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Effect of Processing and Fermentation on Functional Properties and on Anti-nutritional Factors in Horse Gram (*Macrotyloma uniflorum*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author BHS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VCS and HCG checked the first draft and corrected. Authors KHK and PR managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was conducted to understand the Effect of processing and fermentation on functional properties and on anti-nutritional factors in Horse Gram (*Macrotyloma uniflorum*). **Place and Duration of Work:** The study was carried out in Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore during 2019-20. **Methodology:** Horse gram seeds were procured from National seed project, thoroughly cleaned and were subjected to different processing methods (soaking, roasting, cooking and germination) and ground into flour. Then, the processed flours were analyzed for altered functional properties like bulk density, water and oil absorption capacity, foaming capacity and stability etc. The raw seeds were directly milled into flour without any processing and used as control. Further, all processed flours were subjected to fermentation and compared with non fermented flours for reduction of antinutritional factors (tannins and phytates).

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Results: The functional properties of unprocessed (raw) horse gram flour was recorded with values of 0.95 g / g (Bulk density), 1.87 g / mL(water absorption capacity), 1.45 g / mL(oil absorption capacity), 7.56% (foaming capacity) and 70.78% for foaming stability. Whereas, the processing significantly altered the functional properties. When it comes to antinutritional factors, unprocessed flour recorded with 7.9 mg / g of tannins and 0.96 mg / g of phytates. The processing in combination with fermentation facilitate further reduction of antinutrients compared to processing alone (without fermentation). Among them, fermented germinated flour and fermented cooked flour proven their efficiency in reduction of tannins (61.3 and 62.5%) and phytates (54.1 and 46.8%) compared to other processed flours.

Conclusion: Based on results, it is evidenced that processing altered functional properties of horse gram effectively. However, processing combined with fermentation yielded higher reduction in antinutritional factors compared to processing alone. Further, germinated flour and cooked flour on fermentation were found to yield significantly higher reduction in antinutritional factors thereby enhancing its utilization in functional foods as main / partial ingredient.

Keywords: Horse gram; cooking; bulk density; water holding capacity; germination; tannins; phytates; oxalates.

1. INTRODUCTION

Horse gram is one of the underutilized legumes with high protein content besides vitamins and minerals. Since it offers a cheaper protein source for low-income groups, it is popularized as poor man's pulse crop and is considered as great solution to address malnutrition issues in Besides developing countries. balanced nutritional composition, it has been reported continuously for medicinal properties that help in prevention / treating various health issues ranging from simple weight loss, skin disorders to cardiovascular problem including a few types of cancers [1-3]. However, its credentials are masked by prolonged cooking time, less acceptable flavor and taste as well as presence of antinutritional factors like phenols, tannins, phytic acid, oxalates and trypsin inhibitors that may negatively impact nutrient uptake and metabolism resulting in poor protein digestibility [4,5].

Processing is the well established strategy for lowering cooking time and antinutritional factors thereby enhancing its acceptability and nutritional quality. The processes like dehulling, soaking, sprouting, boiling, roasting, cooking and fermentation are routinely practiced at domestic level [6-8]. They may found beneficial in one aspect but, impact negatively on the other. For example, thermal processing methods such as roasting / cooking might be effective in reduction of antinutritional factors but, usually they result in major nutrient loss. However, fermentation was acknowledged for effective reduction of antinutritional factors with improved nutritional value. Simultaneously, it is also reported that processing can influence chemical and functional properties of food, may be desirable to transform raw form into acceptable form, thereby promoting its commercial utilization [9,10].

In this regard, the study was performed to investigate effect of fermentation on functional and antinutritional properties of processed horse gram (*Macrotyloma uniflorum*) flour.

2. MATERIALS AND METHODS

2.1 Sample Procurement

Horse gram (*Macrotyloma uniflorum*) seeds were acquired from National Seed Project (NSP), GKVK, Bangalore in bulk quantity to ensure uniformity and were cleaned thoroughly and stored in glass bottles.

2.2 Processing of Horse Gram

2.2.1 Roasting

The horse gram seeds were dry roasted in metal pan on low flame for 10-15 min, till their color changed to dark brown along with roasted flavor. Then, seeds were allowed to cool and ground to get flour.

2.2.2 Soaking

The seeds were fully covered with distilled water by 1:5 (w/v) ratio and allowed to stand overnight followed by air drying and ground to get flour.

