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Haripriya A
Assistant Professor,
School of Food Science,
MOP Vaishnav College for Women, Chennai, India

Radhika V
MSc Food Technology and Management, School of Food Science, MOP Vaishnav College for Women, Chennai, India

Kavitha K
MSc Food Technology and Management, School of Food Science, MOP Vaishnav College for Women, Chennai, India

Correspondence
Haripriya A
Assistant Professor,
School of Food Science,
MOP Vaishnav College for Women, Chennai, India

Impact of pre-treatments on the selected nutrient and anti-nutrient profile of Horse gram based traditional recipes

Haripriya A, Radhika V and Kavitha K

Abstract
Introduction: The horse gram commonly known as *Kulthi* or *Madras Bean* is a traditional unexploited tropical grain legume. It is well known for its hardiness, adaptability to poor soil and adverse climatic conditions. The horse gram is a cheapest source of protein. It is rich in calcium and iron. But maximum utilization of horse gram is lacking due to the presence of anti-nutritional factors like tannin, trypsin inhibitor, phytic acid which interfere with the bioavailability of nutrients present in horse gram. The present study is to understand the impact of dry roasting, germination and autoclaving in addition to germination on the selected nutrient and anti-nutrient profile of the horse gram based traditional recipes – horse gram chutney powder and rasam mix.
Methodology: Horse gram was subjected to dry roasting, germination and autoclaving in addition to germination. This horse gram flour was used for preparing chutney powder and rasam mix. The prepared horse gram chutney powder and rasam mix with – dry roasted, germinated and germinated & autoclaved horse gram flour was subjected to selected nutrients analysis, α amylase activity and total polyphenols and phytic acid. All the products were subjected to sensory analysis.
Results: Chutney powder and rasam mix prepared with untreated horse gram flour was least accepted and therefore nutrient and other analysis was not done for the same. The horse gram chutney powder and rasam mix developed with all three pre-treatments- dry roasting, germination and germination& autoclaving had a good sensory appeal. There was no significant change in the protein, calcium and iron content on various pre-treatments. In the germinated and germinated & autoclaved samples there was a significant reduction in phytic acid and polyphenol content. Amylase activity has increased on germination. Horse gram chutney powder and rasam mix with either of pre-treatments- germination or germination & autoclaving would maximise 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) [13]. 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., (HariKumar *et al*., 2011) [10] .Although rich in proteins (20 %), due to less acceptable taste and flavour of cooked products, it has remained an underutilized food legume. Maximum utilization of horse gram is lacking due to the presence of anti-nutritional factors like tannin, trypsin inhibitor, phytic acid 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 [6] ; Oloyo, 2004 [15] Sharma *et al* (2013) [16].
One of the traditional recipes that slipped out of Indian cookery over time is horse gram chutney and rasam. The objective of this study is to understand the impact of pre-treatments like dry roasting, germination and autoclaving in addition to germination on selected nutrients and anti-nutrients of horse gram chutney powder and rasam mix. With the hope that present day consumers are health conscious and prefer to make an educated choice in selecting foods

offering a broad nutrient base and large health benefits , horse gram chutney powder and rasam mix prepared with either of the pre-treatments can be commercialised to carry the health benefits of horse gram to people

2. Materials and Methods

2.1 Materials

Horse gram and other ingredients required for the formulation of horse gram chutney powder and rasam mix were purchased from the local market in Chennai.

2.2 Methods

- 2.2.1 Pre-treatment of horse gram flour- dry roasting, germination and germination & autoclaving
- 2.2.2 Preparation of horse gram chutney powder and horse gram rasam mix with all 3 variations and untreated horse gram flour
- 2.2.3 Nutrient analysis of all the samples
- 2.2.4 Sensory analysis of all the samples

2.2.1 Pre-treatment of horse gram flour- dry roasting, germination and Germination & Autoclaving

The method of preparation of dry roasted horse gram, germinated and germinated & autoclaved horse gram powder is explained in the Figure 1 and 2.

