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Effect of Processing and Fermentation on Oxalate, Biochemical and Microbial Properties in Horse Gram

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ABSTRACT

Oxalates are considered to be one among the major antinutritional factors in horse gram, that play a key role in kidney stone formation especially when accumulated in urine**,** predominately derived either by liver synthesis or through absorption of dietary oxalate. Many processing methods are available for reducing these antinutritional factors, thus the present work was carried out to evaluate the best processing methods available for lowering the oxalate content in horse gram. Results showed that fermentation of unprocessed flour (160.12 mg) recorded highest oxalate reduction followed by soaked flour (151.13 mg), germinated flour (132.00 mg), roasted flour (116.25 mg) and cooked flour (103.50 mg /100 g).

Keywords: *Antinutritional factors, Fermentation, Horse gram, Oxalates and Titratable acidity*

Horse gram (*Macrotyloma uniflorum* Lam. [Verdc.]) is one among the most neglected and underutilized legumes with a potential to be developed into a commercial crop. Though this crop is native to Southeast Asia and tropical Africa, Southern India is considered to be the centre of origin of cultivated species. In India it is mainly cultivated in the states of Karnataka, Andhra Pradesh, Odisha, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, Jharkhand and in foot hills of Uttaranchal and Himachal Pradesh in India. It is well known for its nutritional significance, hardiness, adaptability to adverse environmental conditions with better climate resilience, but does not tolerate frost and water logging (Bhartiya *et al*., 2015). It is recognized as super food 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 considered as great solution to address the malnutrition issues in developing nations. Owing to its excellent nutritional composition, it finds place in ayurvedic medicine and has been reported continuously for medicinal properties that help in prevention / treating various health problems ranging from simple skin disorders to jaundice, asthma, bronchitis, piles, kidney stones, leucoderma, urinary discharges and heart diseases including various types of cancers. Due to the presence of beneficial bioactive compounds, it also possesses anti-diabetic, anti-ulcer activity and helps in dietary management of obesity, while high fibre content result in reducing the body fat (Aditya *et al*., 2019). However, its credentials are masked by prolonged cooking time, less acceptable flavor and taste as well as presence of antinutritional factors.

Antinutritional factors (ANF) are the secondary metabolites, synthesized as a part of survival mechanism to rescue themselves from adverse growing conditions. It might be useful in plants point of view but on consumption by humans and animals as food and feed, these anti-nutrients could possibly exert harmful and adverse physiological effects. They are also associated with hard-to-cook phenomenon of legumes (Moktan and Ojha, 2015).

Oxalate is one of such major antinutritional factor, which generally found in plants and animals as Oxalic acid (dicarboxylic acid). Under normal conditions, oxalic acid is confined to separate compartments, but once it got digested they come in contact with the nutrients, interfere with the absorption by forming insoluble salts. The oxalate content of urine plays a role in kidney stone formation**,**

predominately derived either by liver synthesis or through absorption of dietary oxalate which tends to be higher in plant products than in animal products. These antinutritional factors are not only involved in hindering nutrient uptake, metabolism and protein digestibility.

Processing is a well established strategy for lowering cooking time and antinutritional factors thereby enhancing its acceptability and nutritional quality. Simultaneously, it has been reported that processing can influence chemical and functional properties of food that are desired for transforming raw form into acceptable form, thereby promoting its commercial utilization. During food processing, the nutrient content will be affected by the conditions prevailing during processing like pH, heat, oxygen and light. The nutrient retention capability depends on the characteristics of food being processed and quantity of nutrients present in the food. Some of the traditional processing methods commonly practiced at domestic level are dehulling, soaking, boiling, roasting, sprouting, cooking and fermentation processes. All these methods are found beneficial in one aspect but may impact negatively on the other. For example, thermal processing methods such as roasting /cooking might be effective in reduction of antinutritional factors, but usually there will be loss of nutrients. Thus adapting well established home processing methods have the greatest potential for serving high protein based legume food products to a large group of people. Recognition and update of newer and improved processing methods provide the basis and guidelines for development of legume processing technology.

MATERIAL AND METHODS

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.

Processing of Horse Gram

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.

