



Research article

Effects of sprouting of soybean on the anti-nutritional, nutritional, textural and sensory quality of tofu

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ABSTRACT

Soybean (*Glycine max*) is a superior plant food known as the miracle golden bean due to its treasure trove of inexpensive and superior protein to high-priced meat protein. Tofu is typically thought of as a salt or acid-coagulated water-based gel containing soy lipids and proteins trapped within its gel networks. Sprouted tofu is easier to digest and contains more nutrients than regular tofu. The purpose of this study was to evaluate the nutritional and sensory quality of tofu. The study was concerned with the preparation of tofu from non-germinated and germinated soybeans of 0 (T₀), 2 (T₁), 4 (T₂) and 6 (T₃) days. The length of the radicle was 0.8, 1.5, and 2 inches for T₁, T₂, and T₃, respectively. Lipoxigenase activity, phytate inhibitory activity, urease activity, trypsin inhibitory activity, protein solubility, nutritional, and sensory quality of prepared tofu were all assessed. Longer sprouting times reduced phytate, urease, trypsin, and lipoxigenase activity while increasing protein solubility. Tofu prepared from T₃ showed significantly better physicochemical properties than others. Protein, ash, and fat contents were significantly higher in T₃. The level of nutrient content for tofu samples was in the rank of T₃>T₂>T₁>T₀. The L*, a* and b* values were followed the same ranked as nutrient content (T₃>T₂>T₁>T₀). Sensory characteristics indicated that T₃ was significantly more acceptable to the panelist. This study concludes that tofu made from 6 day sprouted soybeans with a height of 2 inches can be a good source of nutrition for consumers.

1. Introduction

Tofu is a soybean (*Glycine max*) curd made by curdling fresh hot soymilk with a coagulant. It can be consumed fresh, pickled and smoked. In terms of nutrition, tofu is cholesterol-free, low in saturated fat, and high in protein (Ojha et al., 2014). On a moisture free basis, tofu comprises about 50% protein and 27% fat, the majority of which is polyunsaturated fatty acids, with the remaining ingredients being carbohydrates and minerals (Rekha and Vijayalakshmi, 2013). Therefore, tofu can be used as an alternative to the products of animal origin (Tripathi and Misra, 2005). Tofu also known as soy cheese which can be generated manually by curdling of soymilk by either salt (calcium chloride or calcium sulphate) or an acid (glucono-δ-lactone) (Obob, 2006). Traditional methods of making tofu use magnesium, sodium, or calcium

chloride as a coagulant. However, calcium sulfate, calcium acetate, calcium lactate, calcium phosphate, calcium carbonate, calcium gluconate, trimagnesium citrate, or sodium sulfate can also be used (Cai and Chang, 1998; Wang et al., 2003, 2018; Joo and Cavender, 2020). In addition, recently commercial proteinases (chymosin-pepsin) have been used (Stanojevic et al., 2011, 2020, 2021). This results in the formation of a soy protein gel, which traps water, soy lipids, and other ingredients in the matrix, resulting in curds. The curds are then compressed into solids (Cai et al., 1997; Cai and Chang, 1998). The quality of the beans, the amount of stirring, the coagulants, and the curd pressing can all have a significant impact on the quality of products (Wang and Chang, 1995). Asia-Pacific (APAC) is the segment of the global tofu market with the highest growth rate. China, Japan, South Korea, Vietnam, the Philippines, Thailand, and Indonesia are the most significant contributors in this region. APAC's tofu

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market has expanded significantly due to the rising demand for nutrient-dense foods and the increase in disposable income. According to the market research by Technavio, during the period from 2017 to 2021, the global tofu market is anticipated to expand by roughly 4% per year. Tofu products can be broadly divided into two categories: fresh or unprocessed tofu (made from soy milk) and processed tofu (produced from fresh tofu). In 2016, the processed segment held close to 85% of the market share. The growth of the vegan population and the number of people with lactose intolerance are influencing the expansion of the market. These individuals favor tofu as an alternative non-meat and non-dairy protein source in their diets (<http://www.technavio.com/>).