2.2.3 Cooking

The presoaked seeds were pressure cooked for 30 min by adding distilled water in 1:7 (w/v) ratio, then dried and milled in to flour.

2.2.4 Germination

The overnight soaked seeds were drained the excess water, tied in muslin cloth and allowed to germinate for 24h. Then seeds were dried and milled in to flour.

2.2.5 Fermentation

All the flours (processed) were added with 1:3 (w/v) distilled water and ground them into batter consistency. Further, the batter was allowed to ferment naturally for nine hours. Batter was dried in an oven to get powder.

2.2.6 Unprocessed flour

A known quantity of raw seeds were directly milled into flour without subjecting them to any kind of processing and considered as control.

2.3 Estimation of Functional Properties of Flour

2.3.1 Packed bulk density

The bulk density was performed by filling 25 g of processed flour into a measuring cylinder and gently taps the cylinder for 25 times until the constant volume was achieved and calculated as described [11].

2.3.2 Water absorption capacity

Processed sample [One gram] was taken in clean centrifuge tube and initial weight was recorded. Then added distilled water (10 mL) to the tube followed by centrifugation at 2,000 rpm for 15 min. The supernatant was discarded and gain in weight with respect to initial weight was calculated as water absorption capacity of flour sample [12].

2.3.3 Oil absorption capacity

The oil absorption capacity was determined by taking initial weight of centrifuge tube with one gram of flour followed by mixing 10 mL of sunflower oil and centrifuged at 2,000rpm for 20 min. The mass gained with respect to initial mass

was taken as oil absorption capacity of horse gram flour [13].

2.3.4 Foam capacity and foam stability

Foaming property was estimated as per the procedure described [14]. Horse gram flour (one gram) was mixed with 50 mL of distilled water in test tube (screw capped) followed by vigorous shaking for 5 min. The foam layer volume was recorded as foam capacity (mL/100 mL). Foam stability was calculated by noting down fall in volume of foam layer after one 60 min.

Foam capacity = Vol. after whipping- Vol. before whipping/ Vol. after whipping X 100

Foam stability = Foam volume after 60 min /Initial foam volume X 100

2.4 Determination of Antinutritional Properties

2.4.1 Tannins

The tannins were quantified by Folin-Ciocalteu method [15]. A sample extract of 0.1 mL taken with subsequent addition of 0.5 mL Folin-Ciocalteu phenol reagent, sodium carbonate solution (1 mL of 35%) and make up the volume (volumetric flask 10 mL) by diluting with distilled water. A set of tannic acid (20, 40, 60, 80 and 100 μ g/ mL) standards were prepared and incubated along with samples for 30 min followed by measuring absorbance at 760 nm.

2.4.2 Phytates

Flour (two grams) was mixed with 2% hydrochloric acid (100 mL) and was soaked for three hours with subsequent filtration. A known quantity (50 mL) of filtrate was diluted with distilled water(107 mL) followed by ammonium thiocyanate solution (10 mL of 0.3%). It was titrated against standard ferric chloride solution (0.00195 g Fe / mL). The persistence of brownish yellow color for five minutes confirmed as an end point and percent phytates was calculated as follows [16].

% Phytates = Titre value X 1.19 X 0.00195 X 100

2.5 Statistical Analysis

The data in five replications was subjected to statistical analysis by Duncan's multiple range test [17].

3. RESULTS AND DISCUSSION

3.1 Processing Effect on Functional Properties of Horse Gram Flour

3.1.1 Bulk density

The bulkiness of a powder is influenced by various treatments. preparation methods. handling and storage packing, conditions administered [18]. There is reduction in bulk density for all processed flours (ranging from 0.95-0.75 g / mL) compared to unprocessed flour (0.95 g / mL). The significant reduction of bulk density was detected in fermentation (0.75 g / mL) and the least was in soaking [Table-1]. Similar kind of results was reported in various studies [19.20]. The reason might be due to breakdown of starch resulting in altering denser compounds into smaller ones during processing [21,22]. The reduced bulk density is one of the desirable traits in weaning food preparations meant for infants.

3.1.2 Water absorption capacity

The processed flours exhibited increased water absorption capacities from 1.98-2.42 (except for cooking) significantly, compared to unprocessed one (1.87). The highest increase was detected in germination, while roasting recorded least, [Table 1] which is supported by many authors [23-25]. The noted difference among the flours to reduced carbohydrate protein owing interaction resulted in exposing of more hydrophilic constituents of protein, which further interacted with water molecules [26,27]. The increased water absorption capacity could maximize its utilization in foods such as dough and sausages and is essential for flavor retention and deliciousness.

