

Research

**Identification of anti-nutritional factors in soya cheese**

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**Abstract:** This study aimed to determine the anti-nutrients of cheese produced from soya beans by chemical processing. Effect of processing using a chemical method on anti-nutritional constituents of soybean seed meal and cheese were carried out. Levels of the anti-nutrients like tannin, oxalate, saponin, and phytate of the raw soya and cheese were determined. The percentage (%) of the studied anti-nutritional substances in the raw seed were obtained as tannin ( $0.168 \pm 0.003$ ), phytate ( $0.470 \pm 0.004$ ), oxaloacetate ( $0.109 \pm 0.002$ ), and saponin ( $5.47 \pm 0.20$ ). Processing, however, significantly reduced the levels of the anti-nutrients like tannin, oxalate, saponin, and phytate in the raw and cheese. The percentage of anti-nutritional levels in the raw seed after processing were found as tannin ( $0.009 \pm 0.002$ ), phytate ( $0.104 \pm 0.004$ ), oxaloacetate ( $0.085 \pm 0.005$ ), and saponin ( $2.53 \pm 0.04$ ). Hence, the processing significantly ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) improved the nutritional quality of the soy cheese and the product can be used as a good protein source.

**Keywords:** Soya beans, Soya cheese, Anti-nutrients, Food processing.

**Introduction**

Tofu, a soybean-based food product, is a kind of traditional food and is a good source of protein. It is generally produced by coagulating the soymilk protein using coagulant. Soymilk can be obtained from soybean extraction. Nowadays, people are found with an increasing interest in tofu due to its good taste, good nutritional, health benefits to human, and affordable. Therefore, tofu has become very popular and embraces a high market potentiality.

The basic reason for purposely processing the milk into cheese is to preserve a portion of perishable food and to convert it into a stable and storable product. It also expands the variety of food (Walther et al., 2008). Cheese has a long history in the human diet. In ancient times, cheese was primarily a concentrated form of milk with the benefit of prolonged shelf life. The high content of fat and protein in cheese made it an energy-rich and nutritious food that was suitable for our hardworking ancestors. Recent advances in nutrition science have highlighted the contribution of cheese to nutrition and health. Cheese is a rich source of essential nutrients; in particular, proteins, bioactive peptides, amino acids, fat, fatty acids, vitamins, and minerals. Soya cheese is free of lactose and therefore suitable for the nutrition of lactose-intolerant individuals (Nazim et al., 2013). The high concentration of essential amino acids in cheese contributes to the growth and development of the human body. Conjugated linoleic acid present in cheese may have anti-carcinogenic properties. The high concentration of calcium in cheese is well known to contribute to the formation and maintenance of strong bones and teeth, but also shows a positive effect on blood pressure and helps in losing weight in combination with low-energy diets. Cheese is an important dairy product and an integral part of a healthful diet due to its substantial contribution to human health. In recent times, the diet has been linked to various diseases such as diabetes, obesity, cardiovascular disease, osteoporosis, and cancer, and the focus of nutrition research has shifted towards specific food ingredients contributing to nutrition and health (Walther et al., 2008).

In the present study, we have investigated the anti-nutritional value of newly developed soya cheese compared to that of normal soya, before and after processing.

## **Materials and methods**

### **Sample preparation**

Soya bean was collected from the local market of the capital city Dhaka, Bangladesh. The cheese was produced by coagulating the soya milk. After production of the soya Cheese, it was preserved by using the solution of citric acid, sodium chloride, and heating in the oven for the appropriate time. After these steps, the Cheese was preserved in the refrigerator until analysis.



**Figure 1:** Appearance of Tofu.

### **Determination of anti-nutrients**

Anti-nutrients composition of the raw soya and cheese was carried out as follows.

