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Biochemical and enzymatic changes during the processing of different millets

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Abstract
The present experiment was conducted to investigate the effect of different processing treatments (germination, soaking, roasting and milling) on different grains (sorghum, pearl millet, finger millet and little millet) with respect to proximate components and enzyme assay. The proximate composition showed significant differences within the millets and between the treatments. Sorghum recorded the highest crude protein. The free amino acid content increased during germination treatment. The enzyme activity declined during roasting treatment due to the denaturation of protein at higher temperature. Overall the different processing treatments led to increased enzyme activity, Thereby the processed millet flour can be used as a functional food component.

Keywords: Crude protein, free amino acid, Enzyme activity, millets, processing treatments

1. Introduction
The term "millet" is derived from the French word "*mille*'' which means thousand, with a handful of millet containing upto 1000 grains (Ramashia *et al*., 2019) [11]. There are eight millets prominently used worldwide *viz*., Sorghum, Finger millet, Foxtail millet, Kodo millet, Proso millet, Pearl millet, Barnyard millet and Little millet. Finger Millet (*Eleusine coracana*) originally from Sudan. Finger millets are high in thiamin, copper, magnesium, phosphorus and selenium. They are also a source of iron. Pearl Millet *(Pennisetum glaucum)* Originating in West Africa. Pearl millets are high in copper, iron, magnesium, phosphorus, selenium and zinc. They are also a source of thiamin and vitamin B6. Little Millet *(Panicum sumatrense*) Evidence points towards the Indian peninsula as the origin of little millets. Little millets are high in copper, magnesium, selenium and sources of thiamin, phosphorus and zinc. Sorghum (*Sorghum bicolor*) The origins of sorghum cultivation were found in the Eastern Sudanese savannah. Sorghum is high in copper, magnesium, phosphorus and selenium, and is a source of iron, zinc, thiamin, niacin, pantothenic acid, and vitamin B6.
Domestic processing methods such as germination, malting, fermentation, thermal and mechanical treatments of grains help improve their digestibility, nutrient bioavailability and sensory property and reducing the anti-nutritional content. The study may provide inputs on possible use of the processed millet flour as a functional food component.

2. Materials and Methods
In this experiment we have used one variety each for each of the four millets *viz*. sorghum, pearl millet, finger millet and little millet. The sorghum variety GNJ-1, pearl millet variety GHB-538, Finger millet variety Phule Nachani and Little millet variety Dudhmogra.

2.1 Treatments
2.1.1 Germination (T_1): It was done by soaking the seeds overnight in distilled water at room temperature (25 °C ± 2). The seeds were placed between the folds of Whatmann No. 1 filter paper. Continuous watering was done for 48 h and the seeds were allowed to germinate. The sprouted seeds were dried at 60 °C, grinded to fine powder (<0.5 mm) and used for further analysis.

2.1.2 Soaking (T_2): In soaking treatment, the raw, clean seeds were soaked in distilled water for 14 h at room temperature (25 °C ± 2). The soaked seeds were then dried at 60 °C, grinded to fine powder (<0.5 mm) and used for further analysis.

2.1.3 Roasting (T_3): In roasting treatment, whole grains were heated in a hot air oven at 110 °C for an hour. The treated seeds were then grinded to fine powder (<0.5 mm) and used for further analysis.

2.1.4 Milling (T_4): The seeds were grinded to fine powder (<0.5 mm) and used for further analysis.

2.2 Proximate Analysis
2.2.1 Crude protein
For the digestion of the samples Gerhardt digestion unit was used. The distillation was carried out in Gerhardt Vapodest automatically. The protein content of the sample was estimated as percent total nitrogen by the Micro Kjeldahl method (A.O.A.C., 2000) [1] and computed by multiplying percent nitrogen using conversion factor 6.25.

2.2.3 Free amino acid
Free amino acid content was estimated as described by Lee and Takahashi (1966) [3]. The extraction was similar to that done in total soluble sugars. Suitable aliquots were taken and volume made up to 1 ml by adding distilled water. To this, 5 ml ninhydrin reagent (1% ninhydrin in 500mM citrate buffer, pure glycerol, and 500mM citrate buffer pH 5.5 in the ratio of 5:12:2) was added, mixed thoroughly and then, tubes were kept in a boiling water bath for 12 minutes. After that, the tubes were transferred to an ice bath for immediate cooling. The tubes were brought to room temperature and the absorbance was measured at 530 nm. The free amino acid content was calculated from reference curve prepared using glycine (10-100 µg) as standard and expressed as appropriate.

