

Effect of germination time on proximate composition, γ amino butyric acid accumulation and trypsin inhibitor activity of soybean (*Glycine max* L. Merr.)

Musa Halilu^{1,*}, Hasanah M. Ghazali², and Yunusa Bello M.³

¹Department of Food Science and Technology, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria

²Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

³Department of Food Technology, Faculty of Agriculture and Life Science, Federal University Wukari, Taraba State, Nigeria (mybello@fuwukari.edu.ng)

*Corresponding author: Musa Halilu) email: musa.halilu@yahoo.com

Abstract

Germination is an inexpensive process that can generally improve the nutrient content, γ - amino butyric acid accumulation (GABA) and digestibility of soybean. Germinated soybean flour can be used by cereal based food processors for supplementation which may also increase the utilization of soybean. This study will provide scientific data on the effect of germination time on the nutritional composition, gamma amino butyric acid accumulation and trypsin inhibitor activity of soybean (*Glycine max* L.) which could be an added advantage to cereal based food industries especially in Northern part of Nigeria where soybean is largely cultivated. Analysis was conducted on non-germinated (control) and germinated soybean for up to 72 h. Results obtained shows that germination led to compositional changes in samples of non-germinated and germinated soybean powder where the moisture content of non-germinated soybean powder decreased from 8.67 to 4.15%, ash, crude protein and carbohydrate were increased from 5.31 to 5.77%, 27.57 to 32.01% and 31.37 to 48.39%, respectively, crude fat decreased from 26.18 to 18.02%. GABA is accumulated with increase in germination time, from 6.36 to 73.43mg/100g. Germination has also decrease trypsin inhibitor activity from 43.26mg/g in non-germinated soybean to 22.28mg/g at 72h of germination.

Keywords: gamma amino butyric acid, germination, proximate composition, trypsin inhibitor.

1. Introduction

Soybean (*Glycine max* L. Merr.) is a legume and native to East Asia. Its cultivation is now worldwide because it is an excellent source of protein and oil (Medic and Hurburgh, 2014; Kim et al., 2013). Soybean is used widely to produce many varieties of food products such as soymilk, tofu, soybean oil and soybean sprouts (Wan et al., 2015). In Nigeria, soybean grows mainly in the middle belt which accounts for 65 to 75 % of the total production in Nigeria (Finelib.com, 2017). In West Africa, Nigeria is the largest producer of soybean and second largest producer in sub-Saharan Africa (Finelib.com, 2017). As such, the development of products from germinated soybean is an important option to further increase the utilization of soybean.

Germination of soybean seeds is now receiving a lot of recognition because it is an effective way to improve the nutritional quality and flavor, and also to reduce anti-nutritional factors (Donangelo et al., 1995). Seed

Effect of germination on soybean quality

germination occurs as a naturally complex biological process that involves catabolic reactions (hydrolysis of reserve substances) and also anabolic reactions such as enzyme synthesis and repair of cellular components to produce new cells of the embryo, thus this process can be very useful at improving the nutritional value of the seeds (Paucar-Menacho et al., (2010). The use of soybean as a protein supplement is one of the methods to reduce malnutrition in developing countries like Nigeria (Kumar et al., 2006). According to Bau, Villaume, and Méjean (Bau et al., 2000), germination can induce modification of biologically active compounds, improve on palatability and nutritional value of soybean seeds. Germination has also been shown to increase protein and starch digestibility, reduce the undesirable flavour and anti-nutritional factors, improve texture and also change the biochemical and nutritional characteristics of the soybean seeds (Kim et al., 2013; Kumar et al., 2006). Besides these, germination can also increase the vitamin content of soybean such as ascorbic acid, riboflavin and thiamine (Mostafa et al., 1987). Trypsin inhibitor, an anti-nutritional factor commonly found in soybean, is heat-labile (Coscueta et al., 2017). However, heat treatment insolubilizes the valuable proteins in the soybean seed, and excessive heat treatment can cause losses of amino acids in soy proteins (Rios and Barnes 1966). The information on nutritional and chemical changes of germinated soybean could be an added advantage to processors of cereal based food products which may require supplementation from cheaper source of protein from plant origin. This study is aimed at assessing changes in the proximate composition, GABA content and trypsin inhibitor activity of soybean during germination in soybeans, the results could be adopted by food processors in Nigeria for nutritional value improvement and the utilization of germinated soybean flour for supplementation in cereal based food products.

2. Materials and methods

2.1. Materials

Soybean (*Glycine max* L.) was sorted manually to remove broken seeds and unwanted materials. All the chemicals used and standards were purchased from Supelco Chemical Co. (Bellefonte, Pennsylvania, USA), Sigma-Aldrich, Inc. (St. Louis, USA) and QREC (New Zealand) and Merck (Darmstadt, Germany).

