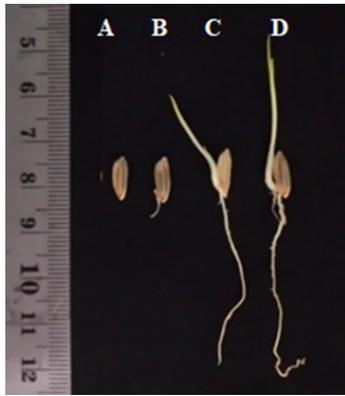
Figure 1. Black rice seeds during germination. A: 0 day, B: 2 days, C: 4 days, D: 6 days

Table 1. Morphology of germinated black rice seeds

Germination period (days)	Plumule (cm)	Radicule (cm)	Germination percentage (%)
0	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 00.00^a
2	0.00 ± 0.00^a	0.36 ± 0.06^b	33.33 ± 11.56^b
4	1.08 ± 0.34^b	3.09 ± 0.38^c	83.33 ± 20.82^c
6	3.56 ± 0.11^c	6.69 ± 0.50^d	86.67 ± 05.77^c

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different.

3.2 Antioxidant amino acid grouping of germinated black rice

The amino acid compositions of germinated rice were analyzed using Ultra-Performance Liquid Chromatography (UPLC). Several amino acids have high capabilities as the antioxidant component was then grouped as antioxidant amino acids, i.e., arginine, lysine, tyrosine, histidine, cysteine, and methionine. The results showed that the amino acid composition increased during the germination period (Figure 2). This indicated that the protein of black rice seeds was degraded during the germination process. Furthermore, the ratio of amino acid antioxidant grouping (TAntAA) to total amino acids (TAA) was analyzed. As a result, the TAntAA/TAA was increased during the germination process (22.16-23.46%) (Table 2). This result is expected to lead to an increase in antioxidant activities.

Table 2. The ratio of amino acid antioxidant grouping to total amino acid composition in germinated black rice

Germination Period (days)	TAA (mg/100 g)	TAntAA (mg/100 g)	TAntAA/TAA (%)
0	103.89	23.02	22.16
2	115.39	25.75	22.32
4	156.26	34.41	22.45
6	161.56	37.91	23.46
CV	0.21	0.23	0.03

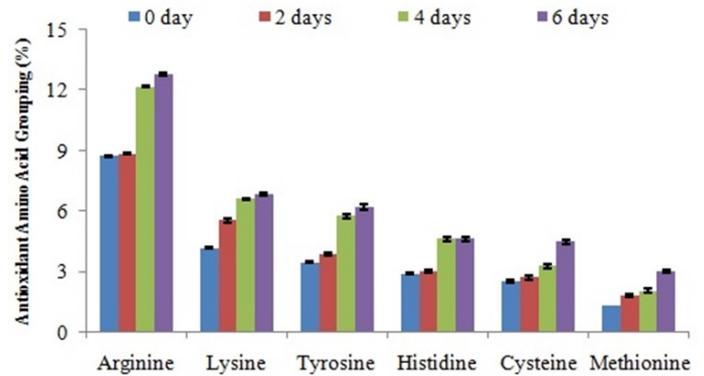TAA: Total amino acids, TAntAA: Total amino acid antioxidant grouping, CV: Coefficient of Variance

Figure 2. Amino acid antioxidant grouping of germinated black rice. The analysis was performed using UPLC with alpha aminobutyric acid as the internal standard.

3.3 Profile of germinated black rice protein

The protein extracted from the germinated pigmented rice for different periods was analyzed by the SDS-PAGE method and degree of hydrolysis (DH). There was polypeptide band degradation during the germination process. The 50 kDa, 37 kDa, and 15 kDa polypeptides were degraded into new polypeptides under 10 kDa (Figure 3A). The degree of hydrolysis was also observed from 0 to 6 days of the germination period (Figure 3B). As a result, the DH was significantly increased until the 4th day of germination and reached the stationary phase on the 6th day of germination.
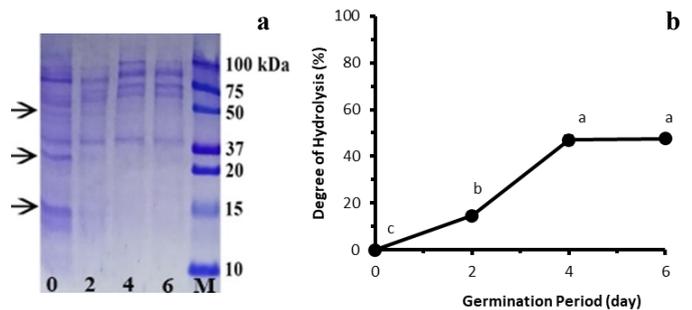

