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Effect of extrusion and flaking on the retention of nutrients and phenolic compounds in millet grains

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Abstract
Millet grains were subjected to extrusion and flaking, analyzed for the nutrient components and phenolic compounds. The present study was focused on the evaluation of retention of chemical constituents in millet grains after processing i.e., extrusion and flaking. The millet grains *viz*., little, proso and barnyard millet were selected for the study, they were dehusked, extruded and then subjected for flaking. They were analyzed for the nutrient components (protein, fat, fibre, minerals) and phenolic compounds. Evaluation for total phenolic content (TPC) was done by Folin-Ciocalteu method and total flavonoid content (TFC) by aluminium chloride colourimetric method. Results revealed that extrusion and flaking had a significant reduction on the chemical constituents. Variation was also observed for TPC and TFC after the processing of grains.

Keywords: Millet, extrusion, flaking, phenolic, flavonoid, antioxidant activity

1. Introduction
Millets are cereal crops with small sized seeds which belongs to family Poaceae. They are superior to rice and wheat, and therefore provide protein, mineral, and vitamins to the poor where the need for such nutrients is in high demand [1]. Since millets are nutritionally superior to several other cereal grains, the non-availability of refined and processed millets has limited their wider use and acceptability. Millets are underutilized even in areas where they grow due to their minimal inclusion in ready-to-use or ready-to-eat convenience food products and lack of research and novel product development processes. Processing them using traditional as well as contemporary methods for preparation of value added and convenience products would certainly diversify their food uses [2].

It is known that fibre intake is associated with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycemic control, reduced risk of certain forms of cancer, and improved gastrointestinal function [3]. Dietary fibre intake has a beneficial role in the prevention of diseases including cardiovascular disease, diabetes, cancer and weight regulation [4-6]. Since these millet grains are rich in fibre content, they may serve in combating several health disorders.

The phytochemicals in plant foods are believed to exert health beneficial effects by combating oxidative stress in the body [7]. Cereal grains contain unique phytochemicals that complement those in fruits and vegetables when consumed together. For instance, various classes of phenolic compounds in grains include phenolic acids, anthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds [8-10]. Millet grain phenolics possess bioactivities against several pathophysiological conditions and may serve as potential natural sources of antioxidants in food and biological systems [11]. It is generally believed that antioxidants scavenge free radicals and reactive oxygen species and thus inhibit oxidative mechanisms which lead to degenerative diseases [12]. An increasing number of epidemiological studies have shown an inverse correlation between the consumption of dietary antioxidants and incidence of various diseases including cancer and heart disease [13]. Fortification of diet with food components rich in phenolic acids has been shown to impart antimutagenic, antiglycemic and antioxidative properties, and this has been exploited for the development of healthy food formulations [14]. Ready to eat cereal flakes are very popular now a days for the convenience and other benefits they offer. The process of flaking involves pearling, hydrothermal treatment, flaking/rolling, blistering. These processes can bring in changes in the nutrient composition and phytochemical contents [15].

Millet grains contain high proportions of husk and bran, which makes the dehusking and debranning prior to consumption of these grains. The health benefits of millets are dependent upon their metabolic profiles, including the types and amounts of natural phenolic compounds present. Much attention has been given for the investigation of the nutrient and nutraceutical properties of major millets like sorghum, finger millet and pearl millet [16-19], also majority of the studies for processing effects in millet grains are on germination, decortication, heating, etc., hence the present research was undertaken to assess the retention of nutrients and phenolics in three different types of millet grains, which is focused on the processing effect of flaking in millet grains. The study will help to know the nutritive value of millet grains after flaking, and may be utilized to develop several convenience and ready to eat food products, which can be replaced for other cereal flakes available.

2. Materials and Methods
2.1 Grain samples
The millet grains *viz*., little, proso and barnyard millets were procured from the local market for the study.

2.2 Chemicals
Aluminium chloride, ethyl acetate, ethyl alcohol, hydrochloric acid, methanol, sodium hydroxide, sodium nitrite, sodium sulphate and sodium carbonate of analytical grade were procured from Merck (Mumbai, India). Folin-Ciocalteu reagent was procured from Merck KGaA, (Darmstadt, Germany). Gallic acid and rutin were purchased from Sigma-Aldrich Co. (St. Louis, USA).

