Polyphenol oxidase activity in white tan-plant type sorghums: An important determinant of the relatively dark colour of their porridges

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Running title: White tan-plant sorghum PPO activity

Summary

The relatively dark colour of food products from white tan-plant (food-grade) sorghums can compromise their acceptability. The relationship between white tan-plant sorghum polyphenol oxidase activity (PPO) and porridge colour was investigated, primarily using lines grown in the same locality over two seasons. Sorghum was intermediate in PPO between wheat and maize. White tan-plant sorghum and white maize whole grain flours were similar in colour. However, with white tan-plant sorghum the transition from flour to porridge caused a much larger reduction in L* value. Further, the correlation between white tan-plant sorghum PPO activity and porridge L* values was highly significantly negative ($p < 0.001$), the relationship accounting for 40-50% of variation. PPO in white tan-plant type sorghums is therefore an important determinant of the relatively dark colour of porridges. Breeding to reduce PPO activity could improve consumer appeal. Cultivar Sima (IS 23520) which had low PPO activity and produced light coloured porridge could be useful for breeding.

Introduction

Sorghum varieties differ widely in grain colour. Genetically, the pericarp of the grain is red, lemon yellow or white (colourless) (Rooney and Miller, 1982). However, whole grain and flour colour, and the colour of food products made from them, is affected by other genetically controlled factors, especially glume colour and the presence of a pigmented (tannin-containing) testa. White tan-plant type sorghums, also referred to as food-grade sorghums (Pontieri *et al.,* 2010), have been developed through breeding for inherently improved sensory attributes of foods produced from them, such as light colour, and bland taste and flavour, in comparison with sorghums with a pigmented pericarp and white tannin sorghums (Miller, 1986a,b; Rooney and Waniska, 2000; Rooney *et al.,* 2011). White tan-plant sorghums have a white pericarp, tan-plant colour and straw or tan coloured glumes, and their endosperm texture ranges from hard to medium (Rooney & Waniska, 2000; Rooney & Awika, 2005).

Notwithstanding this, food products made from white tan-plant sorghum seem to be generally darker in colour and less acceptable than those from other unpigmented cereals. For example, in Botswana low consumer acceptability of the darker coloured porridge produced from white tan-plant sorghum was reported in comparison with porridge made from white maize (Jagwer, 1998). Similarly, when a rice noodle making procedure was used to make sorghum noodles, it was reported that darker noodles were produced from white tan-plant sorghum than those made from rice (Suhendro *et al.,* 2000).

In wheat, the role of polyphenol oxidase (PPO) (EC. 1.14.18.1) in the darkening of wheat-based products has been clearly established (Baik *et al*., 1994; Anderson & Morris, 2001; Bettege, 2004). PPO activity has been shown to be associated with the discoloration and

darkening of wheat products such as noodles (Kruger *et al.*, 1994; Baik *et al.*, 1995; Fuerst *et al.*, 2006; Kang *et al.*, 2008) and pasta (Feillet *et al.*, 2000). Research on the role of PPO activity and quality loss in wheat is advanced (Demeke *et al.*, 2001). Genetic studies indicated that homologous group 2 chromosomes influence PPO activity (Jimenez *et al.*, 1999). This genetic information has resulted in breeding for wheat with low PPO activity (Fuerst *et al.*, 2006) and better acceptance of wheat products and better market value (McCaig *et al.*, 1999).

Research on PPO activity in sorghum grain has been limited (Glennie, 1981; Dicko *et al.*, 2002a,b; Dicko *et al.*, 2006) . Notably, Dicko *et al*. (2002b) compared the phenolic content, PPO and peroxidase (PO) activities of 50 sorghum varieties of various types, mainly originating from Africa. The authors suggested that because cereals do not contain laccase (EC. 1.10.3.2.) and because substrates such as 4-hydroxyanisole and 3,4-dihydroxyphenylpropionic acid are not oxidised by PO in the absence of hydrogen peroxide, it could be inferred that the oxygen-dependent oxidase activity in sorghum was essentially due to PPO. However, on the basis that PO activity in the sorghums was found to be several times higher than PPO activity, it was tentatively concluded that PO was more involved in in vivo oxidation of sorghum polyphenols than PPO. Dicko *et al.* (2006) investigated sorghum characteristics suitable for various products and concluded that white grain with low PPO activity (among other characteristics) was best for Tô (gel-like porridge), couscous, industrial brewing and infant porridges. However, the relationships between sorghum grain oxidative enzymic activities and food product colour was not investigated.

