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Bioactive compounds and antioxidant activity of germinated soybean flour (*Glycine max* L.) at different germination time

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ABSTRACT

Soybean (Glycine max L.) is a rich source of bioactive compounds, and germination is an effective process for improving its nutritional and functional properties. However, the combined effects of germination duration on sprout growth, bioactive compounds, and antioxidant activity in germinated soybean flour remain poorly understood. Therefore, this study aimed to evaluate the effect of different germination times on sprout growth, bioactive compounds, antioxidant activity, and proximate composition of germinated soybean flour. Soybeans were germinated for 0–7 days and analyzed for sprout growth, γ-aminobutyric acid (GABA), total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH), and proximate composition. Germination significantly affected all evaluated parameters ($p < 0.05$). Sprout length increased continuously throughout germination, whereas GABA content reached its maximum on day 3 (416.43 ± 11.69 mg/100 g). The highest TPC (91.88 ± 1.20 mg GAE/100 g) and antioxidant activity (IC_{50} = 10.57 ± 0.01 µg/mL) were obtained on day 5, while the highest TFC (17.30 ± 0.14 mg QE/100 g) was observed on day 4. Germination also increased protein, moisture, and ash contents while reducing fat content. These findings demonstrate that optimizing germination time effectively enhances the nutritional and functional quality of soybean flour, with 3–5 days representing the optimal germination period depending on the targeted bioactive compounds.

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INTRODUCTION

Soybean (*Glycine max* L.) is one of the main food commodities in Indonesia with an estimated amount of use per year 2023 is 2,666,000 tons (Mas'ud et al. 2016). Besides being rich in protein, soybeans also contain several other compounds such as vitamins A, E and K, as well as minerals such as K, Fe, Zn and P. Soybeans also contain about 15% saturated fatty acids and 60% unsaturated fatty acids (Aminah 2020). In her research, Arinanti (2018) suggested that soybeans have high antioxidant compound potential compared to mung beans, green beans, capri beans, cowpeas and kidney beans.

One of the product modifications that can be made using soybeans is soybean flour.. In addition to extending the shelf life of soybeans, this modification was chosen because it has several advantages including a higher protein content of as much as 50% compared to the fresh product and eliminating the unpleasant taste of soybeans (Astawan et al. 2016). Germination is a simple technology that has been widely used to improve nutrition, enrich flavor and reduce antinutritional compounds (Chaudhary et al. 2015). The germination process can improve the quality of soybeans by converting macro molecular compounds into simpler molecules. The germination process can activate enzymes that provide changes to bioactive components in brown rice such as hydrolytic enzymes, phenolics and other essential amino acids such as lysine and niacin and increase the production of the amino acid gamma-aminobutyric acid (GABA) (Munarko et al. 2019).

Bioactive compounds are active compounds in functional foods that are responsible for metabolic reactions and have biological effects on living organisms. One of the of bioactive compounds is antioxidants (Wahjuningsih 2023). Bioactive compounds such as polyphenols and flavonoids have potential as antioxidants that can protect body cells from oxidative damage caused by free radicals (Rasouli et al. 2017)

Therefore, this study aimed to evaluate the effect of different germination times on sprout growth and the simultaneous changes in GABA content, total phenolic content, and antioxidant activity in germinated soybean flour.

METHODS

Materials

The soybean *(Glycine max. L)* of anjasmoro were procured from Bandung, West Jawa, Indonesia. The chemical reagents for analysis were borate buffer pH 9.0, phenol 6%, Sodium hypochlorite (NaOCl) 9%, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, Sodium Carbonate (Na_2CO_3) , folin ciocalteu reagent, gallic acid, potassium acetate, quercatin, ethanol 96% and aluminum chloride. All of the reagen used were

Germination Process and Production of Soybean Flour

The process of making germinated soybean flour refers to Astawan et al. (2016) which was modified. This included the sorting of soybeans, where 100 g of soybeans were selected with whole seeds and foreign objects were removed. Washing was done using running distilled water to remove impurities attached to the soybeans, then the soybeans were soaked in clean water for 12 hours at room temperature using a closed container. The soybeans were placed in seedling containers, and the germination process was carried out for 0, 1, 2, 3, 4, 5, 6, and 7 days. During germination, the soybeans were sprayed with water every 12 hours to maintain moisture and ensure the seeds remained in a humid condition throughout the process. Drying of the germinated soybeans was done using a dryer at a temperature of 50°C for 16 hours. Grinding was performed using a crusher in the form of a miller with a rotational speed of 10,000 RPM for 1 minute, and sieving was carried out using a 60 mesh sieve.

