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Effect of Cooking on the Proximate Composition, Uric Acid content and Anti-Nutritonal factors on insect Bruchid infested Gram at Graded Levels of Infestation

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Abstract

Effect of cooking on the nutritional quality of graded level (20, 40 and 60 percent of infestation) of Insect (*Callosobruchus chinensis (*L) infested *Bengal gram* was seen. The parameters studied were physical characteristics (Weight, per cent damage, colour) and chemical properties i.e. proximate constituents, energy, uric acid and anti nutrients viz, phytic acid and trypsin inhibitors. The results revealed that with increase in the level of infestation, significant increases ($p \leq 0.05$) in all the parameters were observed, except for decreases in values for energy, and true protein. Insect infestation showed an adverse effect on the physical and nutritional quality of *Bengal gram*. Presence of insect body fragments and excreta made the pulse unhygienic. The insect infested *Bengal gram* was damaged qualitatively as well as quantitatively. Even After cooking only, about 50 per cent decrease was noted in uric acid, Phytic acid and Trypsin Inhibitors. Even cooking of insect infested Bengal gram could not improve the quality of the legume.

Keywords: Insect infestation; *Bengal gram*; Uric acid; Calorific value; Phytic acid; Trypsin inhibitor; Cooking; Per cent damage.

Introduction

Legumes belonging to the family leguminosae hold a great promise in meeting the protein requirements of vulnerable groups of vegetarian population. Legumes are economically rich source of proteins, B- complex vitamins and minerals. A significant part of the world population also relies on legumes as a staple food for subsistence, particularly in combination with cereals [1,2]. Legumes provide about 20-24% proteins, 50-60% carbohydrates, 2-3% fats and 2-4% minerals [3]. Pulses are rich source of protein (lysine), low fat, high carbohydrates, fiber, low sulfur containing amino acids, micronutrients and vitamins, which help to get rid of protein malnutrition among vegetarian people especially children and nursing mothers. *Bengal gram* (*Cicer arietinum* L.) is the third most important pulse crop, produced in the world after dry bean and peas (http://www.aicrpchickpea.res.in). *Bengal gram* is widely used as protein rich supplement to achieve better health in developing countries.

Bengal gram is a nutritious legume providing about 360 calories, 17.1 g proteins, 3-5 g fat and 10.2 mg iron per 100 g [3]. The pulse is consumed in various forms, as whole legume, split legume and gram flour. It is also used for the preparation of some sweets. The legume is usually stored for a longer period or until the harvest of the next crop. The farmers generally store legume grains in low cost, locally made storage structures like *Jute bags, Mud bins, Thekka, Pucca kothi, Peru* and *Kaccha* room [4]. Improper storage conditions affect both the quality and quantity of pulses. Deterioration of pulses during storages occurs due to various physical, chemical and biological factors. These are temperature, humidity, storage structure, micro-organism, fungi, insects, rodents and birds. The losses during post harvest handling and storage are up to the extent of 8.5 per cent of total production [5]. The infestation results in quality deterioration including change in B-complex vitamins, protein, minerals and biological protein quality of pulses [6]. Infested grains are likely to contain living insects in different stages of development, their body fragments and excreta make the pulse unhygienic [6-9]; uric acid is one of the excretory end products of insects, when consumed in excess may lead to gout. Routine cleaning removes some of these impurities. The most common methods for consumption of legumes are cooking which improve their nutritional and organoleptic qualities [10]. But insect fragments and invisible larvae and pupal stages remain inside the grains which being hard particles may lead to digestive disturbances. Insect infested Bengal gram is consumed by unwary consumers after cleaning and washing. Work has been done on the nutritional quality of insect infested *Bengal gram* but not much work has been reported on the effect of cooking on nutritional quality of insect infested *Bengal gram*. So the present study was planned to see the effect of cooking on the physico-chemical and nutritional quality of *Bengal gram* at graded levels of infestation.

Materials and Methods

Bengal gram (*Cicer arietinum* L.) was procured from the local market .The pulse beetle *C. chinensis* (L.) (*Dhora*) were obtained from the previously infected *Bengal gram* and released in 500 g of *Bengal gram* in a container covered with muslin cloth. Then the culture of *C. chinenis* was maintained at temperature 28 ± 2°C and relative humidity of 75 ± 2%.

Preparation of sample

Legumes were cleaned manually to get rid of dust/other foreign materials and 1500 g sample was placed in triplicate in stainless steel air tight containers of 2 kg capacity. In each container, 80 (3 to 4 days old) pulse beetles irrespective of sex were released, covered with lids and kept for 28 days at room temperature (33° to 42°C). Depending upon climatic conditions, pulse beetle completes its life cycle in 23 to 28 days. The grains were first observed after 28 days, followed by 15 days interval, for obtaining desired levels of infestation (20, 40 and 60%). The legumes, along with containers, were deep frozen for 72 h to kill the adults as well as the developing insects. The insect debris and frass were removed manually and samples were stored in refrigerator toll further analysis.

