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Changes in α-galactosidase activity and oligosaccharides during germination of soybean seeds

Duong Thi Phuong Lien[1*], Phan Thi Bich Tram[1], Trang Si Trung[2] and Ha Thanh Toan[3]

[1]*College of Agriculture and Applied Biology, Cantho University, Vietnam*

[2]*Faculty of Food Technology, Nha Trang University, Vietnam*

[3]*Biotechnology Research and Development Institute, Cantho University, Vietnam*

[*]*Correspondence: Duong Thi Phuong Lien (email: dtplien@ctu.edu.vn)*

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ABSTRACT

The effect of germination process on α–galactosidase activities, oligosaccharides and reducing sugars contents in soybean seeds was investigated. Soaked soybean seeds were germinated at 25°C in dark condition for 72 hours. Samples were collected every 12 hours during germination for analyzing the α–galactosidase activities, which was monitored with a synthetic substrate ρ-nitrophenyl-α-D-galactopyranoside (ρNPGal). The freeze-dried samples were prepared for determination of raffinose, stachyose, sucrose by thin layer chromatography, and reducing sugars were assayed by reaction with nitro salicylic acid. After soaking, the activity of α–galactosidase increased1.84 times compared to that in the soybean seeds and reached maximum value (164.3±2.5 U/100g, db) after 12 hours of germination. The increase in α–galactosidase activity led to the decrease in raffinose and stachyose contents during soaking and germination. In addition, the degradation of these undesirable components followed the first order exponential equation (R^2 = 0.97–0.99). Sucrose content remained after soaking and up to 12 hours of germination and reduced significantly after that. The final result of the hydrolysis of raffinose, stachyose and sucrose was significant increase in reducing sugars that can be used as energy-source during germination of soybean seeds.

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1 INTRODUCTION

Soybean (*Glycine Max.* (L.) Merril) is an excellent source of proteins for human consumption. However, direct utilization of soybeans is undesirable due to its indigestible oligosaccharides content which may induce flatulence in humanity (Reddy and Salunkhe, 1980; Alani *el al.*, 1990; Wang *el al.*, 2007). The indigestible oligosaccharides in soybean seeds are a group of soluble low molecular weight, mainly raffinose [α–D–galactopyranosyl–(1,6)–α–D–glucopyranosyl–β–D–fructofuranoside] and stachyose[α–D–galactopyranosyl–(1,6)–α–D–galactopyranosyl–(1,6)–α–D–glucopyranosyl–β–D–fructofuranoside] (Guimarães *el al.*, 2001; Wang *el al.*, 2007). Since humans gastrointestinal tract lacks the enzyme α–galactosidases (E.C. 3.2.1.22, α–D–galactosidegalactohydrolase) being necessary for the hydrolysis of the α–1,6 linkages present in oligosaccharides (Reddy and Salunkhe, 1980), these components pass intactly into the large intestine where the fermentation of anaerobic microorganisms occurs, then causing flatulence (Guimarães *el al.*, 2001).

Various processing methods can reduce the flatulence of soybeans inducing seed germination (Markakis, 1987; Alani *el al.*, 1990), cooking (Oboh *el al.*, 2000; Ramadan, 2012), ethanol and aqueous extraction (Rackis *el al.*, 1970; Omosaiye *el al.*, 1978) and fermentation (Akinyele and Akinlosotu, 1991; Egounlety and Aworh, 2003). Among them, germination is a relatively simple and inexpensive one that produces a natural type of food (Shah *el al.*, 2011). Germination causes marked metabolic changes in the seeds resulting in the susceptiblydegradation of carbohydrates (Rackis, 1981; Nnanna and Phillips, 1988). The α–galactosidases were suspected to play an important role in the early stages of germination by hydrolyzing galactose-containing oligosaccharides to provide metabolites for the developing seedling (Dey *el al.*, 1986; Dey and Campillo, 2006), and thereby the oligosaccharides contents were reduced.

This study on changes in α–galactosidase activity and oligosaccharide content during the germination of soybean seeds is to provide practical recommendations for reducing the flatus activity in soybeans.

2 MATERIALS AND METHODS

2.1 Chemicals

The standard raffinose, stachyose and sucrose were purchased from Merck Company (Germany). The synthetic substrate ρNPGal was from Sigma-Aldrich.

