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Effect of Processing Followed by Fermentation on Antinutritional Factors Content of Pearl Millet (*Pennisetum glaucum* L.) Cultivars

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Abstract: Two pearl millet cultivars (Gazira and Gadarif) were processed (grinding, soaking, autoclaving, germinating and debranning). The processed grains were fermented for 12 and 24hrs. Phytic acid, polyphenols and tannin contents were determined. Results obtained showed that phytic acid content was 987.19 and 952.51 mg/100g for Gazira and Gadarif cultivars, respectively. Processing treatments were observed to decrease phytate content significantly (P = 0.05) for both cultivars with a maximum reduction observed when the grains of the cultivars were germinated. Polyphenols and tannin were also decreased significantly after processing of both cultivars. Further reduction in antinutritional factors was obtained when the processed grains were fermented for 12 and 24 hrs. The rate of reduction differs between the cultivars and the processing treatments.

Key words: Phytic acid, polyphenols, tannin, pearl millet

Introduction

The term millet is used for any of several small seeded annual grasses that are important mainly in Asia and Africa. Five millet are common: *Setaria italica, Pennisetum glacum, Pennisetum typhoidium,* (pearl millet) *Eleusine caracan,* (finger millet), *Echinocloa frumentacea* and *Pannicum miliaceum* (proso millet) (Lorenz, 1983). Among millet, pearl millet is one of the most important crops in tropical and subtropical regions of the world, because of its ability to produce good yield of grains under unfavorable condition compared to other cereal crops. Pearl millet is a multipurpose crop, which is grown for food, feed and forage. As a coarse grain cereal for human foods, pearl millet is sustains the live of many millions of people, particularly those of low-income groups in several African and south Asian countries (Kumar and Chauhan, 1993). The presence of all required nutrients in millets make them suitable for large-scale utilization in the manufacture of various food products such as baby foods, snack foods, dietary foods in both grain and flour form. Beside its nourishment, pearl millet contains significant amounts of inositol hexaphosphates (IP6) generally referred to as phytic acid or phytates. Phytate has long been recognized as an antinutritional factor affecting the bioavailability of major minerals such as Ca and P and trace ones such as Zn, Fe, Cu and Mn. Other antinutrients of importance in pearl millet are tannins and polyphenols, which are known to limit the utilization of it as a food or feed. Decreasing of phytic acid is very advantageous, due to its influence on nutrition; therefore interest has grown to reduce its antinutritional effects (Abdelrahaman *et al*., 2005). Soaking, dehulling and fermentation are important traditional methods used to reduce phytic acid. Fermentation is the one of the processes that decrease the levels of antinutrients in food grains and increase minerals extractability (Badau *et al*., 2005), *in vitro* protein digestibility and nutritive value of pearl millet (Elhage *et al*., 2002). Abdelrahaman *et al*. (2005) reported that germination and fermentation enhance the nutritional value of pearl millet by causing significant changes in chemical composition and elimination of antinutritional factors. Therefore, the aim of this study was to evaluate the effect of processing methods followed by fermentation on phytic acid, polyphenols and tannin contents of pearl millet cultivars.

Materials and Methods

Materials: Grain samples of two pearl millet cultivars, (Gazira and Gadarif) were obtained from Khartoum North local market, Sudan. Seeds of the two cultivars were carefully cleaned, freed from foreign material as well as broken and shrunken seeds. The seeds were divided into six parts and kept for processing. All chemicals used in this study were of reagent grade.

Processing treatments

Grinding: The grains were ground to fine and coarse particles to pass 0.4 and 1.0 mm screen, respectively.

Soaking: The grains of the cultivars were soaked in water in a conical flask for 18 hrs. The soaking water was discarded and then the soaked grains were dried at 60°C. The dried seeds were ground to pass a 0.4 mm screen.

Table 1: Effect of processing followed by fermentation on phytic acid content (mg/100g) of pearl millet cultivars

	Cultivars					
	Gazira			Gadarif		
	Fermentation time (hrs)			Fermentation time (hrs)		
Treatment	0	12	24	0	12	24
Finely ground	987.19 (±0.00)^{a}	393.19 (±1.89)^{a}	341.92 (±0.00)^{a}	952.51(±7.72)^{a}	578.27 (±0.00)^{a}	456.10 (±7.46)^{a}
Coarse ground	604.67 (±6.41)^{b}	196.99 (±0.00)^{d}	163.95 (±6.11)^{b}	714.63 (±0.90)^{c}	348.95 (±9.38)^{c}	195.57 (±5.29)^{c}
Soaked	597.50 (±0.00)^{c}	226.08 (±0.00)^{b}	138.31 (±4.70)^{c}	722.20 (±0.00)^{b}	549.49 (±0.00)^{b}	421.07 (±0.00)^{b}
Autoclaved	363.27 (±0.00)^{e}	153.32 (±0.49)^{f}	102.35 (±0.00)^{e}	338.21(±1.58)^{e}	158.61 (±0.00)^{f}	105.25 (±1.15)^{e}
Germinated	327.50 (±0.42)^{f}	175.50 (±0.00)^{e}	111.10 (±0.00)^{d}	329.20 (±1.67)^{f}	165.41 (±1.91)^{e}	108.79 (±0.43)^{e}
Debranned	400.47 (±0.00)^{d}	209.36 (±1.51)^{c}	109.51 (±1.51)^{d}	401.76 (±5.09)^{d}	208.14 (±1.81)^{d}	136.41 (±0.00)^{d}

