

## 2. RESEARCH

The research was in three parts, which addressed the objectives as stated in section 1.5.

- 2.1. Effects of phenolics in sorghum grain on its bitterness, astringency and other sensory properties
- 2.2. Bitterness and astringency of bran infusions of tannin-free and tannin sorghums determined using the dual attribute time intensity sensory method
- 2.3. Consumer acceptability of sorghum rice from tannin and tannin-free sorghums and the influence of PROP taster status

## **2.1. Effects of phenolics in sorghum grain on its bitterness, astringency and other sensory properties**

### **2.1.1. Abstract**

Despite the fact that condensed tannins are potentially important antioxidants, there is a general belief that tannins in sorghum confer objectionable sensory attributes. The objective of this study was to determine differences in the sensory attributes of sorghums containing different levels of total phenolic compounds. A trained sensory panel described and quantified the sensory attributes of sorghum products from different sorghums (tannin-containing and tannin-free). All the sorghum cultivars were perceived as both bitter and astringent. Bran infusions of tannin sorghums were perceived as darker, clearer, more bitter and more astringent than those of the tannin-free sorghums, whilst those of tannin-free sorghums were perceived as sweeter and cloudy. Sorghum whole grain rice from the tannin sorghums, PAN 3860 and Ex Nola 97 GH, which had relatively soft endosperm texture was perceived as dark, hard, chewy, bitter and astringent, whilst that from tannin-free sorghums, Segalane and Phofu, having relatively hard endosperm texture, was perceived as soft, sweet and had a maize-flavour. Surprisingly, the bitterness and astringency, as well as other sensory attributes of another tannin sorghum, NS 5511, were perceived as similar to a tannin-free sorghum, PAN 8564, even though NS 5511 had more than twice the total phenol content of PAN 8564. This suggests not all tannin-containing sorghums have objectionable sensory attributes.

**Key Words:** Bitterness; Astringency; Phenolics; Sorghum; Sensory analysis

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### 2.1.2. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the second most important cereal crop in Africa after maize, with production levels of 22.5 million metric tonnes and 47.7 million metric tonnes in 2005 respectively (FAOSTAT, 2006). Sorghum is prepared into a very wide range of food and beverage products. It is also a rich source of phytochemicals such as phenolic compounds (tannins, anthocyanins and phenolic acids), which are located mainly in the bran (Awika and Rooney, 2004). According to Dykes and Rooney (2006) all sorghums contain phenolic acids, most contain flavonoids and cultivars with a pigmented testa have condensed tannins. Phenolic acids exist as free forms mainly in the bran and bound forms esterified to cell wall polymers (Hahn, Faubion and Rooney, 1983). Some of the phenolic acids that have been identified in sorghum include gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, ferulic, caffeic, *p*-coumaric, and cinnamic acids (Hahn *et al.*, 1983). Some of the anthocyanins found in sorghum include apigeninidin, luteolinidin and their derivatives (Awika, Rooney and Waniska, 2004a). The types of tannins found in sorghums are of the condensed type consisting of polymerized flavan-3-ol and/or flavan-3,4-diols (Dykes and Rooney, 2006). According to Awika and Rooney (2004) sorghums vary widely in their phenolic composition and content due to genetics and environmental factors affecting the type and level of phenolic compounds. Sorghums can be broadly classified by both appearance and total extractable phenols as follows: white tan plant sorghums with no detectable tannins or anthocyanins and very low extractable phenol levels; red sorghums which have no tannins but have a red pericarp and significant levels of extractable phenols; black sorghums with a black pericarp and very high levels of anthocyanins; and tannin sorghums which have a pigmented testa and contain significant levels of condensed tannins with varying degrees of pericarp pigmentation (Awika and Rooney, 2004).

In eastern and southern Africa, traditional sorghum cultivars of moderate tannin content are widely grown and used for staple food and alcoholic beverages (Awika and Rooney, 2004). The agronomic advantages of these cultivars outweigh any negatives such as reduced nutrient availability or astringency. Thus, in southern Africa small farmers intercrop tannin and tannin-free sorghums in areas prone to bird predation in order to reduce grain losses in the field. Some African cultures also prefer tannin sorghums

because the porridge from these sorghums ‘remains in the stomach longer’ and the farmer feels full for most of the day working in the field.

Sorghum bran fractions possess high antioxidant activity *in vitro* relative to other cereals and fruits. Thus they may offer similar health benefits commonly associated with fruits (Awika, Rooney and Waniska, 2004b). Phenolic compounds and their role as antioxidants have been linked to lower incidences of certain forms of cancer and coronary heart diseases (Chung, Wong, Wei, Huang and Lin, 1998). Furthermore, Lakshmi and Vimala (1996) reported significantly lower plasma glucose levels in diabetic subjects after consuming whole grain sorghum foods when compared with consuming decorticated sorghum and wheat foods. However, as phenolic compounds are responsible for the bitterness and astringency of many foods and beverages, they may be aversive to the consumer (Drewnowski and Gomez-Carneros, 2000). The objective of this study was to determine differences in the bitterness, astringency and other sensory attributes of sorghums containing different levels of total phenolic compounds using a trained sensory panel.

### **2.1.3. Materials and methods**

#### **2.1.3.1. Materials**

Six sorghum cultivars were used. Three were tannin sorghums with red pericarp (PAN 3860, Ex Nola 97 GH and NS 5511) and three were tannin-free sorghums, one with a red pericarp (PAN 8564), and two with white pericarp (Segaolane and Phofu). Segaolane and Phofu are open-pollinating cultivars grown in Botswana in 2004, whereas the other four (Ex Nola 97 GH, PAN 8564, PAN 3860, and NS 5511) are hybrids grown in South Africa in 1997, 1999, 2004 and 2004 respectively.

#### **2.1.3.2. Grain characterization**

The pericarp colour of the sorghum kernels was determined by placing them on a white plate and classifying them according to the categories given by Rooney and Miller (1982). Pericarp thickness was determined by the visual examination of a kernel cut longitudinally (Rooney and Miller, 1982). Glume colour was determined by examining the inside of the glume after removing the kernel (Rooney and Miller, 1982). The presence of a pigmented testa was determined using the bleach test as described by Taylor (2001). Endosperm

texture was determined subjectively by visually assessing the relative proportion of corneous to floury endosperm using a scale of 1 (corneous) to 5 (floury) essentially as described by Rooney and Miller (1982). Grain hardness was determined by measuring the decortication yield of 40 g grain decorticated for 4 minutes in a Tangential Abrasive Dehulling Device (TADD; Reichert, Youngs and Oomah, 1982) fitted with a 60 grit sand paper (Norton R284 metalite, Saint-Gobain Abrasives, Isando, South Africa).

#### 2.1.3.3. Bran isolation

Sorghum grain was washed several times with tap water to remove dust, dirt and debris and spread on trays lined with white paper towel and dried in a fume cupboard for 24-36 h. Dried grain was decorticated in a Prairie Research Laboratory (PRL) type dehuller (Rural Industries Innovation Centre, Kanye, Botswana) for 3-4 min. The decorticated grain was sieved manually using a sieve (1400 µm open size) to recover the bran. The sorghum bran was vacuum-packed in food grade polyethylene bags and stored at -18°C until analysis (between one and six months after bran isolation).

#### 2.1.3.4. Determination of phenolics

Total phenolics in the sorghum whole grain, sorghum bran and sorghum bran infusions were extracted with 75% acetone and determined using the Folin-Ciocalteu method as described by Waterman and Mole (1994). Tannic acid (Gallo tannin, 48811 Fluka/Sigma-Aldrich, Atlas Ville, South Africa) was used as a standard. Condensed tannins were extracted with acidified methanol and the vanillin-HCl method with blank subtraction was used to determine the content of condensed tannins in the sorghum grain as described by Price, Van Scoyoc and Butler (1978). Catechin ((+)-Catechin Hydrate, 22110 Fluka/Sigma-Aldrich, Atlas Ville, South Africa) was used as a standard.

#### 2.1.3.5. Descriptive sensory panel selection and training

Twelve panellists (six women and six men) aged 19-39 years were selected from a pool of 42 people after undergoing screening tests. The screening tests included the basic taste test, the PROP test and threshold tests. The one-solution PROP test developed by Tepper, Christensen and Cao (2001) was used to eliminate panellists who could not taste bitterness. A triangle test was used for the threshold tests: two samples with water and an odd sample with a basic taste solution. The concentrations used in the threshold tests were: sour (0.02 and 0.04% citric acid), bitter (0.02 and 0.03% caffeine), salty (0.08 and

0.15% NaCl), sweet (0.4 and 0.6% sucrose) and umami (1.0 and 2.0% mono sodium glutamate [MSG]) dissolved in deionized water. The panellists signed a consent form prior to the training and assessment of the samples, informing them of the nature of the sorghum samples that they would evaluate. The descriptive sensory panel was trained for 1 h a day for a period of three weeks (Fig. 2.1). The training sessions included familiarizing the panellists with the assessment procedures, the computer system and sensory evaluation software (Compusense® Five release 4.6 [1986-2003] Guelph, Ontario Canada) and the sorghum products (sorghum bran infusions and sorghum rice).



**Figure 2.1.** A training session of the descriptive sensory panel.

The panellists were also trained to differentiate between bitterness, sourness and astringency using standards (dissolved in deionised water) and concentrations used by Kallithraka, Bakker and Clifford (1997a): bitterness (1.0 g/l caffeine; food grade), sourness (1.5 g/l citric acid; NCP Food Ingredients, Isipingo Beach, South Africa) and astringency (1.5 g/l tannic acid [Gallotannin]; 48811 Fluka/Sigma-Aldrich, Atlas Ville,

South Africa). Potassium aluminium sulphate [alum] (Fluka/Sigma-Aldrich, Atlas Ville, South Africa) was also used to familiarize the panellists with the astringency sensation using the concentration (0.5 g/l) recommended in ISO 8586 (International Organization for Standardization, 1993). Subsequently, the panellists assessed and described the appearance, aroma, flavour, and mouth-feel attributes of the sorghum bran infusions and the sorghum rice. From the descriptive sensory panel's discussions, descriptive lexicons were developed for the appearance, aroma, flavour and mouth-feel attributes of the sorghum bran infusions (Table 2.1) and sorghum rice (Table 2.2).

**Table 2.1.** Sensory properties of bran infusions from different sorghum cultivars

Sensory Attribute	Definition	Rating scale
Colour	Degree of colour intensity ranging from cream white to dark amber/brown	Light = 1 and Dark = 10
Cloudiness	Degree of cloudiness/opaqueness of solution – cannot see through the solution	Not cloudy/Clear = 1 and Very cloudy = 10
Fruity aroma	Mild sweet and fruity smell	Not intense = 1 and Very intense = 10
Herbal aroma	Smells like grass, bran, herbal tea, straw-like, hay, wheat bran flakes	Not intense = 1 and Very intense = 10
Sweet	Basic sweet taste associated with sucrose	Not intense = 1 and Very intense = 10
Sour	Basic sour taste associated with acidic solutions like citric acid and fermented products like sorghum beer	Not intense = 1 and Very intense = 10
Bitter	Basic bitter taste associated with caffeine and other bitter compounds; bitterness lingers long like an aftertaste	Not intense = 1 and Very intense = 10
Herbal flavour	Herbal flavour (like – bran, herbal tea, yam, malted sorghum porridge and oats).	Not intense = 1 and Very intense = 10
Astringency	A sensation that lingers and coats, dries and numbs the mouth, palate and tongue.	Not intense = 1 and Very intense = 10

**Table 2.2.** Sensory properties of rice from different sorghum cultivars

Sensory Attribute	Definition	Rating scale
Colour	Degree of colour intensity ranging from cream white to dark amber/brown	Light = 1 and Dark = 9
Black specks	Number of black specks on the sorghum rice	Few <sup>1</sup> = 1 and Many <sup>2</sup> = 9
Split kernels	Number of split kernels	Few <sup>1</sup> = 1 and Many <sup>2</sup> = 9
Lumpy	Number of swollen and clustered/clumped together kernels	Few <sup>1</sup> = 1 and Many <sup>2</sup> = 9
Cooked cereal aroma	Smells like cooked cereal	Not intense = 1 and Very intense = 9
Chewy	Length of time required to chew the sorghum rice before swallowing	Not chewy = 1 and Very chewy = 9
Texture (Soft/Hard)	Force required to chew the sorghum rice before swallowing	Soft = 1 and Hard = 9
Sweet	Basic sweet taste associated with sucrose	Not intense = 1 and Very intense = 9
Bitter	Basic bitter taste associated with caffeine and other bitter compounds; bitterness lingers long like an aftertaste	Not intense = 1 and Very intense = 9
Starchy flavour	Pasty, chalky and powdery starch flavour, starchy like potatoes	Not intense = 1 and Very intense = 9
Maize-flavour	Tastes like boiled maize (cobs) maize-meal, and other maize products	Not intense = 1 and Very intense = 9
Residue	Leaves particles of the pericarp in the mouth and teeth	Not much = 1 and Very much = 9
Astringency	A sensation that lingers and coats, dries and numbs the mouth, palate and tongue.	Not intense = 1 and Very intense = 9

Few<sup>1</sup> - barely detectable/noticeable

Many<sup>2</sup> – clearly detectable/noticeable



#### 2.1.3.6. Sample preparation, presentation and assessment

The six sorghum cultivars were assessed by the descriptive sensory panel by descriptive profiling four times per product; with two sessions organized per day (three cultivars assessed in the first session and the other three assessed after two hours) to minimize fatigue and astringency build-up. To balance out any order effect, the sample presentation was randomized for all the four replications and random three digit numbers were used to code the samples, according to Lawless and Heymann (1998).

##### 2.1.3.6.1. *Sorghum bran infusions*

Boiling (96°C) deionised water (300 ml) was added to the sorghum bran (5 g) in a glass beaker and covered with aluminium foil, and then boiled on a hot plate for 20 min. The ratio of 5 g bran to 300 ml water was adopted from tea infusions using a ratio of 1 g tea to 100 ml boiling water (Vinson and Dabbagh, 1998). Bran infusions were weaker than tea; to make them somewhat stronger a ratio of 1:60 (bran to water) was used. Preliminary tests using steeping and boiling were carried out for 5, 10, 15, 20 and 25 min. It was found that boiling was more effective in extracting phenols than steeping. However, boiling for 20 and 25 min was not significantly different. The sorghum bran mixture was centrifuged at 3880 g for 5 min at 20°C. The supernatant (bran infusion) was recovered and kept at 4°C for not more than 12 h before use. The residue was discarded. The bran infusions were brought to room temperature before being served to the panellists. Panellists sat in individual booths and evaluated the samples under white light. The sample (15 ml) was served in a glass tube covered with a lid. Panellists were instructed to place the whole sample in the mouth and swirl it around without swallowing it, and immediately start evaluating the intensity of the attributes. After 15 s the panellists were instructed to expectorate the sample (Kallithraka *et al.*, 1997a). The panellists rated the bran infusion attributes using a ten-point rating scale (Table 2.1). A four minute interval was forced in between samples to minimize the carryover effects from one sample to another. The panellists were given pieces of raw carrots and deionised water to cleanse their mouths thoroughly before tasting and in between samples. Twelve panellists assessed the bran infusions.

##### 2.1.3.6.2. *Sorghum (whole grain) rice*

Sorghum grain (150 g) was washed and soaked in boiled (96°C) deionised water (250 ml) for 1 h in food grade polyethylene bags (150 mm x 200 mm). The soaking water was

drained off at the end of the soaking period. Boiling (96°C) deionised water (500 ml) was added to the soaked grain in polyethylene bags and then cooked for one hour in a boiling water bath (Fig. 2.2 (a & b)). The sorghum rice (15-20 g) was served warm ( $35 \pm 5^\circ\text{C}$ ) in plastic cups (100 ml) covered with a lid (Fig. 2.2 (d)). The panellists rated the sorghum rice attributes using a nine-point rating scale (Table 2.2). The panellists were given pieces of raw carrots and deionised water to cleanse their mouths thoroughly before tasting and in between samples. Ten panellists assessed the sorghum rice.