#### **Tannin content determination**

Quantitative estimation of tannins was carried out using the modified vanillin-HCl method (Abdelseed et al., 2011). A 200 mg of sample was extracted using 10 mL of 1% (v/v) concentrated HCl in methanol for 20 min in the capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL), and the absorbance of the developed colored solution after 20 min 30 °C was measured at 500 nm. A standard curve was prepared to express the results of catechin equivalents, i.e. amount of catechins ( $\text{mg } 100\text{g}^{-1}$ ) which gives a color intensity equivalent to that given by tannins after correcting for blank. Then, tannin content was calculated and expressed in  $\text{mg } 100\text{g}^{-1}$ .

#### **Phytate content determination**

The Wheeler and Ferrel (1971) method was followed to determine the phytate content in the sample. This method relies on the solubilization of phytate by dilute acid and the subsequent precipitation of the phytate with ferric ion ( $\text{Fe}^{3+}$ ). About 4 g of the sample was soaked in 100 mL of 2% HCl for 3 h, and then filtered. 25 mL filtrate was dispensed into a conical flask and 5 mL of

0.3 mL ammonium thiocyanate solution was added as an indicator. Thereafter, 53.5 mL distilled water was added to the mixture to give it a proper acidity and this was titrated with standard iron(III) chloride solution, which contains about 1.95 mg of iron per milliliter (mL) until a brownish-yellow color persisted for 5 min.

### **Oxalate content determination**

This was determined using the modified method employed by Iwuoha and Kalu (1995). The principle involves the digestion of a sample containing oxalate and the precipitation of the oxalate to remove ferrous ions on the addition of  $\text{NH}_4\text{OH}$  solution. Finally titrated against 0.05 M  $\text{KMnO}_4$  solution to a faint pink color, which persisted for 30 s.

### **Saponin content determination**

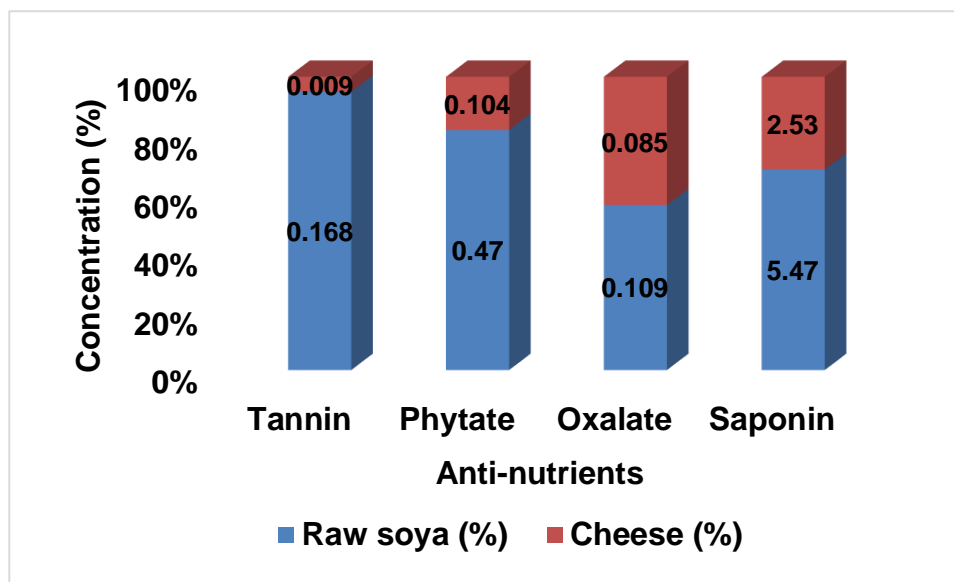
The saponin content in the sample was determined using the modified method of Hudson and El-Difrawi (1979). It was extracted with a polar solvent after removal of lipids with petroleum ether and the saponin was isolated into the polar solvent. 100 g of the milled defatted sample was added into 200 mL of 20% aqueous ethanol in a conical flask and corked tightly to prevent evaporation. The duplicate portions of the extracts were combined and put in a 250 mL separating funnel after the collection of the solvent, then a 20 mL of diethyl ether was added to it and shaken vigorously. The aqueous layer was recovered while the ether was discarded. The process of purification was continued until a colorless aqueous solution was at pH 4.5. Thereafter, 60 mL NaCl (5% aqueous solution) and 30 mL portions of n-butanol were added to the solution and shaken vigorously following each addition. The combined butanol extract was washed using 5% aqueous NaCl and evaporated to dryness to give crude saponin, which was weighed (Saponin content = weight of the sample before extraction – loss in weight after extraction).

