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Effect of soaking, germination and drying on anti-nutrients, minerals and functional properties of horse gram along with its commercial application

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Abstract
The anti-nutritional factors, mineral content and flour functionality of raw and germinated horse gram were evaluated. Horse gram seeds were soaked for 6hrs later germination for 72hrs and dried in cabinet drier at 70˚C for 6hrs. It was found that treatment having 6hrs soaking, 72hrs germination and drying at 70˚C was best where maximum decrease in the anti-nutritional factors i.e. tannin and phytic acid (24.77% and 39.66%), respectively. At the same time mineral content was reduced i.e. calcium and mineral content (31.68% and 20.68%), respectively. Flour functionality of germinated horse gram flour was evaluated for water absorption capacity, oil absorption capacity, swelling capacity, emulsifying activity and emulsifying stability and found to be 323±0.03(g/100g), 252±0.09(g/100g), 16.04±0.24ml, 45.02±0.60% and 43.04±1.23%, respectively. Flour functionality was also improved in treated sample. Above optimized condition was used for the commercial application. Snacks sticks developed with 10% germinated horse gram flour incorporation gave maximum acceptability.

Keywords: horse gram, soaking, germination, drying, functionality

Introduction
Horse gram has a place with family *Fabaceae* is a potential legume having excellent dietary and medicinal properties with better atmosphere versatility to adjust difficult climatic conditions. It is one of the most significant unexploited food legume being developed in everywhere throughout the world (Bhartiya *et al.,* 2015) [1]. In India, horse gram is cultivated as crop impart about 0.33% of total food grain production (Ramteke *et al.,* 2016) [2]. Most extreme use of horse gram is missing because of the components like tannin, trypsin inhibitor, phytic acid which interfere with the bioavailability of supplements present in horse gram (Haripriya *et al.,* 2017) [3]. The procedure of germination is seen as reducing the degree of polyphenols, oxalic acid and phytic acid present in the horse gram. Poor functional properties of horse gram seeds are major limitations to utilize its flour in combination flours. Use of horse gram can be maximized through an understanding of its physical and chemical compounds properties and by the implementation of various processing strategies to encourage the improvement of financially suitable alternative products. Nutritional value and consumption of horse gram could be improved by processing it into new product (Jain *et al.,* 2012) [4].

Materials and Methods
Horse grams (*variety AK 42*) were procured from whole sale vendor, Pune in a bulk quantity to ensure uniformity of the sample all throughout the examination. Horse gram seeds were cleaned manually to remove the dirt particles and some extraneous material present in horse gram seeds. Seeds were washed and soaked in water (1:5 w/v) for 6hrs at room temperature. Later, the same samples were used for germination process. The excess water was drained and the process of germination was carried out for 72hrs at room temperature. All germinated horse gram samples were dried in a cabinet drier (Labfit, India) at 70˚C until the acquirement of consistent moisture content.
Analysis was carried on for raw horse gram sample as well as germinated samples. Raw horse gram seeds without any soaking and germination treatment served as a control. Tannin and phytic acid estimated by (Thimmaiah, 2016) [5].The calcium and iron was estimated by the procedure given in (Ranganna, 2011) [6]. Later on analysis was carried out in the context to functional properties. Water and oil absorption capacity, swelling capacity, emulsifying activity and emulsifying stability were estimated by the procedure given in (Chandra, 2013) [7]. Horse grams were subjected to milling to procure flour. The obtained flour was used for development of Snacks sticks for improving their nutritional quality using horse gram flour for supplementation.