Determination of Soybean Sprout Length and Growth Percentage

The length of the soybean sprouts was measured from the cotyledon through the stem to the root using a ruler, and the sprout length data were recorded for each germination period based on (Munarko et al., 2021). The sprout growth percentage was then determined using (Xue et al. 2016) method by comparing the initial sprout length at day 0 with the sprout length at each subsequent treatment time, multiplied by 100%, as expressed in the Equation (1).

$$Sprout\ Growth\ (\%) = \frac{Sprout\ length\ at\ day\ n}{Sprout\ length\ at\ day\ 0} \times 100\% \quad (1)$$

Determination of Gamma Amino Butyric Acid (GABA)

Analysis of GABA was performed according to the method of (Zhang et al., 2014), A sample of 1 gram of germinated soybean flour in 10 mL of distilled water was extracted using vortex for 10 minutes and then centrifuged for 15 minutes at 4000 rpm. A total of 0.50 mL of extract was put in a centrifuge bottle, added 0.20 mL of borate buffer pH 9.0 and 1 mL of 6% phenol. The sample was chilled for 5 minutes and 0.4 mL of 9% NaOCl was added. The solution was again vortexed and boiled using a waterbath for 10 minutes at 95°C. The sample was cooled for 10 minutes. Samples were analyzed using GENESYS 150 UV-Vis Spectrophotometers THERMO SCIENTIFIC at a wavelength of 645 nm. Standar curve calibration of GABA was prepared to determine GABA concentration in the samples and expressed as mg GABA/100g dried samples.

Determination of Total Phenolic Content

The amount of total phenolic content (TPC) was analysed based on a Folin-Ciocalteu method (Salim et al. 2021). A total of 10 mg of germinated soybean flour in 10 mL of 96% ethanol was homogenized for 10 minutes then extracted using 4000 rpm centrifuged. After that, Germinated soybean flour extract solution of 0.3 mL was added with 1.5 mL of Folin Ciocalteu solution and 1.2 mL of 7% Na_2CO_3 and then incubated for 90 minutes at room temperature. The absorbance of the germinated soybean flour extract solution was measured using a UV-Vis spectrophotometer at a wavelength of 769 nm. The absorbance was compared to the standard curve of gallic acid and expressed as mg galic acid equivalent per 100 g (mg GAE/100g).

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determinated based on colorimetric method (Fawwaz et al.,2017). A total of 10 mg of germinated soybean flour in 10 mL of 96% ethanol was homogenized for 10 minutes then extracted using 4000 rpm centrifuged. A total of 1.5 mL of sample was added to 1.5 mL of 96% ethanol, 0.1 mL of 10% $AlCl_3$ solution and 0.1 mL of 1M potassium acetate then added to 5 mL of distilled water. Incubated for 30 minutes at room temperature. The sample was measured using a UV-Vis spectrophotometer at a wavelength of 444 nm. The total flavonoid content was determined using a standard curve of quercetin and expressed as mg of quercetin per 100 g (mg QE/100g).

Antioxidant Capacity

The antioxidant capacity of germinated soybean flour were evaluated by DPPH radical scavanging capacity according to the method of Lestari et al. (2021). A total of 1 gram of sample was dissolved with 10 mL methanol. The extract of germinated soybean flour 1 mL added with 1mL 0.4 mM DPPH solution. The sample was incubated in the dark for 1 hour. The absorbance was measured at 515 nm and calculate to IC_{50}.

