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Original Research Article

Effect of soaking and sprouting on protein content and transaminase activity in pulses

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ABSTRACT

Background: Pulses belong to the family leguminosae. Pulses provide protein and fibre, as well as vitamins and minerals, such as iron, zinc, folate, and magnesium. In addition, the phytochemicals, saponins, and tannins found in pulses possess antioxidant and anti-carcinogenic effects, indicating that pulses may have significant anti-cancer effects. It is the practice of germinating seeds to be eaten raw or cooked. Sprouts can be germinated at home or produced industrially. The metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Sprouting grains causes increased activities of hydrolytic enzymes like lipase, improvements in the contents of total proteins, fat, essential amino acids, total sugars, B-group vitamins and starch digestibility, and decrease in phytates and protease inhibitors an increase in amino acid lysine is seen after sprouting, increase in proteolytic activity leads to hydrolysis of prolamins and increased lysine.
Methods: Proteins and transaminase activity were estimated from Green mung (*Vignaradiata*), Cowpea (*Vignaunguiculata*), Chick pea (*Cicerarietinum*), Moth bean (*Vignaaconitifolia*) and Black gram (*Vignamungo*) in raw (dried) state, after soaking for 12 hours and on sprouting. Students paired t-test was applied to data and statistical significance was established.
Results: Chick pea showed highest concentration of proteins whereas Black gram showed the least protein content. Concentration of protein after 12 hours of soaking resulted in chick pea showing highest concentration of proteins whereas green gram showed the least protein content. Chick pea was superior amongst the pulses, because it showed highest protein content after sprouting and highest alanine transaminase activity in 12 hours soaked sample as well as in sprouted sample. Raw moth bean showed highest alanine transaminase activity.
Conclusions: Protein content and transaminase activity were found to be highest in sprouted pulses. So, consumption of sprouted pulses should be encouraged. Amongst the pulses studied chickpea (*Cicerarietinum*) gave the maximum nutritional benefit because of its high protein content and high alanine transaminase activity, as compared to other (green gram, cowpea, moth bean and black gram) pulses.

Keywords: Alanine transaminase, Pulses, Proteins, Soaking, Sprouting

INTRODUCTION

Pulses belong to the family leguminosae. The term pulses cover all those grown for their dried seeds. Pulses are a valuable source of energy. The energy content of most pulses has been found to be between 300 and 540 Kcal/100g.[1] Pulses provide protein and fibre, as well as vitamins and minerals, such as iron, zinc, folate, and

magnesium. In addition, the phytochemicals, saponins, and tannins found in pulses possess antioxidant and anti-carcinogenic effects, indicating that pulses may have significant anti-cancer effects. Pulse consumption also improves serum lipid profiles and positively affects several other cardiovascular disease risk factors, such as blood pressure, platelet activity, and inflammation. Pulses are high in fibre and have a low glycaemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels. The carbohydrate content of pulses is high i.e 50 to 60%.[2] Predominant carbohydrate of pulse is starch. About 1.8 - 18% occurs as oligossacharide while 4.3-25%occurs as dietary fibre. Although the oligossacharides, which are made up of raffinose, stachyose, verbascose, cause flatulence in man but they can shorten transit time and promote the growth of bifido bacteria in man. The high dietary fibre content of pulses reduce the transit time in the mammalian gut and help to relieve constipation and diverticular disease, lower the blood cholesterol level due to its ability to bind with cholesterol in the human gut and reduce colonic cancer in man.[3] Pulses also have low glycaemic indices, which makes them valuable foods for diabetics.

Pulses have a high protein content; the value is about twice that in cereals and several times that in root tubers. For this reason, pulses are sometimes called "poor man's meat. Most pulses contain about 1% fat. The fat content besides contributing to the energy needs, provides the needed essential fatty acids for man. The vitamins present in appreciably quantities in pulses are thiamine, riboflavin, pyridoxine and folic acid; vitamin E and K.[4]

Sprouting is the practice of germinating seeds to be eaten raw or cooked. Sprouts can be germinated at home or produced industrially. They are a prominent ingredient of the raw food diet and common in Eastern Asian cuisine.