2.2. Germination of soybean

Soybean seed was germinated in a dark at room temperature (25 ± 2 °C) according to the method described by Huang et al., (2014) with some modifications. Approximately 450 g of the soybean seeds were washed and rinsed with distilled water to remove surface dirt. The seeds were then soaked in distilled water in a covered plastic container (2.9 L capacity) containing 1500 ml of distilled water. After 12 h, the water was drained, and the seeds were rinsed with distilled water. The seeds were then spread over a sterilized wet cotton towel placed on a perforated plastic tray (38.5×23.5 cm) to allow germination to take place in a dark room for 24, 36, 48, 60 and 72 h. The control sample was the soybean seed prior to germination. Sterile distilled water was used to sprinkle the germinating seeds twice a day (morning and evening) to keep the seeds moist. Random samples (90 g) were withdrawn every 12 h of germination for analysis. Germinated samples were first washed using distilled water and rubbed gently between the palms to dehull the seeds such that the hypocotyls were not detached. The dehulled germinated seeds together with their hypocotyls and the control sample (non-germinated soybean seed washed and rinsed with distilled water) were then dried at 45 °C in an oven, (Model FD 115 Binder GmbH, Tuttlingen, Germany) for 15 h to a final moisture content between 4.5 and 6.10 %. The dried seeds were ground using a home blender (Pensonic Model PB-3205DJ, Malaysia) into powder and stored in amber bottles at room temperature (25 ± 2 °C) prior to analysis.

2.3. Determination of proximate composition

The proximate composition of the non-germinated and germinated soybean powders was analysed according to the official methods of the Association of Official Analytical Chemists (2005). The moisture content was determined at 105°C using oven drying method as described in AOAC Official Method 934.06. Crude protein (N = 6.25) was determined using the micro-Kjeldahl method according to AOAC Official Method 984.13 (A - D). Crude fat

Effect of germination on soybean quality

was determined using the Soxhlet method (AOAC, 2005) with hexane as the extracting solvent. Ash was determined using the AOAC Official Method 942.05 where the powder sample was ignited in a muffle furnace at 550 °C. Total carbohydrate was determined by difference and all determinations were done in triplicate.

2.4. Determination of γ -amino butyric acid (GABA)

GABA was determined according to the method described by Yang et al., (2015). Soybean powder was defatted using Soxhlet extraction and 2 g of the defatted powder was soaked in 70 % ethanol for 4 h. The ethanol was then evaporated off and remaining material was freeze dried. The freeze-dried powder (0.1 g) was dissolved in 1 ml of ethanol-water-triethylamine (4:4:2) solution followed by addition of 80 μ l of 95% ethanol-distilled water-triethylamine-phenylisothiocyanate solution (6:1:1:1). The mixture was incubated for 30 minutes at 25 °C and then filtered through a 0.4 μ nylon membrane filter. The standard (0.129 g GABA in 100 ml deionized water) was similarly treated as the sample. Then, 10 μ l each of the filtrate (samples and standard) was injected into a Waters Model 2695 (Waters Corporation, Milford, MA, USA) HPLC equipped with a UV detector Model 2487 set at 254 nm, a Model 2695 pump and fitted with a RP-18 column (5 μ m HX258816 Purospher® STAR analytical column, Merck KGaA, Darmstadt, Germany). Separation was carried out with 80% solution A (0.1M sodium acetate buffer-triethylamine-acetonitrile, pH 5.8) and 20% solution B (acetonitrile: water, 60:40) at a flow rate of 0.8 ml/min. The pH of the solutions was adjusted to 5.8 with 0.1M NaOH, the solutions were then filtered through a 0.45 μ m membrane using filtering apparatus and degassed using a sonicator. The column temperature was set at 40°C.

2.5. Determination of trypsin inhibitor activity

Trypsin inhibitor activity was determined based on the method described by Maetens et al., (2017) where 1 g of each sample powder was mixed with 10 ml of 0.01 mol/L NaOH with constant stirring using an orbital shaker for 3 h. Then, 1.4 ml of the mixture was made up to 2 ml using deionized water such that 2 ml of the sample extract inhibits 40-60 % of the trypsin used as standard. Next, 2 ml of each sample extract was placed in test tubes. To the test tubes, 2 ml of trypsin solution (prepared by dissolving 4 mg of Bovine pancreas trypsin [\geq 10,000 BAEE units/mg protein. T1426 Merck, Germany] in 200 ml of 1 mM HCl) was added. The test tubes were maintained at 37 °C in a water bath for 10 minutes. This was followed by rapid addition and mixing of 5 ml N α -Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPA) solution (prepared by dissolving 40 mg BAPA in 100 ml of 0.05 mol/L Tris buffer containing CaCl₂, pH 8.2) to each test tube. The test tubes were immediately placed in a water bath at 37 °C. The reaction was terminated after exactly 10 min by rapid addition of 1 ml of 30 % acetic acid. A test tube containing sample extract (sample blank) was prepared by the same procedure, except that the trypsin solution was added after the addition of 30 % glacial acetic acid. The absorbance of each solution was measured at 410 nm using spectrophotometer (Model 4001-04 Thermo spectronic Genesys 20, USA). Trypsin inhibitor (TI) was calculated as $TI \text{ (mg/g)} = \text{absorbance of standard} - \text{absorbance of sample} \times \text{dilution factor}/19$

2.6. Statistical analysis

All measurements were done in triplicate and values were presented as mean \pm standard deviation of triplicate determination. One-way analysis of variance (ANOVA) was used to identify the significant differences among the means of different samples and Turkey test was also used to compare the means of the obtained values. The statistical analysis was carried out using Minitab version 17 (Minitab Pty Ltd, Sydney). Probability level of $P < 0.05$ (95% confident level) was identified as significant.