Proximate composition

Proximate compositions (ash, moisture, fat, protein content) of the germinated soybean flour were determinated using standard AOAC methods (AOAC 2005). Moisture content was determined by drying 105ºC to a constant weight in an oven. Lipid was determined by soxhlet extraction method. Ash was determined using a gravimetrical method using furnace for 24h at 550 ºC. Protein was determined by Kjeldahl method. Carbohydrate content was determined by difference (Equation 2).

$$\text{Carbohydrate} = 100\% - (\%\,ash + \%\,fat + \%protein + \%Moisture\ content) \quad (2)$$

RESULTS AND DISCUSSION

Soybean Sprout Length and Growth Percentage

Table 1 and Figure 2 presents the sprout length and sprout growth percentage of soybean during 0 to 7 days of germination. Figure 1 show the picture of germination. The results show that both sprout length and sprout growth percentage increased with increasing germination time. The increase in is attributed to the carbohydrate, protein, and fat content, which serve as an energy source for the morphological process involved in the emergence of plant organs such as roots, leaves, and stems (Ferdiawan et al. 2019). The 12-hour soaking process prior to germination triggers the breakdown of nutrients, leading to the formation or activation of enzymes that enhance metabolic activity. The presence of water within the cells activates several enzymes at the onset of germination. Soaking promotes radicle formation,

as the enzymes required for this process have already been produced (Shofi 2017).

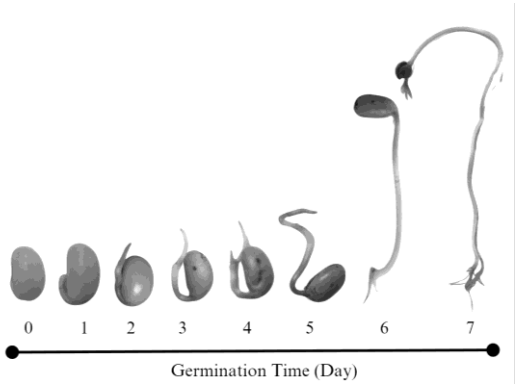
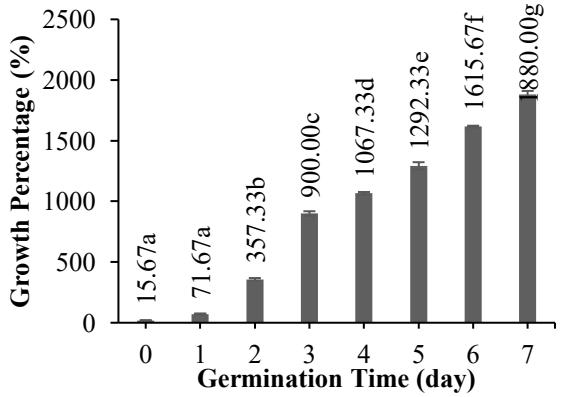


Figure 1 Growth of Soybean Sprout Length During Germination

Table 1 Sprout Length of Soybean Sprouts During Germination Time

Germination Time (Day)	Sprout Length (cm)
0	0.82±0.07[a]
1	1.38±0.05[b]
2	4.23±0.09[c]
3	9.66±0.20[d]
4	11.33±0.11[e]
5	13.58±0.30[f]
6	16.82±0.07[g]
7	19.46±0.29[h]

Note : Different letters indicate values are significantly different (p<0.05)



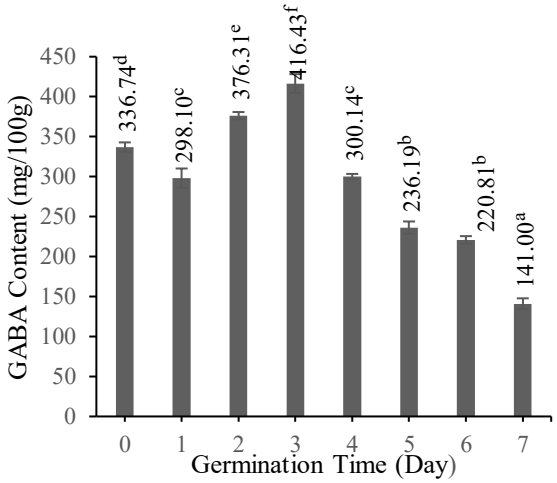
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Figure 2 Percentage Growth of Soybean Sprouts During Germination Time

