

Changes in the antioxidant activities and angiotensin-I converting enzyme inhibitory protein during the germination of green coffee beans

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ABSTRACT

Germinated green coffee beans can be potential dietary sources of bioactive compounds. In this study, two varieties of coffee beans (arabica and robusta) germinated at 28 °C for 0-15 days. The antioxidant and angiotensin-I converting enzyme (ACE-I) inhibitory activities were measured by the antioxidant capacities (ABTS^{••}, DPPH, [•]O₂ and [•]OH) and in vitro ACE-I inhibition assay. Significant differences ($p < 0.05$) in soluble protein and free amino acid contents were noted within and between coffee beans before and after germination. Antioxidant activity increased in robusta and arabica coffee beans after germination when seen from the total amino acid antioxidant (TAAntAA)/total amino acid (TAA) ratio analysis of 0.32 and 2.36%, respectively. The DPPH and ABTS^{••} radical scavenging activity were higher after germination for 10 days in all the coffee beans as compared with the ungerminated beans (0 days); however, [•]O₂ and [•]OH scavenging radical activity did not show the same effect in both treatments. Out of the legumes studied, germinated coffee arabica and robusta beans can be used in the dietary management of hypertension as their hydrolyzed protein showed >82% ACE inhibition, reaching an IC₅₀ value of 2.57 and 1.47 µg/mL, respectively.

Key words: Bioactive protein; robusta; arabica; ACE-Inhibitor; antioxidant activity.

1 INTRODUCTION

Coffee is one of the most widely consumed beverages, and there are a variety of coffee drinks depending on the type of coffee beans, roasting, and brewing methods. The chemical composition of coffee beans has been intensively and continuously studied since the beginning of the century, especially in flavor compounds (Suh et al., 2014). Coffee has also received much attention from researchers involved in health-related research (Cornelis, 2019). Many reports indicate that coffee beans contain many healthful antioxidants, such as volatile heterocyclic compounds (Dippong et al., 2022) and chlorogenic acids (Kim et al., 2018). In recent years, the intake of green coffee products has increased as a healthier option than roasted coffee (Onakpoya; Terry; Ernst, 2011; Ribeiro; Rocha; Prudencio, 2021). The green coffee bean represents potential nitrogenous flavor precursors by free amino acids, peptides, and proteins. The protein content is relatively high, representing 8.7-12.2% of the coffee bean dry weight, based on crude nitrogen and corrected for non-protein nitrogenous components (Rawel; Sagu, 2023). Green coffee proteins, the 11S storage protein (Iriondo-dehond et al., 2022), represent an essential reservoir for free amino acids and peptides. The free amino acid content appears relatively low, 0.3-0.6% on a dry weight basis (Montavon; Mauron; Duruz, 2003). Some studies have reported that storage proteins in legumes and cereals, such as 11S globulin, are peptide precursors capable of carrying out activities in various metabolic pathways that

activate human pathological processes (Cavazos; Gonzalez de Mejia, 2013). Since the main storage protein in green coffee beans is also 11S globulin (Bressani et al., 2023), this protein could potentially be a precursor of a bioactive peptide.

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 (Marcela; Eden; Rosalva, 2017; Bhandari et al., 2020; Malaguti et al., 2014; Susilowati et al., 2022; Zaky et al., 2022). Bioactive peptides, as antioxidants, provide electrons to inhibit an oxidation reaction or prevent the cell destruction process by absorbing free radicals and reactive molecules. Antioxidants are needed to avoid 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, including hypertension. Several studies have reported that bioactive peptides from maize, rice, amaranth, and sorghum seed can potentially be a source of antioxidants (Bhandari et al., 2020; Manfredini et al., 2021; Montoya-Rodríguez et al., 2014; Garzón et al., 2022). Bioactive peptides as antihypertensive can inhibit blood pressure increase by the hormone system regulating blood pressure and body fluid balance, renin-angiotensin-aldosterone (RAAS). Bioactive peptides that are known to have antihypertensive properties are ACE inhibitor peptides. These peptides can inhibit ACE

activity in producing angiotensin II, preventing blood pressure increase. ACE inhibitor peptides found in rice with the amino acid composition are Gln-Phe-Tyr-Ala-Val and Ala-Gly-Pro-Val-Leu-Leu (Kang et al., 2012).