3. Results and Discussion

The result of germination time and the length of hypocotyl produced as compared to non-germinated soybean (control) is presented in Figure 1. The results indicate that as germination time increases, the length of hypocotyl produced is increased from 0.9 cm in the non-germinated seed to 9.8 cm at 72 h of germination time.

Effect of germination on soybean quality

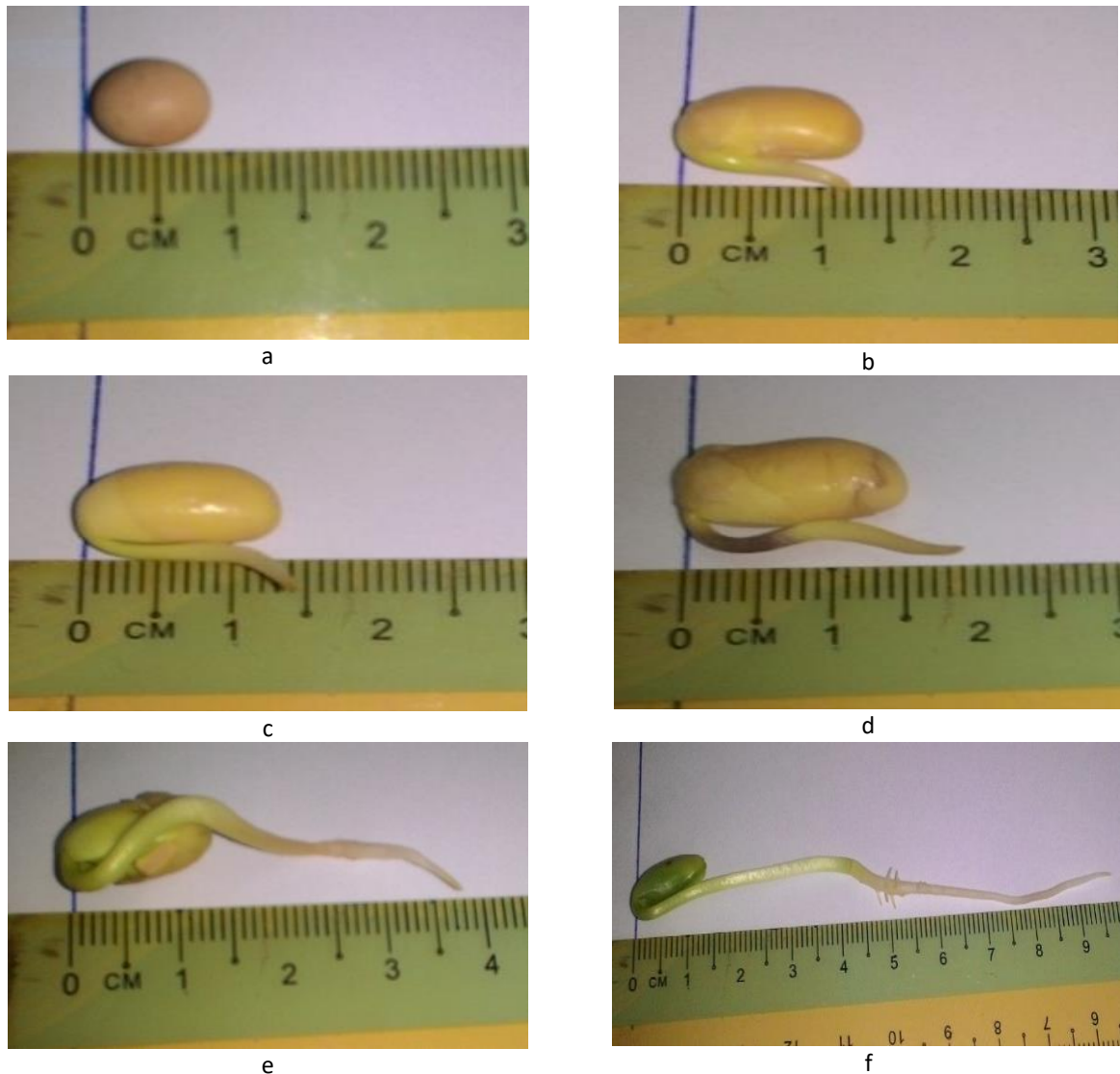


Figure 1: Result of soybean germination (a = 0, b = 24, c = 36, d = 48, e = 60 and f = 72 h, respectively)

