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Food Chemistry 88 (2004) 129–134
Food Chemistry
www.elsevier.com/locate/foodchem

Changes in sorghum enzyme inhibitors, phytic acid, tannins and in vitro protein digestibility occurring during Khamir (local bread) fermentation

Magdi A. Osman

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

Received 5 September 2003; received in revised form 20 December 2003; accepted 20 December 2003

Abstract

Effects of traditional fermentation on enzyme inhibitors, phytic acid, tannin content and in vitro digestibility of three local sorghum varieties were investigated. During a 24 h fermentation, enzyme inhibitory activities were significantly decreased. Trypsin inhibitory activity was reduced by 58%, 43% and 31% in Hamra, Shahla and Baidha, respectively, whereas amylase inhibitory activity was reduced by 74, 75 and in the three varieties after a 24 h fermentation. Phytic acid contents of the three varieties were markedly reduced as a result of fermentation. Tannin content of Hamra, Shahla and Baidha were significantly reduced by, respectively, 31%, 15% and 35% after fermentation. Fermentation significantly improved the in vitro digestibility of sorghum proteins.

Keywords: Trypsin inhibitor; Amylase inhibitor; Phytic acid; Tannins; In vitro protein digestibility

1. Introduction

Sorghum (*Sorghum bicolor L. Moench*) is the most important stable food for millions of peoples in the semi-tropical and tropical of Asia and Africa. Sorghum grows well with limited water and temperature stress. It offers great potential for supplementing the world food resources (House, 1980). The sorghum is the fifth most important cereal in the world production after wheat, rice, maize and barley (FAO, 1977). Grain sorghum besides being a stable food, is also used as a feed for animals and as an industrial raw material. In Saudi Arabia, sorghum is grown mainly in the south-western region (Gizan region) covering about 85% of the cultivated land (Chaudhry, 1989)

Sorghum, like legume and oil seed meal, has some limitations, due to the presence of antinutritional factors, such as trypsin and amylase inhibitors, phytic acid and tannins. These compounds are known to interfere with protein and carbohydrate digestion and mineral bioavialability. Reduction or elimination of these undesirable components is essential for improving the nutritional quality of sorghum and effectively utilizing their full potential as human food. Several methods have been employed to improve the nutritional quality of sorghum. Numerous investigators have proposed germination and fermentation as a way to improve sorghum nutritional value. Fermentation is known to mobilize nutrients and reduce the antinutritonal content in cereal. Chavan, Chavan, and Kadam (1988) reported that fermentation of sorghum increased protein content, soluble protein and free amino acids. Similarly, Kazanas and Fields (1981) found an increase in essential amino acids and nutritive value of sorghum during natural fermentation. Trypsin inhibitor activity, phytate phosphorus and flatus sugars were significantly decreased when corn and corn-soy blends fermented for 4 days (Chompreeda & Fields, 1981). Reddy and Salunkhe (1980) reported almost complete elimination of phytate phosphorus within 8 h fermentation in rice. Natural fermentation of sorghum has also been found to reduce

E-mail address: magdios@ksu.edu.sa (M.A. Osman).

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doi:10.1016/j.foodchem.2003.12.038

tannin content and improve in vitro protein digestibility (Chavan et al., 1988; El-Khalifa & El-Tinay, 1994; Hassan & El-Tinay, 1995; Romo-parada, Simard, & Larrea-Reynoso, 1985; Yousif & El-Tinay, 2001). In Saudi Arabia, grain sorghum is used in the Gizan region for making a fermented bread called Khamir, utilizing the natural microflora in sorghum flour (Gassem, 1998). No nutrient studies have been carried out on nutritional quality of fermented sorghum. The purpose of this study is, therefore, to examine the effect of fermentation on trypsin and amylase inhibitor activities, phytic acid level, tannin contents and in vitro protein digestibility in three local sorghum varieties.

2. Materials and methods

2.1. Materials

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. The sorghum was milled at the local grain market to fine flour using, a Diamant mill, model 500 mm (Denmark). The flour was then transferred to the laboratory in Riyadh and stored at 25 °C until used.

