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Original article

Study of germination, soaking and cooking effects on the nutritional quality of goat pea (*Securigera securidaca* L.)

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ABSTRACT

Effect of traditional processing on the nutrient composition, antinutritional factor and in vivo protein digestibility of goat pea (*Securigera Securidaca* L.) seeds was investigated. Various traditional processing had no significance effect on protein, lipid, fiber and ash except carbohydrate. Essential amino acid analysis revealed high concentration of lysine, leucine and histidine whereas the sulfur containing amino acids are limited. Germination and cooking significantly increased essential amino acid level. Traditional processing resulted in significant decrease of antinutrient phytate contents by 4.32, 1.47, 4.15, 1.9, 1.91 and 4.12% for raw, raw cooked, soaked and cooked, germinated and germinated cooked, respectively. Similarly, tannin was also decreased to 7.1, 4.51, 4.5, 0.75, 0.87, 0.26 and 7.1% for the above samples. Biological parameters showed that the net protein utilization (NPU) for the casein was significantly higher than the raw and processed seeds.

1. Introduction

Legume seeds play an integral role in human nutrition due to their protein content and are consumed as mature and immature seed, pods and leaves. Pulses have been used as the alternative for protein sources, after blending with cereal proteins show good functional properties such as solubility, water and oil holding capacity, emulsion stability and foaming. Due to these properties, legume proteins are used in the preparation of the bakery products, soups, extruded products and ready to eat snacks (Maldonado-Hoil et al., 2011). In order to improve the nutritional quality and organoleptic acceptability of leguminous seeds, processing techniques have been reported by several investigators, to reduce the antinutrients present in them. Some of the commonly used processing techniques include soaking or boiling with water, alkaline or acidic solutions, sprouting, autoclaving, roasting, dehulling, microwave treatment, steam blanching and fermentation (Esenwah and Ikenebomeh, 2008, Slupski, 2011a,b). Ohenhen and Ikenebomeh, (2007) reported that the soaking method was used to improve the nutritional values of raw seed materials in the manufacturing of food products. Soaking and fermentation process occurs simultaneously in the starch containing food grains. Parveen and Hafiz (2003) reported that during soaking process several complex compounds breakdown into simple, which may alter the texture, flavor and aroma. Studies of Dicko et al. (2006) found that the germination can improve the nutrition of raw seeds by reducing starch, induces hydrolytic enzymes synthesis such as phytate reduction and some flavonoid components. This study confirmed that the germination process is very important for the raw seeds to improve the nutrients and preparing for the development of food with low viscosity and high energy. Due to germination process phytic enzymes gets activate and responsible for the hydrolysis of phytic acids (Narish et al., 2012). Soaking and germination at different conditions and time significantly improved the physicochemical and functional properties of horsegram flour (Handa et al., 2017).

Present study, therefore, aims to investigate the nutrient composition of raw seeds and the effect of different traditional processing methods.

Abbreviations: NPU, Net protein utilization; AOAC, American official analytical chemists; RP-HPLC, Reverse phase High performance liquid chromatography; OPA, *O*-phthaldialdehyde; TC, Tannin content; HCl, Hydrochloric acid; PA, Phytic acid; FAO, Food and Agricultural Organization; WHO, World health organization; UNU, United Nations University; IVPD, Invivo protein digestibility; CCAC, Canadian council of animal care; SAS, Statistical analysis software.

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2. Material and methods

Seed of *Securigera securidaca* (goat pea) are procured from local market. Seeds were cleaned and divided in to six groups for traditional processing (Table 1). Samples were air dried under shade, grinded and stored at 4 °C before analysis.

2.1. Proximate analysis

Fat, fiber, and ash contents were determined according to the methods of (AOAC International, 2012). The protein content was determined by the Kjeldhal method (Jiang et al., 2014), using protein-nitrogen coefficient of 6.25. Total carbohydrate of the samples was calculated by subtracting the value of protein, oil, fiber, ash and moisture content from 100. Each sample replicated three times, and data were analyzed by means ± standard deviation.

