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Development and quality assessment of horse gram based instant dosa mix

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Abstract
Introduction: Horse gram is an underutilized pulse crop grown in wide range of adverse climatic conditions. It occupies an important place in human nutrition and has rich source of protein, minerals, and vitamins. The importance of horse gram was well recognized by the folk/alternative/traditional medicine as a potential therapeutic agent. The use of dry seeds of horse gram is limited due to their poor cooking quality and the presence of anti-nutritional factors in horse gram is a matter of concern. The present study is to develop an instant dosa mix with horse gram incorporated in it. It also focus to understand the impact of germination and autoclaving in addition to germination on the selected nutrient and anti-nutrient profile of the dosa mix developed.
Methodology: Horse gram was subjected to germination and autoclaving in addition to germination and this was used to make the dosa mix. The prepared horse gram dosa mix was subjected to selected nutrients analysis, total polyphenols and phytic acid analysis. Functional properties of the dosa mix were also studied. Dosa were prepared from all the variations and subjected to sensory analysis.
Results: The horse gram dosa mix prepared with pre-treated horse gram had a good nutrient profile. There was an increase in the carbohydrate, protein and calcium content, whereas there was a decrease in the iron content due to the pre-treatments. A decrease in the phytic acid and polyphenol content was observed on germination and autoclaving. In the current study a significant decrease ($p<0.05$) in the bulk density and a significant increase ($p<0.05$) in WAC and OAC of germinated horse gram dosa mix and germinated & autoclaved dosa mix was observed. Horse gram dosa mix with either of pre-treatments- germination or germination & autoclaving can be prepared to retain the health benefits of horse gram in our diet.

Keywords: horse gram *(macrotyloma uniflorum),* dry roasting, germination, autoclaving, anti-nutrients

1. Introduction
Horse gram, (*Macrotyloma uniflorum* an underutilized pulse crop native to Southeast Asia has been recognized as potential food source by National Academy of Science (1979) [20].
Horse gram is popularly called "Madras Bean" due to the principal cultivation and production of this legume, especially in rural areas, in the erstwhile Madras Presidency state, India, before the States were separated (Chinnasawamy, 2010) [7]. It is also known as the "poor man's pulse crop" which is extensively used in traditional and ayurvedic medicines to reduce body weight and to treat diseases like jaundice, urolithiasis, skin disorders etc., (Hari Kumar *et al.*,2011) [14]. Horse gram is gaining more attention as legume food due to its high protein content and other nutrients as well (Shasi Jain, 2012) [23].
Although the use of raw horse gram and its flour as food is well known in recent days, maximum utilization is lacking due to the presence of antinutritional factors like tannin, trypsin inhibitor, phytic acid and bowman-birk inhibitors which interfere with the bioavailability of nutrients present in horse gram. However, notable progression has been achieved through dehulling, germination, fermentation, dehydration, soaking and partial hydrolysis of proteolytic enzyme to reduce the anti-nutrient factors and to enhance the nutritive value and functional properties of legumes (Deshpande *et al.*,2002 [10] and Oloyo, 2004) [21].
The present study aims to develop an instant dosa mix with horse gram incorporated in it to tap its potential health benefits. It also focus to understand the impact of germination and autoclaving in addition to germination on the selected nutrient and anti-nutrient profile of the dosa mix developed.

2. Materials and Methods
2.1 Materials
Horse gram and other ingredients required for the formulation of horse gram dosa mix were purchased from the local market in Chennai.

2.2 Methods
- 2.2.1 Pre-treatment of horse gram flour: germination and germination & autoclaving
- 2.2.2 Preparation of horse gram dosa mix with untreated horse gram, germinated and germinated & autoclaved
- 2.2.3 Nutrient analysis
- 2.2.4 Functional Properties
- 2.2.3 Sensory analysis

2.2.1 Pre-treatment of horse gram flour- Germination and Germination & Autoclaving
The method of preparation of germinated and germinated & autoclaved horse gram is explained in the Figure 1.