(a)



(b)



(c)



(d)

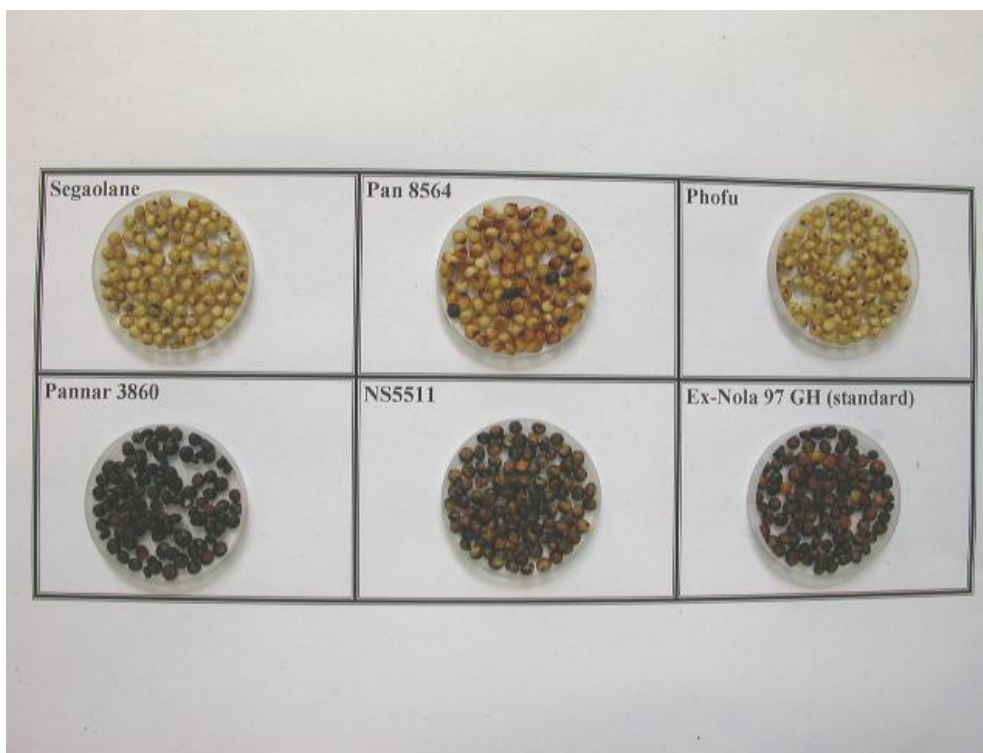
**Figure 2.2.** Sorghum rice preparation for sensory evaluation. Sorghum rice cooking in a boiling water bath (a & b); cooked sorghum rice (c); a panellist tasting the different sorghums (d).

#### 2.1.3.7. Statistical analysis

The effect of sorghum cultivar on grain characteristics, total phenolics content and sensory properties of sorghum bran infusions and sorghum rice were analysed using one-way analysis of variance (ANOVA) and Fischer's least significant difference test ( $p < 0.05$ ) using STATISTICA (StatSoft, Inc. 2005 version 7.1 [www.statsoft.com](http://www.statsoft.com) Tulsa, OK, USA). Principal component analysis (PCA) was carried out on the averaged (four replicate experiments averaged across panellists) sensory data using the correlation matrix option. Descriptive analysis data input was as described by Borgognone, Bussi and Hough (2001): cultivar/sample (rows) by sensory descriptor (columns) matrix using the mean (four replicate experiments) values of the panellists.

#### 2.1.4. **Results and discussion**

Three of the sorghums (PAN 3860, Ex Nola 97 GH and NS 5511) had a pigmented testa and three (PAN 8564, Segaolane and Phofu) did not (Fig. 2.3; Table 2.3). According to the Vanillin-HCl assay, the former contained condensed tannins and the latter did not. The pericarp colour of four of the sorghum cultivars (PAN 3860, Ex Nola 97 GH, NS 5511 and PAN 8564) was red, and the pericarp colour of the other two sorghums (Segaolane and Phofu) was white. All the sorghums had red glumes with the exception of Segaolane which had purple glumes. The endosperm texture of the tannin-free sorghums with a white pericarp (Phofu and Segaolane) was more corneous and these sorghums had significantly higher decortication yields compared to the other sorghums. These findings are consistent with those reported by Awika, McDonough and Rooney (2005) who found that the harder sorghum samples were generally more resistant to bran material removal. The endosperm texture of the tannin sorghums with a red pericarp (PAN 3860 and Ex Nola 97 GH) was relatively softer and they had the lowest decortication yields. PAN 8564 (tannin-free) and NS 5511 (tannin) both had a red pericarp and intermediate endosperm texture and their decortication yields were in between the relatively corneous and softer sorghums.



**Figure 2.3.** Determination of pigmented testa presence in the sorghum grain using the bleach test (Taylor, 2001). Top row sorghums (Segaolane, PAN 8564 and Phofu) have no pigmented testa (no detectable tannins). Bottom row sorghums (PAN 3860, NS 5511 and Ex Nola 97 GH) have a pigmented testa (condensed tannins).

The total phenol content of whole grain and bran of the tannin sorghums was significantly higher, by more than twice, that of the tannin-free sorghums (Table 2.4). This can probably be attributed to the presence of a pigmented testa. Dykes, Rooney, Waniska and Rooney (2005) reported that the presence of the pigmented testa gene  $B_1B_2$  and the spreader gene  $S$  increased total phenols. Grains with  $B_1B_2S$  genes had the highest levels of total phenols. The total phenol content of the sorghum bran was four times that of the sorghum whole grain. This is because phenols are mainly located in the pericarp (bran) of the sorghum caryopsis (Youssef, Bolling, Moustafa and Moharram, 1988; Awika *et al.*, 2005). Aqueous acetone was generally a more efficient extraction solvent of total phenols in sorghum bran than water (bran infusions). Other researchers have also found organic solvents to be better extraction solvents of phenols than water.

**Table 2.3.** Characterization of sorghum grain samples

Sorghum Cultivar	Pericarp		Glume Colour	Presence of Pigmented Testa <sup>1</sup>	Tannin Content (% CE db) <sup>2</sup>	Endosperm Texture <sup>3</sup>	
	Colour	Thickness				Visual Hardness Score <sup>4</sup>	Decortication Yield (TADD) (%) <sup>5</sup>
PAN 3860	Red	Medium	Red	Yes	8.2 <sup>c</sup> (0.1)	3.62 <sup>d</sup> (0.49)	81.6 <sup>a</sup> (1.8)
Ex Nola 97	Red	Thick	Red	Yes	5.7 <sup>b</sup> (0.3)	3.85 <sup>c</sup> (0.78)	80.5 <sup>a</sup> (1.3)
GH							
NS 5511	Red	Medium	Red	Yes	1.8 <sup>a</sup> (0.2)	3.33 <sup>c</sup> (0.48)	86.8 <sup>b</sup> (0.1)
PAN 8564	Red	Medium	Red	No	ND	3.17 <sup>c</sup> (0.46)	86.9 <sup>b</sup> (1.0)
Segaolane	White	Thin	Purple	No	ND	2.50 <sup>b</sup> (0.60)	88.5 <sup>c</sup> (0.8)
Phofu	White	Medium	Red	No	ND	2.23 <sup>a</sup> (0.43)	88.4 <sup>c</sup> (0.4)

<sup>1</sup>Yes = Pigmented testa present, No = Pigmented testa not present.

<sup>2</sup>CE = catechin equivalents dry basis; Means of three replicate experiments and standard deviations; ND = not detected; means in columns with different letter notations are significantly different at  $p \leq 0.05$ .

<sup>3</sup>Means plus standard deviation; means in columns with different letter notations <sup>(a-c)</sup> are significantly different at  $p \leq 0.05$ .

<sup>4</sup>Sixty kernels (3 reps of 20) kernels split in half and endosperm texture subjectively determined using a scale of 1 (Corneous) to 5 (Floury) (Rooney and Miller, 1982).

<sup>5</sup>Grain milled in a Tangential Abrasive Dehulling Device (TADD) for 4 minutes; means of six replicate experiments.

Yu, Ahmedna and Goktepe (2005) reported that methanol and ethanol (80%) were more efficient extraction solvents of total phenolics in peanut skin than water. Zielinski and Kozłowska (2000) also reported methanol (80%) as a better extraction solvent for total phenols in cereals (wheat, barley, rye and oat) than water. Zielinski and Kozłowska (2000) cautioned that the total phenols detected in water extracts may include proteins since the Folin-Ciocalteu assay is not specific to a class of phenols. The solubility of phenolic compounds is governed by the polarity of the type of solvent used, their degree of polymerization, as well as the interaction of phenolics with other food constituents (Naczka and Shahidi, 2004). Condensed tannins complex strongly to metal ions, carbohydrates and proteins (Porter, 1992) and these insoluble complexes are harder to extract (Awika, Dykes, Gu, Rooney and Prior, 2003). In HPLC profiles, Awika *et al.* (2003) observed a significant reduction in the extractability of processed sorghum bran tannins relative to the unprocessed brans. It is probable therefore that during boiling, the tannins bound to proteins making them unavailable to the Folin-Ciocalteu assay. This would account for the aqueous acetone extracts giving higher values than the water extracts.

**Table 2.4.** Total phenol content of sorghum whole grain, sorghum bran and sorghum bran infusions (g kg<sup>-1</sup> tannic acid equivalents db)

Sorghum Cultivar	Whole Grain <sup>1</sup>	Bran <sup>1</sup>	Bran Infusions <sup>2</sup>
PAN 3860	17.5 <sup>h</sup> (1.2)	65.2 <sup>n</sup> (0.3)	48.6 <sup>m</sup> (1.3)
Ex Nola 97 GH	17.1 <sup>h</sup> (1.2)	45.2 <sup>l</sup> (1.2)	33.1 <sup>j</sup> (0.8)
NS 5511	10.6 <sup>e</sup> (1.3)	44.1 <sup>k</sup> (0.3)	28.4 <sup>i</sup> (2.2)
PAN 8564	3.1 <sup>b</sup> (0.5)	16.2 <sup>g</sup> (0.9)	16.8 <sup>gh</sup> (0.4)
Segaolane	1.7 <sup>a</sup> (0.3)	13.3 <sup>f</sup> (0.2)	10.8 <sup>de</sup> (0.9)
Phofu	2.2 <sup>ab</sup> (0.3)	9.9 <sup>d</sup> (0.3)	8.7 <sup>c</sup> (0.7)

Means of six replicate experiments and standard deviations.

Means in rows with different letter notations <sup>(a-n)</sup> are significantly different at  $p \leq 0.05$ .

<sup>1</sup>Extraction solvent – 75% aqueous acetone.

<sup>2</sup>Extraction solvent – deionised water (boiling for 20 minutes).

The colour of the sorghum bran infusions ranged from light to moderately dark (Table 2.5). The infusions from the tannin-free sorghums were perceived as light (2.2–3.4) and the tannin sorghums were perceived as moderately dark (5.0–6.6). It is noteworthy that infusions of the tannin sorghums were all darker than the infusion of PAN 8564 (tannin-free) even though it also had a red pericarp. This was due to the presence of a pigmented testa in these sorghums. According to Awika *et al.* (2005) the pigmented testa is typically darker than the pericarp. The reverse was true for cloudiness. The tannin-free sorghums gave cloudy infusions whereas infusions of the tannin sorghums were clear. The sorghum with the lowest total phenol content (Phofu) was perceived as the cloudiest (7.3) and the sorghum with the highest total phenol content (PAN 3860) was perceived as the clearest (2.5). According to Siebert, Troukanova and Lynn (1996) proteins and polyphenols bind to form soluble colloidal size complexes that are reported to scatter light in solution, and when these protein-polyphenol complexes grow, they sediment out of solution. This probably explains why the infusions from tannin sorghums were perceived as clear and PAN 3860, with the highest total phenol content, being perceived as the clearest. Sorghum condensed tannins form complexes with kafirin, the prolamin protein of sorghum, to form haze (Emmambux and Taylor, 2003).

The sorghum bran infusions were perceived as having both herbal and a slightly fruity aroma and the flavour was described as sweet, sour, bitter and herbal. Infusions from Phofu and Segalane (with the lowest total phenol content) were perceived as significantly sweeter than infusions from PAN 3860 and Ex Nola 97 GH (with the highest total phenol content). The infusions from sorghums with the highest total phenol content (PAN 3860 and Ex Nola 97 GH) were perceived as the most bitter and the infusion from the sorghum with the lowest total phenol content (Phofu) was perceived as the least bitter. The astringency sensation was perceived most strongly in the infusion from PAN 3860 (with the highest total phenol content); followed respectively by Ex Nola 97 GH, NS 5511 and PAN 8564. Infusions from Segalane and Phofu (with the lowest total phenol content) were perceived as least astringent. Thus, the infusion from the sorghum with the highest total phenol content was most bitter and most astringent whilst the infusion from the sorghum with the lowest total phenol content was least bitter and least astringent.

**Table 2.5.** Sensory properties<sup>1</sup> of bran infusions of different sorghum cultivars as evaluated by a trained descriptive sensory panel (n=12)

Sensory Attributes	Tannin Sorghums			Tannin-free Sorghums		
	PAN 3860	Ex Nola 97 GH	NS 5511	PAN 8564	Segaolane	Phofu
Colour	6.6 <sup>c</sup> (1.6)	5.9 <sup>d</sup> (1.4)	5.1 <sup>c</sup> (1.1)	3.4 <sup>b</sup> (1.0)	3.0 <sup>b</sup> (1.2)	2.2 <sup>a</sup> (1.2)
Cloudiness	2.5 <sup>a</sup> (1.6)	3.4 <sup>b</sup> (1.8)	2.8 <sup>ab</sup> (1.7)	4.9 <sup>c</sup> (2.1)	5.0 <sup>c</sup> (2.6)	7.3 <sup>d</sup> (2.1)
Herbal aroma	5.2 <sup>a</sup> (2.0)	5.3 <sup>a</sup> (1.9)	5.6 <sup>a</sup> (2.1)	5.5 <sup>a</sup> (1.9)	5.5 <sup>a</sup> (2.2)	5.7 <sup>a</sup> (1.9)
Fruity aroma	2.8 <sup>a</sup> (2.1)	2.6 <sup>a</sup> (2.0)	2.7 <sup>a</sup> (1.9)	2.7 <sup>a</sup> (2.1)	2.7 <sup>a</sup> (2.2)	3.0 <sup>a</sup> (2.1)
Sweet	1.7 <sup>a</sup> (1.0)	1.6 <sup>a</sup> (1.0)	2.0 <sup>ab</sup> (1.1)	1.9 <sup>ab</sup> (1.1)	2.3 <sup>b</sup> (1.6)	2.3 <sup>b</sup> (1.7)
Sour	2.0 <sup>a</sup> (1.2)	1.6 <sup>a</sup> (1.0)	1.6 <sup>a</sup> (0.9)	1.7 <sup>a</sup> (1.1)	1.7 <sup>a</sup> (1.1)	1.6 <sup>a</sup> (1.1)
Bitter	6.2 <sup>c</sup> (2.0)	5.6 <sup>c</sup> (2.2)	4.4 <sup>b</sup> (2.1)	4.4 <sup>b</sup> (2.3)	3.6 <sup>ab</sup> (1.8)	3.1 <sup>a</sup> (2.0)
Herbal flavour	4.9 <sup>a</sup> (2.0)	4.9 <sup>a</sup> (2.0)	4.9 <sup>a</sup> (1.9)	5.1 <sup>a</sup> (2.0)	5.1 <sup>a</sup> (2.0)	5.0 <sup>a</sup> (2.0)
Astringency	5.9 <sup>c</sup> (2.1)	4.6 <sup>b</sup> (1.9)	4.3 <sup>b</sup> (2.0)	4.2 <sup>ab</sup> (2.1)	3.5 <sup>a</sup> (1.9)	3.4 <sup>a</sup> (2.1)

Means of four replicate experiments and standard deviations averaged across the 12 panellists

Means in rows with different letter notations <sup>(a-e)</sup> are significantly different at  $p \leq 0.05$

<sup>1</sup>Refer to Table 2.1 for bran infusion sensory properties definitions and rating scale



This is consistent with studies that have been carried out on the bitterness and astringency of phenolic compounds in beverages. Phenolic fractions in wine (Arnold, Noble and Singleton, 1980; Kallithraka, Bakker and Clifford, 1997b) and cider (Lea and Timberlake, 1974; Lea and Arnold, 1978) were evaluated for bitterness and astringency. The fractions, ranging from catechin monomers to highly polymerized tannins, were described as both bitter and astringent. The highly polymerized material was primarily responsible for both bitterness and astringency, while the isolated trimers, dimers and monomers contributed only slightly to these sensations. In sorghum, catechin is the most commonly reported monomer and procyanidin B1 is the most common dimer, while tannins in sorghum are mainly polymerized products of flavan-3-ols and/or flavan-3,4-diols (Awika and Rooney, 2004). Thus, the weakly detected bitterness and astringency of infusions from tannin-free sorghums could be attributed to the monomers and dimers. Whilst that from the tannin sorghums, although not very strong, could be attributed to polymerized products of flavan-3-ols and/or flavan-3,4-diols. It is probable that condensed tannins formed irreversible complexes with kafirin as found by Emmambux and Taylor (2003) and sedimented out of solution as described by Siebert *et al.* (1996), and since they are insoluble (Naczka and Shahidi, 2004) they did not contribute to the bitterness and astringency of the infusions. Surprising results were noted for NS 5511 (tannin sorghum) in that the descriptive sensory panel perceived it as similar to PAN 8564 (tannin-free sorghum) in sweetness, bitterness and astringency, even though NS 5511 is a condensed tannin containing sorghum with almost twice the total phenol content of PAN 8564.