3.1. Proximate analysis of soybean powder samples

The results for the proximate composition on dry weight bases of the non-germinated and germinated soybean powder samples is presented in Table 1. The moisture content of the samples ranges from 4.15 to 8.7 %, there is significant difference ($P < 0.05$) between moisture content of samples. The control sample (0 h, non-germinated) had the highest moisture content while the sample germinated after 60 h had the lowest moisture content 4.2 %. The decrease in moisture observed may be due to evaporation during drying process. The crude protein content increased significantly ($P < 0.05$) after 24 h of germination to 32.0 % when compared with the non-germinated sample (27.6 %). The increase in the crude protein content could be attributed to the synthesis of several enzymes which resulted in the production of some non-protein nitrogen containing compounds in the seeds (Moongngarm and Saetung, 2010). It was also observed that as germination progresses, there was a significant decrease ($P < 0.05$) in the protein content of the seeds as evident from 36 h of germination, this may be as a result of leaching of soluble fractions (non-protein nitrogen fractions) during the soaking period as observed by (Mostafa et al., 1987), they reported a slight decrease in total protein during germination of soybean for 6 days and attributed the decrease to leaching of soluble fractions (non-protein nitrogen fractions) during the soaking period. On the other

hand, (Donangelo *et al.* 1995) observed small increase in crude protein after germinating soybean seeds for 48 h. Changes in protein content during germination could be related to protein hydrolysis, synthesis, and re-arrangement. According to (Rodrigues *et al.*, 2009), seeds germination involves mobilization of the protein reserves in cotyledons, coupled with the synthesis of new proteins, necessary for sprout growth. Furthermore, the amino acids produced by hydrolysis of the protein reserves are not used solely in synthesizing new components, but may also be used as an energy source (Chen *et al.*, 1975).

The ash content remains almost the same with slight increase, while the total crude fat content decreased significantly as germination progresses, the lowest value of crude fat content (18.0 %) was obtained after 72h of germination when compared with the non-germinated sample 26.2 %. The decrease could be attributed to the fact that fat may be hydrolysed during germination and used to produce the required energy for the biochemical and physicochemical modifications that occur during germination, (Hahma *et al.*, 2009). Mostafa *et al.*, (1987), also observed a significant change in the oil content during germination of soybean especially in the 5 and 6 days of germination. Kornberg and Beevers (1957), also reported that the depletion of nutrients (Lipids and Carbohydrates) during germination is a process that provides the energy for protein synthesis in plant growth.

The carbohydrate content of the dried germinated soybean powder samples in this study showed a significant increase ($P < 0.05$) after 72h of germination. The value increased from 32.3 % to 49.1 % at 72h germination. The increased could be as a result of the low crude fat and protein which might have been used as source of energy as observed by (Donangelo *et al.*, 1995), instead more of the crude fat and protein were used as evident from the results obtained which recorded a decrease in crude fat and also in protein. Similarly, Tiansawang *et al.*, (2016), also observed that there was no significant change in the carbohydrate content of germinated soybean, black bean and sesame after 48 h of germination which indicates that carbohydrate was not used up as energy source. Suda *et al.*, (1986), also reported that despite the large increase in β -amylase activity as recorded, an increase in starch content in soybean cotyledons during germination was recorded. Kuo *et al.*, (1990), investigated sugar metabolism in germinated soybean seeds and concluded that sucrose must be metabolized into simple sugars, a process that is accomplished by a large increase in glucose and fructose in the tissue.

3.2. Gamma amino butyric acid (GABA) composition

Gamma aminobutyric acid (GABA), is a type of non-protein and water-soluble amino acid that is widely distributed in nature and present in high concentrations in mammalian brains (Kim *et al.*, 2013). GABA is demonstrated to possess many physiological functions; it is an inhibitory neurotransmitter in the mammalian central nervous system (François *et al.*, 2017). Many foods rich in GABA has various functions such as improving cerebral function, regulating blood pressure, and ameliorating type-II diabetes (Abdou *et al.*, 2006, Imam *et al.*, 2012; Adeghate and Ponery, 2002). Today because of the many physiological functions of GABA, it is becoming a trend in the development of pharmaceutical and functional foods enriched with GABA through fermentation process (AbKadir *et al.*, 2016).

The GABA analysis of the non-germinated (0 h) which is the control and the germinated soybean has indicated that GABA is accumulated with increase in germination time, from 6.36 to 73.43 mg/100 g, Figure 2. The chromatograms are also presented in Figure 3. The content of GABA has been reported to have significantly increased during germination in soybean, this may imply that germinated soybean could be a good source of GABA Wan *et al.*, (2015; Quinhone and Ida, 2015).

The GABA content increased from 6.36 mg/100 g in the non-germinated soybean to 73.43 mg/100 g at 60 h of germination which is the maximum accumulation time in this research. However, when germination time was extended to 72 h the GABA content decreased to 60.20 mg/100 g. similar finding was also reported by Luo *et al.*, (2018), they observed a decrease in GABA after 5 days of germination of soybean which maybe an indication that Glutamic acid decarboxylase (GAD) activity has increased and reach its peak at 5 days. Then the enzyme and gene related to degradation of GABA may have been activated. Wan *et al.*, (2015), also reported a similar finding in germinated three Chinese soybean cultivars, where the GABA content increase to a maximum at day 5 of germination and decrease thereafter. Glutamic acid decarboxylase (GAD, EC 4.1.1.15) and diamine oxidase (DAO, EC 1.4.3.6) are the enzymes, responsible for the GABA accumulation (Yang *et al.*, 2011). GABA is biosynthesized by glucose-based fermentation from glutamic acid catalyzed by glutamic acid decarboxylase (Yang *et al.*, 2016).

