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Influence of phenolics in finger millet on grain and malt fungal load, and malt quality

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ABSTRACT

Phenolic type, fungal load, germinative energy (GE) and malt quality of finger millet grain types of varied kernel colour and phenolic content were analysed, to determine if phenolics in finger millet grain influence its malt quality. The fungal load (total fungal count (TFC) and infection levels) of the unmalted grain and malt were negatively correlated ($p < 0.05$) with total phenolics (TP) and amount of phenolic type (condensed tannins, anthocyanins and flavan-4-ols). High-phenol finger millet types had much higher malt quality than the low-phenol types, with respect to diastatic power (DP), and α- and β-amylase activities. GE, DP and α-amylase activity were positively correlated with TP and amount of phenolic type ($p < 0.05$) and negatively correlated with TFC ($p < 0.01$). Phenolics in finger millet grain influence its malt quality positively by contributing to attenuation of the fungal load on the germinating grain.

1. Introduction

Finger millet (*Eleucine coracana* (L.) Gaertn.) is widely grown in Africa and parts of Asia, where it is produced for food and, in a malted form, for the production of weaning foods, opaque beer and other products (Taylor & Emmambux, 2008). In the Southern African Development Community (SADC), the primary use of finger millet is as a malting/brewing grain (Gomez, 1994).

In malting, the moist and warm conditions promote the proliferation of micro-organisms on the germinating grain (Noots, Delcour, & Michiels, 1999). The micro-organisms may have negative effects on malt and beer quality (Noots et al., 1999). Of more concern is that some moulds are potentially toxigenic and can produce mycotoxins, which are a health hazard (Hussein & Brasel, 2001). Rabie and Lübben (1984) reported that South African sorghum malts produced by floor and pneumatic malting were infested with various fungi, including potentially toxigenic types. Odhav and Naicker (2002) showed that South African commercial sorghum beers had significantly high levels of aflatoxins. African home-brewed opaque beers, although not specifically finger millet beer, have been found to contain significant amounts of mycotoxins (Lovelace & Nyathi, 1977; Odhav & Naicker, 2002).

We have reported appreciable levels of phenolics in finger millet grain, and that total phenolics and tannin content varied across grain types (Siwela, Taylor, de Milliano, & Duodu, 2007). Light-coloured grain types had much lower total phenolics and tannins relative to the pigmented types, and types which had a pigmented testa had much higher tannin content. We also established that the tannins were located in the testa layer of the grain, as in sorghum.

Chethan, Sreerama, and Malleshi (2008) suggested that phenolics in finger millet grain were detrimental to its malt quality, as they inhibited malt amylases. On the other hand, Seetharam and Ravikumar (1994) indicated that finger millet grain phenolics, including tannins, may be involved in resistance of the grain to fungal attack, as is widely suggested to be the case in sorghum (reviewed by Chandrashekar & Satyanarayana, 2006). In view of scanty and apparently conflicting information available, the objectives of this study were to determine whether there is a relationship between finger millet grain and malt fungal infection levels and phenolic content, amount of phenolic type and kernel colour, and also to assess whether phenolics in finger millet grain influence its malt quality.

2. Materials and methods

2.1. Finger millet grain

Twenty-two finger millet types of different kernel colour from Kenya and Zimbabwe were grown, processed and stored as de-

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scribed previously (Siwela et al., 2007). The grain was processed by mechanical threshing and then hand cleaning to remove glumes. It was then further cleaned by sieving to remove broken kernels and foreign matter.

The finger millet types were selected to represent the finger millet germplasm found in Southern and East Africa.

2.2. Malting

Twelve grain types, which varied in kernel colour and phenolic content, were malted. Only 12 finger millet types from the whole set of 22 could be malted because there was little grain of the other finger millet types. Three of the grain types that were malted, G35, 95G198W and ICFM95001GMSI, were light (creamy white), had no tannins and had low phenolic content; the others were pigmented (brown) and had high phenolic content. Of the pigmented grain types, two, FNL 0069 and P283, had low tannin content, whilst the rest had high tannin content. Malting was performed according to the South African Council for Scientific and Industrial Research standard method for the laboratory scale malting of sorghum (Dewar, Taylor, & Joustra, 1995). Exactly 50 g of grain was placed in nylon mesh bags and steeped in 25 °C water in a steeping vessel for a total of 24 h. The steeped grain was then germinated in an incubator set at 25 °C and 100% relative humidity for a total of 5 days. The wet malt in the nylon bags was spread out and dried in a forced-draught oven set at 50 °C for 24 h. Two sorghum cultivars, PAN 8564 (non-tannin) and PAN 8225 (high-tannin), were malted in the same way as finger millet (as described above), to obtain sorghum malt standards. Barley malt standards (Megazyme Ceralpha and Betamyl standards) were obtained from Megazyme International, Ireland.