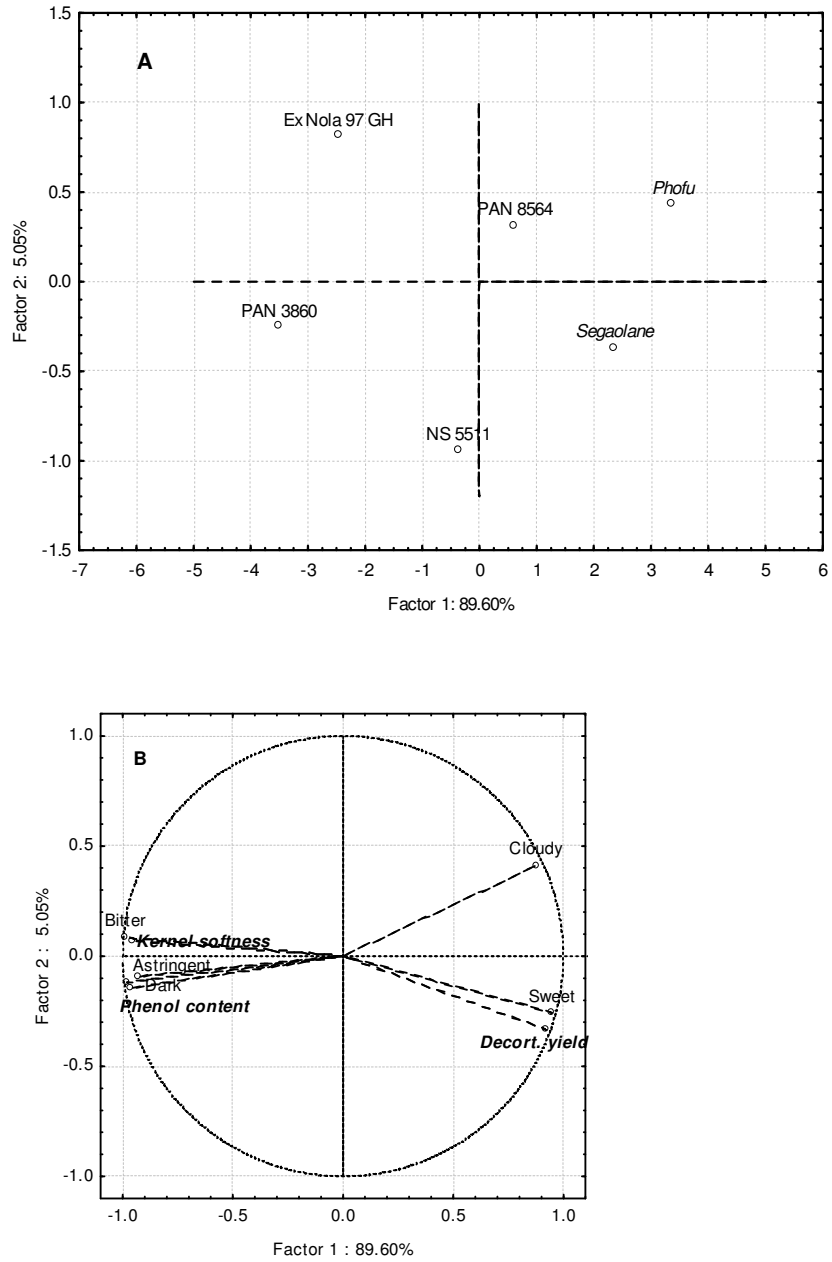
With principal component analysis (PCA) of the bran infusions, the first two principal components (PC) accounted for 95% of the variance in phenol content, endosperm texture and sensory data, with PC1 accounting for 90% and PC2 accounting for only 5% (Fig. 2.4). PC1 accounted for the variance in phenol content, kernel softness, decortication yield, colour, cloudiness, bitterness, sweetness and astringency. The sorghums that clustered to the left, PAN 3860 and Ex Nola 97 GH (tannin) were associated with the sensory attributes: bitter, dark (colour) and astringent as well as high phenol content and softer kernels. Bitterness and astringency were positively correlated and clustered together. Sorghums that clustered to the right, Phofu and Segaolane (tannin-free) were associated with the sensory attributes: cloudy and sweet as well as high decortication yield. NS 5511 and PAN 8564 clustered along the axis origin, with NS 5511 grouping

towards the tannin sorghums (PAN 3860 and Ex Nola 97 GH) and PAN 8564 grouping towards the tannin-free sorghums (Phofu and Segalane).

Concerning the whole grain sorghum rice sensory attributes, with the exception of cooked cereal aroma and starchy flavour, there were significant differences among the sorghums across all the sensory attributes (Table 2.6). The colour trend observed in the bran infusions was repeated in the sorghum rice. The cultivars that gave the lightest sorghum rice colour were the tannin-free sorghums (Fig. 2.2c). The cultivars that gave the darkest sorghum rice colour were tannin sorghums. The sorghum rice from tannin sorghums was darker than that from PAN 8564, even though it also had a red pericarp. This was probably due to the pigmented testa as discussed previously.

Ratings for black specks ranged from few (2.3 - PAN 3860) to many (7.6 - Segalane). Although Segalane had a white pericarp, it had the highest number of black specks. This was probably due to the phenolic pigments of the purple glumes leaching into the grain and causing a discolouration of the sorghum rice. According to Rooney and Miller (1982) there are three main sorghum plant colours: red, tan and purple, and the glumes with intense red and purple colour have a tendency to stain the pericarp under humid conditions because the phenolic pigments leach into the pericarp. The leaching of the pigments into the pericarp can cause discolouration in some of the sorghum food products.

The sorghum rice that was perceived as least chewy and having the softest texture was from sorghums with a relatively corneous endosperm texture and lowest total phenol content, Segalane and Phofu. This was probably due to the fact that many of the kernels split. The sorghum rice that was perceived as most chewy and having a harder texture was from the sorghums with relatively softer endosperm texture and highest total phenol content, Ex Nola 97 GH and PAN 3860. Thus, the perceived texture of the sorghum rice (cooked) seemed to be inversely related to the grain endosperm texture. Although significant differences were noted for residue left in the mouth, there was no pattern or trend.



**Figure 2.4.** Principal component analysis (correlation matrix) of phenol content, endosperm texture and descriptive sensory evaluation of sorghum bran infusions of six sorghum cultivars. (A) Plot of the first two principal component scores of the sorghum cultivars. (B) Plot of the first two principal component loading vectors of phenol content, endosperm texture and sensory attributes.

The trends for the bitterness, sweetness and astringency of the sorghum bran infusions were also found for sorghum rice. The sorghum rice of sorghums with the highest total phenol content (PAN 3860 and Ex Nola 97 GH) were perceived as more bitter and more astringent than the sorghum rice of sorghums with the lowest total phenol content (Phofu and Segaolane). The sorghum rice of sorghums with the lowest total phenol content (Segaolane and Phofu) were perceived as sweeter than the sorghum rice of sorghums with the highest total phenol content (PAN 3860 and Ex Nola 97 GH). As found in the bran infusions, unexpected results were noted for the sorghum rice of NS 5511 in that it was perceived as similar to that of PAN 8564 (tannin-free sorghum) in sweetness, bitterness and astringency. Furthermore, the sorghum rice of NS 5511 was not significantly different in sweetness and astringency from that of Segaolane and Phofu despite the fact that NS 5511 (whole grain) had more than three times the total phenol content of these sorghums and it contains tannins.

For the sorghum rice, the maize-flavour attribute was rated moderate (4.5–6.2) for all the cultivars with PAN 3860 and Ex Nola 97 GH being rated significantly lower for maize-flavour. The herbal-flavour property detected in the sorghum bran infusions was not detected in the sorghum rice probably because it was masked by flavour contributions from the endosperm such as the maize-flavour and cooked cereal aroma.

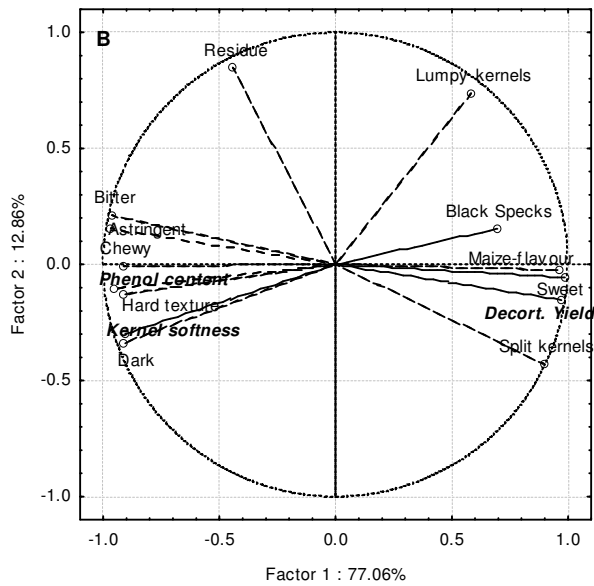
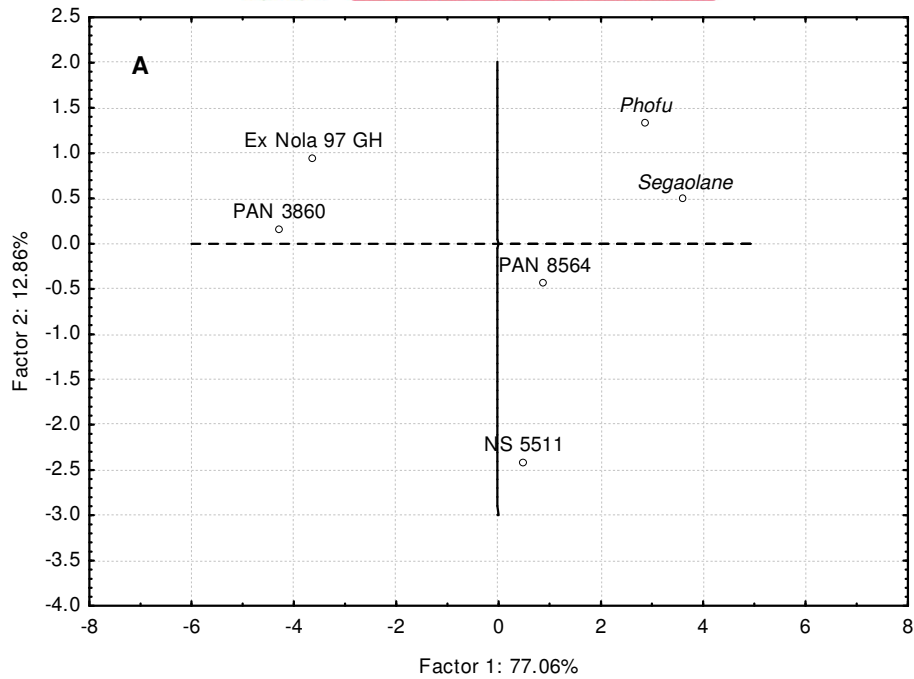
With PCA of the whole grain sorghum rice, the first two principal components accounted for 90% of the variance in phenol content, endosperm texture and sensory attributes of the sorghum rice (Fig. 2.5). PC1 accounted for 77% and PC2 accounting for an additional 13%. PC1 accounted for the variance in phenol content, endosperm texture and sensory attributes: bitterness, sweetness, split kernels, astringency, maize-flavour, colour, hard/soft texture, chewy and black specks. PC2 accounted for the variance in residue left in mouth and lumpy kernels. The sorghum cultivars that clustered to the left had the sensory attributes: bitter, astringent, chewy, hard and dark. These were the tannin sorghums with the highest total phenol content, relatively softer endosperm texture and red pericarp colour (PAN 3860 and Ex Nola 97 GH). The sorghums that clustered to the right had high decortication yield and sensory attributes: sweet, maize-flavour, split kernels and black specks. These were the tannin-free sorghums with the lowest total phenol content, relatively corneous endosperm texture and a white pericarp colour (Segaolane and Phofu).

**Table 2.6.** Sensory properties<sup>1</sup> of sorghum rice of different sorghum cultivars as evaluated by a trained descriptive sensory panel (n=10)

Sensory Attributes	Tannin Sorghums			Tannin-free Sorghums		
	PAN 3860	Ex Nola 97 GH	NS 5511	PAN 8564	Segaolane	Phofu
Colour	7.7 <sup>c</sup> (0.9)	7.9 <sup>c</sup> (0.8)	6.7 <sup>d</sup> (1.7)	4.3 <sup>c</sup> (1.3)	2.8 <sup>b</sup> (1.4)	2.0 <sup>a</sup> (1.5)
Black Specks	2.3 <sup>a</sup> (1.4)	2.8 <sup>ab</sup> (1.7)	2.7 <sup>ab</sup> (1.5)	4.8 <sup>c</sup> (1.9)	7.6 <sup>d</sup> (1.3)	3.2 <sup>b</sup> (1.5)
Split kernels	5.0 <sup>a</sup> (1.7)	4.9 <sup>a</sup> (1.4)	6.2 <sup>b</sup> (1.4)	5.7 <sup>b</sup> (1.5)	6.2 <sup>b</sup> (1.3)	5.8 <sup>b</sup> (1.6)
Lumpy kernels	3.7 <sup>a</sup> (1.8)	4.3 <sup>ab</sup> (1.9)	3.6 <sup>a</sup> (1.9)	3.9 <sup>a</sup> (1.7)	5.0 <sup>b</sup> (1.9)	4.8 <sup>b</sup> (1.8)
Cooked cereal aroma	6.2 <sup>a</sup> (2.1)	6.3 <sup>a</sup> (1.9)	6.3 <sup>a</sup> (2.0)	6.1 <sup>a</sup> (2.0)	6.3 <sup>a</sup> (2.1)	6.8 <sup>a</sup> (1.8)
Chewy	6.3 <sup>b</sup> (2.1)	5.5 <sup>b</sup> (2.2)	5.0 <sup>b</sup> (2.1)	5.3 <sup>b</sup> (2.0)	4.6 <sup>a</sup> (2.1)	4.7 <sup>a</sup> (2.2)
Texture (Soft/Hard)	6.7 <sup>c</sup> (1.9)	5.4 <sup>b</sup> (1.8)	5.0 <sup>ab</sup> (2.0)	5.2 <sup>b</sup> (2.1)	4.2 <sup>a</sup> (2.0)	4.3 <sup>a</sup> (1.7)
Sweet	1.8 <sup>a</sup> (0.8)	1.8 <sup>a</sup> (0.8)	3.0 <sup>bc</sup> (1.2)	2.8 <sup>b</sup> (1.3)	3.5 <sup>c</sup> (2.0)	3.5 <sup>c</sup> (1.9)
Bitter	5.2 <sup>c</sup> (1.8)	5.9 <sup>c</sup> (1.8)	2.8 <sup>b</sup> (1.8)	3.0 <sup>b</sup> (1.8)	1.9 <sup>a</sup> (1.1)	2.5 <sup>ab</sup> (1.5)
Starchy-flavour	4.3 <sup>a</sup> (1.9)	3.9 <sup>a</sup> (2.0)	4.3 <sup>a</sup> (1.9)	4.3 <sup>a</sup> (1.9)	4.7 <sup>a</sup> (1.9)	4.7 <sup>a</sup> (2.3)
Maize-flavour	4.5 <sup>a</sup> (2.0)	4.7 <sup>a</sup> (1.9)	5.5 <sup>b</sup> (1.9)	5.8 <sup>b</sup> (2.1)	5.9 <sup>b</sup> (1.7)	6.2 <sup>b</sup> (2.1)
Residue	5.0 <sup>b</sup> (2.3)	5.0 <sup>b</sup> (2.0)	4.0 <sup>a</sup> (1.9)	4.6 <sup>ab</sup> (2.3)	4.4 <sup>ab</sup> (1.8)	5.0 <sup>b</sup> (2.1)
Astringency	4.8 <sup>b</sup> (1.6)	4.9 <sup>b</sup> (1.9)	3.0 <sup>a</sup> (1.6)	2.8 <sup>a</sup> (1.3)	2.5 <sup>a</sup> (1.3)	2.5 <sup>a</sup> (1.1)

Means of four replicate experiments and standard deviations averaged across the 12 panellists; Means in rows with different letter notations <sup>(a-e)</sup> are significantly different at  $p \leq 0.05$ ;

<sup>1</sup>Refer to Table 2.2 for sorghum rice sensory properties definitions and rating scale.