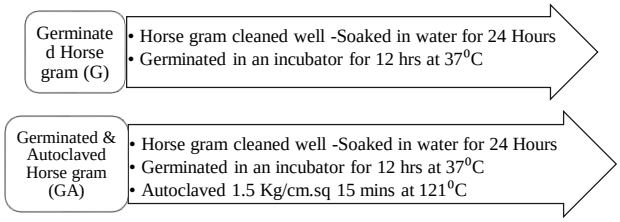

Fig 1: Preparation of Germinated (G) and Germinated & Autoclaved Horse gram powder (GA)

2.2.2 Preparation of horse gram dosa mix

The codes assigned for dosa mix prepared from different pre-treatments of horse gram is given in the Table 1. The method of preparation of horse gram dosa mix is discussed in Table 2

Table 1: Pre-treatment of horse gram and Sample codes

Code	Samples
UTHDM	Untreated Horse gram dosa mix
GHDM	Germinated Horse gram dosa mix
GAHDM	Germinated and Autoclaved Horse gram dosa mix

Table 2: Preparation of Horse gram dosa mix

Ingredients	Sample code		
	UTHDM	GHDM	GAHDM
Rice	400g	400g	400g
Urad dhal	200g	200g	200g
Horse gram	200g	200g	2
Fenugreek seeds	15 g	15g	15g
Method of Preparation of the mix	Horse gram dosa batter was prepared by grinding rice, urad dhal, horse gram and fenugreek seeds. The dosa batter was fermented for 5 hours and the slurry was dried in hot air oven at 60^0C for 12 hours. The dried slurry was pulverized and it was sieved (30mm mesh) in to a fine powder.		
Preparation of Dosa	Water was used to reconstitute the dosa mix. 500g dosa mix requires around 400-450 ml of water. The batter should have a consistency similar to pan cake batter. Salt to be added according to taste. A ladle of batter was poured and spread on a greased non-stick pan and cooked. 500grams of dosa mix can yield 20-25 medium sized dosa.		

2.2.3 Nutrient Analysis

The dosa mix (UTHDM, GHDM, GAHDM) was analysed for moisture, protein, fat, iron, calcium, carbohydrates using standard methods (AOAC 1990) [3]. Total phenolic content was determined by Folin Denis method (AOAC 1990) [3]. Phytic acid content was determined by the method of Davies and Reid (1979) [9].

2.2.4 Functional Properties

The dosa mix (UTHDM, GHDM, and GAHDM) was analysed for bulk density, water absorption capacity and fat absorption Capacity (AOAC 2006) [2]

2.2.5 Sensory Analysis

The dosa mix (UTHDM, GHDM, and GAHDM) was subjected to sensory analysis. Fifteen semi trained panel members were asked to score the product for appearance, colour, texture, flavour, taste and overall acceptability in a 5 point scale.

3. Results and Discussion

3.1 Nutrient Analysis

Table 3: Nutrient content of Horse gram incorporated dosa mix

Parameter	UTHDM	GHDM	GAHDM
Moisture (g/100g)	3.98	3.111 (-21.83)*	4.12 (+3.51)* (+32.43)[1]
Carbohydrates (g/100g))	31.44	39.11 (+24.39)*	39.12 (+24.42)* (+0.025)[1]
Protein (g/100g)	12.12	12.12	15.33 (+26.48)*[1]
Fibre (g/100g)	3.09	3.12 (+0.97)*	3.12 (+0.97)*
Calcium (mg/100g)	123	135 (9.75)*	146 (18.69)* (8.14) [1]
Iron (mg/100g)	2.5	1.2 (-52)*	1.8 (-28)* (50) [1]

Note: * indicates percent increase (+) or (-) decrease over UTHDM values
[1] indicates percent increase (+) or (-) decrease over GAHDM value

The moisture content of UTHDM, GHDM and GAHDM were 3.98g/100g, 3.111 g/100g, 4.12 g/100g. The moisture content decreased by 21.83% in germinated horse gram dosa mix (GHDM) and there was a 3.51% increase in germinated autoclaved dosa mix (GAHDM). The carbohydrate content of UTHDM was 31.44 g/100g. There was a 24% increase in the carbohydrate content in germinated and germinated & autoclaved horse gram dosa mix. Protein content of UTHDM and GHDM was 12.12 g/100g; it had increased by 26.48% in GAHDM. The calcium content of UTHDM was 123 mg/100g. There was an increase of 9.75 % and 18.69 % in the calcium content of the germinated sample (GHDM) and germinated & autoclaved (GHADM). The iron content of UTHDM, GHDM and GAHDM were 2.5. 1.2 and 1.8 mg/100g respectively. There was a 52% and 28% decrease in the iron content in GHDM and GAHDM. This observation was in accordance with other reports

(Enujiugha *et al* 2003) [12]. The reduction in iron contents during soaking and sprouting treatments might be due to the leaching out of the mineral in the soaking water. The fibre content of the samples were in the range of 3.09- 3.12 g/100g

3.2 Phytic acid and Polyphenol content of Horse gram incorporated dosa mix

The actual mechanism of the interactions between phytic acid and minerals are yet to be understood, although it is possible that it could form a complex with a cation on the same or different molecules within a simple phosphate group or between two phosphate groups (Hithamani and Srinivasan, 2014)[15]. Various processing treatments have been reported which can reduce the level of anti-nutrients, such as soaking, germination, steaming, fermentation, microwave heating, etc.

