Research article

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Influence of sorghum grain type on wort physico-chemical and sensory quality in a whole-grain and commercial enzyme mashing process

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To determine the most suitable types of sorghum for whole-grain adjunct in lager beer brewing, 14 cultivars of five different types: white tan-plant, white non-tan-plant, red non-tannin, white tannin (type II) and red tannin (type III) were evaluated. The effects of grain type on wort physico-chemical and sensory quality with raw grain and malt plus commercial enzyme mashing were assessed. Tannin content correlated significantly and negatively with wort extract and fermentable sugars ($p < 0.001$) and free amino nitrogen (FAN; $p < 0.1$). This is attributable to inactivation of the exogenous enzymes by the tannins during the mashing process. However, the type II tannin sorghums had wort quality attributes closer to the non-tannin sorghum types, probably owing to their relatively low tannin content (≤1%). Malting gave a great improvement in wort extract, fermentable sugars and FAN, but substantially influenced wort sensory properties in terms of higher sourness, bitterness and astringency, as well as the expected more malty flavour. Worts from raw red non-tannin sorghums were similar to those of white tan-plant sorghums in both physico-chemical and sensory quality. Thus, red non-tannin sorghums, in view of their better agronomic quality, have considerable potential as a whole-grain adjunct in lager beer brewing.

Keywords: descriptive sensory profiling; mashing; raw whole-grain adjunct; sorghum; wort

Introduction

Sorghum is used in lager beer brewing as malt and/or raw sorghum *(1)*. The successful research that led to the development of commercial sorghum lager beer brewing focused on enzymes in sorghum malting, sorghum malting technology and sorghum brewing technology *(2)*. However, identification of sorghum types with specific grain characteristics suitable for lager brewing remains a major area of concern *(3,4)*. Currently, sorghum types that differ substantially in chemical composition are used for lager beer brewing in Africa, including white pericarp type II tannin sorghums in Nigeria *(5,6)* and white pericarp, type I non-tannin, tan-plant sorghums in Uganda *(1)*.

Mashing with malted sorghum in lager beer brewing yields a high level of free amino nitrogen (FAN) needed to ensure efficient buffering capacity and optimum yeast performance during fermentation *(7)*. However, a low level of fermentable sugars is produced in sorghum malt mashing, which has been attributed to the high starch gelatinization temperature and low β-amylase activity in sorghum compared with barley *(4)*. In practice, sorghum malt mashing requires addition of exogenous enzymes in order to produce fermentable sugars *(3)*. It has been proposed that sorghum in the form of raw grain rather than malt is a more logical and cost-effective approach *(1,4)*. The following grain quality attributes were highlighted as required specifications for sorghum grain suitable for brewing purposes in raw whole-grain brewing: excellent starch content (>72%), protein content (10.0 ± 1.0%), fat content (3.5 ± 0.5%) and tannin (<1%) *(1)*. Particular reference was also made to small grain size, as it negatively influences the above-mentioned grain quality parameters. In barley malt brewing, cultivar differences are a major cause of variability in wort quality *(8)*. Thus, wort quality in sorghum grain brewing is presumably a very important criterion for determining which types of sorghum are most suitable. The objective of this study was therefore to identify which of the major types of sorghum are most suitable as a whole-grain adjunct when mashing with exogenous enzymes.

Materials and methods

Materials

Fourteen improved sorghum cultivars, mainly southern African types, of five different grain types were used. The grain types are classified as described *(9,10)*. They were as follows: red pericarp, type III tannin (dominant spreader gene) – PAN 3860 and NS 5511 (hybrids) (Hs); red pericarp, type I non-tannin – PAN

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8564, NK 283, SNK and MR Buster (Hs); white pericarp, tan-plant – orbit and BSH1(Hs), Macia and KAT 369 (open pollinating varieties, OPVs); white pericarp, type I non-tannin, non-tan-plant (purple plant) – Mmabaitse and Kanye Standard (OPVs); white pericarp, type II tannin (recessive spreader gene) – Feterita (Sudan) and White Tannin sorghum (Zimbabwe) (OPVs).

The whole sorghum grain samples were milled using a hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 1.0 mm opening screen. The flour samples were stored in zip-lock type polyethylene bags at 6–8 °C until analysis. The commercial enzyme preparations used were Cerezyme 2X Sorghum and Fungamyl 4000 BG, which were kindly donated by Novozymes SA, Marlboro, South Africa.

Methods

Malting

Two sorghum cultivars were selected for malting, Macia and White Tannin sorghum (type II). A standard sorghum laboratory malting method was applied *(11)*. Malt was dried at 50 °C, after which the roots and shoots were removed by putting the whole malt grains in a coarse mesh nylon bag and rubbing to break the roots and shoots off, then sieved through the mesh. Macia malt and White Tannin malt had diastatic powers of 11 and 21 sorghum diastatic units (SDU)/g (peptone extract method), respectively, according to SABS Method 235 *(12)*.