**Figure 2.5.** Principal component analysis (correlation matrix) of phenol content, endosperm texture and descriptive sensory evaluation of sorghum rice of six cultivars. (A) Plot of the first two principal component scores of the sorghum cultivars. (B) Plot of the first two principal component loading vectors of phenol content, endosperm texture and sensory attributes.

As observed with the bran infusions, NS 5511 and PAN 8564 grouped together and clustered along the axis origin – though more towards the tannin-free and relatively corneous sorghums. NS 5511 grouped to the bottom of the plot and thus was perceived to leave the least amount of residue in the mouth and looked least lumpy. Thus, the clustering of the cultivars was essentially the same for the sorghum rice as for the infusions.

### **2.1.5. Conclusions**

Phenolics in sorghum grain contribute to the bitterness and astringency of sorghum. It is noteworthy that all the sorghum cultivars (tannin and tannin-free) are perceived as bitter and astringent at least to some extent. Tannin sorghums are more bitter and more astringent than tannin-free sorghums. Infusions of tannin sorghums are clear, whilst infusions of tannin-free sorghums are cloudy. The sorghum rice from the white sorghums which had a relatively harder endosperm texture and was perceived as less chewy (softer) than that from the other sorghums. Surprising results were noted for NS 5511 (tannin sorghum) in that the bitterness and astringency of this sorghum as well as other sensory attributes were perceived as similar to PAN 8564 (tannin-free sorghum) even though NS 5511 had more than twice the total phenol content of PAN 8564. Further research is needed to determine why NS 5511 was perceived similar to PAN 8564. Furthermore, given that tannin sorghums possess high antioxidant activity, it is worth investigating whether tannin sorghums like NS 5511 are equally preferred by consumers compared to tannin-free sorghums like PAN 8564.

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## **2.2. Bitterness and astringency of bran infusions of tannin-free and tannin sorghums determined using a dual attribute time intensity (DATI) sensory method**

### **2.2.1. Abstract**

Although condensed tannins are potentially important antioxidants, it is generally believed that tannins in sorghum confer objectionable sensory attributes. The objective of this study was to use the dual attribute time intensity (DATI) sensory method to determine the intensity and time course of bitterness and astringency of sorghums varying in condensed tannin content. A trained sensory panel assessed the time-course of bitterness and astringency of bran infusions of tannin and tannin-free sorghums. The infusion from PAN 3860, with the highest condensed tannin content (8.2% catechin equivalents [CE] dry basis), was perceived as most bitter and most astringent. The infusion of Ex Nola 97 GH, a tannin sorghum (5.7% CE) was perceived as more bitter than PAN 8564 (tannin-free), whereas the astringency of the infusions of these sorghums were perceived similar. The infusion of NS 5511, a tannin sorghum (1.8 % CE), was perceived similar to tannin-free sorghums in both bitterness and astringency. Bitterness developed and reached maximum intensity significantly faster ( $T_{\max}$  22.5 s;  $p \leq 0.001$ ) than astringency ( $T_{\max}$  27.9 s). The total duration of the astringency ( $D_{\text{tot}}$  69.9 s) sensation lasted significantly longer than bitterness ( $D_{\text{tot}}$  66.3 s). The more bitter and more astringent the sorghum was, the longer the persistence of the bitter and astringent after-taste. There appears to be a condensed tannin threshold level at which the tannins in sorghum products are not 'strongly' perceived and thus are not objectionable.

### 2.2.2. Introduction

Phenolics impart both bitterness and astringency to fruits, vegetables, wine, beer and other foods (Drewnowski and Gomez-Carneros, 2000). In sorghum, condensed tannins are generally believed to impart objectionable sensory attributes (Asante, 1995). A quantitative descriptive analysis study was carried out to profile the sensory properties of tannin-free and tannin sorghums (Chapter 2.1). All the sorghum cultivars (tannin and tannin-free) were, to different degrees, perceived as both bitter and astringent. Sorghums with tannin levels exceeding 5.7% catechin equivalents [CE] dry basis were most bitter and most astringent, whilst the sorghums with no detectable tannins were least bitter and least astringent. Surprisingly NS 5511, with a tannin content level of 1.8% CE was perceived as similar to PAN 8564 (with no detectable tannins) in bitterness and astringency as well as other sensory attributes.

According to Leach (1984), bitterness and astringency are characterized by a persistent after-taste and thus cannot be estimated solely by scalar intensity procedures. Also scalar or point estimates of intensity are inadequate when the sensory properties of samples vary differentially over time (Noble, 1995). For instance, wines that may be equally bitter when first sipped, may vary in the persistence of bitterness after the wine is swallowed (Noble, 1995). Thus, to fully characterize the differences in their sensory properties requires analysis of the time-course of perceived intensity (Noble, 1995). The time intensity sensory evaluation method is useful in continuously capturing, in great detail, the nuances of flavour growth, decay and disappearance (Lawless and Heymann, 1998; Bloom, Duizer and Findlay, 1994). Time intensity sensory evaluation has mostly been used to measure single attributes; however, it is gaining more popularity measuring dual attributes. Duizer, Bloom and Findlay (1997) compared the single attribute time intensity (SATI) method to the dual attribute time intensity (DATI) method in investigating sweetness and peppermint flavour of chewing gum with varying rates of sweetness and peppermint flavour release. No significant differences were observed between the time intensity parameters of the SATI and DATI methods. Zimoch and Findlay (1998) concluded that the DATI method provided a good separation of attributes and was equal or better than the SATI method for differentiating beef samples on the basis of juiciness and toughness. Using the time intensity procedure, Leach (1984) quantified the temporal sequence of astringency and bitterness of phenolic compounds (gallic acid, catechin,

tannic acid and grape seed tannin) in white wine. Tannic acid and grape seed tannin were more astringent than bitter, catechin was equally bitter and astringent and gallic acid was more bitter than astringent. According to Kennedy (2000), of the tannins extracted from grapes in wine production, the low molecular weight tannins are predominantly bitter, while the higher molecular weight tannins are predominantly astringent.

The objective of this study was to use the DATI sensory method to determine the intensity and time course of bitterness and astringency of sorghums varying in condensed-tannin content.

### **2.2.3. Materials and methods**

#### **2.2.3.1. Sorghum grain**

Six sorghum cultivars containing different levels of total phenols were used. Three were tannin-free sorghums (PAN 8564, Segalane and Phofu) with low levels of total phenols; and three were tannin sorghums (PAN 3860, Ex Nola 97 GH and NS 5511) with high levels of total phenols. The tannin sorghums had a red pericarp, so a tannin-free sorghum with a red pericarp (PAN 8564) was used for comparison. The other tannin-free sorghums had a white pericarp (Chapter 2.1).

#### **2.2.3.2. Sorghum bran infusions**

Brans isolated from the sorghum grains were used to prepare the infusions as reported previously (Chapter 2.1). Boiling (96°C) deionised water (300 ml) was added to the sorghum bran (5 g) in a glass beaker and covered with aluminium foil, and then boiled on a hot plate for 20 min. The sorghum bran mixture was centrifuged at 3880 g for 5 min at 20°C. The supernatant (bran infusion) was recovered and kept at 4°C for not more than 12 h before use. The residue was discarded. The bran infusions were brought to room temperature before being served to the panellists.

#### **2.2.3.3. Descriptive sensory panel selection and training**

Twelve panellists (six women and six men) aged 19-39 years participated in the study. These panellists had previously participated in a study to describe the sensory attributes of cooked sorghum rice (Chapter 2.1). The panellists signed a consent form showing willingness to taste the sorghum products, prior to the training and assessment of the

samples. The descriptive sensory panel was trained for 1 h per working day for a period of two weeks to familiarize them with the SATI and DATI sensory evaluation methodology and software (Compusense® Five release 4.6 [1986-2003] Guelph, Ontario Canada) as described by Peyvieux and Dijksterhuis (2001). Initially, the training was carried out measuring a single attribute (bitterness) on a structured horizontal line. A continuous linear scale with 10 markings from 0 = not detectable at the start position to 100 = strongly detected at the end of the line. As the intensity of the bitterness increased, the panellist moved the ‘marker’ to the right, and when the intensity of the bitterness decreased he/she moved the ‘marker’ to the left. The speed with which they moved the ‘marker’ to the right or left was determined by how rapidly the intensity of the attribute developed and increased or how rapidly it decreased. The panellists were also trained to differentiate between bitterness and astringency using standards (dissolved in deionised water) and concentrations used by Kallithraka, Bakker and Clifford (1997a) bitterness (1.0 g/l caffeine; food grade), and astringency (1.5 g/l tannic acid [gallotannin]; 48811 Fluka/Sigma-Aldrich, Atlas-Ville, South Africa). Alum (potassium aluminium sulphate Fluka/Sigma-Aldrich, Atlas-Ville, South Africa) was also used to familiarize the panellists with the astringency sensation using the concentration (0.5 g/l) recommended in ISO 8586 (International Organization for Standardization, 1993).

Training to measure dual attributes simultaneously (bitterness and astringency) was introduced only after the panellists were proficient in measuring the sensations as single attributes. The panellists were trained to measure the intensity of bitterness on a structured vertical line and the intensity of astringency on a structured horizontal line simultaneously, by moving a computer mouse diagonally on a mouse pad; to the right as the attributes developed and increased, and to the left as the attributes decreased. Moving the mouse diagonally moved the ‘marker’ along both lines (vertical and horizontal) simultaneously. During training, time intervals of 2, 3, 4 and 5 min were used in between samples to determine the optimal time interval required to minimize carry over effects. The panellists agreed on a 4 min time interval in between samples to minimize carry over effects. This time interval was also used by Kallithraka, Bakker and Clifford (1997b) in their study using the time intensity methodology to assess the effects of pH on the astringency of model solutions and wines.

#### 2.2.3.4. Sample presentation and assessment

Sorghum bran infusions of the six sorghum cultivars were assessed by the DATI method four times per product, with two sessions organized per day. Three cultivars assessed in the first session and the other three assessed after two hours in order to minimize fatigue and astringency build-up. To balance out any order effect, sample presentation order was randomized over the panellists for all the four replications. Random three digit numbers were used to code the samples.

Panellists sat in individual booths and evaluated the samples under white light. Samples (15 ml) were served in size 8 poly-top glass tubes covered with lids. Panellists were instructed to place the whole sample in the mouth and swirl it around without swallowing it, and immediately start evaluating the intensity of the bitterness and astringency, simultaneously. After 15 s the panellists were instructed to expectorate the sample, following the method of Kallithraka *et al.* (1997a). The panellists measured the intensity of the two attributes, bitterness and astringency, simultaneously and continuously from the time they placed the sample in their mouth to the end of the assessment period of 90 s. The DATI software was programmed to collect responses every 0.5 s for the total duration of 90 s. A four minute interval was enforced between samples to minimize carry over effects from one sample to another. The panellists were given pieces of raw carrots and deionised water to cleanse their mouths thoroughly before tasting and in between samples.

#### 2.2.3.5. HPLC analysis

The sample extraction and procyanidin purification method of Gu, Kelm, Hammerstone, Beecher, Cunningham, Vannozzi and Prior (2002) was adapted and used as described by Awika, Dykes, Gu, Rooney and Prior (2003). The sorghum bran was milled to pass through a 1 mm screen using a hammer mill. A sample (0.1 g) was extracted using 10 ml of a acetone: water: acetic acid (70: 29.5: 0.5) mixture. Samples were sonicated at 37°C for 10 min and left at room temperature for 50 min. The extracts were centrifuged at 1900 g for 15 min. The supernatant was recovered and evaporated to dryness at 25°C under vacuum. The dried residue was dissolved in 6 ml water and applied to a Sephadex LH-20 column (Amersham, UK). The column was prepared by equilibrating 3 g Sephadex LH-20, with water overnight and then manually packed into a burette. The loaded crude extract was washed with 40 ml 30% (v/v) aqueous methanol to wash off the sugars and other low molecular weight phenols. The procyanidins were recovered from the column



using 80 ml 70% (v/v) aqueous acetone. Acetone was evaporated from the eluted liquid under vacuum at 45°C. The remaining sample was freeze dried and vacuum packed until needed for analysis.

The dry residue was dissolved in 70% aqueous acetone and made up to a final volume of 5 ml and filtered using a Whatman nylon membrane filter unit (0.45 µm) (Whatman International, Maidstone, England), before injecting into the HPLC. A Waters HPLC system (Waters, Millford, MA) was used comprising a Waters 717 Plus Autosampler, Waters In-Line Degasser, Waters 600E System Controller and a Waters 474 Fluorescent detector. The system was run using the Waters Empower software.

A modified method of Gu *et al.* (2002) was used to analyze the samples. The mobile phase was (A) dichloromethane, (B) methanol, and (C) glacial acetic acid/water (1:1 v/v). The gradient was 0-30 min, (14.0-28.4% B); 30-45 min, (28.4-39.6% B); 45-50 min, (39.6-86.0% B); 50-55 min, (86.0 B isocratic), 55-60 min, (86.0-14.0% B); followed by 10 min re-equilibration of the column before the next run. A constant 4% C was maintained throughout. The flow rate was 1 ml/min. Separation was on a normal-phase 5-µl Luna silica column (250 x 46 mm) (Phenomenex, Torrance, CA). Fluorescence detection was excitation 276 nm, emission 316 nm.

The HPLC method resolved procyanidins up to pentamers (DP 5), based on molecular weight. Thus procyanidins were reported as oligomers (DP 2-5), and polymers (DP>5) were resolved in a single peak. Total extractable procyanidins were obtained by adding the oligomer and polymer contents.

#### 2.2.3.6. Statistical analysis

Four parameters were extracted from the time intensity curves:  $T_{max}$  (time to reach maximum intensity),  $I_{max}$  (maximum intensity),  $D_{tot}$  (total duration of sensation) and AUC (area under curve). The generalized linear model (GLM) was used to analyze the effects of session, panellist, replicate, sample order and cultivar and designated interaction effects on  $T_{max}$ ,  $I_{max}$ ,  $D_{tot}$  and AUC data for bitterness and astringency using SAS<sup>®</sup> version 8.2 (SAS Institute Cary, NC).

GLM model:

$$y = \mu + \alpha_i + \beta_j + \gamma_k + \delta + \varepsilon_m + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\beta\delta)_{jl} + (\beta\varepsilon)_{jm} + \xi$$

Where:

$\mu$  - mean;  $\alpha_i$  - session;  $\beta_j$  - panellist;  $\gamma_k$  - replicate;  $\delta$  - sample order;  $\varepsilon_m$  - cultivar;  $(\alpha\beta)_{ij}$  - session and panellist;  $(\beta\gamma)_{jk}$  - panellist and replicate;  $(\beta\delta)_{jl}$  - panellist and sample order;  $(\beta\varepsilon)_{jm}$  - panellist and cultivar;  $\xi$  - error.

Fishers' least significant difference test ( $p < 0.05$ ) was used to compare the means. Linear relationships (Pearson's correlation coefficient) between the time intensity parameters ( $T_{\max}$ ,  $I_{\max}$ ,  $D_{\text{tot}}$  and AUC) were calculated. A comparison of the time intensity parameters for bitterness and astringency was performed using ANOVA.

## 2.2.4. Results and discussion

The GLM used was appropriate because it explained 69-84% of the variance in the parameters for bitterness (Table 2.7) and explained 73-83% of the variance in all the parameters for astringency (Table 2.8).

### 2.2.4.1. Main effects

#### 2.2.4.1.1. *Cultivar effect*

There were highly significant cultivar effects ( $p < 0.001$ ) for all the time intensity parameters ( $T_{\max}$ ,  $I_{\max}$ ,  $D_{\text{tot}}$  and AUC) for bitterness (Table 2.7) and astringency (Table 2.8). The most bitter ( $I_{\max}$ ) sorghum infusions were from tannin sorghums, PAN 3860 followed by Ex Nola 97 GH with tannin contents of 8.2 and 5.7% CE, respectively (Table 2.9 and Chapter 2.1). The bitterness intensity of the infusion from NS 5511, a tannin sorghum (1.8% CE; Chapter 2.1), was not significantly different from that of the tannin-free sorghums (Table 2.9). This finding suggests there may be a tannin threshold level at which tannins are not strongly perceived in sorghum based food systems. With the exception of NS 5511, it took approximately 7-10 s longer ( $T_{\max}$ ) to reach maximum bitterness intensity for tannin-containing sorghums (PAN 3860 and Ex Nola 97 GH) than

the tannin-free sorghums. Total duration ( $D_{tot}$ ) of bitterness for the most bitter sorghums (PAN 3860 and Ex Nola 97 GH) generally lasted 9-12 s longer than that of the less bitter sorghums. The more bitter the sorghum, the longer ( $T_{max}$ ) it took to reach maximum intensity ( $I_{max}$ ) and the longer the  $D_{tot}$ . The more bitter the sorghums, the larger the AUC as reflected by the highly significant ( $r = 0.88$ ,  $p < 0.001$ ) positive correlation (Table 2.10).