Table 1: Formulation of snacks sticks

Code	Gram flour (Gm)	Rice flour (Gm)	GHF
A	90	10	0
B	80	10	10
C	70	10	20
D	60	10	30

GHF- Germinated Horse gram Flour

Table 2: Required ingredients (snacks sticks 100gm)

Ingredients	Amount
Flour (g)	100
Salt (g)	2
Chilli powder (g)	3
Black pepper (g)	1
Turmeric (g)	1
Oil (ml)	100 for frying
Water (ml)	20

Germinated horse gram flour, gram flour and rice flour were mixed and added with add red chili powder, coarsely ground black pepper, turmeric powder, salt and oil. Water was added later in small portions. Prepared dough was kept aside for 15 to 20 minutes for serve the rest time. After rest time dough was filled in hand extruder machine and sticks were extruded into hot oil at 170˚C. Fried product was cooled at room temperature and packed in PP bags.

Results and Discussion

Effect on Anti-Nutritional Factors

The tannin content of horse gram seeds was found to be 319mg/100g. Table 3 depicts percent reduction of tannin after soaking, germination and drying at different treatments. It was found that 6hrs soaking, 72hrs germination and 70˚C drying having maximum reduction i.e. 24.77% in tannin content. Germination procedure triggered the enzymatic movement of seeds, which further breaks the starches, proteins and fats into less complex structures. The procedure of germination diminished the amount of polyphenols, oxalic acid, tannin and phytic acid present in the horse gram seeds (Vandarkuzhali *et al*., 2006)[8]. Tannin content of horse gram display a decrease with an increase in time of soaking and germination period (Handa *et al*., 2017; Moktan and ojha, 2016) [9, 10]. The reduction in tannin content is mostly because of the way that these compounds highly present in seed coats (Reddy and Pierson, 1994) [11] and tannins are water soluble (Kumar *et al.*, 1979) [12] and subsequently drain into the liquid medium. Polyphenolase activity during germination causes loss of tannins content in grains (Reddy *et al.*, 1985) [13].

Raw horse gram contained 10.26mg/g of phytic acid. Table 3 depicts percent reduction of phytic acid content after soaking, germination and drying. It concludes that 6hrs soaking, 72hrs germination and 70˚C drying having maximum reduction i.e. 39.66% in phytic acid content. Phytic acid content of horse gram showed a diminishing with increase in soaking time. During sprouting, phytic acid, a phosphate reserve corrupts because of the activity of phytase which is used by growing seed (Mamudu *et al*., 2005) [14]. Phytase activity during germination, resulting in hydrolysis of phytate phosphorus, gave the reduction in phytic acid. The liberated phosphorus is possibly shipped to the embryo for next synthesis of organic phosphates. The rise in phytase action during germination could be because of activation of the pre-existing enzyme. Simultaneously inorganic phosphorus was liberated because of breakdown of phytic acid, (kim *et al.*, 1984) [15]. This has been attributed to a raise of phytase activities. In fact, this enzyme makes the phytates resolvable and discharges dissolvable minerals and protein (Khattak *et al.*, 2007) [16]. The soaking, germination and drying out of horse gram resulted in a decrease in tannin and phytic acid content.

Effect on mineral content

The calcium content of raw horse grams was 281mg/100g. The calcium content of soaked, germinated and dried horse gram is mention in (Table 4). It was found that 6hrs soaking, 72hrs germination and 70˚C drying having reduction i.e. 31.68% in calcium content. It is general to soaked legumes before to processing. Reduction in calcium content at the time of soaking was showed by (duhan *et al*., 2002: Elisa *et al*., 2010; Moktan and ojha, 2015) [17, 18, 10]. Reduction in calcium content with prolongation to soaking (6 and 18 hrs) and this is because of draining of the mineral into liquid medium. (Lagarda *et al*., 2015) [19]. The low ash contents obtained in the germinated horse gram and their reduction could be due to leaching of solid matter in soaking liquid medium (Das *et al*., 1999; Saharan *et al*., 2001) [20,21]. The mineral content was also decreased over sprouting (sadawarte *et al*., 2018) [22]. Major decreases in calcium content of different varieties (Serege, Giza and RO21) as the time of soaking were increase. After a day of soaking a decrease in calcium content was 42, 22 and 33%, although after 3 days of soaking the decrease were (49%, 31% and 41%) (Elmaki *et al*., 2007) [23].

