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Effect of Processing Methods on Secondary Metabolites and Enzyme Inhibitors in Different Developmental Stages of *Parkia roxburghii* G. Don Pods

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ABSTRACT

Secondary metabolites and enzyme inhibitors were studied in different developmental stages of the pods of *Parkia roxburghii,* a lesser known edible leguminous plant in Northeastern India. Flavonoid content in different stages of the pod decreased due to different cooking methods. The percentage removed being 93.18 and 98.48 mg g^{-1} in Tender Pods (TP), 94.39 and 98.13 mg g^{-1} in Immature Pods (IP) and 82.11 and 88.42 mg g^{-1} in Mature Pods (MP) due to ordinary and pressure cooking methods, respectively. Tannin content in TP (98.30 mg g^{-1}), IP (86.47 mg g^{-1}) and MP (56.27 mg g^{-1}) reduced to 12.81, 14.31 and 6.64 mg g^{-1} by Ordinary Cooking (OC) and to 16.69, 18.84 and 8.92 mg g^{-1} by Pressure Cooking (PC). Phytate Phosphorus (PP) in different stages of the pod ranged from 30.9 to 40.5 mg 100 g^{-1} which reduced to 25.2 mg 100 g^{-1} in TP, 24.8 mg 100 g^{-1} in IP and 34.2 mg 100 g^{-1} in MP by Ordinary Cooking (OC) and to 20.7, 17.1 and 25.2 mg 100 g^{-1} by Pressure Cooking (PC). Saponin content in tender (25.20 mg g^{-1}), immature (26.20 mg g^{-1}) and mature raw pod (28.95 mg g^{-1}) decreased to 16.20, 16.70 and 17.0 mg g^{-1} due to OC and to 15.20, 15.80 and 16.50 mg g^{-1} by PC. The different stages of the raw pods recorded 13.37, 7.77 and 6.94 Trypsin Inhibitor Units (TIU) mg^{-1} in tender, immature and mature pods which decreased to 6.02, 5.81 and 4.05 due to OC and to 4.41, 3.88 and 3.02 by PC. Amylase Inhibitor Units (AIU) in mg g^{-1} in TP (4.70), IP (7.90) and MP (8.40) reduced to 3.40, 6.40 and 6.80 by OC and to 3.90, 5.90 and 6.90 by PC. No significant differences were observed in TI and saponin content in different stages of the pod, however, AI increased with the age of the pod. It is reported for the first time.

Key words: Flavonoids, saponins, trypsin inhibitor, amylase inhibitor, processing methods

INTRODUCTION

Although many species and sub-species of legumes are known, only about a dozen of them are important as commercial food crops. Beans and peas each account for about 25% of the total production of legume crops. Chickpea and broad beans rank next in importance. Some of the legumes, however, are of only regional or local importance. There is at least 3000 edible plant species known to man. Out of which, merely 300 crops contribute to more than 90% of the world's calorie intake and only 120 crops are economically important on a national scale which implies that world's food security rest on a slender base of 4% of known edible plants (Cooper *et al*., 1996).