**Table 2.7.** Degrees of freedom (df), R-squared and F-values from analysis of variance of parameters extracted from time intensity curves for bitterness in sorghum bran infusions

Source of variation	df	Time to Max ( $T_{max}$ )	Intensity at Max ( $I_{max}$ )	Total Duration ( $D_{tot}$ )	Area Under Curve (AUC)
		$R^2 - 0.812$	$R^2 - 0.836$	$R^2 - 0.690$	$R^2 - 0.830$
		F	F	F	F
<b>Main effects</b>					
Cultivar	5	11.54***	38.32***	6.16***	34.92***
Panellist	11	35.19***	26.34***	12.29***	28.25***
Session	1	0.88	4.68*	0.14	3.02
Replicate	3	3.05*	3.96*	0.61	3.21*
Sample Order	2	0.25	0.83	1.40	0.04
<b>Interaction effects</b>					
Panellist x cultivar	55	1.63*	2.16***	1.25	2.12***
Panellist x session	11	1.18	2.29*	0.67	1.43
Panellist x replicate	33	1.24	1.80	1.36	1.55*
Panellist x sample order	22	0.89	0.81	0.88	0.99

\*, \*\*, \*\*\* Statistically significant at  $p < 0.05$ , 0.01 and 0.001 respectively.

**Table 2.8.** Degrees of freedom (df), R-squared and F-values from analysis of variance of parameters extracted from time intensity curves for astringency in sorghum bran infusions

Source of variation	df	Time to Max ( $T_{max}$ )	Intensity at Max ( $I_{max}$ )	Total Duration ( $D_{tot}$ )	Area Under Curve (AUC)
		$R^2 - 0.794$	$R^2 - 0.792$	$R^2 - 0.730$	$R^2 - 0.825$
		F	F	F	F
<b>Main effects</b>					
Cultivar	5	4.73***	21.89***	4.89***	22.80***
Panellist	11	33.99***	19.25***	19.15***	29.95***
Session	1	2.38	24.16***	0.01	20.54***
Replicate	3	2.78*	1.94	1.55	1.61
Sample Order	2	0.13	1.27	0.81	0.65
<b>Interaction effects</b>					
Panellist x cultivar	55	1.01	0.99	0.84	1.15
Panellist x session	11	1.34	2.13*	2.6**	1.81
Panellist x replicate	33	1.53*	1.9**	1.30	1.88**
Panellist x sample order	22	0.66	1.71*	0.96	2.18**

\*, \*\*, \*\*\* Significant at  $p < 0.05$ , 0.01 and 0.001 respectively.

**Table 2.9.** Least Square Means ( $\pm$ SE) of parameters extracted from time intensity curves for bitterness of sorghum bran infusions of tannin-containing and tannin-free sorghums

	Tannin sorghums			Tannin-free sorghums		
	PAN 3860	Ex Nola 97 GH	NS 5511	PAN 8564	Segaolane	Phofu
T <sub>max</sub> (s)	27.2 <sup>b</sup> (1.2)	28.2 <sup>b</sup> (1.2)	20.9 <sup>a</sup> (1.3)	20.1 <sup>a</sup> (1.2)	18.3 <sup>a</sup> (1.3)	20.4 <sup>a</sup> (1.3)
I <sub>max</sub>	56.9 <sup>e</sup> (1.9)	49.1 <sup>d</sup> (1.9)	36.4 <sup>bc</sup> (2.2)	37.8 <sup>c</sup> (1.9)	31.4 <sup>b</sup> (2.2)	24.3 <sup>a</sup> (2.1)
D <sub>tot</sub> (s)	75.1 <sup>c</sup> (2.7)	74.1 <sup>bc</sup> (2.7)	63.3 <sup>a</sup> (3.0)	64.9 <sup>ab</sup> (2.7)	60.9 <sup>a</sup> (3.0)	59.6 <sup>a</sup> (2.9)
AUC	2673.7 <sup>d</sup> (123.5)	2381.1 <sup>d</sup> (123.6)	1338.6 <sup>bc</sup> (137.4)	1512.2 <sup>c</sup> (123.6)	1132.5 <sup>ab</sup> (137.4)	845.9 <sup>a</sup> (133.2)

Least Square Means of four replicate experiments and standard errors averaged across 12 panellists.

Least Square Means in rows with different letter notations <sup>(a-e)</sup> are significantly different at  $p \leq 0.05$ .

T<sub>max</sub> – time to maximum intensity, I<sub>max</sub> – maximum intensity, D<sub>tot</sub> – total duration, AUC – area under curve.

**Table 2.10.** Pearson correlation coefficients between parameters extracted from time intensity curves for astringency and bitterness of different sorghums

		Bitterness				Astringency			
		T <sub>max</sub>	I <sub>max</sub>	D <sub>tot</sub>	AUC	T <sub>max</sub>	I <sub>max</sub>	D <sub>tot</sub>	AUC
Bitterness	T <sub>max</sub>	1							
	I <sub>max</sub>	0.37***	1						
	D <sub>tot</sub>	0.29***	0.37***	1					
	AUC	0.43**	0.88***	0.50***	1				
Astringency	T <sub>max</sub>	0.59***	0.24***	0.23***	0.23***	1			
	I <sub>max</sub>	0.17***	0.70***	0.28***	0.66***	0.18***	1		
	D <sub>tot</sub>	0.19***	0.24***	0.64***	0.39***	0.24***	0.27***	1	
	AUC	0.25***	0.64***	0.42***	0.76***	0.16***	0.87***	0.49***	1

\*\*, \*\*\* Significant at  $p < 0.01$  and  $0.001$  respectively

As observed for bitterness, the most astringent infusion (highest I<sub>max</sub>) was from PAN 3860 (Table 2.11), which had the highest tannin content (8.2% CE; Chapter 2.1). Although the infusion of Ex Nola 97 GH (5.7% CE) was significantly more bitter than that of PAN 8564, which had no detectable tannins, the astringencies of these sorghums were not significantly different (Table 2.11). The finding here agrees with the quantitative descriptive analysis (Table 2.5; Chapter 2.1). Thus it appears that bitterness and astringency are generally, but not always, the same in level of strength in individual sorghum cultivars. The bitterness and astringency of PAN 8564 (with no detectable tannins) was perceived similar to that of tannin sorghum NS 5511 (1.8% CE) and its astringency was not significantly different from that of Ex Nola 97 GH (5.7% CE). The astringency (I<sub>max</sub>) of the infusion from NS 5511 was not significantly different from any the tannin-free sorghums.

To determine why the infusion of PAN 8564 was perceived similar to condensed tannin-containing sorghums (Ex Nola 97 GH and NS 5511) in astringency an analysis was carried out by HPLC for condensed tannins. The HPLC chromatogram (Fig. 2.6) clearly shows presence of condensed tannins in PAN 3860, Ex Nola 97 GH and NS 5511, but condensed tannins were not present in PAN 8564. Since PAN 8564 does not contain condensed tannins, the anthocyanins in the red pericarp of this sorghum may be the cause of this sorghum being perceived similar in astringency to Ex Nola 97 GH and NS 5511. Alternatively, when the bran of tannin sorghums was boiled in deionised water to make infusions, some of the condensed tannins would have bound to the proteins in the germ. According to Rooney and Miller (1982) the sorghum germ contains some protein bodies. The formation of condensed-tannin-protein complexes led to a reduction in the quantity of condensed tannins available to bind the salivary proteins during tasting thus explaining the apparent reduction in astringency of Ex Nola 97 GH and NS 5511 that resulted in these sorghums being perceived as similar to PAN 8564.

The  $T_{\max}$  of the least astringent sorghum (Phofu) was shorter than that of the most astringent sorghum (PAN 3860; Table 2.11). Likewise, the  $D_{\text{tot}}$  of the least astringent sorghum (Phofu) was shorter than that of the most astringent sorghum (PAN 3860). The  $T_{\max}$  was generally longer (4-8 s) for the tannin sorghums (PAN 3860, Ex Nola 97 GH and NS 5511) than the tannin-free sorghums (PAN 8564, Segalane and Phofu). With the exception of NS 5511, the total duration ( $D_{\text{tot}}$ ) of astringency of the tannin sorghums (PAN 3860 and Ex Nola 97 GH) lasted significantly longer (7-12 s) than that of the tannin-free sorghums.

In this study, all the time intensity parameters were highly significantly positively correlated with each other (Table 2.10). However, many of the correlations were very weak, showing that they only explained a relatively small percentage of the variability. The strongest correlations were between  $I_{\max}$  and AUC for both bitterness and astringency. Strong positive correlations were also observed between  $I_{\max}$  for bitterness and  $I_{\max}$  for astringency and AUC for bitterness and AUC for astringency; implying that the more bitter the sorghum the more astringent it is.

**Table 2.11.** Least Square Means ( $\pm$ SE) of parameters extracted from time intensity curves for astringency of sorghum bran infusions of tannin-containing and tannin-free sorghums

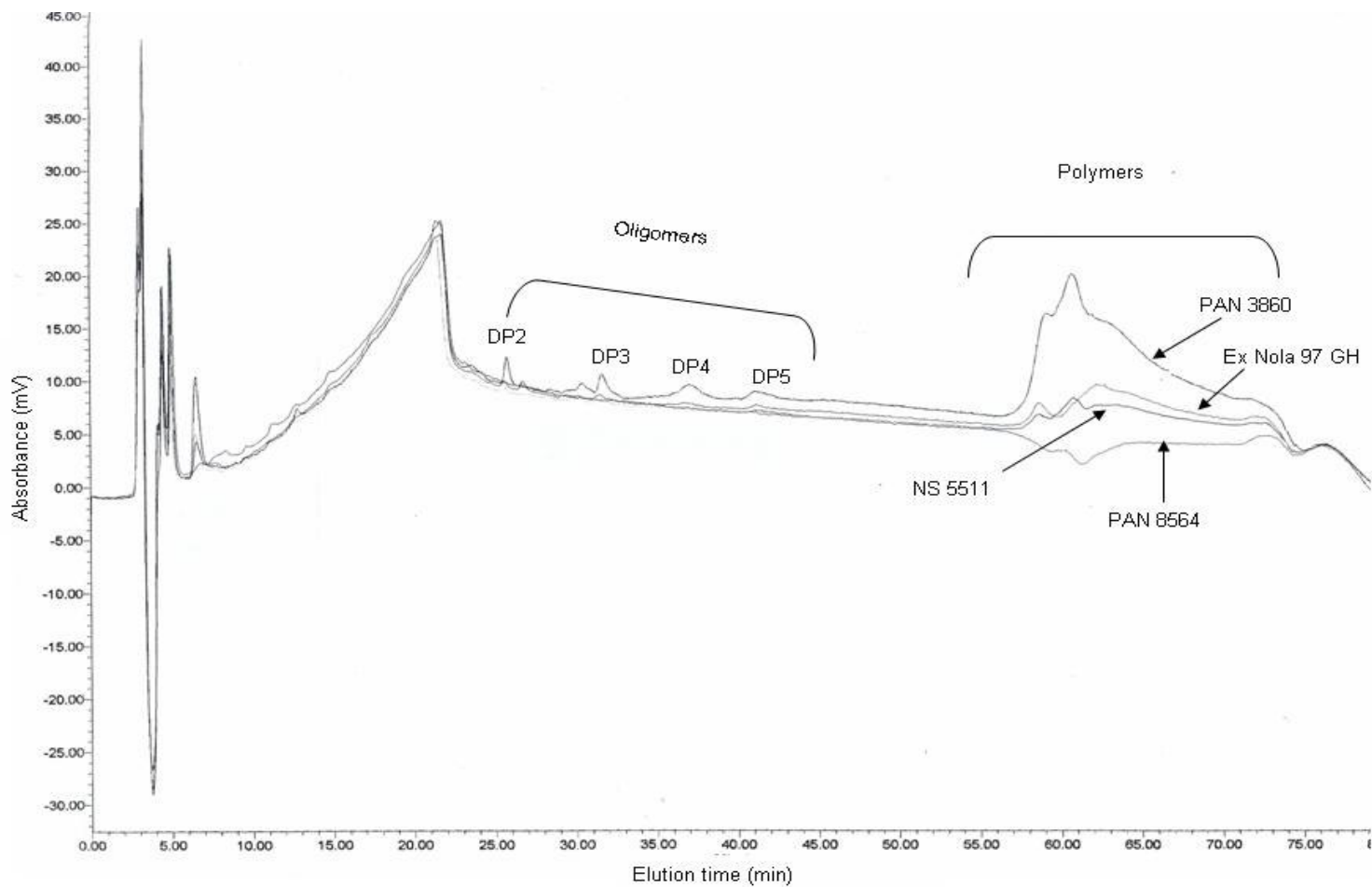
	Tannin sorghums			Tannin-free sorghums		
	PAN 3860	Ex Nola 97 GH	NS 5511	PAN 8564	Segaolane	Phofu
$T_{\max}$ (s)	30.8 <sup>c</sup> (1.4)	30.9 <sup>c</sup> (1.4)	30.3 <sup>c</sup> (1.6)	25.7 <sup>ab</sup> (1.4)	26.9 <sup>abc</sup> (1.6)	23.0 <sup>a</sup> (1.5)
$I_{\max}$	55.1 <sup>d</sup> (2.1)	42.0 <sup>c</sup> (2.1)	33.9 <sup>ab</sup> (2.3)	36.6 <sup>bc</sup> (2.1)	31.4 <sup>ab</sup> (2.3)	28.9 <sup>a</sup> (2.2)
$D_{\text{tot}}$ (s)	76.2 <sup>c</sup> (2.2)	74.2 <sup>c</sup> (2.2)	72.5 <sup>bc</sup> (2.4)	67.2 <sup>ab</sup> (2.2)	65.3 <sup>ab</sup> (2.4)	64.1 <sup>a</sup> (2.4)
AUC	2639.4 <sup>d</sup> (115.5)	1853.1 <sup>c</sup> (115.5)	1436.7 <sup>ab</sup> (128.5)	1562.6 <sup>bc</sup> (115.5)	1319.1 <sup>ab</sup> (128.5)	1102.1 <sup>a</sup> (124.5)

Least Square Means of four replicate experiments and standard errors averaged across 12 panellists.

Least Square Means in rows with different letter notations <sup>(a-e)</sup> are significantly different at  $p \leq 0.05$ .

$T_{\max}$  – time to maximum intensity,  $I_{\max}$  – maximum intensity,  $D_{\text{tot}}$  – total duration, AUC – area under curve.





**Figure 2.6.** Normal phase HPLC procyanidin profiles of PAN 3860, Ex Nola 97 GH, NS 5511 and PAN 8564. Numbers (2, 3, 4, 5) denote degree of polymerization, P = mixed polymers (DP > 5).

Bitterness took a significantly shorter time to reach maximum intensity than astringency (Table 2.12). The mean  $T_{\max}$  for bitterness was 22.5 s, whereas for astringency the mean  $T_{\max}$  was 27.9 s. This might be due to the fact that bitterness is a basic taste (Lawless and Heymann, 1998) that can be detected at very low concentrations (Glendinning, 1994). Bitter taste perception is thought to have evolved to prevent ingestion of potential poisons (Glendinning, 1994; Rodgers, Busch, Peters and Christ-Hazelhof, 2005). Unlike bitterness, astringency is a tactile sensation (Breslin, Gilmore, Beauchamp and Green, 1993). When tannins bind proteins present in the saliva, the conformational changes result with the salivary proteins losing their lubricating power, resulting with a dry and puckery feeling in the mouth (Joslyn and Goldstein, 1964). The tactile sensations caused by increased friction (decrease in salivary lubrication) between oral membranes are the primary basis of astringent sensations (Breslin *et al.*, 1993). The finding here, agrees with the observation that astringency is often the last sensation detected (Kallithraka, Bakker, Clifford and Vallis, 2001).