Sprouted tofu is a great alternative to traditional tofu since it is simpler to the digestion and contains more protein, calcium, iron, and omega-3 fats (Ojha et al., 2014). Sprouted tofu contains more protein and higher percentage of calcium than regular tofu. Sprouting is used to soften the beans which release the phytates that cause troubles. Sprouted tofu is used just like regular tofu. Cooked soybeans are used to form regular tofu, while sprouted soybeans are used to make sprouted tofu. On the other hand, Sprouting is the process where seeds are allowed to soak and then waiting for them to germinate and sprout (Ojha et al., 2014). Sprouting causes a series of metabolic changes that improve the nutritional quality of sprouted legumes while reducing anti-nutritional factors including trypsin inhibitor and phytic acid, as well as flatulent factors (Murugkar and Jha, 2009). It has a reputation for reducing beany and grassy tastes. It can also enhance protein content, vitamin concentration and dietary fiber, mineral bioavailability; cause to the reduction of tannin and phytic acid. As well as the level of calcium, copper, manganese, zinc, riboflavin, niacin, and ascorbic acid may have an improvement (Ojha et al., 2014).

Many people can't afford protein source from meat, fish and milk. As Bangladesh is a developing country, most of her population suffers from malnutrition. Soybeans might be an excellent source of protein in this case. Because soybeans are inexpensive, anybody may buy them and use them to produce tofu, soy milk, and other products. They can be used combined with meat, fish, egg and vegetables, etc. in many dishes. Soy protein or replacing animal protein in diet reduces blood cholesterol. Soy protein is also popular for its health benefits, such as lowering cholesterol levels, alleviating menopause symptoms, and lowering the risk of various chronic diseases, such as cancer, cardiovascular diseases, abdominal body fat, and osteoporosis (Qin et al., 2022). Daily consumption of isolated soy protein is 20–50 g which results reduction in coronary disease risk about 20–30% (Bansal and Kaur, 2014). Tofu has the potential to be the most effective weapon in the fight against global hunger and malnutrition. It is necessary to make sufficient efforts to disseminate its multi-nutritional importance among the general public, as well as to create methods for its industrial production (Gartaula et al., 2013).

Several factors influence tofu yield, quality, and texture, including soybean variety and storage conditions, soaking period of soymilk and temperature, extent of heat-treatment, type and concentration of coagulant, stirring rate, and temperature of coagulation (Noh et al., 2005; Obatolu, 2008; Prabhakaran et al., 2006; Shih et al., 2002). Therefore, the present study was done to optimize the germination days of soybeans and compare the physicochemical properties and organoleptic quality between regular tofu and sprouted tofu.

2. Materials and methods

2.1. Materials

Soybean var BU Soybean 1 was collected from Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. Food grade NaHCO_3 was purchased from Mitali Scientific, Bangladesh.

2.2. Preparation of soymilk from sprouted and non-sprouted soybean

Soybean (1000 g) was soaked in warm water (60 °C) containing 0.5% NaHCO_3 for overnight. The ratio of water and soybean was 2:1. 250 g out of 1000g soybean (T_0) was soaked overnight and discard the water. Soybean was dehulled by manually and cleaned with continuous flow of potable water for three times. Rest (750 g) of soybean was divided into three groups. Each group contains 250 g of soybean and named as T_1 , T_2 and T_3 , respectively. All three samples were kept in dark for 2, 4 and 6 days, respectively. During overnight soaking, water was drained and rinsed by potable water at regular interval of 6 h at 28 °C for 6 to 8 times. After 2, 4 and 6 days of germination, soybean (T_1 , T_2 and T_3) dehulled manually and cleaned with continuous flow of potable water for three times. Both sprouted and non-sprouted soybeans were blanched in 0.5% sodium bicarbonate solution at 80 °C for a period of 10 min. The ratio of water and soybean was 2:1. The blanched soybean was grinded by using super mass collioder and a basket centrifuge with the addition of hot water (100 °C, soybean: water = 1:4). Soy milk from sprouted and non-sprouted soybean was obtained after filtering through a double layer cheese cloth. The soymilk was homogenized and pasteurized at 65 °C for 30 min (Nowshin et al., 2018).

2.3. Preparation of tofu

Soymilk from T_0 , T_1 , T_2 and T_3 was heated to 95 °C for 5 min and then cooled to 80 °C by constant stirring at room temperature (Figure 1). 0.2% (w/v of the milk) CaSO_4 and 2% of lemon juice was added as coagulant. Coagulant was added to soymilk slowly with constant stirring and allowed to settle for 20 min at room temperature. The coagulated milk was transferred to a tofu mould (16 × 18 × 3.5) cm lined with cheese cloth and pressure was applied on tofu by putting weights of 1.0, 1.5 and 2.0 kg/cm² for 45 min. After pressing, tofu was removed from the mould and dipped in chilled water (6 °C) for 30 min. Whey was removed by placing the tofu in the cheese cloth. Processed tofu was packed in aluminum foil and stored at 4 °C for further use.