Figure 3. A: SDS-PAGE of rice seed protein during germination, B: Degree of hydrolysis of rice seed protein during germination. The values are expressed as the means of triplicate measurements. The error bars are defined as the standard deviations. The arrow shows the hydrolysed protein.

3.4 Antioxidant activities

The antioxidant activities were conducted by two different methods, ABTS and hydroxyl. During the germination process, the antioxidant activities were increased both in ABTS and hydroxyl methods. The IC_{50} value of ABTS and hydroxyl on the 6th day of germination was 28.18 µg/mL and 24.84 µg/mL, respectively. While the IC_{50} value of ABTS and hydroxyl of black rice before germination was 50.98 µg/mL and 36.40 µg/mL, respectively (Figure 4).
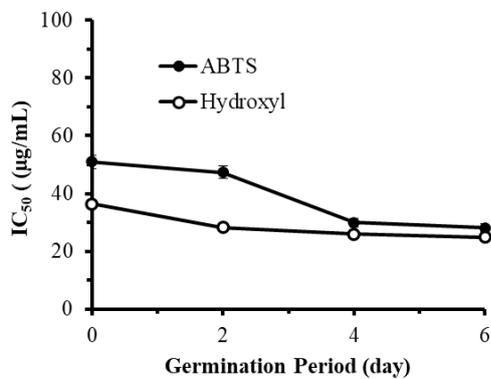
Figure 4. Antioxidant activity of germinated black rice seed protein. The values are expressed as IC_{50} (μg/mL), which is the effective concentration of antioxidants to inhibit 50% activity of radical ABTS and hydroxyl. The values are expressed as the means of triplicate measurements. The error bars are defined as the standard deviations.

3.5 Protecting DNA

A Fenton's reagent was used to assess the oxidative DNA damage defence against hydroxyl. Results showed that the hydroxyl radicals might cause the Fenton reaction to break the single-stranded DNA. It was indicated by the conversion of supercoiled (SC) DNA to open circular (OC) DNA after 15 minutes of incubation of *p*BT7 plasmid in Fenton's reagent (Figure 5). Fortunately, adding the germinated black rice protein at different periods may reduce the conversion of SC to OC. It has a lot in common with the G-SH as the positive control.
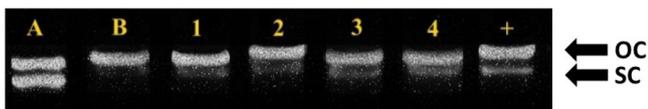

Figure 5. Inhibitory effect of pigmented rice seed protein on DNA nicking treated by hydroxyl radicals. DNA nicking was initiated by mixing plasmid DNA with reaction solution (30 mM H_2O_2, 50 mM ascorbic acid, and 80 mM $FeCl_3$) for 0 min and 15 mins (lanes A and B, respectively). Seed protein of germinated black rice on 0-, 2-, 4-, and 6-days germination were added (lanes 1-4), and G-SH as the positive control (+).

3.6 Angiotensin-I Converting Enzyme (ACE-I) inhibitor activity

The potency of germinated black rice protein to inhibit the activity of ACE-I was also investigated in this study. The ACE-I inhibitor activity was analyzed *in-vitro*, and results demonstrated that the potency of germinated black rice protein until the 4th day of germination was not significantly different. On average, the IC_{50} value was 14.95 μg/mL, while the IC_{50} value on the 6th day of germination was 9.07 μg/mL. However, this value was still significantly different from captopril (2.08 μg/mL) as a positive control (Figure 6).
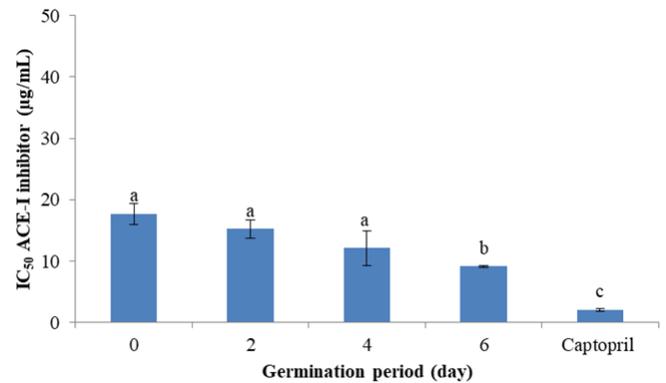