The desirable nutritional changes that occur during sprouting are mainly due to the breakdown of complex compounds into a simpler form, transformation into essential constituents and breakdown of nutritionally undesirable constituents. The metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Complex biochemical changes occur during hydration and subsequent sprouting. The reserve chemical constituents, such as protein, starch and lipids, are broken down by enzymes into simple compounds that are used to make new compounds. Sprouting grains causes increased activities of hydrolytic enzymes, improvements in the contents of total proteins, fat, certain essential amino acids, total sugars. However, improvements in amino acid composition, B-group vitamins, sugars, protein and starch digestibility, and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process. An increase in amino acid lysine is seen after sprouting, increase in proteolytic activity leads to hydrolysis of prolamins and increased lysine. Sprouting increases crude fibre content and lipase activity. Sprouting increases Vitamin content: Especially the B-group vitamins. Certain vitamins such as Vitamin-E and β-carotene are produced during the growth process. When seeds are sprouted, minerals chelate or merge with protein and increase their function. Pulses are rich source of alanine transaminase.[5]

The aim of the study was to assess the nutritional benefits of pulses after soaking and sprouting. The pulses chosen for our study were Green gram (*Vignaradiata*), Cowpea (*Vignaunguiculata*), Chick pea (*Cicerarietinum*), Moth bean (*Vignaaconitifolia*)and Black gram (*Vignamungo*) in raw(dried) state, after soaking for 12 hours and on sprouting.

METHODS

This study was carried out in the Department of Biochemistry, Lokmanya Tilak Municipal Medical College and General Hospital. Pulses were Green gram (*Vignaradiata*), Cowpea (*Vignaunguiculata*), Chick pea (*Cicerarietinum*), Moth bean (*Vignaaconitifolia*) and Black gram (*Vignamungo*) were procured from local market and ground to uniform powder with the help of a mixer grinder. The pulses were soaked for 12 hours and later were subjected to sprouting Proteins were estimated from the dry samples, 12 hours soaked samples and sprouted samples by Biuret method in which alkaline copper sulphate reacts with peptides containing two or more peptide bonds to form a violet coloured complex. The colour of this complex was compared with a standard protein colorimetrically.[6] Alanine transaminase activity was also estimated from these samples by Reitman and Frankel method in which the product pyruvate obtained on transamination reacts with 2,4 dinitro phenyl hydrazine in an alkaline medium to form reddish brown coloured hydrazone which is measured colorimetrically at 530 nm and compared with a pyruvate standard treated similarly. Students paired t-test was applied to the data and statistical significance was established.[7,9]

RESULTS

Pulses have a high protein content, the value of which is about twice that in cereals and several times that in root tubers. For this reason, pulses are called poor man's meat. Pulses when eaten with cereals increase the protein quality of the meal. Specially in children, the consumption of pulses should be encouraged, particularly where animal protein is scarce and expensive, as this would help to provide them with the necessary amino acids required for growth. Exploitation of proteins present in legumes in the diet in combination with cereals will make it nutritionally balanced traditionally, pulses are soaked in water for twelve hours and subsequently subjected to sprouting. It is the practice of germinating seeds to be eaten raw or cooked.

The metabolic activity of seeds increases as soon as they are hydrated during soaking. Improvements in amino acid

composition, starch digestibility, sugars and proteins are the metabolic effects of the sprouting process. These proteins were estimated by Biuret method in the raw pulses, 12 hours soaked pulses and sprouted and findings are shown in Table 1. Highest content of proteins in these pulses were found after sprouting hence, sprouting as a traditional method of processing should be encouraged.

Table 1, It depicts the concentration of proteins in raw, 12 hours soaked and sprouted samples of 5 pulses commonly consumed by the population. Protein calorie malnutrition is a major problem faced by the developing countries, pulses are cheap sources of proteins as compared to the non-vegetarian sources.

Table 1: Highlights the concentration of proteins in raw pulses, 12 hours soaked pulses and sprouted pulses.

Sample	Protein (gm%) (raw pulses)	Protein (gm%) (12 hours soaked pulses)	Protein (gm%) (sprouted pulses)
Green gram	22.5±0.912	30±1.0	36±0.57
Cowpea	30±1.07	32±0.57	40±0.57
Chickpea	32±1.87	40±1.0	48±0.57
Moth bean	30±1.0	36±1.52	40±12.34
Black gram	20±1.52	32±1.52	36±1.54

Results are expressed as mean ± standard deviation.

Therefore Table 1 shows that in raw state of pulses chickpea has the highest concentration of proteins and black gram has the least protein content. In 12 hours soaked state of pulses the concentration of protein showed that chickpea has the highest protein and green gram showed the least protein content. Sprouted state of pulses showed that chickpea has the highest concentration of proteins while green gram and black gram showed the least protein content. Cowpea and moth bean showed equal protein content.

Statistical analysis of proteins in pulses

The data on protein content of each pulse in raw state, soaked and sprouted stewasta subjected to statistical analysis i.e, Paired student's t-test and statistical significance was established.