Cooking

To see the effect of cooking, hundred gram control and insect (*C. chinensis*) infested *Bengal gram* samples at different levels of infestation were soaked and then pressure cooked in pressure cooker with equal amount of water for 30 minutes. The cooked samples were then dried in cabinet tray drier at 50°C ± 3°C for 12 h. The cooked and uncooked samples were milled separately in a Willy mill, to a fine powder so as to pass through a 40 mesh sieve. These samples were then kept in a refrigerator in air tight plastic containers till further analysis was done.

Density

One hundred grains were weighted in triplicate and were put in a graduated cylinder containing 50 ml of water and the rise in water level was noted. Density is weight volume ratio and calculated by formula:

Density=Weight (g)/Volume (ml)

Embedded larvae per cent

Soaked one hundred grains in triplicate for overnight. Next morning, grains were subjected for dissection with the help of a very sharp blade so as to open the grains completely and total numbers of grains with larvae inside were calculated.

Percent embedded larvae=No. of grains with larvae/100 × 100

For calculating the Per cent damaged grains: After properly mixing the grains in containers one hundred grains were taken out and examined for number of grains with holes in them.

Per cent damaged grains=Number of pulse grains with holes × 100

Proximate constituents

Proximate constituents *viz*. moisture, ash, crude fat and crude fiber contents in the samples were determined by standard methods of AOAC [11]. Nitrogen was analyzed by Micro-kjeldhal AOAC [11] and was multiplied by factor 6.25 for converting it into crude protein.

Energy in the samples was determined by chromic oxide method [12]. Non Protein Nitrogen (NPN) in sample was determined by the method of AOAC [11]. Phytic acid was determined by the method of Davis and Reid [13]. Trypsin inhibitor activity in the samples was determined by the method given by Ray and Rao [14]. Extraction of uric acid was conducted by method of Sangle et al. [15] and estimation was done by method of Oser [16].

The data from various laboratory experiments were subjected to statistical analysis for variance in a completely randomized design [7].

Results and Discussion

Physical parameters

Weight of the samples was increased by 1.62 per cent at 20 per cent level which decreased with increase in level of infestation. Maximum percent weight change (31.74%) was observed in the 60 percent infested uncooked *Bengal gram* and 36 percent weight loss observed in similar cooked counterpart. Similar trend was noted for density (Table 1). Weight, density and embedded larvae per cent losses in cooked grains were higher which might be due to reason that during cooking larval and pupal stages of insects emerged out leaving behind mainly the husk portion, which are light in weight and also some soluble nutrients leached out in water.

Table 1. Effect of cooking on physic-chemical characteristics of graded levels of insect (*C. chinensis*) infested Bengal gram.

Attribute	Uncooked (Level of Infestation %)				Cooked (Level of Infestation %)				CD (p ≤ 0.05)
	0	20	40	60	0	20	40	60	
Density	1.22	1.30	1.20	1.10	0.74	0.80	0.72	0.63	0.08
Embedded larvae (%)	0	46.75	45.09	38.92	0	38.16	30.15	25.08	1.87
Damaged grains (%)	0	24.56	48.75	85.92	0	27.08	52.71	90.25	1.12
Moisture (%)	9.51	11.53	13.43	14.53	8.77	10.40	11.45	11.77	1.34
Ash (%)	3.21	3.80	4.57	5.17	3.37	3.57	4.40	5.23	0.27
Crude protein (%)	17.95	18.98	20.31	23.15	16.39	17.99	18.75	20.17	0.34
Crude fat (%)	4.37	4.65	5.18	5.41	4.60	4.75	5.20	5.48	0.11
Crude fiber (%)	3.93	5.20	5.99	7.80	4.35	5.91	6.15	7.90	0.96

Embedded larvae come out during cooking and reduction in weight is noted in grains. Similar results have been reported by Samuel and Modgil [17,18]. Effects of cooking on the density of legumes have been reported by Mankotia and Modgil [19] for moth beans, which decreased when moth bean was cooked. With increase in level of infestation, increase in damage grains has reported, which might have been due to the reason that insects completes their larval and pupal stages inside the grains and adult insects emerged out after creating holes in the grains. Similar results have been reported by Srivastava et al. [20]. According to them the *C. chinensis* caused 88.97 per cent damage in pigeon pea.

Proximate composition

Significant changes in proximate composition of insect infested grains were observed (Table 2). As the levels of infestation increased a significant (P ≤ 0.05) increase was noted in the all the proximate constituents. An increase in moisture content was noted with increase in level of infestation which might have been due to insect and seed respiration which may reduce the structural quality and shelf life of *Bengal gram* [18]. Similar results have been also reported by Vimla and Pushpamma [21]. Increase in the ash content of cooked insect infested grains might have been due to the reason that grains were cooked in tap water and water was not discarded further insect infested grains had more husk. Increase in the crude protein content of uncooked insect infested grains of *Bengal gram* at different level of infestation might have been due to the reason that insect (*C. chinensis* L.) completes its larval and pupal stages inside the grains and even when adult insects emerged out their excreta is left behind in the cavity which causes an increase in crude protein. The increase in crude fiber and ash might have been due to the reason that insect *C. chinensis* has a tendency of consuming endosperm and germ portion of seeds which is rich in carbohydrates and husk portion rich in fiber is left behind. Increase in the crude fiber content of insect infested cooked grains of *Bengal gram* might have been due to the reason that cooking of legume itself increases the crude fiber content which is due to formation of new protein fiber complex [22-24].