Gamma Amino Butyric Acid (GABA) Content

Figure 3. shows that germination time has a significant effect (p<0.05) on the GABA content of germinated soybean flour. The increase in GABA content of germinated soybean flour starts from day 0 and is highest on day 3, then decreases on day 4 to day 7. The highest level of GABA content was significantly (p<0.05) found on day 3 germination of 416.43 ± 11.69 mg/100g, this result increased by 1.27 times compared to day 0 germination. The previous research by Wu et al (2020) also reported the GABA content in non-germinated soybeans (262.42 ± 3.66 mg/100g). They also showed that the germination process in soybeans was able to increase the GABA content.

The decrease in GABA content after prolonged germination observed in the present study is consistent with previous reports. Luo et al. (2018) demonstrated that GABA concentration in soybean reached a maximum on the fifth day of germination and subsequently declined due to increased GABA degradation mediated by GABA transaminase (GABA-T). Likewise, Xu and Hu (2014) reported that prolonged germination shifted GABA metabolism from accumulation toward catabolism through the GABA shunt, supplying carbon intermediates for seedling growth.



Note : Different letters indicate values are significantly different (p<0.05)

Figure 3 GABA Content of Germinated Soybean Flour.

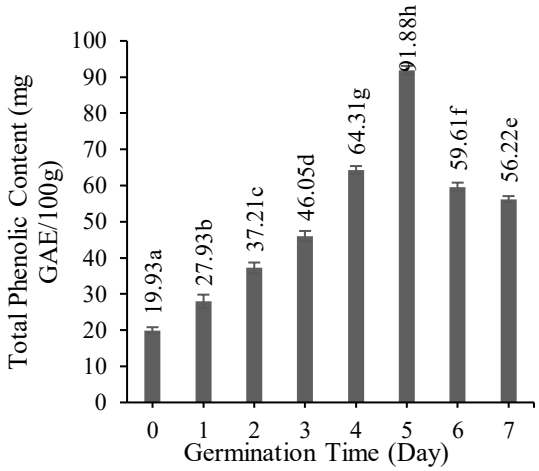
The synthesis of GABA compounds that occurs on day 0 is caused by water absorption which activates the germination process including the glutamate decarboxylase enzyme which converts glutamic acid into GABA (Munarko et al. 2019). The activity of glutamate decarboxylase enzyme that increases as the germination time progresses causes an increase in GABA production. Glutamic acid decarboxylase (GAD)

catalyzes the decarboxylation of glutamic acid into GABA and CO_2 through the GABA shunt pathway at an optimal temperature of 20-40 ° C (Yang et al., 2023). The decrease in GABA content at the day 4 to day 7 germination time is thought to be related to a decrease in GAD activity and an increase in GABA Transaminase (GABA-T) activity, which causes the rate of GABA production to be lower than the rate of conversion of GABA to succinate (Munarko et al. 2022).

Total Phenolic Content

The result in Figure 4 shows that the length of time of germination has a significant effect ($p<0.05$) on the polyphenol content of germinated soybean flour. The polyphenol content of germinated soybean flour increased at the time of germination from day 0 to day 5 and decreased at day 6 to day 7. The highest polyphenol content was significantly ($p<0.05$) at a germination time of 5 days at 91.88 ± 1.20 mg GAE/100g. This result shows an increase of 4.61 times compared to day 0 germination time. Research conducted by Josipovic et al (2016) found that polyphenols in non-germinated soybeans were 31.64 ± 0.14 mgGAE/100g. The results showed that the germination process was able to increase the polyphenol content in germinated soybean.