Values are means of triplicate samples (±SD), Means in a column not sharing a common superscript letter in a column are significantly (P = 0.05) different as assessed by Duncan's multiple range test

Debranning: The grains were soaked in water for 18hr and then hand pounded to separate the bran. The debranned grains were then dried at 60°C and ground to pass a 0.4 mm screen.

Autoclaving: The grains of the cultivars were ground to pass a 0.4 mm screen and then placed in a conical flask and autoclaved at 110°C for 15 minutes.

Germination: The whole grains of both cultivars were immersed in water overnight. The grains were spread on trays lined with cloth and kept wet by frequent spraying of water for 36 hrs. The germinated grains were sun dried and ground to pass a 0.4 mm sieve.

Natural fermentation: The processed grains of the cultivars were ground to pass a 0.4 mm screen and then mixed with distilled water (1:3 w/v). The mixture was incubated at 37°C for 12 and 24 hrs and then the fermented mixture were dried at 60°C and ground to pass a 0.4 mm screen.

Methods

Phytic acid determination: Phytate of each sample was determined according to the method described by Wheeler and Ferrel (1971) using spectrophotometer (JENWAY, Model 6305, London) at 480 nm. A standard curve of different Fe $(NO_3)_3$ concentrations was plotted to calculated the ferric ion concentration. The phytate phosphorus was calculated from the ferric ion concentration assuming 4:6 (iron: phosphorus) molar ratio.

Polyphenols determination: Polyphenols of each sample were estimated using Prussian blue assay, as described by Price and Butler (1977). About 60 mg of ground sample was extracted with 3 mL methanol in a 50 ml conical flask and then poured into a filter paper. The tube was quickly rinsed with additional 3 mL methanol and the content poured once into the filter paper. The filtrate was diluted to 50 mL with distilled water, mixed with 3 mL 0.1M $FeCL_3$ in 0.1N HCL for 3 minutes, followed by timed addition of 3 mL 0.008M $K_3Fe(CN)_6$. The mixture was allowed to stand for 10 minutes. The absorption was read at 720 nm using spectrophotometer (JENWAY, Model 6305, London).

Tannin determination: Tannin content of the samples was determined by the vanillin-HCl method of Price and Butler (1977) using spectrophotometer (JENWAY, Model 6305, London) at 500nm. A standard curve was prepared expressing tannin content as catechin equivalent.

Statistical analysis: Each determination was carried out on three samples and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of variance ANOVA (Snedecor and Cochran, 1987). Duncan's multiple rang test was used to separate means and significance was accepted at P = 0.05.

Results and Discussion

Effect of processing followed by fermentation on phytic acid content: Table 1 shows the effect of processing of grains (finely grinding, soaking, coarsely grinding, autoclaving, germination and debranning) followed by fermentation on phytic acid content (mg/100g) of pearl millet cultivars. Phytic acid content of finely ground grains (control) was found to be 987.19 and 952.51 mg/100g for Gazira and Gadarif cultivars, respectively. Results obtained were within the range reported by Elhage *et al.* (2002) and khetarpaul and Chauhan (1989) but higher than that reported by Elyas *et al.* (2002), Mahajan and Chauhan (1987) and Kumar and Chauhan (1993). Soaking of the grains reduced phytic acid content from 987.19 to 597.50 mg/100g for Gazira cultivar and from 952.51 to 722.20 mg/100g for Gadarif cultivar. Germination of Gazira and Gadarif cultivars caused a significant (P = 0.05) reduction in phytic acid content compared to all other processing methods (Table 1). Phytate content was reduced to 327.5 and 329.2 mg/100g for the cultivars, respectively. Debranning and autoclaving were significantly (P = 0.05)

Table 2: Effect of processing followed by fermentation on tannin content (mg/100g) of pearl millet cultivars

