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Effect of sorghum type (*Sorghum bicolor*) and traditional fermentation on tannins and phytic acid contents and trypsin inhibitor activity

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Abstract

The aim of the present work was to study the effect of traditional fermentation process on the antinutritional factors: tannins, phytic acid and trypsin inhibitor in three sorghum cultivars, Fetarita, Safra and Ahmer, widely used in the Sudan. Investigation showed that tannins, phytic acid and trypsin inhibitor activity levels varied significantly among the three sorghum cultivars. Tannin content of unfermented seeds was 0.32, 0.65 and 1.5 catechin equivalent for Fetarita, Safra and Ahmer, respectively. Safra showed the highest level of phytic acid among the three cultivars, while Fatarita showed the highest trypsin inhibitory activity level. Differences in fermentation characteristics were observed among the three sorghum types. Tannin contents were significantly reduced by 56.3%, 56.9% and 52.7% in Fetarita, Safra and Ahmer, respectively. Phytic acid contents of the three cultivars were markedly reduced by over 50% as results of fermentation. The decrease in the trypsin inhibitory activity levels during fermentation was more obvious in Ahmer (87.4%) than in Safra (77.7%) and Fetarita (76.5%), suggesting that the enzyme inhibitors activity were not correlated with tannin contents.

Key words: Sorghum, fermentation, tannins, phytic acid, trypsin inhibitor, kisra bread.

Introduction

Grain sorghum (*Sorghum bicolor* L. Moench) is an important basic food in many parts of Africa and Asia, it is widely grown in the semiarid regions because of its drought tolerance. Sorghum is the fifth most important cereal in world production, being exceeded by wheat, rice, maize and barley in that order [1]. Sorghum is found to contain many antinutritional factors such as, tannins, phytic acid, proteinase inhibitors and cyanogenic glycosides. Tannins are members of the naturally occurring active nutrients known as polyphenols. Tannins are reported to interact with proteins (both enzymes and non-enzyme proteins) to form tannin–protein complexes resulting in inhibition of digestive enzymes [2]. In feeding studies with growing chicks [3] diets containing high tannins sorghum grain reduced weight gain and feed conversion. Phytic acid is found in plant seeds and many roots and tubers. Phytic acid is found to form complex with minerals in physiological pH, leading to lower mineral bioavailability [4]. In addition phytic acid has been shown to inhibit trypsin [5] and amylase [6]. Trypsin inhibitors are widely spread in plant kingdom, especially in legumes and cereals. They inhibit proteolytic enzymes in digestive system [7] and cause pancreatic hypertrophy and poor growth performance [8]. Several processing methods such as heat-treatment, soaking, germination, fermentation and radiation were found to reduce these antinutritional factor levels and enhance protein and carbohydrates digestibility [9-21].

Grain sorghum is leader cereal crop in Sudan and it is staple food in the diet of most people in the Sudan. Sorghum flour is fermented to make local bread known as kisra. Kisra is a thin pancake – like bread prepared by fermenting a thick paste of flour for 12-24 h and thinning to desire consistency with water before baking. The fermented dough is baked into thin sheets on hot pan. In order to improve the nutritional quality of sorghum products, it is necessary to identify ways of removing these undesirable compounds. Therefore, the aim of this investigation was to study the changes on tannins, phytic acid content and trypsin inhibitory activity during traditional fermentation of sorghum cultivars.

Materials and Methods

Materials: Sudanese sorghum (*Sorghum bicolor*) cultivars, locally known as Fetarita, Safra and Ahmer, were purchased from local grain markets in Omdurman. All samples were carefully cleaned and freed from dirt, stones, chips and other extraneous grains or grits. Sorghum grains were milled at the local grain market to fine flour using a Diamant Mill, model 500-mm (Denmark). The flour was transferred to the laboratory and stored at 25°C until used.

Preparation of fermented dough: Fermented dough was prepared in the traditional way used by Sudanese housewives. In the laboratory, sorghum flour was mixed with sterile distilled water in a 1:2 (wt/vol) ratio. A small amount of the previously fermented dough was then added to the mixture of flour and water to act as a starter (about 5%). This mixture was incubated at 30°C for 24 h in a sterile covered flask (2 kg flour+4 litres water). Fermentation was performed in duplicate and sampled every 4 h during the fermentation period (24 h). For determination of the antinutritional factors, samples were dried in a vacuum oven at 50°C for 24 h [Heraeus LBS-Co]. The dried samples were milled to a fine powder using a coffee mill, passed through a 60 mm mesh and kept at 4°C.

