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Nutrients, phytochemicals and antioxidant activity in whole and dehulled nutri-cereal based multigrain extruded snacks

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Abstract
The extrudates were prepared from the multigrain flour mixture either whole grain flour (WGF) or dehulled grain flour (DGF) comprising of 15% of sweet potato flour, 30% cereal and pulse flour (Bengal gram+ defatted soya bean flour + rice in equal proportion) and 55% of millet flour (Pearl millet+ Foxtail millet + Ragi in equal proportion). It was found that there was no significant difference between WGF/WGE and DGF/DGE in moisture and ash ($P>0.05$). Significantly higher protein (15.31g/100g), dietary fibre (12.5g/100g), Iron (28.70 mg/100g), zinc (6.60mg/100g), higher phenolics (65.8mg/100g), phytates (326.25 g/100g) were found in WGE compared to DGE. While DGE has higher soluble fibre content than that of WGE. The antioxidant activity measured by radical scavenging activity exhibited significantly ($P<0.05$) higher activity in WGE (70.15) than DGE. Thus, utilizing whole grains for extruded products was found to be beneficial in terms of higher nutritional content; more dietary fibre, phenolics and antioxidant activity in whole multi-millet based extruded product. Due to these characteristics, whole grain extrudates can be a potential snack for the health-conscious population suffering from lifestyle diseases and dehulled grain-based extrudates for supplementary nutrition programmes.

Keywords: multi millet, extrusion, antioxidant activity, dietary fibre, phytates, phenolics

1. Introduction
In past few decades, the consumption of foods mainly based on refined flours has resulted in reduced intake of dietary fibre and other micronutrients as a result of urbanized life styles, changes in practices and level of physical activity. This may be associated with rising affluence induced by developmental transition contributed to the increasing prevalence of overweight/obesity. Whole grain millets are known for good dietary fibre, phenolics, phytates and minerals, which will be altered once they are dehulled. Blending of different millets along with pulses and roots not only improve the physicochemical characters but also nutritional quality. Extrusion has been investigated as a means of improving the nutrient profile, for example, increasing levels of dietary fibre (Stojceska *et al.*, 2010) [19].

2. Materials and Methods
The samples were prepared from the multigrain flour mixture either whole or dehulled comprising of 15% of sweet potato flour, 30% cereal and pulse flour (Bengal gram+ soya + rice in equal proportion) and 55% of millet flour (bajra+ foxtail millet + ragi in equal proportion). Sweet potato flour in combination with millet flours will increase the rheological properties up to 30% level.
The twin screw extruder was kept on for 30 min to stabilize the set temperatures and samples were then poured in to feed hopper and the feed rate was adjusted to 15 kg/h for easy and non-choking operation. Feeding of the pre-conditioned composite flour to a twin-screw extruder was accomplished by using a twin screw volumetric gravity feeder. The extrudates were cut into uniform shapes with a cutter 100 rm-1. The product was collected at the die end and kept in a tray drier at 70°C for 2-3 h duration to remove extra moisture from the product so as to contain the moisture content between 5-6 %. After drying, the extruded products were adjusted with a moisture content of less than 6% and tightly packed and stored for nutritional analysis.
Samples were analyzed for moisture, protein, fat, ash, and minerals (AOAC, 1984) [4]. Dietary fiber was analyzed by (AOAC, 1997) [5] method. Antioxidant Activity was determined using 1, 1-diphenyl-2- picryl- hydrazil (DPPH) method by (Dorman *et al.*, 2004) [9].

The phytic acid content was determined by a modification of the method described by (Wheeler and Ferrel, 1971) [25]. Total phenolics was estimated according to the procedure of (Slinkard and Slingleton, 1997) [18].

2.1 Statistical analysis of Data
All the results were statistically analyzed using stratigraphic version 20 software. Multifactor ANOVA technique was used. (Snedecor and Cochran, 1983) [20].

3. Results & Discussions
3.1 Nutrient content
The multi millet extruded snack formulated with three varieties of millets, soya flour, bengal gram flour, rice and sweet potato flour. The nutrient content, phenolics, phytates and antioxidant activity was shown in Table 1 and the percent changes in the contents due to dehulling and extrusion was shown in Table 2. It can be observed from the Table. 1 that the moisture content of the extrudates ranged between 5.3 to 5.4 and there was no significant difference among the treatments (P, 0.05). The moisture content of 6.72% was reported in extrudates from rice, sorghum and soybean Flour (Mary and Symon, 2014). There was no significant difference between the whole and dehulled grain in case of ash content, however, the ash content of the extrudates was lower than their flour counterparts. The mean ash content was 3.75 and 3.55g/100g of flour mix and extrudates respectively (Table 1). The ash content of whole grain flour mix and extrudates was higher than that of dehulled flour which reflects the minerals content. Slightly lower values were reported by (Veronica *et al.*, 2005) for maize and soybean mixture extrudates. The ash content ranged from 2.4 to 4.6g. The ash content depends on the mineral content of the feed mixture used.