2.4 Enzyme Assay
2.4.1 Protease activity
Estimation of general proteases was done using casein as the substrate. Enzymes aliquate (0.2ml) was taken in test tube, to this 2.5 ml phosphate buffer (0.1 M pH 7.2) and 1 ml casein solution (0.5% in 0.1 M phosphate buffer pH 7.2) were added. The tube was incubated in water bath at 37 ^{0}C for one hour. The reaction was terminated by adding 0.5 ml of 10% TCA. The protein precipate was removed by centrifugation at 4000 rpm for 20 minutes. From this 0.5 ml of supernatant was taken for assay of TCA soluble peptides such as Tyrosine. In case of control, TCA was added prior to addition of enzyme extract. The calibration curve was prepared using Tyrosine. The enzyme activity was expressed as mg tyrosine released mg^{-1} protein $hour^{-1}$ (Nayak *et al.*, 1979) [5]

2.4.2 Peroxidase activity
The reaction mixture contained 2.99 ml of 0.03% H_2O_2 in 0.1M phosphate buffer (pH 6.0) containing 0.01% Orthodianisidine dye (freshly prepared, dissolved in methanol). The reaction was initiated by the addition of 10 µl of enzyme extract. The change in color of oxidized dye was read at 460 nm up to 1 minute at the interval of 15 seconds. Blank was run without the addition of enzyme (Malik and Singh, 1980) [4]. The enzyme activity was expressed as $\Delta OD.mg^{-1}$ protein. min^{-1}.

3. Results and Discussion
3.1 Crude protein
The mean values obtained for crude protein of sorghum, pearl millet, finger millet and little millet are ranged from 6.79% to 12.73%. The values for crude protein content were 12.73%, 10.83%, 9.84% and 6.79% for the crops sorghum, pearl millet, finger millet and little millet respectively. The effect of different treatments on the crude protein content are ranged from 9.93% to 10.38%. The crude protein content for roasting and soaking treatments were at par with control but germination led to a significant increase in the protein content.

Table 1: Interaction effect of grains (G) and treatments (T) on change in crude protein content (%)

Sr. No.	Grains/ Treatments	(T_1)	(T_2)	(T_3)	(T_4)
1	Sorghum	13.64	12.22	12.66	12.42
2	Pearl Millet	10.50	11.10	10.69	11.05
3	Finger Millet	9.78	9.91	9.97	9.71
4	Little Millet	7.61	6.53	6.49	6.55
	G x T				
S.Em. ±	0.13	C.D. at 5%	0.37		
CV%	2.20				

T_1=Germination, T_2=Soaking, T_3=Roasting, T_4=Milling

Interaction effects of grains and treatments showed significant difference (Table 1). The mean value for crude protein content ranged from 6.49% to 13.64%. Sorghum and little millet germinated flour showed significantly higher protein content as compared to control; whereas there was a rise for finger millet which was at par with control. On the contrary, pearl millet showed a significant decline in crude protein content upon germination. A significant increase in crude protein content has been reported by Owheruo *et al.* (2018) [9], Tiwari *et al.* (2018) [13], Obadina *et al.* (2017) [8] while on the other hand a significant decline was reported by Choudhury *et al.* (2011) [2]. The increase in protein of germinated flour was due to increased protein synthesis during germination as suggested by Sade, (2009) [12].

3.2 Free amino acids
The mean value for free amino acids content ranged from 1.52mg/g, 1.71mg/g, 1.56mg/g and 1.51mg/g in the crops sorghum, pearl millet, finger millet and little millet respectively. The free amino acids content of germination (T_1), soaking (T_2), roasting (T_3), and milling (T_4) were 2.15mg/g, 1.74mg/g, 0.83mg/g, and 1.58mg/g in the treatments of germination (T_1), soaking (T_2), roasting (T_3), and milling (T_4) respectively.

The interaction effect of grains and treatments is depicted in the (Table 2). The mean value for free amino acids content ranged from 0.73 mg/g to 2.35mg/g. Germination treatment led to a significant rise in free amino acids content whereas the roasting treatment led to a decline in the free amino acid content compared to control. The free amino acid content ranged from 1.92 to 2.35 mg/g and 0.73 to 0.90 mg/g in germination and roasting treatments respectively for various grains. Similar result was reported by Nithya *et al.* (2007) [7] who attributed the rise in free amino acids content during germination and soaking to partial hydrolysis of proteins by endogenous proteases and the decline in free amino acids during roasting to denaturation and degradation of protein to high temperature.