2.2 Soybeans and germination process

Soybeans (MTD 760 variety) were supplied from Department of Genetics and Plant Breeding,College of Agriculture and Applied Biology, Can Tho University. The seeds were cleaned and rinsed with clean water before being soaked for 12 hours to reach the equilibrium moisture content at ambient temperature. Soaking process was carried out in drinkable water with the ratio of soybean seeds and water of 1: 5 and the concentration of gibberellic acid in soaking water of 1 mg/L. The soaked beans were drained, rinsed and placed in a germination chamber in dark condition. Watering the seeds was set up two minutes every 4 hours with clean water automatically. The germination process was carried out at 25ºC for 0, 12, 24, 36, 48, 60 and 72 hours (Duong *el al.*, 2016). α–galactosidases activities were determined in fresh germinated soybeans, and the freeze dried samples were used for analyzing the oligosaccharides and reducing sugar contents.

2.3 Determination of oligosaccharides by thin – layer chromatography (TLC)

Extraction of oligosaccharides: One gram of sample was extracted with 10mL of 70% aqueous ethanol and kept on orbital shaker at 130 rpm for 16 hours. The contents of the flask were filtered through Whatman No.1 filter paper, and the residue was further washed with 5mL of 70% ethanol. The filtrate was evaporated in a rotary vacuum evaporator at 45ºC. The syrup was dissolved in 2 mL of distilled water (Tajoddin *el al.*, 2010a).

TLC of oligosaccharides: Assay of oligosaccharides by TLC was carried out following the method of Tanaka *el al.* (1975)and Tajoddin *el al.* (2010a) with some modifications. Six microliters of syrup were applied on chromatographic plates (20 cm × 20 cm) coated with microcrystalline cellulose powder. Spotting of the sugar samples was done by using capillary tubes. Each sample was spotted thrice separately (2 µL for each spotting) and dried. Plates were developed using solvent system n-propanol:ethylacetate:water (6:1:3). The developed plates were sprayed with 1% α-naphthol in alcohol 95% to locate the sugars spots. For quantitative determination of the amounts of sucrose, raffinose and stachyose, a sugar spot on a chromatogram was scraped off, and the sugar was extracted with 4 mL of distilled water in a test tube overnight at room temperature. One mL of eluent was mixed with 1 mL of 0.02M thiobarbituric acid and 1 mL of concentrated hydrochloric acid. The mixture was heated in a boiling water bath for exactly 6 min, then cooled under running water(Percheron, 1962). The yellow color produced was read at 432.5 nm. The concentration of sugar was calculated from working standards.

2.4 Determination of reducing sugar

The estimation of the reducing water was carried out by nitro salicylic acid reagent (2% NaOH and 20% sodium potassium tartrate). In the estimation, the sample (1 g) was extracted with hot water (70–80ºC), filtered and mixed with nitro salicylic acid reagent in a boiling water bath for 10 minutes. Color intensity was measured at 540 nm, using a standard curve of glucose (Mao *el al.*, 2013). The results were expressed as percent in dry weight (%, db).

2.5 Analysis of α–galactosidases activity

A modified procedure of Guimarães (2001) was used to determine α–galactosidase using ρNPGal as substrate. The sample (0.1 g) with 1 mL of citrate buffer 0.1M (pH 5.5) was shaken for one hour, at 20ºC. After centrifugation with the rate of 13,000 rpm, at 4ºC for 40 min; the supernatant was used directly for enzyme assay (Guimarães *el al.*, 2001). The enzyme was assayed using a reaction system (1 mL final volume) containing 350µL of 0.1 M sodium acetate buffer (pH 5), 100µL of enzyme preparation and 250µl of 2mM ρNPGal in phosphate butter (pH 4.5). Reaction was conducted for 15 min

at 37ºC and stopped by the addition of 1 mL of 0.5 M sodium carbonate. The yellow color produced was measured at wavelength of 420nm. Control sample involved adding enzyme extracts after the sodium carbonate solution had been added. The control was used as the zero calibration reading. The molar extinction coefficient for ρ–nitrophenol was taken as 18,400 (Reid and Meier, 1973) to calculate the amount of ρ–nitrophenol released. A unit of enzyme activity (U) was defined as the amount of α-galactosidase which liberates 1 µmol of ρ–nitrophenol per min under the given assay conditions (Rezende *el al.*, 2005). The results were as U/100 g sample on dry weight.

2.6 Data analysis

All data were performed in triplicate and were submitted to analysis of variance (ANOVA) by Portable Statgraphics Centurion 15.2.11.0. The regression analysis was carried out by Microsoft Excel 2007.