2.2. Amino acid analysis

Essential amino acids were determined according to (AOAC International, 2012). Samples (100 mg) are subjected to acid hydrolysis with 5 ml of 6 M HCl under nitrogen atmosphere for 24 h at 110 °C. The hydrolyzed amino acids were proceeded for RP-HPLC analysis (Shimadzu LC-10AD, Shimadzu Corporation, Kyoto, Japan) after postcolumn derivatisation with *O*-phthaldialdehyde (OPA) and reported as gm amino acid/100 gm of protein.

2.3. Determination of tannin content

Tannin content (TC) of samples was estimated using modified vanillin-HCl in methanol as described by Price et al. (1978). 0.2 g of ground sample was placed in 100 ml conical flask and 10 ml of 1% HCl in methanol (v/v) was added. The contents were mechanically shaken for 20 min and centrifuged at 2500 rpm for 5 min. One ml of supernatant was pipetted out in a test tube and 5 ml of vanillin-HCl reagent (mixing equal volume of 8% concentrated HCl in methanol and 1% vanillin in methanol) was added. The optical density was recorded using Spectrophotometer at 500 nm after 20 min incubation at 30 °C, a blank sample was also analyzed with each run of samples. Standard curve was prepared expressing the result as catechin equivalents, i.e. amount of catechin (mg/ml) which gives color intensity equivalent to that given by tannin after correcting for blank.

$$\text{Calcium} : \cdot\text{Tannin content}(\%) = [C * 10/200] * 100$$

Where: C = concentration corresponding to optical density, 10 = volume of extract in ml, 200 = sample weight in mg.

Table 1
Traditional processing of goat pea seeds.

Group No.	Samples	Traditional processing	Sample weight (g)
1	Raw	Raw seeds	1000
2	Soaked	Raw seeds were soaked in water for 6 h at room temperature (25 °C ± 3 °C)	1000
3	Germinated	Raw seeds were germinated for 48 h at room temperature (25 °C ± 3 °C)	1000
4	Raw cooked	Raw seeds were cooked for 20 min	1000
5	Soaked and cooked	Soaked seeds were cooked for 20 min	500
6	Germinated and cooked	Germinated seeds were cooked for 20 min	500

2.4. Determination of phytate content

Phytate content of the samples was determined according to the modified method of Kakati et al. (2010) using 2.0 gm of a dried sample. Standard curve was prepared expressing the results as Fe $(NO3)^3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

2.5. Net protein utilization (NPU)

2.5.1. Diet formulation

The ingredient composition of the experimental diets are presented in Table 2. The diets were formulated on nitrogen (N) basis to provide 10% protein diet. Casein served as the reference protein. Corn starch, cellulose, soya bean oil, choline, methionine, mineral and vitamin mixture were added to balance the diets (Reeves et al., 1993). The diets were packaged in polythene bags and stored at −20 °C.

2.5.2. Experimental animals

Forty eight white male wistar albino infant rats were used to conduct 10 ays study, weighing 50 ± 10 gm and aged 21 ± 3 days obtained from animal house, College of Pharmacy. Throughout the experimental period individual rat was housed in stainless steel metabolic cage with good aeration at room temperature and normal daily 12-hour light/12-hour dark cycle. All animal procedures are in accordance with the recommendations of the Canadian council of animal care (CCAC, 1998) for the proper care and use of laboratory animals (Olfert et al., 1993). The rats used for experiment were divided into 8 groups containing 6 rats in each group.

2.5.3. Feeding and faeces collection

During the experimental period, 15 gm of feed offered daily for individual rats. Food not eaten during the experimental period was collected and weighed again. Faeces were collected daily from individual rat and cleaned it from left over feed, then dried and grinded to calculate the amount of food utilized by individual rat.

At the end of experiment, rats were slaughtered and divided into two groups. One was experimental animals and second one was control animals (Bender and Miller, 1953). Experimental animal group gives the information about the protein needed for feed. This study was critical in obtaining certain conditions like the age, sex and duration of experiment. Control group gives the same information for the feed without protein.

