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Enhanced Functional Characteristics as a Cholesterol-lowering Bioactive Peptide from *Kara Kratok* Sprouts (*Phaseolus lunatus* L.)

(Ciri Fungsian yang Dipertingkatkan sebagai Peptida Bioaktif Penurun Kolesterol daripada Pucuk Kara Kratok (*Phaseolus lunatus* L.))

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ABSTRACT

HMG-CoA reductase is an enzyme that converts HMG-CoA into cholesterol via the mevalonate pathway, contributing to cardiovascular disease. The germination of brown *kara kratok* (*Phaseolus lunatus*) mixtures, including dark brown, light brown, or brown color pattern, specifically for 0, 24, 48, 72, and 96 h, yielded interesting results. The samples were measured for radicle length, % inhibition of HMG-CoA reductase, type of inhibition, IC_{50} value, total protein content, amino acids, percentage degree of hydrolysis (%DH), and molecular weight (MW), providing valuable insights into the potential use of these biopeptides. The results showed that biopeptides from germinated *kara kratok* exhibited the ability as HMG-CoA reductase inhibitors. Furthermore, the germination process increased its capability, from 46.79% in beans to 83.77% in 72-h germination. The 72-h germination was observed to be the best HMG-CoA reductase inhibitor as a competitive inhibitor, with an IC_{50} value of 335.09 µg/mL. This was supported by a total protein content of 27.76%, with glutamic acid (2.99%) as the dominant amino acid, followed by phenylalanine, aspartic acid, leucine, serine, and arginine, %DH (26.26%), and MW (5-15 kDa). Brown *kara kratok* sprouts possess the potential to inhibit HMG-CoA reductase, and germination increases its capability.

Keywords: Biopeptide; germination; HMG CoA reductase; inhibition; *Phaseolus lunatus*

ABSTRACT

HMG-CoA reduktase ialah enzim yang menukarkan HMG-CoA kepada kolesterol melalui laluan mevalonat, menyumbang kepada penyakit kardiovaskular. Percambahan campuran kara kratok coklat (*Phaseolus lunatus*), termasuk corak warna coklat gelap, coklat muda atau coklat, khusus untuk 0, 24, 48, 72 dan 96 jam memberikan hasil yang menarik. Sampel diukur untuk panjang radikel, % perencatan HMG-CoA reduktase, jenis perencatan, nilai IC_{50}, jumlah kandungan protein, asid amino, peratusan darjah hidrolisis (%DH) dan berat molekul (MW), memberikan pandangan berharga tentang potensi penggunaan biopeptida ini. Keputusan menunjukkan bahawa biopeptida daripada kara kratok yang bercambah menunjukkan keupayaan sebagai perencat reduktase HMG-CoA. Tambahan pula, proses percambahan meningkatkan keupayaannya daripada 46.79% dalam kacang kepada 83.77% dalam percambahan 72 jam. Percambahan 72 jam diperhatikan sebagai perencat reduktase HMG-CoA terbaik sebagai perencat kompetitif dengan nilai IC_{50} 335.09 µg/mL. Ini disokong oleh jumlah kandungan protein sebanyak 27.76% dengan asid glutamat (2.99%) sebagai asid amino dominan, diikuti oleh fenilalanin, asid aspartik, leucine, serin dan arginin, %DH (26.26%) dan MW (5-15 kDa). Pucuk kara kratok coklat mempunyai potensi untuk menghalang HMG-CoA reduktase dan percambahan meningkatkan keupayaannya.

Kata kunci: Biopeptida; HMG CoA reduktase; percambahan; perencatan; *Phaseolus lunatus*

INTRODUCTION

HMG-CoA reductase is an enzyme that converts HMG-CoA as the native substrate to cholesterol via the mevalonate pathway (Murphy, Deplazes & Cranfield 2020). It then changes to LDL-cholesterol that triggers cardiovascular disease (Wang & Patterson 2015), which is inhibited by statins as competitive inhibitors against its native substrate, HMG-CoA (Endo, Kuroda & Tanzawa 1976). Statins have a structure like HMG, which is rigid, hydrophobic, and covalently bonded to HMG-like sites (Istvan 2003). Some legumes, such as soybean hydrolysate, and several others, including pigeon hydrolysate and lupine bean hydrolysate, have been known as bioactive compounds that inhibit HMG-CoA reductase with similar criteria, with a molecular weight of <10 kDa, containing hydrophobic amino acid, including tyrosine, tryptophan, and leucine,

which plays an essential role in binding cholesterol and bile acids (Boachie, Yao & Udenigwe 2018; Hermanto et al. 2021; Lammi et al. 2014). *Phaseolus lunatus* L. is a legume that is believed to have the potential as an HMG-CoA reductase inhibitor because it is rich in leucine, which ranges from 8.84-9.84 g/100 g protein (Palupi, Estiasih & Sutrisno 2021; Seidu, Osundahunsi & Osamudiamen 2018) with 48.19% as globulin fraction (Adewole et al. 2023). However, the current use of this legume has not been optimized in Indonesia, which contributes to its low commercial value.