Mashing

Mashing was conducted using a BRF mashing bath (Brewing Research Foundation, Nutfield, UK). Flour samples (100 g dry weight basis) were mixed with 318 mL distilled water containing 222 mg/L calcium chloride (80 ppm) in pre-weighed mashing beakers and pre-heated to 50 °C, to give a grist–liquor ratio of ~1:3. The pH was adjusted to pH 5.6–5.8. Freshly prepared Cerezyme 2X (1590 ppm with respect to sorghum flour) was added, followed by a protein rest for 30 min, at 50^{0}C. The mash was cooked at 85 °C for 45 min and cooled down to 58 °C. Freshly prepared Fungamyl 4000 BG (1590 ppm with respect to sorghum flour) was added to the mash (pH 5.5) and mashed at 58 °C for 60 min. The mash was heated to 72 °C and rested for 15 min or until starch negative as tested by iodine. The mash temperature was raised to 78 °C, cooled and the mash was weighed. The weight was then made up to 450 g with distilled water. The wort was filtered using cheese cloth and the wort filtrate centrifuged at 2700 ***g*** for 10 min, at 4 °C. The clarified wort was stored frozen at −18 °C prior to analysis.

Analyses

Grain quality attributes

Grain size was determined using sieves with screen opening sizes of 4.0 and 2.36 mm *(13)*. The 1000 kernel weight was determined based on the weight of 1000 sound kernels. The hectolitre (test) weight was determined according to AACC Official Method 55-10 *(14)* but using a 500 mL cup and expressed in kg/hL. Endosperm texture was determined by visual estimation of the grain endosperm texture according to ICC Standard 176 *(15)*. Moisture content was determined according to AACC Method 44-15A *(14)*. Protein content was determined by combustion analysis according to AACC Method 46-30 *(14)*. Total phenol content was determined using a Folin–Ciocalteu method *(16)* and expressed in g catechin equivalents (CE)/100 g. Tannin content was determined using a modified Vanillin-HCl method with blank subtraction *(17)* and expressed in g CE/100 g.

Wort quality attributes

Wort extract was determined by specific gravity using a pycnometer according to European Brewery Convention (EBC) Method 4.5.1 Extract of Malt: Congress Mash (AM) *(18)*. Wort fermentable sugar spectrum was determined by EBC Method 8.7 Fermentable Carbohydrates in Wort by HPLC *(18)*. A high-performance liquid chromatograph fitted with refractive index detector and Rezex RHM-Monosaccharide column, 300 × 7.8 mm, Rezex H + (8%) Monos. (Phenomenex, Torrance, CA, USA) was used. The data are expressed as total fermentable sugars, the sum of maltotriose, maltose, glucose and fructose. Wort FAN was determined by the ninhydrin assay according to EBC Method 8.10 Free Amino Nitrogen in Wort by Spectrophometry (IM) *(18)*. To determine wort colour, wort samples were filtered through kieselguhr and the colour determined by tristimulus colorimetry method, using a Minolta colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Japan) in *L* a* b** values. Wort bitterness was determined by EBC Method 9.8 Bitterness of Beer by Spectrophometry *(18)*. Pure *iso*-octane was used as reference standard to zero the instrument. Wort sensory properties were determined by descriptive sensory profiling performed on two white tan-plant, one red non-tannin, one type II tannin, one type III tannin and one malted white tan plant sorghum worts. The sensory profiling procedure followed the generic descriptive method described by Einstein *(19)*. A trained panel of 10 analysed the wort samples. All the panellists were students at the University of Pretoria, with at least 32 h of previous experience with descriptive sensory analysis of sorghum-based products. Before the evaluation, the panellists participated in a training session, during which they were familiarized with the product, generated descriptors and agreed on attribute definitions and assessment criteria.

Statistical analyses

All experiments were repeated at least once. Data were analysed by one-way analysis of variance (ANOVA). Significant differences among the means were determined by Fischer's least significant difference (LSD) test. Pearson correlations were performed to determine relationships between all the parameters measured. Partial least squares regression (PLS) using The Unscrambler X 10.2 (CAMO Software AS, Oslo Norway 2010-2012) was used to investigate the relationship of the wort quality measures plus protein and tannin content of the grains (*x* group variables) with sensory properties (*y* group variables) of the wort, including full cross validation.