3. Data analysis

The data collected during the experiment was used to calculate the food intake, body weight gain, and protein efficiency ratio. The data regarding stool collection and dry powder of sacrificed rats were calculated the % NPU, nitrogen digestibility, nitrogen retention and biological value with concerned formula.

$$\%\text{NPU} = \text{Nitrogen retained}/\text{Nitrogen intake by feed} \times 100$$

4. Statistical analysis

Each experiment was carried out on three separate sample followed by mean. Data were assessed using SAS software (SAS) (1990). Mean comparisons for the treatments were made using the Duncan's new multiple range tests (DMRT) with a probability $p \leq 0.05$.

Table 2
Diet composition of the rat feed (g/kg).

Group/composition	Protein free diet	Controlled sample	Raw	Soaked	Germinated	Raw cooked	Soaked cooked	Germinated cooked
Casein with 10% protein	–	118	–	–	–	–	–	–
Protein sample 10%	–	–	416	429	418	431	431.4	423
Soya bean oil	70	70	70	70	70	70	70	70
Mineral mixture	35	35	35	35	35	35	35	35
Vitamin mixture	10	10	10	10	10	10	10	10
Choline	2	2	2	2	2	2	2	2
Cellulose	50	50	50	50	50	50	50	50
Starch	832	714	416	403	414	401	400.6	409
DL-Methionine	1	1	1	1	1	1	1	1

5. Results and discussion

Approximate chemical analysis of the raw and processed goat pea (*Securigera securidaca* L.) seeds is presented in Table 3. Raw seed contains protein 25.55%, carbohydrates 60.7%, Fat 1.88%, ash 4.97% and Fiber 6.03%. The protein contents of raw goat pea is similar to the lentil 26.9%, fenugreek 26.30% (Adsule and Akapopunam, 1996) and higher than lima beans 21.5% (Nowokolo and Smartte, 1996) and mung bean 23.6% (Akpapunam, 1996). Various traditional processing (cooking, soaking) did not significantly affect the protein content of goat pea seeds. Raw goat pea contains 1.88% fat which is higher than, mung bean 1.3%, lentil 0.8% and lima bean 0.7%. This decrease in fat is attributed to the disruption of the cell structure during cooking (Koidis, and Boskou, 2015). Carbohydrate (60.7%) of raw goat pea seeds is higher than the fenugreek, Indian green peas and white bean (Nowokolo and Smartte, 1996). Significant increase in carbohydrate was 63.4%, while germinated and cooked 66.6%. There is no significant difference in other processing methods, these results are in agreement with Giami (1993).

5.1. Effect of traditional processing on phytate

Phytic acid (PA) data of raw and processed goat pea is summarized in Table 4. The processing method was reduced the phytic acid level in goat pea seeds. Raw seeds contain 4.32% of PA, after germination reduced to 1.90% corresponding to highest inhibition of 56% (Chau et al., 1997). Soaked and cooked seeds contains 1.90% of PA with 56% inhibition (Alonso et al., 2000, 1998; Deshpande and Cheryan, 1983; Vdal-valaerde et al., 1994). There was no significant reduction of PA in the case of germinated and cooked seeds. The reason for the reduction of PA content in goat pea seeds is due to the leaching out in water.

5.2. Effect of traditional processing on tannin

Data of tannin in raw and processed goat pea is summarized in Table 5. Various processing methods affect the level of tannin in goat pea seeds. The raw seeds contain 7.10% of tannin where germination and cooking reduced the tannin to 0.26% and showed highest inhibition 96% (Reddy and Pierson, 1994). The germinated, soaked and cooked substances also has the low tannins 0.87% and 0.76%, respectively with 88% inhibition. Raw cooked and soaked substances did not show significant change in tannin contents. Reduction of tannin contents in goat pea seeds was due to the hydrolysis of high molecular weight insoluble polymers into soluble low molecular weight polymers (Vernhet et al., 2011)

Table 4
Traditional processing effect on phytate level of goat pea seeds.

Processing	Phytate %	Inhibition %
Raw	4.32 ± 0.38^{a}	–
Raw Cooked	1.47 ± 0.021^{c}	65.97
Soaked	4.15 ± 0.014^{a}	3.94
Soaked and Cooked	1.90 ± 0.11^{c}	56.00
Germinated	1.91 ± 0.014^{c}	56.00
Germinated and Cooked	4.12 ± 0.099^{a}	4.63

Mean ± Standard deviation (N = 3).
Means with the same letters are not significantly different.