The iron content of horse gram seeds was 7.6mg/100g. The iron content of soaked, germinated and dried raw horse gram is shown in (Table 4). Result states that 6hrs soaking, 72hrs germination and 70˚C drying having reduction i.e. 20.68% in iron content. Significant decrease in iron and calcium contents found by sprouting was well covered by (Das *et al.*, 1999) [20]. Iron content was found to be reduced over germination (sadawarte *et al*., 2018) [22]. The low in ash contents were obtained in the germinated horse gram and this reduction because of leaching of solid matter in soaking water (Lestienne *et al*., 2005; Reihaneh and Prakash 2007: Rusydi *et al*., 2011) [24, 25, 26].

Functional Properties Analysis

Water absorption capacity of raw horse gram flour was found to be 143.06±0.12 (g/100g) as stated in table 5. It was found that Water absorption capacity increases after soaking, germination and drying. Water absorption capacity of germinated horse gram flour was found to be 323±0.03 (g/100g). The water and oil absorption capacities are main functional properties of protein which may be defined as the quantity of water or oil retained by a known weight of flour under specific conditions. The reason behind high water absorption capacity was higher protein content in the flour. It is responsible for high hydrogen bonding and high electrostatic repulsion (Bhokre *et al.*, 2015) [27]. The value is found to be in comparison with the values of 340.00 to 345.89 reported by (Handa *et al*., 2017) [9]. Water absorption capacity shows an increasing trend in germinated horse gram flour than raw sample (Vandarkuzhali and Narayanasamy, 2016) [8]. The oil absorption capacity of raw horse gram was found to be 81.03±0.45 (g/100g) as per table 5. It was found that oil absorption capacity increased after soaking, germination and drying. The oil absorption capacity of germinated horse gram flour was found to be 252±0.09 (g/100g). The increased in oil absorption capacity it's due to enhanced hydrophobic character of proteins in the flours. The value is found to be in comparison with the values of 274.67 to 289 (g/100g) reported by (Handa *et al*., 2017) [9].The oil absorption capacity shows an increasing trend in germinated horse gram flour than raw horse gram (Vandarkuzhali and Narayanasamy, 2016) [8].

The swelling capacity of the raw horse gram was found to be 12.54±0.12 ml. and the swelling capacity of the germinated horse gram flour was found to be 16.04±0.24 ml in table 5. It was found that swelling capacity expanded after soaking, germination and drying. The increase in swelling capacity and water absorption capacity can be attributed to the division of polysaccharides into monosaccharide's during sprouting; consequently, the active sites for interaction in the middle of water and molecules increases and thus responsible for increase in these two

functional properties. Swelling capacity of horse gram was comparable with the result reported by (Handa *et al*., 2017) [9] i.e. 16.75 to 17.16 ml.

The results of emulsifying activity and emulsion stability of raw horse gram were found to be 41.25±1.23% and 40.45±0.15% is shown in table 5. It was found that emulsifying activity and emulsion stability increased after soaking, germination and drying. The results of emulsifying activity and emulsion stability of germinated horse gram flour were found to be 45.02±0.60% and 43.04±1.23% is shown in table 5. The emulsion activity shows the ability of a protein to aid in the formation of an emulsion and is connected to the protein's ability to absorb to the interfacial portion of oil and water in an emulsion.

The emulsion stability normally shows the ability of the proteins to provide strength to an emulsion for resistance to stress and changes and therefore this related to the consistency of the interfacial area over a defined time period (Pearce and Kinsella, 1978) [28].

