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In-vitro digestibility of protein, starch and *in-vitro* availability of minerals (iron and zinc) 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 nutri -cereal flour (Pearl millet+ Foxtail millet + Ragi in equal proportion). The *in vitro*-digestibility of protein (IVPD) (70.90%), *in-vitro* availability of Iron (57.85%) and Zinc (38.35%) were higher in DGE than in WGE, while *in-vitro* digestibility of starch (IVSD) was higher in WGE (36.88). 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, *in-vitro* starch digestibility, *in-vitro* protein digestibility, Mineral bioavailability.

Introduction
The rejuvenated nutritional strengths of millets have made them functional grains. Though different millets are available with localised areas of production and usage, their wide spread use can only be possible through food technological approaches leading to globally acceptable products, so that the health benefits of millets reach to everyone everywhere and can be a booster for the growers of millets. Whole grain millets are known for good dietary fibre, phenolics, phytates and minerals, which will be altered once they are dehulled. In addition, the extrusion process denatures undesirable enzymes, inactivates some antinutritional factors (trypsin inhibitors, haemagglutinins, tannins and phytates), sterilises the finished product and retains natural colours and flavours of foods (Fellows, 2000; Bhandari *et al*., 2001) [6, 3]. Prevention or reduction of nutrient destruction, together with improvements in starch or protein digestibility, is clearly of importance in most extrusion applications. Nutritional concern about extrusion cooking is reached at its highest level when extrusion is used specifically to produce nutritionally balanced or enriched foods, like weaning foods, dietetic foods, and meat replacers (Cheftel, 1986; Plahar *et al*., 2003) [4, 9]
Blending of different nutri-cereals along with pulses and roots not only improve the physicochemical characters but also nutritional quality. The work of Siegel and Line back (1976) [11] identified extrusion as a useful process in respect of the development of a high protein snack to overcome issues of protein malnutrition in the developing world. Cereal grains are the commonest raw materials employed in the manufacture of extruded products using multiple composite flours enhances the nutritional composition and increased availability of various nutrients. Though there are various extrusion formulations existing, nutritional quality and health benefits data with respect to multi millet (nutri cereals) were scanty. Therefore, the present study was aimed to assess the effect of extrusion on both whole and dehulled grain feed mixtures in terms of in-vitro digestibility and availability of selected nutrients.

Materials and method
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 r m-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.

***In-Vitro* Starch Digestibility (IVSD):** *In vitro* starch digestibility was estimated according to the procedure of (Singh and Jambunathan, 1982) [13].

***In-Vitro* Protein Digestibility (IVPD):** *In vitro* digestion method was developed which simulates the conditions in the digestive tract of carp. This method has the potential to give useful measures of *in vivo* amino acid and protein digestibility for humans. This was estimated according to the procedure of (Singh and Jambunathan, 1981) [12].

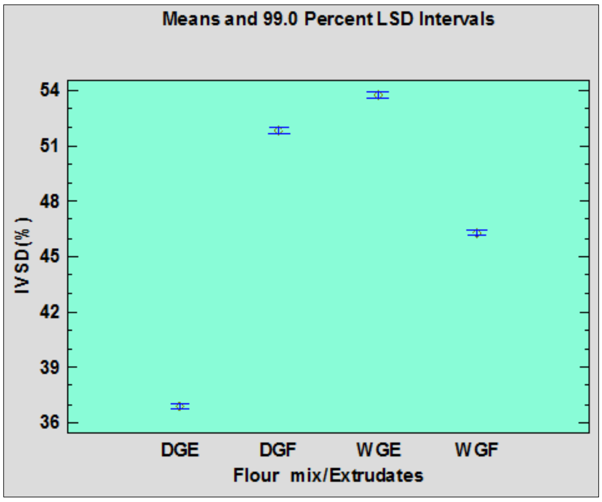
***In-Vitro* availability of Iron and Zinc**: (IV-Iron and Iv-Zinc) The availability of iron and zinc is determined by the *in vitro* digestion method described by (Kiers *et al.*, 2000) [8]. Iron and zinc content was measured by using the Perkin Elmer Atomic Absorption Spectrophotometer.

Result and discussion:

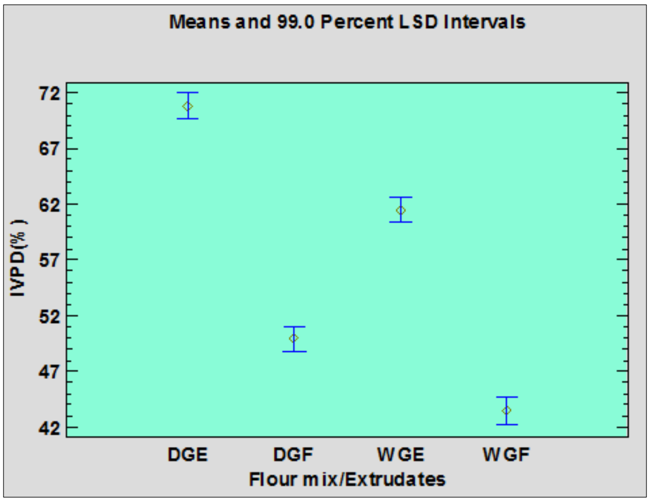
The IVSD of the WGF was 46.40 while that of extrudates was 53.76 with a significant increase by 15.86% (Fig.1a).Extrusion cooking significantly increased the *in vitro* digestibility of barley starch (Altan *et al.*, 2009) [1]. Alonso *et al*, (2000) [2] also found that extrusion treatment significantly increased the *in vitro* digestibility of pea starch. This may be explained in such a way that the increased shearing action develops heat through dissipation of mechanical energy and causes loss of structural integrity and increases enzyme susceptibility. An opposite effect was observed in case of dehulled flour extrudates, dehulling of whole grains, in fact, increased the IVSD from 46.40 to 51.85% (11.72%), but after extrusion of the dehulled flour, the same was decreased to 36.88% (decrease of 28.86%). It was observed that extrusion cooking significantly (p<0.05) reduced *in vitro* starch digestibility from 31.12% in the raw blend I to 28.23% in its extrudates and a reduction of 45.53% in raw blend II to 27.45% in its extruded form. (Samalia and Titus, 2013) [10]. The reduction of digestibility can be attributed to retro gradation or reassociation of gelatinized starch or formation of amylose-lipid complex, starch–protein complex, or starch and thus these complexes caused to reduce the susceptibility of starch to enzyme hydrolysis (Guha *et al.*, 1997) [7].