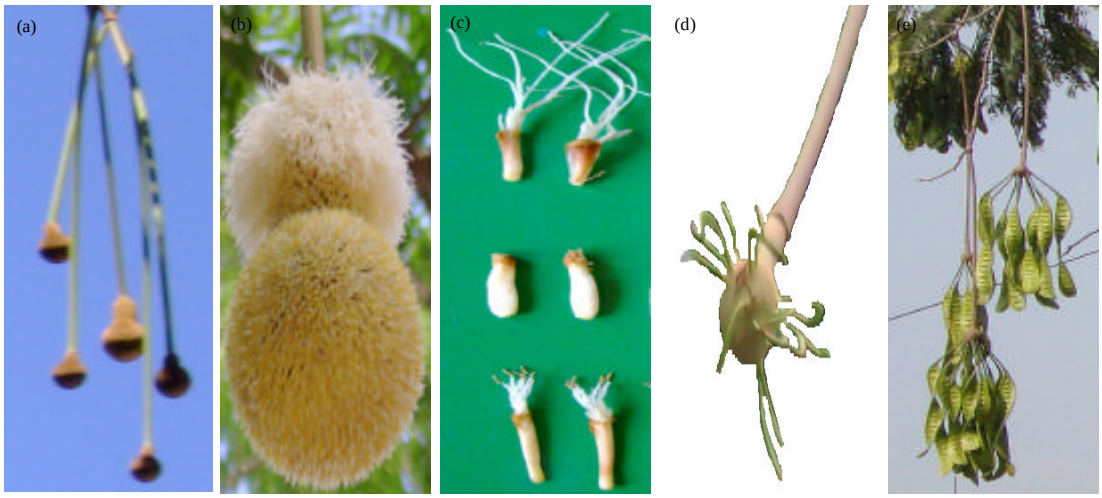

Fig. 1(a-e): Different developmental stages of *Parkia roxburghii* pods (a) Inflorescence (b) Flowering head (c) Different flowers (d) Emerging fruits and (e) Mature fruits

Therefore, from the point of view of meeting the challenges of food security it is necessary to widen the food base by establishing more and more new food crops. One of the most promising areas could be to look for non-conventional legumes. Non-conventional vegetables include those edible plants that grow in the wild or under semi-domesticated condition being grown casually as backyard crop but in any case these form an important component of human food that come from sources other than organized cultivation.

Parkia roxburghii G. Don (Common Name: Parkia or stink bean or tree bean) is considered nutritious and relished by the people of this region (Fig. 1a-e). Various parts of the plant right from the inflorescence and tender pods to the matured seeds are edible. The flowers are taken in the form of salads, whereas pods are used in the preparation of salads, curries, chutnies or in frying items (Salam, 1996). This vegetable is available for use for over seven months (October to April) of the year and can thrive on varied agro-climatic conditions from hotter plains to the colder mountains up to 2000 msl (Meitei and Jayalakshmi, 2005). As a member of the leguminoseae family, *Parkia roxburghii* also shares some of the anti-nutritional substances endemic to legumes. There are a number of reports dealing with such information in the case of popular legumes, whereas no information is available in respect of *Parkia roxburghii* which is considered nutritious in this part of the country especially in the state of Manipur and its adjoining areas (Appelbaum *et al*., 1999; Elias *et al*., 1979; Fenwick and Oakenfull, 1983; Bressani *et al*., 1982). In India, the most important/common domestic processing and cooking methods include soaking, dehulling, germination, ordinary and pressure cooking. These methods are reported to reduce the anti-nutrients in legumes (Adeparusi, 2001; Anderson and Wolf, 1995; Boateng *et al*., 2008; Chitra *et al*., 1996). This study reports part of a series of systematic investigations undertaken to determine the effect of processing methods on this lesser known legume.

MATERIALS AND METHODS

Different stages of *Parkia roxburghii* pods were collected from a plant grown at Iroisemba near the Central Agricultural University campus and classified them into 3 groups based on the mean thickness of the pod measured at the site of seeds as tender (6 mm and below), immature (6.1- 10 mm) and mature (more than 10 mm). All the pods were scrapped to remove the outer green

peel. The margin of the pods were neatly removed with the help of a knife and cut 10 cm (approximately) in length. They were divided into three groups of approximately equal weights and subjected to different methods of cooking following the method of Bishnoi and Khetarpaul (1994) with slight modifications. In Ordinary Cooking (OC), the pods were placed in a round open beaker and cooked on a heater with 4 times double distilled water (w:v) until they became soft. In case of Pressure Cooking (PC), the pods were pressure cooked (3 lit pressure cooker) at the maximum pressure for 2 min. In this case, the sample to water ratio was 1:3 (w:v).The remaining third group was treated as control. All the processed/cooked pods were air dried and kept in the oven at 60±5°C for 48 h. The samples were then ground using a Remi grinder and subsequently sieved (1 mm). The powder samples were collected and kept for various analyses.