2.3 Flaking process
The grain samples were subjected to dehusking to remove the outer husk of the seeds, dehusked grains were steamed for 20minutes, extruded in the laboratory grade extruder and flaked in the flaking machine. The flaked samples were dried in hot air oven (105˚±C) until the samples were completely dried, and subjected for homogenization.

2.4 Sample preparation
Whole grains, dehusked grains and flaked samples were homogenized using a laboratory model homogenizer (Wise Tis, HG-15D, Daihan Scientific Co. Ltd, Korea) and passed trough scientific sieve (180µ). The homogenized samples were dried in hot air oven, sealed in polythene pouches and stored under refrigeration at 4 ˚C until further analysis.

2.5 Nutrient components
Homogenized samples were analyzed for the nutrient components. The following AOAC methods, 2011[20] were used to determine proximate composition; Protein content was determined by the Automated Kjeldahl method (AOAC Method 976.05) using the Kel plus analysis unit (Pelican Equipments, Chennai, India) and was calculated by factor (N x 6.25). Fat content was analyzed by soxhlet method (AOAC Method 2003.05) using automated fat extraction unit (Socs Plus, Pelican Equipments, Chennai, India). The fat free samples were analyzed for the fibre content using standard AOAC Method 973.18. Total mineral content was analyzed by incineration at 600 ˚C for 3h as per the AOAC Method 942.05. All the determinations were done using dried samples, and the values are presented as g/100 g on dry weight basis.

2.6 Phenolic compounds
2.6.1 Extraction of phenolic compounds
The powdered samples were extracted for phenolic components according to the method [15]. Extraction was carried out using 70%(v/v) ethyl alcohol (4 x 50 mL, 3 h each) with agitation; the supernatants were obtained by centrifugation at 15,000 rpm for 15 min at 4 ˚C (Sigma 3K30, Germany) and concentrated under vacuum with rotary evaporator at 50 ˚C, 45 rpm (Hahnvapor, Hahnshin Scientific Co., Korea) and the pH was adjusted to 2.00 with 4 M HCl. Phenolic compounds were separated by ethyl acetate phase separation (4 x 50 mL), and the pooled fractions were treated with anhydrous sodium sulphate to remove moisture, filtered, and evaporated to dryness. Residue obtained is redissolved in methanol (1ml) and stored at -20 ˚C till further analysis.

2.6.2 Determination of total phenolic content (TPC)
TPC was determined by the method described by Singleton & Rossi, 1965 [21]. Briefly, the appropriate dilutions of extracts were reacted with the Folin–Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance was measured at 765 nm after 60 minutes incubation at room temperature under dark using UV-Visible Spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). Gallic acid was used as reference standard, and TPC of the samples were expressed as µg of gallic acid equivalent per gm (µg GAE/g) of sample on dry basis.

2.6.3 Determination of total flavonoid content (TFC)
TFC was determined by aluminium chloride colorimetric method [15] with minor modification. In brief, aliquots (1ml) of appropriately diluted extracts or standard solutions were pipette into 15ml polypropylene conical tubes containing 2 ml double distilled H_2O and mixed with 0.15ml of 5% $NaNO_2$. After 5min, 0.15ml of 10% $AlCl_3.6H_2O$ solution was added and the mixture was allowed to stand for another 5min, and then 1ml of 1M NaOH was added. The reaction solution was well mixed, kept for 15min and the absorbance was determined at 415nm using the UV-Visible Spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). Rutin was used as reference standard, and TFC of the samples were expressed as µg of rutin equivalent per gm (µg RE/g) of sample on dry basis.