Effect of germination on soybean quality

The activity of DAO is also reported to have increased in germinated fava bean (Yang et al., 2011). This indicates that the accumulation of GABA in germinated edible seeds could be due to the increased in activity of endogenous enzymes involved in the metabolic pathways of GABA. GABA can also be transformed via γ -aminobutyraldehyde intermediate from polyamine, in this process diamine oxidase (DAO) and also aminoaldehyde dehydrogenase (AMADH) are the endogenous enzymes involved (Shelp et al., 2012)

Table 1: Proximate composition of non-germinated (control) and germinated soybean powder (dry weight basis) %

Germination time (h)	Moisture	Ash	Protein	Fats	Fiber	Carbohydrate
0 (control)	8.7 ± 0.35 ^a	5.3 ± 0.01 ^b	27.6 ± 0.29 ^b	26.2 ± 0.15 ^a	0.9 ± 0.08 ^a	32.3 ± 0.01 ^e
24	6.1 ± 0.72 ^b	5.3 ± 0.01 ^b	32.0 ± 0.81 ^a	24.3 ± 0.17 ^b	0.9 ± 0.01 ^a	32.3 ± 0.01 ^e
36	5.2 ± 0.17 ^{bcd}	5.4 ± 0.24 ^{ab}	25.0 ± 0.12 ^{bcd}	20.7 ± 0.35 ^c	0.9 ± 0.02 ^a	43.8 ± 0.05 ^c
48	5.4 ± 0.23 ^{bc}	5.7 ± 0.01 ^{ab}	23.5 ± 0.46 ^{cd}	20.5 ± 0.04 ^c	0.9 ± 0.03 ^a	44.9 ± 0.01 ^b
60	4.2 ± 0.35 ^d	5.8 ± 0.19 ^a	26.6 ± 0.93 ^{bc}	20.4 ± 0.53 ^c	0.9 ± 0.02 ^a	43.2 ± 0.02 ^d
72	4.8 ± 0.17 ^{cd}	5.8 ± 0.20 ^a	22.3 ± 0.12 ^d	18.0 ± 0.03 ^d	0.7 ± 0.01 ^b	49.1 ± 0.01 ^a

Means within the same Colum having a common superscript are not significantly different ($P \geq 0.05$), Control= (non-germinated soybean)

3.4. Changes in trypsin inhibitor activity with germination time in soybean samples

Trypsin inhibitor is a type of serine protease *inhibitor* that reduces the biological activity of *trypsin* (an enzyme involves in protein digestion), by forming a trypsin enzyme – trypsin inhibitor complex which is irreversible. This leads to reduction in the function of trypsin as an enzyme to digest protein (Embaby, 2010). The presence of trypsin inhibitor in soybean directly affects the digestibility of soybean protein when consumed. The changes in trypsin inhibitor activity with germination time is presented in Table 2. Results obtained indicates that trypsin inhibitor activity was gradually reduced when the time of germination is increased from 43.26 mg/g in non-germinated soybean to 22.28 mg/g after 72h of germination. This observation was also reported by Maetens et al., (2017) where they observe a reduction in trypsin inhibitor content of germinated soybean flours for 1, 3- and 5-days germination time with a range of 16.6 and 31.1% reduction. They also reported that the non-germinated soybean had a trypsin inhibitor content of 39.8 mg/g.

Similarly, Maetens e al., (2018), also reported a significant reduction in trypsin inhibitor activity of 5 days germinated soy flour treated using UV and IR treatment. (Kumar et al., 2006), also reported reduction in trypsin inhibitor activity of soybean seeds germinated from 0 to 144 h at 25 and 35 °C, a reduction from 85.2 to 46.3 mg/g and 85.2 to 32.3 mg/g, respectively. Bau et al., (1997), showed that germination can reduce the trypsin inhibitor activity in soybean. Similarly, Donangelo et al., (1995), also reported a reduction in trypsin inhibitor by 20 and 25 % for black beans and soybeans, respectively after 24 h germination. This shows that germination can reduce trypsin inhibitor to a highest similar level as obtained in this research.