Estimation of flavonoids: Flavonoid was analyzed following the procedures of Chang *et al.* (2002) with slight modifications. A 50 mg of the powdered sample was transferred into a 50 mL centrifuge tube. Then 10 mL methanol was added and stirred for 4 h in a magnetic stirrer. The solvent was evaporated to about 5 mL at room temperature and centrifuged at 10,000 rpm for 15 min. The supernatant was collected and volume made up to 5 mL with methanol. 0.1 mL of the extract supernatant was transferred into a test tube, 0.1 mL of aluminum chloride and 0.1 mL of 1 M potassium acetate were added and volume made up to 3 mL with double distilled water. This was allowed to stand for 30 min in room temperature and its absorbance was measured at 415 nm in a double beam UV-Visual spectrophotometer. The flavonoid was quantified against a calibration curve prepared using Quercetin (10-100 μg mL^{-1}) in methanol.

Estimation of tannins, phytate phosphorus and cyanogenic substances: Tannins and Phytate Phosphorus (PP) were determined following the procedures of Schanderi (1970) and Wheeler and Ferrel (1971). Cyanogenics determination was carried out as per the method of AOAC (1975).

Estimation of alkaloids and saponins: Alkaloid and Saponin were determined following the procedures of Obadoni and Ochuko (2002) with slight modifications. A 5 g of plant sample was dispensed in 100 mL of 20% ethanol. The suspension was heated over a hot water bath maintained at about 50°C and stirred with a magnetic stirrer for 4 h. The mixture was centrifuged and the residue re-extracted with another 100 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separator funnel and 20 mL of diethylether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated. A 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was evaporated to almost dryness on a water bath. The last traces of moisture were removed by drying in an oven (85°C) to almost constant weight. The difference in weight represents the saponin content.

Estimation of trypsin inhibitor and amylase inhibitor: Trypsin inhibitor activity was determined as per the method of Kakade *et al.* (1974) as modified by Sotelo *et al.* (1999). Amylase inhibitor activity was determined following the method of Deshpande *et al.* (1982) and A-amylase Inhibitory Activity (AIU) was expressed as enzyme inhibitor units in mg g^{-1} sample.

Statistical analysis: Standard errors of Mean differences (S. Ed±) and Critical Differences (CD) were calculated as per standard statistical procedures.

RESULTS

Table 1 indicated changes in secondary metabolites in different stages of the pods. Flavonoid content in the tender stage (13.2 mg g^{-1}) was higher than the other stages which decreased to 0.9 and 0.2 mg g^{-1} in OC and PC while in other stages it decreased to 0.6 and 0.2 mg g^{-1} in immature and to 1.7 and 1.1 mg g^{-1} in mature stages of the pod. The percentage removal of flavonoids in different stages of the pod due to OC and PC were 93.18 and 98.48% in TP, 94.39 and 98.13% in IP and 82.11 and 88.42% in MP (Fig. 2a).

Tannin content in tender (98.30 mg g^{-1}), immature (86.47 mg g^{-1}) and mature raw pods (56.27 mg g^{-1}) reduced to 12.81, 14.31 and 6.64 mg g^{-1} by OC and to 16.69, 18.84 and 8.92 (mg g^{-1}) by PC (Table 1). The percentage removed due to OC and PC was 86.97 and 83.02% in TP, 83.45 and 78.21% in IP and 88.25 and 84.15% in MP (Fig. 2b). Phytate Phosphorus (PP)