The bioactive peptide potential of a plant can be evaluated during the germination period. Susanti, Susilowati and Siswoyo (2022) reported that the protein could be hydrolyzed naturally during germination. 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, which, when increased, provides a hydrolysis effect (Bau; Villaume; Mejean, 2020). The hydrolysis effect produces low molecular weight proteins, simple peptides, and free amino acids. The green coffee bean protein has displayed beneficial properties (Rawe; Sagu, 2023). Nevertheless, no further information about protein or peptide antioxidant activities and ACE-I inhibitors during the germination of green coffee beans was 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 green coffee bean proteins.

2 MATERIAL AND METHODS

2.1 Germination process

Coffee bean germination of robusta (*Coffea canephora*) and arabica (*Coffea arabica*) harvested in Bondowoso were provided by PTPN X, Indonesia, as a single bulk and stored in polypropylene plastic bags for a maximum of two months at room temperature. The parchment is removed, and germination is carried out with the silver skin covered, which will then swell and be peeled off by water during germination. The seeds were kept in water in the dark for seven days at 28

°C. After imbibition, the seeds were sown in perlite at 28 °C in the dark and watered daily. At each sampling day (0, 5, 10 and 15 days after imbibition) (Figure 1), about 40 ungerminated or germinating seeds were collected and finally frozen in liquid nitrogen and stored at -80°C.

2.2 Sample extraction

According to Cao et al. (2010), coffee bean extraction was done. The germinated coffee bean samples were extracted by grinding them with a mortar and then homogenizing them with 50 mM phosphate buffer, pH 6.8, in a 1/10 (w/v) ratio. The mixture was centrifuged (10,000 rpm, 4 °C, 15 min). The supernatant was collected and stored (4°C) to analyze antioxidant and ACE-I Inhibitory activities.

2.3 Gel electrophoresis

Samples of proteins extracted from germinating seeds (robusta and arabica) were analyzed with slight modifications using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to Kupkanchanakul et al. (2018). Samples were added in 525 µL of sample buffer with 75 µL of 5% β-mercaptoethanol (β-ME). Each sample was then heated for 3 min and then cooled. The dissolved protein load to each well is fixed (10 µg) for all samples and separated using SDS-PAGE (a stacking gel containing 4.5% acrylamide and a solvent gel containing 12.5% acrylamide) at 20 mA per gel for 210 min (Mini-PROTEAN® electrophoresis, Bio-Rad, USA). A series of molecular weight markers are used as a standard. After electrophoresis, the gels were stained using 10% Coomassie brilliant blue solution for 24 h.

2.4 Determination of amino acid composition

Amino acid hydrolysis was done by weighing 0.1 g of each sample and adding HCl (5 mL, 6N). The hydrolysis process is carried out at 110 °C for 22 h. After hydrolysis, the mixture was cooled at 26-27°C, transferred to a 50 mL

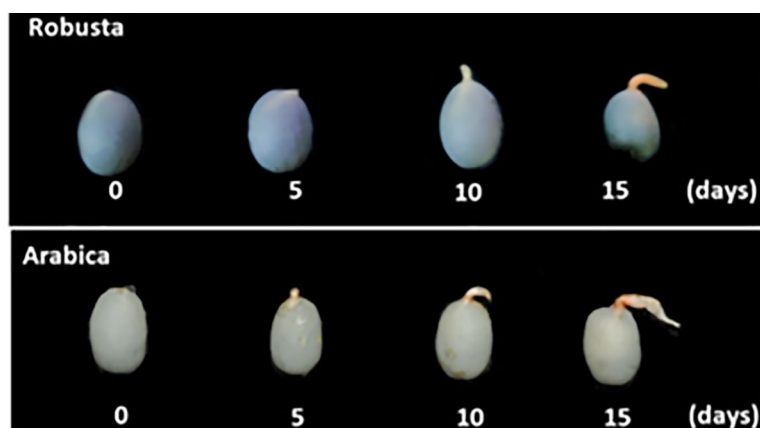


Figure 1: Germination of coffee beans. Parchment was removed and germination was carried out with silver skin covered. Silver skin was swollen and peeled off by water during germination.

measuring flask, added aquabidest to the boundary marker, and filtered using a 0.45 μm filter. It was 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 $\times$ 100 mm) column with Photodiode Array (PDA) as a detector at 260 nm to identify the amino acid compositions.