In view of the increasing importance of sorghum in commercial food manufacture such as gluten-free flour applications (Rooney & Awika, 2005; Pontieri *et al.*, 2010), the relationship

between the relatively dark colour of products from white tan-plant sorghum types and grain PPO activity was specifically studied using whole grain porridge as a simple food product. An objective was also to screen white-tan plant cultivars available in Africa on the bases of PPO activity and food product colour.

MATERIALS AND METHODS

Grain samples

Sixteen sorghum cultivars developed in eastern and southern Africa were cultivated in a controlled field trial at the Golden Valley Research Station, Chisamba, Zambia in two growing seasons, 2008 and 2009. Ten were white tan-plant types: ELT1-16, ELT1-17, Kuyuma, Sima, ZSV-15, SDS 1958-1-3-2, [SDS5006*WSV187]23-2-1 (open pollinating varieties (OPVs)) and MMSH-1040, MMSH-1257, MMSH-1340 (hybrids). ELT1-16 and ELT1-17 share a common parent and Kuyuma, [SDS5006*WSV187]23-2-1 and ZSV-15 have a common background. The other six were pigmented. Five were red/brown type III tannin types: MMSH-375, MMSH-413 and MMSH-740 (hybrids) and [Framida*SDS3843]F6-5 and [Framida*SDS3843]16-2-2 (OPVs), and one was red non-tannin type: MMSH-625 (hybrid).

Eight other white tan-plant sorghum cultivars obtained from commercial cultivation were studied, BSH1 (hybrid), Larsvyt, Sefofu and Segao (OPVs) cultivated in Botswana; NK8828, Orbit and PEX 606/202 (hybrids) cultivated in South Africa and KAT 369 (OPV) cultivated in Kenya. In addition, eight South African white maize hybrids: CRM3508, Panthera, PAN6043, PAN6045, PAN622313, PAN6335, Saffier, and 6Q32113 cultivated at the

Agricultural Research Council Grain Crops Institute, Potchefstroom, and five South African red wheat varieties from the Southern African Grain Laboratory, South Africa: CRM826, Kariega, PAN3118, PAN3355, Steenbras and one South African commercial red bread wheat sample were also studied. White maize was included as a standard as it very commonly used for porridge making in Africa (Rooney & Serna-Saldivar, 2003). Red wheat, the type of wheat which is cultivated in South Africa, was included to establish the PPO activity of sorghum relative to wheat.

Upon receipt, all grain samples were stored at 8^{o}C under dry and dark conditions until analysis. For analysis, approximately 300 g of each grain type was ground with a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) to pass through a 500 µm opening screen. Milled whole grain flour samples (45 g) were vacuum-packaged in polyethylene bags and stored at -18^{o}C in the dark prior to analysis.

PPO analysis

PPO was extracted as described (Dicko *et al.*, 2002b), with modifications. In brief, the enzyme extracts were prepared by mixing 2 g sorghum flour with 9.6 mL 50 mM Tris-HCl pH 7.3 buffer containing 0.5 M $CaCl_2$with 20 g L^{-1} polyvinylpyrrolidone (PVP) at 4^{o}C for 1 h. Insoluble PVP was added to the enzyme extraction buffer to prevent interaction of PPO with endogenous polyphenols. The homogenate was centrifuged (14 000 g, 4^{o}C, 45 min) and the resulting supernatant was used as enzyme extract. A spectrophotometric assay was performed. L-dihydroxyphenylalanine (L-DOPA) was chosen as the phenolic substrate to determine the *o*-diphenolase activity as it has been shown to be an effective substrate for assaying PPO activity for whole cereal grain (Anderson & Morris, 2001). The enzyme extract (80 µL) was incubated with 1.2 mL 50 mM sodium acetate buffer pH 5.5, 80 µL 50 mM 3-

methyl-2-benzothiazolinone hydrazone (MBTH), at 25^{o}C for 5 min. The MBTH was used to trap the *o*-quinones formed in the oxidation of the substrate L-DOPA by the enzyme. N,N'-dimethylformamide (DMF) was added to the assay medium at 2% to dissolve the MBTH-quinone adducts. The reaction was started by addition of 160 µL 100 mM L-DOPA in 0.15 mM phosphoric acid. The reaction was monitored at 475 nm for 25 min. PPO activity was expressed in U/mg whole grain flour dry basis, where a Unit is the amount of enzyme producing 1 µmol of MBTH-quinone-adducts per minute from the oxidation of L-DOPA (Espín *et al.,* 1995).