Germination is known as a cheaper method compared to fermentation or proteolytic enzyme processing. Furthermore, it increases the inhibition activity, bioavailability, nutritional quality, and digestibility of the bean, due to enzyme activation that starts from the bean soaking until two to three days of water imbibition. Proteolytic enzymes will hydrolyse albumin, globulin, glutelin, and prolamin proteins in seeds into short-chain peptides and amino acids (Dattatray et al. 2019; Taylor, Novellie & Liebenberg 1985). Agustia et al. (2023) show the benefit of jack bean sprouts for inhibiting DPPH-IV activity, and the 60-h germination shows the best result (41.57%; half maximal inhibitory concentration=2.24 mg/mL). Thus, this study sought to determine the potential of biopeptide from germinated brown mixtures of *kara kratok* as an inhibitor for HMG-CoA reductase. The results of this study confirm that natural sources such as *kara kratok* sprouts have the potential to inhibit HMG-CoA reductase, and the germination process increases its ability.

MATERIALS AND METHODS

BROWN *Kara kratok* AND CHEMICALS

Brown mixtures of *kara kratok* (light brown, dark brown, and brown color pattern) were obtained from a local market in Bondowoso, in East Java, Indonesia. Some chemical agents and kits were obtained from Merck (o-Phthaldialdehyde (OPA)) and Sigma Aldrich (HMG CoA reductase kit (CS-1090)).

GERMINATION OF *Kara kratok*

The germination time and procedure followed Luis et al. (2012) that found 48 h as the optimum germination duration for developing ACE I inhibitor from *Phaseolus lunatus* with the following steps. The *kara kratok* (*Phaseolus lunatus* L.) seeds were first washed with tap water, then soaked in distilled water for 24 h. Following this, they were soaked for 1 min in 0.2% (w/v) of sodium hypochlorite solution, then washed again for three times with distilled water. The clean beans were then germinated for 0, 24, 48, 72, and 96 h in a dark room with a temperature of 16-18 °C and water sprayed every 8 h. The determination of the maximum germination time of 96 h also considered visual appearance for edible sprouts as preliminary study. The sprouts formed at each germination time dried in a cabinet dryer at 50-55 °C for 8 h followed by grinding to obtain *Phaseolus lunatus* L. sprout flour. The flour was analysed for its % inhibition of HMG-CoA reductase, type of inhibition, total protein content, amino acids, percentage degree of hydrolysis (%DH), and molecular weight (MW). During germination, the radicle length was measured, and visual observation was performed.

HMG CoA REDUCTASE INHIBITION

HMG-CoA reductase inhibition method followed the Sigma Aldrich kit's procedure (CS-1090 kit) and Hermanto et al. (2021). First, 181 µL of 1× assay buffer was added to 1 µL pravastatin or biopeptide's candidate, then, 4 µL NADPH, 12 µL HMG CoA, and 2 µL HMG-CoA reductase were added. This step was repeated for Blanko (without pravastatin and HMG CoA reductase, 184 µL of 1× assay buffer + 4 µL NADPH + 12 µL HMG CoA) and positive reference (without pravastatin, 182 µL of 1× assay buffer + 4 µL NADPH + 12 µL HMG CoA+ 2 µL HMG CoA reductase).

HMG-CoA REDUCTASE INHIBITION KINETICS

Determination of the kinetics of the biopeptide's action of *kara kratok* sprouts on HMG-CoA reductase enzyme was carried out at concentrations of HMG-CoA varied at 0.3, 0.6, 0.9, and 1.2 mmol/L in the absence and presence of biopeptide of *Phaseolus lunatus* at 72 h germination. Subsequently, the model inhibition was determined by Lineweaver-Burk plot curve analysis to determine the Km and Vmax as the measurement of inhibition type (Gholamhoseinian, Shahouzehi & Sharifi-Far 2010). The IC_{50} value was calculated for 50% inhibition of HMG-CoA reductase via regression equation.

TOTAL PROTEIN CONTENT AND AMINO ACID PROFILE

TOTAL PROTEIN CONTENT

The analysis of total protein content of *kara kratok* sprouts was according to the Kjeldahl method (AOAC 2005), with step digestion of 1 g of samples with 15 mL concentrated H_2SO_4 and two copper catalyst tablets, then water was added to cool the solution before neutralization and titration.

AMINO ACID PROFILE

The amino acid profile followed LC-MS methods by Chang, Skauge and Satterlee (1989), column C18 (ACQUITY UPLC® BEH C18, Waters; 1.7 µm, 2.1×50.0 mm) with a Xevo tandem quadrupole detector (Waters) used for this analysis. Two tenths g of samples were mixed with 10 mL HCl 6 N for 12 h at 110 °C hydrolysis, followed by

neutralization with 50 mL NaOH 6 N. Mixed solutions were dissolved with distilled water before filtration with a syringe filter of 0.22 µm. The resulting filtrate was then injected (5 µL) into the system. The eluent (A and B) as a mobile phase was prepared with water: 0.1 % penta-deca-fluorooctanoic acid, where A used a ratio of 0.5:99.5, and B used a ratio of 90:10. The flow rate was determined at 0.6 mL/min at 25 °C using a gradient system with the following schemes: at 0 min, %A (90.00) and %B (10.00); at 4 min, %A (50.00) and %B (50.00); at 4.1 min, %A (95.00) and %B (5.00); and at 6 min, %A (95.00) and %B (5.00).

PERCENTAGE DEGREE OF HYDROLYSIS

Percentage degree hydrolysis followed Agustia, Murdiati and Indrati (2023) using reagent o-phthalaldehyde (OPA) and 340 nm absorbance on spectrophotometry UV-VIS (Dynamica Scientific Halo SB-10). Four tenths mL of peptide sample solution with 3 mL OPA reagent were incubated at room temperature for 20 min, then the absorbance was measured at λ 340 nm using a spectrophotometer (GENESYS 10S). Tryptophan was used to make standard curves and %DH, and the result was calculated using the equation:

$$\%DH = \frac{Ntx - Nt0}{Ntot - Nt0}\, x\, 100$$

where *Ntx* is the hydrolyzed sample at x-h; *Nt0* is the hydrolyzed sample at 0-h; and *Ntot* is the total quantity of the hydrolyzed sample with 10 mL HCl 6 M for 4 h at 105 °C.

