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Research Paper

Proximate composition, total starch and sugar concentrations, and amylase activities of mungbean seeds (*Vigna radiata* L.) as affected by pH-adjusted soaking water and germination period

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ABSTRACT

Variation in pH value of soaking water and germination time alters chemical compositions and enzyme performance of grain seeds. The present study aimed to investigate proximate compositions, starch and sugar concentrations and amylase activity of mungbean seeds under sequential combinations of submersion with acidifying water and a germinating process for 12 h. During germination, the moisture, protein, ash, lost dry matter and reducing sugar concentrations and amylase activity of mungbean seeds increased with increasing germination time, whereas the lipid, total carbohydrate, total starch and total sugar contents significantly reduced. The mungbean seeds soaked in higher pH-adjusted water had higher total carbohydrate and total starch contents, but lower concentrations of moisture, protein, lipid, ash and lost dry matter. When soaking the mungbean seeds in water with a pH of 5.5, the reducing sugar content and amylase activity of the mungbean seeds were the highest, but the total sugar was the lowest. As a result, the mungbean seeds soaked in the acidified water at pH-5.5 before germinating are considered a promising technique to produce high-value-added mungbean seeds.

***Key words:** Amylase activity, Chemical composition, Germination, Mungbean, pH-adjusted water*

INTRODUCTION

Legumes are gaining popularity as an affordable meat alternative and the second most important food supply in human diets worldwide because of their rich protein, vitamins and minerals (Yvonne and Victoria 2017). Among diverse categories of legumes, mungbean (MBs) (*Vigna radiata* L. Wilczek) is acknowledged as the most well-known leguminous crop and is generally planted worldwide, mostly in Asian countries such as Vietnam, Korea, Japan, China, etc (Nasir *et al.* 2022, Zhou *et al.* 2019). It possesses high levels of essential amino acids, crude protein, total carbohydrate, fiber, ash, resistant starch, phytonutrients and water and fat-soluble vitamins and low values of the fat andglycemic index (Nasir *et al.* 2022, Mohan *et al.* 2020, Pratap *et al.* 2022, Schafleitner *et al.* 2016). Owing to its high quality of nutrients, mungbean is confirmed to positively contribute to preventing some chronic diseases, including diabetes, cardiac conditions and cancer (Chen *et al.* 2012). Hence, mungbean has been recognized as the preferred food ingredient for healthy food development (Yvonne and Victoria 2017, Nasir *et al.* 2022, Mohan *et al.* 2020, Pratap *et al.* 2022).

Germinated mungbean seeds contain more proteins, minerals, vitamins, antioxidants and antibacterial activities than other accessible forms of mungbeans, such as raw, fried and boiled seeds (Hanumantha Rao *et al.* 2016, Kim *et al.* 2012). The germination is commonly carried out by steeping mungbean seeds in water and then incubating them under specific temperature and humidity for a period. Principally, this technique aimed to provide basic ingredients and energy for growing seedlings by stimulating and activating endogenous enzymes to break macronutrients (starches, oligosaccharides, lipids, proteins, etc.) down into monomers (glucose, free amino acids, free fatty acids, etc.) that are readily absorbed (Ikram *et al.* 2021). Thus, germination is properly responsible for the significant differences and variations in chemical and nutritional constituents of grain seeds on the grounds. Furthermore, these changes in the chemical composition of seeds were more intense when the seed suffered from seriously stressful conditions such as acid, salt, drought, heat shock, water deficit, hypoxia and anaerobic treatment (Shelp *et al*. 2012). Previous research confirmed that pH value had a critical influence on germination due to its ability to disrupt some physiological processes (Zhang 2013). Thus, a current trend is to apply acidified water for soaking seeds to induce effective germination, resulting in the accumulation of nutrients. Songtip *et al.* (2012), reported that steeping paddy rice in an acidic solution of pH-3.0 positively affected its physicochemical characteristics, reducing sugar and γ-aminobutyric acid contents. Moreover, the employment of citric acid to acidify the submerging water was reported to dramatically enhance the level of γ-aminobutyric acid in the germinated MBs (Yen *et al*. 2022) or significantly improve total phenolic contents and antioxidant activities in rice beans (Sritongtae *et al.* 2017) and adlay seeds (Xu *et al.* 2018). Although the influences of germination conditions on the changes in chemical constituents of MBs have been previously reported, the lack of information on the impact of a combination of the treatment with pH-adjusted soaking water and germination time on changes in proximate compositions, total starch and sugar concentrations, which are related to the activities of hydrolyzed enzymes in the germinated MBs, has been manipulated. Hence, this study aims to examine the fluctuations in proximate compositions, starch and sugar concentrations and amylase activities of the MBs pretreated with different pH-adjusted soaking water and germinated at various periods.