2.4. Analysis of prepared tofu

AOAC methods (2019) were used for the identification of different factors such as moisture (method 950.46) (AOAC International, 2019a), ash (method 920.153) (AOAC International, 2019b), crude protein (method 981.10) (AOAC International, 2019c) and fat (method 922.06) (AOAC International, 2019d). Total carbohydrate content was measured following the procedure of (Maclean et al., 2003). The tofu yield was determined as the weight of fresh tofu produced from the amount of soybeans used in its preparation (Yasin et al., 2019). Tofu yield (g/mL), Tofu strength (N), Tofu protein (g/kg), and Whiteness index (Wi) are added together to form the Tofu index (Murugkar, 2014).

2.5. Determination of anti-nutritional factors

Lipoxygenase (LOX), and urease activity were determined according to modified methods of some previous studies (Croston et al., 1955; Erlanger et al., 1961; Rounds and Nielsen, 1993; Gökmen et al., 2002; Mazumder, 2016). Oxalate content was determined according to the process of Ojha et al. (2014). In a 100ml volumetric flask, 1g oven dried material, 10ml 3N HCl, and 65 ml distilled water were combined and heated for 1 h. The material was diluted to 100ml and filtered after cooling. Two aliquots of 50ml extract containing 20ml 6N HCl were evaporated to half their original volume and filtered numerous times. 3–4 drops of methyl red indicator were added to the filtrate, followed by a strong ammonia solution until the solution became pale yellow. The solution was heated to 90–100 °C in a water bath, and 10mL of 5% CaCl_2

2.7. Texture analysis of tofu

The texture analyzer (TA-XT plus, Stable Micro System, UK) with a 5 kg load cell, 75 mm flat plate probe, 50 percent compression, and a test speed of 1.0 mm/s was used to measure the hardness, cohesiveness, springiness, and chewiness of tofu (2.54 cm, 2.54 cm, 2.54 cm) from the center section of tofu. The pre-test and post-test speed was 2.0 mm/s having trigger force 5 g. A two cycle compression was used and 5 s time gaps were maintained between these two compressions (Tasnim et al., 2020). Texture expert 1.05 software (Stable Microsystem) was used to program a double cycle to calculate the textural properties of prepared tofu.

2.8. Sensory evaluation

The consumer acceptability i.e. color, flavor, texture, taste and overall acceptability of developed tofu was evaluated by a taste panel 15 semi-trained panelists at a 9-point hedonic rating scale (Amerine et al., 1965). Randomly coded samples were presented to the panelists and asked to rate color, flavor, texture, taste and overall acceptability of prepared tofu samples. The hedonic rating scale was arranged as 1 = extremely dislike, 2 = very much dislike, 3 = moderately dislike, 4 = slightly dislike, 5 = neither like nor dislike, 6 = slightly like, 7 = moderately like, 8 = very much like and 9 = extremely like. The scorecard for hedonic rating test is provided in the supplementary section (Table S1).

2.9. Statistical analysis

The developed tofu was statistically examined using Fischer's LSD multiple comparison test to see whether there were any differences among the tofu samples. Using the software Microsoft Office Excel 2013 and STATA v15, a single component analysis of variance (ANOVA) was used to determine the significant difference between the data acquired wherever necessary at a 5% level of significance, following the approach of Gomez and Gomez (1984).

Ethical approval

The study was approved by the institutional review board of Bangladesh Agricultural University, Bangladesh. The experiments were conducted according to established ethical guidelines and each panelist was asked to provide informed written consent before progressing with the process. It is good to mention that, in Bangladesh, there are no strict requirements for ethical approval for sensory tests.

3. Results and discussion

3.1. Effect of sprouting on yield and tofu index

Table 1 shows the length of radicle for germinated and non-germinated soybeans. There was no radicle in sample T₀ (control). However, sample T₁ had a length of 2.03 cm. Sample T₂ and T₃ showed 3.81 and 5.08 cm radicle length, respectively. The yield of tofu from non-

Table 1. Characteristics of tofu from sprouted and non-sprouted soybean.

Sample	Germination time (days)	Length of radicle (cm)	(%) Yield of tofu	Tofu index	Comments
T ₀	0	0	40.33 ^c ± 0.50	530.35 ^c ± 7.5	Good
T ₁	2	2.03 ^c ± 0.05	42.44 ^b ± 0.60	536.48 ^b ± 9.6	Good
T ₂	4	3.81 ^b ± 0.07	54.39 ^a ± 1.00	565.80 ^a ± 8.2	Best
T ₃	6	5.08 ^a ± 0.04	39.99 ^c ± 0.95	525.10 ^c ± 6.5	Better

Mean ± SD, means are the average of three replicates for each analysis, different letters in the same column are significantly different ($p < .05$), T₀ = Control (Tofu prepared from non-sprouted soybean; T₁ = Tofu prepared from 2 days sprouted soybean; T₂ = Tofu prepared from 4 days sprouted soybean; T₃ = Tofu prepared from 6 days sprouted soybean.