Table 2: Trypsin inhibitor activity of soybean during germination (mg/ g)

Germination time (h)	Trypsin inhibitor activity
0 (control)	43.26±0.46 ^a
24	41.16±0.06 ^b
36	33.87±0.11 ^c
48	30.63±0.24 ^d
60	26.46±0.07 ^e
72	22.28±0.03 ^f

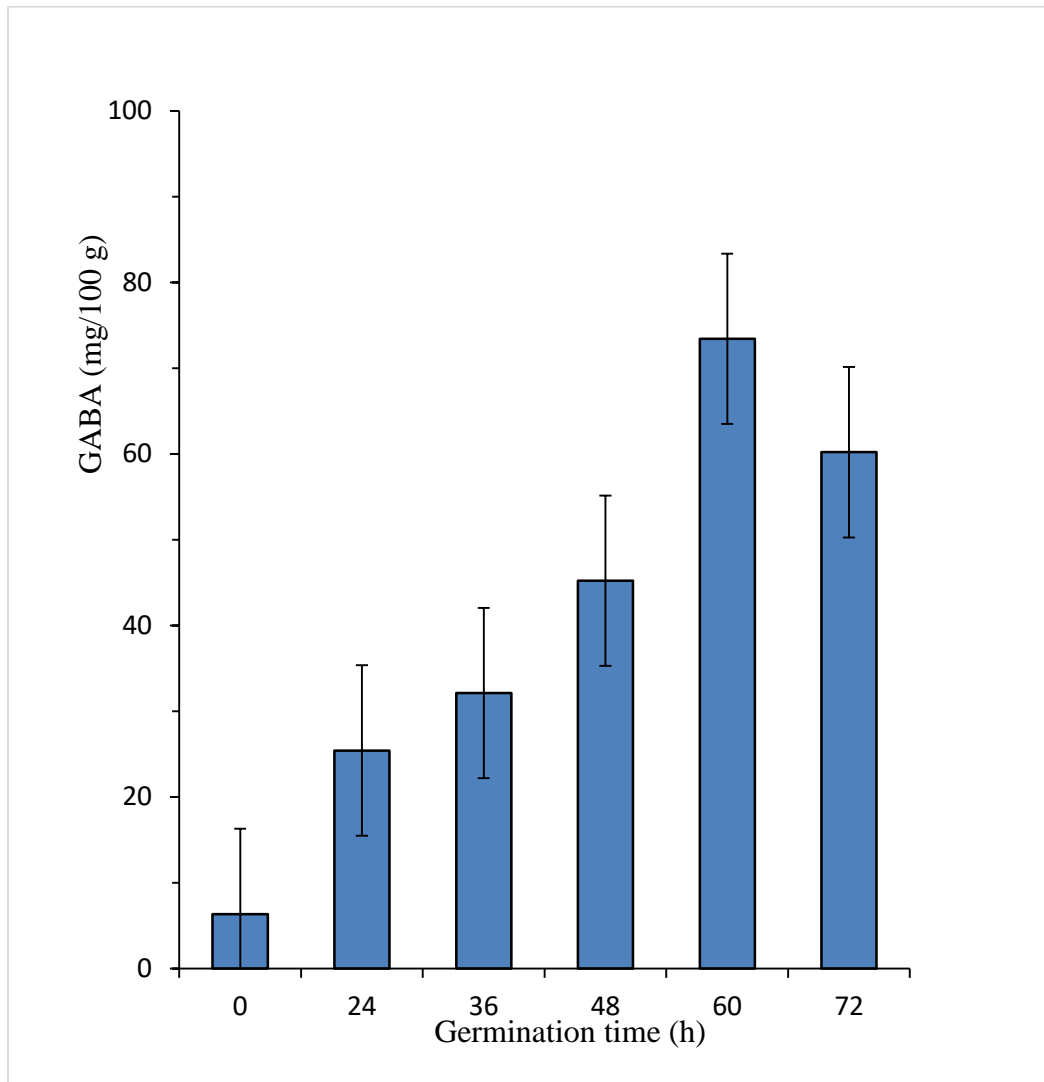


Figure 2: Effect of germination time on GABA content of soybean

4. Conclusion

This study has shown that germination can improve the nutritional composition of soybean. The study compared non-germinated soybean and germination from 0 to 72 h. Results obtained has indicated that 24 h germination was the best in terms of increase in crude protein content. Thus, this germination time could be adopted by food processors in Nigeria for nutritional value improvement and the utilization of germinated soybean flour for supplementation in cereal based food products. There was significant improvement in gamma amino butyric acid accumulation, a positive indication that germinated soybean could be used in functional foods and also additional information to food processors in Nigeria. Germination significantly ($P \leq 0.05$) decreased crude fat from 26.18 to 18.02 %, this suggest that germination could be an advantage to reduce fat content of soybean at the same time increasing carbohydrate content. Germination has also reduced the trypsin inhibitor activity in soybean, this information may increase the utilization of germinated soybean in Africa especially Nigeria.