MATERIALS AND METHODS

Materials

Mungbean grains [*Vigna radiata* (L.) Wilczek], a commercial product named DX208, was provided by Southern Seed Co., Vietnam. All seeds chosen for germination in this study were dried, intact, and full-grown. All chemicals, solvents and standard reagents were of analytical grade and were purchased from Sigma-Aldrich Pte. Ltd. (Germany).

Germinating procedure of mungbean grains

The germination procedure was conducted according to the previous research revealed by (Yen *et al.* 2022). The MBs were first prepared and immersed in anacidified water solution having various pH values (4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, adjusted using 2.8 M citric acid) and a grain-to-soaking water ratio of 1:4 (g/ml). They were steeped at 40ºC for 4 h, then all liquid was decanted, and the seeds were germinated in a germinating machine (Kangaroo KG-262, China) at 30ºC under dark cloth as a top cover. The germinated seeds were carefully collected at every 2-h interval and then ground into fine powder passing through a sieve of 0.105 mm in aperture size for determining their chemical compositions and enzyme activities.

Evaluation of proximate compositions of germinated mungbean seeds (MBs)

The standard AOAC procedures 950.46, 920.39, 2001.11 and 942.05 were employed to analyze the moisture, crude lipid, crude protein and ash contents of the MBs, respectively (AOAC International 2000). Briefly, approximately 3 g of ground mungbean flour was weighed into a pre-dried aluminum dish and heated in the oven at 105ºC for 1.5 h to determine the moisture content of the flour by the loss in weight of the sample after heating. The crude protein content in the samples was analyzed based on the total nitrogen content in 2.5g of ground MBs, determined by the micro-Kjeldahl method. The crude protein content was calculated from the total nitrogen by multiplying a nitrogen-to-protein conversion constant of 6.25. The crude lipid content was extracted from 5g of ground MBs with diethyl ether and petroleum ether, using Soxhlet extraction. The crude fat content was determined based on the loss in weight of the sample due to the solubility of lipids in solvents. Regarding the ash content, 2g of ground MBs were placed in the furnace preheated to 600ºC for 2h and then the loss in weight of the sample under the high-temperature-controlled furnace was recorded. The concentration of total carbohydrate was computed by taking 100% and subtracting the protein, lipid and ash contents (FAO 2003). Additionally, the dry matter was estimated from the total mass of the MBs minus the moisture content, while its loss during the germination period was the difference between the dried weights of the raw and germinated seeds (Hung *et al*. 2020).

Determination of starch, reducing sugar and total sugar contents of germinated mungbean seeds (MBs)

The total sugar, reducing sugar and starch contents of the MBs were investigated according to the modified technique of (Tian *et al.* 2010). The MBs powder (5g) was first extracted with 50 ml of 80% ethanol solution (v/v) at ambient temperature for 60 min and then centrifuged at 4,000×g for 10 min to obtain the supernatant and solid parts separately. The ethanol-insoluble segment was liquefied with 50% aqueous perchloric acid (v/v) and the phenol-sulfuric acid technique was conducted to analyze the total sugar content of the whole mixture (Taylo 1995). Afterwards, this value was utilized to estimate the approximate total starch content by multiplying it by 0.9 (Li 1996). On the other hand, the supernatant was evaporated at 50ºC to collect the residue, which was subsequently dissolved in 50 ml of distilled water and centrifuged at 4,000×g for 10 min. After centrifuging, the supernatant was collected and used to measure reducing sugar content by the procedure of the 3,5-Dinitrosalicylic acid (Miller 1959) and total sugar content by the approach of phenol-sulfuric acid methods (Taylor 1995). The absorbance of the colored reaction solutions was measured at 520 nm for reducing sugar and at 490 nm for total sugar using a spectrophotometer (UVD-2960, Labomed, USA). All values were expressed as grams glucose per 100 grams dry matter. Glucose was used as a standard to form a calibration curve and the concentrations of reducing sugar and total sugar were determined based on the calibration curve. All experiments were carried out in triplicate.