3.1.3 Oil absorption capacity

In case of oil absorption capacity, all processed flours showed higher values ranging between 1.6 - 2.52 (g / mL) compared to unprocessed flour (1.45 g / mL). The germinated flour had the highest increase, while the least was in roasted sample [Table 1]. The increase might be due to solubilization and dissociation of proteins thereby exposing non-polar constituents resulting in binding of fat molecules to hydrophobic ends by physical entrapment [28,29]. This could be a required factor for preparation of meat extenders since it improves the mouth feel besides flavor retention.

3.1.4 Foaming capacity and stability

The processed flours were reported with lower values for foaming capacity than unprocessed flour (7.56%) except for soaking (9.12%) and fermentation (10.58%). A lower foaming capacity was exhibited by cooking process (2.23%). The unprocessed flour had good whipping property of 107.56 mL and produced less foam stability compared to processed flours except for cooking. The foam stability values after 1h were 70.7, 67.7, 84.9, 11.9, 63.9 and 72.5% respectively for unprocessed. roasted. soaked. cooked. germinated and fermented flours. Similar kind of reductions have been reported [30], especially for heat processed flours [31] and were opinioned to be ruled by nature of protein and its denaturation may be the basis for its decrease [32].

3.2 Effect of Fermentation on Antinutritional Factors Reduction

The highest tannin reduction in non-fermented flours was observed with cooking process (3.79 mg / g) followed by germination (4.97 mg / g) and roasting (5.28 mg / g), while soaking was observed with minimum effect (5.85 mg / g). However, the combined effect of fermentation with other processed flours showed further more significant reduction [Table 2] and were recorded as 5.05, 3.49, 3.68, 2.96 and 3.05 mg / g for unprocessed, roasted, soaked, cooked and germination respectively.

The tannin content of unprocessed flours reported by [33,34] was noticed as higher than the values obtained in present study (7.90 mg / g), that might be owing to varietal difference. In case of phytates, the fermented processed flours exhibited significant reduction than the non-fermented flours. The highest reduction for fermented flours was observed with germinated flour (0.44 mg / g) followed by cooking (0.51 mg / g) and soaking (0.62 mg / g) compared to non-fermented flours as 0.56, 0.65, 0.71 mg / g with respect to germinated, cooked and soaked respectively [Table 3].

The results were in accordance with the reports [35,36], heating and germination were found to reduce tannin and phytate contents among legumes and cereals. Many authors have given detailed report on mechanisms / pathways involved in reduction of antinutritional factors. During soaking process, the reduction of antinutritional factors (phytates, oxalates and

tannins) in horse gram flour was due to leaching loss [37]. The denaturation of protein besides complexes formation might be the clue for reduction in roasting [38,39] and polyphenol oxidase activation for tannin reduction was also reported [40,41]. During germination, the reduction was due to activation of endogenous enzymes such as phytase, polyphenol oxidase and oxalate oxidase besides complex formation.

The non-fermented flours [Fig. 1] per cent reduction values were ranged between 25.94 - 37.08% for tannins, while fermented flours were between 36.07 - 62.53%. For phytates, the per cent reduction values of fermented

Processing Method	Bulk Density (g / mL)	Water absorption capacity (g / mL)	Oil absorption capacity (g / mL)	Foam capacity (%)	Foam stability (%)
Unprocessed	0.95±0.02 ^a	1.87±0.06 ^e	1.45±0.01 [†]	7.56±0.07 ^c	70.78±0.10 ^c
Roasted	0.89±0.03 ^b	1.97±0.03 ^d	1.60±0.00 ^e	7.06±0.09 ^d	67.74±0.07 ^d
Soaked	0.95±0.01 ^ª	2.00±0.06 ^c	2.01±0.01 ^d	9.12±0.09 ^b	84.90±0.11 ^ª
Cooked	0.92±0.01 ^{ab}	1.59±0.01 ^f	2.46±0.01 ^c	2.23±0.10 ^f	11.92 ±0.10 ^f
Germination	0.81±0.03 ^c	2.42±0.07 ^a	2.52±0.01 ^a	4.18±0.07 ^e	63.90±0.11 ^e
Fermentation	0.75±0.01 ^d	2.25±0.02 ^b	2.48±0.04 ^b	10.58±0.03 ^ª	72.5±0.05 ^b
CV	3.98	1.34	0.91	2.81	0.58
CD _(0.01)	0.06	0.04	0.03	0.33	0.63
CD _(0.05)	0.04	0.03	0.02	0.24	0.46