2.2. Fermentation

Fermented dough was prepared in the traditional way. Natural fermentation was carried out by mixing sorghum flour with sterilized dionized water and other ingredients (onion, garlic, lemon juice and fenugreek) in a 1:0.8 (w/w) ratio (traditionally known as ajeen). The dough was incubated at 37 °C for 24 h in sterilized covered beaker. Two consecutive fermentations were carried out using 3% inocula (traditionally called shetiah) from a previous fermentation to start each subsequent batch. Each fermentation was performed in duplicate and sampled every 6 h during the fermentation period (24 h). Samples were dried in a vacuum oven at 50 °C (Heraucus LBS Co.) the dried samples were milled to fine powder using a coffee miller, then passed through a 60mm mesh and kept at 4 °C in polyethylene bags for analysis.

2.3. Trypsin inhibitor activity (TIA)

Trypsin inhibitor activity was assayed according to the Kakade, Simon, and Lierer (1969) using BAPA (*N*-benzoyl-DL-arginine-*P*-nitroanilide hydrochloride) and trypsin, type III, from bovine pancreas. TIA, expressed as trypsin inhibitor units per milligramme of sample (TIU/mg sample) was calculated from absorbance read against blank in a spectrophotometer. One trypsin unit is defined as an increase of 0.01 for an absorbance unit at 410 nm per 10 ml of reaction mixture.

2.4. Amylase inhibitor activity

Amylase inhibitor activity was determined according to Dephande, Sathe, Salunkhe, and Cornforth (1982). Triplicate samples, weighing 1g, were extracted in 10 ml distilled water for 12 h at 4 °C in a shaker. The extract was centrifuged at 5000*g* for 10 min. The supernatant was incubated with α-amylase for 15 min at 37 °C, then starch was added to the mixture and this was incubated for a further 3 min at 37 °C. One unit of enzyme activity was defined as that which liberated, from soluble starch, 1 mg of mallose/min at 37 °C and pH 7.0 under specific conditions.

2.5. Phytic acid

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

2.6. Tannin

The modified vanillin – HCl method of Price, Socoyoc, and Butler (1987) was followed with minor modification. 1 g of sample was extracted with 10 ml 1% HCl in methanol for 24 h at room temperature, then centrifuged at 5000 rpm. Vanillin HCl reagent was prepared by mixing, prior to use, equal volumes of 8% HCl in methanol with 2% vanillin in methanol. 1 ml of supernatant was mixed with 5 ml of vanillin HCl reagent. The absorbance was read at 500 nm after 20 min incubation at room temperature.

2.7. In vitro protein digestibility

In vitro digestibility was determined, following Hsu, Vauak, Satterlee, and Miller (1977), as modified by Satterlee, Marshall, and Temyson (1979). The drop of pH of casein (control) and the sample after 20 min hydrolysis by proteolytic enzymes was measured using an Orion pH meter. The enzymes used were trypsin type IX from porcine pancrease, chymotrypsin type II from bovine pancreas, peptidase type III from porcine intestine and protease type VI from streptomyces griseus. All enzymes were supplied by Sigma chemical company (St. Louis, Mo. USA). The in vitro digestibility was calculated according to the Satterlee et al. (1979) equation.

$$\%\ \text{In vitro digestibility} = 234.84 - 22.56X,$$

where X is the pH of suspension after 20 min hydrolysis of the protein.

2.8. Statistical analysis

The data were analyzed used one way ANOVA with means separated by least significance differences (LSD) at $P \leqslant 0.05$ (Steel & Torrie, 1960).