Table 5
Traditional processing effect on tannin level of goat pea seeds.

Processing	Tannin %	Inhibition %
Raw	7.10 ± 0.063^{a}	–
Raw Cooked	4.51 ± 0.077^{b}	36.50
Soaked	4.50 ± 0.028^{b}	36.62
Soaked and Cooked	0.76 ± 0.021^{a}	89.30
Germinated	0.87 ± 0.007^{c}	87.70
Germinated and Cooked	0.26 ± 0.007^{e}	96.30

Mean ± Standard deviation (N = 3).
Means with the same letters are not significantly different.

5.3. Effect of traditional processing methods on essential amino acids

Essential amino acid data is reported in Table 6. In general essential amino acids in raw goat pea are within the recommended limits for the adults, whereas lower than the pre-school children, (Joint FAO/WHO Expert Consultation on Protein Quality Evaluation, 1991). Raw goat pea seed is rich in histidine (4.12%)

Table 3
Proximate analysis of raw and processed goat pea seeds.

Sample	Protein %	Fat %	Ash %	Fiber %	Carbohydrates%
Raw	25.55 ± 0.15^{ba}	1.88 ± 0.27^{a}	4.97 ± 0.65^{a}	6.03 ± 1.11^{a}	60.70 ± 0.59^{dc}
Raw cooked	23.97 ± 0.37^{b}	1.55 ± 0.52^{a}	4.78 ± 0.62^{a}	5.50 ± 1.66^{a}	63.04 ± 0.51^{ba}
Soaked	25.95 ± 1.34^{a}	1.80 ± 1.41^{a}	4.53 ± 0.73^{a}	5.67 ± 0.29^{a}	62.30 ± 0.56^{d}
Soaked and Cooked	24.95 ± 0.49^{ba}	1.43 ± 0.86^{a}	4.88 ± 1.54^{a}	5.57 ± 2.26^{a}	65.63 ± 1.31^{bc}
Germinated	25.45 ± 0.78^{ba}	1.60 ± 0.50^{a}	5.10 ± 0.40^{a}	5.87 ± 1.38^{a}	61.05 ± 0.78^{dc}
Germinated and cooked	24.10 ± 0.42^{b}	1.87 ± 1.58^{a}	4.63 ± 0.23^{a}	4.60 ± 1.73^{a}	66.60 ± 0.57^{a}

Mean ± Standard deviation (N = 3).
Means with the same letters are not significantly different.

Table 6
Goat pea seeds essential amino acids (g AA/100 g protein).

Amino acid (AA)	Raw	Soaked	Germinated	Soaked and cooked	Germinated and cooked	Raw cooked	Reference protein
Lysine	5.30 ± 0.10^{a}	5.13 ± 0.058^{abc}	4.40 ± 0.058^{d}	4.90 ± 0.23^{c}	5.23 ± 0.15^{ba}	5.00 ± 0.10^{bc}	5.80
Methionine	0.77 ± 0.058^{b}	0.80 ± 0.00^{b}	0.60 ± 0.00^{d}	0.70 ± 0.00^{c}	0.93 ± 0.058^{a}	0.80 ± 0.00^{b}	2.50
Threonine	2.17 ± 0.058^{b}	2.10 ± 0.058^{b}	1.90 ± 0.10^{c}	2.10 ± 0.058^{b}	2.50 ± 0.15^{a}	2.10 ± 0.058^{b}	3.40
Isoleucine	1.10 ± 0.00^{d}	1.20 ± 0.00^{cb}	1.13 ± 0.058 ^{cd}	1.23 ± 0.058^{b}	1.40 ± 0.058^{a}	1.10 ± 0.00^{d}	2.80
Leucine	4.40 ± 0.058^{d}	4.80 ± 0.12^{c}	4.20 ± 0.10^{e}	5.00 ± 0.20^{b}	5.70 ± 0.00^{a}	4.80 ± 0.00^{c}	6.60
Valine	1.70 ± 0.00^{c}	1.77 ± 0.058^{cb}	1.83 ± 0.058^{b}	1.80 ± 0.00^{b}	2.13 ± 0.058^{a}	1.70 ± 0.00^{c}	3.50
Phenyl alanine	2.70 ± 0.00^{d}	2.80 ± 0.058^{c}	2.60 ± 0.12^{e}	3.00 ± 0.00^{b}	3.50 ± 0.12^{a}	2.90 ± 0.00^{cb}	6.30
Histidine	4.13 ± 0.12^{d}	4.60 ± 0.15^{c}	5.23 ± 0.253^{a}	4.90 ± 0.15^{b}	5.43 ± 0.15^{a}	4.30 ± 0.058^{d}	

