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## Effects of domestic processing on trypsin inhibitor, phytic, acid, tannins and *in-vitro* Protein Digestibility of three sorghum varieties

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**Magdi A. Osman<sup>\*</sup> and Mustafa Gassem**

Food Science and Nutrition Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

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Effects of soaking, germination and fermentation on trypsin inhibitors, phytic acid, tannins content and *in-vitro* protein digestibility of three local sorghum varieties Hamra, Shahla and Baidha were investigated. Soaking process significantly decreased trypsin inhibitory activities (TIA), phytic acid and tannins contents, whereas *in vitro* protein digestibility was not significantly improved. Germination for 3 days of Hamra, Shahla and Baidha sorghum varieties significantly decreased TIA and phytic acid levels, but it significantly increased tannins content that coincided with decrease in protein digestibility. During 24 hrs fermentation TIA, phytic acid and tannins contents of the three varieties were significantly reduced. Fermentation also significantly improved the *in-vitro* digestibility of sorghum proteins. Among the three domestic processes, fermentation was the most effective in reducing antinutritional factors and improving protein digestibility.

**Keywords:** sorghum, domestic processes, trypsin inhibitor, phytic acid, tannins, *in-vitro* protein digestibility.

### Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the most important staple food for millions of peoples in the semi-and tropics of Asia and Africa. Sorghum is important source of proteins, carbohydrates, and minerals. The plant grows and produces well at limited water and temperature stress conditions. The sorghum is the fifth most important cereal of the world production after wheat, rice, maize and barley FAO (2005), and is known to contain antinutritional factors such as trypsin inhibitors, phytic acid and tannins. These compounds are known to interfere with protein and carbohydrate digestion and mineral bioavailability. Several investigators have reported that soaking and germination can be used

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<sup>\*</sup> Corresponding author: Magdi A. Osman; e-mail: [magdios@ksu.edu.sa](mailto:magdios@ksu.edu.sa)

effectively for improving nutritional quality of cereal grains by increasing protein content and availability of essential amino acids nutrients ( Traore *et al.*, 2007; Obizoba and Atii, 1994; Dalby and Tasi, 1970) Soaking and germination were also found to decrease enzyme inhibitors, phytic acid, and tannin and improve in vitro protein digestibility of cereals and legumes (Mbithi-Mwikya, et al 2000 and Alonso *et al.*, 2000 ). However, certain studies have shown some contradictory results. Tannins content was found to increase significantly in sorghum after germination (Ahmad *et al.*, 2010; Oloyo, 2004). Fermentation is known to mobilize nutrients and reduce the antinutritional content in cereal. Chavan *et al.* (1988) reported that fermentation of sorghum increased protein content, soluble protein and free amino acids. Natural fermentation of cereal grains has been also found to reduce trypsin inhibitors, phytic acid and tannin content and improve in-vitro protein digestibility (Abdel Rahman and Osman 2011; Abdelhaleem *et al.*, 2008).

In Saudi Arabia, the sorghum is grown in South -West region of Jazan. The sorghum is utilized to certain extent to prepare bread and stiff porridge from germinated and fermented flour in some part of the region (Gassem, 1998). No information is available regarding nutritional quality of domestically processed sorghum. Therefore, this study was undertaken to evaluate the effect of soaking, germination and fermentation on trypsin inhibitor activities, phytic acid level, tannins content and in-vitro protein digestibility of three local sorghum varieties.

## **Materials and methods**

The three sorghum varieties, Hamra, Shahla and Baidha used for this study were obtained from a grain market in Abu-Arish (South-west Saudi Arabia). The grain was thoroughly cleaned from dust and other extraneous material prior to use. Sorghum soaking and germination was carried out according to Ahamad *et al.* (1996). Fermented dough was prepared in the traditional way according to Gassem (1998).

### ***Trypsin inhibitor activity (TIA)***

Trypsin inhibitor activity was assayed according to the Kakade *et al.* (1969) using BAPA N-benzoyl-DL-arginine-P-nitroanilide hydrochloride and trypsin type III from bovine pancreas.