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.

Cooking

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

Germination

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

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.

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.

Estimation of Oxalate content

Sample (2 g) extraction was done in 250 mL volumetric flask with 6 M HCl (10 mL) followed by digestion (60 min). The volume was made up to 250 mL (distilled water) and filtered. From this, two portions of filtrate was made (125 mL each) and mixed with methyl red (3-4 drops) and conc. NH_4OH drop (salmon pink to faint yellow). Further, each portion was heated (90 °C), cooled and filtered. The extract obtained was heated again followed by $CaCl_2$ addition (10 mL of 5 %) followed by decanting. The precipitate was taken and dissolved in H_2SO_4 (10 mL of 20 %) followed by volume makeup (300 mL). An aliquot (125 mL) was heated to near boiling and titrated against potassium permanganate (0.05 M) till pink color persists for 30 sec (Adeniyi *et al*., 2009).

Physical and biochemical analysis of processed and fermented flours

Batter volume

A known quantity (50 mL) batter was transferred into a 100 mL measuring cylinder, sealed

it with aluminum foil followed by fermentation. Initial batter volume was noted down and the rise in batter volume was recorded.

Titratable acidity (TA)

Sample (10 mL) was boiled in water bath at 70 °C followed by titration against 0.1 N NaOH after adding phenolphthalein indicator (3 drops). The end point was noted down after pink colour appearance and was calculated and expressed in terms of Per cent of lactic acid.

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times 90.01 \times 100}{\text{mL of sample taken} \times 1000}$$

pH

The digital pH meter was calibrated with standard buffer solutions (pH 4.0, 7.0 and 9.2) before measuring the pH of the batter.

LA bacterial population analysis

The enumeration of LA bacterial population was carried out initially and after fermentation using standard plate count technique. A known quantity (10 g) of processed flour samples were taken into 100 mL sterile saline (0.85 %) and homogenized. Aliquot of 10 mL was transferred serially to next saline blanks to get 10^{-9} dilution. Aliquots (1 mL) of each dilution were dispensed into sterile Petri plates followed by pouring MRS agar medium and then incubated at room temperature for two days. Plates having 30-300 colonies were taken for enumeration.

Results and Discussion

Oxalate content

Table 1 explains the significance of processing and fermentation on oxalate reduction in horse gram. The oxalate content in unprocessed flour was found to be 554.25 mg, which was reduced to 397.50 mg (roasting), 536.63 mg (soaking), 375.00 mg (cooking) and 339.38 mg (germination) per 100 g flour during processing. Fermentation of unprocessed and processed flours had an additional reduction in oxalate content up to 394.13 mg (unprocessed flour), 281.25 mg (roasting), 385.50 mg (soaking), 271.50 mg (cooking) and 207.38 mg (germination) per 100 g flour.

The overall impression with oxalate content alone is that, fermentation of germinated flour followed by cooked flour had 207.38 and 271.50 mg of oxalates /100 g respectively (Table 1). However, these two methods significantly reduced oxalates prior to fermentation itself (339.38 and 375.00 mg /100 g during processing), which got reduced further during fermentation. Since, main interest of the study was reduction of antinutritional factors exclusively due to fermentation, the actual reduction due to fermentation is obtained from difference between initial and after fermentation oxalate content. By considering this, the highest oxalate reduction was reported in fermentation of unprocessed flour (160.12 mg) followed by soaked flour (151.13 mg), germinated flour (132.00 mg), roasted flour (116.25 mg) and cooked flour (103.50 mg /100 g).

Fig. 1 clearly depicts the per cent reduction (%) in oxalate content during processing and fermentation. The per cent reduction values ranged between 0 – 38.77 % in processed flours, while fermentation of processed flours ranged between 28.89 – 62.58 %. The actual per cent reduction in oxalate content owing to fermentation is clearly shown in Fig. 2, where the highest per cent reduction in oxalate content was observed in fermentation of unprocessed flour (28.89 %) followed by soaked flour (27.27 %), germinated flour (23.81 %), roasted flour (20.98 %) and cooked flour (18.67 %).