3. Results and discussion

3.1. Enzyme inhibitory activities

The effect of fermentation on trypsin inhibitory activity (TIA) of Hamra, Shahla and Baidha varieties is presented in Table 1. In general, there was a progressive decrease in TIA with increase of fermentation time of the three varieties. There was significant differences ($P \leqslant 0.05$) in TIA within and among the varieties. The loss of TIA was greater in Shahla (58%) than in Baidha (43%) and Hamra (42%). This finding agrees with that of Abdel-Rahman (2000), who reported a reduction in TIA level during fermentation of three sorghum cultivars by 76.5, 77.7 and 87.4 g. Similarly Chompreeda and Fields (1981) also observed a significant decrease in TIA in corn and corn soybean blend after fermentation. In the same way, Yassmin and Pattabiraman (1988) reported reduction in trypsin and chymotrypsin inhibitory activities in preparation of gruels. Other processes such as heat treatment and germination in sorghum grain (Mulimani & Vadiraij, 1993), irradiation of soybean defatted flour (Abu-Tarboush, 1998), heat treatment of beans (Kabbara, Abbas, Scheeren, Tinsly, & Berry, 1987; Kadam, Ghorpade, Adsule, & Salunkhe, 1986; Ologhobo & Fetuga, 1983; Osman, Reid, & Weber, 2002; Vidal-Valverde et al., 1994) and germination of beans. (El-Hag, Haard, & Morse, 1987; Sathe, Deshaprde, Reddy, Goll, & Slunkhe, 1983) were also found to reduce TIA level. Trypsin inhibitors have been reported to cause growth depression and pancreatic hypertophy in mice, rats and chicks (Gertler, Birk, & Bondi, 1967; Gumbmann, Dugan, Spangler, Baker, & Rackis, 1989; and Osman, Reid, & Weber, 2003). The reduction of trypsin inhibitor may be useful in improving nutrition quality of sorghum varieties with respect to protein digestibility.

The loss of amylase inhibitory activity during fermentation of the three varieties is shown in Table 2. During a 24 h fermentation, amylase inhibitory activity was significantly reduced. Generally, it decreased from 50.8 to 18.1, from 47.8 to 12.0 and from 43.5 to 12.7 g for Hamra, Shahla and Baidha varieties, respectively. Shahla showed the greatest reduction (75%), followed by Hamra (74%) and Baidha (71%). Sharma and Kapoor (1996) reported that natural fermentation of processed pearl millet for 48 h reduced amylase inhibitory activity to a non-detectable level. Heat treatment by 100 °C cooking of presoaked seed and roasting was also found to eliminate amylase inhibitory activity (Mulimani & Supriya, 1993; Singh, Khaerkar, & Jambunathan, 1982). Germination of great northern beans for 5 day also decreased amylase inhibitory activity (Sathe et al., 1983). In contrast, dehulling of dry bean was found to increase amylase inhibitory activity (Dephande et al., 1982).

3.2. Phytic acid content

Data on the effect of fermentation on phytic acid contents, for Hamra, Shahla and Baidha (Table 3)

Table 1
Effect of fermentation on trypsin inhibitor activity content (TIU/mg sample) of Sorghum

Varieties	Trypsin inhibitor activity after different fermentation periods (h)					% of reduction after a 24 h fermentation
	0	6	12	18	24	
Hamra	$29.8^{ax} \pm 0.7$	$26.3^{bx} \pm 0.4$	$22.3^{cy} \pm 1.3$	$19.7^{dy} \pm 1.2$	$18.7^{dx} \pm 1.2$	37
Shehla	$26.3^{ay} \pm 0.3$	$22.7^{by} \pm 0.7$	$14.7^{cz}0.2$	$13.1^{dz} \pm 0.08$	$10.9^{ey} \pm 0.4$	58
Baidha	$29.9^{ax} \pm 1.9$	$26.7^{bx} \pm 0.5$	$24.1^{cx}0.8$	$22.4^{cx} \pm 1.2$	$17.2^{dx} \pm 1.8$	43

All values are means of four replicates ± SD. Values with same letters (a, b, c, d within rows) are not significantly different at $P < 0.05$. Values with same letter (x, y, z within columns) are not significantly different at $P < 0.05$.