**Table 2.12.** Time intensity parameters extracted from time intensity curves (mean) for bitterness and astringency of sorghum bran infusions.

	$T_{\max}^{***}$ (s)	$I_{\max}$	$D_{\text{tot}}^*$ (s)	AUC
Bitterness	22.5 <sup>a</sup>	39.3 <sup>a</sup>	66.3 <sup>a</sup>	1647.3 <sup>a</sup>
Astringency	27.9 <sup>b</sup>	38.0 <sup>a</sup>	69.9 <sup>b</sup>	1632.2 <sup>a</sup>

Means in columns with different letter notations <sup>(a-b)</sup> are significantly different: \*, \*\*\* at  $p < 0.05$  and  $0.001$  respectively.

$T_{\max}$  – time to maximum intensity,  $I_{\max}$  – maximum intensity,  $D_{\text{tot}}$  – total duration, AUC – area under curve.

The mean duration of the astringency sensation was significantly ( $p \leq 0.05$ ) longer by 3.6 s than the duration of bitterness (Table 2.12). The findings of the sorghum bran infusions agree with the observations of Leach (1984) who determined the bitterness and astringency of gallic acid, catechin, grape seed tannin and tannic acid using the time intensity sensory method, and reported that the duration ( $D_{tot}$ ) of the astringent after-taste was generally longer by 10-15 s than that of bitterness. A significantly shorter time ( $T_{max}$ ) was required to reach  $I_{max}$  for less astringent compounds, gallic acid and catechin, than for the more astringent compounds, tannic acid and grape seed tannin. Furthermore, duration ( $D_{tot}$ ) of the bitter and astringent after-taste increased with increasing intensity ( $I_{max}$ ) of bitterness and astringency. King and Duineveld (1999) studied the bitterness in beer during ageing and observed a significant positive correlation between  $I_{max}$  and AUC ( $r = 0.95$ ,  $p < 0.05$ ). Sensory bitterness generally decreased with the age of the beer, resulting in lower  $I_{max}$  and a smaller AUC. Similarly in this study, there was a highly significant positive correlation between  $I_{max}$  and AUC ( $r = 0.88$ ,  $p < 0.001$ ) for bitterness (Table 2.10).

François, Guyot-Declerck, Hug, Callemien, Govaerts and Collin (2006) studied the influence of pH and accelerated ageing of beer on its astringency by the time intensity method and quantitative descriptive analysis. Contrary to the findings of Leach (1984) and those reported here, they found a significant ( $p < 0.05$ ) inverse relationship between  $T_{max}$  and  $I_{max}$  ( $r = -0.820$ ) for the astringency of beer. In other words, the more intense the astringency of beer, the less time it took for panellists to perceive the maximum intensity of astringency. This difference might be due to the media matrix of astringency of the sorghum bran infusions compared to the beer tested in the study by François *et al.* (2006). The astringencies of the sorghum bran infusions were perceived as only mild to moderate during quantitative descriptive analysis (Chapter 2.1). François *et al.* (2006) also observed a high ( $p < 0.01$ ) positive correlation between  $I_{max}$  and AUC ( $r = 0.914$ ) for astringency in beer. Intensification of astringency led to a longer and/or higher persistence. Similarly in this study, a high ( $p < 0.001$ ) positive correlation between intensity ( $I_{max}$ ) and AUC ( $r = 0.87$ ) for the astringency sensation (Table 2.10).

#### 2.2.4.1.2. Panellist effect

There was a highly significant ( $p < 0.001$ ) panellist effect for all the time intensity parameters ( $T_{max}$ ,  $I_{max}$ ,  $D_{tot}$  and AUC) for both bitterness (Tables 2.7) and astringency (Tables 2.8). This is related to the fact that there was variation between the ratings of

panellists (Table 2.13). Some of the panellists (4 and 11) routinely used the upper end of the scale, whilst others (3, 7 and 9) used the lower end of the scale. Four panellists (6, 9 and 11) experienced the bitterness and astringency of the sorghum bran infusions a lot longer ( $D_{tot} > 80$  s) than panellists 1 and 12 ( $D_{tot}$  between 50 and 60 s). The astringency and bitterness sensations developed very slowly for Panellists 1 and 11, as a result their  $T_{max}$  for astringency and bitterness was the longest. Panellist effects on  $T_{max}$ ,  $I_{max}$  and AUC for astringency and bitterness of the sorghum bran infusions are discussed under the interaction effects.

According to Tomic, Nilsen, Martens and Næs (2007), a source of individual differences in time intensity data among panellists may be due to panellists using the time intensity scale differently, the panellists experiencing sensory attributes differently, and/or random variation error. These factors might also apply to the findings of this study. The fact that the panellists used the time intensity scale differently and experienced the sensory sensations differently is demonstrated by the different shapes of their time intensity curves (Fig. 2.7 a-l) and consequently the time intensity parameters (Table 2.13) extracted from their curves. This phenomenon has been observed by other researchers (Leach and Noble, 1986; Noble, Matysiak and Bonnans, 1991; Kallithraka *et al.*, 2001; François *et al.*, 2006) and is referred to as the individual panellist's 'signature'. Different curve shapes among panellists has been demonstrated as the major cause of large standard deviations in time intensity tests (Noble *et al.*, 1991). Leach and Noble (1986) compared the bitterness of caffeine and quinine by time intensity procedure. Judges differed significantly ( $p < 0.001$ ) in the responses to all time intensity parameters for bitterness of caffeine and quinine, and were consistent among replications. Kallithraka *et al.* (2001) observed significant differences ( $p \leq 0.001$ ) between panellists for  $T_{max}$ ,  $I_{max}$  and  $D_{tot}$  for astringency of wine. Their panel had been trained extensively and had considerable experience (2 years). Thus, these differences were not attributed to inconsistent performance of the panel. Rather, two possibilities were considered. Some subjects habitually used the higher end of the scale, and in the case of astringency, differences in salivary flow rates among panellists also attributed to the differences. François *et al.* (2006), in their study of the influence of pH and accelerated ageing of beer on its astringency, also observed extremely diverse individual panellist time intensity curves. Each panellist presented exactly the same pattern ('signature'). They also attributed these differences to different salivary flow rates.

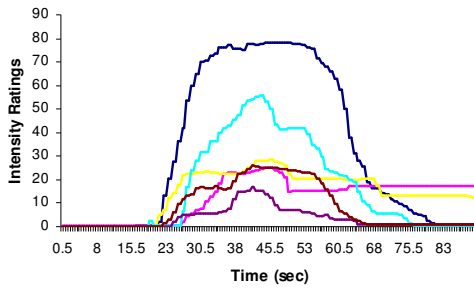
**Table 2.13.** Panellists' Least Square Means of parameters extracted from time intensity curves for astringency and bitterness of sorghum bran infusions

Panellists	Bitterness				Astringency			
	T <sub>max</sub> (s)	I <sub>max</sub>	D <sub>tot</sub> (s)	AUC	T <sub>max</sub> (s)	I <sub>max</sub>	D <sub>tot</sub> (s)	AUC
1	36.9 <sup>e</sup>	42.3 <sup>de</sup>	52.4 <sup>a</sup>	1339.7 <sup>bc</sup>	44.2 <sup>d</sup>	47.9 <sup>fg</sup>	60.9 <sup>b</sup>	1355.0 <sup>ab</sup>
2	20.5 <sup>c</sup>	48.0 <sup>e</sup>	66.2 <sup>cd</sup>	1737.0 <sup>cd</sup>	24.6 <sup>b</sup>	34.0 <sup>cd</sup>	58.2 <sup>b</sup>	995.0 <sup>a</sup>
3	13.8 <sup>a</sup>	18.3 <sup>a</sup>	64.4 <sup>bcd</sup>	578.5 <sup>a</sup>	20.0 <sup>ab</sup>	30.6 <sup>bcd</sup>	66.2 <sup>b</sup>	1223.0 <sup>a</sup>
4	14.3 <sup>ab</sup>	64.3 <sup>f</sup>	72.8 <sup>de</sup>	3243.1 <sup>e</sup>	20.1 <sup>ab</sup>	67.7 <sup>h</sup>	82.5 <sup>c</sup>	3899.0 <sup>e</sup>
5	14.7 <sup>ab</sup>	32.3 <sup>bc</sup>	56.9 <sup>abc</sup>	1082.0 <sup>b</sup>	16.7 <sup>a</sup>	43.4 <sup>efg</sup>	64.0 <sup>b</sup>	1813.4 <sup>c</sup>
6	25.3 <sup>d</sup>	39.5 <sup>cd</sup>	82.9 <sup>e</sup>	1905.6 <sup>d</sup>	20.7 <sup>ab</sup>	34.3 <sup>de</sup>	87.6 <sup>c</sup>	1707.5 <sup>bc</sup>
7	23.0 <sup>cd</sup>	31.3 <sup>b</sup>	53.9 <sup>ab</sup>	1036.6 <sup>ab</sup>	27.2 <sup>b</sup>	24.8 <sup>ab</sup>	62.7 <sup>b</sup>	969.2 <sup>a</sup>
8	17.6 <sup>abc</sup>	32.0 <sup>bc</sup>	60.3 <sup>abc</sup>	1260.4 <sup>bc</sup>	16.0 <sup>a</sup>	26.1 <sup>abc</sup>	66.3 <sup>b</sup>	1072.5 <sup>a</sup>
9	15.7 <sup>ab</sup>	27.1 <sup>b</sup>	85.5 <sup>e</sup>	1285.3 <sup>bc</sup>	32.3 <sup>c</sup>	23.1 <sup>a</sup>	88.0 <sup>c</sup>	1375.8 <sup>abc</sup>
10	22.6 <sup>cd</sup>	28.7 <sup>b</sup>	55.1 <sup>ab</sup>	1248.3 <sup>b</sup>	46.8 <sup>d</sup>	35.6 <sup>de</sup>	65.1 <sup>b</sup>	1390.5 <sup>ac</sup>
11	46.7 <sup>f</sup>	61.0 <sup>f</sup>	89.4 <sup>e</sup>	3686.5 <sup>e</sup>	46.4 <sup>d</sup>	48.0 <sup>g</sup>	89.0 <sup>c</sup>	2897.9 <sup>d</sup>
12	18.9 <sup>bc</sup>	47.2 <sup>e</sup>	56.1 <sup>abc</sup>	1365.1 <sup>bc</sup>	20.2 <sup>ab</sup>	40.0 <sup>def</sup>	48.3 <sup>a</sup>	1127.3 <sup>a</sup>

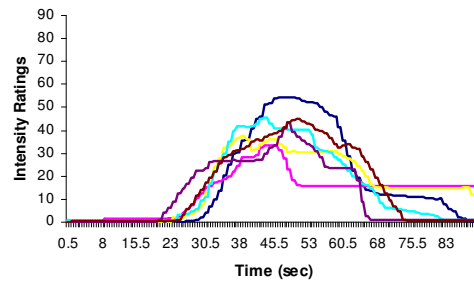
Least Square Means of four replicate experiments.

Least Square Means in columns with different letter notations <sup>(a-e)</sup> are significantly different at  $p \leq 0.01$

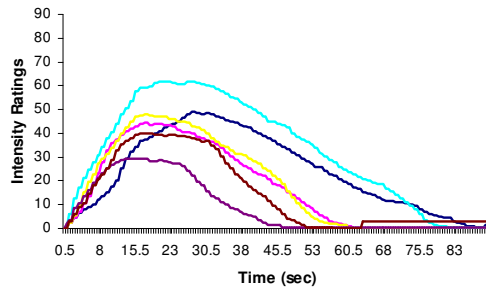
T<sub>max</sub> – time to maximum intensity, I<sub>max</sub> – maximum intensity, D<sub>tot</sub> – total duration, AUC – area under curve.



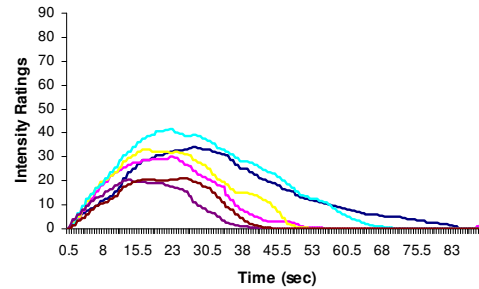
Panellist 1 - Bitterness



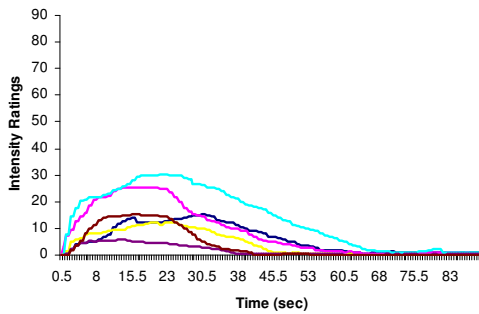
Panellist 1 - Astringency



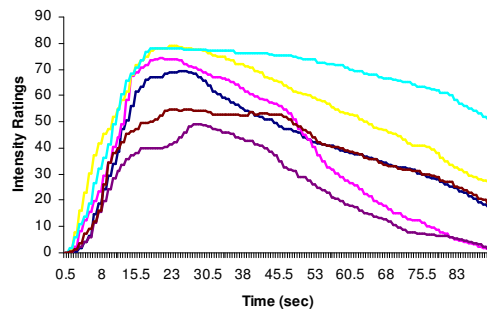
Panellist 2 - Bitterness



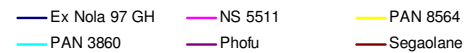
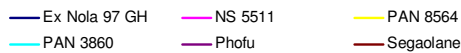
Panellist 2 - Astringency



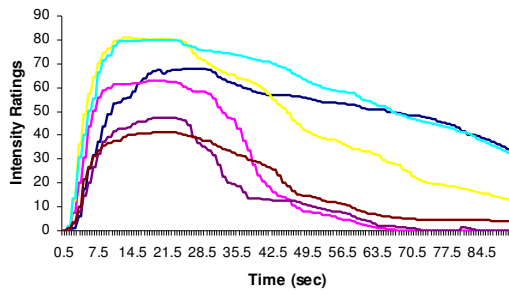
Panellist 3 - Bitterness



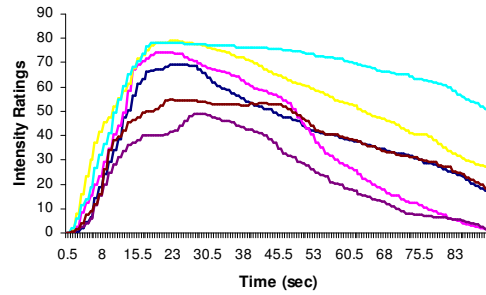
Panellist 3 - Astringency



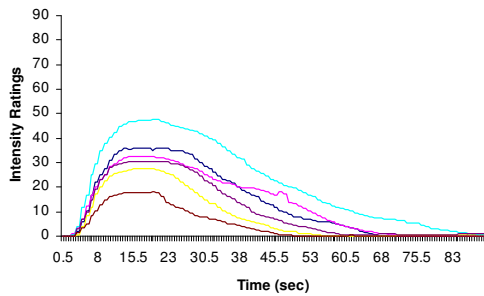
**Figure 2.7.** Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 1 – 3.



