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Changes in tannin and cyanide contents and diastic activity during germination and the effect of traditional processing on cyanide content of sorghum cultivars

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Investigation of three sorghum cultivars showed that tannin content of ungerminated seeds were 220, 410 and 400 mg/100 g for Waznashra, Feterita and Gadamelhamam cultivars, respectively. For all cultivars, ungerminated seeds were found to contain no free or total cyanide. Their diastatic activities (the combined α- and β-amylase activities) were found to be zero. For all cultivars, tannin content was slightly increased when seeds were germinated for different periods. Both free and total cyanide were significantly increased with the length time of germination for all cultivars. Fermentation and heat treatment of germinated seeds significantly reduced cyanide content and only traces were detected in the final product (Hulu-murr and Merissa). Fermentation alone was found to reduce cyanide content, although not as much as when it was accompanied by heat treatment. Diastatic activity markedly increased with time of germination for Feterita and Gadamelhamam cultivars while for Waznashra it increased markedly up to day 3 after which it started to decline rapidly.

Results indicated that germination of sorghum seeds for different periods increased tannin and cyanide contents, while changes in diastatic activity depend on type of cultivar. Traditional processing was found to reduce cyanide contents of germinated seeds. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Sorghum has been a vital staple food for millions of people in the semi-arid tropics. In different parts of the world, vigorous efforts are directed towards coupling the beneficial effects of harmful constituents such as tannin and cyanide in sorghum seeds by direct removal of seed testa, inactivation, fermentation or by extraction. Tannin content of low-tannin cultivars was slightly increased when seeds were germinated for different periods (Glennie, 1983). In feeding trials with rats (Yasaman *et al.*, 1990) and chicks (Teeter *et al.*, 1986), tannins reduced weight gain and feed conversion. Cyanide content was significantly increased when sorghum seeds were germinated for different periods, and the consumption of germinated sorghum seed or its dried products is potentially hazardous, particularly in circumstances where sorghum is a staple food for persons who are malnourished and are suffering from chronic dietary exposure to cyanide (Panasiuk & Bills, 1984). Hydrocyanic acid or prussic acid (HCN) is one of the most toxic and rapidly acting of the common poisons with a lethal dose of 50–60 mg/100 g (Panasiuk & Bills, 1984). Traces of cyanide have been detected in alcoholic beverages prepared by sorghum fermentation (Nartey, 1981). The malting process of sorghum seeds that generates the fermentable mono- and disaccharides is dependent upon the activity of α-and β-amylases that develop in sorghum seeds during germination (Hulse *et al.*, 1980). The activity of amylases, but not α-glucosidase increased appreciably during malting (Bureng & Worgan, 1982). The study described aims to investigate whether toxic constituents in sorghum seeds could be reduced when seeds are germinated and fermented for different periods.

MATERIALS AND METHODS

Seeds of three sorghum cultivars, Waznashra, Feterita and Gadamelhamam, obtained from the Agricultural Research Station, Wad Medani, were carefully cleaned.

Germinated seeds (1–5 days) were dried at room temperature (25–30°C) for 2–3 days. Germinated and ungerminated seeds and the dry samples of Hulu-murr were ground to pass through a 0.4 mm screen. Hulu-murr and Merissa are usually manufactured from seeds of the Feterita cultivar.

Germination

Germination was carried out by soaking seeds in water for 20 h. The soaked seeds were spread on a metal box, the lower side of which was a mesh covered with a piece of Jute sack; another piece of Jute sack was used to cover the box. The box was kept at room temperature (25–30°C) in the dark. Seeds were watered once a day for 5 days. Samples for analysis were taken everyday.

