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Germination of jack bean [*Canavalia ensiformis* (L.) DC.] and its impact on nutrient and anti-nutrient composition

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Abstract

The nutrient and anti-nutrient composition of jack bean sprouts were investigated as a local alternative protein source. This study used six germination time treatments of 12, 24, 36, 48, 60, and 72 hrs. The morphology of the sprouts was studied using a descriptive approach, while the nutrition and anti-nutritional components were analyzed by analysis of variance. The results indicate that germination time significantly increased protein solubility and amino acid concentration, whereas HCN, phytic acid, trypsin inhibitors, and tannin decreased with germination time. Sprouts germinated for 60 and 72 hrs had radicle lengths of about 8 to 9 cm, respectively, and were categorized at a safe cyanide level for consumption according to the WHO. These sprouts are rich in hydrophobic amino acids, such as phenylalanine, isoleucine, leucine, and valine, which are potential bioactive peptides.

1. Introduction

Jack bean seeds [*Canavalia ensiformis* (L.) DC.] have been developed in Indonesia as a local alternative protein source to replace soybeans. Many studies have been conducted to diversify jack beans for food processing (Ningrum *et al*., 2018; Agustia *et al*., 2019). Jack beans are a potential alternative vegetable protein source because of their rich protein level. However, jack beans contain many anti-nutritional components, including Concanavalin A, trypsin inhibitors, tannins, phytic acid, and toxic HCN (Ahirwar and Nahar, 2015). The anti-nutritional contents of jack beans can be reduced by processing, particularly through heat treatment (Agbede and Aletor, 2005; Doss *et al*., 2011; Alagbaoso *et al*., 2015; Okomoda *et al*., 2016). Many studies have reported the toxicity of HCN. Ramli *et al*. (2021) determined that the HCN content of raw jack beans is 52.78 mg/kg or 52.78 ppm. Studies about fermentation (Puspitojati, Indrati, Cahyanto *et al*., 2019) and soaking following fermentation (Ramli *et al*., 2021) have been conducted to reduce the anti-nutrient components, including HCN in jack beans. Ramli *et al*. (2021) reported that soaking jack beans in 1% $NaHCO_3$ for 36 hrs following fermentation reduces the anti-nutrient factors, particularly HCN, to an acceptable level. Puspitojati, Indrati, Cahyanto *et al*. (2019) determined that two soaking boiling treatments removed 97.95% of the HCN from jack beans. This treatment following fermentation reduced HCN content to 0.71 ppm.

Germination has been studied as a simple food processing technique to enhance the nutritional profile and reduce the anti-nutritive content of grains (Ikram *et al*. 2021). Changes in the nutritional and anti-nutritional components during germination depend on a variety of factors, such as germination time (Masood *et al*., 2014). Germination time varies for each type of bean. For example, soybean and mung bean seeds take about 36–60 hrs to develop into sprouts suitable for consumption (Megat *et al*., 2016), while cowpea and komak beans take 96 hrs (Benitez *et al*., 2013). The length of the germination time strongly affects proteolytic activity in *Vicia faba* L. (Kirmizi and Guleryus, 2006), the trypsin inhibitor in cowpea (Malomo *et al*., 2014), dietary fibre in soy, kidney, and mung beans, and peanut (Megat *et al*., 2016); proximate components in lentils, black beans, and green beans (Sattar *et al*., 2017); phytate content in maize grains (Mihafu *et al*., 2017), and protein solubility in pigeon pea (Sharma *et al*., 2019).

Free amino acid contents increase and the type of

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amino acid changes during bean germination (Kuo *et al*., 2004; Kanetro, 2018). For example, soybean germinated for 36 hrs has high specific free amino acids such as alanine, leucine, phenylalanine, and lysine, popularly known as insulin-stimulated secretion (Kanetro, 2018). According to Kirmizi and Guleryus (2006), germination of broad bean (*V. faba* L.) for 7 days enhances free amino acid content. Damayanti *et al*. (2019) studied the physicochemical and HCN range of jack bean sprout flour for 48 hrs. However, few studies have been performed on the effect of germination time on nutritional components in jack beans, particularly the amino acid profile. Morphological studies of jack bean sprouts are also scarce. Therefore, this study was conducted to provide the nutritional and anti-nutritional components, including the amino acid profile and a morphological study of jack beans during germination.