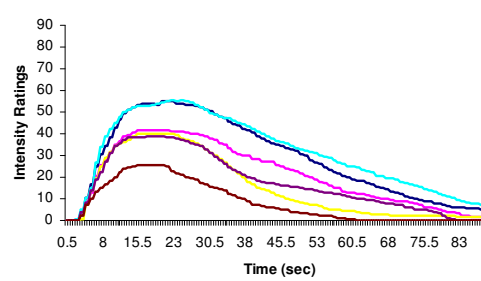
Panellist 4 - Bitterness



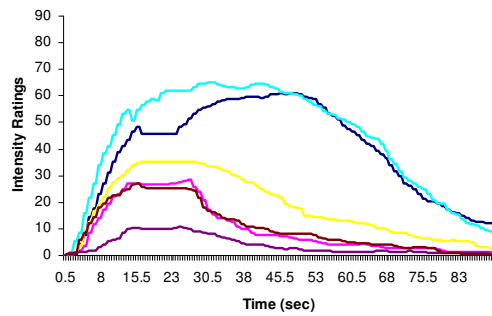
Panellist 4 - Astringency



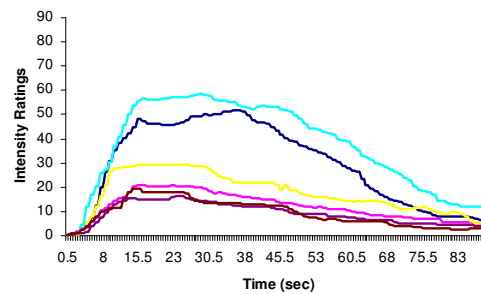
Panellist 5 - Bitterness



Panellist 5 - Astringency



Panellist 6 - Bitterness

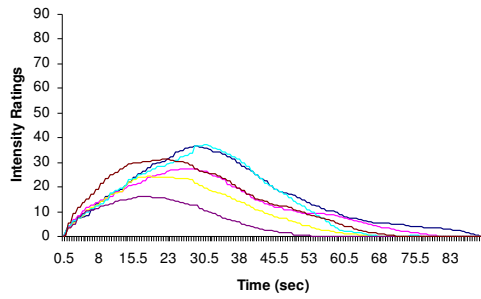


Panellist 6 - Astringency

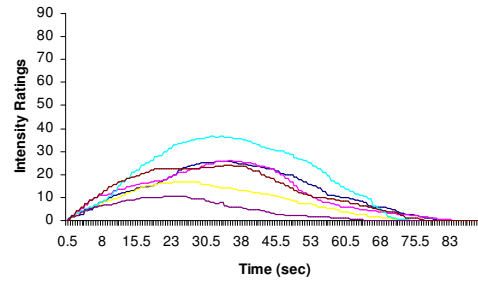
— Ex Nola 97 GH    — NS 5511    — PAN 8564  
— PAN 3860    — Phofu    — Segaothane

— Ex Nola 97 GH    — NS 5511    — PAN 8564  
— PAN 3860    — Phofu    — Segaothane

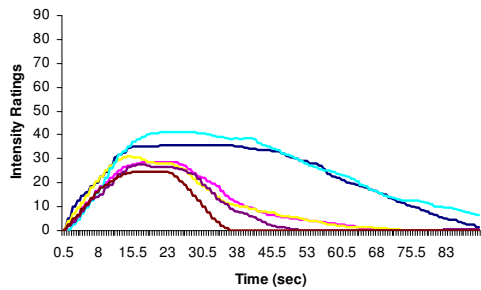
**Figure 2.7.** Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 4 – 6 (continued).



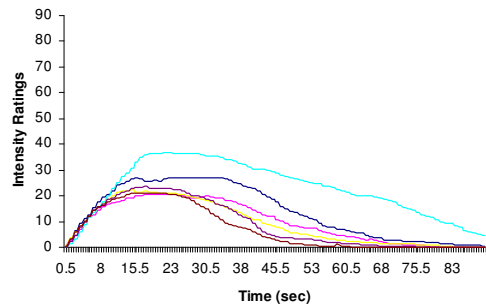
Panellist 7 - Bitterness



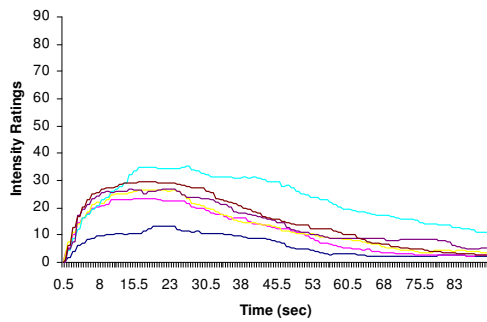
Panellist 7 - Astringency



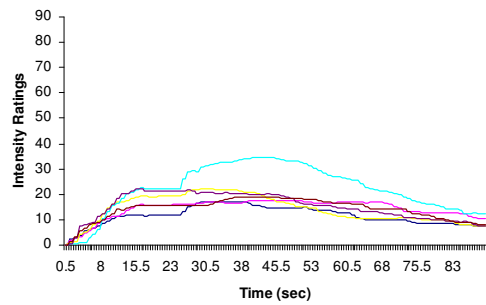
Panellist 8 - Bitterness



Panellist 8 - Astringency



Panellist 9 - Bitterness



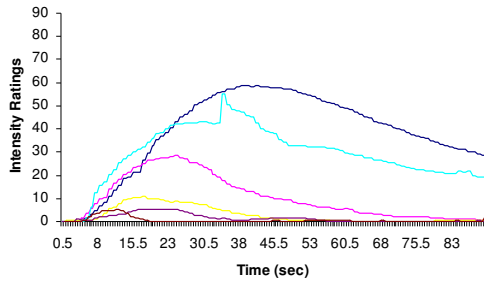
Panellist 9 - Astringency

— Ex Nola 97 GH    — NS 5511    — PAN 8564  
— PAN 3860    — Phofu    — Segaolane

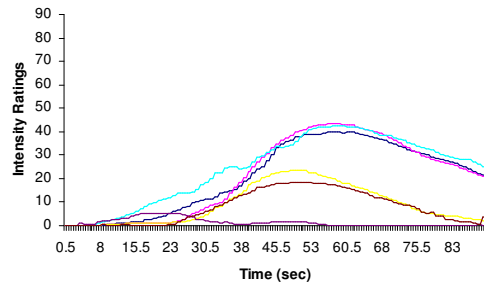
— Ex Nola 97 GH    — NS 5511    — PAN 8564  
— PAN 3860    — Phofu    — Segaolane

**Figure 2.7.** Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 7 – 9 (continued).

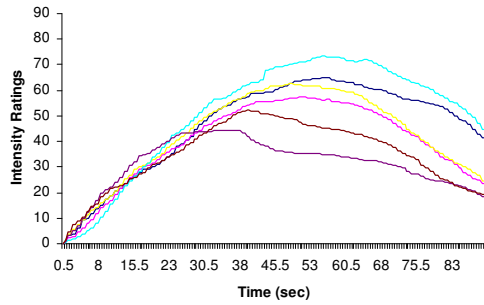




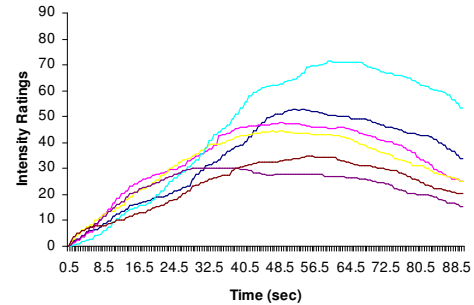
Panellist 10 - Bitterness



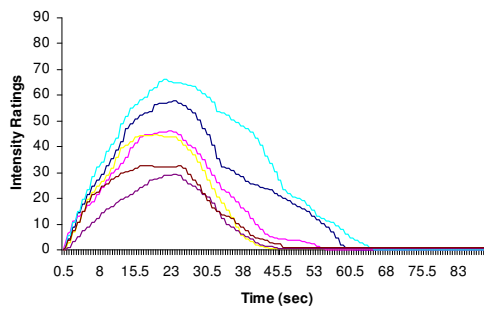
Panellist 10 - Astringency



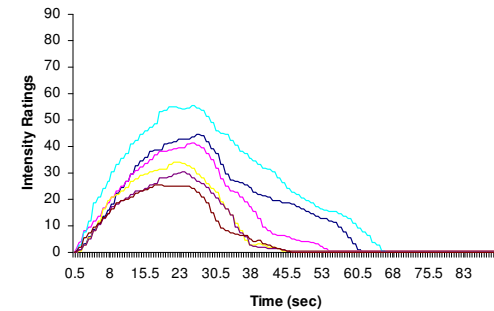
Panellist 11 - Bitterness



Panellist 11 - Astringency



Panellist 12 - Bitterness



Panellist 12 - Astringency

— Ex Nola 97 GH      — NS 5511      — PAN 8564  
— PAN 3860      — Phofu      — Segalane

— Ex Nola 97 GH      — NS 5511      — PAN 8564  
— PAN 3860      — Phofu      — Segalane

**Figure 2.7.** Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 10 – 12 (continued).

This study did not determine the panellist's salivary flow rates. However, it is probable that differences in astringency perceptions could be related to different individual salivary flow rates, as was found by Kallithraka *et al.* (2001) working with wine, François *et al.* (2006) working with beer, Fischer, Boulton and Noble (1994) working with wine; and Ishikawa and Noble (1995) studying red wine. Guinard, Pangborn and Lewis (1986) determined the time-course of astringency in wine upon repeated ingestion and reported that the time required to return to normal mouth lubrication after removal of tannin-protein precipitate by saliva determines the duration of the astringency sensation in the mouth. This might explain why subjects with lower salivary flow rates experience the astringency sensation longer than subjects with higher salivary flow rates.

In this study, another consideration could be the extent of training of panellists in the use of the DATI sensory method. The length of training may not have been sufficient and thus could have also contributed to the inconsistencies observed within and between panellists. The task of paying attention to two different attributes, and simultaneously tracking their changes is a complex one (Dijksterhuis and Piggott, 2001). Notwithstanding the fact that measuring two different attributes simultaneously is complex, it was worthwhile to determine them this way, because this method revealed differences in the rates of bitterness and astringency development and persistence.

#### 2.2.4.1.3. *Session effect*

The only highly significant variations ( $p < 0.001$ ) noted between sessions were for  $I_{\max}$  and AUC for astringency (Table 2.8). The panel rated the maximum intensity ( $I_{\max}$ ) of astringency of samples in session 2 significantly higher than samples in session 1 (Table 2.14). If this was due to astringency build up from session 1, it would mean that the two hour gap between the sessions was not adequate. Guinard *et al.* (1986) studied the time-course of astringency of wine upon repeated ingestion. Maximum intensity of astringency increased ( $p < 0.001$ ) upon repeated ingestion. The ingestions were seconds apart. The increase was greater (although not significantly) when 20 s compared to 40 s was programmed between ingestions. Thus, in the work reported here the significant variations between sessions were probably due to random error.

**Table 2.14.** Least square means of time intensity parameters of different sessions for astringency and bitterness of sorghum bran infusions.

Session	Bitterness				Astringency			
	T <sub>max</sub> (s)	I <sub>max</sub>	D <sub>tot</sub> (s)	AUC	T <sub>max</sub> (s)	I <sub>max</sub>	D <sub>tot</sub> (s)	AUC
1	22.0 <sup>a</sup>	37.5 <sup>a</sup>	66.8 <sup>a</sup>	1555.9 <sup>a</sup>	27.0 <sup>a</sup>	33.7 <sup>a</sup>	70.0 <sup>a</sup>	1428.9 <sup>a</sup>
2	23.0 <sup>a</sup>	41.1 <sup>a</sup>	65.9 <sup>a</sup>	1738.8 <sup>a</sup>	28.9 <sup>a</sup>	42.3 <sup>b</sup>	69.8 <sup>a</sup>	1875.4 <sup>b</sup>

LS Means of four replicate experiments averaged across two sessions.

LS Means in columns with different letter notations <sup>(a-b)</sup> are significantly different at  $p \leq 0.001$ .

T<sub>max</sub> – time to maximum intensity, I<sub>max</sub> – maximum intensity, D<sub>tot</sub> – total duration, AUC – area under curve.

#### 2.2.4.1.4. Replicate effect

There were significant ( $p \leq 0.05$ ) replicate variations for bitterness for T<sub>max</sub>, I<sub>max</sub> and AUC (Table 2.7). For astringency, the only significant ( $p < 0.05$ ) replicate effect was for T<sub>max</sub> (Table 2.8). There was no trend to suggest that samples were stronger or weaker in astringency and/or bitterness on one day than other days (Table 2.15). The lack of a trend in the differences probably implies that the observed significant differences were due to random variation.

**Table 2.15.** Least square means of time intensity parameters of different replicates for astringency and bitterness of sorghum bran infusions.

Replicates	<u>Bitterness</u>				<u>Astringency</u>			
	T <sub>max</sub> (s)	I <sub>max</sub>	D <sub>tot</sub> (s)	AUC	T <sub>max</sub> (s)	I <sub>max</sub>	D <sub>tot</sub> (s)	AUC
1	24.8 <sup>b</sup>	37.5 <sup>ab</sup>	68.0 <sup>a</sup>	1592.3 <sup>ab</sup>	30.6 <sup>b</sup>	35.9 <sup>a</sup>	72.1 <sup>a</sup>	1576.8 <sup>a</sup>
2	22.8 <sup>ab</sup>	36.2 <sup>a</sup>	67.2 <sup>a</sup>	1523.6 <sup>a</sup>	27.7 <sup>ab</sup>	36.8 <sup>a</sup>	68.1 <sup>a</sup>	1625.5 <sup>a</sup>
3	21.1 <sup>a</sup>	40.6 <sup>ab</sup>	64.0 <sup>a</sup>	1562.0 <sup>ab</sup>	27.6 <sup>ab</sup>	38.0 <sup>a</sup>	67.9 <sup>a</sup>	1580.2 <sup>a</sup>
4	21.3 <sup>ab</sup>	43.0 <sup>b</sup>	66.1 <sup>a</sup>	1911.5 <sup>b</sup>	25.9 <sup>a</sup>	41.2 <sup>a</sup>	71.5 <sup>a</sup>	1826.2 <sup>a</sup>

Least Square Means of four replicate experiments averaged across four replicates.

Least Square Means in columns with different letter notations <sup>(a-b)</sup> are significantly different at  $p \leq 0.01$ .

T<sub>max</sub> – time to maximum intensity, I<sub>max</sub> – maximum intensity, D<sub>tot</sub> – total duration, AUC – area under curve.

#### 2.2.4.1.5. *Sample order effect*

As expected, there was no significant main effect differences observed related to the order in which the sample were evaluated for both bitterness and astringency (Tables 2.7 and 2.8, respectively).

#### 2.2.4.2. Interaction effects

There were significant interaction effects: panellist x cultivar, panellist x session and panellist x replicate for bitterness (Table 2.7); and panellist x session, panellist x replicate, and panellist x sample order for astringency (Table 2.8). An interaction exists when the impact of one independent variable depends on the value of another independent variable (Lewis-Beck, 1993).

#### 2.2.4.2.1. *Panellist x cultivar*

Although there was a significant panellist x cultivar interaction effect for  $T_{\max}$ ,  $I_{\max}$  and AUC for bitterness, it was not strong (Table 2.7 and Fig. 2.8). All the panellists ( $n = 12$ ) were sensitive to 6-n-propyl-2-thiouracil (PROP) tasters and thus PROP taster status could not have accounted for the variations. There was no significant panellist x cultivar interaction effect for astringency, indicating that the individual panellists agreed on the relative difference in astringency of the sorghum cultivars (Table 2.8).

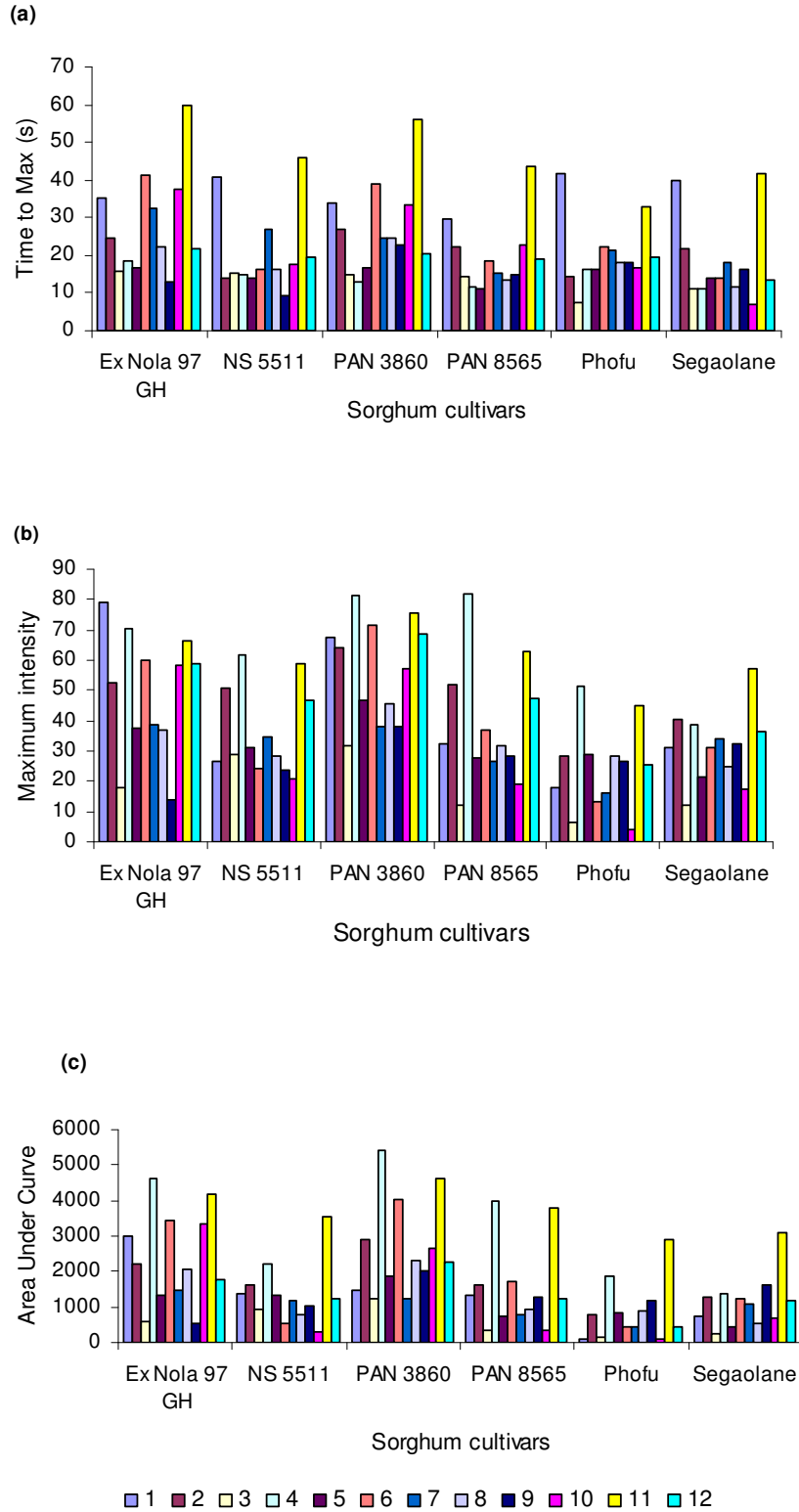
#### 2.2.4.2.2. *Panellist x session*

Although there was a significant ( $p < 0.05$ ) session x panellist interaction effect for bitterness  $I_{\max}$  (Table 2.7) and a significant ( $p < 0.05$ ,  $p < 0.01$ , respectively) session x panellist interaction effect for astringency  $I_{\max}$  and  $D_{\text{tot}}$  (Table 2.8), it was not strong. For bitterness  $I_{\max}$  some panellists rated higher in session 1 than session 2 whilst others rated higher in session 2 than session 1 (Fig. 2.9). Panellists 5 and 10 showed much more variation compared to others, particularly in the second session.