The increase of total phenolic content during the early stage of germination may be attributed to the hydrolysis of bound phenolics into free forms. This is consistent with findings in germinated black soybean, where bound polyphenol, flavonoid, phenolic acid, and isoflavone contents decreased while their free counterparts increased with germination time, indicating a conversion from bound to free forms through catabolic activity (Kim et al., 2016). In addition there is possibility day 0 germination time it caused by the increased activity of enzymes involved in biosynthetic processes in the early stages of germination such as phenylalanine ammonia-liase (PAL) which has a role in polyphenols, especially in the production of flavonoid and lignin compounds. PAL converts the amino acid phenylalanine into trans-sinnamic acid which is the main precursor in the polyphenol biosynthesis pathway by releasing ammonia. Trans-cinnamic acid then enters the phenylpropanoid pathway and is converted into various phenolic compounds (Xu, et al., 2020). The decrease in polyphenols that occurs on the day 6 dan 7 of germination is due to the activation of polyphenol oxidizing enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) and phenol compounds begin to be converted into lignin which together with cellulose and other polysaccharides as cell wall reinforcing materials (Xue et al. 2016). In the early stages of germination, soybeans require high amounts of polyphenols as a defense against pathogens, oxidative stress and other environmental factors. However, once soybean seeds start to develop further and get more protection from physical structures such as cell wall lignification, the need for polyphenols as a chemical defense will decrease, causing thesprouts to produce less polyphenols and divert esources to other processes (Wang et al. 2018).



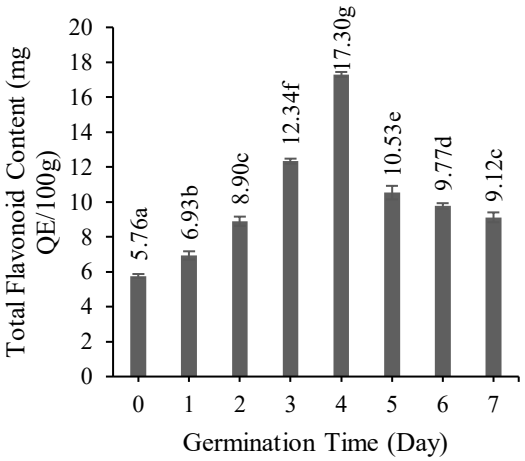
Note : Different letters indicate values are significantly different ($p<0.05$)

Figure 4 Total Phenolic Content (TPC) of Germinated Soybean Flour.

Total Flavonoid Content

The results from Figure 5 shows that the length of time of germination has a significant effect ($p<0.05$) on the content of flavonoid compounds in germinated soybean flour. The content of flavonoid compounds in germinated soybean flour began to increase from day 0 and reached a peak on day 4. The decrease occurred on day 5 to day 7. The highest level of flavonoids was significantly ($p<0.05$) obtained on the 4th day germination time of 17.30 ± 0.14 mg QE/100g, with an increase of 3.05 times compared to the polyphenol content on the 0th day germination. Research conducted by Hasanah et al (2019) on flavonoid content in non-germinated soybeans was 3.54 ± 0.024 mg QE/100g. The results showed that the germination process was able to increase the content of flavonoid compounds in soybeans.

The increase in flavonoid contents that occurred at day 0 of germination was due to the presence of shikimic acid, which is the result of the glycolysis pathway that does not involve pyruvic acid. Shikimic acid is produced by the reaction between erythrose and phosphoenolpyruvate (PEP) through the phenylpropanoid pathway (Ulfaniah et al. 2014). The phenylpropanoid pathway begins in the early stages of germination mainly after imbibition when the cells begin to activate, where this pathway is the biosynthetic pathway of polyphenols and flavonoids. Shikimic acid is further transformed into phenylalanine and tyrosine through several enzymatic processes. Phenylalanine will release NH_3 to form cinnamic acid. Furthermore, cinnamic acid is oxidized into aromatic ring which is part of the basic structure of flavonoids (Arifin, 2018).

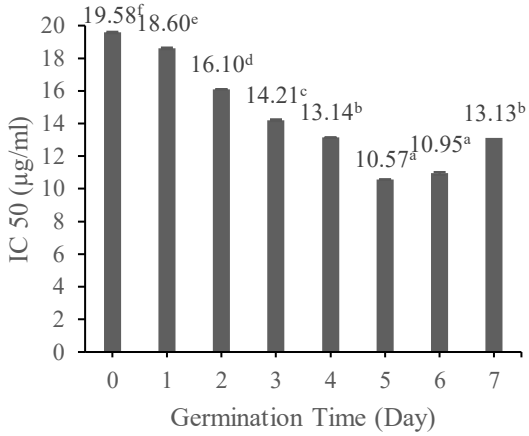


Note : Different letters indicate values are significantly different ($p<0.05$)

Figure 5 Total Flavonoid Content (TFC) of Germinated Soybean Flour.

