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Studies on *Vigna mango* L. - Effect of Processing on Carbohydrate Fractionation and Influence of Grain Starch on Protein Utilization in Albino Rats

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Abstract
The effect of black gram starch on the utilization of casein was also tested in albino rats using PER, digestibility coefficient, biological value and net protein utilization as parameters. Soaking reduced the levels of sugars, starch and oligosaccharides but starch digestibility was improved significantly. Cooking increased the level of sugars while starch and verbascose content was decreased. Starch digestibility was also improved on cooking. Stachyose and raffinose contents were increased when unsoaked seeds were cooked, but decreased when the seeds were soaked before cooking. Germination decreased starch and oligosaccharide contents thereby raising the level of the soluble sugars. Starch digestibility was increased appreciably on germination. On cooking, the black gram starch promoted growth similar to corn starch as no difference was noticed in the parameters studied in albino rats.

Introduction
Legumes contribute a considerable amount of carbohydrates to the diet in the form of starch. Availability of energy from the dietary legumes is, therefore, dependent to a significant extent on the contents as well as digestibility of carbohydrates in these foods. Starch in legumes possesses low digestibility due to presence of a number of antinutritional factors, such as alpha-amylase inhibitors (Mulimani and Supriya, 1993; Feng *et al.*, 1996). Other antinutrients like phytic acid, saponin and polypheriols also have significant effect on the digestibility of legumes (Kataria *et al.*, 1989). Poor digestibility of starch have negative effects on the utilization of proteins and minerals and decrease in the rate of starch digestion may have therapeutic application (Dreher *et al.*, 1984). The digestibility can be improved through different processing and cooking methods (Kataria *et al.*, 1992; Shashikala and Prakash, 1995). The legumes are also regarded as notorious inducers of flatulence when these are consumed in large quantity (Singh *et al.*, 1982). Human digestive system lacks the enzyme, α-glactosidase, and the oligosaccharides pass into the large intestine where they are fermented anaerobically to produce gas (Sosulski *et al.*, 1982). Such oligosaccharides include verbascose, raffinose and stachyose. Legumes are also considered as a good source of protein by a great segment of population but the utilization of legume starch effects the utilization of legume protein (Gahlawat and Sehgal, 1994; Geervani and Theophilus, 1981). This study was conducted to see the effect of processing and cooking on the carbohydrate content, oilgosaccharides and starch digestibility in the domestic variety of the grain legume *Vigna mungo* L. commonly called as black gram. The influence of black gram starch on protein utilization in albino rats was also studied and reported in this paper.

Materials and Methods
The grain legume *Vigna mungo* L. (black gram) was collected from the Department of Plant Breeding and Genetics. University of Agriculture, Faisalabad, Pakistan.

Processing included the following methods

Soaking: Seeds were freed from dust and other foreign material and then soaked in water for 12 and 18 hours at room temperature. A seed to water ratio of 1.5 (w/w) was used. The seeds were washed and dried in an oven at 70°C for 36 hours.

Ordinary Cooking: The seeds after soaking for 12 h were rinsed in distilled water and put in round flasks fitted with condensers. Having added water the soaked seeds were cooked until soft. The seeds were then dried at 70°C for 36 h to a constant weight. The unsoaked seeds were rapidly rinsed with water and then cooked and dried in the same manner as mentioned above for the soaked seeds.

Pressure Cooking: The seeds soaked for 12 hours and unsoaked seeds were autoclaved for 15 min at 1.05 kg/cmy and then dried at 70°C for 36 hours.

Sprouting: The seeds soaked for 12 h were germinated in sterile petri dishes lined with wet filter papers for 24, 48 and 60 h at 25°C, with frequent watering. The sprouts were then dried at 70°C.

Chemical Analysis: Total soluble sugars were extracted by refluxing in 80 percent ethanol and starch from the sugar free pellet was extracted in 52 percent perchloric acid at room temperature. Soluble sugars, reducing sugars and starch were determined colorimetrically (Jood *et al.*, 1988). Non-reducing sugars were determined as the difference between total soluble sugars and reducing sugars. Starch digestibility (*in vitro*) was assessed by employing pancreatic amylase and incubating at 37°C for two hours. Liberated maltose was measured colorimetrically by using DNS reagent (Singh *et al*., 1982).

The oligosaccharides stachyose, verbascose and raffinose were extracted with 80 percent ethanol for 6 hours in a soxhlet apparatus. Butanol-pyridine-water (5:1:4 v/v) solvent was used to separate the oligosaccharides by descending chromatography run for 80 hours. The paper, after drying, was sprayed with ammonical silver nitrate. The strips were heated at 110°C until dark spots appeared which indicated the oligosaccharides. The sugars from the strips were eluted with water and their concentration estimated colorimetrically by the phenol-sulphuric acid method (DuBois *et al*., 1956).