Mean ± Standard deviation (N = 3).
Means with the same letters are not significantly different.

which is higher than the required by children, also higher than chick pea (Bryant et al., 1988). Goat pea seed contains 5.3% lysine which is similar to reference protein of 5.8% (Joint FAO/WHO/UNU Report, 1985). These values are higher than the peanut protein (Oke et al., 1975), sunflower seed and similar to Okra (Bryant et al., 1988), cotton seed and sesame (Betschart et al., 1975). Lysine is reduced due to the processing methods, soaking causes a significant increase in isoleucine and phenylalanine, however germination increases the isoleucine. The soaking and cooking cause an increase in valine, phenylalanine, isoleucine and leucine, while decrease in methionine and lysine. Unlike the process of germination the germinated and cooking had increased essential amino acids except lysine. Raw cooked process caused an increase in methionine and leucine, while decreases the lysine. Germination and cooking process is therefore increased the ratio of essential amino acids.

5.4. Net protein utilization (NPU)

Invivo protein digestibility (IVPD) values of goat pea seeds are shown in Table 7. Germinated and cooked process significantly increased the rat weight with highest percentage of pure protein i.e. 85.96% compared to other treatments. It may be due to the germination process leading to denaturation of proteins as well as reduction of tannin and phytic acid (El-Beltagy, 1996). Feeding of soaked goat pea seeds to rats gain lowest weight i.e. 0.96 gm compared to other process with lower level of pure protein. Raw cooked, soaked and cooked, and soaked goat pea seeds were having the low levels of pure protein, 22.15, 11.58 and 17.88%, respectively. Almost same amount of feed was provided in the cases of casein and raw-soaked goat pea seeds, rats gained almost same weight, but casein has higher percentage of pure protein (66.88) than the raw and cooked seeds (22.15). During cooking process, reduction will occur in some essential amino acids reduction will occurs. Rats had ingested more feed of soaked and cooked i.e. 1.74 gm than the germinated and cooked i.e. 1.42 gm, but net gain was observed by germinated and cooked goat pea seeds, because germinated and cooked goat pea seeds are having the highest percentage of pure protein 85.96%. Therefore, it indicates that the pure protein percentage is responsible for weight gain, not the amount of feed.

Table 7
Biological evaluation of raw and processed goat pea seeds.

Group	Daily rat feed (g)	Rat weight gain (g)	Protein %
Casein	1.61 ± 1.40	1.12 ± 5.27	66.88 ± 4.78^{a}
Raw	1.006 ± 1.21	0.80 ± 3.26	32.20 ± 3.96^{b}
Soaked	1.08 ± 1.71	0.74 ± 3.98	17.88 ± 5.06^{bc}
Raw Cooked	1.67 ± 1.26	1.25 ± 4.31	22.15 ± 3.47^{b}
Soaked and Cooked	1.74 ± 1.36	0.96 ± 3.41	11.58 ± 1.73^{bc}
Germinated and Cooked	1.42 ± 1.58	2.06 ± 5.36	85.96 ± 18.0^{a}

Mean ± Standard deviation (N = 3).
Means with the same letters are not significantly different.

6. Conclusion

Overall, the results of this study showed that cooking, soaking and germination affects the composition, antinutrients and nutritional quality of goat pea (*Securigera Securidaca* L.) seeds. Phytate content was decreased from 4.32 to 1.47% due to the traditional processing, similarly the tannin was decreased from 7.1 to 0.26%. Traditional processing have increased essential amino acid which improved net protein utilization (NPU). Therefore, the proteins of the goat pea (*Securigera Securidaca* L.) seeds are useful for the food industry in order to fulfill the demand of protein consumption by the human being.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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