3 RESULTS AND DISCUSSION

Germination begins with the water uptake of dry seed, and ends with the emergence of the radicle (Bewley, 1997).The images of soybean seed during germination were illustrated in Figure 1. According to Han *el al.* (2013), the first stage of germination is a rapid water uptake phase (about 12 hours after soaking), the second stage is a plateau phase and for soybean seeds germination, plateau phase was defined as 12–24 hours after imbibition. The last phase is the post-germination stage in which the radicle begins to grow (Figure 1).

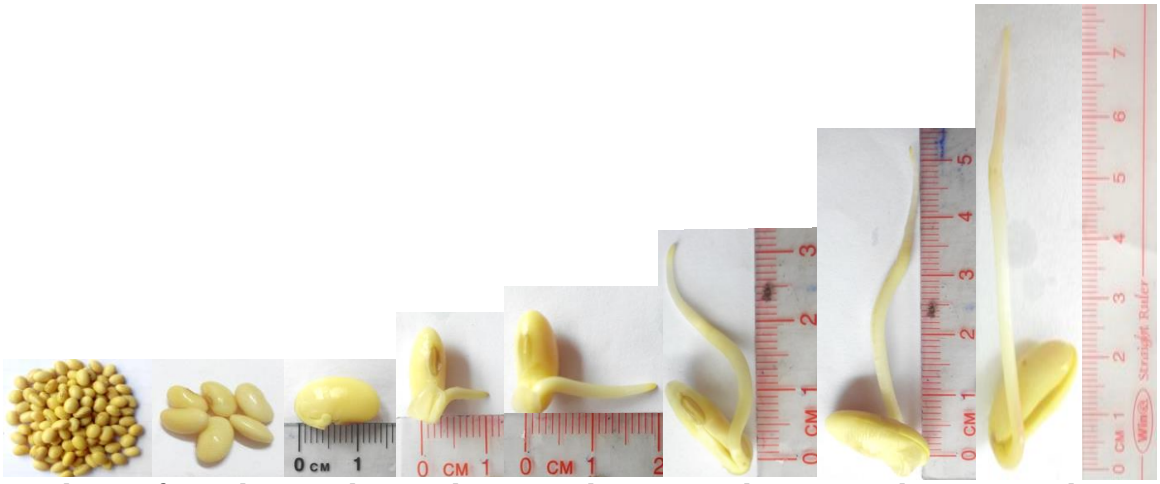

Figure 1: Images of soybean seeds during germination

Figure 2 showed the TLC results depicting the separation pattern of standard sugars and oligosaccharides of soybean seed and germinated soybeans. The identity of sucrose, raffinose and stachyose in soybean samples was confirmed by spots of the corresponding standard sugars (Figure 2A). The spots in Figure 2B are the oligosaccharides of soybean samples. The change of the contents of raffinose, stachyose, sucrose and reducing sugars of soybeans after soaking and during germination were shown in Table 1. It is doubted that α–galactosidasein seeds involved in the hydrolysis galactose-containing oligosaccharides leading to the change in oligosaccharide content (Dey *el al.*, 1986; Dey and Campillo, 2006). Therefore, the activity of α–galactosidase in soybean seeds and germinated soybeans were determined and shown in Table 1.