2. Materials and methods

The jack bean [*Canavalia ensiformis* (L.) DC.] white variety was obtained from farmers in Kulon Progo Yogyakarta. The germination treatment included six time points (12, 24, 36, 48, 60, and 72 hrs). The treatments were repeated three times resulting in 18 repeated treatments. Jack bean that did not germinate (0 hr) was used as the control.

2.1 Jack bean soaking process

White jack bean seeds (700 g) were sorted, washed, and divided into seven groups of 100 g each. The groups of seeds were placed on a tray (20 cm in diameter and 3.5 cm high) and arranged so that they did not overlap with each other. Then, the seeds were soaked in 500 mL of warm water (50°C) containing $NaHCO_3$ (1% w/w), at a seed: water = 1:5 w/v. The soaking process was carried out at room temperature for 24 hrs, which was the maximum absorption time (water soaking). The water was replaced every 6 hrs, using plain water, and the seeds were removed from the tray to be rinsed and drained before germination.

2.2 Germination

The seeds were germinated at room temperature and at a relative humidity of ~100% by placing the seeds in a 25 × 30 × 2 cm incubation tray with holes. The seeds were arranged on the tray so that they did not overlap each other and were covered with a wet napkin. The napkin was kept damp during the incubation and care was taken not to drip water from the damp napkin. The relative humidity in the room was maintained at ~100% under these conditions. The sprouts were harvested according to the treatment time.

2.3 Processing of jack bean sprout flour

The sprouts were sliced, frozen, freeze-dried (−60°C; 20 hrs), and ground to pass through a 60-mesh sieve. The components of the jack bean sprout flour were determined, including proximate and anti-nutritional contents, soluble protein, dietary fibre, and amino acid concentration, as well as HCN, phytic acid, trypsin inhibitor, and tannin contents.

2.4 Analysis of nutritional components

The ash, fat, and protein content (Kjeldahl) of jack bean sprout flour were tested according to Association of Official Analytical Chemists (AOAC) (2019) standard procedures. Soluble protein content was examined according to the Lowry method, and dietary fibre was determined according to the multiple enzyme method (McCleary *et al*., 2012). All jack bean sprout flour analyses were carried out in triplicate.

2.5 Analysis of the amino acid profile

2.5.1 Sample preparation

The samples (0.2 g) were hydrolyzed with 10 mL of 6 N HCl for 12 hrs at 110°C. Briefly, 50 mL of 6 M NaOH was used to neutralize the solution. The mixture was filtered (0.22 µm), diluted ten-fold with H_2O, and 5 µL was injected into the liquid chromatography/tandem mass spectrometry system (LC/MS-MS).

2.5.2 Preparation of the mobile phase

To prepare mobile phase A, 0.1% pentadecafluorooctanoic acid (PDFOA) was mixed with water and CH_3CN (99.5% PDFOA: 0.5% water/CH_3CN with formic acid (0.1%). Mobile phase B was prepared as 10% 0.1% PDFOA and 90% water/CH_3CN with 0.1% formic acid.

2.5.3 LC/MS-MS conditioning

The amino acid profile was analyzed using an LC/MS-MS system equipped with a Waters Xevo Tandem Quadrupole Detector and a C18 column. The Xevo TQD features T-wave ™ collision cell technology to provide fast MRM and assessment of acquisition information known as RADAR ™. The mass analyzer specifications were quadrupole analyzers (MS1/MS2) with two high-resolution high stability plus pre-filters. The detector (long-life photomultiplier) specifications were digital dynamic (range 4×10^6), low noise, and off-axis. The running specifications were capillary voltage of 3.5 kV, desolvation rate of 1,000 L/h at a temperature of 500°C, and collision energy of 15 V. The amino acids were separated using a gradient system at a flow rate of 0.6 mL/mins. Table 1 shows the gradient elution scheme.