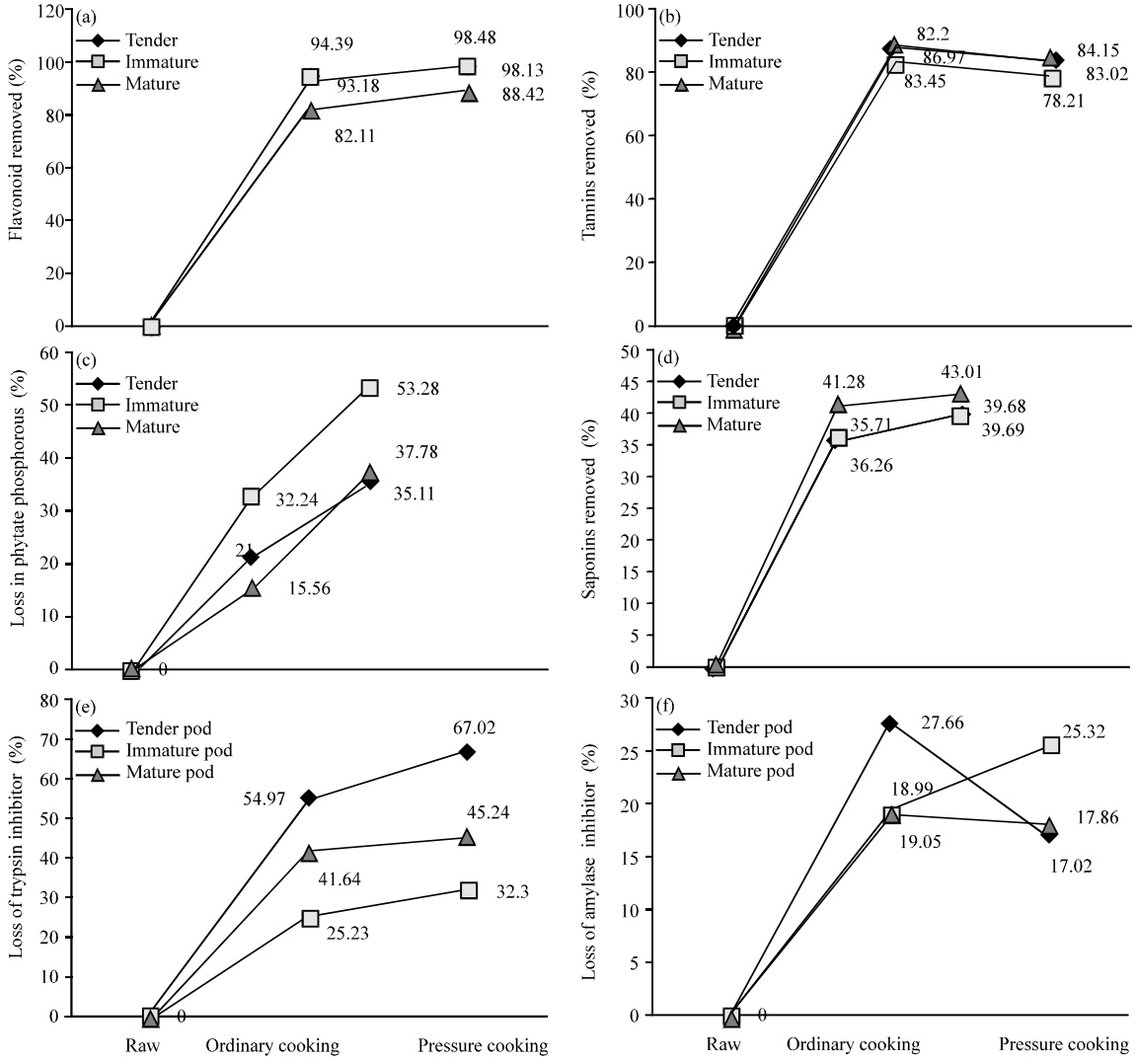

Fig. 2(a-f): Percentage removal of secondary metabolites and enzyme inhibitors due to different processing methods in different stages of *Parkia roxburghii pods,* (a) Flavonoids (b) Tannins (c) Phytate phosphorus (d) Saponins (e)Trypsin inhibitors and (f) Amylase inhibitors

Table 1: Effect of processing methods on some secondary metabolite substances in different developmental stages of *Parkia roxburghii* pods (mg g^{-1})