Influence of starch on protein utilization: Black gram seeds were cooked as described earlier. Starch was extracted by method B described by Schoch and Maywald (1968). It was dried and powdered before use. The effect of black gram starch (cooked and uncooked) on protein utilization was assessed on the basis of protein efficiency ratio (PER), digestibility coefficient (DC), biological value (BV) and net protein utilization (NPU). Three groups of weanling rats of 15-20 days old were selected and distributed randomly with ten rats in each group. The weight range of the rats was between 28-32 g. Composition of the diet is given in Table 1. Rats were fed ad libitum daily for four weeks. PER was calculated on the basis of weight gain and protein consumed. After termination of growth study the rats were transferred to metabolic cages. One set of ten adult rats was put on a 4 percent egg albumin diet for determining the metabolic faecal nitrogen and urine nitrogen. Nitrogen in the food, faeces and urine was determined by microkjeldhal method (AOAC, 1990). The DC, BV and NPU were calculated on the basis of nitrogen absorbed and retained after correcting for metabolic nitrogen (Mitchell, 1964).

Results and Discussion

Carbohydrate Fractionation

Sugars: Soaking of dry seeds in water decreased the level of total soluble sugars, reducing sugars and non-reducing sugar of the black gram, significantly (Table 2). The loss aggravated with increase in the period of soaking. The seeds when soaked for 18 h lost 29.42 percent total soluble sugars, 35.92 percent reducing sugars and 22.22 percent non-reducing sugars. Simple diffusion of sugars. after getting solubilized, from the seed to the soaking medium may account for the losses of the sugars during soaking. The greater losses of sugars during the longer periods of soaking may be because of enhanced solubility of sugars. The disparaging effect of soaking on sugars in some pulses has been reported earlier (Jood *et al*., 1986, 1987).

After soaking for 12 h, the seeds were cooked until soft. Level of the total soluble sugars in the cooked seeds was significantly higher than that in the soaked seeds but still less than that in the unprocessed controls (Table 3). The cooking seemed to raise the level of reducing sugars to a greater extent than that of non-reducing sugars. Cooking of unsoaked seeds, as expected raised the concentration of the sugars significantly above the level found in unprocessed controls. Pressure cooking of soaked seeds not only recovered the loss of sugars encountered during soaking but also raised the level of sugars above controls. As a result of pressure cooking the total soluble sugars, reducing sugars and non-reducing sugars were higher by 14.20, 30.69 and 3.51 percent than those in the controls. Hydrolysis of starch to monosaccharides may be responsible for increased concentration of sugars in the cooked pulses. Rao and Belavady (1978) observed an increase in sugars contents of some pulses during cooking.

Table 1: Composition of casein diet supplemented with black gram starch

Ingredients	Casein	Black gram Starch
Casein	10.0	10.0
Corn starch	75.0	---
Black gram starch	---	75.0
Ground nut oil	10.0	10.0
Salt mix	4.0	4.0
Vitamin mix	1.0	1.0
Total	100.0	100.0
% Protein	9.0	9.1

When the 12 h soaked seeds were germinated there was decrease in the sugars but a gradual increase in concentration of sugars with increase in germination time was noticed (Table 4). Mobilization and hydrolysis of seed polysaccharides during germination may account for increased sugar content of germinated seeds. Reddy and Salunkhe (1980b) have also shown the decrease in total sugars during germination but it was found that the sugar content was increased with increase in germination time.

Starch: Soaking in plain water decreased the starch content of the black gram seeds (Table 2); the loss being highly significant ($p<0.01$) only after 12 and 18 h of soaking. When compared with decrease in soluble sugars starch was affected to a much less extent.

Ordinary cooking of soaked as well as unsoaked seeds reduced starch content significantly (Table 3); soaking or no soaking did not cause a significant difference in starch content of cooked beans. Starch content of the soaked and unsoaked pressure cooked seeds was less than that of unprocessed seeds by 41.98 and 35.04 percent respectively. Similar decrease in starch content due to autoclaving and ordinary cooking was studied by Jood *et al*. (1988). Sprouting resulted in considerable reduction in starch, extent of reduction was more when the germination time was prolonged (Table 4). A loss of 20.56 percent observed after 24 h germination went upto 35.59 percent when germination continued for 60 h. Jood *et al*. (1986) have also shown the decrease in starch content after germination in chickpea, green gram, bengal gram, black gram, red gram and brood bean.