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Table 1. Gradient elution scheme used to determine the amino acid profile.

Time (mins)	Solvent A (%)	Solvent B (%)
Initial	90.00	10.00
4.00	50.00	50.00
4.10	95.00	5.00
6.00	95.00	5.00

2.6 Analysis of anti-nutritional components

2.6.1 HCN content

HCN content was measured using the method described by Nwokoro *et al*. (2010) with slight modifications. The samples were prepared by adding distilled water to jack bean sprouts, followed by 4 hrs incubation. The filtrate (1 mL) was diluted with 2% KOH (1 mL) and alkaline picrate (5 mL). The solution was incubated for 15 s in boiling water (100°C). The resulting solution was analyzed with a UV-VIS spectrophotometer at 510 nm, with KCN as the standard.

2.6.2 Phytic acid content

Phytic acid content was analyzed using the method described by Fitriani *et al*. (2021). The sample solution was prepared by adding 0.1 g of the sample to 20 mL 0.5 M HNO_3. The mixture was placed in a shaker water bath and shaken for 4 hrs at 28–30°C. The extract was filtered through Whatman filter paper No. 1. The extract (1 mL) was mixed with distilled water (0.4 mL), 1 mL of 0.005 M $FeCl_3$ was added, and the solution was dropped in boiling water (100°C) for 20 mins. After the solution cooled, amyl alcohol (5 mL) and ammonium thiocyanate (0.1 mL, 0.1 M) was added and the mixture was centrifuged at 3,000 rpm. Absorbance was measured with a UV-VIS spectrophotometer at 495 nm. A phytic acid standard curve was prepared by mixing Na-Phytate solutions (50, 100, 150, and 200 ppm) with HNO_3. The phytic acid content is shown as % of mg/g dry matter.

2.6.3 Trypsin inhibitor

Trypsin inhibitor (T.I.) activity was determined based on Malomo *et al*. (2014) and Coscueta *et al*. (2017). The determination was started by providing a T.I. extract, substrate (BAPNA solution), and a trypsin solution. The T.I. extract was prepared by mixing jack bean sprout flour (0.5 g) and NaOH (25 mL, 0.01 M), followed by a 3-h room temperature incubation. The extract was centrifuged at 3,500 rpm for 10 mins, and the activity of the filtrate was examined. The BAPNA stock solution was prepared by dissolving BAPNA (40 mg) in 1 mL dimethyl sulfoxide (DMSO), followed by a dilution in Tris-buffer (100 mL, 0.05 M) pH 8.2. The stock was stored at 37°C and always provided fresh before assay. Trypsin (2 mg) was dissolved in HCl (100 mL, 0.001 M) to prepare a trypsin solution. This stock was stored at 4°C and provided fresh before assay. The control solution consisted of BAPNA, distilled water, and the trypsin solution, and the sample solution consisted of BAPNA, the sample, and the trypsin solution. The control solution was prepared by mixing distilled water (2 mL) and BAPNA (5 mL) with the trypsin solution (2 mL), which were incubated in a shaker water bath for 10 mins (37°C). The trypsin reaction was stopped by adding acetic acid (1 mL, 30%) followed by centrifugation for 10 mins (3,500 rpm). UV-Vis spectrophotometry at 410 nm was used to determine the absorbance values. The sample solution was the same as the control solution, as BAPNA and trypsin were mixed into the sample extract (2 mL). The blank was prepared by adding acetic acid (1 mL, 30%), trypsin solution (2 mL), and BAPNA (5 mL). Trypsin inhibition was expressed as TUI/mg sample and calculated using the following formula:

$$(\text{TUI/mg sample}) = \frac{(C - S) \times (100/df)}{t/df}$$

Where C is the absorbance of the control solution, *S* is the absorbance of the sample solution, *df* is the dilution factor (mL), and *t* is the weight of the sample.