2.5 Antioxidant capacity

The antioxidant capacity of the protein extracts was determined using the 2,2'-azino-bis (3-ethyl benzothiazole-6-sulfonic acid) (ABTS⁺), 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl ($\cdot\text{OH}$), and superoxide ($\cdot\text{O}_2$) radical scavenging methods. The ABTS⁺ radical scavenging 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 h in the dark at room temperature. Before starting the test, the ABTS reagent was diluted with 0.2 M phosphate buffer saline (PBS), pH 7.4, to an absorbance of 0.70 $\pm$ 0.02 at a wavelength of 734 nm. Subsequently, 50 μL of the protein extracts solution in various concentrations ranging from 0.5-10 mg/mL were mixed with 950 μL of ABTS solution in sodium phosphate buffer saline. The absorbance was measured at 734 nm. Blank controls were made in the same way without adding samples. The percentage of inhibition of ABTS⁺ oxidation was calculated using the following Equation 1 :

$$\text{ABTS}^{++} \text{ radical scavenging (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (1)$$

Where: A_{control} is the absorbance value without adding the sample, and the A_{sample} is the absorbance value with adding the sample.

Based on Kumar et al. (2013), hydroxyl radical scavenging was carried out. Sample solutions were prepared with 0.5-10 mg/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 $^\circ\text{C}$, 1 h). After incubation, TBA (100 μL , 1%) and TCA (400 μL , 2.8%) were added to reveal a pink chromogen color. The test tube containing the sample was then heated at 80 $^\circ\text{C}$ for 30 min. Then, it cooled, and absorbance was read at a wavelength of 532 nm. Antioxidant activity through the reduction of hydroxyl radicals ($\cdot\text{OH}$) was expressed as percent (%) of inhibition against hydroxyl radicals and IC_{50} (mg/mL). The calculation of hydroxyl radical reduction is determined by following the Equation 2:

$$\text{Hydroxyl radical scavenging (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (2)$$

Where: A_{control} is the absorbance value without adding the sample, and the A_{sample} is the absorbance value with adding the sample.

Superoxide anion radicals ($\cdot\text{O}_2$) was analyzed by Tang et al. (2010), 0.2 mL of sample solutions (0.5–10 mg/mL) were mixed with 1.7 mL of 50 mM Tris-HCl pH 8.2, then incubated at 25 $^\circ\text{C}$ for 10 min, followed by the addition of 0.1 mL of 10 mM pyrogallol (in 10 mM HCl). Measured slope for 4 min at wavelength 320 nm.

$$\text{Superoxide (\cdot O}_2\text{) radical scavenging (\%)} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100\% \quad (3)$$

The oxidation rate of pyrogallol for samples was calculated as the slope of the absorbance line (ΔA_{sample}). The autooxidation rate of pyrogallol for control was measured with 1.0 mL of double-distilled water ($\Delta A_{\text{control}}$).

2.6 Determination of ACE-I inhibitory activity

The ACE inhibitor activity was based on Arihara et al. (2001). The test was carried out by adding 50 μL of the sample (1-15 $\mu\text{g/mL}$) to 125 μL of buffer substrate (7.6 mM HHL and 0.6 M NaCl in 10 mL borate buffer pH 8.3). The mixture was incubated at 37 $^\circ\text{C}$ for 15 min. Then 50 μL of ACE enzyme 50 mU/mL was added to the mix and incubated for 30 min. 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 min. Finally, 1000 μL of supernatant was taken and dried at 95 $^\circ\text{C}$ for 90 min. 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.

2.7 Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) and Duncan's Multiple Range Test with a significance level ($p < 0.05$).

3 RESULTS

The electrophoresis profile of proteins extracted from germinated period (0-15 days) seeds defatted with hexane in a Soxhlet apparatus for 24 h was shown in Figure 2. Around 20 and 37 kDa, two main bands dominate robusta coffee beans. This protein is also seen in arabica coffee, but a different protein band with a molecular weight of around 30 kDa was found in this type of coffee. During germination, the 30 and 37 kDa bands decrease in intensity (indicated by arrows). Low molecular weight proteins are more stained or begin to be visible only at later stages of germination. However, the most visible observation in the protein profile is a strong colored band of around 30 kDa, which changes at 5 days after germination (shown by the arrow), more specifically, the arabica coffee beans appear to be very faint.

