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RESEARCH ARTICLE

Comparative Biochemical Analysis of Raw and Processed Soybean

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Abstract

Glycine max is considered as a wonder crop of the twentieth century. It is a high yielding legume and also an excellent source of protein, isoflavones and phytoestrogens, leading to its inclusion in the diet of common masses. For cooking, various common methods like pressure cooking, soaking, roasting, etc. are used. Many processing methods increase the nutritive value while some cause a decrease in it. The work plan included the comparative biochemical estimation of protein and anti- nutritional factors in raw and processed soybean grains of the variety JS-335. For biochemical estimation, quantities of protein, trypsin inhibitor activity and total phytate content were determined by standard procedures described. Protein content was observed to increase by pressure cooking and soaking whereas by roasting, a decrease in protein content was observed. The increase in the amount of protein was 2.72% and 6.19% by pressure cooking and soaking respectively. A decrease of 17.20% of total protein was observed by roasting. The anti- nutritional factors like trypsin inhibitor activity and total phytate content decreased significantly on roasting, pressure cooking and soaking. This has led us to conclude that different cooking methods have different effects on different nutrients. This may be due to the difference in biochemical properties and needs to be evaluated thoroughly for maximising the benefit of soybean.

Keywords

Biochemical estimation, Protein content, Soybean

Introduction

Legumes or pulses constitute an important part of diet in Indian population. Of all the legumes, soybean has gained global recognition as a food source of immense importance to health and well being of mankind. It represents the paradigm of a dietary source possessing excellent nutritional and prophylactic profiles. It is grown as a food crop in many countries of Asia, South America and United States. It contains more protein (about 40-45%) but less fat (about 16-20%) than most of the common oilseeds and legumes. The protein content of soybean has been reported to range between 30% - 46% depending on the variety. It contains a greater amount of all essential amino acids than groundnut and other common legumes. It helps in lowering total serum cholesterol while raising HDL which helps in decreasing the risk of cardiac diseases. Soybean is also a rich source of calcium, iron and phosphorus. However, legumes also contain several anti- nutritional factors in the raw seeds like haemagglutinins, saponins, goitrogens, trypsin inhibitors, phytate, etc. which have a negative influence on the human dietary system and is needed to be reduced so as to enhance the digestibility and nutritive value. These factors can be minimised by various processing methods. (Satya, 2010; Mbah *et al,* 2012).The effect of common processing techniques viz. ordinary cooking, pressure-cooking, microwave cooking, germination and soaking on the nutritional parameters and the antinutrients have been studied. (Jogyabathi *et al,* 2001) The degree of elimination of toxic compound depends on the type of pulses and the processing techniques (Jain *et al,* 2009) The processing methods adopted for common domestic cooking, besides reducing anti-nutritional factors also deteriorate its nutritional qualities and needs to be analysed thoroughly. Heat treatment has also been well established to destroy antinutrients such as protease inhibitors and lectins, but also destroys some of the amino acids and vitamins as well. For maintaining the nutritional value of food, it is necessary that heating temperature and length of processing do not exceed the optimum temperature required to eliminate the effect of inhibitors. Some of the processing techniques are even known to increase several nutritional advantages and produce edible products having a higher nutritional value and lower toxic compound.

Thus simple cooking is not sufficient to make soybean edible in terms of anti-nutritional factors and precise processing methods are needed. Each has a variable effect on the nutritional composition. A comparison of the various techniques hence allows for the selection of the best processing technique that enhances the nutritional

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value with minimum loss of nutrients and maximum reduction in antinutrients.

Various processing methods change the nutritional composition of the food drastically and needs to be thoroughly assessed. In recent years considerable attention has been paid to the effect of processing on the nutritional quality of soybean but still a thorough study in this field is needed. Keeping this perspective in mind, the present study was undertaken to estimate the total protein content, trypsin inhibitor and phytate content in soybean seeds in raw and processed state.

Materials and Method

The experiments were conducted using soybean seeds (*Glycine max*) of the variety JS-335. Certified seeds of soybean were procured from Durgapura Agricultural Research Station, Jaipur. Seeds were biochemically tested to analyse the content of protein and some anti-nutritional factors like trypsin inhibitor and phytate. Seeds were subjected to three processing methods- soaking, pressure cooking and roasting as these are the practises which are commonly used in Indian homes. Roasting was done at 100ºC for 5 minutes in the oven. The samples were pressure cooked at 15 psi pressure at 121 ºC for 20 minutes. For soaking seeds were soaked in water for 24 hours at room temperature with seeds to water ratio of 1:10(w/v)

Biochemical estimations were done in both raw and processed soybean seeds by standard methods described in the lab manual of National Institute of Nutrition (Raghuramulu *et al*, 1983). In each case a standard curve was prepared by using known concentrations of the nutrient to be tested and then the quantity of the nutrient in the given sample of soybean was calculated by extrapolating on the standard curve. Total protein content was estimated by the method of Lowry *et al*, (1951). A minimum of five replicates of the sample were taken in each case. All the tests were performed simultaneously in raw and processed seeds so that variations in climatic conditions do not affect the results. All the results were analysed statistically.