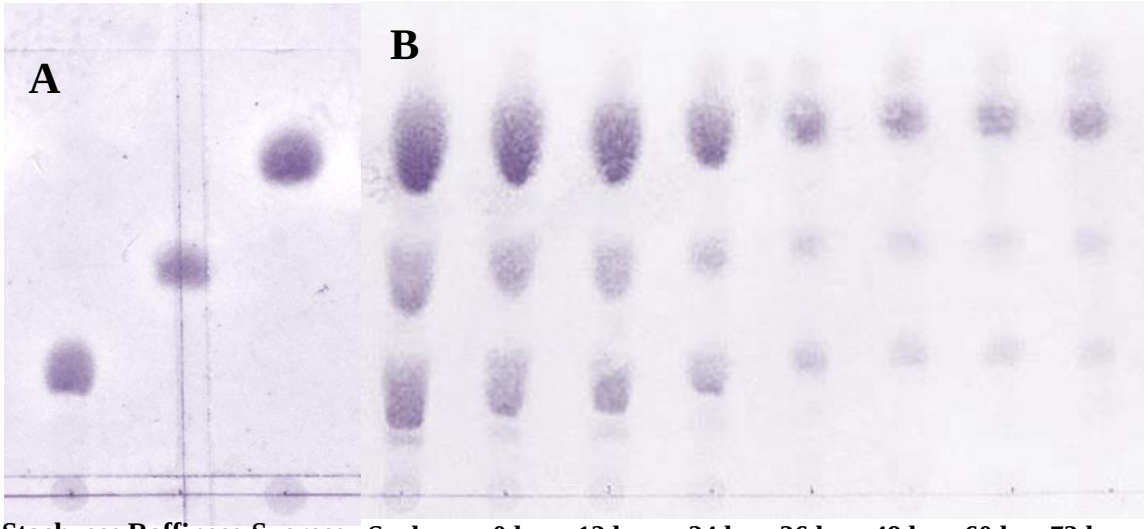

Figure 2: TLC analysis of standard oligosaccharides (A) and soybean samples (B)

Table 1 showed that the raffinose and stachyose level in the soybeans were 1.05±0.07 and 4.75±0.09%, respectively. This is in agreement with the previous study which reported that the raffinose and stachyose contents were 0.99 and 4.87% in soybean seeds (Abdullah *el al.*, 1984). The sucrose content of soybean seeds was 5.96±0.08% (Table 1) and was in range of 2.69–8.63% that resulted from the study on the sucrose content of 14 soybean genotypes (Teixeira *el al.*, 2012). Other authors found that the ranges of raffinose, stachyose and sucrose of three soybean cultivars were 0.75–1.16, 3.28–4.09 and 3.97–4.81%, respectively (Silva *el al.*, 1990).

Table 1: Changes in sugars contents and α-galactosidase activity during germination of soybeans

Germination time (hours)	Raffinose (%)	Stachyose (%)	Sucrose (%)	Reducing sugar (%)	α-galactosidase activity (U/100 g)
Soybeans	1.05±0.07^{a}	4.75±0.09^{a}	5.96±0.08^{a}	2.50±0.18^{f}	47.8±1.1^{f}
0 (soaked)	0.95±0.02^{b}	4.45±0.09^{b}	5.79±0.08^{a}	2.41±0.18^{f}	87.9±1.1^{bc}
12	0.86±0.02^{c}	3.78±0.04^{c}	5.77±0.06^{a}	3.14±0.09^{e}	164.3±2.5^{a}
24	0.66±0.02^{d}	3.14±0.03^{d}	5.16±0.07^{b}	3.45±0.10^{e}	93.8±7.1^{b}
36	0.44±0.02^{e}	2.53±0.02^{e}	2.67±0.09^{c}	5.93±0.17^{c}	84.2±2.1^{cd}
48	0.34±0.02^{f}	1.99±0.01^{f}	2.47±0.06^{c}	6.32±0.13^{b}	85.2±6.3^{cd}
60	0.27±0.01^{g}	1.55±0.04^{g}	2.35±0.07^{c}	7.88±0.14^{a}	74.5±4.4^{e}
72	0.21±0.01^{g}	1.15±0.02^{h}	2.34±0.04^{c}	5.22±0.14^{d}	78.1±2.8^{de}

Data are expressed as mean ± standard deviation (with n=3). In a column, values followed by the same letter are not significantly different (p< 0.05) by LSD test.

The activity of α-galactosidase was detected in the seeds even before imbibitions, suggesting that this enzyme is pre-existing and present in the ripe seeds (Lahuta *el al.*, 2000; Ataíde *el al.*, 2013). In the present study, the α-galactosidase activity was 47.8±1.1 U/100g. The α-galactosidase activity was found 36 U/100g in soybean seeds (McCleary and Matheson, 1974). Soaking may activate α–galactosidase, leading to the breakdown of the oligosaccharides (Rakshit *el al.*, 2015). Indeed, after soaking, α–galactosidase activity increased 1.84 times compared to that in the soybean seeds (Table 1). From the spots on TLC, it is evident that the raffinose and stachyose contents decreased after soaking. The percent of loss of raffinose and stachyose after soaking were 9.2 and 6.4%, respectively. The losses of raffinose and stachyose in the soaking water were represented 3.32 and 0.37% of these contents in starting soybeans (Wang *el al.*, 2007). However, according to other result, up to 56.3% of oligosaccharides in soybeans was removed by soaking in water for 12 hours (Han and Baik, 2006). Significant reduction of oligosaccharides by soaking has also been reported in cowpea, mung bean, chickpeas, yellow peas, green peas and soybeans (Han and Baik, 2006; Agbenorhevi *el al.*, 2007; Tajoddin *el al.*, 2010b).