Sharma *et al* (2013) [22] studied the effect of soaking and cooking on polyphenols, tannins, and phytates and reported approximately 14.7–45.1% reduction in soybeans

Table 4 depicts the phytic acid, polyphenol content, amylase inhibitor activity of horse gram incorporated dosa mix. The phytic acid content of UTHDM, GHDM and GAHDM are 140mg/100g, 110mg/100g and 80 mg/100g respectively. There was a 27.27% decrease in phytic acid content on germination in GHDM and 42.85% decrease on germination and autoclaving in GAHDM.

The results observed were in accordance with similar studies by Borade *et al* 2009 [5]. The reduction in the phytic acid can be attributed to leaching out of this anti-nutrient in to the soaking medium and the enzymatic hydrolysis of phytic phosphate during germination (Dave *et al* 2008) [8].

Polyphenols act as anti-nutrients and chelates divalent metal ions like iron and zinc and reduce their bioavailability. They also inhibit digestive enzymes and may also precipitate proteins. Hithamani and Srinivasan (2014) [11] investigated the effect of domestic processing on the polyphenol content in pearl millet (*Pennisetum glaucum*) and observed that sprouting and pressure cooking reduced 33.52 and 41.66% polyphenols, respectively.

The polyphenol content of UTHDM, GHDM and GAHDM are 2mg/100g, 1.5mg/100g and 0.5 mg/100g respectively. There was a 25% decrease in polyphenol content on germination in GHDM and 75% decrease on germination and autoclaving in GAHDM.

Germination of food blends followed by autoclaving resulted in significant reduction in polyphenol content in similar studies. Decrease in polyphenols during germination may be ascribed to the presence of polyphenol oxidase and enzymatic hydrolysis (Jood *et al* 1998 [16] and Arora etal 2008 [4])

Table 4: Phytic acid and Polyphenol content of Horse gram incorporated dosa mix

Parameter	UTHDM	GHDM	GAHDM
Phytic acid (mg/100g)	140	110 (27.27)*	80 (42.85)* (27.27)[1]
Polyphenol (mg/100g)	2	1.5 (25)*	0.5 (75)* (66.66)[1]

Note: * indicates percent increase (+) or (-) decrease over UTHDM values

[1] indicates percent increase (+) or (-) decrease over GAHDM values

3.3 Functional Properties of Horse gram incorporated dosa mix

The bulking property of a powder alters according to the preparation methods, different treatments administered and storage (WHO, 2012) [26].The density of the processed products or the uniqueness of its container determines the amount and strength of packaging material (Wilhelm *et al.*, 2004) [25]. In the study by Vandarkuzhali and Narayanasamy Sangeetha (2016) [24], gradual increase in germination periods showed decrease ($p < 0.05$) in the bulk density of horse gram flour. Germination of horse gram flour at 24 h, 48 h and 72 h significantly reduced the bulk density of horse gram flour by 4 % (0.69 – 0.74g/ml) when compared to the control sample (0.75 g/ml).

The bulk density (g/100ml) of UTHDM, GHDM and GAHDM were 76.45±1.25, 67.8 ±1.2, 73.05 ±0.15. In the current study a significant decrease ($p<0.05$) in the bulk density of germinated horse gram dosa mix and germinated & autoclaved dosa mix was observed.

This result is in agreement with Akubor and Obiegbuna (1999) [1] who observed lesser bulk density value for germinated millet flour. Similarly, Ghavidel and Prakash (2006) [13] reported reduced bulk density for germinated green gram, cowpea, lentil and bengal gram.

As an effective traditional technology, germination enhances the feasibility of incorporation of horse gram flour in weaning foods (Malleshi *et al* 1989) [18].

Table 5: Functional Properties of Horse gram incorporated dosa mix

Parameter	UTHDM	GHDM	GAHDM
Bulk Density (g/100ml)	76.45±1.25	67.8 ±1.2*	73.05 ±0.15**
Water absorption capacity (g/100ml)	69.4 ±8.04	72.5±7.3*	58.4±8.07**
Fat absorption capacity (%)	79.6±13.8	82.9±12.3*	82.5±13.7**

* t value significant at($p<0.05$) on comparing UTHDM and GHDM

** t value significant at($p<0.05$) on comparing UTHDM and GAHDM

The water and oil absorption capacities are essential functional properties of protein which may be defined as the amount of water or oil retained by a known weight of flour under specific conditions.

