Original Research Article

ALKALINE PRETREATMENT AND FERMENTATION MODEFIES PROTEIN FRACTIONS AND TANNINS CONTEN OF KARAMAKA-A HIGH-TANNINS SORGHUM CULTIVAR

ABSTRACT

Aims: to study the effect of soaking in 0.20% NaOH for 8 h, followed by fermentation for 16 h on tannins content and protein fractions.
Study design: Factorial Experimental design.
Place and Duration of Study: Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Sudan and Food Research Center, Shambat, Sudan, between July 2007 and May 2008

Methodology: A high-tannin sorghum (*Sorghum bicolor* L . *Moench*) cultivar (Karamaka) was soaked in 0.20% NaOH for 8 h followed by fermentation for 16 h. The effect of soaking and fermentation was studied on tannins content and protein fractions, and untreated sorghum was used as a control.

Results: Results showed that soaking the sorghum grains in NaOH for 8 h caused a decrease in tannins content by 41.96%. Combining soaking and fermentation further lowered the tannins content of sorghum by 59.09%, with a maximum reduction of 81.44%. Soaking in NaOH and fermentation caused a significant ($P \leq 0.05$) increase in the albumins fraction by 40.97%, accompanied by a significant reduction in the glutelins fraction by 46.44%.
Conclusion: This modification of sorghum protein fractions along with the decrease in the tannins content would indicate improvement in the quality of sorghum proteins and therefore will improve the nutrition status of populations where sorghum is staple food. .

Keywords: Sorghum, tannins, soaking, sodium hydroxide, fermentation, protein fractions

1. INTRODUCTION

Grain sorghum is a staple food for the people in Africa and India. It is the most important cereal crop in Sudan, and various Sudanese foods are made from sorghum. The rural Sudanese traditionally divide these foods into two major groups. One group encompasses the foods and beverages involving the use of germinated grain and the other group is composed of the foods and beverages prepared from the non-germinated grain [1]. The non-malt sorghum foods are the major foods in Sudan, and include the staple dishes of Aceda and Kisra that are prepared from fermented sorghum flour. Aceda is a stiff porridge prepared by boiling water and then adding sorghum dough with stirring until a well-cooked stiff porridge is obtained. Kisra is a fermented sorghum flour baked on a hot plate to give thin bread sheets [2]. Apart from having substantial amounts of micro and macronutrients, e.g. protein, carbohydrates, fiber,some minerals and vitamins, some sorghum contains high amounts of phenolic acids, flavonoids, and condensed tannins [3]. Different studies have shown that these compounds have numerous health benefits to humans including the ability to decrease the risk of cardiovascular disease by improving endothelial function and inhibiting platelet aggregation [4-5], and having anticarcinogenic properties [6].

Sorghum use in food is limited (except in traditional foods) by its poor digestibility and lack of functionality, which are exacerbated during wet cooking. Protein modification studies have been undertaken in attempt to overcome these problems, and these can be classified into 3 broad categories: biochemical/chemical,

enzymatic, and thermo-mechanical [7]. Fermentation is known as a simple and inexpensive method for production, preservation, and improving nutritional quality of plant foods. Various studies have shown that fermentation can increase in the concentrations of vitamins, minerals and protein [8], increase soluble protein [9]. Moreover, the nutritive value of sorghum can be increased by fermentation, which leads to increase in lysine content and methionine and tryptophan [10]. Fermentation of foods also has been practiced for improving flavour, texture and palatability [11]. According to Kazanas and Fields [12], fermentation can help enrich the nutritive content of essential nutrients through microbial synthesis and improvement in protein and carbohydrates digestibility. This is probably due to both the enzymatic breakdown of the proteins by microorganisms in the fermentation medium and the effects of decreased pH during fermentation [13]. Elkhalifa et al. [2] and Yousif and El Tinay [14] reported that there was partial degradation of complex storage proteins into simpler and more soluble products after sorghum fermentation.

Other studies also showed that fermentation could help to remove ant-nutrients, natural toxicants and mycotoxins [11]. It can improve nutrient density and increase the amount and bioavailability of nutrients through degradation of anti-nutritional factors, pre-digestion of certain food components, synthesis of compounds that improve absorption and by influencing the uptake of nutrients in the intestine [15].