Results and Discussion

Soybean constitutes a very nutritious food being rich in proteins. Although it has high nutrient content but the bioavailability of the nutrient is low owing to the presence of anti-nutritional factors such as phytate and trypsin inhibitor. To reduce the effect of these anti-nutritional factors, mostly it is consumed after processing such as soaking, roasting, sprouting and pressure cooking. The processing methods besides affecting anti-nutritional factors also have an effect on the nutrients. Many processing methods increase the nutritive value while some cause a decrease in it (Palande *et al,* 1996). Nutrient loss on cooking depends on condition of cooking and the stability of nutrients (Rajyalakshmi and Geervani,1990). This aspect of Soybean is still unexplored and needs to be studied thoroughly to standardise cooking conditions for normal human diet with an idea of maximising nutritional qualities and minimising anti-nutritional qualities.

In the present study, seeds of the hybrid variety JS 335 of soybean were selected and biochemical estimation of the content of protein, phytate and trypsin inhibitor was done in raw and processed (pressure cooked, roasted and soaked) soybean seeds.

Effect on Protein Content

The amount of total protein observed in raw soybean was 12.67g/100g of seeds. Considerable changes were observed by giving various treatments. Upon pressure cooking and soaking an increase was observed, whereas by roasting a decrease in protein content was observed. In case of roasting, the decrease was significant i.e. 17.20% of the total protein ($P<0.05$) whereas increase by pressure cooking and soaking was non- significant i.e. 2.72 % and 6.19% of the total protein respectively (Table 1; Fig.1).

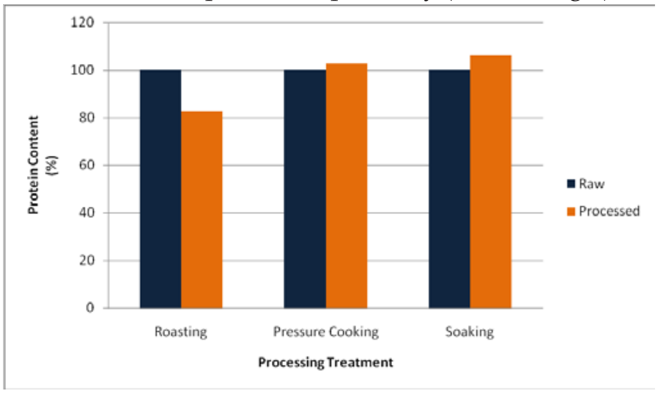

Fig. 1. Protein content in raw and processed soybean

Similar studies had also been conducted by several other workers. Richard and Puwastian, 1984 also observed an increase in protein content by soaking. Antony and Chandra, 1998 observed similar results in finger millet. They found that soaking for 12 hours significantly increased the protein content. Similar were the observations of Khader (1983); *Ertas(2011);* Xu and Chang (2011), in their studies on soybean. According to them this increase might be due to increased mobilisation of protein by water uptake. Similar were the results of Sattar *et al,* 1989 in the study performed in mung beans. Contrary to our results, Kasson and Bakowpsi (1986); Faldet *et al,* (1991) observed a decrease in protein content by soaking.

Table 1. Effect of processing treatments on protein content of soybean seeds

Protein content in raw soybean seeds (g/100g) Mean ± S.D.	Processing Treatment	Protein content in processed soybean seeds (g/100g) Mean±S.D.	%Variation (Gain/ Loss)
12.67 ± 0.04	Roasting	10.50 ± 0.48	- 17.20%[a]
	Pressure cooking	13.01 ± 0.25	+ 2.72%[b]
	Soaking	13.45 ± 0.15	+ 6.19%[b]

+/- = Gain/Loss

a = significant difference (P<0.05)

b = non significant difference (P<0.05

According to them, the decrease might be due to leaching out of proteins from seeds. They also observed that losses in protein increased with the increase of temperature of the water which further supports their conclusion.

In our study a significant decrease in protein content was observed by roasting (17.20%). Similar were the results of Egbe and Akinyele, 1990. The observed decrease in protein content was 3.4%. They concluded this decrease might be due to structural deterioration of proteins by heat. (Clemente *et al,* 1998)

Data available on the effect of roasting and pressure cooking is very limited and needs to be investigated thoroughly.