Assay of α-amylase activity in *germinated mungbean seeds (MBs)*

The α-amylase activity of the MBs was measured according to the methods published by (Hung *et al*. 2020), with minor adjustments. The MB powder (2g) was blended with 20 ml of 0.1 M acetate buffer (pH 5.5) and 20 ml of 0.2% calcium chloride solution (w/v) at room temperature for 60 min and β-amylase was later inactivated at 70ºC for 15 min. Subsequently, the mixture was centrifuged at 3,000×g for 10 min to obtain the supernatants containing α-amylase and made up to a final volume of 100 ml with acetate buffer. Afterwards, 1 ml of the diluted extract containing α-amylase was mixed with 0.5 mL of 1% soluble starch solution (w/v) and 0.5 ml of acetate buffer and then incubated at 40ºC for 15 min. After incubation, 2 ml of 1 N HCl was added to the end of the reaction. The amount of maltose liberated was assayed with the 3,5-dinitrosalicylic acid reagent at 100ºC for 20 min. The absorbance was measured at 540 nm. Maltose was employed as a standard for this assay. The capacity of alpha-amylase was signified as mg of maltose released per minute per gram of sample. All experiments were carried out in triplicate.

Statistical analysis

All values attained were the average of triplicate performance. SPSS software (version 20, SPSS Inc., USA) was used to interpret and compare the results ($p < 0.05$) with the aid of analysis of variance and Duncan's post-hoc multiple comparisons.

RESULTS AND DISCUSSION

Effect of the pH-adjusted soaking water and germination time on moisture contents and dry matter losses of the MBs during germination

Figures 1 and 2 present the effect of pH-adjusted soaking water and germination time on moisture content and dry matter loss of the germinated MBs, respectively. Both moisture content and dry matter losses in the MBs were reduced when increasing the pH values of the soaking water from pH-4.5 to pH-7.0 for 12h of germination. However, the moisture content and dry matter loss of the MBs significantly increased with increasing germination time. These outcomes coincided with the previous study, which confirmed that the moisture content of the MBs increased from 53.9% to 67.8% after germinating for 24h (Wongsiri *et al*. 2015). (Hung *et al*. 2020), also affirmed that the dry matter losses of the germinated MBs increased from 5.9% to 21.7% after germinating for 96h. The water imbibition probably contributed to the rise in the water content of the germinated seeds (Wongsiri *et al*. 2015), where as the decline indry weight of the germinated MBs might be due to the physiological metabolisms, especially respiration (Wu *et al.* 2020). Tian *et al.* (2010), proved that respiration was responsible for 57% of dry matter loss in germinated oat seeds. (Wu *et al.* 2020), claimed that the endogenous enzymes were activated during respiration to degrade storage macromolecules into smaller monomers and provide energy for life activities. Particularly, sugars were degraded into water and carbon dioxide during respiration, which escaped into the surroundings, resulting in a significant reduction in the dry matter (Mbithi-Mwikya *et al*. 2000). Moreover, protons donated by exogenous acid solutions stimulated the metabolic activities of organs such as chloroplasts, mitochondria, etc. (McCue *et al*. 2000), leading to the loosening of the cell walls, improving water absorption and increasing the losses of soluble dry matterin the acid-pretreated seeds (Zhang 2013, McCue *et al*. 2000).

Effect of pH-adjusted soaking water and germination time on proximate compositions of the MBs *during germination*

Tables 1 – 4 illustrate tendencies for proximate compositions of the germinated MBs. Amounts of total carbohydrate and crude lipidin all germinated seeds soaked with acidifying water markedly declined over the 12-h germinating period, whereas the opposite tendencies were experienced with ash and crude protein contents, regardless of pH values of the soaking water. These results could be clarified by the activities of endogenous enzymes such as amylase, protease, etc., intensively increased during germination (Hung *et al.* 2020). Particularly, the changes in the chemical compositions of the germinated seeds occurred as a result of the catalytic activities of endogenous enzymes to provide energy sources and materials for commencing the germination process (Wu *et al*. 2020, Mubarak 2005). Lipids and carbohydrates were recognized as the preferred sources of energy development during seed germination. Hence, the lower contents of crude lipids and carbohydrates were witnessed with longer germination time.

