ISSN:2319-2607 IISUniv.J.Sc.Tech. Vol.2(1),54-59 (2013)

RESEARCH ARTICLE

Nutritional Changes in Germinated Soybean, Green Gram and Bengal Gram

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Abstract

Legumes are the important source of protein, carbohydrate, dietary fiber, minerals and oil content. Germination causes some important changes in the biochemical and nutritional characteristic of the legumes that may be beneficial to human health and nutritional status. In the present study estimation of biochemical constituents present in legumes -soyabean, green gram and bengal gram was carried out. Further, an effort was made to find out the effect of germination on the nutritional profile of the selected legumes at 24 hours and 48 hours respectively. The content of nutrients - moisture, protein, fat, fiber, ash, carbohydrate, iron, calcium and ascorbic acid was estimated and comparison of these was made in the raw and processed forms. Moisture, Protein, fiber, ash, mineral and ascorbic acid content of all the three legumes were found to increase significantly ($p < 0.05$) after germination for 48 hours. While fat content was found to decrease. Carbohydrate content increased in soybean and a decrease was observed in both, green gram and bengal gram.

Keywords

Biochemical estimation, Germination, Legumes

Introduction

Legumes provide a valuable source of protein in the diet of many of the world's poor population. These are relatively inexpensive compared to meat foods, have high carbohydrate content and along with cereals contribute to meet the essential fatty acid (EFA) needs of an adult. It is well established that the protein of legumes and cereals mutually supplement each other and thereby improve the quality of the protein (Dikshit *et al,* 2003; Yasmin *et al*, 2008; Rusydi *et al,* 2011). The limited utilization of unprocessed legumes is owing to the poor nutritive value of its protein due to the presence of certain anti nutritional factors, such as hemagglutin, trypsin inhibitor, polyphenol, tannins, phytatis, etc. (Khalil, 2006). Processing to remove these anti nutritional compounds is an essential stage in the preparation of most legumes. Further bioavailability of protein and trace elements is also improved as a result of processing.

The major pulses and legumes, which find an important place in vegetarian diet, are bengal gram, black gram, red gram, soybean, green gram and lentil. Bengal gram (*Cicer aritinum L.*), like other legumes, is a valuable source of folate, iron, manganese and protein. It is also a healthy source of complex carbohydrates and is high in fiber and low in fat. The lecithin, a type of fat, which plays a major role in the control of blood cholesterol and triglycerides, is higher in bengal gram as compared to other legumes. Another legume, well known as a major source of edible oil, is soybean (*Glycine max*). It contains the oil in the range of 18.0 - 22.0% (Dikshit *et al,* 2003; Rusydi *et al,* 2011). Its fat characteristics and fatty acid components have also been extensively studied. Besides oil, it is also a good source of energy, protein, vitamins, minerals and dietary fiber. The high content of protein finds its use as a protein supplement in the European countries (Khattak *et al,* 2008). One of the additional popular pulses among vegetarian population is green gram (*Vigna radiata*), which is commonly referred to, as 'nutritional powerhouse' due to its high protein content. Along with protein, it is also rich in fiber, which aids in digestion and absorption of food. It plays a vital role in cholesterol metabolism, and thus controls blood cholesterol levels. The carbohydrate content is reported to be low in bengalgram, making it a good choice for people with diabetes. Owing to the nutritional attributes and important use in vegetarian diet these three legumes are selected in the present study to investigate the nutritional changes in them on germination.

As mentioned earlier the nutrient content of legumes changes as a result of different processing techniques like soaking, germination and roasting. Many legumes are consumed in sprouted form as it improves eating quality

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along with nutritional quality by not only reducing the anti nutritional factors but also increasing bioavailability of protein, essential amino acids, vitamins, carbohydrates and polyvalent minerals like calcium, iron and magnesium (Sangronis *et al*, 2006).

Through the present study, an effort has been made to investigate the effect of germination process on the biochemical composition of raw and processed bengal gram, green gram and soybean. Since germination is cheap and more effective in improving nutritional value, it is hoped that this can contribute to providing nutrition to general people (Rusydi *et al,* 2011).

Materials and Method

Procurement of Samples

Mature and dried seeds of all three selected legumes/ pulses were taken from different sources.

- Soybean seeds (*Glycine max*) of variety JS-205 were procured from an agriculturist at Heerapur village near Kota district, Rajasthan.
- Bengal gram seeds (*Cicer arietinum*) of variety RSG-895 and green gram (*Vigna radiata*) seeds were procured from Agricultural Research Station Durgapura, Jaipur, Rajasthan.

All the procured legumes were cleaned of dust, grid, damaged seeds and other foreign matter, before storing in polybags.