Results from the study clearly indicated that each method has its own way of significance in reduction of antinutritional factors, where fermentation and germination were found to be the best possible ways out of all. The presence of antinutritional factors in horse gram not only hindered mineral absorption, protein digestibility, but also associated with hard-to-cook phenomenon resulted in restricting its usage (Moktan and Ojha, 2015; Amalraj and Pius, 2015). The horse gram is known to have several antinutritional factors such as tannins, phytates, oxalates, trypsin inhibitor and hemagglutinin activities, which can be eliminated by adopting various traditional household practices such as dehusking, soaking, cooking, roasting, germination and fermentation (Ahmed *et al*., 2006; Akande and Fabiyi, 2010). reported that soaking and cooking were effective for phytates and tannins reduction in kidney bean, lentil, chick pea and white gram.

Table 1: Effect of processing methods and fermentation on oxalate content of horse gram

Treatment details	Oxalate content (mg /100 g)		Reduction exclusively due to fermentation (mg /100 g)
	BF	AF	
Unprocessed flour	554.25	394.13	160.12[a]
Roasted flour	397.5	281.25	116.25[d]
Soaked flour	536.63	385.5	151.13[b]
Cooked flour	375	271.5	103.50[e]
Germinated flour	339.38	207.38	132.00[c]

Note: Means followed by different letters indicate that they differ significantly at 1 % level of significance as per DMRT analysis.
BF= Before fermentation; AF = After fermentation

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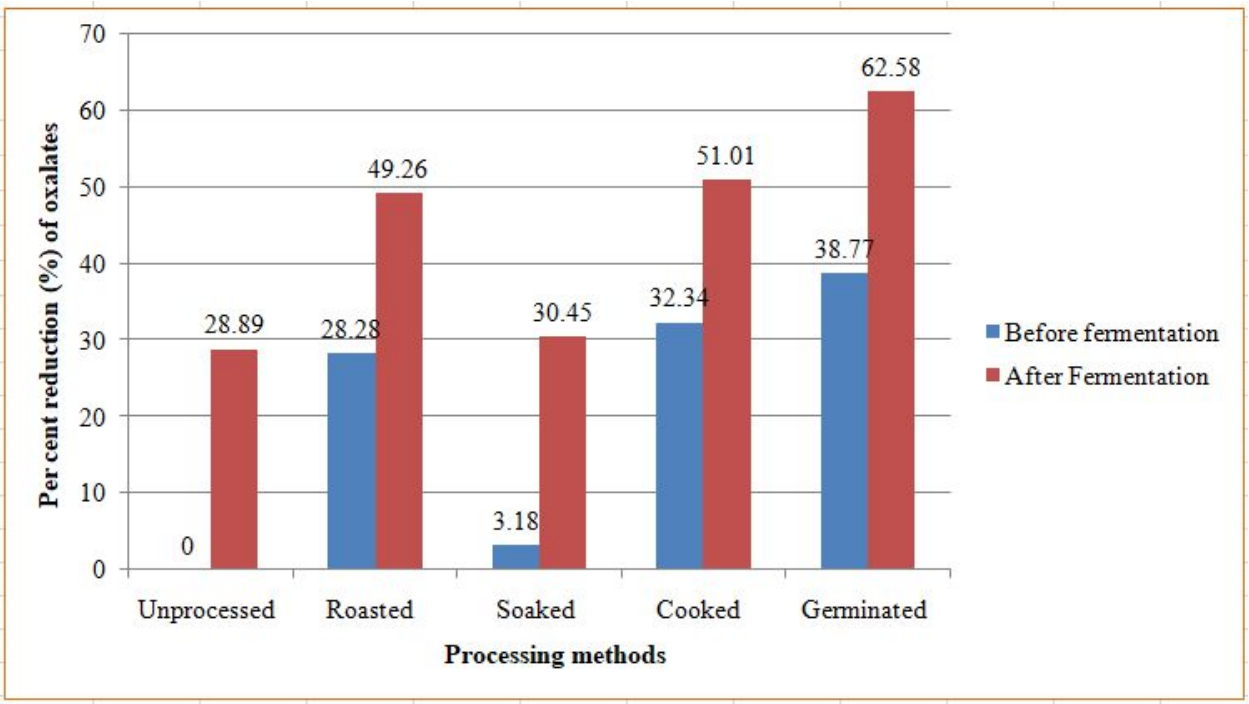