The slight increase in total protein and ash contents might be due to the loss of dry weight of the MBs during the germination period. In addition, the phytase enzyme was responsible for breaking down the bond in protein-enzyme-mineral compounds to liberate the minerals, resulting in a higher ash concentration in the germinated seeds with a longer germination time (Hung *et al.* 2020, Zhang *et al.* 2015, Elobuike *et al.* 2021).

Regarding the effects of soaking in acidifying water on changes in levels of primary chemical composition in the germinated seeds at the same germination stage, a slight reduction in crude protein and lipid contents was witnessed as the pH values increased from pH-4.5 to pH-7.0. The reduction of dry matter loss of the MBs soaked with water having higher pH values contributed to the higher dry weight of the MBs, resulting in lowered protein and lipid contents.

As shown in Table 3, a reduction in the pH values of the soaking water from pH-7.0 to pH-5.5 induced the germinated seeds torelease minerals, increasing the ash content. When further reducing the pH value to pH-5.0 or pH-4.5, the ash contents noticeably dropped compared to those at pH-5.5. This phenomenon occurred because citric acid acts as a competitive chelator and a strong organic acid, which enhances enzyme phytase activity to considerably disassociate minerals and phytic acid from phytate (Romano and Kumar 2018). Besides, the optimal pH for phytase activity in beans ranged from pH-5.0 to pH-5.5 (Chang and Schwimmer 1977). Hence, the highest ash content was obtained in the germinated MBs soaked with the acidifying solution of pH-5.5.

The changes in the concentrations of crude protein, lipid and ash in the germinated MBs were responsible for varying carbohydrate contents. Therefore, an increasing tendency in carbohydrate level was experienced with the increase in pH values of the soaking water from pH-4.5 to pH-7.0 (Table 4).

Effect of the pH-adjusted soaking water and germination time on starch, reducing sugar and total sugar contents of the MBs during germination

Changes in starch, total sugar and reducing sugar contents of the germinated MBs are shown in Figures 3-5. Generally, the raising germination time contributed to a gradual decline in quantities of total starch and sugar contents and a remarkable increase in reducing sugar content of the germinated MBs. These results coincided with the preceding study by (Tian *et al*. 2010), who claimed that the reducing sugar content of oat seeds enormously improved after germinating for 144h, whereas their amount of starch noticeably diminished. Additionally, other studies also proved that the germination time authenticated a sharp climb in sugar contents, whereas there was a corresponding reduction in starch levels, such as in finger millet (Mbithi-Mwikya *et al*. 2000), brown rice (Songtip *et al*. 2012, Charoenthaikij *et al*. 2009), mungbean (Wongsiri *et al.* 2015), and soybean (Kaczmarska *et al*. 2017). Furthermore, the total starch and sugar contents steadily reduced along with the rising pH values of the soaking water from pH-4.5 to pH-5.5, but significantly boosted when the pH value was greater than pH-5.5. However, the reducing sugar content was in the opposite direction. These results were agreeable with previous research of (Songtip *et al*. 2012), who reported that brown rice seeds showed significantly higher reducing sugar content in seeds steeped at pH-3.0 than at pH-6.8.

Carbohydrate constituents in the germinated MBs are commonly monosaccharides (glucose, fructose, galactose, xylose, etc.), disaccharides (maltose, sucrose, etc.) and oligosaccharides (raffinose, stachyose, verbascose, etc.). (Sudhakaran and Bukkan 2021), also revealed that raffinose, stachyose, verbascose and ajugose contents occupied 0.07-2.60%, 0.30-2.83%, 1.57-5.48% and 0.03-0.11% of total sugar content, respectively. Other previous studies also confirmed that the germination process could probably trigger a significantly reduced content of oligosaccharides (Mubarak 2005, Elobuike *et al.* 2021, Kaczmarska *et al*. 2017, El-Adawy *et al.* 2003), contributing to the

Table 1. The changes in protein content (g/100 g, dry basis) of MBs pre-treated with acidifying water and germinated for 12 h[1]