2.6.4 Tannin content

Tannin content was analyzed using the method of Chanwitheesuk *et al*. (2005). Jack bean sprout flour (0.3125 g) was diluted with distilled water (62.5 mL) and boiled (100°C) for 2 hrs. The cooled extract was filtered through Whatman filter paper No. 1. One mL of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and Na_2CO_3 (2 mL, 20%). The solution was incubated for 30 mins at room temperature, and absorbance was read by UV-Vis spectrophotometry at 748 nm. Tannic acid (0.00, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) standard was used to prepare a standard curve in the same manner as the samples.

2.7 Sprout morphology

Morphological changes during germination were monitored by photographing the bean sprout samples after different germination times.

2.8 Statistical analysis

Data on the nutritional and anti-nutritional components were evaluated by analysis of variance. If the germination time treatment effect was significant, Duncan's multiple range test was used to detect the differences. A p-value < 0.05 was considered significant.

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3. Results and discussion

3.1 Jack bean sprouts morphology

Germinated seeds, grains, and beans develop into sprouts, which are grown in water or other media and harvested before the true leaves develop. All parts of the sprout, including the seeds and radicles, are consumed (AACC, 2008). The maximum germination time in this study was determined to be 72 hrs because the radicle grew fine roots after that time and the seedlings did not qualify as sprouts. Most sprouting studies observed seed development during the first germination phase (Benincasa *et al*., 2019). In this study, the germination process began with soaking the jack bean seeds in 1% $NaHCO_3$.

The morphology of the jack bean sprouts during germination is shown in Figure 1. The first radicle began to appear after 20 h rsof germination with a length of a few mm. The first seed picture was taken after 24 hrs of germination when the sprouts were about 1 cm long. Benincasa *et al*. (2019) reported that the first visible sign of germination is the emersion of the radicle, which is generated by the lengthening of cells. The radicle can appear in a few hours or a few days under favourable conditions. The appearance of the radicle indicates the end of the first phase (activation stage) of germination. Benincasa *et al*. (2019) showed that the second phase of germination involves metabolic reactivation, while the third phase is associated with the prolongation of cells to complete germination. The length of the radicle increased from 3 to 12 cm from 36 to 96 hrs of germination. Prolonging the germination time increased root length. The sprouts grew fine roots after 84 and 96 hrs of germination. The seeds looked withered, the skin was peeling, and the grain split ready to grow new leaves. Similar to mung bean, soybean, and black bean sprouts, Xu *et al*. (2016) reported that the length of jack bean radicles increases during germination but the radicle was shorter after 6 days of germination.

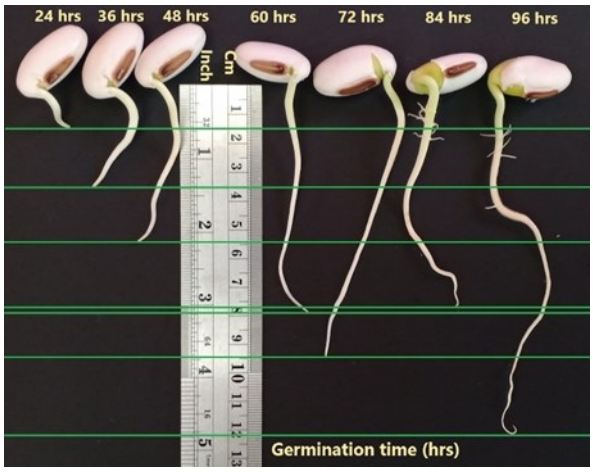

Figure 1. The lengths of the jack bean sprouts at various germination times.

3.2 Nutritional components in jack bean sprout flour

Table 2 shows the ash content of jack bean sprout flour at different germination times. The highest ash content occurred at 0 hr of germination, while the lowest content occurred at 72 hrs of germination. No significant difference ($p < 0.05$) in ash content was detected at any of the germination times. However, a slight decrease of 10.22% was observed at 72 hrs of germination, which was probably due to the leaching of micro and macro elements, leading to reduced ash content (Kassegn *et al*., 2018). This result is similar to Sattar *et al*. (2017), who reported a downward trend (8–20%) in ash content in three different legume cultivars after germination. Table 2 shows that there was no significant difference in fat content ($p < 0.05$). Germination resulted in a slight reduction in fat content (6.25%), which was probably caused by lipase activity that produces free fatty acids during germination (Benincasa *et al*., 2019). Jack bean sprouts germinated for 24 hrs had the highest fat content (3.88% db), while those at the 60 and 72 hrs germination times had the lowest average value (3.60%). The increase in fat content after 24 hrs was not significantly different from the other treatments.