As the levels of infestation increased, total nitrogen and NPN also increased in uncooked grains. Total nitrogen ranged from 2.87-3.70 g/100 g, 148 whereas NPN ranged from 0.001-2.06 g/100 g. True protein decreased with increase in the infestation levels. It ranged from 17.93 to 10.25 per cent. Cooking of insect infested *Bengal gram* resulted in a significant (p ≤ 0.05) decrease in total nitrogen, non-protein nitrogen and true protein content. Uric acid, which is one of the major end products of protein metabolism in insects, increased significantly (p ≤ 0.05) with increase in level of infestation. There was a negligible amount of uric acid in uncooked control samples. As the levels of infestation increased, manifold increase in the uric acid was observed. In insect infested uncooked control samples the maximum uric acid content (3575.27 mg/100 g) which was at 60 per cent infestation level. Cooking of insect infested grains of *Bengal gram* resulted in a decrease in uric acid content. Modgil and Mehta [24] reported an increase in NPN of insect infested legumes. As the levels of infestation increases, there was decrease in the true protein content of *Bengal gram*. The decrease in true protein content might have been due to the reason that more non protein nitrogenous substances are produced by insect *C. chinensis*. Proteins are also utilized by insects for their growth. The insect's saliva and urine in the seeds also contributes towards an increase in crude protein [20]. Cooking of insect infested grains resulted in a decrease in uric acid content, but uric acid was not destroyed completely even after cooking.

Anti-nutritional factors phytic acid and trypsin inhibitors increased with the increase in levels of infestation of *Bengal gram*. The maximum phytic acid content was in *Bengal gram* at 60 per cent level (357.06 mg/100 g). In uncooked insect infested grains of *Bengal gram* trypsin inhibitor ranged from 291.05 to 301.30 (TIU/g). Cooking of insect infested grains of *Bengal gram* resulted in a slight decrease in phytic acid and trypsin inhibitor. Insect infestation results in a significant (p ≤ 0.05) increase in the phytic acid content of *Bengal gram*. Insect consuming endosperm portion, as a result more husks is left, intact causing in an increase in phytic acid content. Modgil and Mehta [24] have revealed that graded levels of insect infestation resulted in increase in the phytic acid content of *Bengal gram*. Cooking of insect infested legume resulted in a decrease in phytic acid content, and trypsin inhibitor activity which might have been due to the reason that phytic acid and trypsin inhibitors are heat labile, anti nutritional factor which were destroyed during cooking. But

Table 2. Effect of cooking on energy, total nitrogen, non protein nitrogen, true protein, uric acid and anti nutritional content of graded levels of insect (*C. chinensis*) infested *Bengal gram*.

Attribute	Uncooked (Level of Infestation %)				Cooked (Level of Infestation %)				CD (P ≤ 0.05)
	0	20	40	60	0	20	40	60	
Energy (Kcal/100 g)	368	351	335	306	301	280	260	240	3.50
Total nitrogen (g/100 g)	2.87	3.04	3.25	3.70	2.62	2.88	3.00	3.23	0.15
Non protein nitrogen (g/100 g)	0.0001	0.51	1.05	2.06	0.00	0.23	0.86	1.87	0.10
True protein (%)	17.93	15.81	13.75	10.25	16.37	16.56	11.88	8.50	0.22
Uric acid (mg/100 g)	0.00	785.05	1620.66	3575.27	0.00	340.45	832.34	1650.96	511.80
Trypsin inhibitors (TIU/g)	290.97	293.80	295.01	301.25	165.32	193.05	235.32	181.27	12.54
Phytic acid (mg/100 g)	284.21	309.80	332.77	356.16	173.35	196.51	235.79	339.30	8.54

as the levels of infestation increases, increase in trypsin inhibitor activity was there. Cooking had resulted in a slight decrease in trypsin inhibitor activity of insect infested legume. Insect infestation has caused an increase in TIA which was reduced by cooking. Similar results have been reported by Sangle et al. [10] and Estervez et al. [25], who have found that the heat treatment reduced the anti nutrient factors.

Conclusion

Insect infestation showed an adverse effect on the physical and nutritional quality of *Bengal gram*. Even cooking of insect infested *Bengal gram* could not improve its quality due to presence of insects (*C. chinensis*) larval and pupal stages being inside the grains. Their body fragments and excreta made the pulse unhygienic. After cooking insect infested grains, about 60 per cent decrease was noted in uric acid and anti-nutritional factors. Insect infested legumes should not be consumed even after cooking. The insect infested *Bengal gram* was damaged qualitatively as well as quantitatively. Such grains should not be consumed by human beings.

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