Figure 6. The IC_{50} value of ACE-I inhibitor activity of germinated black rice seed protein. IC_{50} value (μg/mL) is the effective concentration of protein to inhibit 50% activity of ACE-I. Bars with different notations are significantly different. The error bars are defined as the standard deviations. Captopril was used as the positive control.

4. Discussion

Rice seeds are rich in proteins that contain bioactive fragments, which have been shown to have antihypertensive, antioxidant, anti-inflammatory, and anti-cholesterol properties (Saisavoey *et al*., 2016; Wang *et al*., 2017; Shobako and Ohinata, 2020; Chanput and Lawyer, 2020). Several methods are used to produce food bioactive peptides, one of which is through the germination process (López-Barrios *et al*., 2016; Ohanenye *et al*., 2020; Noviyanti *et al*., 2020). The germination stage may influence some biochemical processes and improve the nutritive value of black rice seeds. According to Sefatie *et al*. (2013), the germination process can cause significant changes in the biochemical properties of seeds by activating enzymatic processes that convert storage macromolecules into nutrients for seed growth. Thus, the protein source and degree of hydrolysis determine the properties and bioactivities of the peptides in the hydrolysate. The hydrolyzed protein is the product of the hydrolysis reaction or the breaking of the peptide bonds in the protein molecule, containing various peptides and free amino acids depending on the type of enzyme and substrate concentration (Ramakrishna and Ramakrishna-Rao, 2006).

This study on Indonesian black rice found that total amino acids increased during the germination process (Table 2). SDS-PAGE profile of proteins from Indonesian black rice seeds revealed three major bands previously observed by other authors (De Souza *et al*., 2016; Wang *et al*., 2016; Amagliani *et al*., 2017; Likittrakulwong *et al*., 2021). The SDS-PAGE profile and degree of hydrolysis were used to determine how the protein of the Indonesian black rice profile changes during the germination process (Figure 3). Although the protein pattern remains unchanged, some bands are lost

or have their intensity reduced after germination. The germination process has been shown to degrade the black rice protein into smaller polypeptides or amino acids. Singh and Matta (2014) discovered that small molecular weight peptides were produced during various stages of rice seed germination (rice line 'Pusa-HH'). Furthermore, Coa *et al.* (2010) reported that germination of brown rice (*Oryza sativa L wuyunjing NO.2.*) seeds for up to three days increased the soluble protein content while decreasing the amount of higher molecular weight (22.3, 36.3 and 50.9 kDa) proteins. Singh and Matta (2014) demonstrated that the intensity (rice line 'Pusa-HH') protein bands with molecular weights of 65 kDa, 21.5 kDa, 20 kDa, 18 kDa, 16 kDa and 13 kDa in rice seed vanished by the fifth and sixth day of germination. Hydrolyzed protein is a product of the peptide bonds breaking in protein molecules, resulting in a mixture of various peptide components and free amino acids depending on the degree of hydrolysis (Cao *et al.*, 2010; Noviyanti *et al.*, 2020). The amino acid constituents, the presence of polar groups, and the presence of ionizable polar groups all contribute to the length and characteristics of the peptides formed. It also determines the plant's functional and bioactive properties, which are affected not only by the degree of hydrolysis but also by the protein used as a substrate (Cao *et al.*, 2010; Piotrowicz *et al.*, 2020). Furthermore, Gao *et al.* (2019) explained that protein DH was linked to an increase in the antioxidant capacity of germinated seeds. As a result, the antioxidant activity of the hydrolyzed protein was also examined. It was previously known that free radical scavenging activities were increased during the germination period. In the previous study, there exists a lack of consensus on the size of the peptides and the corresponding antioxidant activity. According to some studies, smaller peptides have higher antioxidant potential (Ajibola *et al.*, 2011; Girgih *et al.*, 2011).