Table 2: Effect of soaking on total soluble sugars, reducing sugars, non-reducing sugars, starch and *in vitro* starch digestibility of black gram

Soaking period(h)	Total soluble sugars (g/100g)	Reducing sugars (g/100g)	Non-reducing sugar (g/100g)	Starch (g/100g)	Starch digestibility(*)
0	6.80±0.20	2.67±0.10	4.13±0.22	42.32±1.31	73.88±1.41
12	4.95±0.00	1.92±0.18	3.02±0.23	38.89±2.11	80.99±1.89
18	4.80±0.14	1.71±0.11	3.08±0.20	36.68±1.24	96.10±2.80

($p<0.01$) * = mg maltose released/g. Values are means±SD of three independent determinations

Table 3: Effect of cooking on total sugars, reducing sugars, non-reducing sugars, starch and in vietro starch digestibility

Soaking period(h)	Total soluble sugars (g/1009)	Reducing sugars (g/100g)	Non-reducing sugar (g/100g)	Starch (g/100g)	Starch digestibility(*)
Ordinary cooking of soaked seeds	5.90±0.11	2.62±0.10	3.28±0.11	33.97·-0.48	245.32±10.1
Ordinary cooking of nsoaked seeds	7.16±0.11	2.99±0.11	4.17±0.13	35.17-0.57	148.68±12.8
Pressure cooking of soaked seeds	7.77±0.21	3.49±0.13	4.27±0.13	24.55=-0.52	262.30±9.7
Pressure cooking of unsoaked seeds	8.02+0.19	3.61+0.12	4.41±0.12	27.49±.0.32	236.82±10.2

($p<0.01$) * = mg maltose released/g. Values are means ±SD of three independent determinations

Table 4: Effect of germination on total soluble sugars, reducing sugars, non reducing sugars, starch and *in vitro* starch digestibility

Soaking period(h)	Total soluble sugars (g/100g)	Reducing sugars (g/100g)	Non-reducing sugar (g/100g)	Starch (g/100g)	Starch digestibility(*)
24	5.20±0.21	2.20±0.11	2.99±0.00	33.62±0.72	148.04±8.70
48	5.45±0.21	2.79±0.13	2.69±0.19	29.68±0.73	173.34±9.20
60	5.68±0.22	2.94±0.13	2.74±0.24	27.26±0.94	163.79±10.4

($p<0.01$) * = mg maltose released/g. Values are means ± SD of three independent determination

Table 5: Effect o processing and cooking on the oilgosaccharide content of *Vigna mango* L.

Treatment	Verbascose (mg/g)	Stachyose (mg/g)	Rafinose (mg/)
Raw seeds	31.4±0.30'	19.2±0.19	10.3±0.12
Ordinary cooking (Unsoaked seeds)	29.8±0.25	20.5±0.18	11.4±0.11
Ordinary cooking (Soaked seeds)	26.2±0.32	18.7±0.18	9.7±0.11
Pressure cooking (Unsoaked seeds)	27.3±0.20	18.8±0.19	12.3±0.12
Pressure cooking (Soaked seeds)	24.2±0.21	16.8±0.18	10.1±0.13
12 h Soaded	28.9±0.30	14.4±0.11	7.7±0.10
18 h Soaded	28.3±0.27	13.6±0.12	7.3±0.11
24 h Sprouted	13.1±0.18	4.2±0.10	1.7±0.10
48 h Sprouted	9.3±0.11	3.4±0.11	1.4±0.11
60 h Sprouted	9.1±0.12	3.3±0.11	1.4±0.10

Values are means ±SD of three independent determinations

Table 6: Effecet of black gram starch on PER, DC, BV, and NPU of casein fed to rats at 9 percent protein level

Source of setarch	PER	DC	BV	NPU
Corn	2.65±0.19a	81±4.3a	89±5.1a	76±4.7a
Black gram (uncooked)	2.25±0.18b	69±2.3b	85±2.9a	63±3.2b
Black gram (cooked)	2.41±0.23a	79±4.5a	87±3.8a	72±4.1a

Mean carrying the same letters are not significantly ($p>0.05$) different

Leaching out of soluble portion of starch from seed to the soaking medium is possible. Cooking may rupture the starch granules followed by amylolysis, Starch may also get hydrolyzed to monosaccharides during germination. All these factors may account for the decreased amount of starch in the processed legumes.

Oligosaccharide Content: Black gram seeds contained stachyose, raffinose and verbascose 19.2, 10.3 and 31.4 mg/g respectively (Table 5). Ordinary cooking decreased the verbascose content upto 16.5 percent in case of soaked seeds. Stachyose and raffinose increased a little bit for unsoaked seeds but slight decrease was found in case of soaked seeds. Pressure cooking decreased the verbascose content upto 23 percent. Stachyose content was also decreased, but to a lesser extent. Raffinose content was increased significantly in case of unsoaked pressure cooked seeds i.e. upto 19.4 percent. Increase was also found in case of soaked seeds but was very small (1.94% only). The increase in stachyose and raffinose may be due to hydrolysis of higher polysaccharides and verbascose during cooking. Decrease in verbascose content and a slight increase in the stachyose content have been reported earlier (Reddy and Salunkhe, 1980a; Reddy *et al*., 1980). Reddy and Salunkhe (1980b) noticed that cooking reduced the oligosaccharide content of black gram upto 74.5 percent. In contrast, Rao and Belavady (1978) observed a significant increase in the oligosaccharide content after cooking for 15 minutes at 15 psi.