Process of Germination

The legumes were first soaked in water (seed-to-water ratio of 1:5, w/v) at the room temperature for a period of 12 hours. At the end of the period, the water was drained away and soaked legumes were subjected to 24 hours and 48 hours of germination, respectively, in dampened cotton cloth. The germinated seeds were then placed in a hot air oven for drying at a temperature of 70±5ºC until the weight of samples is constant. All the dried samples were then ground to fine powder and packed separately in zip lock bags, later to be used for estimation of different nutrients present in raw and processed legumes. The chemicals used for estimation were of analytical grade and estimation of nutrient content was carried out using standard techniques as describe below.

Nutrient Analysis

The raw and germinated legume seeds were analyzed for various nutrients using standard techniques. The analysis of various nutrients i.e moisture, protein, fat, fiber and ash was done by the standard AOAC (2005) methods. The moisture content was determined using hot air oven method. The nitrogen was estimated by micro Kjeldhal method (KEL PLUS KES-04L digestion unit, ELITE EX distillation unit). The nitrogen content thus determined was multiplied with a factor of 6.25 to arrive at the crude protein content. Soxhlet extraction method was used to estimate fat content (SOCS PLUS SCS-4). The estimation of crude fiber content was carried out by acid alkaline method using fibra plus (FIBRA PLUS FES-2). Estimation of ash content was done by incineration of the plant material in silica crucible in a muffle furnace. Carbohydrate was calculated by difference, after subtracting the values of moisture, protein, fat, fiber and ash from 100. Besides these, estimation of micronutrients like calcium, iron and ascorbic acid was done flame photometrically, spectrophotometrically (Raghuramunu, 2003) and titrimetrically (Sharma, 2005) respectively. The values of various nutrients analyzed are calculated on dry weight basis and are given in Table 1 and 2.

Statistical Analysis

All the data of biochemical analysis were statistically analyzed. Mean standard deviation and t-scores were calculated for five replicate samples of each nutrient.

Results and Discussion

Proximate Composition

Nutrient analysis of raw and germinated forms (24 hours and 48 hours) of selected legumes green gram, bengal gram and soybean were carried out using standard techniques (Table 1).

Moisture

On comparing the values of moisture content of raw and germinated samples it is observed that there is a overall significant increase in the moisture content. However the moisture content in soaked and germinated samples showed that there is a significant increase in the values on germination in case of green gram and Bengal gram as reported by Mubarak, 2005 in *mung* beans. While in case of soybean there is a decrease from soaking to germination as reported in case of kidney beans, *mung* beans, soybeans and peanuts by Rusydi *et al,* 2011; in *mung* beans by Shah *et al,* 2011; and in chickpea by Joshi *et al,* 2009. The increase in moisture is due to the absorption of water by legumes for the commencement of metabolic processes. The increase in water uptake with time is due to the increasing number of cells within the seed becoming hydrated (Rusydi *et al,* 2011; Shah *et al,* 2011). In green gram, Bengal gram the reason for gradual increase in moisture content from raw to soaking and germination may be attributed to the absorption of water from the dampened cloth, which is used for germination.

Protein

A significant increase in protein content was observed in green gram, bengal gram and soybean after 24 hours and 48 hours germination, respectively with a prior decrease in its content after soaking. This is in accordance to number of studies carried out to the effect of germination on protein content in variety of legumes/pulses like green gram (Shah *et al,* 2011), mucuna beans (Mugendi *et al,* 2010), black and white beans (*Phaseolus vulgaris*) (Sangronis *et al,* 2006), which have also reported an increase in protein content after germination. The increase has been explained due to extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components that take place during germination. The seedlings are sites of high amino acid biosynthesis activity, resulting in high content of free amino acids supporting the synthesis of proteins and development of the plant (Mugendi *et al,* 2010). Dikshit *et al,* (2003) too reported germination process to increase the availability of free amino acids and amino nitrogen content by 4 to 8 folds, respectively. Increase in protein was also attributed to loss of dry matter through respiration and microbial spoilage (Mugendi *et al,* 2010). Some of the additional studies carried out on pigeon pea (Onimawo and Asugo, 2004), however have reported a decrease in protein content due to the inadvertent loss of accumulated amide in the developing axis during washing of the sprouted seeds before analysis.

Fat

Unlike protein, fat content decreased significantly in all the three legumes on soaking and with increase in germination time. Similar significant decrease in fat content in *mung* bean (Shah *et al,* 2011), peanut, soy bean (Rusydi *et al,* 2011; Dikshit *et al,* 2003), chickpea (Joshi *et al,* 2009) and pigeon pea flour (Onimawo and Asugo, 2004), has also been reported in the literature. The decrease in fat content could be due to depletion of stored fat as a result of catabolic activity of the seeds during germination (Onimawo and Asugo, 2004). Rusydi *et al,* (2011) has also suggested that reduction in fat is due to the use of fat as the major source of carbon for seed germination, and it is also suggested that fatty acids are oxidized to carbon dioxide and water to generate energy for germination.