There was a significant difference in the IVPD of flour and extrudates, a higher IVPD was observed in extrudates than that of their flour counterparts (fig. 1b). An increase of 40.91 and 41.94 percent was recorded in whole and dehulled extrudates respectively. There was a difference of 8.9 percent change from whole to dehulled flour based extrudates. It was reported that the protein digestibility of raw sorghum variety CS3541 was 44.8% in the pepsin assay. This was increased to 74.6% by extrusion at 200°C. Likewise, the U.S. market class sorghum had a digestibility value of 43.3% and increased to 68.2% by extruding at 200°C. (Fapojuwo *et al.*, 1987) [5]

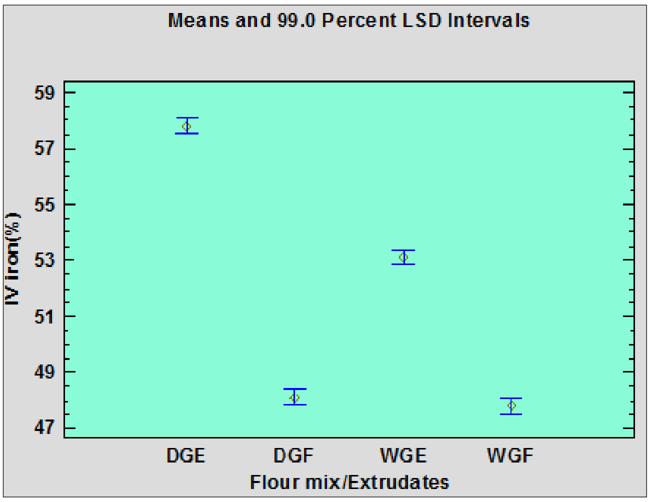
The percent bioavailability of both iron (57.85) and zinc (38.35) was significantly high in dehulled flour based extrudates compared to whole grain flour based extrudates. Higher difference was observed in Zinc (34.51) than in Iron (8.74) (fig 1c and d). However, there was no significant difference in *in-vitro* availability of iron and zinc between DGF and WGF (P>0.01). Though WGF has higher iron content, the bioavailability was high in dehulled extrudates. The lower content in dehulled extrudates might be compensated by the higher bioavailability. Almost similar trend was observed in zinc also where the bioavailability was higher in DGE followed by WGE, DGF and WGF. It has been observed that extrusion cooking can enhance the absorption of minerals by decreasing other factors that inhibit absorption. Extrusion cooking increases the amount of iron and zinc available for absorption, almost in all cases. The percent available iron content was significantly higher in dehulled flour (48.20%) as well as extrudates (57.85%) than that of whole grain counterparts (47.90 and 48.20 respectively). There was a 11.06 percent increase in the bioavailability of iron when whole grain flour converted into extrudates, while that of dehulled flour was 20.02 percent. This clearly indicates that extrusion significantly improves the iron bioavailability.



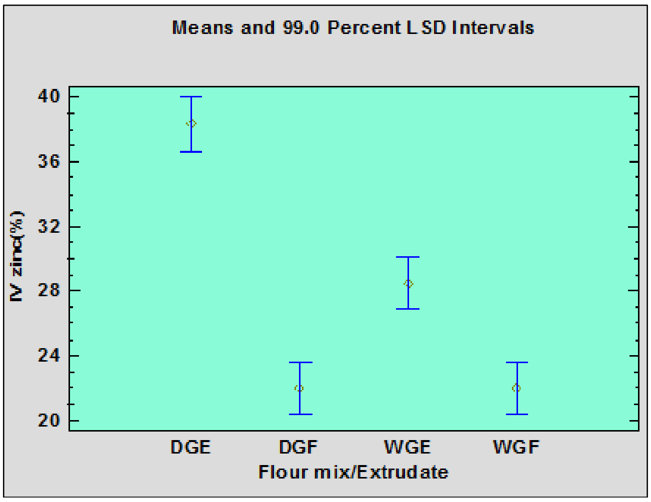
a. IVSD



b. IVPD



c. IV Iron



d. IV Zinc

Fig 1: *In-vitro* Digestibility of Starch (a), protein (b), and *in-vitro* availability of Iron (c) and Zinc (d) in nutri-cereal based multi-grain whole and dehulled mixture and extrudates

Conclusion

The study demonstrated that utilization of multi millet (nutri-cereal) grains is more beneficial for extrudates production. The dehulled extrudates have a higher digestibility of protein, iron and zinc. Depending upon the target population either whole grain or dehulled grain-based extrudates can be prepared.

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