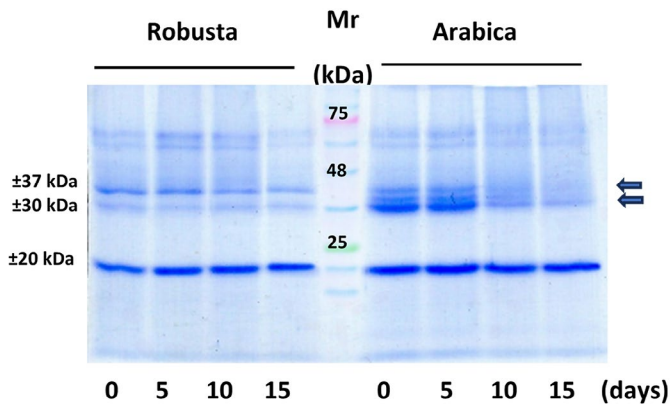


Figure 2: Electrophoretic profile of proteins extracted from robusta (*Coffea canephora*) and arabica (*Coffea arabica*) coffee beans during the germination period (0-15 days). Mr. Protein marker.

The total soluble protein content of robusta and arabica coffee beans increased during germination from 0 to 5 days

and decreased after 15 days (Table 1). The soluble protein content at the beginning of germination (0 days) was 8.78 mg/g (robusta) and 3.52 mg/g (arabica). The levels decreased to 8.21 mg/g (robusta) and 3.28 mg/g (arabica) at the end of 15 days of germination. When compared, the changes in soluble protein content during germination from 0 to 15 days between arabica and robusta coffee showed a slight difference of 6.5% and 6.8%, respectively.

The total amino acid composition of robusta and arabica coffee beans is shown in Table 2 and presented as TAA (g/100 g Protein). TAA detection using the UPLC system using PDA as a detector at 260 nm in both coffee beans decreased along with the length of germination (10 days). It was found that the presence of seven types of amino acids increased in robusta coffee beans, such as Glu, Phe, Arg, Lys, Asp, Tyr, and His, whereas in arabica coffee beans, were found to increase six amino acids, such as Phe, Arg, Gly, Tyr, His, and Cys.

Table 1: Biochemical characteristics of robusta (*Coffea canephora*) and arabica (*Coffea arabica*) coffee beans during the germination period.

Germination Period (days)	Robusta		Arabica	
	Soluble Protein Content (mg/g)	Free Amino Acid (mg/g)	Soluble Protein Content (mg/g)	Free Amino Acid (mg/g)
0	8.78 ± 0.16 ^b	0.89 ± 0.01 ^b	3.52 ± 0.06 ^b	0.90 ± 0.01 ^{ab}
5	9.53 ± 0.33 ^a	0.90 ± 0.00 ^a	4.50 ± 0.24 ^a	0.91 ± 0.00 ^a
10	9.52 ± 0.21 ^a	0.91 ± 0.01 ^a	3.89 ± 0.32 ^a	0.88 ± 0.01 ^b
15	8.21 ± 0.34 ^c	0.88 ± 0.02 ^b	3.28 ± 0.33 ^b	0.85 ± 0.00 ^c

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p < 0.05$).

Table 2: Amino Acid Composition of Robusta and Arabica coffee beans during germination.

Amino Acid	Robusta		Arabica	
	0 days	10 days	0 days	10 days
Serine (Ser)	3.38	3.24	3.48	3.36
Glutamic Acid (Glu)	9.21	9.40*	10.68	10.55
Phenylalanine (Phe)	3.56	3.58*	4.55	4.89*
Isoleucine (Ile)	2.43	2.28	2.67	2.40
Valine (Val)	3.42	3.33	3.62	3.37
Alanine (Ala)	2.53	2.30	2.52	2.23
Arginine (Arg)**	3.18	3.56*	3.53	3.95*
Glycine (Gly)	5.29	5.28	5.53	5.57*
Lysine (Lys)**	2.62	2.88*	2.72	2.46
Aspartic Acid (Asp)	4.16	4.28*	4.33	4.33
Leucine (Leu)	5.19	5.03	5.49	5.20
Tyrosine (Tyr)**	1.94	2.27*	1.93	2.40*
Proline (Pro)	3.00	2.77	3.09	2.89
Threonine (Thr)	2.61	2.27	2.79	2.59
Histidine (His)**	1.99	2.22*	2.55	2.66*
Cysteine (Cys)**	3.39	2.14	6.27	7.05*
Methionine (Met)**	0.11	0.10	0.11	0.10
Total AA(g/100 g protein)	57.99	56.93	66.16	65.98