Table 1. Proximate composition of raw and germinated soybean, green gram and bengal gram (dry weight basis - g/100g)

Legumes	Processing	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
Green gram	Raw	9.31±0.45 ^{a}	24.48±0.33^{a}	1.77±0.33^{a}	4.51±0.25^{a}	3.82±0.04^{a}	56.11±0.22^{a}
	12 hours soaking	11.49±0.44 ^{b}	22.97±0.04^{b}	1.69±0.31^{b}	3.93±0.42^{b}	3.7±0.14^{a}	55.27±0.74^{b}
	24 hours germination	12.58±0.45^{c}	28.3±0.83^{c}	1.22±0.21^{c}	4.87±0.21^{c}	3.73±0.3^{b}	49.62±0.93^{c}
	48 hours germination	12.92±0.03^{d}	32.29±0.91^{d}	1.08±0.10^{d}	4.99±0.01^{c}	4.12±0.39^{c}	44.59±0.86^{d}
Bengal gram	Raw	9.424±0.43^{a}	17.5±0.32^{a}	5.42±0.22^{a}	4.42±0.2^{a}	3.15±0.1^{a}	60.08±0.50^{a}
	12 hours soaking	12.03±0.16^{b}	15.17±0.28^{b}	4.95±0.05^{b}	4.12±0.19^{b}	2.81±0.53^{b}	59.99±0.57^{b}
	24 hours germination	13.04±0.16^{c}	18.07±0.77^{c}	4.97±0.55^{b}	4.77±0.10^{c}	3.4±0.22^{c}	56.26±0.98^{c}
	48 hours germination	13.61±0.32^{d}	19.11±0.45^{d}	4.58±0.17^{c}	5.12±0.22^{d}	3.43±0.43^{d}	53.55±0.29^{d}
Soybean	Raw	6.96±0.04^{a}	42.87±0.2^{a}	19.77±0.03^{a}	5.06±0.3^{a}	5.59±0.08^{a}	19.75±0.12^{a}
	12 hours soaking	8.55±0.06^{b}	41.76±0.8^{b}	19.52±0.2^{b}	4.9±0.18^{a}	5.24±0.05^{b}	25.27±0.17^{b}
	24 hours germination	7.42±0.16^{c}	45.12±0.32^{c}	15.18±0.17^{c}	5.0±0.06 ^{a}	5.39±0.14 ^{c}	27.28±0.04^{c}
	48 hours germination	8.75±0.12^{d}	46.45±0.25^{d}	12.60±0.16^{d}	4.8±0.14 ^{a}	5.13±0.13^{b}	27.4±0.08^{c}

Mean ± standard deviation
Significant at $p < 0.05$ level
Means followed by the same superscripts within a column do not differ significantly ($p<0.05$)

Fiber

In case of green gram and bengal gram, fiber content was found to increase significantly, while in case of soybean the change was insignificant. The effect of time duration of germination is also noteworthy. In case of green gram and bengal gram the increase was 7.98% and 10.6%, and in case of bengal gram increase was 13.03% and 21.32% from raw sample at 24 hours and 48 hours germination respectively. In earlier studies the sprouting has shown an increase in total fiber content of peanut, soybean (Rusydi *et al,* 2011), *mung* beans (Shah *et al,* 2011). This increase could be attributed to the synthesis of more of cell wall material like cellulose and hemicelluloses to support the shoots and rootlets during germination. Onimawo and Asugo (2004) on the other hand reported fiber content to be not significantly affected by germination in pigeon pea. Contradictory results are also available in the literature, reporting significant decrease in fiber content in green gram and kidney bean. This may typically be caused by to either genotypic differences (Shah *et al,* 2011) or on account of removal of some amount of hull and sprouts into soaked water (Rusydi *et al*, 2011).

Ash

The ash content of green gram, bengal gram and soybean decreased significantly ($p < 0.05$) on soaking, however there was a significant increase after 24 and 48 hours germination, in case of green gram and bengal gram, and a non significant decrease in case of soybean. The increase in case of green gram and bengal gram may be explained due to decrease in fat and carbohydrate content which may have led to the apparent increase in ash. Similarly significant increase was reported with increase in sprouting time in *mung* bean during germination by Shah *et al,* (2011). While the decrease in its content soaking may be explained due to leaching out of organic and inorganic compounds into soaked media (Akubor, 2004).