Flour and porridge colour

Twenty g flour was weighed into a 90 mm Petri dish and covered with the Petri dish lid. Flour colour measurements were performed using a Konica Minolta Chrome Meter C R 400 (Sensing IN, Japan), using the L* scale (Oliver *et al.,* 1992). For porridge preparation, a flour to water ratio of 1:5 was used. Porridge was prepared in two stages in order to optimise enzyme activity. Flour (45) g was mixed with 225 g water to make a slurry, which was held at 45^{o}C in a water bath for 15 min. Thereafter, the slurry was then transferred to an electric hot plate where it was cooked for 15 min at 90^{o}C. After cooking, the porridge was poured into 90 mm Petri dishes and left to cool to ambient temperature. Then the lids were placed on the Petri dishes and porridge colour was measured as described for flour.

Statistical analysis

PPO activities were analyzed by analysis of variance using Fischer's Least Significant Difference (LSD) test. Relationships between PPO activity and flour and porridge L* were assessed by linear regression correlation.

RESULTS AND DISCUSSION

Effects of grain type on PPO activity ~~and flour and porridge colour~~

All the sorghum samples had intermediate PPO activity between red wheat and white maize, with activities ranging from 86.8 to 185.5 U/mg (Table 1). This relatively small range is in agreement with the findings of Dicko *et al.* (2002b), but contrasts with the much greater range in the red wheats (229 to 1147 U/mg). The level of PPO activity in the white maize samples was very low (10.5-17.1 U/mg) and there was no significant difference in PPO activity between the samples ($p \geq 0.05$).

Overall, there was no difference in PPO activity between white-tan plant sorghums and the pigmented types, again in agreement with findings of Dicko *et al.* (2002b). However, the actual level of PPO activity found in this study was higher than that found by Dicko *et al.* (2002b), with these authors reporting a maximum activity of approx. 60 U/mg. The difference is possibly due to the authors using 3,4-dihydroxyphenylpropionic acid (DHPPA) as a substrate, whereas L-DOPA was used in this study.

The finding that PPO activity is present in mature sorghum grain is also in agreement with Dicko *et al.* (2002b). However, these findings are in contrast to work by Glennie (1981) where no PPO activity was found in mature red sorghum grain. The probable reason is that this author assayed for PPO activity simply by measuring reduction in absorbance in the UV range. This type of assay is not specific for PPO activity as compound with a phenolic group, even amino acids like tyrosine, absorb in the UV range (Lundblad, 2009).

Concerning the level of PPO activity measured, relative to what may occur during sorghum porridge making in practice, the processes of making sorghum porridges in Africa are highly variable. Some involve straight cooking of meal in water, while others first involve incubation of the meal for up to 3 days at room temperature (approx. 25^{o}C), as in the case of the *ting* porridge of southern Africa (Taylor and Dewar, 2000). These incubations would enable considerable PPO activity, probably much more than in the model system where the incubation was for 15 minutes at 45^{o}C.

Colour of white tan-plant sorghum and white maize flours and porridges

The white tan-plant sorghum whole grain flours were slightly darker than the white maize whole grain flours, mean L* values of 85.38 and 91.57, respectively (Table 2). Visually, both the white tan-plant sorghum and the white maize flours appeared white. With both sorghum and maize the L* values of the porridges were lower than those of the flours, mean L* values of 57.53 and 74.46, respectively. However, significantly the transitions from flours to porridges with the white tan-plant sorghums, resulted in much greater darkening in colour than with the white maize samples, with mean L* value reductions of 27.85 and 17.11, respectively. Visually, the white tan-plant sorghum porridges were buff coloured, whereas the white maize porridges were white in colour. These dark porridges from white tan-plant sorghums have also been observed in work in Botswana (Jagwer, 1998) and Niger (Aboubacar *et al*., 1999). The researchers, however, did not attribute a cause to this. Since the colour change for white tan-plant sorghums was from white flour to a buff coloured

porridge, the colour change when making porridges from red/brown sorghums was not investigated. This is because the 3-deoxyanthocyanin pigments responsible for the strong colour of these sorghums (*Awika et al*., 2004) would have completely masked this change.