The total dietary fibre (TDF) content was in the range of 10.5 to 12.5% in flour mix and extrudates. Whole grain flour and extrudates found to have significantly ($p<0.01$) higher TDF than their dehulled counterparts (Table 1). The mean TDF content of 11.0 % in flour mix was increased to 12.1% in extrudates. There was a decrease in TDF content by 8.6% due to dehulling of whole grains and an increase of 8.5% due to extrusion. The increase in the dietary fibre after extrusion is due to the increase in the SDF component, 111.0, 144.7 and 131.1 percent from WGF to WGE, DGF to DGE and WGE to DGE respectively (Table 2). It can also be observed from the same table that there is no change in IDF even after extrusion in both WGF and DGF, however, there was a reduction in WGF to DGF and WGE to DGE by 8.9 and 3.62 percent. Westerlund and Theander (1983) [26] have reported an increase in dietary fibre after extrusion of wheat flour; the more severe the extrusion conditions, the greater the increase in fiber. Formation of amylose-lipid complexes by extrusion cooking of cereal starches have been observed (Mercier, 1980) [14]. Compared to rice (3.81%) and corn (7.15%) (Pastor-Cavada *et al.,* 2011) [16] The dietary fibre content in the present extrudates was high, which can be attributed to the higher percentage of millets. In Pearl millet based extruded snack, there was a shift in the components of dietary fiber in raw flour from 3.4. and 5.5 to 3.6 and 5.7 of soluble and insoluble dietary fibre respectively (Sumathi *et al.*, 2007) [17]. The mean protein content of the flour and extrudates was 13.19. Higher protein was found in WGE followed by DGE, WGF and DGE. The protein content in the wholegrain extrudates and dehulled grain extrudates was 15.31 and 14.78 g/100 product (Table 1). This was much higher than many other extrudates reported earlier. Over all extrudates have higher protein (15.04 g/100g) content than their flour (11.47g/100g) counterparts. There was an increase in protein content by 31.59 % and 34.06 % from whole flour and dehulled flour to extrudates respectively. There was a reduction of 5.25 and 3.46 in protein from WGF to DGF and WGE to DGE respectively (Table 2). Colonna *et al.,* (1989) reported a mild increase in the protein content by extrusion cooking due to the increase in digestibility. That can be due to the inactivation of enzyme inhibitors and denaturation of protein present in raw ingredients that exposes them to the attack of enzymes (Colonna *et al.,* 1989). The study indicates that extrusion can be employed for maximizing the utilization of protein in the extrudates flour mix. The mean fat content of flour and extrudates was 4.75 and 4.05 respectively with a significant difference ($p<0.01$) (Table 1). The present fat value was higher than the rice (0.53 %) and corn (2.18%) extrudates, rice with *Lathyrus* (0.54%) and corn with lathyrus (1.65%) (Pastor-Cavada *et al.,* 2011) [16]. The fat content can be attributed to the pearl millet flour in the feed mix.

The minerals, zinc and iron content were significantly higher in extrudates than that of flour. Significantly higher content was found in whole grain flour and extrudates than in their dehulled counterparts (Table 1). The iron content of 11.87 mg/100g of WGF was increased to 28.87 mg/100g extrudates. DGF has 8.94mg/100 mix which was increased to 19.90 mg/100 extrudates. Dehulling caused a reduction in the total iron content by 24.68%, however, after extrusion, the content increased by 123 % (Table 2). Overall, there was an increase of 141.79 and 122.60 per cent of iron from whole and dehulled flour to extrudates respectively. The higher increase was observed in whole flour than that of dehulled flour. The mean zinc content was 6.13 mg/ 100g product. A higher Zinc was found in whole grain flour (6.62 mg/100g mix) and extrudates (6.40 mg/100g extrudate) than in dehulled flour (5.56 mg/100g mix) and extrudates (5.95 mg/100g extrudate). The study demonstrated that to retain higher content of minerals extrusion of whole grain flour is beneficial. However, extrusion cooking resulted in higher percentage of zinc and iron when dehulled flour mix is extruded. Dehulling effected iron content at a higher percentage than that of zinc.