Note: Mean values having same superscript letter are not significantly different at P<0.05 CV: Coefficient of Variation; CD: Critical Difference

Processing	Tannins (mg/g	3)	Per cent re	duction (%)
Method	BF	AF	BF	AF
Unprocessed	7.90±0.03 ^a	5.05±0.06 ^a	0.00	36.07
Roasted	5.28±0.03 ^c	3.49±0.05 ^c	33.16	55.82
Soaked	5.85±0.02 ^b	3.68±0.04 ^b	25.94	53.41
Cooked	3.79±0.02 ^e	2.96±0.07 ^d	52.02	62.53
Germinated	4.97±0.04 ^d	3.05±0.07 ^d	37.08	61.39
CV	1.114	3.648		
CD _(0.01)	0.111	0.239		
CD _(0.05)	0.082	0.176		

Note: Mean values having same superscript letter are not significantly different at P<0.05 BF: Before fermentation; AF: After fermentation; CV: Coefficient of Variation CD: Critical Difference

Table 3. Effect of	processing on	phytate reduction

Processing	Phytates (mg/g)	Per cent reduction (%	
Method	BF	AF	BF	AF
Unprocessed	0.96±0.02 ^a	0.80±0.02 ^a	0.00	16.66
Roasted	0.81±0.01 ^b	0.69 ± 0.02^{b}	15.62	28.12
Soaked	0.71±0.01 ^c	0.62±0.01 ^c	26.04	35.41
Cooked	0.65±0.02 ^d	0.51±0.01 ^d	32.29	46.87
Germinated	0.56±0.01 ^e	0.44±0.02 ^e	41.66	54.16
CV	4.359	6.621		
CD _(0.01)	0.058	0.073		
CD _(0.05)	0.043	0.054		

Note: Mean values having same superscript letter are not significantly different at P<0.05

BF: Before fermentation; AF: After fermentation; CV: Coefficient of Variation

CD: Critical Difference

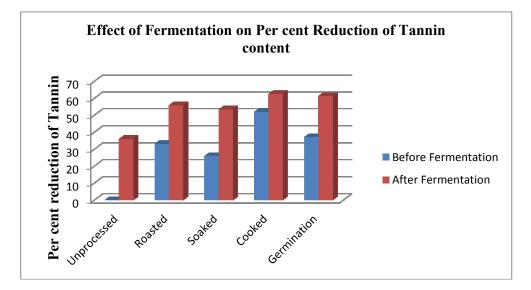


Fig. 1. Effect of Fermentation on Per cent Reduction of Tannin content

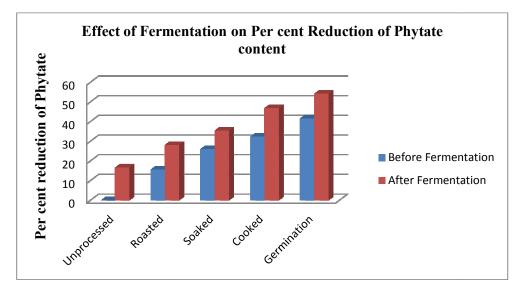


Fig. 2. Effect of fermentation on per cent reduction of phytate content

flours (16.66 - 54.16%) were higher as compared to non-fermented flours (15.62 - 41.66%) and were explained in Fig. 2.

4. CONCLUSION

It is confirmed from the present study that processing of horse gram alters the functional properties significantly to a desirable level, thereby improving its acceptability compared to raw sample. Among all processing methods, germination and cooking were found to be the best methods to enhance its suitability as functional food. The antinutritional factors will form complexes with minerals making them unavailable thereby interfere with mineral absorption., It is evident from results obtained that, each processing method has its own mechanism / pathway in reducing antinutritional factors. However, fermentation of germinated as well as cooked flour showed highest reduction of antinutritional factors compared to non-fermented processed flours, resulting in direct enhancement of nutritional value. Finally, it leads to a conclusion that, processing of horse gram, particularly fermentation encourages to promote and maximize its utilization as functional ingredient in food preparations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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