Germination time did not affect protein content ($p < 0.05$). Table 2 shows a decrease in protein content of about 2.5% in the samples over time due to an increase in the protease levels during germination (Masood *et al*., 2014). The average protein content of jack bean sprouts after 72 hrs of germination was 33%. The decrease in protein content was probably due to the immense water absorption that occurs during germination, leading to the

Table 2. Nutritional components of jack bean sprout flour during germination.

Components	0 hr	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs
Ash (%db)	3.13±0.38	3.11±0.35	2.83±0.13	2.92±0.21	2.85±0.21	2.85±0.23	2.81±0.10
Fat (%db)	3.84±0.23	3.63±0.10	3.88±0.19	3.57±0.08	3.78±0.20	3.60±0.07	3.60±0.09
Protein (%db)	33.97±0.09	34.34±0.05	33.38±0.06	33.37±0.05	33.72±0.05	33.60±0.07	33.12±0.05
Soluble protein (%db)	$5.09±0.45^{b}$	$5.06±0.22^{b}$	$4.99±0.13^{b}$	$5.23±0.17^{ab}$	$5.54±0.36^{ab}$	$5.85±0.53^{a}$	$5.34±0.40^{ab}$
Dietary fibre (%db)	$23.37±0.27^{a}$	$21.49±0.16^{b}$	$19.43±0.05^{d}$	$19.86±0.11^{c}$	$19.92±0.14^{c}$	$18.60±0.44^{e}$	$18.46±0.09^{e}$

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). db: dry basis.

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dilution of the protein (Ramli *et al*., 2021). However, Kassegn *et al*. (2018) and Shreeja *et al*. (2021) reported that protein is produced throughout germination. The protein synthesis that occurs during water uptake before germination leads to enhanced protein content in sprouts (Kirmizi and Guleryuz, 2006). Kassegn *et al.* (2018) showed that the increase may be due to losses of other components and enzymatic synthesis.

Germination significantly increased the protein solubility of the jack bean sprout flour ($p < 0.05$) (Table 2) by 2.75–14.93%. Samples at 0 hr of germination (control) had a protein solubility value of 5.09%. However, protein solubility decreased during the early phase of germination (first 24 hrs), and the 24-h sample contained the lowest soluble protein content (4.99%). Subsequently, a significant increase in soluble protein content was observed until 60 h which decreased at 72 hrs. The decrease during the early germination phase (until 24 hrs) was probably due to adaptation. Afify *et al*. (2012) reported that this decline in protein solubility was likely due to the leaching of water-soluble peptides. Furthermore, Benincasa *et al*. (2019) discovered an increase in the respiration rate during the early phase of germination, which required energy and decreased soluble protein content. The significant increase in soluble protein content that occurred from 36 to 72 hrs of germination could be due to the degradation and hydrolysis of macromolecules, such as proteins and free amino acids, which enhance protein solubility (Singh *et al*., 2017). The increase in soluble protein content was similar to that reported by Sharma *et al*. (2019). The protein solubility of pigeon pea also increases after 48 hrs of germination. Novel food products can be developed based on jack bean sprout flour protein when higher protein solubility is required.

The dietary fibre content in jack bean sprout flour decreased significantly throughout germination (Table 2). The lower dietary fibre content was probably due to the degradation of complex molecules during germination so some molecules became soluble components, resulting in lower fibre content. The 60 and 72 hrs germinated samples contained the lowest dietary fibre content. The germination time, genotype, and fibre fraction affect the dietary fibre content of sprouts (Nelson *et al*., 2013). However, Benitez *et al*. (2013), Megat *et al*. (2016), and Shreeja *et al*. (2021) reported an increase in dietary fibre throughout germination. The breakdown of the starch may be attributed to enhanced fibre content (Shreeja *et al*., 2021).