Due to its reactivity with various classes of antioxidants, the ABTS radical is widely applied for antioxidant assays. The ABTS radicals use proton donors to assess the stability of free radicals. The antioxidant activity is measured by removing the colour of ABTS, which was originally blue-green, but will fade in colour when reduced by free radicals (Moon and Shibamoto, 2009). The hydroxyl radical determines the sample`s ability to absorb the most reactive hydroxyl radicals among the reactive oxygen species—produced by the Fenton reaction. The antioxidant activities of polypeptides are related to the amino acid composition, type, and hydrophobicity (Guo *et al.*, 2009). In addition, the amino acid composition of Indonesian black rice during germination was found to have more Arg, Lys Tyr, His, Cys, and Met (Figure 2) and these amino acids have been linked to the higher radical scavenging

activity, according to several studies (You *et al.* 2010; Guo *et al.*, 2009; Xu *et al.*, 2017). Furthermore, the antioxidant amino acid group (Arg, Lys Tyr, His, Cys, and Met) was analyzed, and the results revealed that the ratio of TAntAA/TAA increased (Table 2). During germination, it may have resulted in increased antioxidant activity on Indonesian black rice protein (Figure 4). Our findings are consistent with Noviyanti *et al.* (2020) and Gao *et al.* (2019), who found an increase in the inhibition of $ABTS^{2+}$ activity of germinated *melinjo* seed and soybean seed. Hydrolysis proteins donate more electrons and hydrogen atoms from each active peptide during this process, converting free radicals to more stable radicals (Pazinatto *et al.*, 2013). Hydrolysis will result in the formation of shorter peptides and free amino acids. Thus, peptides become more hydrophilic, effectively inhibiting hydroxyl ABTS radicals (Chen *et al.*, 1998).

Reactive oxygen species (ROS) or free radicals are highly reactive species produced in the body during normal metabolic functions or introduced from the environment. Hydroxyl radicals are highly reactive oxygen species that cause damage to biomolecules, such as lipids, proteins, and nucleic acids, and are a factor in degenerative diseases (Reshmi and Nandini, 2018). Consequently, the defence of the hydroxyl radical is necessary for many metabolic disorder protections associated with the hydroxyl radical (Ajibola *et al.*, 2011). However, antioxidants can prevent the formation of ROS. Before attacking cells, antioxidants deactivate free radicals (Rahman, 2007). However, in our study, the DNA damage was also evaluated on pBT7 plasmid using Fenton's reagent treatment. The findings indicated that germinated Indonesia black rice protein could reduce the conversion of SC to OC plasmid conformation (Figure 5). The germinated Indonesian black rice seed protein has been shown to have high antioxidant activity. Furthermore, it may protect the DNA from hydroxyl radicals.

The ability of germinated black rice protein to inhibit the activity of ACE-I was also investigated. The results revealed that germinated black rice protein extracted on the 6th day of germination had the greatest ability to inhibit the activities of ACE-I (Figure 6). It has been suggested that the germination process can produce peptides with ACE-I inhibitor properties. Previous studies revealed the presence of ACE-I inhibitor peptides in rice plants. The amino acid compositions were Gln-Phe-Tyr-Ala-Val and Ala-Gly-Pro-Val-Leu-Leu (Gu *et al.*, 2012). The mechanism of ACE-I inhibition by bioactive peptides is that amino acid residues bind ACE-I in the peptide via hydrogen bonds, hydrophobic interactions, hydrophilic interactions, electrostatic

interactions, and Zn^{2+} binding (Pan *et al*., 2011). The structural properties of ACE-I, such as chain length, composition and sequence, can influence its inhibitory activity. The inhibition of ACE-I activity is also affected by the size of the peptide. ACE-I inhibiting peptides must have gastrointestinal stability and reach the cardiovascular system to demonstrate bioactivity. ACE-I inhibiting peptides generally consist of short chains with 2-12 amino acids (Dikmen *et al*., 2017).

5. Conclusion

During the germination process, Indonesian black rice seed (*Oryza sativa* L.) decreased the amino acid value due to protein storage hydrolysis, which resulted in small-sized peptides that strongly inhibit free radicals and ACE-I activities. The results indicated that germination time has positively affected antioxidant activities, protected hydroxyl radical-induced oxidative DNA damage and ACE-I inhibitory activity. It might be used in future nutraceuticals and human health applications.

Conflict of interest

The authors declare no conflict of interest.

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