* The type of amino acid that has increased, **Antioxidant amino acid grouping.

The results showed that the robusta and arabica coffee beans with the long period of germination have antioxidant activity in IC_{50} that tends to decrease in $ABTS^{++}$ and DPPH, but not with $\cdot OH$ and $\cdot O_2$, which increases after the 10 days germination period, as shown in Figure 3. As shown in Table 3, the ratio amount of antioxidant amino acids and total amino acid is presented as TAntAA/TAA. Both types of germinated coffee beans contain higher TAntAA/TAA than ungerminated (0 days). This is represented in the ratio of TAntAA/TAA, in which ungerminated (0 days) has a ratio of 22.81% (robusta) and 25.86% (arabica), a lower value than the germinated coffee bean at 10 days with its high TAntAA/TAA ratio of about 23.13% (robusta) and 28.22% (arabica) with a difference in the ratio of 0.32 and 2.36%, respectively.

This shows that arabica beans have stronger antioxidant activity than robusta beans.

The ability of germinated two coffee bean proteins to inhibit the activity of ACE-I was also investigated and compared in this study. The ACE-I inhibitor activity was analyzed in vitro, and results demonstrated the low potency of ungerminated robusta bean, almost 2.86 times compared to arabica. However, during the germination period, there was a rapid increase in ACE-I inhibitor activity compared to Arabica beans until the 5th day of germination, after which the increase was not significantly different. On average, the IC_{50} value on the 10th day of germination was 2.57 $\mu g/mL$ of robusta and 1.47 $\mu g/mL$ of arabica. However, this value was still significantly different from captopril (2.08 $\mu g/mL$) as a positive control (Figure 4).