Table 2: Green gram.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	-14.03	4.30	NS
12 hours versus sprouted	-16.65	4.30	NS
Raw versus sprouted	-50.0	4.30	NS

Table 3: Cowpea.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	15.46	4.30	S
12 hours versus sprouted	-0.50	4.30	NS
Raw versus sprouted	2.33	4.30	NS

Table 4: Chickpea.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	-19.06	4.30	NS
12 hours versus sprouted	-19.15	4.30	NS
Raw versus sprouted	13.77	4.30	S

Table 5: Moth bean.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	15.82	4.30	S
12 hours versus sprouted	2.31	4.30	Ns
Raw versus sprouted	-7.76	4.30	Ns

Table 6: Black gram.

Sample	T value (calculated)	T value (tabulated)	P value
Raw versus 12 hours	-9.66	4.30	NS
12 hours versus sprouted	-3.02	4.30	NS
Raw versus sprouted	-26.5	4.30	NS

NS =not significant, s = significant.

Pulses are rich in transaminase activity. There is a close relationship between transaminase activity and protein synthesis in plants. Germinating seeds are seat of rigorous metabolic activity where proteins are constantly broken down and amino acids formed are taken up the growing plant for the de novo synthesis of proteins. The Transaminase activity in raw, 12 hours soaked and sprouted pulses was estimated by Reitman and Frankel method.

Table 2 depicts the activity of alanine transaminase in raw, 12 hours soaked and sprouted samples of pulses. It showed that raw moth bean has the highest alanine transaminase activity while raw black gram has the least. In 12 hours soaked state of pulses the activity of alanine transaminase was found to be highest for chickpea as compared to other pulses. Chickpea had the highest alanine transaminase activity in the sprouted state while black gram showed the least.

Table 7: Highlights the activity of transaminase in raw, 12 hours soaked and sprouted pulses.

Sample	Transaminase activity (unit/ml) (raw pulses)	Transaminase activity (unit/ml) (12 hours soaked pulses)	Transaminase activity (unit/ml) (sprouted pulses)
Green gram	18152±0.1	32515±0.01	36859±0.02
Cowpea	21665±0.01	36020±0.02	38715±0.17
Chickpea	16490±0.01	38715±0.02	43865±0.11
Moth bean	24240±0.01	26784±0.02	31000±0.02
Black gram	13885±0.02	26784±0.03	36890±0.02

Results are expressed as Mean ± Standard Deviation

Statistical analysis of transaminases activity in pulses

The data on alanine transaminase activity of each pulse in raw state, soaked and sprouted states was subjected to statistical analysis i.e, Paired student's t-test and statistical significance was established.

Table 8: Green gram.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	-1.41	4.30	NS
12 hours versus sprouted	-8302.2	4.30	NS
Raw versus sprouted	41.49	4.30	S

Table 9: Cowpea.

Sample	t value (calculated	t value (tabulated)	P value
Raw versus 12 hours	-4200.11	4.30	NS
12 hours versus sprouted	-1508.61	4.30	NS
Raw versus sprouted	-1488.60	4.30	NS

Table 10: Chickpea.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	-66672.32	4.30	NS
12 hours versus sprouted	-276.70	4.30	NS
Raw versus sprouted	-15804.96	4.30	NS

Table 11: Moth bean.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	-276.04	4.30	NS
12 hours versus sprouted	-1094.92	4.30	NS
Raw versus sprouted	-100.69	4.30	NS

Table 12: Black gram.

Sample	t value (calculated)	t value (tabulated)	P value
Raw versus 12 hours	-1843.71	4.30	NS
12 hours versus sprouted	-127.80	4.30	NS
Raw versus sprouted	-68896.29	4.30	NS

NS =not significant, s = significant

DISCUSSION

Pulses are a rich source of proteins. These provide good quality proteins to the consumers. Pulses are cheap and readily available in abundance. They serve as source of

proteins in patients suffering from protein calorie malnutrition. Traditional methods of processing pulses are soaking them for 12 hours and subsequently subjecting them to sprouting. Soaking and sprouting improves the nutritional values of pulses. In our study, five pulses which were commonly consumed were analysed for protein content in raw state, after soaking for twelve hours in water and after sprouting. These results are highlighted in Table 1. Chickpea had the highest concentration of proteins in raw, soaked and sprouted states.

Our research findings are in agreement with the observations made by Harmuth-hoene AE et al and Syed Shah A et al. They have reported that sprouts of green gram and chickpea have a greater nutritional value because of high proteins, calcium and phosphorus.[9,10,12] The values of proteins of black gram in the raw state compare with the findings of Tresina PS et al.[13] Amongst the 5 pulses studied chickpea showed highest protein content in raw, 12 hours soaked and sprouted samples. Chickpea renders maximum nutritional benefit to the consumers in the sprouted state hence, chickpea consumption in the sprouted state should be encouraged. Preparations with sprouted chickpea should be given to patients with protein malnutrition.