3. Results and Discussions
3.1 Nutrient composition
The nutrient composition of the native millet grains is presented in Table 1. Little millet and barnyard showed high content of protein, fibre and minerals, whereas proso millet had least content of these nutrients comparatively. Fat content was in the sequence; barnyard (10.13) > little (12.54) > proso (3.68). Dehusking of the millet grains decreased the nutrient contents in the analyzed samples which is presented in Table 2. The nutrients in dehusked grains ranged from 8.01 to 9.88 for protein, 2.65 to 3.68 for fat, 3.46 to 7.89 for fibre and 1.83 to 3.63 for minerals. The high protein content of 9.88 g/100 g is observed in dehusked proso millet flour, which is in agreement with the earlier reports [22, 23]. The high content of protein may be due to the bran layer which is rich in nutrients. Dehusked little millet sample reported high content of fibre and dehusked barnyard millet had high mineral content. There was no significant difference observed for fat content in all the millet grains after dehusking, this may be due to the presence of bran layer which is rich in fat content, which was in agreement with the research which reported that dehulling does not have a significant effect on the fat content of the millets, debranning of millets results in a significant decrease in the fat content of polished grains due to the removal of the lipid-rich bran layers [24].

Table 1: Nutrient composition of raw millet grains

Millet	Protein	Fat	Fibre	Minerals
Little	12.54	4.26	11.83	3.19
Proso	9.11	3.68	7.29	3.79
Barnyard	10.13	4.54	8.65	4.86

*All values are for g/100g of sample & mean of three replicate experiments

Table 2: Nutrient composition of dehusked millet grains

Millet	**Protein**	**Fat**	**Fibre**	**Minerals**
Little	8.37	4.18	7.89	2.47
Proso	9.88	3.55	4.46	1.83
Barnyard	8.01	4.27	4.21	3.63

*All values are for g/100g of sample & mean of three replicate experiments

Table 3: Nutrient composition of flaked millet grains after dehusking

Millet	Protein	Fat	Fibre	Minerals
Little	6.32	2.58	4.11	2.14
Proso	4.06	2.47	2.33	1.29
Barnyard	6.11	1.72	3.64	3.26

*All values are for g/100g of sample & mean of three replicate experiments

The processing of flaking in millet grains significantly decreased the nutrient composition, hence still the nutrient recovery of fibre, protein and mineral content was acceptable after the processing effect. As comparatively in other cereal grains, even without any processing they lack in these nutrients. The results after the flaking process revealed that little millet sample had high content of protein and fibre, whereas barnyard millet had high content of minerals. Fat content reduced significantly in all the three millet grains. A similar study [24] reported that milling has a significant effect on the mineral content of millet fractions wherein highest and lowest mineral contents were observed in whole and polished grains, respectively, also dehulling of millets results in a significant impact on the total dietary fibre content.

3.2 Phenolic compounds

Phenolic compounds and flavonoids have been of interest to researchers due to the health beneficial effects they impart. Total phenolic content (TPC) in the analyzed samples is presented in Figure 2. TPC ranged from 326.14 to 451.34 GAE µg/g in native grains, 229.05 to 369.01 GAE µg/g in dehusked grains and 204.91 to 316.23 GAE µg/g in flakes.