MOLECULAR WEIGHT

SDS-PAGE with a slight modification of Laemmli's method (Laemmli 1970) was used to determine the molecular weight of germinated *kara kratok* samples. This method used 5% resolving gel and 13% separating gel. The SDS sample buffer, which contained 0.5M Tris-HCl at pH 6.8, 87% glycerol (w/v), 10% SDS (w/v), 0.5% bromophenol blue (w/v) and distilled water, was used to dilute the peptide extracts at a ratio of 1:2. After heating the sample solution for 4 min at 100 °C, 20 µL of the samples was loaded into each well together with 5 µL of standard protein marker. The gel ran for 60 min at 220V, followed by a soaking period for 30 min with distilled water, which was repeated three times. Finally, the gel was stained with 0.2% Coomassie brilliant blue R-250, which contained 50% methanol, 10% acetic acid, and 40% distilled water.

STATISTICAL ANALYSIS

The experimental method for this study was a completely randomized design with three replications of batch and analysis. SPSS IBM 23 software was used to analyse the statistical data with a 95% confidence interval for significant differences ($P \leq 0.05$) in each parameter as impacted by various germination duration. A one-way analysis of variance (ANOVA) was used for the data analysis to determine if any significant difference continued with Duncans' multiple range test (DMRT).

RESULTS AND DISCUSSION

RADICLE LENGTH OF GERMINATED *Kara kratok*

Visual observation showed the formation of radicles during germination as the first growth of the plant's embryonic root after water imbibition to the seed (Recek et al. 2021). The radicle length of *kara kratok* increased during longer germination time with a maximum length of 5.38 cm at 96-h germination. During germination, the radicle length of *kara kratok* sprouts increased two times from 2.58 cm to 5.38 cm after 72-h germination (Figure 1). In contrast, after 24-h germination, the radicle length was only around 0.75 cm, indicating difficulties in shoot growth despite the rapid growth observed after the radicles emerged from the seed. This condition is caused by the inhibition of gibberellin as a growing hormone by the chloroform phase compound in the seed (Köhler & Lang 1963). The formation of radicles during germination indicates a protein breakdown into smaller peptides that have the benefit of inhibiting the HMG-CoA reductase enzyme (Bueno et al. 2020).

HMG CoA REDUCTASE INHIBITION

Germinated *kara kratok* (*Phaseolus lunatus* L.) demonstrated the capability as an HMG-CoA reductase inhibitor, and its capability increased over the course of the germination period. The inhibition of HMG-CoA reductase observed in the biopeptides of *kara kratok* germinated for 0-h and 24-h was lower than that of pravastatin as a reference. However, this was not applicable for germination after 48-h (Figure 2). Statistical calculations showed that all germination times were significantly different ($P \leq 0.05$), including that with pravastatin. During germination, complex proteins such as albumin, globulin, glutelin, and prolamin change to short-chain peptides and amino acids (Dattatray et al. 2019; Taylor, Novellie & Liebenberg 1985), subsequently increasing its bioactivity (Bueno et al. 2020). Short-chain peptides with 3-20 amino acids are essential for inactivating the active site of HMG-CoA reductase (Zioudrou, Streaty & Klee 1979). This includes biopeptide LPYP from soybean hydrolysate (Lammi, Zanoni & Arnoldi 2015), or biopeptide GGV, IVG, and LVG from *Amaranthus cruentus* hydrolysate (Soares et al. 2015).

HMG-CoA REDUCTASE INHIBITION KINETICS

Based on Figure 3(A), the sample without biopeptide compounds intersected on the Y axis with the added