Determination of anti-nutritional factors: Tannin content was determined using the vanillin – HCl method as described by Price

et al. [22]. The phytic acid content was determined by the ion-exchange method [23]. Trypsin inhibitor activity was determined according to Kakade *et al.* [24] using N-benzoyl-arginine-p-nitroanilide hydrochloride (Sigma Chemical Co., St. Louis, MO).

Statistical analysis: Data, expressed as mean ±SD, were statistically analyzed using one-way ANOVA [25]. Duncan's multiple tests were used to compare means, and significance was accepted at $P \leq 0.05$.

Results and Discussion

Anti-nutritional factors: Tannins, phytic acid and trypsin inhibitor levels of Fetarita, Safra and Ahmer cultivars are shown in Table 1. In tannin contents of the sorghum cultivars, expressed as catechin equivalent (CE), there were significant differences ($P < 0.05$) among the three cultivars. Ahmer (1.5%) contained the highest amount of tannins, followed by Safra (0.65%), whereas Fetarita (0.32%) had the lowest value. These results were in agreement to those reported in earlier studies [9, 26]. The phytic acid content varied significantly among the three cultivars. It varied from 267.3 mg/100 g in Fetarita to 369.1 mg/100 g in Safra, with Safra variety showing the highest phytic acid content while the Fetarita the lowest one. The phytic acid content of the three cultivars falls within the reported range of phytic acid in sorghum[27]. The phytic acid content of Fetarita was comparable to that found by Mahgoub and El-Hag [28]. Similarly to tannins and phytic acid, the three cultivars showed a significant difference in trypsin inhibitor activity levels among them. The trypsin inhibitory activity levels (TIA) were 35.7, 16.6 and 23.0/100 mg for Fetarita, Safra and Ahmer variety, respectively. The results agree well with those reported for sorghum [29, 30]; barley and fall and spring rye [1 31], but lower than those reported in soybean [32].

Table 1. Antinutritional factor contents of sorghum cultivars.

Cultivar	Tannin (catechin equivalent)	Phytic acid (mg/100g sample)	Trypsin inhibitor (TUI/mg sample)
Fetarita	0.32 ± 0.1^{c}	276.3 ± 1.4^{c}	35.7 ± 0.1^{a}
Safra	0.65 ± 0.2^{b}	369.1 ± 2.0^{a}	16.6 ± 0.2^{c}
Ahmer	1.50 ± 0.4^{a}	339.0 ± 1.0^{b}	23.0 ± 0.3^{b}

* All values are mean of four replicates. * Results are expressed on dry weight basis.
* Means not sharing a common superscript letter in a column are significantly different at P<0.05, as assessed by Duncan's multiple–range test.

Tannin content: During 24 h fermentation, tannin content was significantly reduced (Fig. 1). Generally, it decreased from 0.65% to 0.28, from 0.32% to 0.14% and from 1.5% to 0.71 for Safra, Fetarita and Ahmer variety, respectively. The percent of decreases in the tannin content after 24 h fermentation were 56.3%, 56.9% and 52.7% for Fetarita, Safra and Ahmer, respectively. These results agree with that of Abdel Haleem *et al.* [33] who reported that natural fermentation of high tannin cultivar (Karamaka) and low tannin cultivar (Mugud) decreased their tannin content by 68.0% and 74.5%, respectively. Traditional African processing of sorghum-based food such as fermented and unfermented porridge, were also found to decrease tannin contents in high tannin sorghum [34]. Decrease, in proanthocynidins were also reported during natural fermentation of sorghum for preparation of opaque beer and mahewu, a non-alcoholic beverage [35]. The reduction in tannins can be attributed to the microbial degradation compounds during fermentation or due to less extractable tannin-protein complex [36]. This reduction in tannins may improve the nutritional quality of sorghum and effective utilization to their full potential as human food. Contradictory reports on tannins in fermented cereals are also available. An increase in total phenolic content was observed during brewing process of opaque sorghum beer[37], whereas a decrease and then increase in polyphenol content were reported for pearl millet when fermented for 14 h [12]. The increase in tannins was attributed to the hydrolysis of condensed tannins.

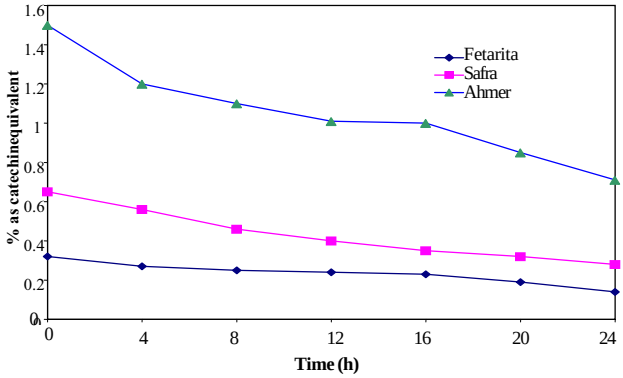

Figure 1. Effect of fermentation on tannin content (%catechin equivalent) of sorghum cultivars.