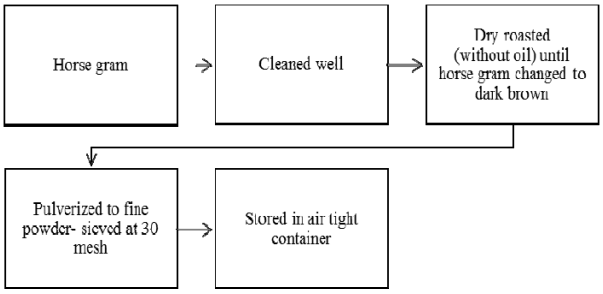

Fig 1: Preparation of Roasted Horse Gram Powder (DR)

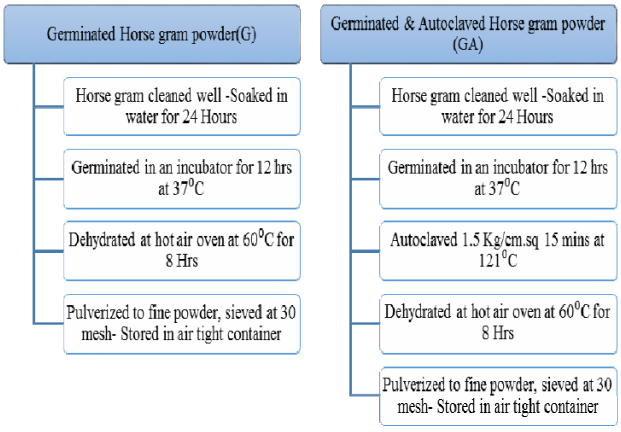

Fig 2: Preparation of Germinated (G) and Germinated & Autoclaved Horse gram powder (GA)

2.2.2 Preparation of horse gram chutney powder and horse gram rasam mix

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

Table 1: Preparation of Horse gram Chutney powder and rasam mix

Recipe for Horse gram Chutney powder	Recipe for Horse gram Rasam mix
Ingredients • Horse gram – 30g (Variations : UT,DR, G & GA) • Urad dhal -20g • Curry leaf-3 g • Asafoetida a pinch • Salt- 2.5g	**Ingredients** • Horse gram – 30g (Variations : UT,DR, G & GA) • Red gram dhal -5g • Coriander seeds-4g • Cumin seeds-4g • Red chillies-3g • Black pepper-4g • Asafoetida a pinch
Method Urad dhal, red chilli and curry leaf were roasted and powdered. Mixed them with each variation of horse gram powder (30 g of DR, G & GA).Added asafoetida and salt. Mixed well. **For sensory analysis-** Horse gram chutney powder was mixed with hot rice and gingely oil and served.	**Method** Red gram dhal, coriander seeds, cumin seeds, red chillies, black pepper and asafoetida were dry roasted. Mixed them with each variation of horse gram powder (30 g of DR, G & GA).Mixed together. **For sensory analysis-** Horse gram rasam was served hot

Table 2: Pre-treatment of horse gram and Sample codes

Code	Samples
UTCP	Untreated Horse gram chutney powder
UTRM	Untreated Horse gram rasam mix
DRCP	Dry Roasted Horse gram chutney powder
GCP	Germinated Horse gram chutney powder
GACP	Germinated and Autoclaved Horse gram chutney powder
DRRM	Dry Roasted Horse gram rasam mix
GRM	Germinated Horse gram rasam mix
GARM	Germinated and Autoclaved Horse gram rasam mix

2.2.3 Nutrient Analysis

The dry roasted, germinated and germinated & autoclaved horse gram chutney powder and rasam mix were analysed for moisture, protein, fat, iron, calcium and carbohydrates using standard methods (AOAC 1990) [1]. Total phenolic content was determined by Folin Denis method (AOAC 1990) [1]. Phytic acid content was determined by the method of Davies and Reid (1979) [5].The activity of α-amylase of flour samples was determined using Megazyme α-amylase assay kit (Ireland)

2.2.4 Sensory Analysis

The untreated, dry roasted, germinated and germinated & autoclaved horse gram chutney powder and rasam mix were 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.

Note: Horse gram chutney powder and rasam mix with untreated horse gram flour was not subjected to nutrient & anti-nutrient analysis and it was not used as standard for comparison as it had an unacceptable earthy taste and flavour on sensory analysis.

3. Results and Discussion

3.1 Nutrient Analysis

Table 3 depicts the nutrient content of dry roasted, germinated and germinated autoclaved horse gram chutney powder and rasam mix.

The moisture content of DRCP was 3.12g/100g. The moisture content increased by 3% in germinated chutney powder (GCP) and there was a 27.8% decrease in germinated autoclaved chutney powder (GACP). The moisture content of dry roasted rasam mix (DRRM) and germinated autoclaved rasam mix (GARM) was 2.11g/100g. There was a 179% increase in moisture content of the germinated sample (GRM).