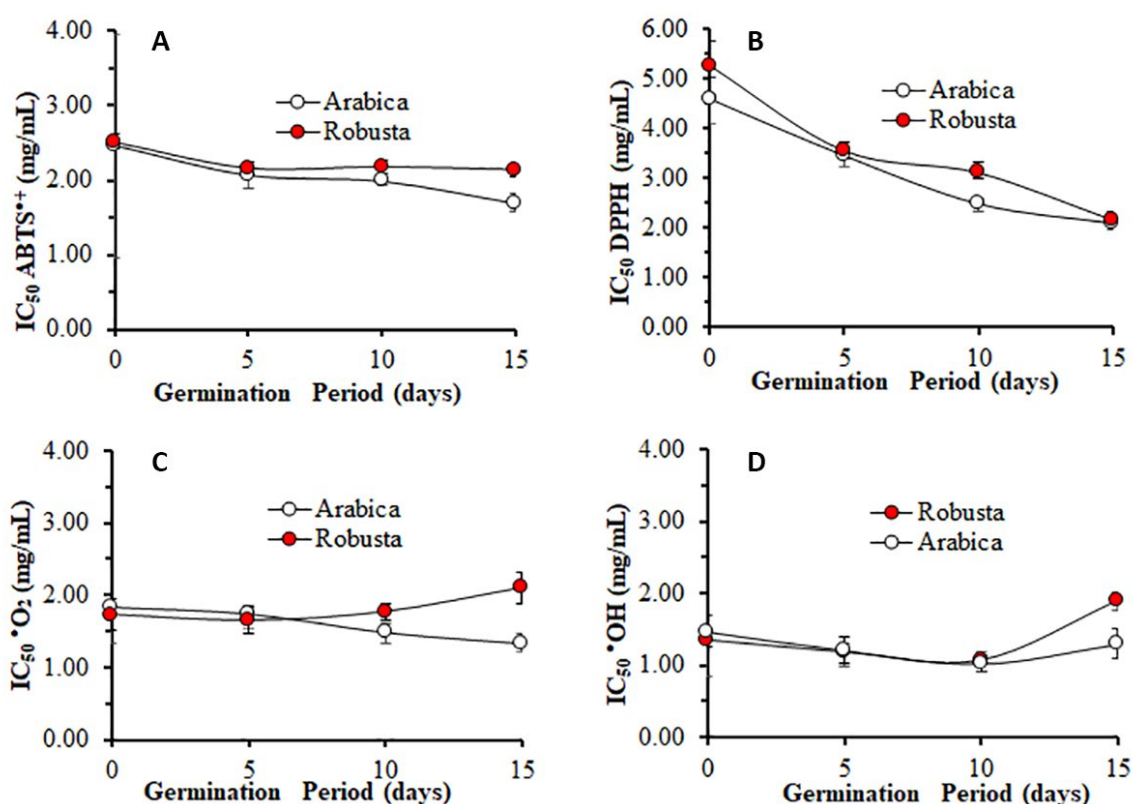


Figure 3: Change of antioxidant capacities (IC_{50}) in green coffee beans protein with different germination times. $ABTS^{++}$ scavenging ability (A), DPPH scavenging ability (B), superoxide ($\cdot O_2$) scavenging ability (C), and Hydroxyl ($\cdot OH$) scavenging ability (D).

Table 3: Average amino acid groupings of robusta and arabica coffee beans during germination and some ratios.

Amino Acid	Robusta		Arabica	
	0 days	10 days	0 days	10 days
TAA (g/100 g protein)	57.99	56.93	66.16	65.98
TAntAA (g/100 g protein)	13.23	13.17	17.11	18.62
TAntAA/TAA (ratio, %)	22.81	23.13	25.86	28.22

TAA=Total Amino Acid; TAntAA=Total Amino Acid Antioxidant.

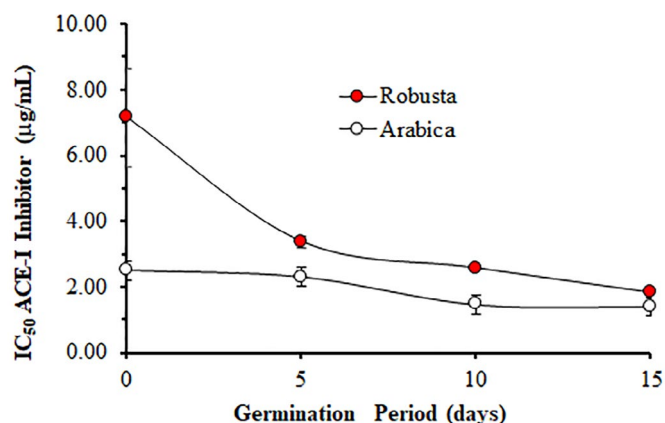


Figure 4: The IC₅₀ value of ACE-I inhibitor activity of germinated green coffee beans protein with different germination times.

4 DISCUSSION

Germination is a natural biological process in all superior plants. Seeds emerge from the latency stage when minimal environmental conditions, such as variety, humidity, temperature, and nutrients, are necessary for their growth and development (Sangronis et al., 2006). As a result of the germination process, coffee beans experience large metabolic changes in their protein storage. Although the total protein content showed slight variations (Table 1), the electrophoretic profiles showed significant changes. The Electrophoretic profile of coffee bean proteins showed two main bands previously observed by other authors (Ali; Rawel; Hellwig, 2023; Shimizu; Mazzafera, 2000). Changes in the intensity of protein bands during germination indicate that these bands undergo hydrolysis. The partial hydrolysis of proteins during germination was reflected in the decreased intensity of several bands, particularly the high-intensity bands. However, the total protein content did not show significant changes. This can be explained by the appearance of small polypeptides as degradation products of proteins. Similar results were observed during the germination of mung bean (Wintersohle et al., 2024) and lupins (Rumiyati; James; Jayasena, 2012), where the loss of high molecular weight proteins was attributed to their decomposition into short-chain peptides and amino acids, which are not detectable under electrophoretic conditions (Rumiyati; James; Jayasena, 2012).