Hulu-murr manufacture

Malt and sprouts of seeds germinated for 2 days were sun-dried and ground to a fine flour. A slurry was made by mixing the flour with water, which was then heated and made in the form of porridge. The porridge was cooled to room temperature and mixed thoroughly with a flour of ungerminated sorghum seeds in the ratio 1:2. The mixture was placed in a round earthenware container, water and spices, such as cinnamon, ginger, cloves, coriander and pepper powder, were added to the mixture. After thorough mixing, the dough was left to ferment overnight (12–24 h) at room temperature. At the end of fermentation, tamarind or roselle "karkadi" was added. The fermented dough was then baked on a hot steel plate (150–160°C) in a process known as "Aowasa". A small amount of the fermented dough was spread over the hot plate using a 4–5 inch strip of wood. The process of spreading was continued until the dough covered the plate forming a very thick, dry and brown sheet after 1–2 s; this was then taken out. The sheets were spread on a flat surface to dry at room temperature. Hulu-murr samples were stored for up to 3 months.

Merissa manufacture

The process was more or less similar to that of Hulu-murr except that in Merissa manufacture a dough was used as starter instead of natural fermentation, and the fermented dough was sieved. The supernatant is normally used as an alcoholic beverage.

Fermentation

Milled sorghum seeds were made into a paste by mixing the flour of seeds germinated for 3 days with that of ungerminated seeds in the ratio of 1:2 and left to ferment. Fermentation was carried out for 3 days. Samples were taken every day for cyanide analysis.

Moisture and tannin determination

To express results on a 105°C dry matter basis, moisture was determined according to the AOAC (1965) method. Tannins were estimated by the modified procedure of Maxon and Rooney, as described by Price *et al.* (1978). A 200 mg sample was extracted with 10 ml 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the colour developed after 20 min at 30°C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, that is the amount of catechin (mg/ml) which gives a colour intensity equivalent to that given by tannins after correcting for the blank.

Diastatic activity measurement

Diastatic activity, i.e. the combined α-and β-amylases activity, was determined by the alkaline ferricyanide method "Recommended methods of analysis of Barley, Malt and Adjunct" as described by Anon (1971).

Results are expressed as 10 units (10 u), i.e. the amount of enzyme that catalyses the conversion of 10 μmol of starch per min under defined conditions.

Cyanide content determination

Cyanide content was determined by the enzymatic assay of Cooke (1979).

Each sample was analysed in duplicate and the figures were then averaged.

RESULTS

Figure 1 shows the effect of germination on tannin content (mg/100 g) of sorghum cultivars. Tannin content of ungerminated seeds were 220, 410 and 400 mg/100 g for cultivars Waznashra, Feterita and Gadmelhamam, respectively. Germination of seeds for 1 day slightly increased tannin content to 250, 420 and 420 mg/100 g for the cultivars, respectively. Increase in the

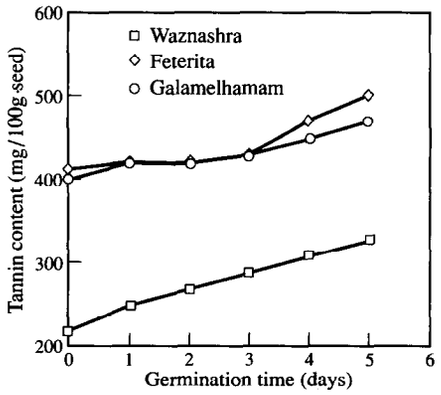

Fig. 1. Effect of germination on tannin content of sorghum cultivars.

germination period up to 5 days markedly increased tannin content to 330, 500 and 470 mg/100 g for the cultivars, respectively.

Figure 2 shows the effect of germination on diastatic activity, i.e. the combined α- and β-amylase activities. For all cultivars diastatic activity (as IOU) of ungerminated seeds was found to be nil. For cultivar Waznashra (Fig. 2) diastatic activity markedly increased up to 41 when seeds were germinated for 2 days after which it markedly decreased to 8.0 after 5 days germination.

For both Feterita and Gadamelhamam diastatic activity was significantly increased up to 73.0 and 72.0 for the two cultivars respectively, when seeds were germinated for 5 days.