Table 2
Effect of fermentation on α-amylase inhibitor activity content (μmole/g sample) of Sorghum

Varieties	Amylase activity after different fermentation periods (h)					% of reduction after a 24 h fermentation
	0	6	12	18	24	
Hamra	$50.8^{ax} \pm 0.6$	$37.6^{by} \pm 0.3$	$28.5^{cx} \pm 1.2$	$16.5^{dy} \pm 0.7$	$13.1^{ex} \pm 0.3$	74
Shehla	$47.8^{ay} \pm 0.4$	$40.3^{bx} \pm 0.4$	$19.1^{cz} \pm 0.3$	$15.1^{dz} \pm 0.6$	$12.0^{ey} \pm 0.4$	75
Baidha	$43.5^{az} \pm 0.6$	$33.9^{bz} \pm 0.5$	$22.8^{cy} \pm 0.3$	$18.2^{dx} \pm 0.5$	$12.7^{exy} \pm 0.7$	71

All values are means of four replicates ± SD. Values with same letters (a, b, c, d within rows) are not significantly different at $P < 0.05$. Values with same letter (x, y, z within columns) are not significantly different at $P < 0.05$.

Table 3
Effect of fermentation on phytic acid content (mg per 100 g) of Sorghum

Varieties	Phytic acid content after different fermentation periods (h)					% of reduction after a 24 h fermentation
	0	6	12	18	24	
Hamra	$301.6^{ay} \pm 4.9$	$268.1^{by} \pm 6.5$	$244.0^{cy} \pm 2.0$	$210.2^{dy} \pm 5.5$	$175.3^{ex} \pm 8.4$	42
Shehla	$366.5^{ax} \pm 3.5$	$294.7^{bx} \pm 14.6$	$269.4^{cx} \pm 1.4$	$242.2^{dx} \pm 3.2$	$184.3^{ex} \pm 1.9$	58
Baidha	$308.5^{ay} \pm 5.4$	$239.8^{bz} \pm 7.3$	$230.9^{cz} \pm 4.6$	$187.3^{dz} \pm 6.1$	$158.6^{ey} \pm 7.7$	43

All values are means of four replicates ± SD. Values with same letters (a, b, c, d within rows) are not significantly different at $P < 0.05$. Values with same letter (x, y, z within columns) are not significantly different at $P < 0.05$.

indicated that, as the period of fermentation increased, a significant decrease in phytic acid content occurred. Among the three varieties, Baidha showed the greatest reduction (58%) compared to Hamra (42%) and Shahla (43%). These results were similar to those observed for sorghum (Mahgoub & El-Hag, 1998), corn (Lopez, Gordon, & Fields, 1983), rice, cassava, cocoyam, sorghum, maize, soybean, cowpea and yam (Marfo, Simpson, Idowu, & Oke, 1990), soybean (Sutardi & Buckle, 1985), pearl millet (Dhankher & Chauhan, 1987; Khetarpaul & Chauhan, 1989) and rice (Reddy & Salunkhe, 1980). The reduction in phytic acid during fermentation was attributed to the action of the microbial enzyme phyatase. This reduction in phytic acid may be useful in improving nutritional quality of sorghum with respect to mineral bioavailability.

3.3. Tannins content

Table 4 shows that the tannin contents decreased significantly ($P \leqslant 0.05$) during a 24 h fermentation in the three sorghum varieties. At 24 h fermentation, the tannin contents of Hamra, Shahla and Baidha were reduced by 31%, 15% and 35%, respectively. Similar observations on decreased tannins have been reported by other investigators. Abdel-Rahman (2000) reported reduction in tannin contents during fermentation of three sorghum varieties, by 56.3%; 56.9% and 52.7%. Hassan and El-Tinay (1995) also found that natural fermentation of high and low tannin content sorghum varieties, decreased their contents by 63% and 61.4%, respectively. In addition, El-Khalifa and El-Tinay (1994) and Romo-Parada et al. (1985) were able to achieve a decreased content of high tannin sorghum cultivar by 92% through fermentation. The lower reduction in tannin after a 24 h fermentation in the present study may be due to differences in cultivars and fermentation procedures. A similar trend was observed in pearl millet (Khetarpaul & Chauhan, 1989). Tannins are known to be responsible for decreased feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. Therefore, reduction of tannin content of the sorghum varieties through fermentation would improve the nutritional value.