Results and discussion

Grain quality attributes

All 14 sorghum cultivars had a very high percentage of medium size grains, ranging between 85.4 and 99.9% (Table 1). The grain size data correspond to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) data *(13)*. This implies that all the sorghum types were of a grain size potentially suitable for beer

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Table 1. Physical and chemical quality attributes of the sorghum cultivars

Sorghum type	Cultivar	Relative percentage medium grain size (2.36–4.00 mm)	Test weight (kg/hL)	1000 kernel weight (g)	Grain endosperm texture (relative percentage)			Protein ($N \times 6.25$) (g/100 g dwb*)	Total phenols (g catechin equiv./ 100 g dwb)	Tannins (g catechin equiv./ 100 g dwb)
					Corneous	Intermediate	Floury			
White tan-plant	BSH1	98.4^{defg}	76.4^{e}	31.2^{i}	90.0^{g}	5.0^{a}	5.0^{abc}	12.95^{j}	0.10^{ab}	0.0^{a}
	Orbit	98.1^{def}	72.6^{c}	29.7^{h}	55.0^{d}	42.5^{g}	2.5^{ab}	8.55^{d}	0.12^{b}	0.0^{a}
	KAT 369	98.3^{def}	77.5^{f}	29.1^{h}	80.0^{ef}	10.0^{ab}	10.0^{c}	9.40^{e}	0.09^{a}	0.03^{abc}
	Macia	98.9^{efg}	78.1^{fg}	20.3^{c}	52.5^{cd}	40.0^{fg}	7.5^{bc}	6.90^{a}	0.12^{b}	0.01^{ab}
White non-tan-plant	Mmabaitse	99.9^{g}	69.6^{a}	23.2^{d}	7.5^{b}	45.0^{gh}	47.5^{f}	14.25^{k}	0.23^{d}	0.18^{abcd}
	Kanye Standard	97.6^{de}	71.9^{bc}	31.9^{i}	5.0^{ab}	77.5^{i}	17.5^{d}	11.25^{i}	0.15^{c}	0.17^{abc}
Red non-tannin	PAN 8564	89.4^{a}	71.3^{b}	16.7^{a}	10.0^{b}	52.5^{h}	37.5^{e}	8.20^{c}	0.33^{f}	0.25^{cde}
	NK 283	93.8^{c}	74.1^{d}	26.7^{g}	85.0^{fg}	15.0^{bc}	0.0^{a}	10.95^{h}	0.30^{e}	0.40^{de}
	SNK	99.4^{fg}	73.6^{d}	24.3^{e}	47.5^{c}	42.5^{g}	10.0^{c}	12.80^{j}	0.28^{e}	0.20^{bcd}
	MR Buster	97.2^{d}	78.5^{g}	29.1^{h}	77.5^{e}	20.0^{cd}	2.5^{ab}	10.50^{g}	0.21^{d}	0.25^{cde}
White tannin (type II)	Feterita	91.9^{b}	69.3^{a}	24.1^{e}	0.0^{a}	27.5^{de}	72.5^{h}	15.10^{l}	0.37^{g}	0.46^{e}
	White Tannin	99.3^{efg}	72.2^{c}	23.0^{d}	0.0^{a}	27.5^{de}	72.5^{h}	7.25^{b}	0.75^{h}	1.04^{f}
Red tannin (type III)	NS 5511	98.3^{def}	72.2^{c}	19.3^{b}	7.5^{b}	42.5^{g}	47.5^{f}	10.85^{h}	1.22^{i}	3.82^{g}
	PAN 3860	95.2^{c}	71.8^{bc}	25.5^{f}	5.0^{ab}	32.5^{ef}	62.5^{g}	10.25^{f}	1.34^{j}	4.53^{h}

* Dry weight basis; mean values in the same column but with different letters are significantly different ($p < 0.05$).

brewing, as recommended *(1)*. The thousand kernel weight (TKW) and the test weight (TW) properties for all the sorghum types ranged from 16.7 to 31.9 g and 69.3 to 78.5 kg/hL, respectively, similar to TKW and TW commercial hybrid sorghum data recently reported *(20)*. Thus, grain size was not a factor that influenced the findings. Endosperm hardness, as estimated on the relative proportions of corneous to floury endosperm of these sorghum types, revealed that most of the non-tannin sorghum types had a hard (corneous) endosperm. The tannin sorghum types had a predominantly soft (floury) endosperm texture, as expected. Tannin sorghum grains generally have a soft endosperm as the tannins serve as a defence mechanism against mould attack *(21)*.

Grain protein content ranged between 6.9 and 15.1 g/100 g (Table 1). This very large variation between the sorghum types was valuable to determine the influence of protein content on wort quality. However, there was no clear difference in protein content between the types. Total phenol content also varied widely and distinguished clearly between the non-tannin types (0.09–0.33 CE g/100 g) and the tannin types (0.37–1.34 g CE/100 g). Tannin content of the sorghum types varied very widely and distinguished clearly between all five types. The range was from 0 g CE 100 g in the white tan-plant types, to <0.2 in the white non-tan-plant (purple plant) types, to 0.2–0.4 in the red non-tannin types, to 0.5–1.0 in the white type II tannin types, to 3.8–4.5 in the red type III tannin types. It should be noted that the vanillin–HCl assay is not entirely specific for condensed tannins and that non-tannin polyphenols are also measured to a limited extent *(22)*. Hence, the tannin data additionally give an indication of the levels of other polyphenols in the sorghums.