Table 2. Physicochemical composition of tofu prepared from different samples.

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
T ₀	12.51 ^c ± 0.36	1.32 ^a ± 0.13	34.71 ^d ± 0.64	18.19 ^a ± 0.15
T ₁	13.15 ^c ± 0.34	1.05 ^b ± 0.06	36.47 ^c ± 0.61	17.31 ^b ± 0.20
T ₂	15.22 ^b ± 0.23	0.94 ^c ± 0.03	39.66 ^b ± 0.42	16.47 ^c ± 0.27
T ₃	17.22 ^a ± 0.41	0.83 ^d ± 0.03	41.96 ^a ± 0.73	14.88 ^d ± 0.38

Mean ± SD, means are the average of three replicates for each analysis, different letters in the same column are significantly different ($p < .05$), T₀ = Control (Tofu prepared from non-sprouted soybean; T₁ = Tofu prepared from 2 days sprouted soybean; T₂ = Tofu prepared from 4 days sprouted soybean; T₃ = Tofu prepared from 6 days sprouted soybean.

germinated (T₀), and germinated (T₁, T₂ and T₃) soybeans was shown in Table 1. Germination of soybean (4 days) shows the highest yield percentage and tofu index followed by T₂ and T₁, respectively. However, soybean germinated for 6 days shows the lowest yield percentage and tofu index. This might be due to the leaching of 60% water and carbohydrate, 6% lipids and crude protein and also the components can be metabolized during germination and, therefore, reduce the verified content (Nowshin et al., 2018). However, Ojha et al. (2014) suggested that sprouting of soybean reduce dry matter content ranged from 7 to 13%. The result of this research is an agreement with Ojha et al. (2014) who found the yield percentage of tofu was 42.33, 37.69, and 39.70% for regular, sprouted, and combined tofu, respectively. Differences in soybean varieties and treating processes could explain the variation in percent tofu yield.

3.2. Effect of sprouting on physicochemical composition of tofu

The chemical composition of prepared tofu from sprouted and non-sprouted soybean was analyzed to determine their moisture content, total solid, ash, protein and fat. Table 2 shows the results of the triplicate determination.

Mean ± SD, means are the average of three replicates for each analysis, different letters in the same column are significantly different ($p < .05$), T₀ = Control (Tofu prepared from non-sprouted soybean; T₁ = Tofu prepared from 2 days sprouted soybean; T₂ = Tofu prepared from 4 days sprouted soybean; T₃ = Tofu prepared from 6 days sprouted soybean.

Tofu prepared from sprouted soybean had higher moisture content than control sample. It was seen that increasing the germination time increased ($p < 0.05$) the moisture content of tofu. This might be due to the leaching of dry matter content from soybean while soaking for a long period of time (6 days). The moisture content of sample T₃ was higher than the others and T₀ shows the lowest moisture content. The hydration of soybeans may account for the rise in moisture content of sprouted soybean tofu. During germination process, legumes (soybean) absorbed moisture from the surrounding so as to commence metabolic process, which increased the moisture content of the sprouted soybean tofu.

Total ash content of tofu was ranged from 1.08 ± 0.03 to 1.32 ± 0.13%. Control sample (T₀) shows higher amount of ash content than other tofu sample. Tofu from 6 days of sprouted soybean had significantly

($p < 0.05$) lower extent of ash content than others. This might be due to the leaching of mineral matter during long time soaking of soybean. However, ash content in the regular tofu was a range from 5.2-7.9% (Obatolu, 2008) and for sprouted tofu was $3.93 \pm 0.24\%$ for (Ojha et al., 2014). The result is an agreement with Blessing and Gregory (2010) and Mostafa and Rahma (1987), both of them reported the reduction of total ash content for mungbean and soybean during germination.