In order to improve the quality of sorghum grain, it is desirable to separate the proteins of sorghum and study the individual fractions in detail. Traditionally, proteins are classified into four types, albumins, globulins, prolamins and glutelins, according to their solubility [16]. Many studies on amino acid composition of sorghum protein fractions showed that the albumins and globulins fractions contain high amounts of lysine and tryptophan.

In our recent study [16] we studied the effect of soaking in alkali and cooking on the protein fractions of a high tannin sorghum cultivar, and we reported that the effect of pretreatment of sorghum grains in alkali followed by cooking appears to bring about modification in the protein fractions; favouring the conservation of the albumins and globulins fractions, which are rich in the amino acids lysine and tryptophan. Studies on the modifications induced by soaking of sorghum grain in alkali prior to natural fermentation on the protein fractions are very limited.

The aim of the present investigation is to determine the effect of soaking in alkali for 8 h, with and without fermentation for 16 h, on tannins content and protein fractions of a high-tannin sorghum cultivar.

2. MATERIALS AND METHODS

2.1 Samples: A Sudanese sorghum cultivar (Karamaka) high in tannins was obtained from Kadogly Research Station, Sudan, where this cultivar is mainly cultivated there. The sample was carefully cleaned from husk, damaged grains and foreign matter.

2.2 Soaking: The cleaned sorghum grains were immersed in 0.20% NaOH (1:3 w/v, sorghum:NaOH) for 8 h at room temperature (30 ± 2 ℃). The wet gr ains were then sun dried, and finely ground to pass through a 0.4 mm sieve, before being stored at 4 ºC in tightly-closed containers. Untreated sorghum sample was used as a control.

2.3 Fermentation: Fermented dough was prepared in the traditional way as described by El Tinay et al. [17]. Sorghum flour (1 kg) was mixed with 2 litres of water in a round earthenware container. Previously fermented dough (300 g) was then added to the mixture of flour and water to act as starter. After a thorough mixing, samples were taken at 4 h intervals until the end of fermentation, which was terminated after 16 h (pH 3.8-3.9) at ambient temperature (30 + 2℃). These samples were dried in an oven at 70℃ and finely ground.

2.4 Determination of tannins: Quantitative estimation of tannins, as catechin equivalent, was carried out using the modified vanillin-HCl method of Price *et al*. [18]. The reagent was prepared daily.

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2.5 Determination of protein fractions: The sequential extraction of flour proteins was carried out, according to the method described by Mendel and Osborne [19], with slight modification. Duplicate 2.5 g samples were taken in plastic bottles provided with screw caps. The sample was extracted twice with 50 ml of distilled water. Extraction was carried out for 30 min with continuous shaking on a shaker. The extract was separated from residue by centrifugation at 2000g for 30 min. The clear supernatant liquids were collected. The residues were then extracted successively in a similar manner with 1.0M NaCl solution, 70% ethanol and 0.2% NaOH solution and the extracts collected, in the same way as described above. The residues remaining after these successive extractions with the four solvents were the insoluble residues. The protein contents of the four extracts and the residues were determined by the micro-Kjeldahl method.

2.6 Statistical analysis: Triplicate samples were analysed for each determination and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) [20] and by Duncan's multiple range test (DMRT) with probability ($P \leq 0.05$) level of significance [21].

3. RESULTS AND DISCUSSION

Figure 1 shows the tannins content of untreated soaked and fermented Karamka sorghum cultivar. The unprocessed sorghum flour had a tannins content of 2.86%. Tannins content significantly ($P \leq 0.05$) decreased by 41.96% when the grains were soaked in NaOH for 8 h, and the reduction was more pronounced (59.09%) when the grains were fermented after soaking in sodium hydroxide (figure 1). Tannins readily dissolve in water and alcohol to form colloidal solutions [22]. The active group on tannins is the phenolic hydroxyl group. Phenols dissolve readily in dilute NaOH. Babiker [23] showed that when 0.05 M NaOH was used it removed 84% of tannins from sorghum after 24 h at 30 ℃. The marked reduction in tannins after 16 h of fermentation may be due to the effect of the microorganisms during fermentation. These results are in accordance with the results obtained by Hassan and El Tinay [11] and Idris et al. [24], who reported that fermentation, decreased the tannins content of sorghum cultivar by 66% after 14 h of fermentation. Combination of soaking in alkali and fermentation greatly enhances the removal of the antinutritional factor (tannins), which is believed to be responsible for unavailability of both proteins and divalent minerals.