Another reason for the reduction in oligosaccharide content after soaking of soybeans may be leaching of oligosaccharides into soaking water. When soybean is soaked, the water is absorbed into the bean, the oligosaccharides dissolved and leached into the surrounding water (Han and Baik, 2006; Agbenorhevi *el al.*, 2007).

The statistical results from Table 1 and the spots in Figure 2B demonstrated that raffinose and stachyose contents of soybeans significantly decreased ($p < 0.05$) during germination. As the period of germination was prolonged, significant and successive reduction in oligosaccharides was observed. Highest loss of raffinose and stachyose was observed in soybeans respectively after 72 hours of germination, which was corresponding to 79.6 and 75.8%. Diminishing effect of germination on raffinose and stachyose has been noticed for black gram, mung bean, cowpeas and other legume seeds (Reddy and Salunkhe, 1980; Alani *el al.*, 1990; Oboh *el al.*, 2000; Tajoddin *el al.*, 2010a). During germination, raffinose and stachyose levels decreased in all three soybean cultivars, and it was possible to measure small amounts of these oligosaccharides after six days of germination (Silva *el al.*, 1990). In other studieson soybean germination, raffinose was concluded to disappearon the fourth day (East *el al.*, 1972) and on the third day (Chen and Luh, 1976), and stachyose was found to disappear on the sixth day (East *el al.*, 1972) and on the fourth day (Chen and Luh, 1976). The wide variance in the findings may be due to differences in type of bean, conditions of germination or activity of enzymes, particularly α-galactosidase (Abdullah *el al.*, 1984). The increase in α-galactosidase activity in soybeans during early stage of germination in the present study was able to explain the degradation of indigestible oligosaccharides. The highest activity of α-galactosidase in germinated soybeans was 164.3±2.5 U/100g corresponding to the 12-hour germination. After that, it reduced significantly, however, the activity of α-galactosidase in soybeans after 72 hours of germination was still significantly higher than that of soybean seeds (Table 1). The observed rule of α-galactosidase activity change during germination is similar to that of cowpeas (Agbenorhevi *el al.*, 2007), rosewood seeds (Carrijo *el al.*, 2010)and black gram seeds(Reddy and Salunkhe, 1980). The α-galactosidase catalyzed the hydrolytic removal of α–1,6–linked–galactose residues from simple oligosaccharides including stachyose and raffinose forming sucrose that can be further hydrolyzed to obtain glucose and fructose (Zhang *el al.*, 2015). The breakdown of oligosaccharides by active α-galactosidases in seeds took place during germination and even soaking stage before germination (Blöchl *el al.*, 2008; Tajoddin *el al.*, 2010a). The above mechanism of reaction and the rule of α-galactosidases activity change explained why the sucrose content remained high and unchanged after soaking and in the early stages of germination (12 hours from beginning), after that the sucrose content decreased significantly (Table 1 and Figure 2B). Sucrose content in barley was unchanged during first 2 day germination, but it diminishes markedly after 3 days (MacLeod *el al.*, 1953). Some authors reported an increase in sucrose content during the first stage of germination in black gram (Reddy and Salunkhe, 1980) and mung beans (Tajoddin *el al.*, 2010a). Alani*el al.* (1990) stated that sucrose content in cowpeas declined during the first 6 hours of germination, then increased after 24 hours of germination (Alani *el al.*, 1990). The alteration in sucrose content during germination was the result from the following events: the hydrolysis of raffinose and stachyose forming sucrose by α-galactosidase enzyme; the hydrolysis further of sucrose forming glucose and fructose used as energy-source for the projection of the radicles and promote development of seedlings (Sett, 2016). In the first stage, the α–galactosidase activity and content of oligosaccharides were high, so the hydrolysis of oligosaccharides to form sucrose predominated that resulted in high content of sucrose. After this stage, oligosaccharides content decreased the hydrolysis of sucrose to form glucose, and fructose became remarkable. The hydrolysis of oligosaccharides as well as sucrose led to increase in reducing sugars during germination. The reducing sugars content in soybeans was highest after 72 hours of germination, which increased 2.1 times compared to that of soybean seeds (Table 1). This was similar to results reported for fenugreek seeds during 96 hours of germination (El-Mahdy and El-Sebaiy, 1983).