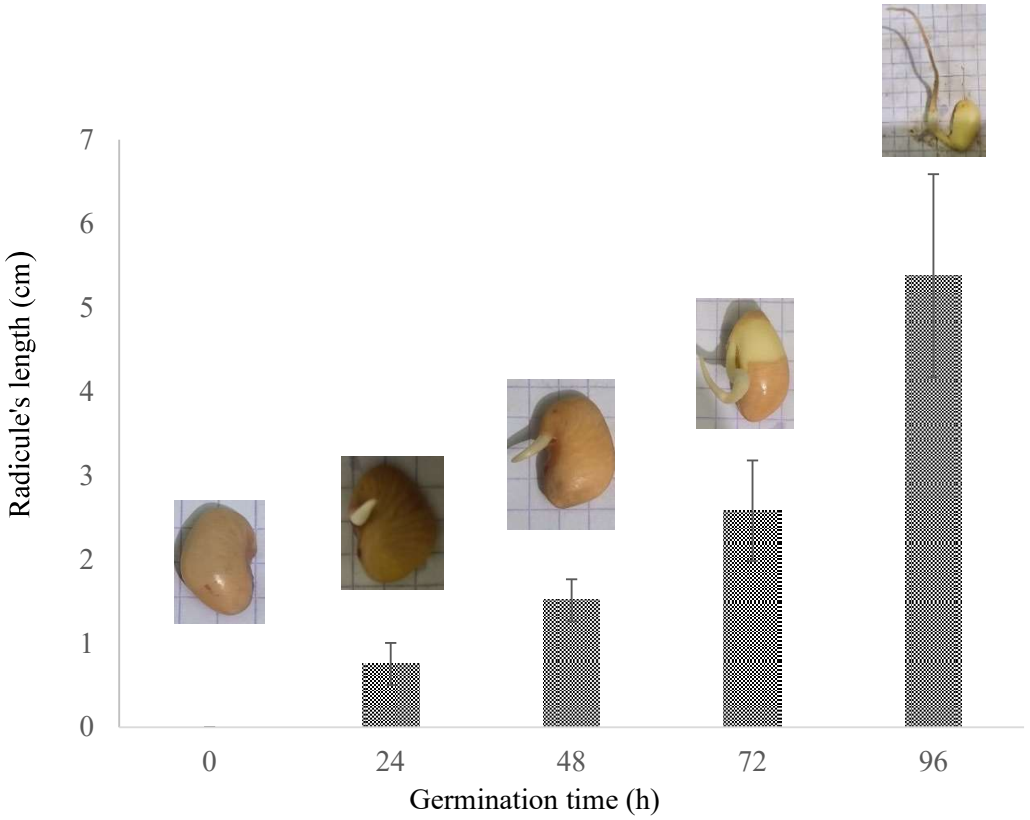
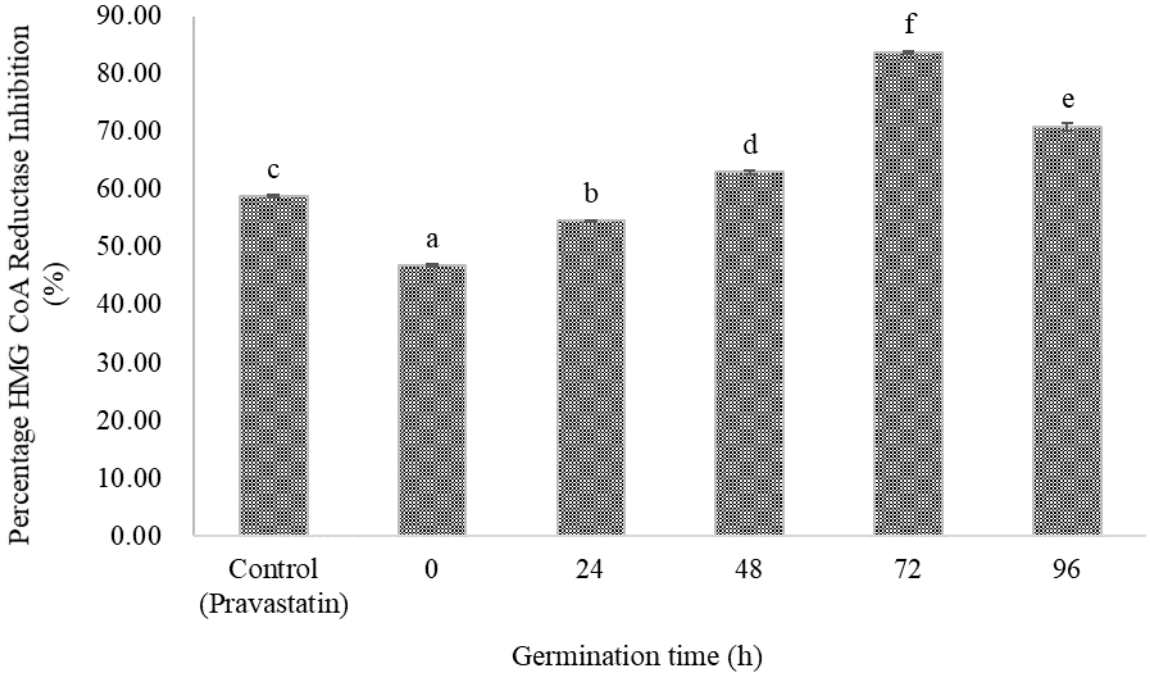


FIGURE 1. Radicle length and visual appearance *kara kratok* (*Phaseolus lunatus* L.) during germinated for 0, 24, 48, 72, and 96 h



The data from an average of six replicates ± standard deviation; a different letter is a significant difference at P≤0.05

FIGURE 2. Percentage of HMG-CoA reductase inhibition of brown *kara kratok* (*Phaseolus lunatus* L.) germinated for 0, 24, 48, 72, and 96 h

biopeptide compounds of 72-h germinated *kara kratok* flour. Based on the linear graph and equation, the Michaelis-Menten constant (Km) of no inhibitor was 0.772, while that with biopeptide inhibitor was 1.882. From the linear line in Figure 3(A), the Vmax values of both were almost the same, at 1.027 for the sample without inhibitor and 1.030 for the sample with the inhibitor of biopeptide compounds. Attaallah and Amine (2021) stated that competitive inhibitor compounds do not affect the maximum activity (Vmax) of the enzyme, but there is an increase in the Michaelis-Menten constant (Km) of the enzyme because a higher substrate concentration is needed to achieve semi-maximal activity. Therefore, based on these Km and Vmax parameters, biopeptide compounds derived from 72-h germinated *kara kratok* were competitive inhibitors with natural substrate HMG-CoA to the active site of HMG-CoA reductase enzyme. This inhibition pattern is consistent with that observed for soybean peptides YVAE at concentrations of 0.04M and 0.08M, as reported by Pak et al. (2007). Vmax value for non-inhibitor was 19.7 µM/min, and that with YVAE biopeptide inhibitor at both concentrations was 20 µM/min. Furthermore, the KMapp/KM ratio showed an increase, with values for non-inhibitor, YVAE peptide 0.04M, and 0.08M being 1, 3.4, and 6.6, respectively. A competitive inhibitor is a molecule like a substrate that binds to the same active site of an enzyme reversibly and prevents the binding of its natural substrate molecules. It can reduce the enzyme's catalytic efficiency towards the appropriate substrate.