Figure 1: Effect of processing methods and fermentation on per cent reduction (%) of oxalate content of horse gram

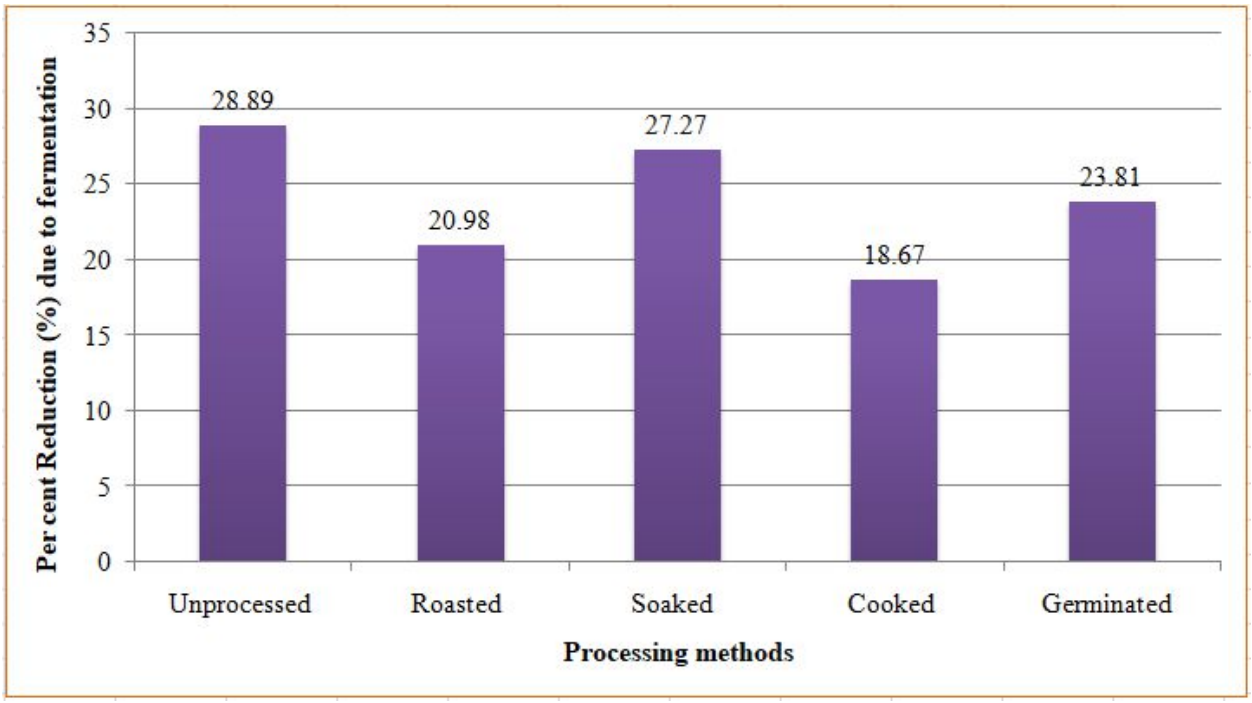

Figure 2: Per cent reduction (%) in oxalate content exclusively due to fermentation of processed horse gram samples

Biochemical and microbial analysis of processed and fermented flours of horse gram

The biochemical and microbial changes like pH, TA, batter volume and LA bacterial population are listed in Table 2. The batter volume was rose significantly during fermentation, where germinated flour fermentation (70 mL), recorded the highest volume rise followed by soaked (63 mL) and unprocessed flour (60 mL) fermentations respectively. While, fermentation of roasted and cooked flours reported with less volume rise.

A drop in pH was observed in both processed as well as fermentation of processed flour samples. A significant reduction in pH was observed in fermentation of processed flours, ranged between 5.00 - 4.48 compared to processing alone (6.79 - 6.02). Nevertheless, the actual pH reduction exclusively due to fermentation was noticed higher in unprocessed flour (1.93) followed by soaked (1.89), roasted (1.78), cooked (1.60) and germinated (1.53) flours.