Germination time	pH-adjusted value					
(h)	4.5	5.0	5.5	6.0	6.5	7.0
0	27.1 ± 0.2^{fF}	26.6 ± 0.2^{eE}	26.3 ± 0.1^{dD}	25.9 ± 0.1^{cC}	25.5 ± 0.1^{bB}	25.2 ± 0.1^{aA}
2	27.6 ± 0.1^{gG}	27.2 ± 0.4^{fF}	26.7 ± 0.1^{eE}	26.2 ± 0.1^{dD}	25.8 ± 0.1^{cBC}	25.4 ± 0.1^{bAB}
4	28.3 ± 0.1^{hH}	27.7 ± 0.3^{gG}	27.0 ± 0.1^{fF}	26.5 ± 0.2^{eE}	26.1 ± 0.1^{dC}	25.6 ± 0.1^{cBC}
6	29.0 ± 0.3^{gI}	28.3 ± 0.2^{hH}	27.6 ± 0.2^{gG}	27.1 ± 0.1^{fF}	26.5 ± 0.2^{eD}	25.9 ± 0.1^{dC}
8	29.7± 0.3^{jJ}	29.0 ± 0.2^{iI}	28.4 ± 0.2^{hH}	27.9 ± 0.3^{gG}	27.1 ± 0.4^{fE}	26.5± 0.1^{eD}
10	29.8 ± 0.3^{kJ}	29.3 ± 0.3^{jIJ}	28.6 ± 0.2^{iHI}	28.0 ± 0.3^{hGH}	27.4 ± 0.2^{gEF}	26.6 ± 0.3^{fD}
12	30.3 ± 0.2^{lK}	29.5± 0.2^{kJ}	28.8 ± 0.2^{jI}	28.2 ± 0.2^{iH}	27.6 ± 0.2^{hF}	26.8 ± 0.2^{gD}

[1]The same lowercase letters signified no considerable difference within the same germination period (p>0.05), while the same uppercase letters expressed no remarkable difference within the same pH-adjusted steeping water (p>0.05).

Table 2. The changes in lipid content (g/100 g, dry basis) of MBs pre-treated with acidifying water and germinated for 12 h[1]

Germination time (h)	pH-adjusted value					
	4.5	5.0	5.5	6.0	6.5	7.0
0	1.95 ± 0.01	1.91 ± 0.01	1.86 ± 0.02	1.80 ± 0.01	1.75 ± 0.02	1.72 ± 0.01
2	1.92 ± 0.01	1.86 ± 0.04	1.78 ± 0.04	1.68 ± 0.02	1.64 ± 0.01	1.61 ± 0.02
4	1.88 ± 0.02	1.82 ± 0.01	1.71 ± 0.01	1.63 ± 0.02	1.55 ± 0.02	1.50 ± 0.03
6	1.84 ± 0.01	1.78 ± 0.01	1.66 ± 0.02	1.56 ± 0.01	1.49 ± 0.02	1.43 ± 0.01
8	1.79 ± 0.01	1.71 ± 0.02	1.60 ± 0.03	1.51 ± 0.02	1.43 ± 0.02	1.37 ± 0.01
10	1.73 ± 0.03	1.61 ± 0.02	1.53 ± 0.01	1.45 ± 0.01	1.36 ± 0.01	1.29 ± 0.03
12	1.65 ± 0.02	1.54 ± 0.01	1.48 ± 0.02	1.38 ± 0.04	1.26 ± 0.01	1.20 ± 0.03

[1]Means values significantly different ($p < 0.05$) in either the same column or the same row.

Table 3. The changes in ash content (g/100 g, dry basis) of MBs pre-treated with acidifying water and germinated for 12 h[1]

Germination time	pH-adjusted value					
(h)	4.5	5.0	5.5	6.0	6.5	7.0
0	2.39 ± 0.03	2.60 ± 0.07	2.80 ± 0.05	2.48 ± 0.02	2.31 ± 0.01	2.23 ± 0.05
2	2.54 ± 0.04	2.69 ± 0.04	2.93 ± 0.05	2.60 ± 0.02	2.45 ± 0.02	2.34 ± 0.03
4	2.63 ± 0.02	2.84 ± 0.03	3.03 ± 0.05	2.73 ± 0.02	2.53 ± 0.05	2.44 ± 0.02
6	2.73 ± 0.05	2.94 ± 0.06	3.15 ± 0.04	2.85 ± 0.01	2.65 ± 0.01	2.58 ± 0.01
8	2.92 ± 0.07	3.13 ± 0.01	3.32 ± 0.05	3.03 ± 0.05	2.82 ± 0.03	2.74 ± 0.02
10	3.02 ± 0.03	3.24 ± 0.04	3.45 ± 0.01	3.13 ± 0.02	2.90 ± 0.01	2.83 ± 0.02
12	3.11 ± 0.02	3.33 ± 0.03	3.56 ± 0.01	3.23 ± 0.03	2.98 ± 0.02	2.92 ± 0.03

[1]Means values significantly different ($p < 0.05$) in either the same column or the same row.