Germination reduced indigestible oligosaccharides contents in soybeans, and there is a positive correlation between the decline in oligosaccharides contents and the depression of seed longevity (Górecki *el al.*, 2001). Evaluation the degradation efficiency of indigestible oligosaccharides during germination is important for estimation of the changes their contentsand the rate of reaction. These changes can be described by the application of the following zero order (Eq. I), first order (Eq. II) orsecond order (Eq. III) equations(Kadlec *el al.*, 2008).

$$C = C_o - kt \quad \text{(Eq. I)}$$

$$C = C_o\exp(-kt) \quad \text{(Eq. II)}$$

$$1/C = 1/C_o + kt \quad \text{(Eq. III)}$$

where, C_o– initial content (%); t – germination time (min); k – reaction rate constant (per min) and C–calculated content (%).

Table 2: Correlation coefficients calculated for the equations applied to oligosaccharides degradation during soybean germination

Raffinose			Stachyose		
Eq.	**Models**	**R^2**	**Eq.**	**Models**	**R^2**
(I)	$C = 0.93434 – 0.01123 \times t$	0.95	(I)	$C = 4.312 – 0.0462 \times t$	0.99
(II)	$C = 1.04 \exp(–0.02284 \times t)$	0.97	(II)	$C = 4.74 \exp(–0.0188 \times t)$	0.99
(III)	$1/C = 0.585 + 0.0543 \times t$	0.93	(III)	$1/C = 0.151 + 0.0086 \times t$	0.92

Table 2 showed the correlation coefficients (R^2) calculated using all equations. In case of raffinose, the best approximation ($R^2 = 0.97$) was achieved using Eq. II, and the fitted models used for estimation stachyose content were Eq. 1 and Eq. 2 ($R^2 = 0.99$). The changes in total oligosaccharides content with germination time for mung bean and lentil were simulated by Eq. I ($R^2 = 0.96$ and 0.95, respectively) and for chickpea by Eq. II ($R^2 = 0.97$)(Kadlec *el al.*, 2008). In the present study, there was not any model that could be stimulated the changes in sucrose and reducing sugar contents as well as α–galactosidase activity with germination time. Besides, there was no relationship between α–galactosidase activity and oligosaccharides contents.

4 CONCLUSIONS

Germination causes significant decreases in indigestible oligosaccharides. This desirable change is mostly due to the action of α–galactosidase in soybean seeds which was activated during soaking and germinating. The first order equation was the best approximation for the degradation of both raffinose and stachyoseduring soybean germination. The hydrolysis of undigestible oligosaccharides leading to increase in reducing sugars was used as energy-source for the development of seedlings. Reduction of these undesirable components by germination promises an effective method for reducing both flatus-causing factors and antinutrients in soybeans. Germination is one of the most promising methods for reducing flatus-causing factors and improving nutritional value for soybeans.

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REFERENCES

Abdullah, A., Baldwin, R. E. and Minor, H., 1984. Germination effects on flatus-causing factors and antinutrients of mungbeans and two strains of small-seeded soybeans. Journal of Food Protection. 47(6): 441-444.

Agbenorhevi, J. K., Oduro, I., Ellis, W. O., Abodakpi, V. D. and Eleblu, S. E., 2007. Effect of soaking, autoclaving and repeated boiling on oligosaccharides in cowpea. Nigerian Food Journal.25(2): 88-94.

Akinyele, I. O. and Akinlosotu, A., 1991. Effect of soaking, dehulling and fermentation on the oligosaccharides and nutrient content of cowpeas *(Vigna unguiculata)*. Food Chemistry. 41(1): 43-53.

Alani, S. R., Smith, D. M. and Markakis, P., 1990. Changes in alpha-galactosidase activity and oligosaccharides during germination and incubation of cowpeas (*Vigna unguiculata*). Food Chemistry. 38(2): 153-158.

Ataíde, G. d. M., Gonçalves, J. F. d. C., Guimarães, V. M., Bicalho, E. M. and Flores, A. V., 2013. Activities of α-galactosidase and polygalacturonase during hydration of Dalbergia nigra (*(Vell.) Fr All. ex Benth*.) seeds at different temperatures. Journal of Seed Science. 35(1): 92-98.

Bewley, J. D., 1997. Seed germination and dormancy. The Plant Cell. 9(7): 1055-1066.