Crude protein content in the tofu prepared from sprouted and non-sprouted was ranges from 34.71 ± 0.64 to $41.96 \pm 0.73\%$. These values were lower than the values suggested by Shokunbi et al. (2011) who found 56.89–59.98% and Oboh and Omotosho (2005) who obtained 57.85%. The variations might be due to variety difference and tofu processing methods. Statistical study revealed a significant difference between products ($p < 0.05$). Sample T₃ (tofu prepared six days germinated soybean) contained higher protein content than others. The protein content of sample T₀ was lowest which was prepared from non-germinated soybean. Protein content of tofu increased with the increasing the germination time of soybean. The breakdown of reserve protein during sprouting to produce NH₃, which accumulates in the form of amides such as glutamic acid and aspartic acid, could account for these observations (Murugkar, 2014). Total fat content of tofu were ranges from 14.88 ± 0.38 to $18.19 \pm 0.15\%$. However, Ojha et al. (2014) found $21.15 \pm 0.79\%$ total fat for non-sprouted tofu and $15.81 \pm 1.72\%$ for sprouted tofu. This may be due to the variety of soybean. Sample T₃ shows the lowest in fat content than other samples. The depletion of stored fat is caused by the catabolic activities of the seeds during sprouting (Murugkar, 2014), consistent with our findings. Similar as ash content, total fat decreased with increasing the germination time. The action of lipolytic enzymes during soybean sprouting may be responsible for the considerable reduction in fat content of sprouted tofu (Frank et al., 2009). Fat could be used as an energy source to start germination, which would explain the decrease in fat. The reduction in fat may be due to a decrease in total solids. The result is an agreement with Ghavidel and Prakash (2007).

3.3. Effect of sprouting on anti-nutritional factors of soybean

Table 3 shows the values of different anti-nutritional factors of tofu prepared from non-germinated and germinated soy seeds at different times. The LOX activity of 2 day germinated soy seeds was reduced by roughly 85%, but 4 and 6 day germinated soy seeds were shown to inactivate more than 90% of the LOX activity. T₂ and T₃ did not give any significant difference ($p > 0.05$). However, for the best quality of fruits and vegetable processing and frozen storage, a reduction of >90% LOX activity was suggested (Gökmen et al., 2005). The urease activity of non-germinated soybean was found significantly higher ($p < 0.05$) than that of germinated soybean. Germination of soybean at 4 and 6 days revealed maximum 96% reduction of urease activity (not significantly different with each other). This result suggested that germination of soybean was almost sufficient to destroy more than 90% of LOX and urease activity. Heat treatment is necessary to destroy LOX and urease activity (Nowshin et al., 2018). According to the study of Nowshin et al.

Table 4. Textural properties of tofu prepared from germinated and non-germinated soybeans.

Sample	Textural properties of tofu			
	Hardness	Cohesiveness	Springiness	Chewiness
T ₀	11.4 ^a ± 1.3	0.37 ^c ± 0.07	0.45 ^c ± 0.2	2.5 ^a ± 0.30
T ₁	7.0 ^b ± 1.1	0.44 ^b ± 0.02	0.55 ^b ± 0.3	1.7 ^b ± 0.14
T ₂	6.6 ^c ± 1.2	0.45 ^b ± 0.13	0.56 ^b ± 0.2	1.5 ^c ± 0.27
T ₃	5.7 ^d ± 1.2	0.47 ^a ± 0.05	0.60 ^a ± 0.1	1.2 ^d ± 0.44

Mean ± SD, means are the average of three replicates for each analysis, different letters in the same column are significantly different ($p < .05$), T₀ = Control (Tofu prepared from non-sprouted soybean; T₁ = Tofu prepared from 2 days sprouted soybean; T₂ = Tofu prepared from 4 days sprouted soybean; T₃ = Tofu prepared from 6 days sprouted soybean.

(2018) germination and blanching at 80 °C for 10 min could significantly reduce the urease activity by 100%. Yalcin and Basman (2015) found a 99% reduction in the urease activity of soybean samples after infrared (IR) treatment. In addition, they preferred the infrared treatment over the regular heat treatments for the reduction of urease activity in soybeans. In the same study, the LOX activity in Nazlican soybean samples was reduced by 98% (for 30 min) and 99% (for 45 min) after an IR treatment at 814 W (Yalcin and Basman, 2015). Mazumder et al. (2018) found that, soaking at 65 °C for 2 h resulted in a maximum 50% reduction in urease activity of raw soybeans. However, he thought that blanching (100 °C for 10min) and soaking (65 °C for 2 h) could significantly reduce 100% urease activity. In another study, the LOX content in soluble fractions was significantly reduced after the HTC process. Soymilk has more LOX compared to okara and tofu whey (Stanojević et al., 2017). Besides all these, there are other factors that may contribute to the LOX inactivation such as pH, and genotype (Stanojevic et al., 2014). Green bean LOX was discovered to be more heat sensitive and easily inactivated by a mild blanching treatment such as 70 °C for 2 min (Bahçeci et al., 2005). Increase in germination time of soybean leads to decrease the oxalate content of tofu. According to Shimi and Haron, germination and seed soaking reduced the oxalate content of soybeans. Soybean oxalate content also varies due to biological variation, which includes cultivar, growing environment, and harvesting time (Shimi and Haron, 2014). Removal of oxalates from fruits and vegetables to a safe level was recommended via germination (Khokhar et al., 2003). Hence, Paul et al. (2012) reported that cooking can reduce around 24.9% of oxalate from green pea and Bengal gram. Khan and Ghafoor (1978) discovered that germination contributed to the decrease in oxalate in soybean in his study.