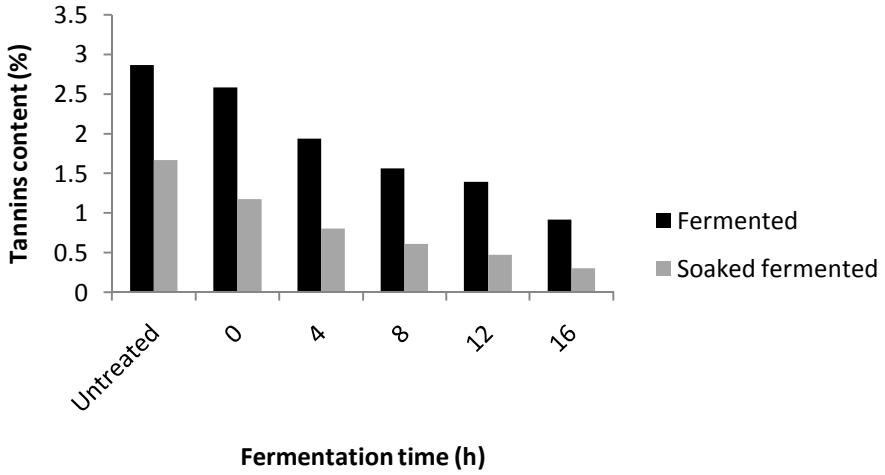

Fig.1. Tannins content (%), as catechin equivalent, of soaked (0.20% NaOH for 8 h) **and soaked fermented (16 h) sorghum grain**

Mean ± SD = Mean values ± Standard deviation of three replicates.

Tables 1 and 2 shows the protein fractions of fermented and soaked fermented sorghum grains. The total protein of the sorghum cultivar was fractionated, on the basis of solubility for each treatment, into

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albumins, globulins, prolamins and glutelins. The albumins fraction of untreated fermented sorghum increased by 2.69% after 16 h of fermentation, the prolamins fraction significantly ($P \leq 0.05$) increased from 55.35 to 58.88% at the end of the fermentation process. The glutelins decreased from 21.22 at zero time to 16.16% after 16 h of fermentation, but the globulins and the residue protein were fluctuated during the fermentation process (Table 1). Yousif and El Tinay [14] reported a sharp increase in prolamins fraction of sorghum after 36 h of fermentation. According to Ibrahim et al. [25] the albumins plus globulins fraction was shown to increase significantly ($P \leq 0.05$) during the first 8 h of fermentation, while the other fractions fluctuated during the fermentation process. Kazans and Fields [12] reported that nutritional value of sorghum could be increased by fermentation, which increases the content of lysine and methionine.

When sorghum grains soaked in NaOH for 8 h and then fermented, the albumins fraction significantly ($P \leq 0.05$) increased by 40.97% (Table 2). The increase was by 23.16% after 16 h fermentation. A significant ($P \leq 0.05$) increase was observed also in the prolamins fraction, reaching its maximum value (63.84%) after 16 h of fermentation (Table 2). The glutelins fraction significantly ($P \leq 0.05$) decreased by 46.44% in the soaked fermented sorghum grains. The soaking of sorghum in NaOH before fermentation had no effect on the globulins and the residue protein, as they are still fluctuated during the fermentation process (Table 2).

Considering the experimental conditions of soaking sorghum grains in dilute NaOH solution at room temperature (28–30 ℃), they are unlikely to cause appreciable hydrolysis of the glutelins fraction, yielding significant increases in albumins fraction (Table 2). It is plausible that the alkaline-soluble glutelins are leaching into the soaking solution, causing reconstitution of the sorghum proteins in favour of the nutritionally superior albumins fraction [16]. In the present work, soaking of sorghum in alkali followed by fermentation conserved the albumins fraction significantly, compared to the control (Table 2).