Wort quality attributes

Wort extract from raw grains of the sorghum types also varied widely, from 63.8 to 81.0% (Table 2). The red type III tannin sorghums gave the lowest wort extract. In fact, wort extract was highly negatively correlated with tannin content ($p < 0.001$, $r = -0.846$; Table 3). Low extract yield of the type III tannin sorghum types was primarily owing to interaction between condensed tannins and the amylase enzymes during mashing *(23)*. Condensed tannins also complex irreversibly with the sorghum grain kafirin storage proteins *(24)* and this may have also contributed to poor starch hydrolysis in the type III tannin sorghum types. The white tannin (type II tannin) sorghum gave similar extract to the non-tannin sorghums and this was probably related to its low protein content (7.25 g/100 g; Table 1). Notably, all of the white non-tan-plant and red non-tannin sorghums gave extracts in the same range as the white tan-plant sorghums. Further, Kanye Standard (white non-tan-plant) and PAN 8564, NK 283 and MR Buster (red non-tannin) gave similar extracts (78.5–80.9%) to the three highest white tan-plant sorghums (79.6–81.0%).

The level of total fermentable sugars in the worts also varied widely, from 5.0 to 10.8 g/100 mL (Table 2). The type III tannin sorghums gave the lowest fermentable sugars. This is also an indication of tannin inactivation of amylase enzymes during mashing *(23)*. Also, as with extract, grain tannin content correlated highly significantly and negatively with wort fermentable sugars ($p < 0.001$, $r = -0.810$; Table 3). However, the white type II tannin sorghums, as well as the red non-tannin and white non-tan-plant sorghums, all gave similar total fermentable sugars to the white tan-plant types (Table 2).

Table 2. Wort quality attributes from the different sorghum cultivars

Sorghum type	Sorghum cultivar	Extract (percentage dwb*)	Total fermentable sugars (g/100 mL)	FAN (mg/L)	Colour			Bitterness (BU)
					*L**	*a**	*b**	
White tan-plant (WTP)	BSH1	75.5^{c}	9.66^{cde}	73.5^{e}	58.7^{e}	0.1^{a}	3.4^{abc}	1.7^{abc}
	Orbit	81.0^{gh}	9.08^{cd}	69.6^{de}	56.4^{bcd}	0.7^{abcd}	4.5^{abcde}	2.7^{d}
	KAT 369	79.6^{efg}	8.64^{c}	62.3^{d}	57.8^{cd}e	0.1^{a}	2.5^{ab}	1.5^{abc}
	Macia	80.8^{fgh}	9.86^{cde}	65.4^{de}	55.9^{bcd}	0.5^{abc}	5.5^{bcde}	3.7^{e}
White non-tan-plant	Mmabaitse	76.2^{c}	8.86^{cd}	139.1^{g}	52.7^{a}	2.7^{g}	5.9^{cde}	3.8^{e}
	Kanye Std	79.9^{efg}	10.79^{ef}	68.2^{de}	56.9^{bcde}	1.1^{cde}	3.5^{abcd}	2.1^{bcd}
Red non-tannin	PAN 8564	80.9^{fgh}	9.77^{cde}	50.2^{c}	55.1^{b}	2.3^{g}	7.1^{e}	1.7^{abc}
	NK 283	78.5^{de}	9.51^{cde}	42.2^{bc}	55.7^{bc}	1.4^{ef}	6.5^{cde}	1.6^{abc}
	SNK	75.9^{c}	10.36^{def}	104.9^{f}	56.5^{bcd}	0.9^{bcde}	4.5^{abcde}	1.4^{abc}
	MR Buster	79.0^{def}	10.73^{ef}	50.7^{c}	57.4^{cde}	0.7^{abcd}	3.3^{abc}	2.3^{cd}
White tannin (type II)	Feterita	72.2^{b}	8.73^{c}	63.9^{de}	56.8^{bcde}	0.7^{abcde}	2.0^{a}	1.6^{abc}
	White Tannin	77.1^{cd}	9.35^{cde}	43.6^{bc}	58.6^{e}	1.3^{cde}	3.9^{abcde}	1.8^{abc}
Red tannin (type III)	NS 5511	70.4^{b}	7.04^{b}	37.4^{ab}	52.5^{a}	2.4^{g}	6.7^{de}	1.0^{a}
	PAN 3860	63.8^{a}	4.95^{a}	31.2^{a}	57.9^{de}	1.3^{de}	5.4^{bcde}	1.3^{ab}
WTP	Macia (malted)	84.5^{i}	12.43^{g}	196.1^{i}	57.2^{cde}	0.2^{ab}	5.6^{bcde}	2.2^{cd}
Type II	White Tannin (malted)	81.9^{h}	11.34^{fg}	180.4^{h}	57.3^{cde}	2.1^{fg}	4.9^{bcde}	1.5^{abc}

* Dry weight basis; mean values in the same column but with different letters are significantly different ($p < 0.05$).
FAN, free amino nitrogen.