All these studies lead us to conclude that this aspect of soybean study needs immediate and intensive study as soybean is an important part of the human diet.

Effect on Trypsin Inhibitors Activity

One of the limitations to an increased use of grain legumes as food is the presence of diverse compounds in their grain, commonly referred to as anti-nutritional factors, that both decrease nutritive value of grain legumes and, if taken in larger amounts, cause health problems that may be fatal for both human and animals. By this reason, studies of all grain legumes is aimed at decreasing the content of anti-nutritional factors to a safe extent (Egbe and Akinyele,1990 ; Yadav and Khetarpaul, 1990; Reddy and Pierson,1994; Vig and Walia, 2001; Yasmin, *et al,*2008; Khattab and Arntfield, 2009; Kaushik *et al,* 2010; Mugendi *et al,* 2010; Güzel and Sayar, 2012)

Soybean contains large quantities of natural toxins or antinutritients like trypsin inhibitor and phytate. Trypsin inhibitors block the action of trypsin and other enzymes needed for protein digestion. They can produce serious distress, reduced protein digestion and reduced amino acid uptake.

The study was conducted in raw as well as processed soybean seeds. In raw seeds, the amount of trypsin inhibitor was 114.16mg/100g of seeds. Considerable changes were observed by giving various treatments. A decrease was observed by all the processing methods, but, in case of soaking the decrease was comparatively less. The decrease in trypsin inhibitor content was 46.21%, 66.10% and 30.09% by roasting, pressure cooking and soaking respectively which were significant (P<0.05) (Table 2; Fig.2). Several other experts also conducted similar studies. Decrease in trypsin inhibitor activity after soaking was also studied by Kataria *et al,*(1988); Ramnani *et al,* (1996); Egounletya and Aworhb (2009). Embaby (2010) also observed that cooking, soaking and dehulling soybean, cowpea and groundbean for 30, 7 and 15 min respectively resulted in 82.2%, 86.6% and 76.2% decrease in trypsin inhibitor

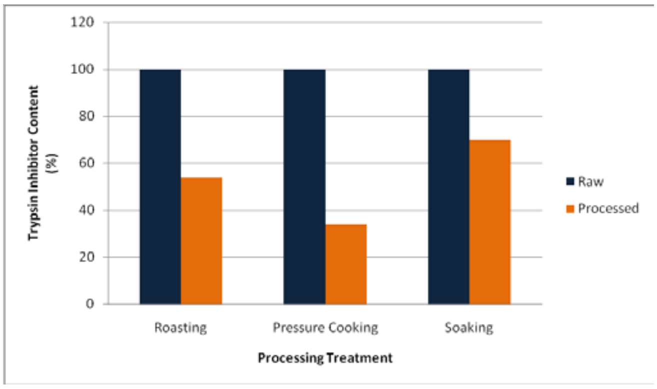

Fig. 2. Trypsin inhibitor content in raw and processed soybean

According to Ramnani *et al,* (1996), trypsin inhibitor can be also be inactivated upto 95%-100% by roasting and dehulling. Decrease in trypsin inhibitor activity by pressure cooking and roasting were also observed by Kumar *et al,* (2001); Khokhar and Chauhan (1986). According to them loss in trypsin inhibitor activity is due to heat labile nature of trypsin inhibitor . They observed that heat treatments were beneficial for improving the

Table 2. Effect of processing treatments on trypsin inhibitor content of soybean seeds

Trypsin inhibitor content in raw soybean seeds (mg/100g) Mean±S.D.	Processing Treatment	Trypsin inhibitor content in processed soybean seeds (mg/100g) Mean±S.D.	%Variation (Gain/ Loss)
114.16 ± 0.62	Roasting	61.4 ± 0.18	-46.21% [a]
	Pressure cooking	38.7 ± 0.25	-66.10% [a]
	Soaking	79.8 ± 0.54	-30.09% [a]

+/- = Gain/Loss
a = significant difference (P<0.05)

nutritive value of soybean. At 100°C steaming for 15 minutes was found to be sufficient to inactivate the trypsin inhibitor activity. El-Adawy (2002) compared the effects of cooking treatments and germination and observed that germination was less effective than cooking treatments in reducing trypsin inhibitor.

Effect on Phytate Content

Phytate is a hexaphosporic acid derivative of inositol and exists mainly in soybean seeds as insoluble non-nutritional complex. It is quite high in soybean and has a strong binding affinity to minerals such as calcium, magnesium, iron and zinc; this results in precipitation, making the minerals unavailable for absorption. About two thirds of the total phosphorus from soybean seed is bound to phytic acid. Phytic acids are common in the hulls of nuts, seeds and grains.