The water absorption capacity depends on capillary, pore size and the charges on the protein molecules. The oil absorption capacity is also due to enhanced hydrophobic character of proteins in the flours.

Oil absorption capacity (OAC) of legume flours is an essential property to develop novel food products and store them for a long period. Flavor and mouth feel of food depends on the fat molecules present in the flour to some extent (Kinsella, 1976) [17].

Table 5 depicts the water and oil absorption capacity of horse gram dosa mixes. The water absorption capacity (g/ml) of UTHDM, GHDM and GAHDM were 69.4±8.04, 72.5±7.3 and 58.4±8.07 respectively. A significant increase ($p<0.05$) in WAC was observed in germinated and germinated autoclaved horse

gram dosa mix compared to untreated horse gram dosa mix.
WAC also indicates the gelling capacity of the starch and also very important in the texture of food systems. Horse gram starch can contribute greatly to the textural properties of many foods and in industries as a thickener, gelling agent and bulking agent. The oil absorption capacity (%) of UTHDM, GHDM and GAHDM were 79.6±13.8, 82.9±12.3 and 82.5±13.7 respectively. A significant increase (p<0.05) in OAC was observed in germinated and germinated autoclaved horse gram dosa mix compared to untreated horse gram dosa mix
The results are in agreement with the report of oil absorption capacity of 80.76 ± 0.03 (%) and water absorption capacity 142.14 ± 0.10 (g /100g) in horse gram flour by Marimuthu and Krishnamoorthi (2013) [19].
The result is also in excellent agreement with the reports outlined by Elkhalifa *et al.,* (2010) [11] for germinated sorghum flour and Chinma *et al.,* (2009) [6] for germinated tiger nuts. In a study by Vandarkuzhali and Narayanasamy Sangeetha (2016) [24], OAC of germinated horse gram flour for 48h and 72h exhibited significant increase (p<0.05) than the control sample
Therefore, germinated horse gram flour finds applications in the preparation of emulsion type food products with enhanced mouth feel (Kinsella, 1976) [17].

3.4 Sensory Analysis

The overall acceptability score of the UTHDM, GHDM and GAHDM were found to be 2.93, 3.86 and 3.4 respectively. The rating of GHDM was found to be high for all parameters when compared to UTHDM and GAHDM. (Table 6)
Analysis of variance revealed that there was no significant difference in the sensory quality on either of the pre-treatments.

Table 6: Sensory attributes of Horse gram dosa mix

Parameter	UTHDM	GHDM	GAHDM
Appearance	3.06±0.25	3.6±0.73	3.6±0.63
Colour	2.8±0.67	3.3±0.61	2.86 ±0.51
Taste	2.93±0.88	3.4±0.63	3.0±0.84
Texture	3.26±0.79	3.73±0.45	3.26±0.96
Odour	2.86±0.91	3.33±0.48	3.8±0.77
Flavour	2.66±0.89	3.86±0.63	3.93±0.79
Mouth feel	2.46±0.83	3.93±0.7	3.8±0.77
Over all acceptability	2.93±0.79	3.86±0.81	3.4±0.73

4. Conclusion

The horse gram dosa mix prepared with pre-treated horse gram had a good nutrient profile and sensory appeal. The carbohydrate content of the dosa mix was in the range of 31.44 - 39.11 g/100g. There was a 24% increase in the carbohydrate content in germinated and germinated & autoclaved horse gram dosa mix. Protein content of UTHDM and GHDM was 12.12 g/100g; it had increased by 26.48% in GAHDM. There was an increase of 9.75 % - 18.69% in the calcium content of the germinated sample (GHDM) and germinated & autoclaved (GHADM). There was a 52% and 28% decrease in the iron content in GHDM and GAHDM.
There was a 27.27% decrease in phytic acid content on germination in GHDM and 42.85% decrease on germination and autoclaving in GAHDM. There was a 25% decrease in polyphenol content on germination in GHDM and 75% decrease on germination and autoclaving in GAHDM
In the current study a significant decrease (p<0.05) in the bulk density of germinated horse gram dosa mix and germinated & autoclaved dosa mix was observed. A significant increase (p<0.05) in WAC and OAC was observed in germinated and germinated autoclaved horse gram dosa mix compared to untreated horse gram dosa mix.
Horse gram dosa mix with either of pre-treatments- germination or germination & autoclaving can be prepared to retain the health benefits of horse gram in our diet.

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