The IC_{50} value of the HMG-CoA reductase inhibitor indicated the maximum inhibitory concentration of 50% HMG-CoA reductase enzyme inactivation. It measured the compound efficiency in inhibiting biological processes by half of its activity (Aykul & Martinez-Hackert 2016). The IC_{50} value of 72-h germinated *kara kratok* hydrolysed by pepsin-pancreatin was 335.09 µg/mL (Figure 3(B)), indicating that 50% HMG-CoA reductase enzyme was inhibited by 335.09 µg/mL of biopeptide from 72-h germinated *kara kratok* flour. This IC_{50} value is lower than the hydrolysate of lentils with pepsin pancreatin (1.2 mg/mL), green peas with alcalase (1.4 mg/mL), and green peas with pepsin pancreatin (1.5 mg/mL). A lower value suggests that the peptide inhibitor is more efficient in inhibiting HMG-CoA reductase (Moreno et al. 2020). However, the IC_{50} is higher than that of soybean which ranges from 59 to 229 µg/mL, depending on its variety at high or low glycinin: β-conglycinin ratio (Rebollo-Hernanz, Bringe & de Mejia 2023).

TOTAL PROTEIN CONTENT AND AMINO ACID PROFILE

TOTAL PROTEIN CONTENT

Germination time showed incremental total protein content in germinated *kara kratok* from 0-h to 72-h but decreased at 96-h. The highest level of protein content was identified at 72-h, although it was not significantly different ($P \leq 0.05$) from the protein content at 48-h (Figure 4). This trend aligns with HMG-CoA reductase inhibition of *kara kratok* sprouts germinated from seeds for 72-h as the highest inhibitor. Longer germination time increases the total protein content. This indicates the start of proteolytic activity in the seed after water imbibition (Wang et al. 2007), while a decreasing protein content is due to the breakdown of storage protein into small peptides, such as amino acids, to support seedling growth.

AMINO ACID PROFILE

Amino acid profiling was measured to determine the protein composition of *kara kratok*, facilitating the development of bioactive peptides that serve as HMG-CoA reductase inhibitors (Table 1). Glutamic acid was the highest amino acid found in the germinated *kara kratok* (*Phaseolus lunatus* L.), followed by phenylalanine, aspartic acid, leucine, serine, and arginine. This finding is consistent with the finding of Palupi et al. (2021) which similarly identified glutamic acid as the highest amino acid, followed by phenylalanine, aspartic acid, leucine, serine, and arginine. Our observation on hydrophobis amino acids in the germinated *kara kratok* is consistent with the experiment of Agustia, Murdiati and Indrati (2023) which used jack bean (*Canavalia ensiformis* L.) rich in phenylalanine, isoleucine, leucine, and valine for 60 - 72 h of germination. The amino acid concentration of *kara kratok* germinated for more than 24-h was also higher than that for 0-h due to amino acid reaching the maximum level of a single amino acid over the course of germination time (Bautista-Expósito et al. 2020). Statistically, 24-h germination showed significantly ($P \leq 0.05$) higher glutamic acid than 0-h and 96-h, but it was not significantly different ($P \leq 0.05$) with 48-h and 96-h.

DEGREE OF HYDROLYSIS

The hydrolysis degree of protein of *kara kratok* during germination showed the incremental, which is in line with the longer time of germination and statistically significant difference ($P \leq 0.05$) each between germination time (Figure 5). The maximum percentage of hydrolysis degree was 31.99% at 96-h germination due to proteolytic activity as a biological activity during the germination process (Sandoval-Sicairos et al. 2020). Bueno et al. (2020) reported that 18 h of *P. sativum* germination increases the protein hydrolysis to amino acids and small peptides, while Fitriani et al. (2022) show the same incremental trend of hydrolysis degree until 48 h of *lamtorogung* germination.

PROTEIN MOLECULAR WEIGHT FRACTION

The band thickness of the gel was the indicator range of the germination sample's molecular weight. Germination of *kara kratok* for 0-h showed a molecular weight in the