Blöchl, A., Peterbauer, T., Hofmann, J. and Richter, A., 2008. Enzymatic breakdown of raffinose oligosaccharides in pea seeds. Planta. 228(1): 99-110.

Carrijo, L. C., Borges, E. E. d. L., Pontes, C. A., Lopes, M. R. and Brune, A., 2010. α-galactosidase activity and carbohydrate mobilization in seeds of *Dalbergia nigra (vell.) Allemão ex Benth.-fabaceae (Brazilian rosewood)* during germination. Cerne. 16(3): 283-289.

Chen, A. O. and Luh, B. S., 1976. Oligosaccharides and nutrients in soybean sprouts. Food Science. 3: 13-25.

Dey, P. M., Naik, S. and Pridham, J. B., 1986. Further characterization of α-galactosidase I-glycoprotein lectin from Vicia faba seeds. Phytochemistry. 25(5): 1035-1041.

Dey, P. M. and Campillo, E. D., 2006. Biochemistry of the multiple forms of glycosidases in plants. Advances in Enzymology and Related Areas of Molecular Biology. 56: 141-249.

Duong, T. P. L., Tran, M. P., Phan, T. B. T. and Ha, T. T., 2016. Time and temperature dependence of antioxidant activity from soybean seeds (*Glycine max* L. *Merr*.) during germination. International Journal of Food Science and Nutrition. 1(5): 22-27.

East, J. W., Nakayama, T. and Parkman, S., 1972. Changes in stachyose, raffinose, sucrose, and monosaccharides during germination of soybean. Crop Science. 12(1): 7-9.

Egounlety, M. and Aworh, O. C., 2003. Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max Merr*.), cowpea (*Vigna unguiculata L. Walp*)

and groundbean (*Macrotyloma geocarpa Harms*). Journal of Food Engineering. 56(2): 249-254.

El-Mahdy, A. R. and El-Sebaiy, L. A., 1983. Changes in carbohydrates of germinating fenugreek seeds *(Trigonella foenum graecum L.)*. Journal of the Science of Food and Agriculture. 34(9): 951-956.

Górecki, R., Fordonski, G., Halmajan, H., Horbowicz, M., Jones, R. and Lahuta, L., 2001. Seed physiology and biochemistry. In*:* Hedley C. L. (Ed). Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics. CABI Publishing.117-143.

Guimarães, V. M., de Rezende, S. T., Moreira, M. A., de Barros, E. G. and Felix, C. R., 2001. Characterization of α-galactosidases from germinating soybean seed and their use for hydrolysis of oligosaccharides. Phytochemistry. 58(1): 67-73.

Han, C., Yin, X., He, D. and Yang, P., 2013. Analysis of proteome profile in germinating soybean seed, and its comparison with rice showing the styles of reserves mobilization in different crops. PloS one. 8(2): 1-9.

Han, I. H. and Baik, B.-K., 2006. Oligosaccharide content and composition of legumes and their reduction by soaking, cooking, ultrasound, and high hydrostatic pressure. Cereal Chemistry. 83(4): 428-433.

Kadlec, P., Dostalova, J., Bernaskova, J. and Skulinova, M., 2008. Degradation of alpha-galactosides during the germination of grain legume seeds. Czech Journal of Food Sciences. 26(2): 99.

Lahuta, L. B., Górecki, R. J., Michalczyk, D. and Piotrowicz-Cieślak, A. I., 2000. Alpha-D-galactosidase activity in stored yellow lupin (*Lupinus luteus* (L.)) seeds. Electron Journal Polish Agriculture University. 3(1): 1-7.

MacLeod, A. M., Travis, D. and Wreay, D., 1953. Studies on the free sugars of the barley grain. Journal of the Institute of Brewing. 59(2): 154-165.

Mao, C., He, G., Du, X., Cui, M. and Gao, S., 2013. Biochemical changes in the fermentation of the soy sauce prepared with bittern. Advance Journal of Food Science and Technology. 5(2): 144-147.

Markakis, P., 1987. Tempeh prepared from germinated soybeans. Journal of Food Science. 52(6): 1736-1737.

McCleary, B. V. and Matheson, N. K., 1974. α-D-Galactosidase activity and galactomannan and galactosylsucrose oligosaccharide depletion in germinating legume seeds. Phytochemistry. 13(9): 1747-1757.