Phytic acid content: The effect of fermentation on phytic acid content of Fetarita, Safra and Amher is shown in Fig. 2. A progressive and significant decrease in phytic acid contents of Fetarita, Safra and Ahmer was observed due to fermentation. The phytic acid contents were reduced by 54.6 for Faterita, 50.6 for Safra and 51.8 mg/100 g for Ahmer, respectively. A similar trend was observed during preparation of ben–saalga, fermented millet –based gruel [38]. Natural fermentation of sorghum to khamir bread was also found to decrease in phytic acid [39]. The fermentation treatment has been also found to decrease phytic acid content in sorghum gruels [40], pearl millet [41], soybean temph [42,] and opaque sorghum beer [37]. Enzymatic hydrolysis of phytic acid by endogenous phytase of sorghum and/or by phytase which was produced by the microorganism, may account for most of the reduction of PA during fermentation. The low pH of fermented product and temperature of fermentation may also provide favorable conditions for phytase activity.

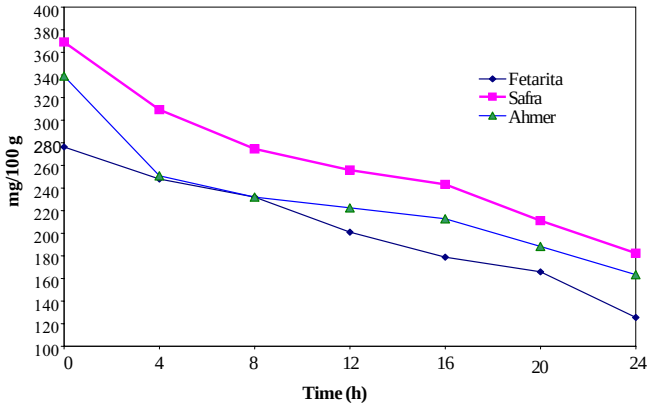

Figure 2. Effect of fermentation on phytic acid content (mg/100g) of sorghum cultivars.

Trypsin inhibitor activity: The loss of trypsin inhibitory activity (TIA) during fermentation of Faterita, Safra and Amher is shown in Fig 3. Compared to zero time, a significant ($P<0.005$) reduction in TIA level in the three sorghum cultivars was observed after 24 h fermentation, TIA decreased to 8.4, 3.7 and 2.9/100 mg sample for Faterita, Safra and Amher, respectively. Ahmer showed the greatest reduction (87.4%) followed by Faterita (77.7%) and Safra (76.5%). Osman [39] studied changes in the TIA in three sorghum cultivars, during preparation of khamir bread. He found that fermentation for 24 h significantly reduced trypsin inhitor activity level. Similar results were also obtained for soybean, cowpea and ground bean during 48 h of fermentation for tempe processing [42]. Chompreeda *et al.* [43] observed also 32% reduction in TIA of corn meal fermented at 32ºC for four days. Yasmin and Pattabiraman [44] not only found significant reduction in TIA, but also observed appreciable reduction in chymotrypsin activity during preparation of gruels. The reduction in TIA can be attributed to microbial degradation of trypsin inhibitor during lactic acid fermentation. The decrease of TIA will be useful in improving nutritional quality of sorghum with respect to protein utilization.

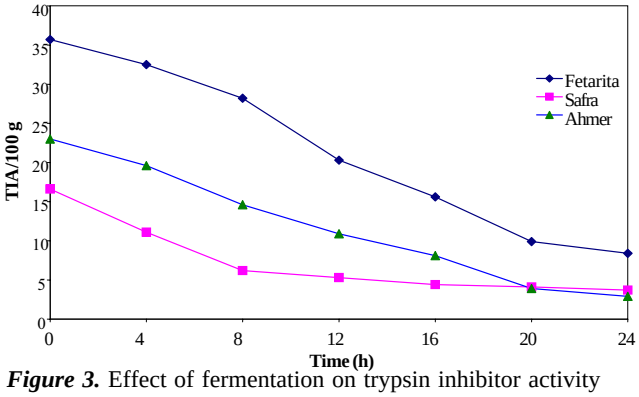

Figure 3. Effect of fermentation on trypsin inhibitor activity (TIA/100 g) of sorghum cultivars.

Conclusions

Traditional Sudanese processing of kisra bread markedly reduced tannins, phytic acid and trypsin inhibitory activity. The reductions in these antinutritional factors may be useful in improving the nutritional quality of the kisra bread with respect to protein and starch digestibility and mineral bioavailability.

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