The study reveals that emulsion capacity and stability of raw and germinated horse gram flour increased with germination time (Vandarkuzhali and Narayanasamy, 2016) [8]

Tables and Figures

Table 3: Anti-nutritional factors

Anti-nutritional factors	Raw horse gram	Germinated horse gram flour 6hrs soaking, 72 germination, 70˚C drying
Tannin content (mg/100g)	319	239.98
Phytic acid content (mg/g)	10.26	6.19

Table 4: Mineral content

Mineral content	Raw horse gram	Germinated horse gram flour 6hrs soaking, 72 germination, 70˚C drying
Calcium content (mg/100g)	281	191.98
Iron content (mg/100g)	7.6	6.10

Table 5: Functional properties

Functional properties	Raw horse gram	Germinated horse gram flour 6hrs soaking, 72 germination, 70˚C drying
Water absorption capacity (g/100g)	143.06±0.12	323±0.03
Oil absorption capacity (g/100g)	81.03±0.45	252±0.09
Swelling capacity (ml)	12.54±0.12	16.04±0.24
Emulsion activity (%)	41.25±1.23	45.02±0.60
Emulsion stability (%)	40.45±0.15	43.04±1.23

The obtained flour was used for development of snacks sticks for improving their nutritional value and increase use of horse gram pulse flour for supplementation. Snacks sticks were prepared in different combinations i.e. A: Control (Gram flour and rice flour), B: GHF (10%), Gram flour and rice flour, C: GHF (20%), Gram flour and rice flour, D: GHF (30%), Gram flour and rice flour.

Sensory Analysis

Sensory evaluation of snacks sticks colour and appearance, flavour, texture, taste and overall acceptability were carried out using 9-point hedonic scale with different semi-trained panelists. Sensory attributes were rated on a scale of 1 (dislike extremely) to 9 (like extremely). The mean scores for sensory parameters of sample B were found to be maximum on the 8 scale indicating their acceptability.

Table 6: Sensory evaluation of snacks sticks

Sensory attributes of samples	Colour and appearance	Flavour	Texture	Taste	Overall Acceptability
A (control)	8.00	7.50	8.50	8.50	8.60
B	7.50	7.30	8.24	8.10	8.00
C	6.95	7.00	7.50	7.45	7.30
D	5.55	6.50	6.75	6.30	6.65

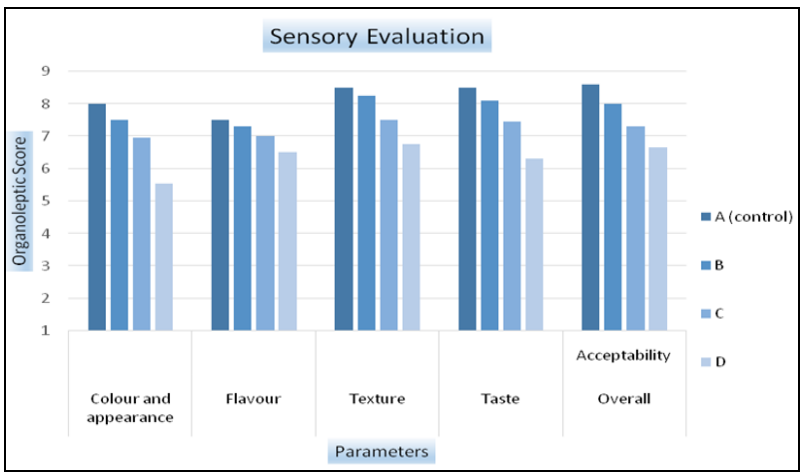

Fig 1: Graphical representation of sensory evaluation

Conclusions

Soaking, germination and drying performed on horse gram shows influenced in the properties of the horse gram. Tannin content and phytic acid content of horse gram showed a decrease with soaking, germination and dehydration i.e. (24.77% and 39.66%), respectively. Calcium and iron content of horse gram was reduced after soaking, germination and drying i.e. (31.68% and 20.68%), respectively. Based on the properties, it was found that treatment having 6hrs soaking, 72hrs germination and drying at 70˚C was the best where maximum decrease in the anti-nutritional factors at the same time flour functional properties got enhanced due to the soaking and germination. Thus, it can be concluded that 6hrs soaking, 72hrs germination and drying at 70˚C can be considered as the optimum condition which can further be utilized for preparation of snacks sticks. Commercial product (Snacks sticks) developed with 10% GHF incorporation gave maximum acceptability.

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