Antioxidant Activity Compound (DRSA, DPPH Radical Scavenging Activity)

The results from Figure 6 shows that the germination time has a significant effect ($p<0.05$) on the antioxidant activity of germinated soybean flour. The IC_{50} value of germinated soybean flour increased on day 0 to day 5 and decreased on day 6 to day 7. The lowest IC_{50} was significantly ($p<0.05$) at 5 days germination time of 10.57 ± 0.01 µg/mL. This result shows an increase of 1.85 times compared to day 0 germination time. Research conducted by Prvulović et al (2017) found that the IC_{50} value of non-germinated soybeans was 45.89 ± 2.17 µg/mL. The results showed that the germination process was able to increase antioxidant activity in germinated soybean.



Note : Different letters indicate values are significantly different ($p<0.05$)

Figure 6 DPPH Radical Scavenging Activity of Germinated Soybean Flour.

The high antioxidant activity at day 5 germination time is thought to be due to an increase in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD). Where enzymes have a role in fighting free radicals generated during intensive metabolic processes in growing cells (Xue et al. 2016). The germination process is a critical phase in the life cycle of plants, to protect themselves from oxidative stress that occurs seeds increase their defense system including antioxidant mechanisms (Aminah 2020). In addition, the increase in antioxidant activity is also influenced by the increase in phenolic and flavonoid compounds along with the length of time of germination. Phenolic compounds have the ability as antioxidants due to the influence of OH groups contained in phenolic compounds. The hydroxyl group is substituted in the molecule which increases the ability to capture free radicals will be stronger with the increase of donor hydrogen atoms (Tsalissavrina et al., 2022).

The decrease in antioxidant activity that occurs in germination occurs because antioxidant compounds such as vitamin C, phenolic compounds and flavonoids are used in various forms in metabolic processes to support embryo growth. This intensive use will reduce the amount of antioxidant compounds available, which in turn will reduce the overall antioxidant activity (Susanti et al., 2022). In addition, the decrease in

antioxidant activity is possible because some antioxidant compounds are degraded due to exposure to light, temperature or pH changes during the germination process (Diniyah, 2020).

Table 2 Proximate Compounds of Germinated Soybean Flour

Germination Time (Day)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
0	5.44 ± 0.18^{a}	5.53 ± 0.03^{a}	44.38 ± 0.30^{b}	18.35 ± 0.03^{e}	26.31 ± 0.12^{d}
1	5.66 ± 0.29^{a}	5.67 ± 0.23^{ab}	42.08 ± 0.04^{a}	19.18 ± 0.19^{f}	27.43 ± 0.74^{e}
2	6.29 ± 0.06^{b}	5.74 ± 0.06^{b}	45.62 ± 0.29^{c}	19.03 ± 0.01^{f}	23.34 ± 0.28^{a}
3	6.14 ± 0.04^{b}	6.14 ± 0.01^{c}	45.51 ± 0.37^{c}	17.26 ± 0.04^{d}	24.96 ± 0.44^{bc}
4	7.77 ± 0.20^{c}	6.50 ± 0.04^{c}	47.28 ± 0.26^{e}	14.22 ± 0.06^{c}	24.24 ± 0.56^{ab}
5	8.18 ± 0.07^{c}	6.87 ± 0.04^{d}	48.67 ± 0.16^{f}	12.26 ± 0.06^{a}	24.03 ± 0.33^{ab}
6	7.85 ± 0.13^{c}	6.69 ± 0.01^{d}	45.94 ± 0.06^{c}	13.42 ± 0.17^{b}	26.11 ± 0.37^{d}
7	7.87 ± 0.24^{c}	6.64 ± 0.02^{d}	46.66 ± 0.06^{d}	13.41 ± 0.10^{b}	25.43 ± 0.30^{cd}