Liquid chromatography-mass spectrometry was used to investigate the amino acids. Table 3 shows that the

Table 3. Amino acid concentrations of jack bean sprout flour during germination.

Amino acid	Amino acid concentration (g/100 g protein D.W.)						
	0 hr	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs
Essential amino acid							
L-Arginine	3.93±0.08^{c}	4.16±0.05^{b}	3.14±0.02^{d}	4.02±0.07^{bc}	4.01±0.06^{c}	5.26±0.08^{a}	5.39±0.02^{a}
L-Threonine	0.12±0.00^{c}	0.12±0.00^{c}	0.12±0.00^{c}	0.15±0.00^{b}	0.17±0.01^{a}	0.14±0.01^{b}	0.15±0.00^{b}
L-Methionine	0.08±0.00^{a}	0.03±0.00^{b}	0.08±0.01^{a}	0.09±0.01^{a}	0.03±0.00^{b}	0.08±0.01^{a}	0.04±0.00^{b}
L-Phenylalanine	2.99±0.03^{c}	2.97±0.07^{c}	3.31±0.07^{bc}	3.94±0.09^{ab}	3.87±0.76^{ab}	4.09±0.00^{a}	4.33±0.1^{a}
L-Isoleucine	2.66±0.03^{e}	2.66±0.06^{de}	2.79±0.03^{d}	3.31±0.04^{c}	3.74±0.02^{a}	3.47±0.04^{b}	3.62±0.11^{a}
L-Leucine	4.44±0.03^{b}	4.51±0.09^{b}	4.75±0.01^{b}	5.53±0.12^{a}	5.66±0.02^{a}	5.69±0.03^{a}	4.52±0.59^{b}
L-Valine	0.98±0.03^{d}	0.99±0.03^{d}	1.03±0.04^{d}	1.25±0.01^{b}	1.09±0.01^{c}	1.28±0.02^{b}	1.37±0.02^{a}
L-Thryptophan	0.01±0.00^{b}	0.01±0.00^{ab}	0.02±0.00^{ab}	0.02±0.00^{a}	0.01±0.00^{ab}	0.01±0.00^{ab}	0.02±0.00^{a}
L-Histidine	2.61±0.01^{c}	2.77±0.09^{c}	2.12±0.01^{d}	2.77±0.13^{c}	2.83±0.04^{c}	3.87±0.15^{b}	4.54±0.10^{a}
Total	17.82	18.22	17.36	21.08	21.42	23.89	23.98
Non-essential amino acid							
L-Aspartic acid	0.14±0.01^{c}	0.15±0.01^{c}	0.17±0.00^{c}	0.21±0.01^{b}	0.26±0.02^{a}	0.22±0.01^{b}	0.21±0.01^{b}
L-Glutamic acid	0.19±0.00^{d}	0.19±0.00^{d}	0.21±0.02^{d}	0.26±0.01^{bc}	0.30±0.01^{a}	0.27±0.01^{b}	0.24±0.00^{c}
L-Tyrosine	0.20±0.01^{d}	0.21±0.00^{d}	0.24±0.01^{c}	0.31±0.01^{a}	0.27±0.00^{b}	0.29±0.00^{a}	0.29±0.01^{a}
L-Glycine	0.04±0.01^{ab}	0.03±0.00^{b}	0.04±0.00^{ab}	0.04±0.01^{ab}	0.06±0.02^{a}	0.05±0.01^{ab}	0.05±0.01^{ab}
L-Alanine	0.07±0.00^{ab}	0.07±0.00^{b}	0.06±0.00^{b}	0.07±0.00^{a}	0.06±0.00^{b}	0.07±0.00^{ab}	0.07±0.00^{a}
L-Serine	0.06±0.00^{d}	0.07±0.00^{cd}	0.08±0.01^{bc}	0.09±0.01^{bc}	0.12±0.02^{a}	0.10±0.00^{b}	0.09±0.00^{bc}
L-Proline	0.001±0.00^{b}	0.001±0.00^{b}	0.003±0.00^{b}	0.013±0.00^{a}	ND	0.002±0.00^{b}	0.009±0.1^{ab}
Total	0.70	0.72	0.80	0.99	1.07	1.00	0.96
Total (Essential and Non-essential amino acid)							
	18.52	18.94	18.16	22.07	22.49	24.89	24.94

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). ND: not detected.