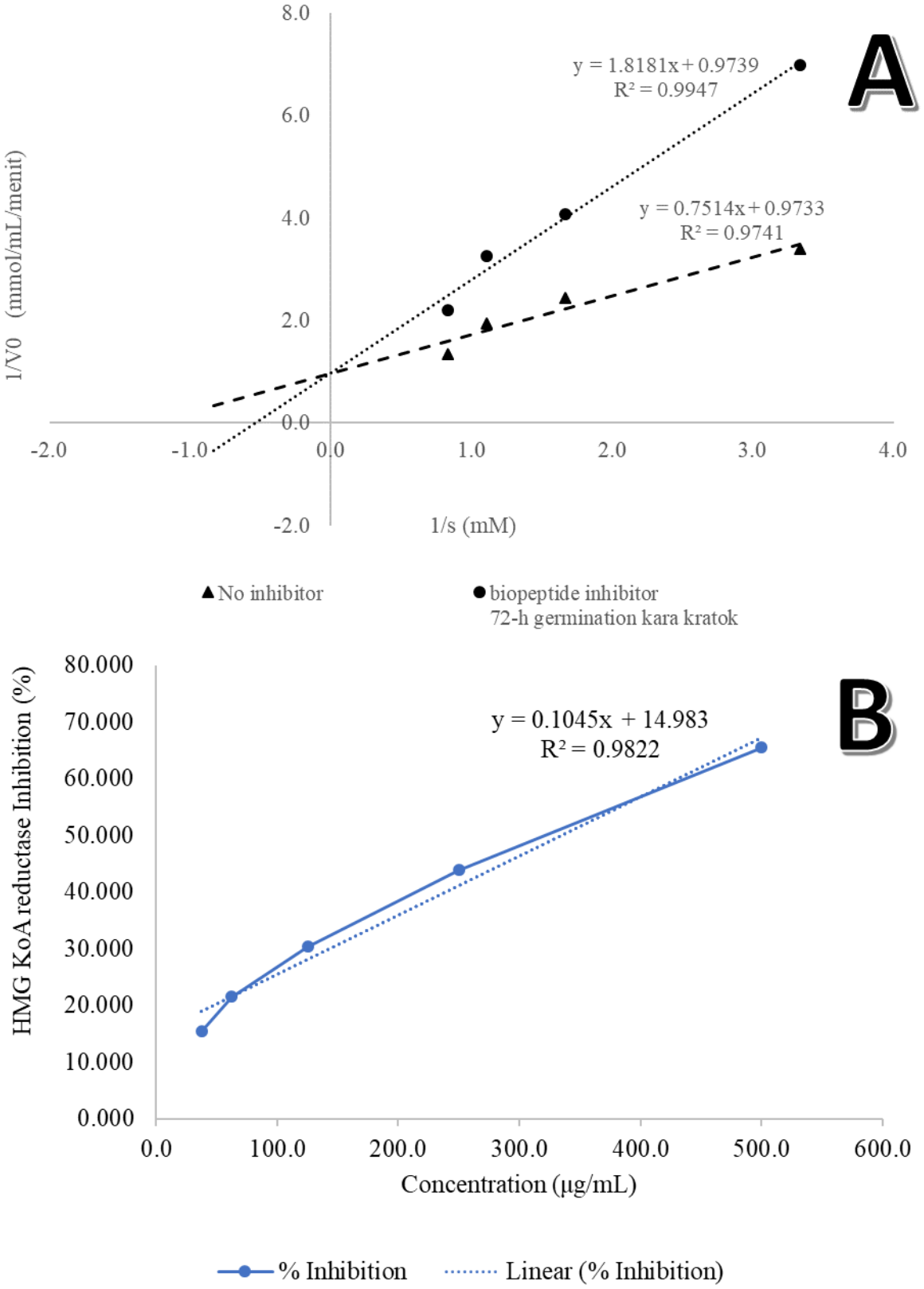
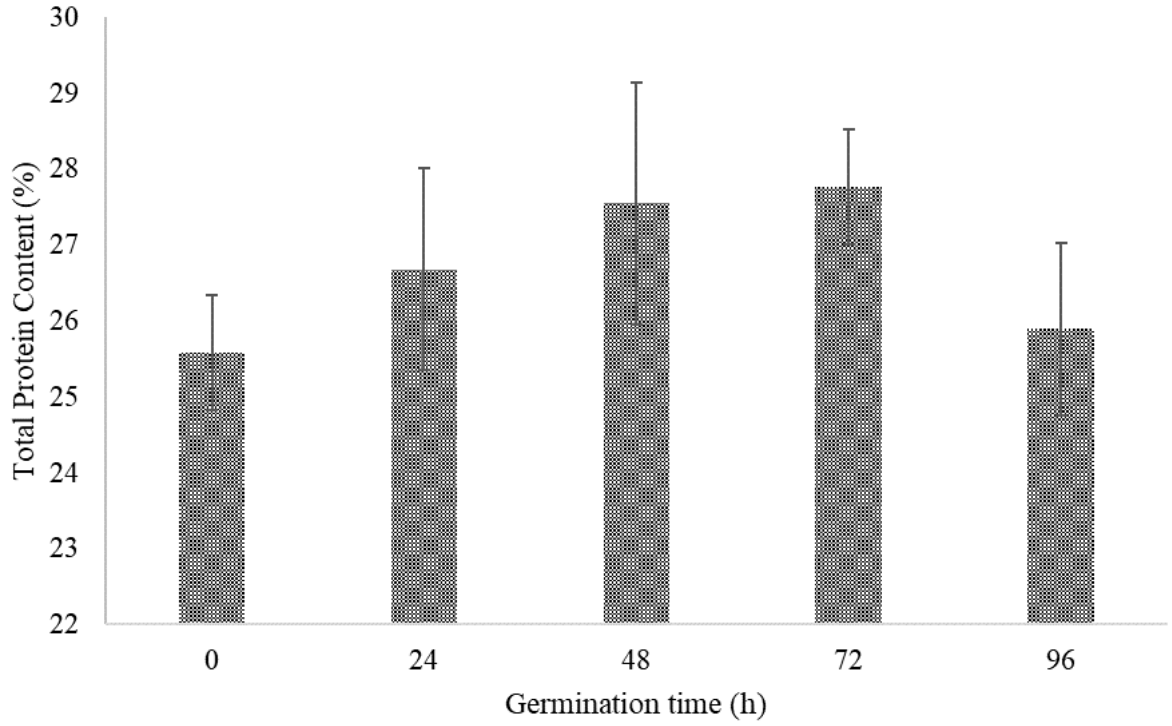