Effect of germination on soybean quality

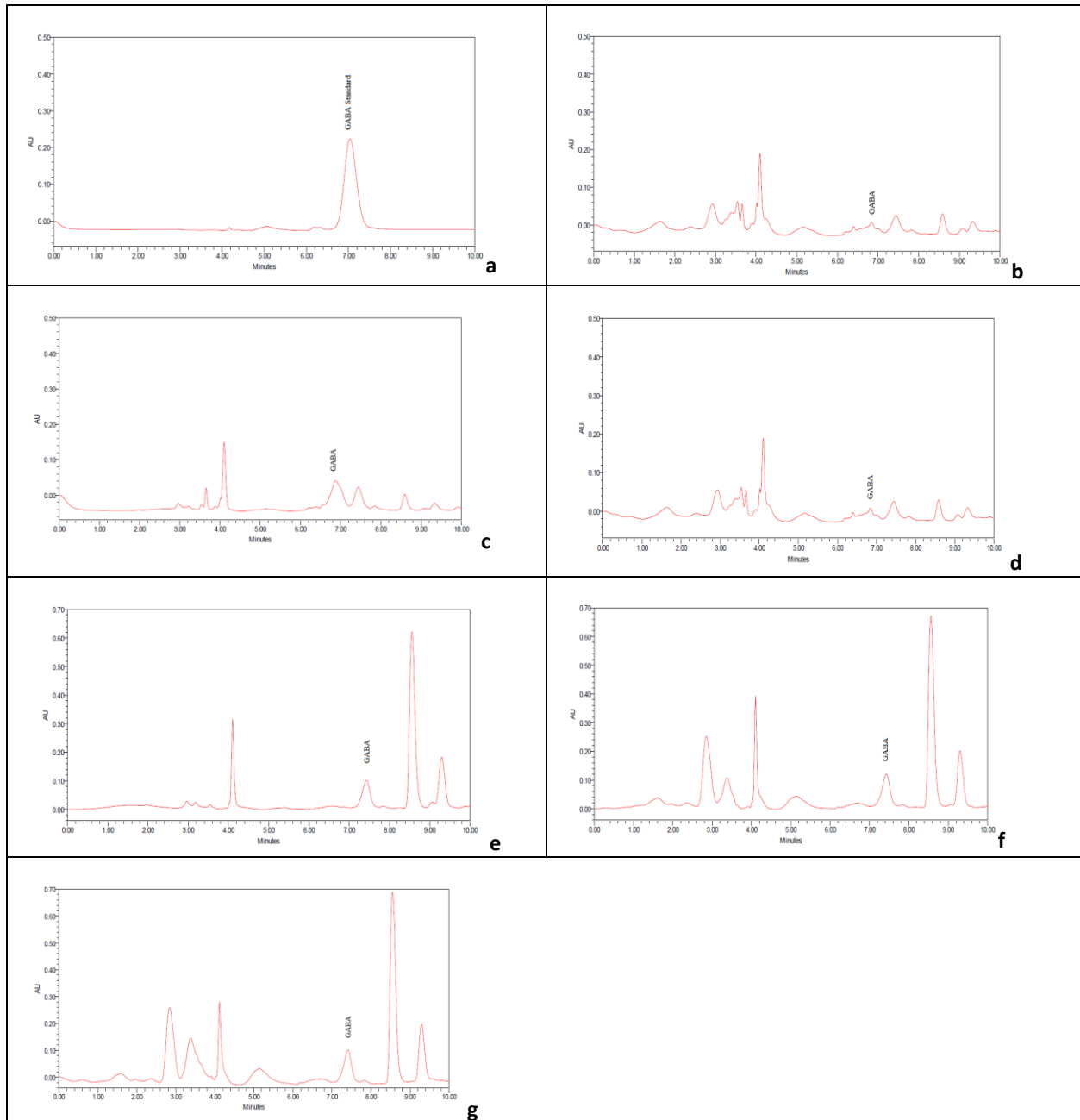


Figure 3: Chromatogram for GABA content of non-germinated and germinated soybean, a= GABA Standard, b= 0 h (non-germinated), c= 24, d= 36, e= 48, f= 60 and g= 72 h, respectively.

References

- Abdou, A. M., Higashiguchi, S., Horie, K., Kim, M, Hatta, H, and Yokogoshi, H. (2006). Relaxation and immunity enhancement effects of γ -Aminobutyric acid (GABA) administration in humans. *Biofactors*, 26(3), 201-208.
- AbKadir, S, Wan, WAA. Q. I., Mohammad, R., Lim, S. A. H., Mohammed, A. S., and Saari, N. (2016). Evaluation of commercial soy sauce koji strains of *Aspergillus oryzae* for γ -aminobutyric acid (GABA) production. *Journal of*