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amino acid concentrations (both essential and nonessential amino acids) increased significantly (2.27–34.67%, $p < 0.05$) after germination. All samples were rich in essential amino acids, such as arginine, phenylalanine, leucine, isoleucine, valine, and histidine. The highest essential amino acid concentration was found at 72 hrs, and the lowest occurred at 0 hr of germination. Afify *et al*. (2012) found that sprouting increases the levels of essential amino acids, such as leucine, valine, threonine, lysine, isoleucine, and phenylalanine, in sorghum. The protein quality of bean products improves and better digestibility occurs after germination due to the increase in amino acid contents (Nelson *et al*., 2013). A substantial change in whole grain biochemical composition occurs during germination, including changes in amino acid composition that favour the growth of the shoots (Benincasa *et al*., 2019; Ikram *et al*., 2021).

Hydrophobic amino acids, such as phenylalanine, valine, isoleucine, and leucine, were detected in substantial quantities after 72 hrs of germination in jack beans. These four hydrophobic amino acids increased 25.02% and to their highest level at 72 hrs of germination. The 60 and 72-hrs phenylalanine concentrations were not significantly different, while the 72 hrs sample contained slightly more isoleucine and valine than that at 60 hrs. In contrast, the 60-h leucine concentration was higher than that at 72 hrs . Bioactive peptides, which are inhibitors of angiotensin-converting enzyme, dipeptidyl peptidase-IV, α-amylase, and α-glucosidase, can be synthesized from these hydrophobic amino acids (Gonzalez-Montoya *et al*., 2018; Puspitojati, Cahyanto, Marsono *et al*., 2019). This result was similar to that of Kanetro (2018). Specific amino acids increase during 36 hrs of soybean sprout germination, including phenylalanine, leucine, lysine, and alanine. These specific amino acids are known to stimulate insulin secretion.

3.3 Anti-nutritional components of jack bean sprout flour

The HCN content of jack bean sprout flour decreased significantly ($p < 0.05$) by 72 hrs of germination (30.83 to 8.95 ppm), which was a 70.97% decrease (Table 4). This level is safe for consumption, and the WHO suggested that the limit of cyanide for consumption is 10 ppm. Soluble cyanide could leach into the water during soaking. According to Ramli *et al*. (2021), soaking jack beans in 1% $NaHCO_3$ for 36 hrs after fermentation reduced the HCN level to 7.43 ppm. Leonir-da-Silva *et al*. (2019) reported that hydrolysis of β-glucosidase during germination might be attributed to the decline in HCN content. Hydroxynitrile lyase hydrolyzes cyanogenic glucoside (linamarin) to free cyanide and cyanohydrin. This form of free cyanide is lost significantly through leaching and is absorbed by other developing parts, such as shoots, roots, and leaves as the plant grows.

The phytic acid content in jack bean sprout flour during germination is shown in Table 4. Phytic acid content decreased gradually during germination. The 72-hrs germinated sample had the lowest phytic acid content of 0.2 mg/g dry matter. The highest phytic acid content occurred at 0 hr of germination (0.57 mg/g dry matter), which was a decrease of 64.91%. These data correspond to Mehanni *et al*. (2021), who discovered that phytic acid content in faba beans was 63.4% after 72 hrs of germination. Mihafu *et al*. (2017) reported a similar reduction of 34.1% in maize grains after 72 hrs of germination. The reduction of phytic acid in legumes indicates that the hydrolysis of phytates during germination leads to increased inorganic phosphates for plant growth (Mihafu *et al*., 2017). One of the factors responsible for reducing mineral bioavailability in beans is phytic acid as a mineral or metal-chelating agent. The nutritional quality of beans may increase with reduced phytic acid content.