Phytic acid (mg/100 g dry basis) of tofu T₀, T₁, T₂ and T₃ were 110.0 ± 2.37 , 93.5 ± 1.03 , 90 ± 0.5 and 87 ± 1.5 , respectively (Table 3). Sprouting decreased the phytic acid content, which has been confirmed by other researchers due to the possibility of a 3 to 5- fold increase in phytase activity in certain cereal grains and legume seeds (Dave et al., 2008; Egli et al., 2002).

Table 3. Anti-nutritional factors of tofu prepared from non-germinated and germinated soy seed.

Sample	Lipoxygenase Inhibition %	Urease activity inhibition %	Oxalate (%)	Phytic acid (mg% db)	Trypsin inhibitor (mg/g db)
T ₀	65.85 ^c ± 2.5	70.25 ^c ± 1.8	0.045 ± 0.05	110.0 ± 2.37	7.10 ± 0.03
T ₁	85.25 ^b ± 3.0	86.20 ^b ± 2.0	0.018 ± 0.04	93.50 ± 1.03	3.05 ± 0.05
T ₂	93.75 ^a ± 2.8	96.10 ^a ± 3.0	0.014 ± 0.07	90.50 ± 0.5	1.10 ± 0.08
T ₃	94.60 ^a ± 2.0	96.70 ^a ± 2.0	0.011 ± 0.01	87.30 ± 1.5	0.78 ± 0.06

Mean ± SD, means are the average of three replicates for each analysis, different letters in the same column are significantly different ($p < .05$), T₀ = Control (Tofu prepared from non-sprouted soybean; T₁ = Tofu prepared from 2 days sprouted soybean; T₂ = Tofu prepared from 4 days sprouted soybean; T₃ = Tofu prepared from 6 days sprouted soybean.