Secondary metabolites	Cooking methods	Stages of the pod			Mean	S.Ed±		$C.D_{0.05}$		$C.D_{0.01}$	
		Tender	Immature	Mature	(Stages)	Methods	Stages	Methods	Stages	Methods	Stages
Tannins	Raw	98.30[a]	86.47[a]	56.27[a]	80.35	8.34	8.34	23.16	ns	38.42	-
	Ordinary cooking	12.81[b]	14.31[b]	6.640[b]	11.25						
	Pressure cooking	16.69[b]	18.84[b]	8.920[b]	14.82						
	Mean (Methods)	42.60	39.87	23.94							
Phytate	Raw	31.90[a]	36.60[a]	40.50[a]	36.33	1.89	1.89	5.23	5.23	8.68	8.68
Phosphorus	Ordinary cooking	25.20[b]	24.80[b]	34.20[b]	28.07						
(mg 100 g^{-1})	Pressure cooking	20.70[b]	17.10[c]	25.20[c]	21.00						
	Mean (Methods)	25.93	26.17	33.30							
Flavonoids	Raw	13.20	10.20	9.500	11.13	1.07	1.07	2.98	ns	4.95	-
	Ordinary cooking	0.900	0.600	1.700	1.07						
	Pressure cooking	0.200	0.200	1.100	0.50						
	Mean (Methods)	4.770	3.830	4.100							
Saponins (mg g^{-1})	Raw	25.20[a]	26.20[a]	28.95[a]	26.78	0.69	0.69	1.92	ns	3.18	-
	Ordinary cooking	16.20[b]	16.70[b]	17.00[b]	16.63						
	Pressure cooking	15.20[b]	15.80[b]	16.50[b]	15.83						
	Mean (Methods)	18.87	19.57	20.82							
Alkaloids	Raw	ND	ND	ND	-	-	-	-	-	-	-
Cyanogens	Raw	ND	ND	ND	-	-	-	-	-	-	-

ND: Not detected. Different letters in a column indicate significance at 5% level and different letters with different colours indicate significance at 1% level

in different stages of the raw pod ranged from 30.9 to 40.5 mg 100 g^{-1} which reduced to 25.2 mg 100 g^{-1} in TP, 24.8 mg 100 g^{-1} in IP and 34.2 mg 100 g^{-1} in MP by OC and to 20.7, 17.1 and 25.2 mg 100 g^{-1} by PC while percentage removed out of the original content by OC and PC was 21 and 35.11% in tender stage, 32.24 and 53.28% in immature stage and 15.56 and 37.78% in mature stages of the pod (Fig. 2c). Saponin content in tender (25.20 mg g^{-1}), immature (26.20 mg g^{-1}) and mature (28.95 mg g^{-1}) raw pods decreased to 16.20, 16.70 and 17.0 (mg g^{-1}) due to OC and to 15.20, 15.80 and 16.50 (mg g^{-1}) by PC (Table 1) while degree of removal of saponin due to different cooking methods ranged from 35.71 to 39.68% in TP, 36.26% to 39.69% in IP and 41.28 to 43.01% in MP, respectively (Fig. 2d). Alkaloids and cyanogenetic factors were not detected in the *Parkia roxburghii* pods.

Trypsin inhibitor content in terms of Trypsin Inhibitor Units (TIU) mg^{-1} in tender (13.37), immature (7.77) and mature (6.94) raw pods decreased to 6.02, 5.81 and 4.05 TIU mg^{-1} in OC and to 4.41, 3.88 and 3.02 TIU mg^{-1} in PC (Table 2). Similarly, Amylase Inhibitor Units (AIU) in mg g^{-1} in tender (4.70), immature (7.90) and mature raw pods (8.40) reduced to 3.40, 6.40 and 6.80 in OC and to 3.90, 5.90 and 6.90 in PC. Percent destruction of TI in tender, immature and mature stages of the pod recorded was 67.02, 45.24 and 32.3% of the original content (Fig. 2e) while destruction of AI due to different cooking methods was comparatively lesser and recorded 27.76% in tender, 25.32% in immature and 17.86% in mature stages, respectively (Fig. 2f).