Soaking decreased all the oligosaccharides and the decrease was higher after 18 hours soaking. Verbascose content decreased upto 9 percent and stachyose and raffinose upto 29 percent. Iyengar and Kulkarni (1977) also noted considerable losses of all saccharides when the seeds were soaked for 12 hours in water.

Germination upto 48 h resulted in a considerable reduction in the oligosaccharide content. Verbascose, stachyose and raffinose decreased upto 58.28, 78.12 and 83.49 percent respectively after 24 hour sprouting (Table 5). This suggests that flatus producing potential of these sprouted seeds should be considerably less than the original seeds. There was no or little decrease in the oligosaccharide content after 48 hour germination. The hydrolysis of these oligosaccharides is carried out by α-galactosidases present in the kernel (Reddy and Salunkhe, 1980b). Reddy and Salunkhe (1980b) also showed that a - galactosidase activity in black gram seeds was high and increased during the first two days of germination. Decrease in oligosaccharides, after germination has also been reported by Rao and Belavady (1978) and Reddy and Salunkhe (1980a). Disappearance of 86.8 percent of the total oligosaccharides of the raffinose family after germination of the seeds was found (Reddy *et al*., 1980).

Starch Digestibility: Soaking of the seeds improved the starch digestibility, the longer the period of soaking the higher was the starch digestibility (Table 2). Starch digestibility of the seeds soaked for 18 h was higher than that of unsoaked seeds. Factors like amylase inhibitors, phytic acid and polyphenols have been reported to inhibit alpha amylase (Deshpande and Cheryan, 1984). Level of these antinutrients in the legume seeds decreases during soaking (Jood *et al*., 1987) which may account for increased starch digestibility of soaked beans seeds (Table 2).

A significant negative correlation between phytic acid content and digestibility of starch has been shown by Sharma and Khetarpaul (1995). Such improvement in starch digestibility with reduction in phytic acid might be due to the fact that this antinutrient is known to inhibit amylolysis and proteolysis.

Starch digestibility increased more than three fold as a result of ordinary cooking of soaked legumes (Table 3). Ordinary cooking of unsoaked seeds also improved the starch digestibility but the digestibility was significantly higher when soaked instead of unsoaked seeds were cooked. Pressure cooking was more effective in improving the starch digestibility than ordinary cooking. The digestibility was higher when soaked rather than unsoaked seeds were pressure cooked. Enhancement of starch digestibility in cooked legumes may be attributed to rupturing of starch granules which facilitates more randomized configuration for alpha amylase to affect hydrolysis. Increased *in vitro* digestibility of available carbohydrates after cooking has been reported for other legumes like moth bean, cowpea and chickpea (Rao and Deosthale, 1982). Germination of seeds increased the starch digestibility which was raised upto 48 h and then decreased (Table 4). Similar results have been reported earlier (Jood *et al*., 1988). Kataria *et al*. (1992) also observed significant increase in starch digestibility in four varieties of amphidiploids by cooking and germination.

Influence of black gram starch on protein utilization: The protein efficiency ratio, and nitrogen balance data are presented in the Table 6. The black gram starch was found inferior to the corn starch in promoting growth ($p<0.05$) but the treated black grams starch supported grow equivalent to corn starch. Similarly the digestibility of the cooked black gram increased significantly and no difference was observed in the biological value, digestibility coefficient and NPU of the black gram starch from cooked seeds and corn starch. Present findings are in contradiction with those of Geervani and Theophilus (1981) who found that black gram starch supported growth similar to corn starch. They further showed that the red gram and Bengal gram starches had significantly lower PER as compared to the corn starch. These differences in growth were attributed to the greater in vitro digestibility of black gram starch than red gram and Bengal gram starches. It may be presumed from the present studies that the starch component of the diets influenced the utilization of protein. It has been pointed out earlier (Booher *et al*., 1951) that apparent digestibility of protein varies with the starch component of the diet. As the *in vivo* digestibility of cooked black gram starch increased with increase in starch content, the influence of starches on protein utilization may be through its interference in digestibility (Geervani and Theophilus, 1980). On the basis of these findings it can be postulated that the PER of black gram protein can be improved if the digestibility of the respective carbohydrates can be improved on par by adequate processing.

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