Table 4. The changes in total carbohydrate content (g/100 g, dry basis) of MBs pre-treated with acidifying water and germinated for 12 h[1]

Germination time (h)	pH-adjusted value					
	4.5	5.0	5.5	6.0	6.5	7.0
0	68.6 ± 0.2^{gF}	68.9 ± 0.1^{hG}	69.1 ± 0.1^{iH}	69.8 ± 0.1^{jI}	70.4 ± 0.1^{kJ}	70.9 ± 0.1^{lK}
2	67.9 ± 0.1^{fE}	68.3 ± 0.3^{gF}	68.6 ± 0.1^{hG}	69.5 ± 0.1^{iH}	70.1 ± 0.1^{jI}	70.7 ± 0.1^{kJ}
4	67.2 ± 0.1^{eD}	67.7 ± 0.3^{fE}	68.3 ± 0.1^{gF}	69.1 ± 0.1^{hG}	69.8 ± 0.1^{iH}	70.4 ± 0.1^{jI}
6	66.4 ± 0.3^{dC}	67.0 ± 0.1^{eD}	67.6 ± 0.2^{fE}	68.5 ± 0.1^{gF}	69.4 ± 0.2^{hG}	70.1 ± 0.1^{iH}
8	65.6 ± 0.2^{cB}	66.1 ± 0.1^{dC}	66.7 ± 0.2^{eD}	67.6 ± 0.2^{fE}	68.6 ± 0.4^{gF}	69.4 ± 0.1^{hG}
10	65.4 ± 0.3^{bB}	65.9 ± 0.3^{cBC}	66.4 ± 0.2^{dCD}	67.4 ± 0.2^{eDE}	68.3 ± 0.1^{fEF}	69.3 ± 0.2^{gFG}
12	64.9 ± 0.2^{aA}	65.6 ± 0.1^{bB}	66.2 ± 0.2^{cC}	67.2 ± 0.1^{dD}	68.1 ± 0.1^{eE}	69.1 ± 0.1^{fF}

[1]The same lowercase letters signified no considerable difference within the same germination period (p>0.05), while the same uppercase letters expressed no remarkable difference within the same pH-adjusted steeping water (p>0.05).

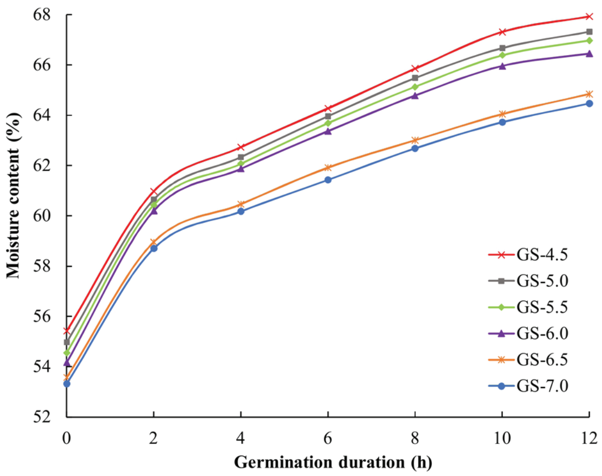

Fig. 1. The fluctuations in moisture content of MBs pre-treated with acidifying water and germinated for 12 h. [1]GS-4.5, GS-5.0, GS-5.5, GS-6.0, GS-6.5 and GS-7.0 were MBs pretreated with acidifying solution of pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, respectively.