The decline in pH correlated with increased titratable acidity (TA) of processed flours. The initial TA values of processed flour samples ranged between 0.08 - 0.28 %, whereas after fermentation it was increased to 0.63 – 0.88 %. The actual rise in TA exclusively due to fermentation was the highest in unprocessed flour (0.75 %) followed by soaked (0.65 %) and germinated flour (0.60 %).

The observations from microbial analysis confirmed that each processing method has significantly affected LA bacterial population (Table 2). During initial stage, soaked sample was reported with the highest population (6.40 x 10^4 log cfu /g) followed by unprocessed (6.36 x 10^4 log cfu /g) and germinated (6.24 x 10^4 log cfu /g) flours. However, after fermentation, the highest population was observed in unprocessed flour with 8.32 x 10^6 log cfu /g followed by soaked (8.25 x 10^6 log cfu /g) and germinated (8.11 x 10^6 log cfu /g) flours. Since, roasting and cooking methods involved with heat treatment, lower LA bacterial population was reported during both initial and after fermentation.

The decline in pH with concomitant rise in TA and batter volume was observed during the study, might be due to high metabolic activity of microorganisms and was reflected in increased LA bacterial population. Similar trend was observed by Jood *et al*. (2012), where two food mixtures were developed using raw and germinated sorghum flour and were autoclaved and fermented with *Lactobacillus acidophilus* curd for 12 h. The biochemical analysis revealed that the pH of ungerminated and germinated raw mixtures was 6.23 and 5.24, which finally reduced to 4.43 and 3.65 after autoclaving and fermentation. The initial TA of non germinated and germinated flours was 1.71 and 2.04

g /100 mL respectively, which increased to 2.22 and 3.14 g /mL after fermentation for 12 h. Nefale and Mashau (2018) attempted germinatation of finger millet flour naturally at 0, 24, 48 and 72 h and studied for physico-chemical properties. A significant reduction in pH (6.24 - 5.72) with increased TA (0.50 – 1.34 %) was found in germinated flour (72 h) than in soaked flour. The reason might be germination involved in production of organic acids through induction of hydrolytic enzymes with release of sugars and amino acids, thereby responsible for pH decline.

Table 2: Biochemical and LA bacterial population changes of processed and fermented horse gram flour samples

Treatment details	Batter volume (mL)		pH		Fermentation effect	TA (%)		Fermentation effect (%)	LA bacteria population (Log cfu /g)*	
	BF	AF	BF	AF		BF	AF		BF (10^4 cfu /g)	AF (10^6 cfu /g)
Unprocessed flour	50	60.00^{c}	6.8	4.9	1.93^{a}	0.1	0.8	0.75^{a}	231.33 -6.36	210.33 (8.32^{a})
Roasted flour	50	54.00^{d}	6.5	4.7	1.78^{c}	0.2	0.7	0.53^{d}	77 -5.89	50.67 (7.70^{e})
Soaked flour	50	63.00^{b}	6.4	4.5	1.89^{b}	0.1	0.8	0.65^{b}	249.33 -6.4	178.67 (8.25^{b})
Cooked flour	50	52.00^{e}	6.6	5	1.60^{d}	0.2	0.6	0.44^{e}	37 -5.57	84.67 (7.93^{d})
Germinated flour	50	70.00^{a}	6	4.5	1.53^{e}	0.3	0.9	0.60^{c}	174.33 -6.24	129.33 (8.11^{c})

Note: Means followed by different letters indicates that they differ significantly at 1 % level of significance by DMRT analysis.

BF = Before fermentation;
AF = After fermentation;
TA = Titratable Acidity;
LA bacteria = Lactic acid bacteria;
*(Figures in parenthesis are log transformed values).

From the present investigation, it is concluded that horse gram is having high levels of oxalates that result in kidney stone formation especially when accumulated in urine. However, many household practices were proven for lowering their levels of which fermentation was evidenced as the best approach not only for reducing the oxalate content, but also for improving the biochemical and microbial properties of horse gram.

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