Table 2: Interaction effect of grains (G) and treatments (T) on change in free amino acid content (mg/g)

Sr. No.	Grains/Treatments	(T_1)	(T_2)	(T_3)	(T_4)
1	Sorghum	2.14	1.67	0.84	1.43
2	Pearl Millet	2.35	1.88	0.90	1.70
3	Finger Millet	2.20	1.78	0.73	1.52
4	Little Millet	1.92	1.61	0.85	1.66
	G x T				
S.Em. ±	0.01	C.D. at 5%	0.03		
CV%	1.24				

T_1=Germination, T_2=Soaking, T_3=Roasting, T_4=Milling

3.3 Protease activity

The mean values obtained for protease activity in sorghum, pearl millet, finger millet and little millet ranged from 1.77 to 2.26. The values for protease activity were 2.15, 2.26, 1.77 and 1.90 in the grains sorghum, pearl millet, finger millet and little millet respectively. The highest protease activity was recorded in germination (T_1) (2.68) and lowest was observed in roasting (T_3) treatment (1.50).

The interaction effects of grains and treatments showed significant differences (Table 3). The mean value for protease activity ranged from 1.33 to 3.53. The germination treatment resulted in the maximum protease activity which ranged from 2.15 to 3.53 while the lowest activity was observed in roasting treatment (1.60- 1.33). Similar results were obtained by Nithya *et al*. (2006) [6] in pearl millet. The decrease in proteolytic enzyme activity during roasting treatment was due to enzyme deactivation. Significant increase in free amino acids contents were observed with soaking and germination treatments, germination treatments recorded higher values of free amino acids over soaking treatments. This could be attributed to the partial hydrolysis of storage proteins by endogenous proteases during the germination of seeds. Ramana and Radhakrishnan (1987) [10] suggested the rise in protease activity may be due to *de novo* synthesis of protease during germination.

Table 3: Interaction effect of grains (G) and treatments (T) on change in protease activity (mg^{-1} protein $hour^{-1}$)

Sr. No.	Grains/Treatments	(T_1)	(T_2)	(T_3)	(T_4)
1	Sorghum	3.53	2.15	1.33	1.59
2	Pearl Millet	2.76	2.51	1.60	2.17
3	Finger Millet	2.15	1.79	1.54	1.60
4	Little Millet	2.27	2.01	1.51	1.81
	G x T				
S.Em. ±	0.03	C.D. at 5%	0.08		
CV%	2.50				

T_1=Germination, T_2=Soaking, T_3=Roasting, T_4=Milling

3.4 Peroxidase activity

Peroxidases are known to be involved in multiple functions ranging from reactive oxygen species generation and regulation, H_2O_2 level regulation and oxidation of various substrates. The mean values obtained for peroxidise activity in sorghum, pearl millet, finger millet and little millet were 7.00, 6.63, 8.79 and 6.63 in the grains of sorghum, pearl millet, finger millet and little millet respectively. The mean value of peroxidase activity ranged from 3.67 to 9.88. The values for peroxidase activity were 9.88, 8.42, 3.67, and 7.08 in the treatments germination (T_1), soaking (T_2), roasting (T_3) and milling (T_4) respectively. The interaction effects of grains and treatments for peroxidase Activity are as shown in (Table 4.). The mean value for peroxidase activity ranged from 2.50 to 12.00. The highest peroxidase activity was observed in germination (12.00- 7.50) while the lowest was observed in roasting (5.67- 2.50) in different grains.

Table 4: Interaction effect of grains (G) and treatments (T) on change in Peroxidase activity ($\Delta OD.mg^{-1}$ protein. $min.^{-1}$)

Sr. No.	Grains/Treatments	(T_1)	(T_2)	(T_3)	(T_4)
1	Sorghum	10.50	8.00	3.00	6.50
2	Pearl Millet	9.50	8.00	2.50	6.50
3	Finger Millet	12.00	10.67	3.50	9.00
4	Little Millet	7.50	7.00	5.67	6.33
	G x T				
S.Em. ±	0.93	C.D. at 5%	2.67		
CV%	2.11				

T_1=Germination, T_2=Soaking, T_3=Roasting, T_4=Milling

As compared to control, there was a significant rise in peroxidase activity in soaked and germinated seeds whereas roasting treatment led to a significant decline which may be associated with the denaturation of the enzyme at high temperature.

4. Conclusions

The germination treatment led to a significant increase in crude protein content which ranged from 7.61 to 13.64% as compared to milling (6.55 to 12.42%). The highest free amino acid content was found in pearl millet (1.71 mg/g) whereas the least content was found in little millet (1.51 mg/g). The soaking and germination treatment led to a significant incline in the free amino acid content while the roasting treatment led to a significant decline amongst the various grains. The protease enzyme activity showed a significant increase in soaking and germination treatment while the roasting treatment led to a significant decline in the various grains. The results can be correlated with the corresponding increase in free amino acid content recorded due to the hydrolytic action of proteases on the protein. The peroxidase enzyme activity also showed a rise during soaking and germination while the activity showed significant decline during roasting treatment amongst various grains.

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