Table 2: Effect of different processing methods on trypsin inhibitor and amylase inhibitor content in different stages of the *Parkia roxburghii* pods

	Stages					
	Trypsin inhibitor (TIU mg^{-1})			Amylase inhibitor (AIU mg^{-1})		
Processing methods	Tender pod	Immature pod	Mature pod	Tender pod	Immature pod	Mature pod
Raw	13.37	7.77	6.94	4.70	7.90	8.40
Ordinary cooking	6.02	5,81	4.05	3.40	6.40	6.80
Pressure cooking	4.41	3.88	3.02	3.90	5.90	6.90
	Methods	Stages		Methods	Stages	
S.Ed±	1.38	1.38		0.27	0.27	
C.D. at 5%	3.82	NS		0.74	0.74	
C.D. at 1%	-	-		1.23	1.23	

DISCUSSION

Though legumes are an excellent source of proteins and essential dietary minerals its utility to human is limited by the presence of certain secondary metabolites and enzyme inhibitors (Savage and Deo, 1989). Flavonoid content in different stages of the pod decreased due to different cooking methods, the maximum loss being recorded in pressure cooking (Table 1 and Fig. 2a). Processing methods reported to affect phenolic, flavonoid and antioxidant contents (DPPH, FRAP) of dry beans (Boateng *et al*., 2008). Xu and Chang (2009) also reported significant decreases in Total Flavonoid Content (TFC), DPPH free-radical scavenging activity and ferric-reducing antioxidant power by different processing methods. Tannins were removed greater than the other secondary metaabolites and recorded loss of 91.56% in tender, 90.98% in immature and 87.76% in mature stage of the *Parkia* pods (Fig. 2b). Processing methods are reported to be effective in reducing tannin content of winged bean, soya bean and common beans (Tan *et al*., 1984; Bressani *et al*., 1982; Elias *et al*., 1979). Higher loss of tannins may be due to its solubility in water as tannins are known to be water soluble. Tannin reduction in *Parkia* seeds may improve its nutritional value by increasing protein digestibility (Savelkoul *et al*., 1992). Though presence of high amounts of tannin interferes with the digestive system, presence of tannin in food sometimes gives body and fullness of flavour to the food (Valier, 1951).

Loss of Phytate Phosphorus (PP) is lesser compared to tannins and accordingly its removal out of the total content is also lesser recording 33.01 to 53.28% in different stages of the pod (Table 1 and Fig. 2c). Cooking methods significantly ($p<0.05$) reduced PP in *P. roxburghii*. This observation agrees with the results of Ologhobo and Fetuga (1984) and Sutardi and Buckle (1985). Loss of phytate is due to its solubility in hot processing water during cooking operations and also partly due to the activities of endogenous phytases (Lolas and Markakis, 1975). Reduction of Phytate may increase the bioavailability of protein and minerals of *Parkia* pods. Appreciable amounts of saponins are detected in *P. roxburghii* pods. Saponin content in the pods increases as the pod matures (Table 1). However, loss of saponins due to different processing methods increases with the age of the pods, maximum loss (43.01%) being recorded in MP (Fig. 2d). Saponins occur in a wide variety of food plants, bengal gram, soybean, navy beans, haricot beans but most of them are removed by processing methods (Fenwick and Oakenful, 1983; Oakenfull, 1981; Varsney, 1969).

Trypsin Inhibitor Units in (TIU mg^{-1}) and Amylase Inhibitor Units (AIU mg g^{-1}) decreased due to OC and PC (Table 2). No significant differences were observed in TIU and saponin content in different stages of the raw pods, however, AIU increased with the age of the pods. Percent

destruction of TIU due to processing methods recorded up to 67.02% in the tender stage of the pod (Fig. 2e) while destruction of AI was comparatively lesser, the maximum loss being 27.76% in tender stage of the pod (Fig. 2f). Heat inactivation of enzyme inhibitors in legumes has been reported by many workers (Ravindran and Ravindran, 1988; Ogun *et al*., 1989).

CONCLUSION

Parkia roxburghii pods recorded lesser values of phytate phosphorus and enzyme inhibitors to compare with the conventional legumes. The mean values of all the anti-nutrients decreased due to processing and cooking methods as compared to raw pods. Pressure cooking brought higher reduction in anti-nutritionals than ordinary cooking. Tannin reduction in *parkia* seeds may improve its nutritional value by increasing protein digestibility. Reduction of phytate may increase the bioavailability of protein and minerals of *Parkia* pods. Alkaloids and cyanogenetic factors are not detected in *Parkia roxburghii* pods.

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