Table 1. Protein fractions (%) of Karamaka sorghum cultivar (control) fermented for 16 h

Fermentation time (h)	pH	Albumins	Globulins	Prolamins	Glutelins	Residue	Total Protein Recovered
Untreated	6.21 (±0.00)^{a}	11.13 (±0.06)^{f}	6.27 (±0.07)^{d}	55.35 (±0.03)^{e}	20.11 (±0.03)^{b}	6.33 (±0.4)^{b}	99.26 (±0.31)^{e}
0	5.85 (±0.00)^{b}	11.44 (±0.10)^{e}	6.46 (±0.08)^{c}	56.18 (±0.10)^{c}	21.22 (±0.08)^{a}	6.20 (±0.06)^{cd}	101.50 (±0.33)^{c}
4	4.94 (±0.02)^{c}	11.97 (±0.02)^{d}	6.73 (±0.09)^{b}	55.73 (±0.08)^{d}	20.09 (±0.04)^{b}	8.03 (±0.03)^{a}	102.55 (±0.21)^{b}
8	4.17 (±0.02)^{d}	12.49 (±0.15)^{c}	7.04 (0.03)^{a}	55.23 (±0.08)^{e}	19.82 (±0.08)^{d}	6.22 (±0.08)^{bc}	100.80 (±0.40)^{d}
12	3.69 (±0.01)^{e}	13.32 (±0.09)^{b}	6.52 (±0.09)^{c}	58.08 (±0.05)^{b}	19.97 (±0.03)^{c}	6.32 (±0.07)^{b}	104.21 (±0.32)^{a}
16	3.33 (±0.00)^{f}	14.12 (±0.07)^{a}	7.10 (±0.10)^{a}	58.88 (±0.10)^{a}	16.16 (±0.05)^{e}	6.10 (±0.10)^{d}	102.36 (±0.37)^{b}

Values are means (± SD)

Means in a column not sharing a common superscript letter are significantly ($P \leq 0.05$) Different as assessed by Duncan's multiple range test.

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Table 2. Protein fractions (%) of Karamaka sorghum cultivar soaked in 0.20% NaOH for 8 h and fermented for 16 h

Fermentation time (h)	Albumins	Globulins	Protamins	Glutelins	Residue	Total Protein Recovered
Untreated	11.13 (±0.07)^{f}	6.27 (±0.03)^{f}	55.35 (±0.03)^{f}	20.11 (±0.03)^{a}	6.33 (±0.04)^{d}	99.19 (±0.21)^{e}
0	12.11 (±0.11)^{e}	6.50 (±0.10)^{c}	58.21 (±0.09)^{e}	18.13 (±0.13)^{b}	6.01 (±0.01)^{f}	100.97 (±0.08)^{d}
4	12.88 (±0.03)^{d}	6.78 (±0.10)^{b}	59.30 (±0.11)^{d}	16.22 (±0.04)^{c}	6.21 (±0.09)^{e}	101.39 (±0.09)^{c}
8	14.03 (±0.03)^{c}	6.88 (±0.03)^{a}	61.77 (±0.13)^{c}	13.17 (±0.10)^{d}	7.01 (±0.01)^{c}	102.89 (±0.34)^{b}
12	15.76 (±0.03)^{b}	6.12 (±0.07)^{e}	63.13 (±0.06)^{b}	11.02 (±0.02)^{e}	7.44 (±0.14)^{e}	102.82 (±0.31)^{b}
16	15.76 (±0.07)^{a}	6.02 (±0.02)^{e}	63.84 (±0.07)^{a}	10.77 (±0.07)^{f}	7.12 (±0.07)^{b}	103.51 (±0.27)^{a}

Values are means (± SD)
Means in a column not sharing a common superscript letter are significantly ($P \leq 0.05$) Different as assessed by Duncan's multiple range test

4. CONCLUSION

The effect of pretreatment of sorghum grains in alkali followed by fermentation improved the protein fractions, favouring the conservation of the albumins fraction, which is rich in the amino acids lysine and tryptophan. This, together with a significant reduction in tannins, is likely to improve sorghum protein digestibility. This will indicate that soaking in NaOH followed by fermentation of sorghum for 16 h results in improvement in the nutritional value of sorghum.

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