Wort FAN varied very widely between the sorghum grain types, from 31 to 139 mg/L (Table 2). However, the FAN levels were low (<74 mg/L), with the exception of Mmabaitse and SNK, in agreement with other work on mashing with 100% raw sorghum *(7)*. The low wort FAN is attributable to poor protein hydrolysis during mashing. Poor protein hydrolysis is related to the intrinsic structure of the sorghum kafirin storage protein *(25)*. An earlier study in our laboratory showed that kafirin cross-linking by disulphide bonding was a major factor contributing to poor sorghum protein digestibility *(26)*.

The type III tannin sorghum cultivars gave the lowest FAN levels (Table 2). This is due to high levels of interaction between condensed tannins and protein *(24)*. FAN somewhat negatively correlated with tannin content ($p < 0.1$, $r = -0.498$) (Table 3). However, Feterita (a white type II tannin sorghum) gave a FAN level similar to the white tan-plant sorghums, presumably owing to its high protein and relatively low tannin contents (Table 1). The high level of FAN from Mmabaitse (white no tan-plant) and SNK (red non-tannin) is probably linked to their high protein contents (14.3 and 12.8 g/100 g), respectively, as shown by a sign slightly significant positive correlation ($p < 0.1$, $r = 0.511$) between grain protein content and wort FAN (Table 3).

Wort colour *L** value (lightness) varied only slightly, from 52.5 to 58.7 (Table 2). There was no clear effect of grain type. It was observed that the spent grain from the red non-tannin and red type III tannin sorghum types was highly coloured. Adsorption of the grain colour pigments to the spent grain presumably explained why the sorghum type did not affect wort *L** value. The wort colour *a** value (redness-greenness) and *b** value (yellowness-blueness) ranged from 0.1 to 2.7 and from 1.4 to 7.1, respectively. The white tan-plant sorghum types generally gave lower wort colour *a** values (0.1–0.7) than the other sorghum types. This is presumably due to white tan-plant sorghums not containing anthocyanin pigments *(27)*. Wort colour *b** value was not obviously affected by sorghum type.

Wort bitterness ranged between 1.0 and 3.8 BU (Table 2). Surprisingly, the tannin sorghum types generally gave lower wort bitterness (1.0–1.8 BU) than the non-tannin types (1.4–3.8 BU). This result was not expected as phenolics in beer contribute significantly to the bitterness attribute *(28)*. Also, the trends in wort bitterness in this study did not agree with findings of a study using sorghum bran infusions *(29)*. This work showed that infusions from tannin sorghum cultivars were more bitter than those from the non-tannin sorghums, as perceived by a trained sensory panel. Also, the wort bitterness results do not agree with the sensory data in this present work (Table 4), which showed that the wort from the type III tannin sorghum was perceived as being significantly more bitter ($p < 0.05$; see next section). The probable reason for these anomalous results is that iso-octane is too non-polar to be a good solvent for the sorghum condensed tannins. These are normally extracted with more polar organic solvents such as aqueous acetone or acidified methanol *(30)*. Therefore the EBC Bitterness Method *(18)* appears not to be applicable to determination of bitterness owing to sorghum condensed tannins.

Malting the sorghums increased wort extract by ~5%, total fermentable sugars by >20% and FAN 2- to 3-fold (Table 2). The improvement in extract was probably due to endosperm modification as a result of malting. Higher sorghum extract yield with malting agrees with findings of other work which reported higher wort extract in malted sorghum mashed with exogenous enzymes compared with unmalted sorghum *(3)*. The large increase in fermentable sugars is undoubtedly due to the combined activity of the malt amylase enzymes and the exogenous amylases. Similarly, the very large increase in FAN was due to the combination of malt and exogenous protease enzymes *(31,32)*.

Wort sensory quality attributes

The trained panel detected significant differences ($p < 0.05$) between the sorghum worts for all the sensory attributes (Table 4).

Table 3. Correlation matrix of sorghum grain and wort quality attributes[a]