Relationships between white tan-plant sorghum flour and porridge colour and PPO activity

Table 3 shows that with the white-tan plant sorghums there were significant negative correlations between PPO activity and flour and porridge L* values, There were also significant positive correlations between flour L* and porridge L* values. For the 2008 cultivation season sorghums, all the parameters were significantly correlated. With the 2009 season sorghums only PPO activity and porridge L* value were significantly correlated. However, considering all the Zambia cultivated white plant-plant sorghums together (n = 20) and the whole set of white tan-plant sorghums (n = 28), all the parameters were correlated, with highly significant negative correlations ($p < 0.001$), $r = -0.710$ and $r = -0.641$, respectively between PPO activity and porridge L value. Their R^2 (coefficient of determination) values indicate that some 40-50% of the variation was accounted for by the relationship. This indicates that the porridge discoloration was related to PPO activity, as with wheat PPO activity and noodle dough discoloration (Baik *et al.*, 1995).

Figure 1 shows that the data points of PPO activity verses porridge L value for all three white tan-plant sorghum data sets (Zambia 2008, Zambia 2009 and commercially cultivated) were well distributed and therefore the data were meaningful. There was a clear negative relationship between PPO activity and porridge L* value in all cases. Figure 1 also reveals that with the Zambia 2008 and 2009 data sets, the same white tan-plant sorghum cultivar, Sima (points A and B) had among the lowest PPO activity and gave the lightest

coloured porridge. In the commercially cultivated data set, cultivar KAT 369 (point C) had among the lowest PPO activity and gave the lightest coloured porridge. Sima (IS 23520) and KAT 369 (KARI/Mtama 1) were both developed by ICRISAT in partnership with national agricultural research institutes in Africa (Bantilan *et al.*, 2004). Conversely, with the Zambia 2008 and 2009 data sets, the same white tan-plant sorghum cultivar, SDS 1958-1-3-2 (points E and D) had among the highest PPO and gave the darkest coloured porridge. The Zambia data clearly indicate that PPO activity in white tan-plant sorghums is genetically controlled, as is the case in other cereals such as wheat (Jimenez & Dubcovsky, 1999) and barley (Quinde-Axtell *et al.*, 2005).

CONCLUSIONS

In view of the relatively high PPO activity of white tan-plant sorghum cultivars in comparison with white maize and the significant negative correlations between white tan-plant sorghum PPO activity and porridge L* value, accounting for 40-50% of the variation, it is clear that PPO activity in white tan-plant sorghums significantly adversely affects the colour of ~~their~~ food products such as porridges. Since PPO activity is genetically controlled, breeding can be used to reduce levels of PPO in white tan-plant sorghum, as done with wheat, to improve the aesthetic appeal of food products from these sorghums and improve their acceptability among consumers. From this first screening of white tan-plant sorghum cultivars, Sima (IS 23520) had low PPO activity and produced light coloured porridge, and thus appears to be useful in such a breeding programme.

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LEGENDS TO FIGURE

Figure 1. Relationships between white tan-plant sorghum PPO activity and porridge colour (L* values) for the three data sets. Diamonds = Zambia controlled field trial 2008, Squares = Zambia controlled field trial 2009, Triangles = Commercially cultivated sorghums. A and B = cultivar Sima, C = cultivar KAT 369, D and E = cultivar SDS 1958-1-3-2.

Table 1. Summary data of polyphenol oxidase activity of different sorghum, maize and wheat cultivars