Table 1 shows that coffee beans free amino acid content is relatively low in robusta and arabica coffee beans. However, the levels increase during germination for up to 5 days and decrease after that. The increase in free amino acid content up to 5 days is probably caused by rapid protein hydrolysis, thereby releasing free amino acids. After that, a decrease in total amino acids indicates rapid utilization and translocation of amino acids to the growth axis or respiratory impairment or may result in the accumulation of free amino acids in the cotyledons. Similar results were reported by Ehrhardt-

Brocardo, Coelho and Souza (2021) on *Phaseolus vulgaris* seedlings, Qi et al. (2022) on soybean seeds, and Yan et al. (2022) on sesame seeds (*Sesamum indicum* L). Differences in the amino acid composition of coffee beans reflect different amounts of TAA. The amount of TAA in the sample was dominated by the composition of Glu, Gly, and Leu, which was similar to the total amino acid composition of coffee bean protein in accordance with previous studies (Hwang; Chen; Ho, 2012; Shimizu; Mazzafera, 2000). The same was observed for free amino acids (Ribeiro; Rocha; Prudencio, 2021; Ali; Rawel; Hellwig, 2023), although the Cys content determined here was slightly higher in arabica coffee beans.

According to Donkor et al. (2012), higher antioxidant activity, as shown in low IC₅₀, is likely to contribute to each phytochemical compound and result in metabolic activity. The germination process can significantly change the seeds biochemical properties due to the activation of enzymatic processes that convert protein storage into bioactive peptides. The hydrolyzed protein is the product of the hydrolysis reaction or the breaking of the peptide bonds in the protein molecule containing a mixture of components of various peptides and free amino acids depending on the amount and kinds of amino acids. According to Xu, Guanqun and Hui (2017), several types of amino acids, such as Arg, Lys, Tyr, His, Cys, and Met, have great potential as antioxidants. Some amino acid types fall into this group in coffee beans, but the amounts and types vary between the two coffees (robusta and arabica).

Chen et al. (2017) reported that the degree of hydrolysis of protein storage was highly related to increased antioxidant capacity in the germination seed. They have observed antioxidant activity in hydrolyzed proteins (small-size peptides) due to the exposure of amino acids, resulting in high free radical scavenging activity, reducing power, inhibiting lipid oxidation and metal chelation capacity. The variety of peptides or free amino acid coffee beans was higher than the rest and comparable to germination. This can be attributed to the differential activity of hydrolases and oxidase during germination and differences in seed matrices control in antioxidant capacity.

The results revealed that germinated coffee bean protein extracted on the 10th day of germination had the greatest ability to inhibit the activities of ACE-I. Angiotensin-I converting enzyme assay was performed to evaluate ACE-I inhibitory activity by bioactive proteins produced during germination (Magaña et al., 2015). This suggests that decreasing IC₅₀ ACE-I inhibitors increases the formation of bioactive proteins during the germination process and reaches an optimal value on the 10th day of the germination process. This suggests that relatively fewer specific peptides are produced during the germination process and undergo further cleavage into smaller, more bioactive peptides during the 10th-day germination process. Further protein during the longer germination process results in

smaller peptides and fewer bioactive peptides. 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 Zn²⁺ binding (Li et al., 2024). The structural properties of ACE-I, such as chain length, composition, and sequence, can influence its inhibitory activity. The size of the peptide also affects the inhibition of ACE-I activity. ACE-I inhibiting peptides must have gastrointestinal stability and reach the cardiovascular system to demonstrate bioactivity. ACE-I peptides generally contain short chains with 2-12 amino acids (Daskaya-Dikmen et al., 2017).

5 CONCLUSION

The present study successfully explained and revealed that the germination of two varieties of green coffee beans (arabica and robusta) is related to their potential to affect the protective agent with high content of antioxidant amino acid groups, scavenging ABTS•⁺, DPPH, •O₂, and •OH, and inhibiting ACE-I activity. These results provide information related to nutraceutical sources in preventing cellular disease, including hypertension.

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7 AUTHORS CONTRIBUTION

Conceptual Idea: Siswoyo, T.A; Methodology design: Siswoyo, T.A; Noviyanti, E; Isnainun, A; Data collection: Noviyanti, E; Isnainun, A; Data analysis and interpretation: Siswoyo, T.A; Isnainun, A and Writing and editing: Siswoyo, T.A.

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