### **Statistical analysis**

The significant difference between the mean results was determined by student's t-test where the values of  $p < 0.05$  were considered as significant and those of  $p < 0.01$  and  $P < 0.001$  were highly significant. The outcome was calculated using the SPSS software.

### **Results and discussion**

The anti-nutritional factors such as phytic acid, tannin, saponin, and oxalic acid harm human health through inhibition of protein digestion, growth, iron, and zinc absorption (V.S. Rathod et al, 2011). It was found that anti-nutrients are significantly decreased (tannin 94.64 %, phytate 77.87%, oxalate 22.03%, and saponin 53.74%) in the developed product (Figure 1). It may be concluded that this soya product has a low amount of anti-nutrients than the raw soya (Table 1).



**Figure 1:** Comparison of the concentration (%) of anti-nutrients in the raw soya and cheese.

**Table 1:** Anti-nutrients composition (%) of cheese and soya seed.

Anti-nutrients	Raw soya (dry basis)	Cheese (dry basis)	% of change
Tannin	0.168±0.003	0.009±0.002***	94.64
Phytate	0.470±0.004	0.104±0.004***	77.87
Oxalate	0.109±0.002	0.085±0.005*	22.03
Saponin	5.47±0.20	2.53±0.04**	53.74

N.B.: Values are means of three determinations  $\pm$  SD (standard deviation). Values along the same row with different stars are significantly different (\* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$ , and \*\*\* indicates  $P < 0.001$ ). To test the level of significance, data were expressed as mean  $\pm$  SD of the mean and were subjected to t-test. Significant differences between treatment means were determined at the 5% level using the t-test.

Polyphenols are commonly known as tannins, form complexes with proteins. Tannin-protein complexes are responsible for low protein digestibility and decreased amino acid availability. A decrease in the tannin content therefore improves the protein digestibility (Srivastava and Srivastava, 2003). Tannins are known to undergo hydrolysis by acids, bases, or some hydrolytic enzymes. Tannins have been shown to precipitate proteins (Bate-Smith and Swain, 1962; Hemingway, 1989; Reed, 1995), which inhibits in some ruminant animals the absorption of nutrients from high-tannin grains such as soya. Tannins interfere with iron absorption through a complex formation with iron when it is in the gastrointestinal lumen which decreases the bioavailability of iron (Brune et al., 1989). For insensitive individuals, a large intake of tannins may cause bowel irritation, kidney irritation, liver damage, irritation of the stomach, and gastrointestinal pain. A correlation has been made between esophageal or nasal cancer in humans and regular consumption of certain herbs with high tannin concentrations (Lewis and Elvin-Lewis, 2003).

Phytic acid has a strong binding affinity to important minerals such as calcium, magnesium, iron, and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates, and will be absorbable in the intestines. This process can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries (Hurrell, 2003).

Oxalate is widely distributed in plant foods in a readily water-soluble form as potassium, sodium, and ammonium oxalates and as insoluble calcium oxalates (Holloway et al., 1989). Oxalate forms strong chelates with dietary calcium, thus rendering the complex unavailable for absorption and assimilation. It precipitates as insoluble salts accumulating in the renal glomeruli and contributes to the development of the renal disorder. While other factors have to be considered in the development of a renal disorder, it is being recommended to limit the intake of oxalate-rich foods, specifically for individuals at risk for kidney stone formation (Bsc and Bsc, 1999).

Phytate, tannin, and oxalate are known to chelate mineral elements especially the divalent ions of iron, calcium, zinc, manganese, magnesium, and potassium (Reed, 1995; Webb, 1999; Adeyemi and Muhammad, 2008), thereby rendering them unavailable in a biological system.