	Corn.	Interm.	Floury	Med.	TW	TKW	Protein	Tannin	TP	Extract	*L**	*a**	*b**	FAN	Bitter
Interm.	−0.640**														
Floury	−0.846****	0.132													
Med.	0.195	−0.033	−0.232												
TW	0.808****	−0.486*	−0.706***	0.319											
TKW	0.498*	−0.228	−0.478*	0.304	0.310										
Protein	−0.076	−0.076	0.150	−0.026	−0.383	0.233									
Tannin	−0.445	0.046	0.530*	−0.056	−0.277	−0.283	−0.042								
TP	−0.543**	0.00.047	0.657**	−0.092	−0.369	−0.394	0.078	0.968****							
Extract	0.404	0.137	−0.611**	0.075	0.375	0.133	−0.377	−0.845****	−0.819****						
*L**	0.229	−0.392	−0.104	0.064	0.343	0.524*	−0.172	−0.139	−0.139	0.027					
*a**	−0.594**	0.461*	0.440	−0.220	−0.614**	−0.627**	0.081	0.362	0.439	−0.209	−0.767***				
*b**	−0.177	0.275	0.032	−0.208	−0.195	−0.541**	−0.220	0.297	0.317	−0.100	−0.656**	0.713***			
FAN	0.002	0.208	−0.140	0.423	−0.183	0.079	0.511*	−0.499*	−0.508*	0.233	−0.292	0.109	−0.073		
Bitter	0.032	0.232	−0.195	0.331	0.080	−0.039	−0.100	−0.439	−0.467*	0.434	−0.200	0.029	0.011	0.548**	
TFS	0.342	0.125	−0.519*	0.112	0.298	0.210	−0.020	−0.810****	−0.764***	0.768***	0.162	−0.230	−0.206	0.331	0.264

[a]Correlation matrix excludes the malted samples data.
Significant at * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$ and **** $p < 0.001$, respectively. TW, Test weight; TKW, thousand kernel weight; TP, total phenols; FAN, free amino nitrogen; TFS, total fermentable sugars; Corn., Corneous; Interm., Intermediate; Med., Medium grain size.

Table 4. Sensory properties of wort from the different sorghum types, as evaluated by a descriptive sensory panel

Sensory attributes	Turbidity	Cooked sorghum smell	Malty aroma	Viscosity	Sweet	Sour	Bitter	Malty flavour	Cooked sorghum flavour	Sweet Aftertaste	Duration of sweet aftertaste	Astringent aftertaste
White tan-plant (Macia)	58.4^{c} (19.0)	28.9^{b} (23.2)	35.1^{bc} (25.4)	27.5^{b} (15.3)	49.6^{cd} (19.3)	3.1^{ab} (2.7)	1.5^{a} (0.9)	34.9^{ab} (22.8)	27.1^{ab} (22.5)	35.4^{c} (18.5)	30.4^{bc} (22.6)	8.2^{ab} (6.5)
White tan-plant (BSH-1)	24.4^{a} (15.7)	21.7^{a} (18.2)	25.4^{a} (19.0)	13.8^{a} (11.2)	44.3^{c} (16.6)	1.1^{a} (0.8)	0.9^{a} (0.7)	28.5^{a} (21.7)	26.9^{ab} (19.3)	27.1^{b} (20.5)	24.4^{ab} (22.6)	8.7^{ab} (10.2)
Red non-tannin (PAN 8560)	49.8^{b} (17.1)	29.1^{b} (22.5)	28.7^{ab} (26.9)	24.5^{b} (18.1)	49.1^{cd} (18.5)	2.5^{a} (2.3)	2.1^{a} (2.0)	30.9^{a} (27.9)	29.6^{b} (19.5)	30.7^{bc} (21.1)	28.3^{b} (23.5)	5.5^{a} (4.5)
White type II tannin	43.6^{b} (14.3)	27.9^{b} (19.8)	47.8^{de} (26.5)	22.6^{b} (10.9)	53.3^{d} (20.6)	4.2^{ab} (5.3)	1.6^{a} (1.2)	42.5^{b} (28.7)	27.3^{ab} (23.2)	29.6^{bc} (20.7)	25.4^{b} (23.1)	11.1^{bc} (7.9)
Red type III tannin (PAN 3680)	24.9^{a} (15.3)	27.9^{b} (23.1)	39.7^{cd} (26.4)	14.1^{a} (10.3)	19.9^{a} (11.9)	8.5^{bc} (8.0)	5.8^{b} (3.3)	35.4^{ab} (28.2)	20.7^{a} (19.6)	14.1^{a} (11.5)	18.6^{a} (16.5)	11.9^{bc} (11.1)
Malted Macia	71.8^{d} (21.9)	39.2^{c} (24.1)	54.2^{e} (26.7)	37.8^{c} (25.2)	35.6^{b} (16.2)	9.4^{c} (7.5)	9.9^{c} (5.6)	52.9^{c} (25.8)	38.5^{c} (21.6)	25.1^{b} (19.9)	35.2^{c} (23.9)	14.1^{c} (11.0)

Scale: 0 = not intense and 100 = very intense; mean values in the same column but with different letters are significantly different ($p < 0.05$); values in parentheses are standard deviations ($n = 3$).

Notably, the wort from the type III tannin sorghum was perceived to have low sweetness, sweet aftertaste and short sweet aftertaste duration. It was more bitter than the non-tannin raw grain worts. These findings were expected owing to presence of a high level of condensed tannin in the grain (Table 1). Surprisingly, the wort from the white tan-plant sorghum malt (cultivar Macia) was more bitter, with an equally astringent aftertaste and as sour as the wort from the type III tannin sorghum, although it had the expected higher malty aroma and flavour. The bitterness may be a result of caramelization and browning reactions, which take place during drying of the green malt *(33)*. This is likely since its wort had the highest malty aroma and flavour. The sourness was probably from lactic acid produced by lactic acid bacteria, which are very common in sorghum malting *(5)*.