	Cultivars					
	Gazira			Gadarif		
	Fermentation time (hrs)			Fermentation time (hrs)		
Treatment	0	12	24	0	12	24
Finely ground	415.38 (±1.90)^{a}	339.71 (±0.80)^{c}	348.15 (±0.00)^{d}	420.25 (±0.17)^{a}	402.89 (±0.00)^{a}	391.02 (±0.00)^{b}
Coarse ground	347.68 (±0.77)^{f}	316.64 (±0.19)^{e}	337.2 9 (±0.11)^{f}	360.71 (±0.00)^{f}	354.32 (±0.17)^{c}	321.01 (±0.16)^{e}
Soaked	392.16 (±0.37)^{c}	365.59 (±0.17)^{a}	357.55 (±0.10)^{c}	376.25 (±0.17)^{d}	354.46 (±0.27)^{c}	322.50 (±0.00)^{d}
Autoclaved	390.20 (±0.17)^{d}	320.87 (±1.20)^{d}	345.88 (±0.90)^{e}	397.27 (±0.00)^{b}	343.28 (±0.00)^{d}	339.06 (±0.03)^{c}
Germinated	407.91 (±0.66)^{b}	364.39 (±0.18)^{b}	415.45 (±0.38)^{a}	369.47 (±0.00)^{e}	384.85 (±0.18)^{b}	410.22 (±0.00)^{a}
Debranned	378.68 (±0.80)	339.38 (±0.50)^{c}	371.21 (±0.70)^{b}	390.69 (±0.19)^{c}	332.08 (±0.00)^{e}	338.63 (±0.19)^{c}

Values are means of triplicate samples (±SD), Means in a column not sharing a common superscript letter in a column are significantly (P = 0.05) different as assessed by Duncan's multiple range test

decreased phytic acid content to 400.47 and 363.27 mg/100g, respectively for Gazira cultivar and to 401.76 and 338.21 mg/100g, respectively for Gadarif cultivar. Results obtained were in agreement with those reported by Gupta and Sehgal (1991) who found that phytic acid content of cereal grains was significantly decreased during soaking and germination. Badau *et al.* (2005) reported that germination of pearl millet was significantly (P = 0.05) reduced phytic acid content. Reduction in the level of phytic acid during germination could be attributed to leaching out during hydration as well as activation of phytase during germination (Michael and Wiebs, 1983). Elhage *et al.* (2002) reported that dehulling of pearl millet was significantly deceased phytic acid content. Alonos *et al.* (2000) reported that soaking in water was significantly reduced phytic acid content of faba bean seeds. The Reduction in phytic acid during soaking could be attributed to leaching out in soaking water under concentration gradient (Kataria *et al.*, 1989). Results revealed that processing methods applied were significantly (P = 0.05) decreased phytic acid content. Further reduction in phytate was observed when finely ground grains were fermented for 12 hrs, it decreased to 393.19 and 578.27 mg/100g for Gazira and Gadarif cultivar, respectively. Fermentation for 12 hrs of coarse ground, soaked, autoclaved, germinated and debranned grains was significantly decreased phytic acid content and was found to be ranged from 196.99 to 153.50 mg/100g for Gazira cultivar and from 348.95 to 158.61 mg/100g for Gadarif cultivar. Fermentation of processed grains for 24 hrs caused an additional reduction in phytic acid content (Table 1). Results obtained were similar to those observed during fermentation of pearl millet (Khetarpaul and Chauhan, 1990). The reduction in phytic acid during fermentation could be attributed to the action of the enzyme phytase released by microorganisms during fermentation.

Effect of processing followed by fermentation on polyphenols content: The effect of processing followed by fermentation on polyphenols content of pearl millet cultivars is presented in Table 2. Polyphenols of finely ground grains were 415.38 and 420.25 mg/100g for Gazira and Gadarif cultivars, respectively. Results obtained in this study were within the range reported by Abdelrahman *et al.* (2005), but lower than that reported by Khetarpaul and Chauhan (1989). The processing methods applied were significantly (P = 0.05) reduced the level of polyphenols for the two cultivars. Debranning was significantly (P = 0.05) decreased polyphenols to 378.68 and 390.69 mg/100g for Gazira and Gadarif cultivars, respectively. The reduction obtained was comparable to that reported by Elhage *et al.* (2002). Reduction in polyphenols may be attributed to the removal of the outer layer, which reported to be rich in polyphenols. The results showed that, germination of the cultivars grains was significantly reduced polyphenols to 407.91 mg/100g for Gazira cultivar and to 369.47 mg/100g for Gadarif cultivar. The results obtained agree with those reported by Borade *et al.* (1984). Reduction in polyphenols may be attributed to the presence of phenolic oxidase during germination (Kataria *et al.*, 1989). However, soaking and autoclaving methods to less extend reduced polyphenols content compared to other processing methods as reported by Sehga and Kawatra (2004). Fermentation for 12 hrs significantly decreased the polyphenols of finely ground grains of Gazira to 339.71 mg/100g and Gadarif to 402.25 mg/100g. Polyphenols of Gazira Gadarif cultivars were further significantly decreased after fermentation for 24 hrs. Similar result was obtained by Elyas *et al.* (2002) who reported a decrease in polyphenols after 36 hrs fermentation. Also results showed that fermentation of soaked, autoclaved and dehulled grains for 12 and 24 hrs significantly (P = 0.05) reduced polyphenols for both cultivars. The decrease in polyphenols during fermentation of processed grains indicates the ability of microflora to ferment phenolics, as reported by Bravo (1998).