### ***Phytic acid***

Phytic acid analysis was performed according to the method of Latta and Eskin (1980), using chromophore reagent. Phytic acid (Dodecasodium salt) from corn was supplied by Sigma chemical company and used as a standard.

### ***Tannin***

The tannins content of the samples was determined according to modified vanillin – HCl method of Price *et al.* (1978).

### ***In-vitro protein digestibility***

In-vitro digestibility was determined following Hsu *et al.* (1977) as modified by Satterlee *et al.* (1979). The in-vitro digestibility was calculated according Satterlee *et al.* (1979) equation.

$$\% \text{ In - vitro digestibility} = 234.84 - 22.56 X$$
where, X = the pH of suspension after 20 min hydrolysis protein.

### ***Statistical analysis***

The data was analyzed using one way ANOVA with mean separated by least significance differences (LSD) at  $P \leq 0.05$  (Steel and Torrie, 1960).

## **Results and discussions**

### ***Trypsin Inhibitory Activity***

The effect of traditional processes on trypsin inhibitory activity (TIA) of Hamra, Shahla and Baidha sorghum varieties is presented in Table 1. Trypsin inhibitors levels varied among the three sorghum varieties. Hamra, showed the highest (31.06) followed by Baidha (29.4) and Shahla (23.8). These results are in agreement with those reported earlier (Gassem and Osman 2004). Soaking sorghum grains in water overnight at room temperature significantly reduced TIA level in the sorghum varieties compared to the raw grains. The loss of TIA was greater in Shahla (33.6%) than in Baidha (22.1%) and Hamra (12%). The results observed, are in agreement with those reported for soybean, mung bean and kidney bean (Mohamed et al 2011), pea, chickpea, faba bean and kidney bean (Abd El-Hady and Habiba 2003) and Lablab bean (Osman 2007). Germination for three days also significantly reduced TIA level in the three

sorghum varieties. It decreased from 31.6 to 19.9, from 23.8 to 13.3 and from 29.4 to 17.6 for Hamra, Shalha and Baidha respectively. Similar trend was observed in sorghum, finer millet, lablab bean and great northern. (Al-Jasser, 2005; Osman, 2007; Sathe *et al.*, 1983). Fermentation treatment was the most effective in decreasing TIA compared to soaking and germination. The fermentation process reduced the TIA in Hamra, Shalha and Biadha by 44.9%, 52.9% and 42.9% respectively. A decrease in TIA during fermentation has been reported in sorghum (Osman, 2004), yellow maize (Ejigui *et al.*, 2005), pearl millet (Yassmin and Pattabirman, 1988), corn and corn soy bean blend (Chompreeda and field, 1981) and soy bean (Ari *et al.*, 2012). The decrease in TIA may be attributed to the utilization of trypsin inhibitor as energy source or degradation by hydrolytic enzymes during the processes.

**Table 1.** Effect of processing methods on trypsin inhibitory activity of sorghum

Treatments	Hamra		Shahla		Baidada	
		Reduction%↓		Reduction %↓		Reduction %↓
Raw	31.6±0.29 <sup>a</sup>	00.00	23.8±1.00 <sup>a</sup>	00.00	29.4±0.39 <sup>a</sup>	00.00
Soaking	27.8±0.10 <sup>b</sup>	12.02	15.8±0.30 <sup>b</sup>	33.61	22.9±0.12 <sup>b</sup>	22.11
Germination	19.9±0.23 <sup>c</sup>	37.03	13.3±0.20 <sup>c</sup>	44.12	17.6±0.30 <sup>c</sup>	40.14
Fermentation	17.4±0.21 <sup>d</sup>	44.94	11.2±0.40 <sup>d</sup>	52.94	16.8±0.40 <sup>c</sup>	42.86

All values are means of three replicates ±SD values with same letters abcd within column are not significantly different.