Table 1: Nutritional composition antioxidant activity of multi millet flour mix and extrudates

Parameters	WGF	WGE	DGF	DGE	P -Value
Moisture (%)	5.4±0.88	5.3±0.92	5.5±0.87	5.3±0.89	NS
Ash (g)	3. 8±0.34	3. 7±0.54	3.6 ±0.99	3.5±0.85	NS
Total Dietary Fiber(g)	11.5±0.55	12.5 ±0.32	10.5 ±0.22	11.7±0.45	0.001**
Insoluble Dietary Fiber(g)	10.6±0.34[a]	10.6±0.12 [a]	9.65±0.34 [b]	9.62±0.22 [b]	0.01**
Soluble Dietary fiber(G)	0.9±0.11	1.8±0.42	0.85±0.23	2.08±0.11	0.001**
Protein (g)	11.63±0.56	15.31±0.53	11.03±0.56	14.78±0.54	0.001**
Fat (g)	5.0±0.23	4.1±0.22 [c]	4.5±0.32	4.0 ±0.43 [c]	0.001**
Zinc (mg/100)	6.62±0.22	6.40±0.12	5.56±0.33	5.95±44	0.001**
Iron(mg/100)	11.87±0.46	28.70±0.21	8.94±0,43	19.90±87	0.001**

Antioxidant activity %	75.22	70.15	72.10	65.30	0.001*

*Significant at p,0.05 ** Significant at p, 0.01, NS- not significant at 0.05
WGF- whole grain flour mix, WGE- whole grain extrudates, DGF-dehulled grain flour mix. DGE- dehulled grain extrudates

Table 2: Percent change in the selected nutrients, minerals, phytates and antioxidants

Components	Change from WGF to WGE	Change from DGF-DGE	Change from WGF-DGF	Change from WGE-DGE
Protein (g)	31.59(+)	34.06(+)	5.24(-)	3.46(-)
Dietary fibre(g)	8.60(+)	11.42(+)	8.6(-)	1.73(+)
IDF	0.00	0.31(-)	8.9(-)	3.629(-)
SDF	111.0(+)	144.7(+)	5.5(-)	131.1(+)
Phenolics(mg)	67.22(-)	70.80(-)	8.63(-)	18.60(-)
Phytates (mg)	27.01(-)	67.10(-)	55.37(-)	79.88(-)
Iron(mg)	141.79(+)	122.60(+)	24.68(-)	30.66 (-)
ZINC (mg)	3.32(-)	7.05 (+)	16.01(-)	7.00 (-)
Antioxidant Activity (%)	6.75 (-)	9.30 (-)	4.26(-)	6.91 (-)

3.2 Phytate content

The phytate content of the whole grain flour mix was significantly higher than dehulled flour mix and their extrudates counterparts (Fig 1). A significant reduction by 55.37 % was observed due to dehulling of the grains which was further reduced by 67.10 due to extrusion. In case of whole grain flour, the reduction due to extrusion was only 27.01%, indicating that for the reduction of phytates dulling followed by extrusion can be employed.

It was also observed that the phytates content was 79.88 % higher in extrudates from whole grain flour than that of dehulled flour extrudates (Fig 1 and Table 3). (Gualberto *et al.,* 1997) [10] Reported that extrusion of raw oat bran and raw wheat bran resulted in the phytate content from1.42 to 1.07 and 5.27 to 5.03 g/100g and stated that extrusion did not cause any significant effect on the phytate content. (Alonso *et al.,* 2000) [1] Reported a reduction of phytates in both faba beans and kidney beans after extrusion. Decrease in phytate content due to the hydrolysis of inositol hexa phosphates into lower phosphates or due to formation of insoluble complexes between phytate and other charged components. Much higher reduction was observed in extruded product of the present study. (Wani and Kumar, 2015) [24] reported that extrusion of rice and peas showed a decrease in phytic acid (5.02mg/g).

The phytate: Iron molar ratios of both whole (0.96) and dehulled (0.28) based extrudates are below the critical value (<1.0), indicating a good absorption of the mineral, while the same were above the critical value in raw flour mixes. With respect to phytate zinc molar ratio both raw flour mix and extrudates had lower values than the critical value, indicating good availability, extrudates had much lower ratios. These present phytate /mineral ratios are reemphasizing the advantages of extrusion cooing for better mineral bioavailability. The phytate/minerals molar ratios are used to predict its inhibitory effect on the bioavailability of minerals. The phytate/iron molar ratio >1 is regarded as indicative of poor iron bioavailability (Hallberg *et al.,* 1989) [11]. Zinc absorption is greatly reduced and results in a negative zinc balance when the phytate/ zinc molar ratio is 15. In human studies, phytate: Zn molar ratios of 15: 1 have been associated with reduced zinc bioavailability.