2.3. Analyses

The Folin–Ciocalteu method of Singleton and Rossi (1965) was used to measure total phenolics (TP), as described previously (Siwela et al., 2007). Exactly 0.4 g flour was extracted with 20 ml acidified methanol (1% HCl in methanol) for 1 h at room temperature (approx. 25 °C), with vortex mixing at 5-min intervals. The samples were centrifuged for 10 min at 1200*g*, using a temperature-controlled centrifuge set at 25 °C. Three replicate supernatants were obtained. Sample extracts (0.5 ml) were mixed with 2.5 ml Folin–Ciocalteu phenol reagent in a 50-ml volumetric flask; 7.5 ml 20% (w/v) sodium carbonate was added within 8 min after addition of the Folin–Ciocalteu phenol reagent. The contents were mixed and the flask made up to volume with distilled water, stoppered and thoroughly mixed. Sample blanks were included, in which the sample was replaced by distilled water. The flasks were left to stand at room temperature (approx. 25 °C) for 2 h, after which absorbance at 760 nm was measured. Gallic acid was used as a standard.

Condensed tannin content was measured using the vanillin–HCl method of Price, Van Scoyoc, and Butler (1978) with blank subtraction for extract colour as described previously (Siwela et al., 2007). Extraction was as for determination of total phenolics except that 100% methanol was used in place of acidified methanol. The extracts and the vanillin reagent (4% HCl in methanol and 0.5% (w/v) vanillin in methanol) were maintained at 30 °C in a thermostat-controlled water bath before mixing the reactants. Sample extracts (1 ml) were mixed with 5 ml vanillin reagent in test tubes and then maintained at 30 °C in the water bath for 20 min. Sample blanks in which the vanillin reagent was replaced by 4% HCl in methanol were included. Absorbance at 500 nm was measured. Catechin was used as a standard. Three replicates were analysed.

Anthocyanins were estimated as 3-deoxyanthocyanidin pigments, apigeninidin and luteolinidin, which are the forms in which anthocyanins occur in sorghum grain (Dykes & Rooney, 2006). Analysis was according to Menkir, Ejeta, Butler, and Melake-Berhan (1996). Exactly 0.25 g of finger millet flour was extracted with 15 ml of absolute ethyl acetate for 30 min and centrifuged for 10 min at 1200*g*, using a temperature-controlled centrifuge set at 25 °C. The residue was extracted further with 15 ml of 0.05% (v/v) methanol in HCl. The extracts were pooled together. Exactly 0.4 g of acid-treated polyvinylpyrrolidone was added to 8 ml of the extract, mixed thoroughly by vortexing, incubated at room temperature (approx. 25 °C) for 10 min, and centrifuged. The absorbance of the supernatant was read at 475 nm for apigeninidin and at 495 nm for luteolinidin. Two replicates were analysed.

Flavan-4-ols were analysed according to Dykes, Rooney, Waniska, and Rooney (2005). Extraction was as for the determination of total phenolics. Exactly 1 ml of extract was reacted with 5 ml of HCl–butanol reagent (solution of 0.0616 g $FeSO_4{\cdot}7H_2O$ in 5% HCl in *sec*–butanol). The reaction was allowed to stand for 1 h at room temperature and absorbance was then read at 550 nm. Two replicates were analysed.

Fungi infecting the finger millet grains were enumerated, isolated and identified by the direct plating method explained by Rabie and Lübben (1984). Kernels of each grain type were surface-disinfected by shaking them in a flask containing 76% (v/v) ethanol and then rinsing them three times with sterile distilled water. Five kernels were placed on plates (10 each) of potato dextrose agar (PDA), malt salt agar (MSA) and pentachlorobenzene agar (PCNB) and incubated at 25 °C for 2 to 14 days. Kernels with mycelial growth of any fungal type were counted and expressed as a percentage of the total kernels plated. The fungal colonies were isolated and purified on fresh PDA plates and then identified on the basis of morphological features of their fruiting bodies by referring to standard mycology literature.

Total fungal count (TFC) (yeasts and moulds) on the surface of the unmalted finger millet grain and malt was determined by the standard plate count method. Exactly 10 g of either unmalted grain or malt were placed in a 250-ml conical flask and 90 ml peptone water (0.1% (w/v) peptone, 0.85% (w/v) NaCl) were added. The flask was swirled by hand and then shaken for 5 min on a lateral shaker to suspend any micro-organisms on the surface of the kernels in peptone water. Ten-fold serial dilutions were prepared in test tubes and then spread plated, in duplicate, on PDA and incubated at 25 °C for 2 to 14 days. Counting was done on plates that had 15–300 colonies. Fungal infection of the unmalted finger millet grain and malt was determined as described earlier.

GE of the finger millet and sorghum grain was determined according to Dewar et al. (1995).

Diastatic power (DP) (joint α- and β-amylase activity) of the finger millet malts, and sorghum and barley malt standards was determined according to South African Bureau of Standards (SABS) Method 235 (SABS 1970), with modifications according to Dewar et al. (1995). Finger millet and sorghum malts were milled into flour using a 1093 Cyclotec sample mill (Foss Tecator, Höganäs, Sweden) fitted with a 1-mm opening screen. The modification to the SABS Method 235 for the determination of DP was that 5 g of malt was used and the extraction volume was 100 ml, instead of 25 g and 500 ml, and in addition to extraction with 2% peptone as per SABS method 235, extraction was also made with distilled water. The apparatus size and reagent volumes were reduced accordingly.

The α- and β-amylase activities of the finger millet malts, and sorghum and barley malt standards were determined by the Alpha Amylase Procedure (Ceralpha Method) and Betamyl Method, respectively (Megazyme International, Ireland).

2.4. Statistical analysis

STATISTICA (StatSoft, Inc. 2005 version 7.1 www.statsoft.com Tulsa, OK) was used to analyze the data. Tukey's studentised range