Pulses are rich in transaminase activity. In our study, five pulses which were commonly consumed were analysed for transaminase activity in raw state, after soaking for twelve hours in water and after sprouting. These results are highlighted in Table 7. Chickpea had the highest transaminase activity in soaked and sprouted states. Moth bean showed highest transaminase activity in raw state.

Transaminase activity co relates positively with protein synthesis in the seeds. It is clearly evident that chickpea had the highest protein content hence shows high alanine transaminase activity.[14] No comparative study of these 5 pulses is reported in the literature.

Statistical analysis of proteins in all five pulses was carried out, for each pulse raw sample was compared with 12 hours soaked sample and 12 hours soaked sample was compared with sprouted sample. The last sample compared was raw v/s sprouted. As seen from the results most of the values showed statistical non-significant values only in case of cowpea and moth bean raw versus 12 hours, in chickpea raw versus sprouted statistical significant values were observed. Statistical analysis of proteins in all five pulses is depicted in Table 2, 3, 4, 5 and 6.

Statistical analysis of Transaminase activity in pulses showed all the values to be statistically non-significant except for green gram (raw versus sprouted). Statistical analysis of alanine transaminase activity in all five pulses is depicted in Table 8, 9, 10, 11 and 12.

CONCLUSION

Soaking and germination affected the protein, transaminase activity of the pulses. Protein content and transaminase activity were found to be highest in sprouted pulses. The nutritional benefits in terms of protein and transaminases increases on sprouting, so consumption of sprouted pulses should be encouraged. Amongst the pulses studied chickpea (*Cicerarietinum*) gave the maximum nutritional benefit because of its high protein content and high transaminase activity, as compared to other (green gram, cowpea, moth bean and black gram) pulses. However, study of antinutrient factor in these pulses needs to be done.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Tiwari, Brijesh K, Gowen A, McKenna B. ed. Pulse Foods: Processing, Quality and Nutraceutical Applications; 2011:366.
2. Reddy NR, Pierson MD, Sathe SK, Deshpande SS, Salunkhe DK. Dry bean tannins- a review of Nutritional implicationa. J Am Oil Chem Soc. 1985;62(3):541-9.
3. Hangen L, Bennink MR. Composition of beans and navy neans (phaseolus vulgaris) reduced azoxymethane - induced colon cancer in rats. Cancer. 2002;44(1):60-5.
4. Tiwari, Brijesh K, Singh, Naripinder, Tiwari, Brijesh. Pulse Chemistry and Technology, RSC Publishing; 2012:54.
5. Safron, Jeremy A. The raw truth: the art of preparing living foods. Berkeley: Celestial Arts. 2013 ISBN 1-58761-172-4:8-18.
6. Alonso R, Orue E. Marzo F. Effects of extrusion and conventional processing methods on protein and anti-nutritional factor contents in pea seeds. Food Chem. 1998;63:505-12.
7. Sathe SK, Deshpande SS. Effects of germination protein, raffinose oligosaccharides, and anti-nutritonal factors in the great Northern Beans (Phaseolus vulgaris L). J Food Sci. 2006;48(6):1796-800.
8. Mahajan BK. Methods in biostatistics for medical students and research workers, 5th edition, Jaypee Brothers; 2002:83.
9. Shah SA. African journal of agricultural Research African Journal of Agricultural Research. 2011;6(22):5091-7.
10. The influence of germination on the nutritional value of wheat, mung beans and chickpeas Harmuth-Hoene AE, Bognar AE, Kornemann U, Diehl JF. Z Lebensm Unters Forsch. 1987;185(5):386-93.

11. Bhandal A. Effects of fermentation on in vitro digestibilities and the level of antinutrients in moth bean (Vignaaconitifolia (jacq. Marechal). Int J Food Sci Tech. 2008;43(11):2090-4.
12. Mudryj AN, Yu N. Nutritional and health benefits of pulses Aukema HM. Appl Physiol Nutr Metab. 2014;39(11):1197-204.
13. Trasina PS, Daffodil ED, Linsy P, Mohan VR. Assessment of biochemical composition and nutritional potential of three varieties of Vignaradiata Wilezek. Int J Biol Lifesci. 2014;2(2):655-67.
14. Giri KV, Radhakrishnan AN, Vaidyanathan CS. Transaminase activity in plants. J Indian Inst Sci. 1952;34:305-13.

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