The worts from the red non-tannin sorghum grain (cultivar PAN 8564) and the two white tan-plant sorghum grains were similarly sweet with low bitterness, sourness and astringent aftertaste (Table 4). This was despite the fact that the former grain contained three times more total phenols (Table 1). Interestingly, the wort from the type II tannin sorghum grain had a similar sweetness profile and low bitterness to worts from the two white tan-plant sorghums, despite the fact that it contained 1% tannins (Table 1). However, probably because of this, its astringent aftertaste was similarly as strong as the wort from type III tannin sorghum, in agreement with a previous report *(27)*, which showed that tannin sorghums were more astringent than non-tannin sorghums. PLS regression was applied to identify how the wort quality measures plus protein and tannin content of the grains (*x* group variables) predict sensory properties (*y* group variables) of the wort. The PLS regression variances for four PLS factors including the 12 sensory attributes and the 10 physico-chemical attributes are presented in Table 5. Figure 1 shows the PLS maps. Factor 1 explained 48% of the variance in samples owing to the *x* variables and 39% of the variance owing to the sensory properties (*y* variables). Factor 1 separated the sorghums left to right from the type III tannin sorghum (PAN 3680) to malted Macia (the white tan-plant cultivar) based on percentage extract, total fermentable sugars, tannin content, total phenols and protein content of grains. The sensory properties that best explained the differences in samples on Factor 1 were longer duration of sweet aftertaste, turbidity, more intense cooked sorghum flavour and more viscous worts for the samples to the left of the plot. Factor 2 explained an additional 10% of the variance in samples owing mainly to two X variables, FAN and total phenol content. Factor 2 explained an additional 49% of the variance in samples owing to the sensory properties with malted Macia and PAN 3680 at the top described as more bitter, sour, astringent with more malty aroma and flavour (particularly malted Macia) and cooked sorghum aroma, while the sorghum types lower on the plot (the white type II tannin, the two white tan-plant types and the red non-tannin type) were characterized by more intense sweet taste and aftertaste. Overall, four sensory properties were relatively well predicted by the measured wort quality attributes plus protein, tannin and total phenol content of the grains (root mean square error (RMSE)/regression coefficient r^2): viscosity = 1.55/0.96, turbidity = 4.61/0.93, duration of sweet aftertaste = 0.29/0.99 and cooked sorghum flavour = 2.00/0.86.

Table 5. Partial least squares regression used to study relationships between the sorghum wort quality measures plus protein and tannin content of the grains (*x*-block) variables and sensory properties (*y*-block) of sorghum worts

	Percentage explained variances			
	Factor 1	Factor 2	Factor 3	Factor 4
x-Block variables				
Extract	97.2	97.9	97.9	97.9
*L**	23.9	25.4	95.3	99.1
*a**	5.4	10.0	72.6	78.3
*b**	5.9	10.4	95.3	98.6
FAN	44.2	81.0	90.9	99.1
Bitterness	40.4	41.9	43.0	44.7
Total fermentable sugars	89.3	89.4	95.9	96.4
Protein	37.4	47.7	64.4	99.7
Tannin content	76.7	92.6	97.7	99.2
Total phenol content	60.9	82.6	97.5	97.6
Cumulative explained *x*-block variance	48.1	57.9	85.1	91.1
y-Block variables				
Turbidity	79.1	92.7	97.4	99.9
Cooked sorghum porridge aroma	31.7	94.0	98.2	98.8
Malty aroma	5.4	69.0	72.0	98.9
Viscosity	74.7	96.4	98.3	99.9
Sweet	34.4	84.6	85.1	98.3
Sour	1.2	99.0	99.4	100.0
Bitter	1.9	95.3	95.3	98.8
Malty flavour	18.4	81.4	85.6	98.0
Cooked sorghum flavour	72.6	85.6	87.9	89.9
Sweet aftertaste	56.0	93.1	93.3	98.2
Duration of sweet aftertaste	95.6	99.7	99.7	100.0
Astringent aftertaste	0.6	74.3	95.7	99.9
Cumulative explained *y*-block variance	39.3	88.8	92.3	98.4