Figure 3 shows the effect of germination on free and total cyanide content. For all cultivars ungerminated seeds were devoid of free (Fig. 3A) and total (Fig. 3B) cyanide. Germination of seeds for 1 day markedly increased free cyanide content to 1.5, 1.3 and 1.1 mg HCN/100 g for the cultivars Waznashra, Feterita and Gadamelhamam, respectively (Fig. 3A). Germination for 5 days increased free cyanide to 6.0, 8.3 and 8.7 mg HCN/100 g for the cultivars, respectively. Germination of seeds for 1 day significantly increased total cyanide to 7.6, 12.2 and 14.4 mg HCN/100 g for the cultivars, respectively (Fig. 3B). Germination for 5 days rapidly increased total cyanide to 61.4, 85.8 and 89.5 mg HCN/100 g for the cultivars, respectively.

Figure 4 shows the effect of traditional processing, storage and fermentation on free (Fig. 4A) and total (Fig. 4B) cyanide content of a malt of Feterita seeds germinated for 3 days. A malt of sorghum seeds germinated for 3 days was found to contain 7.1 and 58.0 mg HCN/100 g free and total cyanide, respectively. Fermentation and heat treatment, during the preparation of Hulu-murr, and storage were found to decrease free cyanide significantly to 0.5 and 0.4 mg HCN/100 g when Hulu-murr was stored for 1 and 3 months, respectively. When Hulu-murr was stored for different periods (1–3 months) total cyanide was significantly decreased with minimum and maximum values of 1.0 and 3.3 mg HCN/100 g compared to that in malt of germinated seeds. Alcoholic beverage (Merissa, unsieved) processing was found to decrease free and total cyanide to 0.2

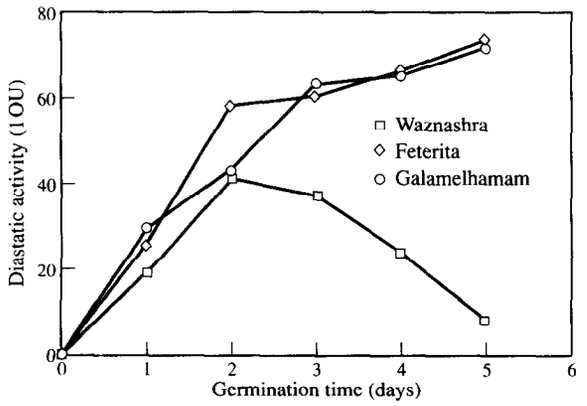

Fig. 2. Effect of germination on diastatic activity of sorghum cultivars.

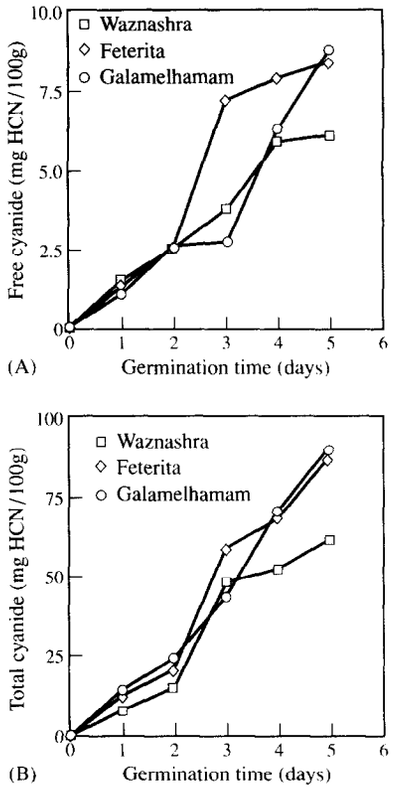

Fig. 3. Effect of germination on (A) free and (B) total cyanide content of sorghum cultivars.