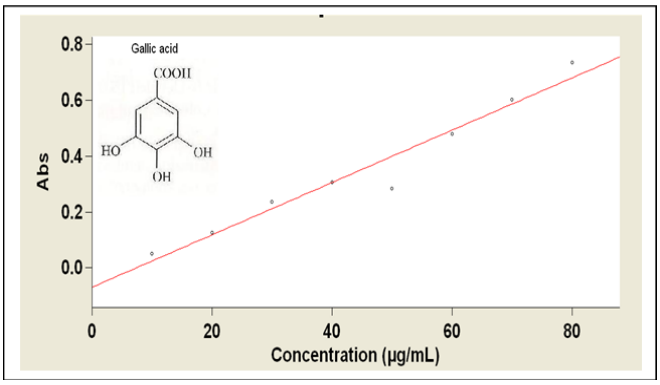

Fig 1: Calibration curve for TPC

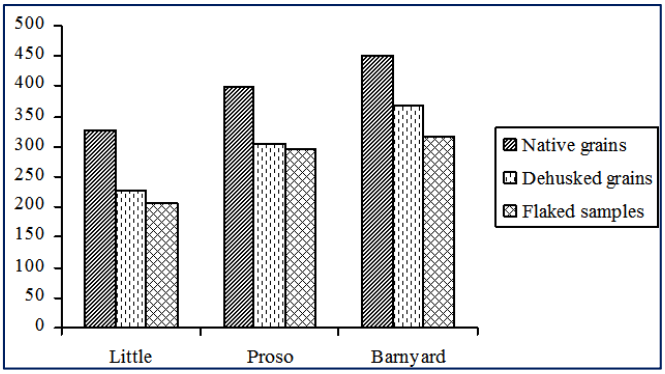

Fig 2: TPC of millet samples expressed as µg/g of Gallic acid equivalent (GAE)

Total flavonoid content (TFC) in the analyzed samples is presented in Figure 4. Wherein TFC decreased significantly after dehusking of the grain samples which was ranging from 279.49 to 381.73 RE µg/g in native grains, 209.11 to 313.91 RE µg/g in dehusked grains and 176.59 to 248.52 RE µg/g in flakes.

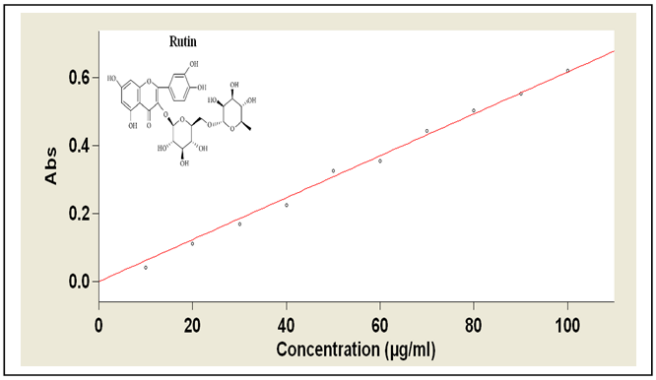

Fig 3: Calibration curve for TFC

Barnyard millet had a high content of TPC and TFC in all the fractions i.e., native, dehusked and flaked samples compared to other two millet grains. Reduction in the TPC and TFC was observed after flaking in all the millet samples, which was in agreement with the research on sorghum phenolics which also reported decrease in the TPC and TFC after the flaking process of the grains [15], process of flaking reduces the phenolic compounds as most of these components are concentrated in outer layers of the grain [25], which is lost during flaking process.

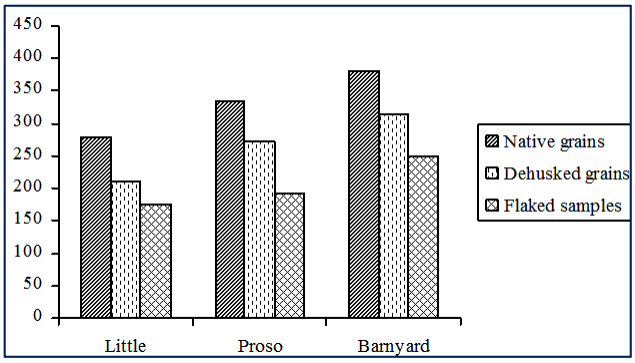

Fig 4: TPC of millet samples expressed as µg/g of Gallic acid equivalent (GAE)

4. Conclusion

The present study revealed that the processing effect of extrusion and flaking significantly affected the nutrients, total phenolics and total flavonoids in three different millet grains. In all the type of millets analyzed changes was observed. In the present study even after processing of millets, there was sufficient retention of nutrients and phenolic compounds, which when compared to other cereals is significantly more in concentration. Evaluation of processing effects such as dehusking, extrusion and flaking in the present study has contributed to the understanding of these underutilized cereals and proper processing methods to enrich their biologically active phytochemicals and nutrients. These processing effects have not only affected the nutrient components, also phenolic compounds were reduced after processing. These results have provided useful information for effective utilization of millets as functional food ingredients for promoting health. Also their usage in commercial food systems such as multigrain and gluten-free products would help in increasing the consumption of millets amongst non-millet consumers. Due to their favourable nutritional properties and their phytochemical associated health benefits, these millets offer an enormous

potential for use as ingredients in functional food product development [2].

5. References

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