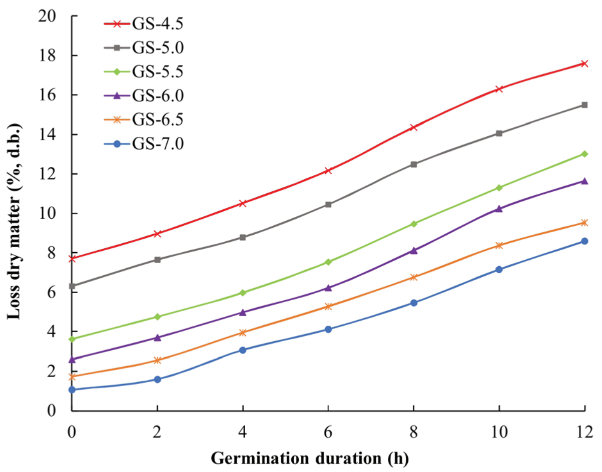

Fig. 2. The fluctuations in dry matter losses of MBs pre-treated with acidifying water and germinated for 12 h. [1]GS-4.5, GS-5.0, GS-5.5, GS-6.0, GS-6.5 and GS-7.0 were MBs pretreated with acidifying solution of pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, respectively.

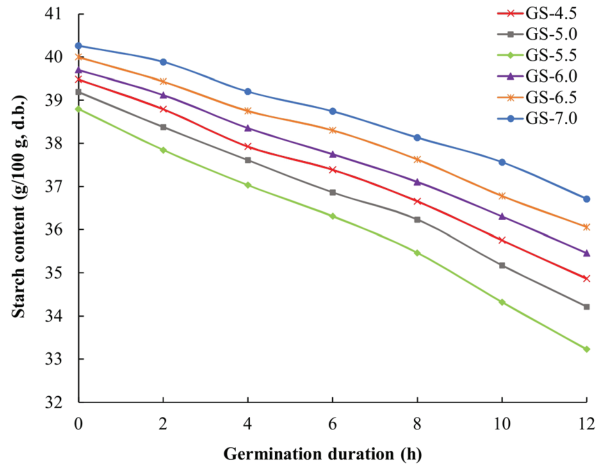

Fig. 3. The fluctuations in starch content of MBs pre-treated with acidifying water and germinated for 12 h. [1]GS-4.5, GS-5.0, GS-5.5, GS-6.0, GS-6.5 and GS-7.0 were MBs pretreated with acidifying solution of pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, respectively.

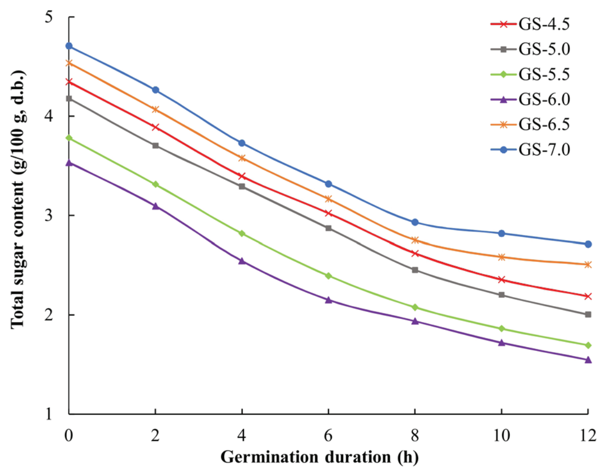

Fig. 4. The fluctuations in total sugar content of MBs pre-treated with acidifying water and germinated for 12 h. [1]GS-4.5, GS-5.0, GS-5.5, GS-6.0, GS-6.5 and GS-7.0 were MBs pretreated with acidifying solution of pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, respectively.

changes in starch, total sugar and reducing sugar contents, which could be explained by the activity of enzymes including both amylase and galactosidase. Particularly, (Kadlec *et al*. 2008), affirmed that galactosidase was activated during germination, leading to the robust hydrolysis of oligosaccharides, resulting in a reduction in the quantity of total sugars from 4.98g/100g db to 1.55g/100g (dried basis) after first soaking in water with pH-6.0 and then germinating for 12 h. Moreover, (Tripathi *et al*. 2007), announced that pH-5.6 was recognized as the optimum pH condition for amylase catalysis in the germinated MBs, leading to the fact that the highest reducing sugar and lowest starch contents were attained in the germinated MBs pre-treated with an acidifying solution of pH-5.6.