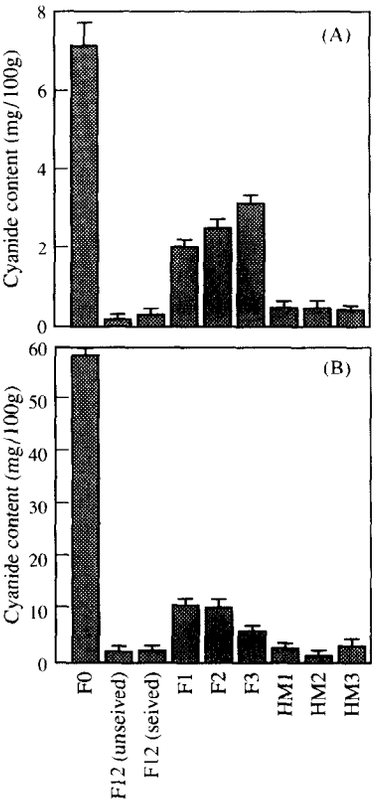

Fig. 4. Effect of traditional processing, storage and fermentation on (A) free and (B) total cyanide content of a malt of Feterita cultivar. F0, malt before processing; F12, malt fermented for 12 h (alcoholic drink, Merissa); F1–F3, malt fermented for 1–3 days; HM1–HM3, Hulu-murr samples stored for 1–3 months.

and 2.0 mg HCN/100 g, respectively, and it declined to 0.3 and 2.3 mg HCN/100 g, respectively, when Merissa was sieved. Free cyanide content increased slowly with length of fermentation time up to day 3 (3.1 mg HCN/100 g). For all periods of fermentation, free cyanide content was significantly lower than that of the malt (7.1 mg HCN/100 g). Total cyanide was slightly decreased with length of fermentation time to 5.6 mg HCN/100 g when seeds were fermented for 3 days. For all periods of fermentation, total cyanide content was found to be lower than that of the malt (58.0 mg HCN/100 g).

DISCUSSION

For all cultivars, results revealed that tannin content was slightly increased when seeds were germinated for different periods (Fig. 1). This could be a result of solubilization of tannin when the seeds were soaked in water and migration of tannin to the outer layer as a result of germination, as indicated by the browing of the germinated seeds. Diastatic activity for Wazanashra cultivar increased for a certain period and then started to decline (Fig. 2). This could be due to the fact that the germination of seeds caused an increase in tannin content which has an inhibitory effect on enzyme activity by forming an insoluble complex with them. Kock *et al.* (1985) reported that polyphenols in the testa and pericarp of sorghum seed have an inhibitory effect on enzyme activity during hydrolysis of starch. Although for Feterita and Gadamelhamam cultivars tannin content increased with germination time, diastatic activity also increased (Fig. 2). This observation is a departure from an otherwise good correlation between tannin content and enzyme activity. The explanation for this difference may lie in chemical (as well as quantitative) differences between the tannins of the cultivars and the amounts and concentrations of amylases. Free and total cyanide markedly increased when seeds were germinated for different periods (Fig. 3A and 3B). Cyanide content rapidly increased to a level that may be toxic for humans and animals when seeds were germinated for longer periods (4–5 days). This is possibly due to the fact that when seeds are placed in an environment favourable to germination, the rate of enzyme activity is markedly accelerated, which converts cyanogenic glycosides to hydrocyanic acid (HCN) (Conn, 1973). Traditional processing of germinated seeds for making alcoholic beverages, such as Merissa, or non-alcoholic beverages, such as Hulu-murr, was found to reduce cyanide content to a level far below than of germinated seeds (Fig. 4). Fermentation of germinated seeds (Fig. 4) was found to give results similar to those obtained when they were fermented and heated (Hulu-murr and Merissa). Reduction of cyanide content in processed germinated seeds occurred as a result of fermentation, which creates acidic conditions that may help in hydrolysing the cyanoglycosides; accordingly HCN content of the ferment can be detected as free cyanide.

Germination of sorghum seeds should be coupled with traditional processing, that is, fermentation or fermentation and heating for making alcoholic or non-alcoholic beverages which reduces cyanide content rapidly and improves its nutritional quality.

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