Nnanna, I. A. and Phillips, R. D., 1988. Changes in oligosaccharide content, enzyme activities and dry matter during controlled germination of cowpeas (*Vigna unguiculata*). Journal of Food Science. 53(6): 1782-1786.

Oboh, H. A., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M. M., Ayet, G. and Osagie, A. U., 2000. Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. Plant Foods for Human Nutrition. 55(2): 97-110.

Omosaiye, O., Cheryan, M. and Matthews, M., 1978. Removal of oligosaccharides from soybean water extracts by ultrafiltration. Journal of Food Science. 43(2): 354-360.

Percheron, F., 1962. Dosage colorime'trique du fructose et des fructofuranosides par l'acide thiobarbiturique. Comptes Rendus de l'Academie des Science. 255: 2521-2522.

Rackis, J. J., Honig, D. H., Sessa, D. J. and Steggerda, F. R., 1970. Flavor and flatulence factors in soybean protein products. Journal of Agricultural and Food Chemistry.

Rackis, J. J., 1981. Flatulence caused by soya and its control through processing. Journal of the American Oil Chemists' Society. 58(3): 503-509.

Rakshit, M., Sharma, A., Saha, J. and Sarkar, P. K., 2015. Optimization of soaking condition of blackgram to minimize flatogenic sugar content in blackgram-based products. LWT-Food Science and Technology. 63(2): 814-820.

Ramadan, E. A., 2012. Effect of processing and cooking methods on the chemical composition, sugars and phytic acid of soybeans. Food and Public Health. 2(1): 11-15.

Reddy, N. R. and Salunkhe, D. K., 1980. Changes in oligosaccharides during germination and cooking of black gram and fermentation of black gram/rice blend. Cereal Chemistry. 57(5): 356-360.

Reid, J. S. G. and Meier, H., 1973. Enzymic activities and galactomannan mobilisation in germinating seeds of fenugreek (*Trigonella foenum-graecum L. Leguminosae*). Planta. 112(4): 301-308.

Rezende, S. T. d., Guimarães, V. M., Rodrigues, M. d. C. and Felix, C. R., 2005. Purification and characterization of an alpha-galactosidase from *Aspergillus fumigatus*. Brazilian Archives of Biology and Technology. 48(2): 195-202.

Sett, R., 2016. Changes in levels of soluble sugar, reducing sugar and lipid during germination of seeds of *Albizia procera*. International Journal of Plant & Soil Science. 12(1): 1-15.

Shah, S. A., Zeb, A., Masood, T., *et al*., 2011. Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties. African Journal of Agricultural Research. 6(22): 5091-5098.

Silva, H. C., Braga, G. L., Bianchi, M. d. L. P. and Rossi, E. A., 1990. Effect of germination on oligosaccharide and reducing sugar contents of Brazilian soybean cultivars. Alimentos e Nutrição Araraquara. 2: 13-19.

Tajoddin, M., Shinde, M. and Junna, L., 2010a. Raffinose, stachyose and sucrose contents of mungbean cultivars differing in seed coat color from Hyderabad-Karnataka region of India: Effect of soaking and germination. The Bioscar. 5(3): 343-346.

Tajoddin, M., Shinde, M. and Junna, L., 2010b. Raffinose, starchyo and sucrose contents of mung bean

cultivars differing in seed coat color from Hyderrabad-Karnatara region of India: Effect of soaking and germination. The Bioscar. 5(3): 343-346.

Tanaka, M., Thananunkul, D., Lee, T. C. and Chichester, C., 1975. A simplified method for the quantitative determination of sucrose, raffinose and stachyose in legume seeds. Journal of Food Science. 40(5): 1087-1088.

Teixeira, A. I., Ribeiro, L. F., Rezende, S. T., Barros, E. G. and Moreira, M. A., 2012. Development of a method to quantify sucrose in soybean grains. Food Chemistry. 130(4): 1134-1136.

Wang, Q., Ke, L., Yang, D., Bao, B., Jiang, J. and Ying, T., 2007. Change in oligosaccharides during processing of soybean sheet. Asia Pacific Journal of Clinical Nutrition. 16(S1): 89-94.

Zhang, W., Du, F., Wang, L., Zhao, L., Wang, H. and Ng, T. B., 2015. Hydrolysis of oligosaccharides by a thermostable α-galactosidase from *Termitomyces eurrhizus*. International Journal of Molecular Sciences. 16(12): 29226-29235.