In our study the amount of phytate in raw seeds of soybean was 16.03mg/100g of seeds. Considerable changes were observed by all the cooking methods adopted for the study but maximum decrease was observed by roasting i.e. a decrease of 14.71mg/100g of seeds which accounts to about 88.65% decrease (Table 3; Fig.3).

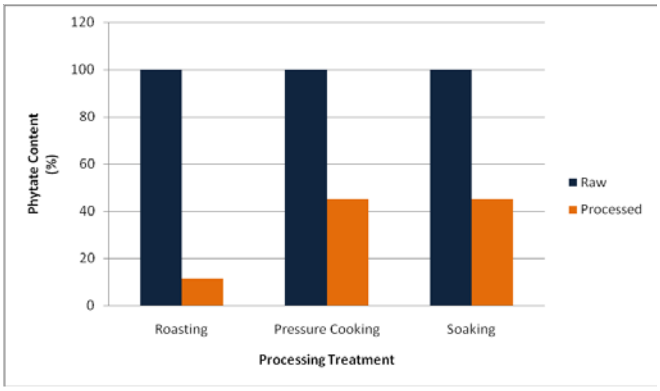

Fig. 3. Phytate content in raw and processed soybean

Sanbarg and Andlid, 2002 also had similar observation in their study. But the observations of Egounletya and Aworhb, 2009 were opposite to this. According to them soaking the beans for 12–14 h increased the phytic acid content to 1.7% in soybean and to 0.8% and 0.7% in groundbean and cowpea .

Kataria *et al*, (1989), in their study on mungbean (*Vigna radiata* L.) seeds, adopted domestic processing and cooking methods including soaking, ordinary cooking, pressure cooking of soaked and unsoaked seeds and sprouting.

Table 3. Effect of processing treatments on phytate content of soybean seeds

Phytate content in raw soybean seeds(mg/100g) Mean ± S.D.	Processing Treatment	Phytate content in processed soybean seeds (mg/100g) Mean±S.D.	%Variation (Gain/ Loss)
16.03 ± 0.16	Roasting	1.82 ± 0.06	- 88.65% [a]
	Pressure cooking	7.23 ± 0.13	- 54.90% [a]
	Soaking	7.23 ± 0.23	- 54.90% [a]

+/- = Gain/Loss
a = significant difference (P<0.05)

They reported a significant lowering of phytic acid, saponin and polyphenols. Soaking for 18 h removed 30% phytic acid and extent of removal was still higher when the period of soaking was raised An increase in the period of pressure cooking was more effective in reducing saponins and polyphenols than phytic acid. Soaking the seeds in plain water and mineral salt solution for 12 hr decreased phytic acid to the maximum (46–50%). Soaked and dehulled seeds showed significant reductions in phytic acid (4%) Loss of antinutrients was at a maximum when soaked and dehulled seeds were autoclaved for 25 min. Antinutrient concentrations declined during germination; the longer the period of germination the greater was the reduction (Khokhar and Chauhan, 1986; Sharma and Sehgal, 1992).

Duhan *et al,* (1989), also reported almost similar results in legume grains. Phytic acid was lowered significantly by the common methods of domestic processing and cooking including soaking, cooking, autoclaving and sprouting. Sprouting had the most marked phytic acid lowering effect followed by autoclaving and soaking. Cooking of soaked seeds lowered phytic acid by 20-26% in chickpea and 35-40% in black gram grains whereas the loss was 7-11% and 6-9% in these pulses, respectively, when unsoaked seeds were cooked.

The reduction in content of phytic acid was found to be greater with distilled water soaking (14%) than with salt water soaking (8%). Cooking and autoclaving significantly reduced (32–35%) the phytic acid content (Vijayakumari *et al,* 1997).

According to Shah *et al,* 2011 not only soaking but germination promoted a significant reduction in phytates. These changes were attributed to an increase of phytase activities. In fact, this enzyme would cause solubilization of phytates and would release soluble protein and minerals. Phytates are present in soybeans in concentrations up to 2.3% of the grain's dry weight. Despite its possible deleterious effects on human nutrition, phytate is also an effective antioxidant. Based on the results, germination (day 2) should be popularised as a simple process for naturally fortifying food with essential minerals and vitamins, specially soybean.

Conclusion

The above results led us to conclude that maximum increase in protein content occurred by soaking. The antinutrient activity needs to be minimised to increase the palatability of a food. In the present study the effect of processing treatments on two antinutrients i.e trypsin inhibitor and phytate content were analysed. Utmost reduction in trypsin inhibitor and phytate content was observed by pressure cooking and roasting respectively. We thus reached a conclusion that it is best to consume soybean after pressure cooking and soaking.

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