Note : Different letters in the same coloum indicate values are significantly different ($p<0.05$)

Proximate Composition

Moisture Content

The results in Table 2 shows that the germination time has a significant effect ($p<0.05$) on the moisture content of germinated soybean flour. The highest moisture content was obtained on the 5th day of germination at 8.18 ± 0.07%. The results showed an increase of 1.50 times compared to the length of time of day 0 germination. Research conducted by Astawan et al (2016) showed that the water content in non-germinated soybeans was 6.71 ± 0.73. The results showed that the germination process was able to increase the water content in germinated soybean.

Ash Content

The results in Table 2 also shows that the germination time has a significant effect ($p<0.05$) on the ash content of germinated soybean flour. The highest ash content was obtained on the 5th day of germination at 6.87±0.04%. The results showed an increase of 1.24 times compared to the germination time of day 0. Research conducted by Mitharwal (2022) showed that the ash content in non-germinated soybeans was 5.71 ± 0.03%. The results showed that the germination process was able to increase the ash content in germinated soybean flour. The increase in ash content in germinated soybean flour is due to the increase in mineral content of Ca, Mg and P (Özcan, 2014).

Fat Content

The results of fat content in germinated soybean flour ranged from 12.26% - 19.18%. Based on Table 2, the results shows that the germination time has a significant effect ($p<0.05$) on the fat content of germinated soybean flour. The highest fat content was obtained at day 1 germination time of 19.18 ± 0.19%. The results showed a decrease of 1.56 times in the day 5 of germination compared to the day 0 of germination. Research conducted by Astawan et al (2016) on soybean fat content was 38.65 ± 3.69%. The degradation of fat content in germinated soybean occurs due to fat being converted into energy during the germination process (Astawan et al., 2016). Germination causes lipolytic enzyme activity to increase which involves the hydrolysis of fat which is converted into simpler components such as fatty acids and glycerol (Yang et al. 2021).

Protein Content

Based on Table 2, the results shows that the germination time has a significant effect ($p<0.05$) on protein levels in germinated soybean flour. The highest protein content was obtained on the 5th day germination time of 48.67 ± 0.16%. The results showed an increase of 1.16 times compared to the day 0 of germination. Research conducted by Mitharwal et al (2022) showed protein levels in non-germinated soybeans were 38.88 ± 0.06%. The results of the study increased protein levels in germinated soybean. The increase in protein levels in germinated soybean is produced by endogenous enzyme activity in the germination process which results in protein biosynthesis in soybeans (Aparicio-García et al., 2021). Please link this with GABA. Since GABA is also amino acid.

Carbohydrate Content

Based on Table 2, the results shows that the germination time has a significant effect ($p<0.05$) on the carbohydrate content of germinated soybean flour. The highest carbohydrate content was obtained on day 1 germination time of 27.43 ± 0.74%. The results showed an increase of 1.16 times compared to the length of time of day 0

germination. Research conducted by Mitharwal (2022) showed a decrease in carbohydrate content after the germination process. This is due to the process of hydrolysis of carbohydrates into simpler sugars during germination due to increased α-amylase activity (Handa et al. 2017).

CONCLUSIONS

Based on the result, the germination time of soybean (Glycine Max L.) showed significant effects ($p<0.05$) on the content of polyphenols, flavonoids, gamma amino butyric acid (GABA), antioxidant activity, sprout length and percentage of sprout growth. The best germination time treatment is on day 3 to day 5 with the highest polyphenol content of 91.88 ± 1.20 mg GAE/100g and the best antioxidant activity of 10.57 ± 0.02 µg/mL on germination time of 5 days, the highest flavonoid content on germination time of 4 days at 17.30 mg QE/100g and the highest GABA content of 416.43 ± 11.69 mg/100g on germination time of 3 days. Germination process resulted in significant ($p\leq0.05$) for proximate compound, increase in moisture (8,18±0.07%), ash (6.87±0.04%), fat (19.18 ± 0.19%), protein (48.67±0.16%), carbohydrate (27.43±0.74%.).

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