The carbohydrate content of DRCP was 50.21g/100g .There was an 11% decrease in carbohydrate in germinated chutney powder and 22% decrease in germinated autoclaved chutney powder. There was 14-27% decrease in carbohydrate content on germination and germination & autoclaving compared to dry roasting in rasam mix samples. The results were comparable to the findings of similar works (Ghavidel and Prakash, 2006) [8].This decrease can be attributed to the increase in amylase activity. (Reference Table 4)

Protein content of DRCP was 20.98g/100g; it had increased by 27.6% in GCP and GACP. On comparing the protein content of DRRM, GRM and GARM, the protein content was in the range of 20- 28g/100g. In the rasam powder samples, there was 40% increase in protein content on germination over dry roasting. Autoclaving the germinated sample led to a decrease of 7% in the protein content compared to germination alone.

The calcium content of DRCP was 288.5 mg/100g. There was an increase of 1% and 2% in the calcium content of the germinated sample (GCP) and germinated & autoclaved (GACP). The iron content of all the chutney powder samples was in the range of 5.9-6.0 mg/100g. There was a 33-34% decrease in the fibre content on germination (GCP) and germination & autoclaving (GACP) on a value of 7.6g/100g in DRCP

In all the rasam mix, there was not any significant change in the calcium and fibre content on germination and autoclaving. There was a 17% decrease in the iron content on germinated and autoclaved sample (GARM).This observation was in accordance with other reports.(Enujiugha *et al* 2003) [7]

Table 3: Nutrient content of Horse gram Chutney powder and Rasam mix

Parameter	DRCP	GCP	GACP	DRRM	GRM	GARM
Moisture (g/100g)	3.12	3.225 (+3.36)*	2.25 (-27.8)* (+30.23)[1]	2.11	5.89 (+179)[#]	2.11 (-64.17)[$]
Carbohydrates (g/100g))	50.21	44.56 (-11.25)*	39.11 (-22.10)* (-12.23)[1]	54.41	46.7 (-14.17)[#]	39.4 (-27.5)[#] (-15.6)[$]
Protein (g/100g)	20.98	26.78 (+27.6)*	26.78 (+27.6)*	20.24	28.34 (+40)[#]	26.12 (+29.05)[#] (-7.83)[$]
Fibre (g/100g)	7.6	4.98 (-34.98)*	5.11 (-33.28)* (+2.61)[1]	5.46	5.89 (+7.8)[#]	5.44 (-0.36)[#] (-7.64)[$]
Calcium (mg/100g)	288.5	291.5 (+1.03)*	296 (+2.59)* (+1.54)[1]	255	256 (+0.39)[#]	248 (-2.74)[#] (-3.12)[$]
Iron (mg/100g)	5.9	6.0 (+1.69)*	5.95 (+0.84)* (-0.83)[1]	6.44	6.44	5.33 (-17.23)[#$]

Note: * indicates percent increase (+) or (-) decrease over DRCP values
[1] indicates percent increase (+) or (-) decrease over GCP values
[#] indicates percent increase (+) or (-) decrease over DRRM values
[$] indicates percent increase (+) or (-) decrease over GRM values

3.2 Phytic acid, Polyphenol content, Amylase Activity of Horse gram Chutney powder and Rasam 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)[11]. Similarly, polyphenols also 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. Various processing treatments have been reported which can reduce the level of anti-nutrients, such as soaking, germination, steaming, fermentation, microwave heating, etc. Several researchers have studied the effect of various processing treatments on the content of anti-nutrients of different cereals/legumes (Goyal *et al* 2014) [9]

Sharma *et al* (2013) [16] studied the effect of soaking and cooking on polyphenols, tannins, and phytates and reported approximately 14.7–45.1% reduction in soybeans. Similarly, 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.

Table 4 depicts the Phytic acid, Polyphenol content, Amylase Activity of Horse gram Chutney powder and Rasam mix

The phytic acid content of DRCP was 500mg/100g. There was 40% decrease on germination and 70% decrease on germination followed by autoclaving in GCP and GACP samples respectively. On comparison with dry roasted sample (DRCP) the polyphenol content decreased by 4% and 7.5% on germination and germination & autoclaving in GCP and GACP samples respectively.

Similar to chutney powder, in both germinated (GRM) and germinated & autoclaved (GARM) rasam mix samples there were a significant reduction in phytic acid and polyphenol content.

The results observed were in accordance with similar studies by Borade *et al* 2009 [3]. 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) [4]. 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 [12] and Arora etal 2008 [2]).
Amylase activity has increased on germination. Similar results were obtained in other studies. Seed germination process triggers the enzymatic activity of sprouting seeds, which further breaks the carbohydrates, proteins and fats into simpler forms (Nout and Ngoddy, 1997) [14]. The process of germination is found to be decreasing the level of polyphenols, oxalic acid and phytic acid present in the horse gram seeds (Sudha, 1995) [17].