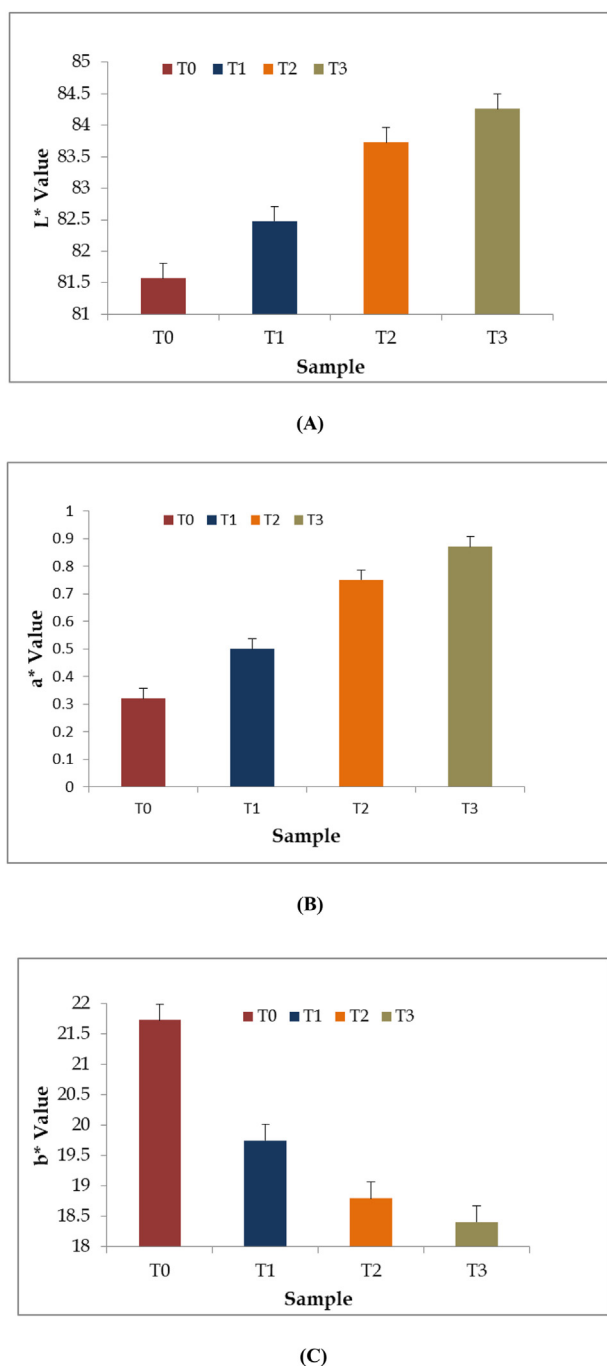


Figure 2. Effect of germination time on A) Lightness (L*) value, B) Redness (a*) value, C) Yellowness (b*) value of tofu prepared from soybean.

It was seen that trypsin inhibitors were decreased with increasing germination time. T₃ sample showed the lowest trypsin inhibitor content of 0.78 ± 0.06 trypsin inhibitor unit (TIU) per g of protein whereas in T₀ sample obtained the highest content of 7.10 ± 0.03 TIU per g of protein, respectively. The result is an agreement with Mostafa et al. (1987). There was a significant difference between sprouted and non-sprouted tofu in terms of inhibition of trypsin inhibitors. This could be related to the fact that reserve protein is broken down during sprouting to produce NH₃, which accumulates in the form of amides such as glutamic acid and aspartic acid (Hsu et al., 1980), while fat storage is depleted due to the catabolic activities of the seeds. Due to the proteolytic activity of enzymes produced during sprouting, both Kunitz soybean trypsin inhibitor

(Collins and Sanders, 1976) and the main Bowman-Birk soybean trypsin inhibitor can be degraded (Chauhan and Chauhan, 2008).

3.4. Effect of sprouting on color of tofu

The color parameters (L*, a*, b* value) of four different tofu prepared from germinated and non-germinated soybean were illustrates in Figure 2. Figure 2(A) represented L* (lightness) of tofu increased with increasing time of germination (from 81.57 ± 0.13 to 84.26 ± 0.23). Lightness (L*) values of T₃ was the highest followed by T₂, T₁ and T₀, respectively. Choi et al. (2013) suggested that L* value of *Eunhakang* (*Glycine max*) was considerably increased at the early stage of germination. The a* value represented that all of the samples were significantly reddish as the values were positive. Figure 2 (B) suggested that germination time significantly affected the redness (a*) value of tofu prepared from soybean. The a* value of soybean was rapidly increased at the beginning of germination and decreased thereafter (Choi et al., 2013). From Figure 2 (C), it was seen that b* (Yellowness) value of tofu decreased with increasing of germination time of soybean (from 21.73 ± 0.24 to 18.41 ± 0.27). Color changes in tofu samples could be caused by non-enzymatic browning, specifically the Maillard reaction (Murugkar, 2015). Soybean testa colors (yellow, green, brown and black) vary according to the soybean variety. Because of the presence of chlorophyll, all soybean varieties are green in the early stages of maturity. As the bean matures, chlorophyll is lost and flavonoid pigments take over (Yousif, 2014). A potential reason for the decrease in the soybean b* color value is that one of the impacts of soybean storage at high moisture content and temperature is the hydrolysis of β -Carotenes (Yousif, 2014).

3.5. Effect of sprouting on textural properties of tofu

Hardness of tofu was significantly lower in sample in T₃ followed by others. Tofu hardness may be connected to tofu volume in an indirect way. The capacity of any food material to attach to itself is measured by its cohesiveness, which quantifies the food structure's internal resistance. The cohesiveness of different tofu was in the range of 0.37–0.47. However, there was no significant ($p > 0.05$) difference between sample T₂ and T₁ in terms of cohesiveness of tofu. The springiness of germinated and non-germinated soy tofu was in the range of 0.45–0.60. Tofu prepared from 6 days germinated soybean had lower springiness ($p < 0.5$) than others. There was no significant ($p > 0.05$) difference between sample T₂ (4 days germinated tofu) and T₁ (2 days germinated tofu) (see Table 4).

3.6. Effect of germination time on sensory characteristics of tofu

Taste, flavor, and general acceptability of tofu from germinated and non-germinated soybeans differed significantly ($p < 0.5$) on a 9-point hedonic scale. Tofu's taste and flavor are the two most essential factors in determining whether or not it is liked or disliked. One of the most

Table 5. Mean score for color, flavor, texture and overall acceptability of tofu.