Cereal	Specific type	Country of cultivation	Polyphenol oxidase activity (U/mg)
Sorghum	**White tan-plant**		
		Zambia 2008 season (n = 10)	
		Minimum	111.8 efgh
		Maximum	185.5 m
		Zambia 2009 season (n = 10)	
		Minimum	106.6 bcdef
		Maximum	184.2 m
		Botswana (n = 4)	
		Minimum	121.1 fghi
		Maximum	157.8 kl
		South Africa (n = 3)	
		Minimum	118.7 fghi
		Maximum	152.6 jk
		Kenya (n = 1)	107.9 cdefg
	Red/Brown tannin		
		Zambia 2008 season (n = 5)	
		Minimum	86.8 b
		Maximum	136.8 ijk
		Zambia 2009 season (n = 5)	
		Minimum	88.2 bc
		Maximum	140.8 ijk
	Red non-tannin		
		Zambia 2008 season (n = 1)	173.7 lm
		Zambia 2009 season (n = 1)	176.3 lm
Wheat	**Red**		
		South Africa (n = 6)	
		Minimum	229.0 n
		Maximum	1147.4 s
Maize	**White**		
		South Africa (n =8)	
		Minimum	10.5 a
		Maximum	17.1 a

Values followed by different letters are significantly different ($p < 0.05$)

Table 2. Summary data of L* colour values of whole grain flour and porridge from white tan-plant sorghum and white maize cultivars

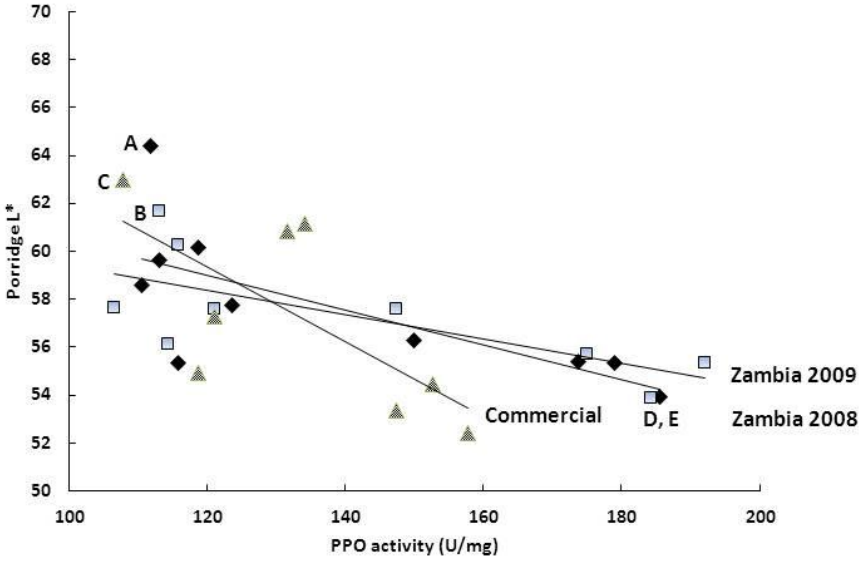
	Flour	**Porridge**
White tan-plant sorghums		
Zambia cultivated 2008 season (n = 10)		
Minimum	82.70 bc	55.33 de
Maximum	87.50 lm	64.40 o
Zambia cultivated 2009 season (n = 10)		
Minimum	82.17 ab	55.36 de
Maximum	86.20 klm	61.65 mn
Botswana cultivated (n = 4)		
Minimum	81.48 a	52.41 a
Maximum	85.79 hi	61.13 mn
South Africa cultivated (n = 3)		
Minimum	82.13 ab	53.37 b
Maximum	83.97 de	54.92 de
Kenya cultivated (n = 1)	88.17 lm	62.99 n
Mean	85.38	57.53 (27.85)
White maize		
South Africa (n =8)		
Minimum	90.40 a	72.94 a
Maximum	92.58 e	77.76 d
Mean	91.57	74.46 (17.11)

Values followed by different letters for white tan-plant sorghum and for white maize within a column are significantly different ($p < 0.05$)
Values in parentheses are reductions in L* value from flour to porridge

Table 3. White tan-plant sorghum correlation matrices for PPO activity, flour colour L* values and porridge colour L* values

Variable	**PPO**	**L* flour**
Zambia 2008 cultivated (n = 10)		
L* flour	-0.876**	
L* porridge	-0.714*	0.830**
Zambia 2009 cultivated (n = 10)		
L* flour	-0.216	
L* porridge	-0.734*	0.494
All Zambia cultivated (n = 20)		
L* flour	-0.524*	
L* porridge	-0.710***	0.674**
All white tan-plant sorghums (n = 28)		
L* flour	-0.489**	
L* porridge	-0.641***	0.750***

Level of statistical significance *p<0.05, **p < 0.01, ***p<0.001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Zambia 2008

Figure 1