### ***Phytic acid content***

Table 2 shows the effect of soaking, germination and fermentation on phytic acid contents of sorghum cultivars. The three sorghum varieties showed significant difference in phytic acid contents. The phytic acid content was 311.6, 290.7 and 327.7mg/100gm for Hamra, Shalha and Baidha respectively. For all varieties, over-night soaking significantly reduced phytic acid content. Generally, it dropped to 291.8, 258.8 and 299.8 for the three varieties respectively. Soaking of sorghum, millet, soybean, brown rice, at room temperature for 24 hrs was also found to reduce phytic content by 4- 51% (Mahgoub and Elhag, 1998; Lestienne *et al.*, 2005; Liang *et al.*, 2008). Germination of the soaked sorghum for 3 days resulted in further reduction in phytic acid content. Shalha showed highest reduction (35.6%) followed by Baidha (28.3%) and Hamra (18%). The decrease in phytic acid during soaking and germination could be attributed to leaching out of phytic acid during hydration. Similar to soaking and germination, fermentation process also reduced phytic acid content significantly. During 24 hrs fermentation phytic acid content decreased from 311.6 to 183.8, from 290.7 to 118.7 and from 327.7 to 182.5 for Hamra, Shalha and Biadha respectively. A decrease in phytic

acid contents was observed in pearl millet (El-Tayeb *et al.*, 2007), sorghum (Kayode *et al.*, 2007), rice (Liange *et al.*, 2008), and maize (Abedel Hady *et al.*, 2005) after fermentation. Phytic acids are commonly found in seeds and grains cereals, legumes and other plants where they constitute the main storage form of total phosphorus. Because of the high negative charges, phytic acids can form complex with protein and minerals and lead to a decrease in protein solubility and minerals availability. The reduction in phytic acid during fermentation was attributed to the action of microbial enzyme phatase.

**Table 2.** Effect of processing methods on phytic acid content (mg/100gm) of sorghum

Treatments	Hamra		Shahla		Baidada	
		%reduction		%reduction		%reduction
Raw	311.6±3.7 <sup>a</sup>	00.00	290.7±0.5 <sup>a</sup>	00.00	327.7±3.5 <sup>a</sup>	00.00
Soaking	291.4±2.3 <sup>b</sup>	6.48	2588±2.6 <sup>b</sup>	10.97	299.8±2.5 <sup>b</sup>	8.51
Germination	255.5±2.7 <sup>c</sup>	27.63	187.3±4.5 <sup>c</sup>	35.57	235.0±6.5 <sup>c</sup>	28.29
Fermentation	183.8±4.9 <sup>d</sup>	41.01	118.7±6.1 <sup>d</sup>	59.17	182.5±4.6 <sup>c</sup>	44.40

All values are means of three replicates ±SD values with same letters abcd within column are not significantly different.

**Table 3.** Effect of processing methods on Tannins content of sorghum expressed as catchin equivalent (CE)

Treatments	Hamra		Shahla		Baidada	
		%↑↓		%↑↓		%↑↓
Raw	0.866±0.02 <sup>b</sup>	00.00	0.392±0.005 <sup>a</sup>	00.0	0.058±0.00 <sup>b</sup>	00.0
Soaking	0.823±0.01 <sup>c</sup>	5.00↓	0.327±0.05 <sup>c</sup>	16.6↓	0.044±0.01 <sup>c</sup>	24.1↓
Germination	1.174±0.05 <sup>a</sup>	35.60↑	0.364±0.07 <sup>b</sup>	7.2↓	0.084±0.04 <sup>a</sup>	44.0↑
Fermentation	0.502±0.03 <sup>d</sup>	42.03↓	0.251±0.01 <sup>d</sup>	36.0↓	0.032±0.05 <sup>d</sup>	44.8↓

All values are means of three replicates ±SD values with same letters abcd within column are not significantly different.

### **Tannins content**

Table 4 shows the effects of domestic processing on tannins content of Hamra, Shehla and Baidha. The tannins content is expressed as catchin equivalent (CE). Tannins content varied significantly among the three the sorghum varieties. It varied from 0.866 CE in Hamra to 0.058 CE in Shahla, Soaking process resulted in significant reduction in tannins content in the three sorghum varieties. The tannins content of Hamra, Shahla and Baidha were reduced by 5.0 %, 16.6 % and 24.0 %, respectively. In contrast, germination of soaked Hamra and Baidha grains for three days resulted in significant increase in tannins content, whereas Shahla showed significant decrease in tannin content. Several investigators have reported the change in tannins content in