3.4. In vitro protein digestibility (IVPD)

The results of IVPD of Hamra, Shahla and Baidha varieties as a function of fermentation time are presented in Table 5. IVPD progressively increased in the three varieties. Significant increases ($P \leqslant 0.05$) in IVPD occurred in Hamra (79.4%), Shahla (74.6%) and Baidha (78.1%) at 24 h when compared to 75%, 69.6% and 74.6% for 0 h for the three varieties. In both Hamra and Baidha varieties there was no significant increase in IVPD after 18 h of fermentation, whereas Shahla showed a significant increase. These results clearly demonstrate that fermentation improved protein digestibility. Several workers have observed significant increase of IVPD by natural fermentation. Yousif and El-Tinay (2001) showed that natural fermentation of sorghum increased IVPD from 51.8% to 75.6%. Similarly, Chavan et al. (1988) reported significant increase in IVPD of sorghum, green grain and sorghum plus green grain blend within the first 24 h of fermentation. The improvement of protein digestibility after fermentation can be attributed to the reduction of antinutritional factors such as trypsin inhibitor and tannins.

In conclusion, this study has demonstrated that the traditional fermentation of the local sorghum varieties

Table 4
Effect of fermentation on tannin content (% as catechin equivalent) of Sorghum

Varieties	Tannin content after different fermentation periods (h)					% of reduction after a 24 h fermentation
	0	6	12	18	24	
Hamra	$0.83^{ax} \pm 0.03$	$0.77^{bx} \pm 0.01$	$0.74^{bx} \pm 0.01$	$0.71^{ca} \pm 0.009$	$0.57^{dx} \pm 0.02$	31
Shehla	$0.38^{ay} \pm 0.005$	$0.36^{by} \pm 0.005$	$0.34^{cy} \pm 0.005$	$0.32^{dy} \pm 0.005$	$0.31^{ey} \pm 0.05$	15
Baidha	$0.068^{az} \pm 0.002$	$0.062^{bz} \pm 0.0005$	$0.058^{cz} \pm 0.001$	$0.057^{dz} \pm 0.001$	$0.045^{ez} \pm 0.004$	35

All values are means of four replicates ± SD. Values with same letters (a, b, c, d within rows) are not significantly different at $P < 0.05$. Values with same letter (x, y, z within columns) are not significantly different at $P < 0.05$.

Table 5
Effect of fermentation on in vitro protein digestibility of Sorghum

Varieties	In vitro digestibility after different fermentation periods (h)				
	0	6	12	18	24
Hamra	$75.1^{cx} \pm 0.3$g	$77.6^{bx} \pm 0.3$	$77.5^{bx} \pm 0.3$	$79.1^{ax} \pm 0.1$	$79.4^{ax} \pm 0.3$
Shehla	$69.6^{ay} \pm 0.6$	$70.2^{cz} \pm 0.1$	$70.5^{cy} \pm 0.1$	$72.9^{bz} \pm 0.4$	$74.6^{bz} \pm 0.2$
Baidha	$74.6^{ax} \pm 0.1$	$76.1^{by} \pm 0.6$	$77.4^{ax} \pm 0.4$	$78.1^{ay} \pm 0.4$	$78.1^{ay} \pm 0.3$

All values are means of four replicates ± SD. Values with same letters (a, b, c, d within rows) are not significantly different at $P < 0.05$. Values with same letter (x, y, z within columns) are not significantly different at $P < 0.05$.

resulted in a significant reduction trypsin and amylase inhibitory activities, phytic acid and tannin contents. Fermentation was also found to significantly improve in vitro protein digestibility. These results clearly indicate that fermentation may be useful for improving the nutritional quality of the sorghum with respect to protein and carbohydrate utilization as well as mineral bioavailability.

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