FIGURE 3. HMG-CoA reductase inhibition kinetics (A) Inhibition type via Lineweaver-Burk method (B) IC_{50} value of *kara kratok* (*Phaseolus lunatus* L.) germinated for 72-h



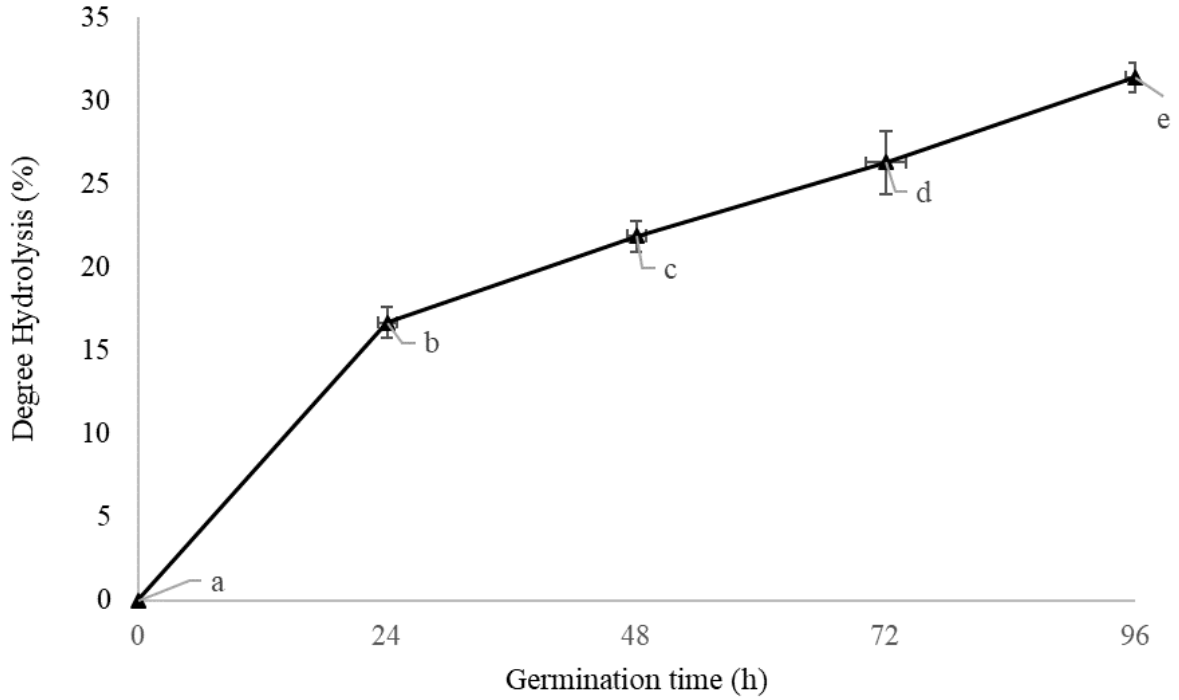
The data is an average of six replicates ± standard deviation; a value followed by a different letter is a significant difference at P≤0.05

FIGURE 4. Protein total (%) of *kara kratok* (*Phaseolus lunatus* L.) germinated for 0, 24, 48, 72, and 96 h

TABLE 1. Amino acids profile of brown *kara kratok* (*Phaseolus lunatus* L.) germinated for 0, 24, 48, 72, and 96 h