3.3 Phenolics

The phenolic content was significantly higher in whole grains than in dehulled grains (Fig.1), it was reduced by 8.63 % after dehulling and after extrusion by 67.22 % and 70.80 % respectively in whole and dehulled extrudates (Table 3). Higher reduction was found in DGF converted extrudates than that of WGF. Due to dehulling the phenolics content significantly reduced from 200.75.42 to 183.75 mg/100g. Whole grain flour retained higher phenolics (65.8mg) than that of dehulled flour (53.56mg) after extrusion, which can be attributed the dehulling action followed by extrusion. Total phenols were reduced on average 10% in starch and navy bean extrudates, in comparison to the raw mixtures. In a study conducted by (Deepali *et al.,* 2016) [7] among the whole dehulled grain and hull of different foxtail millet varieties, highest phenolics were in 17 % hull and least were in 17 % dehulled grain indicating that dehulling has a direct effect on TPC of different grain fractions. Dehulling to 10 and 17 % resulted in the loss of 25 and 43 % TPC respectively. (Tian *et al.,* 2004) [21] Reported that phenolic compounds are concentrated in the bran layers and are liable to losses during the separation of seed coat in the milling process. More important reductions occurred in small red bean extrudates, which had their total phenols content decreased in approximately 70% (Anton *et al.,* 2009) [3]. Several studies have shown that extrusion processing significantly reduces measurable bioactive compounds in food products. (Korus *et al.,* 2007) investigated the effect of extrusion on polyphenol content and antioxidant activity of common bean they observed a significant decrease in polyphenol content and antioxidant activity. Similarly, (Delgado *et al.,* 2009) [8] observed a significant decrease in the total polyphenols and antioxidant activity during extrusion of bean/corn mixture. Hence from this and others study it can be said that extrusion cooking of whole grain flour is advantageous to obtain products with higher levels of polyphenols provided products possess sensorial acceptance.

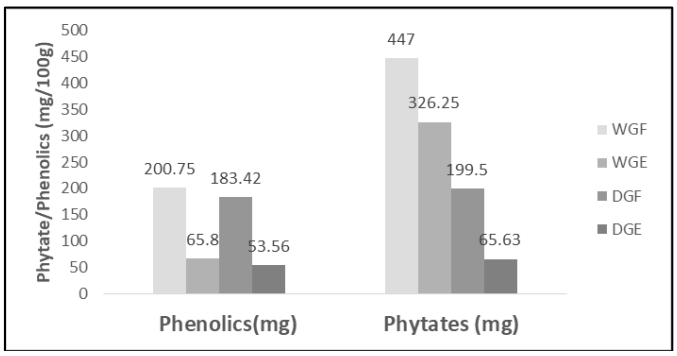

Fig 1: Phenolics and Phytate content of whole and dehulled grain multi millet flour mix and extrudates.

3.4 Antioxidant activity (AOX)

Antioxidant activity (AOX) quantifies the ability of a

complex biological material to scavenge free radicals and evaluates reducing property related to their health promoting eff etc. Antioxidant activity was measured by radical scavenging capacity of the flours and extrudates. Higher scavenging activity was observed in WGF (75%) followed by DGF (72%), WGE (70%) and DGE (65%) (Table. 1). This phenomenon can be attributed to the higher phenolic content of the whole grain flour compared to the dehulled grain flour. The percent reduction from WGF to WGE is 6.75, while that of DGF to DGE is 9.30, higher reduction was observed in dehulled grain than in whole grain flour mix (Table 3). Conversion of WGF to DGF itself caused a 4.26 percent reduction in the antioxidant activity. Compared to DGE, WGE exhibited 6.91 percent higher antioxidant activity. Similar reduction in AOX after extrusion has been reported in barley (Altan *et al.,* 2009) [2]. The extrusion cooking process might cause a decrease in the concentration by structural changes of compounds with AOX such as carotenoids and polyphenols (Obradovic *et al.,* 2015), attributed mainly to the high process temperature. Short exposure time of the components could lead to a less degradation of betalains and polyphenols resulting in higher AOX of the extrudates (Martha *et al.,* 2015) [13].

4. Conclusion

The study demonstrated that utilization of multi millet grains is more beneficial for extrudates production. Whole grain based extrudates are better in protein, ash, insoluble fibre, zinc and Iron as well as higher antioxidants. The dehulled extrudates has lower content of nutrients but has higher protein, iron and zinc, lower phytates. The whole grain based extrudates are best suited for the functional food development after conducting clinical trials in targeted population.

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