- Industrial Microbiology and Biotechnology*, 43(10), 1387-1395.
- Adeghate, E, and Ponery, AS. (2002) GABA in the endocrine pancreas: cellular localization and function in normal and diabetic rats. *Tissue and Cell*, 34(1), 1-6.
- AOAC International (2005) Official Methods of Analysis of AOAC International (18th Ed.). Gathersburg, MD, USA: Association of Analytical Communities.
- Bau, HM, Villaume, C, and Méjean, L. (2000) Effects of soybean (*Glycine max*) germination on biologically active components, nutritional values of seeds, and biological characteristics in rats. *Die Nahrung*, 44(1), 2–6.
- Bau, HM., Villaume, C, Nicolas, JP., and Méjean, L. (1997) Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. *Journal of the Science of Food and Agriculture*, 73(1), 1-9.
- Chen, LH, Wells, CE, & Fordham, JR. (1975) Germinated seeds for human Consumption. *Journal of food science*, 40(6), 1290-1294.
- Coscueta, ER, Pintado, ME, Picó, GA, Knobel, G, Boschetti, CE, Malpiedi, LP, and Nerli, BB. (2017) Continuous method to determine the trypsin inhibitor activity in soybean flour. *Food chemistry*, 214, 156-161.
- Donangelo, C. M., Trugo, L. C., Trugo, N. M. F., and Eggum, B. O. (1995). Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats. *Food chemistry*, 53(1), 23-27.
- Embaby, HES. (2010) Effect of heat treatments on certain antinutrients and in vitro protein digestibility of peanut and sesame seeds. *Food Science and Technology Research*, 17(1), 31-38.
- Finelib.com (2017). Groundnut producing states in Nigeria and its commercial uses. <https://www.finelib.com/about/nigeria-cash-crops/groundnut...in-nigeria...uses/90> Retrieved on 01 July, 2018.
- François, A., Low, SA, Sypek, EI, Christensen, AJ, Sotoudeh, C, Beier, KT, and Delp, SL. (2017) A brainstem-spinal cord inhibitory circuit for mechanical pain modulation by GABA and enkephalins. *Neuron*, 93(4), 822-839.
- Hahma, T, Park, SJ, and Lo, YM. (2009). Effects of germination on chemical composition and functional properties of sesame (*Sesamun indicum* L.) seeds. *Bioresource Technology*, 100, 1642–1647.
- Huang, X, Cai, W, and Xu, B (2014) Kinetic changes of nutrients and antioxidant capacities of germinated soybean (*Glycine max* L.) and mung bean (*Vigna radiata* L.) with germination time. *Food Chemistry*, 143, 268-276.
- Imam, MU, Azmi, NH, Bhangar, MI, Ismail, N, and Ismail, M. (2012). Antidiabetic properties of germinated brown rice: a systematic review. *Evidence-Based Complementary and Alternative Medicine*, 2012, 1-12.
- Kim, SL, Lee, JE, Kwon, YU, Kim, WH, Jung, GH, Kim, DW, and Hwang, TY. (2013) Introduction and nutritional evaluation of germinated soy germ. *Food Chemistry*, 136(2), 491-500.
- Kornberg, HL, and Beevers, H. (1957) A mechanism of conversion of fat to carbohydrate in castor beans. *Nature*, 180(4575), 35-36.
- Kumar, V, Rani, A, Pandey, V, and Chauhan, GS. (2006) Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures. *Food Chemistry*, 99(3), 563-568.
- Kuo, TM., Doehlert, DC., and Crawford, CG (1990) Sugar metabolism in germinating soybean seeds: evidence for the sorbitol pathway in soybean axes. *Plant Physiology*, 93(4), 1514-1520.
- Luo, Y, Liang, J, Zeng, G, Chen, M, Mo, D, Li, G, and Zhang, D. (2018). Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. *Waste Management*, 71, 109-114.
- Maetens, E, Hettiarachchy, N., Dewettinck, K., Horax, R., and Moens, K. (2018) Reductions of anti-nutritional factors of germinated soybeans by ultraviolet and infrared treatments for snack chips preparation. *LWT-Food Science and Technology*, 90, 513-518.
- Maetens, E, Hettiarachchy, N, Dewettinck, K, Horax, R, Moens, K, and Moseley, DO. (2017) Physicochemical and nutritional properties of a healthy snack chip developed from germinated soybeans. *LWT-Food Science and Technology*, 84, 505-510.
- Medic, J, Atkinson, C, and Hurburgh Jr, CR. (2014) Current knowledge in soybean composition. *Journal of the American Oil Chemists' Society*, 91(3), 363-384.
- Moongngarm, A, and Saetung, N (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chemistry*, 122(3), 782-788.
- Mostafa, MM, Rahma, EH, and Rady, AH. (1987) Chemical and nutritional changes in soybean during germination. *Food Chemistry*, 23(4), 257–275.

Effect of germination on soybean quality

- Mostafa, MM, Rahma, EH, and Rady, AH. (1987). Chemical and nutritional changes in soybean during germination. *Food Chemistry*, 23(4), 257–275.
- Paucar-Menacho, LM, Berhow, MA, Mandarino, JMG, Chang, YK, and De Mejia, EG. (2010) Effect of time and temperature on bioactive compounds in germinated Brazilian soybean cultivar BRS 258. *Food Research International*, 43(7), 1856-1865.
- Quinhone Junior, A. and El Ida (2015) Profile of the contents of different forms of Soybean isoflavones and the effect of germination time on these compounds and physical parameters in soybean sprouts. *Food Chemistry* 166(2015), 173- 178.
- Rios, I, & Barnes, RH. (1966. The effect of overheating on certain nutritional properties of the protein of soybeans. *Food Technology*, 20, 835-838.
- Rodrigues, RR, Lima, RAF, Gandolfi, S and Nave, AG. (2009). On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. *Biological Conservation*, 142:1242 – 1251.
- Shelp, BJ, Bozzo, GG, Trobacher, CP, Zarei, A., Deyman, KL. and Brikis, CJ. (2012) Hypothesis/review: Contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. *Plant Science*, 193, 130-135.