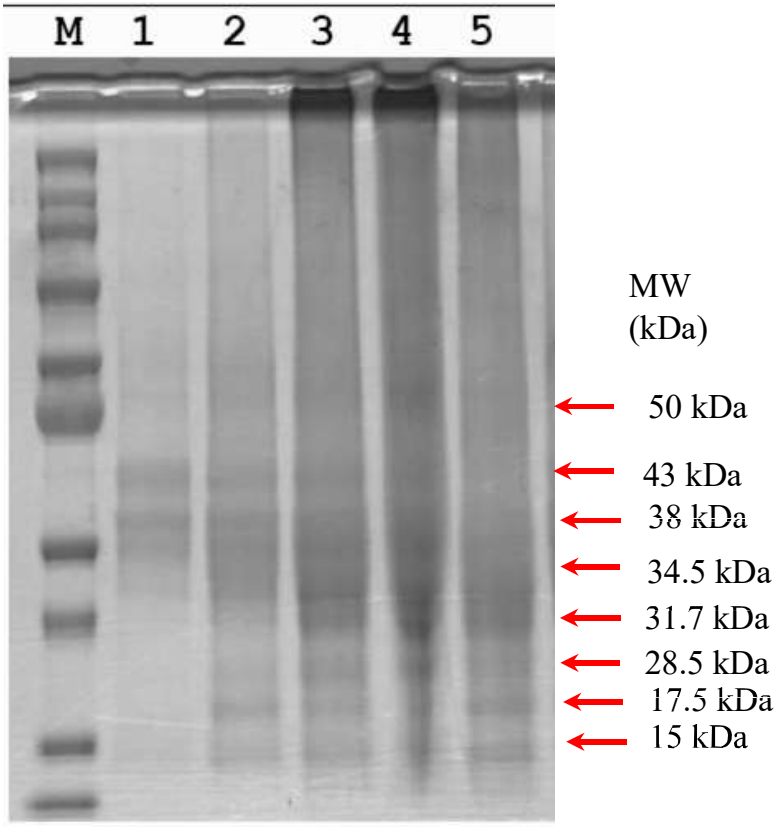
Parameter	Germination time (h)				
	0	24	48	72	96
L-Alanine	1.01±0.02^{a}	1.12±0.01^{c}	1.05±0.01^{b}	1.12±0.02^{c}	1.05±0.03^{b}
L-Arginine	1.93±0.09^{bc}	1.96±0.04^{bc}	1.91±0.03^{ab}	2.02±0.02^{c}	1.82±0.10^{a}
L- Aspartic acid	2.20±0.07^{a}	2.52±0.14^{b}	2.28±0.04^{a}	2.64±0.10^{b}	2.63±0.27^{b}
L- glutamic acid	2.77±0.08^{a}	3.14±0.09^{b}	3.02±0.02^{b}	2.99±0.12^{b}	2.67±0.16^{a}
Glycine	1.26±0.02^{c}	1.16±0.05^{ab}	1.20±0.01^{b}	1.15±0.01^{ab}	1.13±0.04^{a}
L-Proline	1.06±0.02^{a}	1.09±0.03^{a}	1.05±0.01^{a}	1.07±0.04^{a}	1.08±0.02^{a}
L-Serine	2.27±0.02^{a}	2.33±0.08^{ab}	2.34±0.03^{abc}	2.42±0.03^{bc}	2.44±0.12^{c}
L-Tyrosine	0.99±0.05^{a}	1.05±0.10^{ab}	1.01±004.^{a}	1.12±0.06^{b}	0.97±006.^{a}
L-Histidine	1.45±0.07^{ab}	1.52±0.06^{ab}	1.49±0.08^{ab}	1.57±0.08^{b}	1.39±0.11^{a}
L-Isoleucine	1.35±0.02^{a}	1.45±0.04^{b}	1.45±0.01^{b}	1.47±0.06^{b}	1.44±0.02^{b}
L-Leucine	2.30±0.03^{a}	2.36±0.10^{a}	2.35±0.04^{a}	2.35±0.09^{a}	2.31±0.02^{a}
L-Lisin	1.05±004.^{bc}	1.12±0.03^{c}	1.02±0.06^{b}	1.02±0.04^{b}	0.95±0.05^{a}
L-Phenylalanine	2.35±0.17^{a}	2.56±0.23^{ab}	2.53±0.19^{ab}	2.76±0.19^{b}	2.33±0.16^{a}
L-Threonine	1.57±0.02^{a}	1.56±0.04^{a}	1.58±0.01^{a}	1.57±0.04^{a}	1.52±0.09^{a}
L-Valine	1.50±0.02^{a}	1.58±0.04^{bc}	1.55±0.01^{b}	1.60±0.04^{c}	1.60±0.01^{c}

Values are given as the Mean of four replicates ±SD. Numbers followed by different letters in the same row are statistically different $p \leq 0.05$



The data is an average of six replicates ± standard deviation; a value followed by a different letter is a significant difference at P≤0.05

FIGURE 5. Degree of hydrolysis of brown *kara kratok* (*Phaseolus lunatus* L.) germinated for 0, 24, 48, 72, and 96 h



M= Marker, Lane 1-5: samples germinated for 0, 24, 48, 72, and 96 h

FIGURE 6. Molecular weight via SDS-PAGE of brown *kara kratok* (*Phaseolus lunatus* L.) germinated for 0, 24, 48, 72, and 96 h

range of 30 - 50 kDa, for 24-h and 48-h germination, the molecular weight was in the range of 23 - 25kDa, while for 72-h and 96-h germination, it was in the range of 17 - 18 kDa (Figure 6). Phaseolin as a storage protein in *kara kratok* (*Phaseolus lunatus* L.) exhibited a molecular weight of around 54 - 58 kDa, containing two types of subunits of 32.0 - 38.5 kDa dan 21 - 27 kDa (Bonita, Shantibala Devi & Singh 2020). This phaseolin was detected in the sample germinated for 0-h that later changed into smaller peptides at longer germination time, a process similar to the proteolysis reaction observed in the germination of *Mexican amaranth* (Sandoval-Sicairos et al. 2020).

In this study, longer germination time gave a thin band in the highest and lowest molecular weight as the proteolytic activity result. This result is consistent with that of Rumiyati, James and Jayasena (2012), which shows that *Lupinus angustifolius* germination for nine days increases the total protein content and changes the high molecular weight to 40, 50, 65, and 90 kDa, which inhibits HMG-CoA reductase due to small molecules of the peptides. Villalobos, Nicolas and Trinidad (2023) found biopeptide from lemongrass (*Cymbopogon citratus* Stapf.) as an HMG-CoA reductase inhibitor with molecular weight of 10-27 kDa or higher.

CONCLUSION

The germinated brown mixture of *kara kratok* beans (*Phaseolus lunatus* L.) used in this study demonstrated HMG-CoA reductase inhibition ability, and the inhibition was slightly higher than that of pravastatin, with the highest being in the 72-h germination samples. Similar to pravastatin, the biopeptide *kara kratok* sprouts from 72-h germinated seeds was also a competitive inhibitor with an IC_{50} value of 335.09 µg/mL. This capability was supported by small molecular weight development, an incremental total protein with glutamic acid as the highest content of amino acids, and an incremental of degree hydrolysis as proteolytic activity result. This study is a preliminary experiment to explore the potential of germinated *kara kratok* (*Phaseolus lunatus* L.) as an HMG-CoA reductase inhibitor. Currently, a study is being conducted to identify the peptide fraction which may result in the digestion via *in vitro* simulation for germinated and ungerminated *kara kratok* (*Phaseolus lunatus* L.) that has the highest HMG-CoA reductase inhibitory activity.

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