Table 4: Phytic acid, Polyphenol content, Amylase Activity of Horse gram Chutney powder and Rasam mix

Parameter	DRCP	GCP	GA	DRRM	GRM	GARM
Phytic acid (mg/100g)	500	300 (-40)*	150 (-70)* (-50)[1]	589.5	330 (-44.02)[#]	170 (-71.16)[#] (-48.48)[$]
Polyphenol (mg/100g)	1.2	1.15 (-4.1)*	0.3 (-75)* (-56.66)[1]	0.96	0.42 (+56.25)[#]	0.42 (+56.25)[#]
Amylase Activity (ceralpha unit/ g flour)	0.34	25	25	0.29	35	35

Note: * indicates percent increase (+) or (-) decrease over DRCP values
[1] indicates percent increase (+) or (-) decrease over GCP values
[#] indicates percent increase (+) or (-) decrease over DRRM values
[$] indicates percent increase (+) or (-) decrease over GRM values

3.3 Sensory Analysis

Table 5: Sensory attributes of Untreated (UT) Horse gram chutney powder and Rasam mix

Parameter	UTCP	UTRM
Appearance	2.1±0.7	2.16±0.7
Colour	2.33±0.8	2.76±0.9
Taste	1.2±1.0	1.6±0.9
Texture	1.2±0.7	1.33±0.8
Odour	1.06±0.9	1.03±0.8
Flavour	1±1.0	1.06±0.9
Mouth feel	1.13±0.9	1.09±0.7
Over all acceptability	1.1±0.9	1.23±1.1

Table 5 depicts the sensory score of untreated horse gram chutney powder and rasam mix.
Chutney powder and rasam mix prepared with untreated horse gram flour was least accepted and therefore nutrient and other analysis was not done for the same.
Dry roasting is an inevitable step in the traditional recipe of horse gram chutney powder and rasam. Probably, this is to overcome the earthy taste and flavour of horse gram.

Table 6: Sensory attributes of pre-treated Horse gram chutney powder and rasam mix

Parameter	DRCP	GCP	GACP	DRRM	GRM	GARM
Appearance	3.2±0.7	3.46±0.7	3.73±1.0	3.06±0.5	3.0±0.9	2.93±1.1
Colour	3.33±0.8	3.6±0.9	3.6±0.8	2.73±0.9	2.73±0.8	2.93±1.1
Taste	3.6±1.0	3.46±0.9	3.73±1.0	2.93±1.0	2.66±0.8	2.8±1.2
Texture	3.2±0.7	3.33±0.8	3.36±1.0	2.66±1.1	2.8±0.7	2.6±1.1
Odour	3.06±0.9	3.13±0.8	3.73±1.0	2.93±0.8	3.13±1.2	3.06±1.1
Flavour	3±1.0	3.4±0.9	3.53±0.9	3.0±1.0	3.0±0.9	2.93±1.1
Mouth feel	3.13±0.9	3.0±0.7	3.46±0.9	2.93±1.0	2.86±0.9	2.86±1.1
Over all acceptability	3.4±0.9	3.53±1.1	4.75±0.8	2.86±0.8	2.86±0.8	2.93±1.1

The overall acceptability score of the dry roasted,(DRCP) germinated horse gram chutney powder (GCP) and germinated autoclaved horse gram chutney powder(GACP) were found to be 3.4, 3.53 and 4.75 respectively. The rating of GACP was found to be high for all parameters when compared to DRCP and GCP. (Table 6)
The sensory evaluation of DRRM, GRM and GARM inferred that DRRM and GRM had an overall acceptability score of 2.86. The overall acceptability of GARM was 2.93 (Table 6)
Analysis of variance revealed that there was no significant difference in the sensory quality on either of the pre-treatments.

4. Conclusion

The traditional horse gram chutney powder and rasam mix developed with all three pre-treatments- dry roasting, germination and germination& autoclaving had a good sensory appeal. There was a decrease in carbohydrate content on germination and germination & autoclaving when compared to dry roasting. The protein content of all the samples were in the range of 20-28 g/100g. All the products had an appreciable amount of calcium (248-288mg/100g) and iron (5.3-6.0 mg/100g).In the germinated and germinated & autoclaved samples there was a significant reduction in phytic acid and polyphenol content. Amylase activity had increased on germination. Horse gram chutney powder and rasam 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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