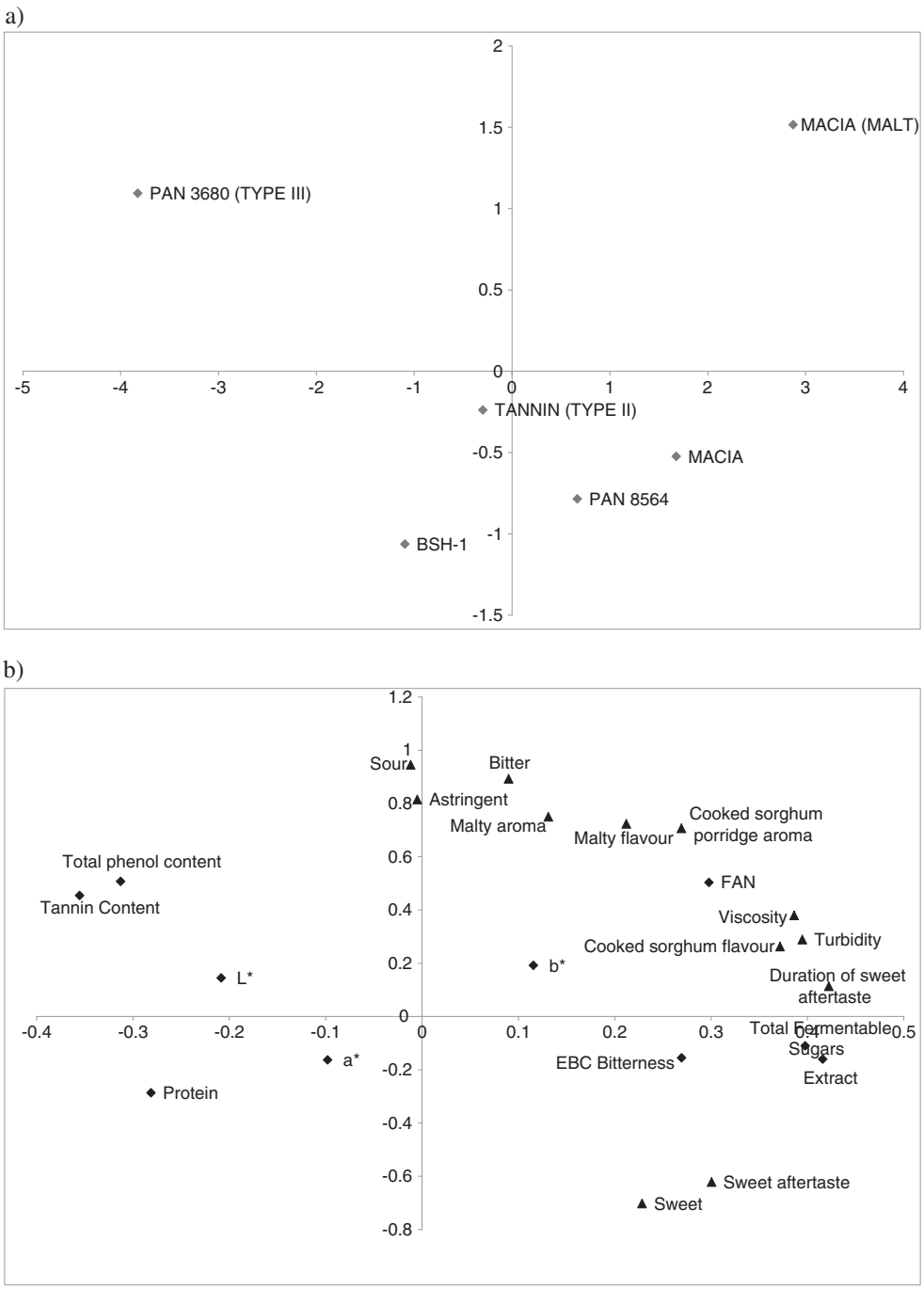

Figure 1. PLS regression plots of factors 1 and 2 showing sorghum samples: (a) sample scores and (b) loadings for sensory properties and wort quality measures plus protein and tannin content of the grains of the wort.

Conclusions

This study shows that the tannin property is the major sorghum grain quality attribute that determines suitability of sorghum types in whole grain and commercial enzyme type lager beer brewing. Tannins in sorghum strongly negatively affect major wort quality attributes such as extract, fermentable sugars and FAN, as a result of enzyme inactivation. Thus, the high tannin content of type III tannin sorghums renders them unsuitable for lager brewing without either treatment to inactivate the tannins *(34)* or admixing with a high proportion of non-tannin sorghum. The situation with regard to the type II tannin sorghums is not so clear. The findings suggest that there could be a tannin threshold level involving other factors, such as protein content, below which enzyme activity is not significantly inhibited and whereby this type of sorghum could be used without either treatment to inactivate the tannins or admixing. Concerning the red non-tannin sorghum type, it has better agronomic characteristics than white tan-plant type sorghum *(35)* owing to the presence of substantial levels of flavonoids *(27)*. In view of this and the finding that there was no apparent difference in wort quality parameters and sensory attributes

compared with the white tan-plant sorghum type, red non-tannin sorghum appears to have excellent potential in whole sorghum grain and commercial enzyme type lager brewing. However, further study is necessary in regard to comparative fermentability and final product quality of suitable cultivars of both white tan-plant and red non-tannin sorghum types.

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