Effect of the pH-adjusted soaking water and germination time on amylase activity of the MBs during germination

(Figure 6) demonstrates the effect of the pH-adjusted soaking water and germination period on the activity of amylase in the germinated

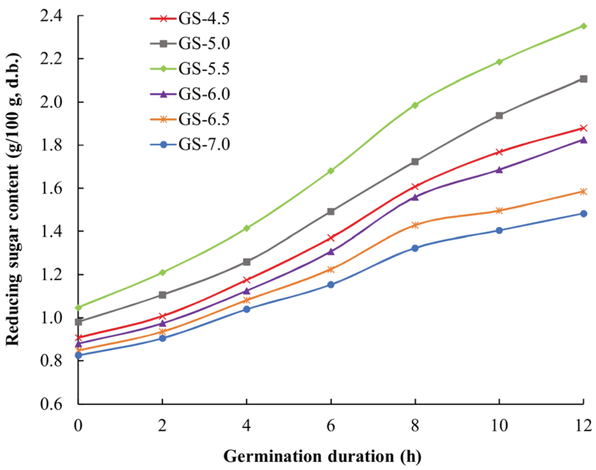

Fig. 5. The fluctuations in reducing sugar of MBs pretreated with acidifying water and germinated for 12 h. [1]GS-4.5, GS-5.0, GS-5.5, GS-6.0, GS-6.5 and GS-7.0 were MBs pretreated with acidifying solution of pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, respectively.

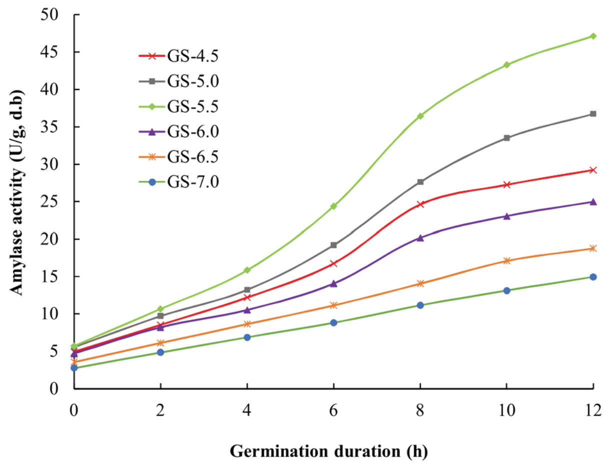

Fig. 6. The fluctuations in amylase activity of MBs pretreated with acidifying water and germinated for 12 h. [1]GS-4.5, GS-5.0, GS-5.5, GS-6.0, GS-6.5 and GS-7.0 were MBs pretreated with acidifying solution of pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0, respectively.

MBs. Among all kinds of seeds in this study, the highest amylase activity (47.12 U/g, db) belonged to germinated MBs soaked with the acidifying solution of pH-5.5 and germinated for 12 h, while dry ungerminated MBs almost rarely performed amylase activity (0.79 U/g, db) (Data not shown). Generally, amylase activities of MBs constantly escalated along with the rising germination period, while these data regularly decreased when the pH value was higher than pH-6.0. These results indicated that amylase activity positively correlated with reducing sugar levels, whereas starch levels showed a negative relationship at the same germination time. These results were agreeable to previous publications, which claimed that germination time contributed to the intensification in amylase activity of oat seeds (Tian *et al*. 2010), finger millet (Mbithi-Mwikya *et al*. 2000), brown rice (Songtip *et al*. 2012, Charoenthaikij *et al.* 2009), mungbean (Wongsiri *et al*. 2015) and soybean (Kaczmarska *et al*. 2017). (Tripathi *et al*. 2007), also confirmed that pH-5.6 was the optimum pH for amylase activity in the germinated mungbean.

CONCLUSION

Effects of pH values of soaking water and germination time on chemical compositions and amylase activities were investigated in this study. Both pH values of soaking water and germination time significantly influence in concentrations of moisture, protein, lipid, ash, total carbohydrate, lost dry matter, total starch, total sugar and reducing sugar of the mungbean seeds. The amylase activities of the germinated mungbean seeds significantly increased with increasing germination time. The amylase activities of the mungbean seeds soaked in the acidifying water at pH 4.5-pH 5.5 were higher than those of the mungbean seeds soaked in the water at pH 6.0-pH 7.0. As a result, the mungbean seeds soaked in the acidified water at pH-5.5 before germinating are considered a promising technique to produce high-value-added mungbean seeds.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to the publication of this article.

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