cereal grains during germination. Some studies have reported an increase, (Ahmad *et al.*, 1996; Eltayeb *et al.*, 2007), while other has shown a decrease in tannins content (Elmaki *et al.*, 1999, Sharma and Kapoor, 1996). The increase in tannins content could be attributed to hydrolysis of condensed tannins such proanthocyanidin. While the decrease may be to their binding with cotyledon endosperm that are usually undetected by routine method due to their insolubility in solvent or may be due to microbial phenyl oxidase action. Fermentation of Hamra, Shahla and Biadha for 24 hrs significantly decreased its tannins content. It reduced the tannins content by 20.4 to 38 % for the three varieties. A decrease in tannins content during natural fermentation was observed for sorghum (Hassan and El-Tinay, 1995), pearl millet (Osman, 2010) and maize (Abdel- Hady *et al.*, 2005). Tannins are known to have stringent taste which affects palatability and decreases feed intake. Tannins also form complex with protein and reduce their digestibility. Therefore, reduction of content of sorghum varieties through fermentation would improve its nutritional value.

**Table 4.** The effect of domestic processes on protein digestibility

	<b>Hamra</b>	<b>Shahla</b>	<b>Baidada</b>
Raw	77.1±0.3 <sup>b</sup>	72.4±0.3 <sup>b</sup>	74.6±0.6 <sup>b</sup>
Soaking	77.0±0.1 <sup>b</sup>	72.3±0.1 <sup>b</sup>	74.2±0.4 <sup>b</sup>
Germination	75.3±0.4 <sup>c</sup>	71.2±0.3 <sup>c</sup>	72.3±0.4 <sup>c</sup>
Fermentation	81.5±0.6 <sup>1</sup>	77.6±0.4 <sup>a</sup>	78.0±0.3 <sup>a</sup>

All values are means of three replicates ±SD values with same letters abcd within column are not significantly different.

#### ***In-vitro protein digestibility (IVPD)***

The results of IVPD of Hamra, Shahla and Baidha varieties as a function of soaking, germination and fermentation are shown in Table 4. Protein digestibility was not significantly ( $p < 0.05$ ) changed when sorghum were soaked over-night. However, germination of soaked sorghum caused significant decrease ( $p < 0.05$ ) in protein digestibility from 77.3 to 75.3, from 70.2 to 71.2, and from 74.6 to 72.3 for Hamra, Shahla and Biadha respectively. This agrees with the results of Osman (2010) in pearl millet and Elmaki *et al.* (1999) in sorghum who reported the decrease in protein digestibility during germination. The decrease in protein digestibility may be due to increase in tannins content during germination processes. Tannins have been found to inhibit digestive enzymes such as trypsin, chymotrypsin, amylase and *B* galactosidase and lower protein and starch digestibility as well. In contrast to germination, fermentation significantly improved protein digestibility in the three varieties. The

magnitude of IVPD increase, upon fermentation ranged from 4.5% to 7.5%. Several workers have observed significant increase of IVPD by natural fermentation. Youssif and El-Tinay (2000) showed that natural fermentation of sorghum increased IVPD from 51.8% to 75.6%. Similarly, Osman, 2004, observed an improved in protein digestibility during natural fermentation of sorghum for Khamir bread preparation. An increase in protein digestibility has also been reported in finger millet and pearl millet when fermented for 24 hrs (Mbithi-Mwikya *et al.*, 2000; Elyas *et al.*, 2002). The improvement of protein digestibility after fermentation can be attributed to the reduction in antinutritional factors such as trypsin inhibitor and tannins.

## Conclusion

In conclusion this study has demonstrated that, soaking and fermentation processes resulted in a significant reduction in trypsin inhibitory activities, phytic acid and tannins of the local sorghum varieties. Germination process also significantly reduced trypsin inhibitor activity and phytic acid levels, but increased tannins content in two varieties that affected protein digestibility. Fermentation was also found to improve in-vitro protein digestibility. These results clearly indicate that fermentation may be useful in improving the nutritional quality of sorghum with respect to protein and carbohydrate utilization as well as mineral bioavailability.

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