Sample	Sensory attributes			
	Color	Flavor	Texture	Overall acceptability
T ₀	5.1 ^d ± 0.32	5.1 ^d ± 0.34	5.3 ^c ± 0.22	5.4 ^c ± 0.26
T ₁	5.9 ^c ± 0.21	5.8 ^c ± 0.23	5.9 ^b ± 0.31	6.5 ^b ± 0.25
T ₂	6.5 ^b ± 0.24	6.3 ^b ± 0.14	6.3 ^b ± 0.23	6.6 ^b ± 0.21
T ₃	7.3 ^a ± 0.42	7.3 ^a ± 0.30	7.4 ^a ± 0.08	7.7 ^a ± 0.12

Mean ± SD, means are the average of three replicates for each analysis, different letters in the same column are significantly different ($p < .05$), T₀ = Control (Tofu prepared from non-sprouted soybean; T₁ = Tofu prepared from 2 days sprouted soybean; T₂ = Tofu prepared from 4 days sprouted soybean; T₃ = Tofu prepared from 6 days sprouted soybean).

significant impediments to soy's marketing as a nutritious food is its beany flavor and off smell.

A one-way ANOVA revealed a significant difference ($p < 0.05$) in the color of all samples, indicating that the samples were not accepted equally. Sample T₃ showed the highest color values followed by T₂, T₁, and T₀. It was observed that the color of tofu was changed with the increasing time of germination. The sample T₀ had a low acceptability as color and was not well accepted to the panelists. Table 5 showed that T₃ had the highest flavor and texture values and like moderately by the panelists. A significant difference ($p < 0.05$) was found between the sample T₃ and sample T₁. These two samples were prepared from six days and two days germinated soybean, respectively. Sample T₃ was more accepted than Sample T₁. The obtained results of sensory analysis have shown that there was a statistically significant difference ($p < 0.05$) between the samples. Sample T₃ showed the highest value and was ranked like moderately. The overall acceptability of the samples differed significantly ($p < 0.05$). Sample T₃ had the highest overall acceptability while sample T₀ had lowest value. Hence, the sample T₃ was accepted mostly by the panelists followed by sample T₂, T₁ and T₀, respectively. The longer germination time of soybean sample for tofu was more acceptable than whole soybean for the making of tofu. With the increasing of germination time of soybean for making of tofu sample, the acceptability of color, flavor, texture as well as the overall acceptability was also increased. One of the most significant impediments to soy's marketing as a nutritious diet is the existence of a beany flavor and unpleasant odor. Sprouting is known to minimize undesired qualities such as beany and grassy to a minimum and enhance complete acceptability for tofu made from two-day-germinated soybeans, as well as improve nutty odor and taste (Murugkar, 2014), which is consistent with our findings. In other factors, such as tofu texture, a decrease in hardness as measured by texture analysis did not appear to have a comparable impact on sensory acceptance. Actually, the marginal difference of 1.5% indicates that some respondents liked the softer tofu.

4. Conclusion

Soybean germination increases protein content while lowering fat and anti-nutritional factors such as lipoxygenase, urease activity, oxalate, phytic acid, and trypsin inhibitor content. Meanwhile sprouting is natural, non-chemical and non-thermal process, resulted high quality processed soy products such as tofu. Apart from germination of soybean for 2, 4 and 6 days improved nutritional quality of tofu with higher tofu index. Tofu prepared from germinated soybeans for six days contained high protein content but low in fat, and ash content. The tofu which was prepared from six days germinated soybean had highest L* and a* value but lowest b* value. Tofu made from six days germinated soybean was found to be the best in terms of sensory analysis after sprouting aided to reduce beany odor. Tofu prepared from T₃ had better physicochemical properties than others. Protein, ash, and fat contents were significantly higher in T₃. Similarly, tofu made from sprouted beans that had been sprouted for six days exhibited good color and texture features, as well as high acceptability on the 9-point hedonic scale. In conclusion, among the tofu samples prepared, we can declare that the T₃ is the most acceptable one.

5. Limitations

The best analysis of the tofu parameters would be done if the starting samples were also analyzed. In this way, it would be easier to determine which changes were caused by seed germination and which were caused by the applied technological process. In this study, this analysis has not done and we take this as a limitation of the study.

Declarations

Author contribution statement

Sumaiya Afrin Zinia, MS: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Asmaul Husna Nupur, MS: Performed the experiments; Analyzed and interpreted the data.

Poly Karmoker, PhD; Abir Hossain, MS: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Md Fahad Jubayer, MS: Conceived and designed the experiments; Wrote the paper.

Delara Akhter, PhD: Analyzed and interpreted the data; Wrote the paper.

Md. Anisur Rahman Mazumder: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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