Table 4 shows the T.I. activity of jack bean sprout flour. Only slightly reduced T.I. activity was detected ($p > 0.05$) in jack bean sprout flour during germination. The highest T.I. activity in jack bean sprout flour was observed at 0 hr of germination, while the lowest was observed at 60 hrs of germination. This reduction was 5.25%–41.29%. The decline in T.I. activity throughout germination was probably due to leaching at the onset of germination. This result is similar to that reported by Malomo *et al*. (2014) who discovered a decrease in T.I.

Table 4. Anti-nutritional components of jack bean sprout flour during germination.

Components	0 hr	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs
HCN (ppm)	30.83±0.24^{a}	26.65±0.61^{b}	23.70±0.77^{c}	21.86±0.39^{d}	16.85±0.44^{e}	9.62±0.62^{f}	8.95±0.34^{f}
Phytic acid (mg/g dry matter)	0.57±0.01^{a}	0.44±0.01^{b}	0.35±0.02^{c}	0.29±0.02^{d}	0.29±0.01^{d}	0.21±0.00^{e}	0.20±0.00^{e}
Trypsin inhibitor (TUI mg dry matter)	0.035±0.01	0.033±0.01	0.029±0.01	0.027±0.01	0.028±0.01	0.016±0.01	0.021±0.01
Tannin (mg/g dry matter)	1.84±0.16	1.86±0.00	1.84±0.09	1.81±0.16	1.78±0.07	1.76±0.19	1.75±0.16

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). ppm: parts per million, TUI: trypsin unit inhibited.

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activity in cowpea during 5 days of germination. Germination also reduced the tannin level (Table 4) from 1.84 mg/g dry matter (0 hr germination) to 1.75 mg/g dry matter (72 hrs germination), which was a 4.89% decrease. This pattern of decline is similar to that reported by other studies and is possibly due to enzymatic hydrolysis, such as polyphenol oxidase or other catabolic enzymes (Mihafu *et al*., 2017; Mehanni *et al*., 2021).

3.4 The optimum jack bean sprout germination time

The optimum jack bean sprouts germination time was determined using the effectivity index (DeGarmo *et al*., 1984). The 60 hrs germination time was selected based on its nutritional and anti-nutritional components. These sprouts exhibited increased protein solubility and amino acid contents by 2.75–14.93% and 2.27–34.67%, respectively. The results of the six jack bean sprout replicate at 60 hrs of germination are shown in Figure 2. The 60 hrs sprouts were 7–9 cm long, which agreed with Xu *et al*. (2016), who discovered that the length of the radicles from 60 hrs sprouts of mung bean, soybean, and black bean was 6–7 cm. Germination is a simple, cheap, and easily adaptable technology, so 60 hrs germinated jack bean can be easily applied on a household scale.

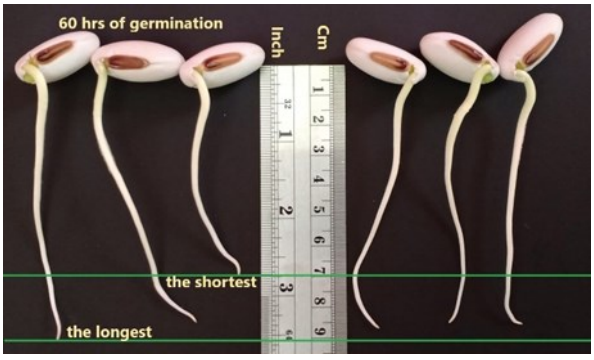

Figure 2. The appearance of the six replicates of jack bean sprouts at 60 hrs of germination.

4. Conclusion

The results of this study indicate the significant effects of germination on the nutritional and anti-nutritional components in jack bean sprout flour. Germination increased the nutritional contents, such as soluble protein and amino acid concentrations. It also reduced the anti-nutritional components. The 60-hour germinated jack bean had a maximum amino acid concentration of 24.89 g/100 g protein, and the maximum soluble protein content was 5.85%db; HCN levels were within safe limits (<10 ppm) for consumption. The 60-hour germinated jack beans contained bioactive compounds, such as hydrophobic amino acids. Therefore, 60 hrs was the optimum germination time for jack beans.

Conflict of interest

The authors declare no conflicts of interest.

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