Boiling and heating are the most important two steps in cheese production. Boiling can reduce the soluble oxalate content of food if the water used for boiling is discarded (Oscarsson and Savage, 2007). Boiling may also cause considerable skin (epidermal) rupture and facilitate the leakage of soluble oxalate into the cooking water. Therefore, the reduction in the levels of oxalate in this study following boiling may be due to its solubility in hot water. Heat treatment is an effective measure in reducing the oxalate levels in this soya product; thus, making the food prepared from these accessions safe for human consumption. This is particularly beneficial because oxalic acid and its salts can have deleterious effects on human nutrition and health, mainly by decreasing calcium absorption and aiding the formation of kidney stones. Besides, the risk of absorbing excess soluble oxalates in food can be reduced by the consumption of high calcium-containing food like milk and milk products. When extra calcium was eaten together with spinach, an oxalate-rich food, the uptake of oxalates was reduced (Lewu et al., 2009).

A saponin is a group of substances that occur in plants and can produce soapy lather with water (Rathod and Valvi, 2011). Saponin is a naturally occurring oily glycoside occurring in a wide variety of plants when eaten, they are dangerous when injected into the bloodstream and quickly haemolyse red blood corpuscles (Rathod and Valvi, 2011).

The processing of the soya to cheese dramatically reduces the concentration of anti-nutritional factors. Several steps may be involved in the reduction of anti-nutritional factors. Dehulling or boiling or coagulating or preservative (citric acid) or sodium chloride or heating maybe reduces the concentrations of anti-nutrition factors. Our findings have also supported some authors. For example, Iyayi et al. (2008) showed that heat treatment resulted in a reduction in all the ANF in the seeds. Ghavidel and Prakash (2007) showed that phytic acid and tannin reduced by 47–52% and 43–52%, respectively in dehulled samples over control. Significant ( $P < 0.05$ ) negative correlations were found between anti-nutritional factors, and nutrients bioavailability and digestibility.

The cooking of legumes removes or significantly reduces the harmful anti-nutrients, improves mineral bioavailability, protein digestibility, sugar availability, and crude fiber which are beneficial for the human body. The cooking of soaked seeds enhances the nutrient and anti-nutrient losses. The addition of sodium bicarbonate to soaking or cooking media enhances the nutrient and anti-nutrient losses (Satya et al., 2010). The introduction of sodium salts into the soaking medium alters the integrity of seed cellular walls, thus favoring diffusion of intracellular matter to the outer liquid. In contrast, soaking in citric acid solution preserves the nutrients (Satya et al., 2010).

The decrease in phytic acid is due to leaching during soaking and cooking (Satya et al., 2010). Soaking results in a decline in sugars, starch, protein, vitamins, minerals, and anti-nutrients such as phytic acid, tannins, oligosaccharides, and TIA on account of their leaching into soak water (Satya et al., 2010). The use of additives such as citric acid also minimizes the precious nutrient loss in legumes so more nutrients are available to the human body. Therefore, processed legumes significantly improve the nutritional quality of legumes and improve their potential as human food (Satya et al., 2010).

Several studies indicate that soaking can reduce the levels of total sugars,  $\alpha$ -galactosides, minerals, phytic acid, and proteolytic enzyme inhibitors (Frias et al., 2000) which can be partly or solubilized and eliminated with the discarded soaking solution. During soaking some metabolic processes can take place and usually affect the soluble carbohydrate and riboflavin contents (Prodanov et al., 2004).

## **Conclusions**

Humans need a wide range of nutrients to lead a healthy and active life. The required nutrients for different physiological groups can only be derived from a well-balanced diet. Components of the diet must be chosen judiciously to provide all the nutrients to meet the human requirements in proper proportions for the different physiological activities. We concluded that this soya product may provide a low amount of anti-nutritional factors for reducing different harmful physiological activities.



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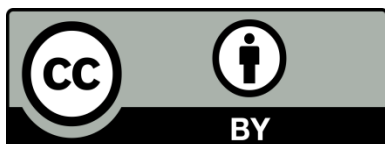
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## Conflicts of Interest

The authors declare that they have no conflict of interest.



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