Effect of processing followed by fermentation on tannin content: Table 3 shows tannin content of

Table 3: Effect of processing followed by fermentation on polyphenols content (mg/100g) of pearl millet cultivars

	Cultivars					
	Gazira			Gadarif		
	Fermentation time (hrs)			Fermentation time (hrs)		
Treatment	0	12	24	0	12	24
Finely ground	220 (±1.00)^{b}	120 (±0.90)^{c}	90 (±0.10)^{c}	170 (±2.00)^{b}	160 (±1.10)^{a}	110 (±1.00)^{c}
Coarse ground	180 (±0.00)^{c}	160 (±1.10)^{a}	90 (±0.00)^{c}	110 (±1.00)^{d}	60 (±0.60)^{f}	40 (±0.08)^{e}
Soaked	170 (±1.00)^{c}	100 (±2.00)^{d}	120 (±3.00)^{b}	150 (±2.00)^{c}	130 (±0.80)^{c}	120 (±1.20)^{b}
Autoclaved	100 (±0.70)^{d}	70 (±1.20)^{e}	80 (±0.00)^{d}	80 (±2.00)^{e}	90 (±2.10)^{d}	40 (±1.10)e
Germinated	250 (±3.00)^{a}	130 (±1.30)^{b}	240 (±1.00)^{a}	210 (±1.00)^{a}	150 (±0.00)^{b}	270 (±2.00)^{a}
Debranned	80 (±0.00)^{e}	70 (±2.00)^{e}	80 (±1.00)^{b}	80 (±1.00)^{e}	80 (±1.20)^{e}	70 (±0.00)^{d}

Values are means of triplicate samples (±SD), Means in a column not sharing a common superscript letter in a column are significantly (P = 0.05) different as assessed by Duncan's multiple range test

processed pearl millet grains before and after fermentation. Tannin content of Gazira cultivar was found to be 220 mg/100g while that of Gadarif cultivar was 170 mg/100g. The processing treatments were significantly (P = 0.05) decreased tannin content of both cultivars, except germination, which was found to increase tannin content and was found to be 250 and 210 mg/100g for Gazira and Gadarif cultivars, respectively. Similar finding was reported by Oloyo (2004) who found that germination of pigeon pea seeds for 3 days caused an increment in tannin content. Ahmed *et al.* (2006) reported an increase in tannin content during germination of guar grains. The increment in tannin content during germination may be attributed to solubilization of tannins and the soluble tannin migrates from the seed coat to the core of the seeds. Debranning significantly decreased tannin content of Gazira and Gadarif cultivars. Other processing methods (coarse grinding, soaking and autoclaving) were significantly (P = 0.05) decreased tannin content in the range from 100 to 180 mg/100g for Gazira cultivar and from 80 to 150 mg/100g for Gadarif cultivar. This in accord with an earlier investigation on different varieties of *Pisum sativum* (Bishnoi *et al.*, 1994) and *Vicia faba* (Sharma and Sehgal, 1992). Fermentation of finely ground grains for 12 hrs significantly (P = 0.05) decreased tannin content of Gazira cultivar to 120 mg/100g while that of Gadarif was decreased to 160 mg/100g. Fermentation of germinated grains for 12 hrs significantly decreased tannin content of Gazira (130 mg/100g) and Gadarif (150 mg/100g) cultivars. Fermentation of soaked, autoclaved and debranned seeds decreased tannin content and was found to be ranged from 70 mg/100g to 100 mg/100g for Gazira and from 80 to 130 mg/100g for Gadarif cultivar. Fermentation of processed grains for 24 hrs was found to be fluctuated depending on the processing method. Fermentation of autoclaved grains significantly decreased tannin content of Gazira to 80 mg/100g while that of Gadarif cultivar was decreased to 90 mg/100g. It seems likely that autoclaving solubilized tannin and further solubilization was occurred during fermentation (Osman, 2004).

Conclusion: The processing of pearl millet cultivars significantly decreased phytic acid content as well as polyphenols. Tannin content was decreased during processing; however, germination caused an increment. Further decrease in antinutritional factors was observed after fermentation of the processed grains.

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