Carbohydrate

Carbohydrate content increased significantly ($p < 0.05$) in germinated soybean samples, while decreased significantly in green gram and bengal gram samples. Similar increase in the carbohydrate content has been reported by Rusydi *et al,* (2011) in germinated *mung* and kidney beans. The carbohydrate content was estimated on calculation basis so the increase in its content in soybean was due to decrease in other nutrients i.e. fat, fiber and ash. As in case of green gram and bengal gram the decrease was due to increase in other nutrients i.e. moisture, protein, fiber and ash content. Onimawo and Asugo, 2004; Dikshit *et al,* 2003 have also been reported a decrease in carbohydrate content in pigeon pea and in soybean respectively. No significant difference was observed in chickpea samples after germination (Joshi *et al,* 2009). The carbohydrate content in all the three samples were calculated after subtracting the values of moisture, protein, fat, fiber, ash from 100.

Table 2. Effect of germination on mineral and vitamin contents of soybean, green gram and bengal gram
(dry weight basis- mg/100g)

Legumes	Processing	Iron	Calcium	Ascorbic acid
Green gram	Raw	$3.68±0.17^{a}$	$120.58±1.98^{a}$	$3.46±0.4^{a}$
	12 hours soaking	$2.58±0.44^{b}$	$115.24±0.43^{b}$	$2.41±0.15^{b}$
	24 hours germination	$2.86±0.41^{c}$	$118.06±0.83^{c}$	$2.16±0.23^{c}$
	48 hours germination	$4.06±0.25^{d}$	$124.10±0.41^{d}$	$5.53±0.58^{d}$
Bengal gram	Raw	$5.04±0.13^{a}$	$201.13±2.3^{a}$	$3.57±0.41^{a}$
	12 hours soaking	$4.11±0.15^{b}$	$194.11±1.9^{b}$	$2.33±0.31^{b}$
	24 hours germination	$4.11±0.1^{c}$	$198.9±0.5^{c}$	$2.16±0.23^{c}$
	48 hours germination	$5.63±0.2^{d}$	$205.2±1.1^{d}$	$5.53±0.58^{d}$
Soybean	Raw	$9.6±0.10^{a}$	$245±0.17^{a}$	$3.09±0.08^{a}$
	12 hours soaking	$8.1±0.30^{b}$	$239±0.05^{b}$	$2.99±0.007^{b}$
	24 hours germination	$11.0±0.12^{c}$	$259.8±0.02^{c}$	$3.66±0.191^{c}$
	48 hours germination	$11.8±0.02^{d}$	$263±0.05^{d}$	$3.982±0.0238^{d}$

Mean ± standard deviation
Significant at $p < 0.05$ level
Means followed by the same superscripts within a column do not differ significantly ($p<0.05$)

Micronutrient Analysis

Micronutrient analysis of raw and germinated forms (24 hours and 48 hours) of selected legumes green gram, bengal gram and soybean, were carried out using standard techniques (Table 2).

Results showed that germination increased the calcium and iron content significantly ($p < 0.05$) in all the three legumes, when compared to their raw forms. Since the calculations are done on dry weight basis, g/100g, the increase may be attributed to the loss of water soluble constituents during steeping and washing, thereby increasing the concentration of minerals in 100g.

Germination showed a significant ($p < 0.05$) increase in ascorbic acid content in all the three legumes with a prior decrease after soaking. Shah *et al*, 2011 also reported a significant increase in ascorbic acid content in germinated green gram. During germination several enzyme systems become active and bring about profound changes in the nutritive value of pulses. Ascorbic acid, which is practically absent in dry legume seeds, increases in significant amount after germination. The increase in ascorbic acid content after germination may be brought about by enzyme L-gulonolactose oxidase (GULO), which becomes active during germination. Also it is reported that during germination through enzyme driven steps monosaccharides like mannose and galactose convert to ascorbic acid (Harris, 1996; Wheeler *et al*, 1998).

Conclusion

The process of germination affects the biochemical composition of soybean, green gram and bengal gram. It is found that protein, fiber, calcium, iron and ascorbic acid content increased in all the three legumes while fat content decreased in soybean after germination. Besides this the carbohydrate content increased in soybean and decreased in bengal gram and green gram. The processing technique used here is simple and can easily be adapted at domestic level, thereby improving the nutritional quality of legumes. The germinated soybean, green gram and bengal gram, therefore have good potential as cheap and alternate sources of protein especially soybean. These can be included in the diet and can be utilized to improve food security.

Acknowledgement

The authors would like to thank the Department of Foods and Nutrition and Chemical Sciences, The IIS University, Jaipur for granting permission to carry out this study and providing facilities and materials to conduct the research.

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