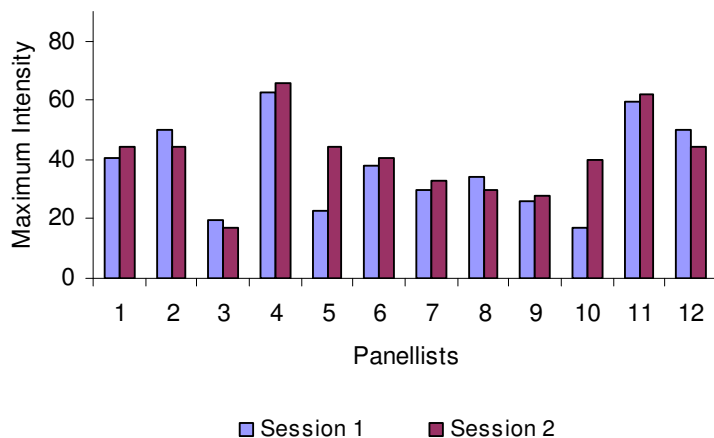
For astringency, some of the panellists rated  $I_{\max}$  of samples in session 2 much higher than those in session 1 (Fig. 2.10a); and total duration ( $D_{\text{tot}}$ ) of some of the panellists was rated longer in session 1 than session 2, whilst for some it was shorter in session 2 than session 1 (Fig. 2.10b). These differences account for the significant session x panellist interaction effect observed and seem to be due to random variation.

#### 2.2.4.2.3. *Panellist x replicate*

Although there was a significant ( $p < 0.05$ ) panellist x replicate interaction effect for bitterness AUC (Table 2.7; Fig. 2.11), and a significant ( $p < 0.05$ , 0.01, 0.01, respectively) panellist x replicate interaction effect for astringency  $T_{\max}$ ,  $I_{\max}$  and AUC and (Table 2.8; Fig. 2.12), it was not strong. Panellists rated the samples differently on different days (replications). However, there was no trend observed indicating that samples evaluated during initial replicates were perceived to be stronger or weaker than those served in latter replicates. The lack of a trend suggests that the significant differences observed are due to random variation.



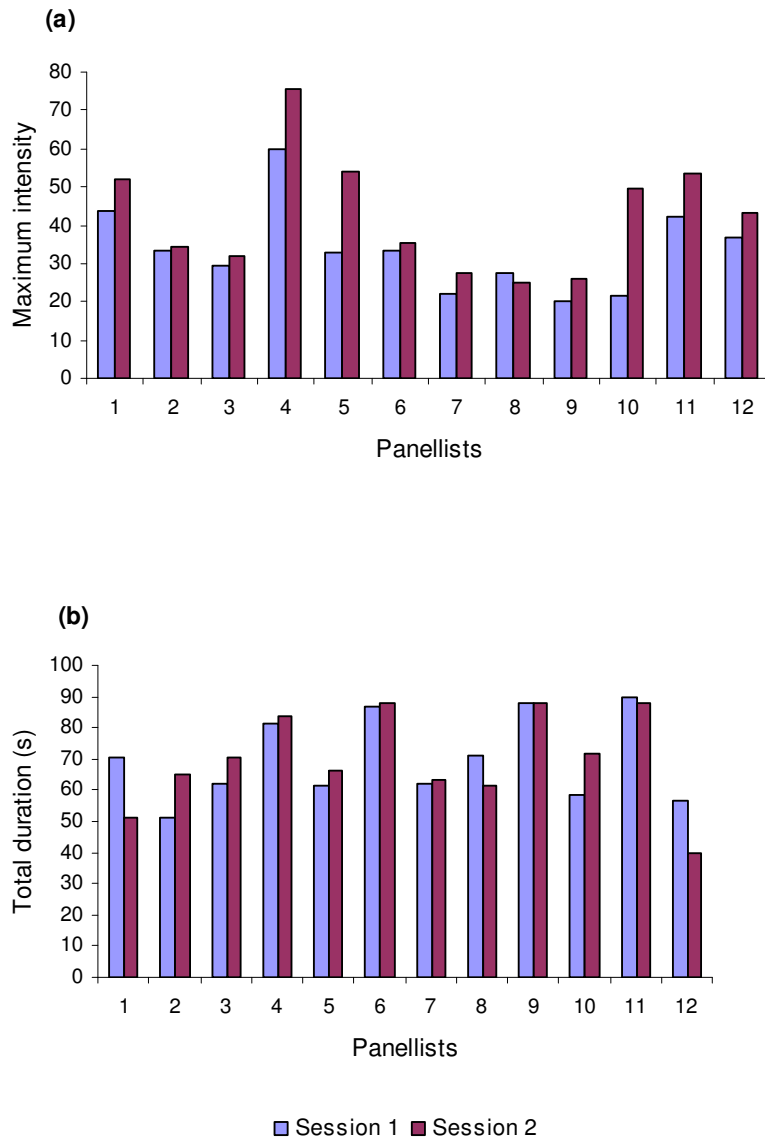
**Figure 2.8.** Least square means of panellist x cultivar interaction effect on (a)  $T_{max}$ , (b)  $I_{max}$  and (c) AUC for bitterness. Numbers 1 to 12 refer to individual panellists.



**Figure 2.9.** Least square means of panellist x session interaction effect for  $I_{\max}$  for bitterness.

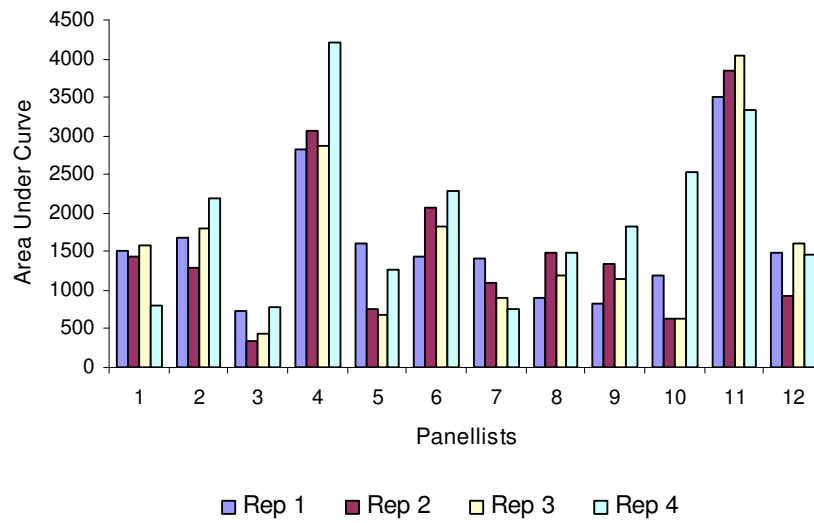
#### 2.2.4.2.4. Panellist x sample order

Although there was a significant ( $p < 0.05$ ,  $0.01$  respectively) panellist x sample order interaction effect for astringency  $I_{\max}$  and AUC, it was not strong (Table 2.8). Generally, panellists' ratings were not influenced by sample order. However, some panellists rated samples in certain positions much higher or lower than samples in other positions (Fig. 2.13). Nonetheless, there was no trend of certain sample positions being rated higher or lower than others.

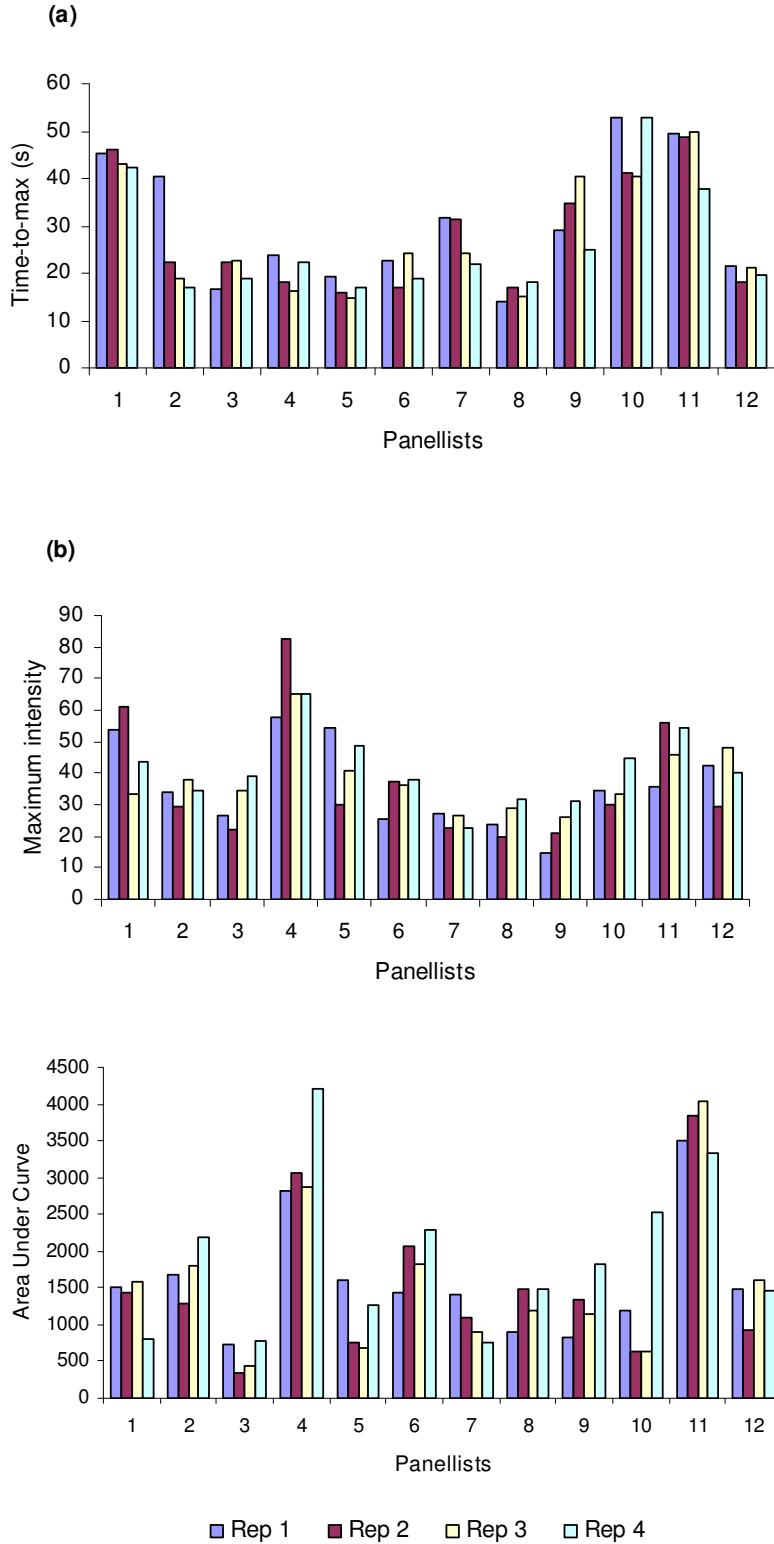


**Figure 2.10.** Least square means of panellist x session interaction effects for (a)  $I_{\max}$  astringency and (b)  $D_{\text{tot}}$ .

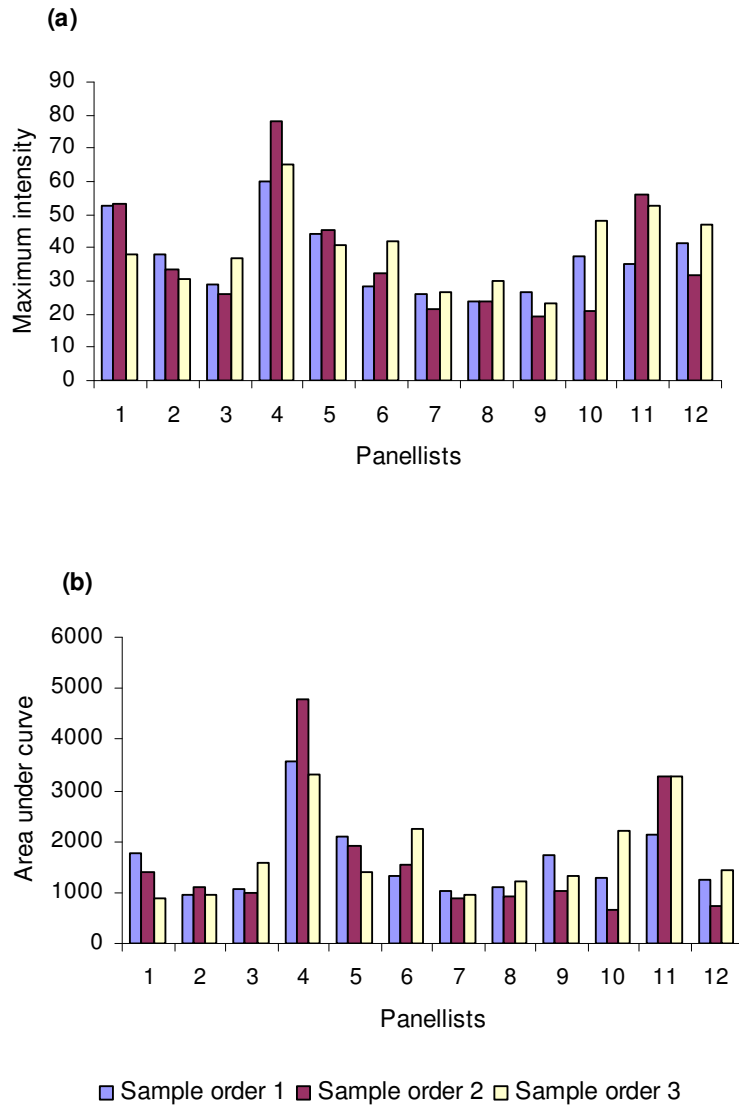




**Figure 2.11.** Least square means of panellist x replicate interaction effects for AUC for bitterness.



**Figure 2.12.** Least square means of panellist x replicate interaction effects for (a)  $T_{max}$ , (b)  $I_{max}$  and (c) AUC for astringency.



**Figure 2.13.** Least square means of panellist x sample order interaction effects on (a)  $I_{\max}$  and (b) AUC for astringency.

### **2.2.5. Conclusions**

The more bitter the sorghum the more astringent it is. It appears that bitterness and astringency are generally, but not always, the same in level of strength in individual sorghum cultivars. For some tannin sorghums, bitterness seemed more predominant than astringency. As NS 5511 was perceived similar to the tannin-free sorghums, it seems there is a condensed tannin threshold level at which the tannins are not 'strongly' perceived. The findings suggest that in sorghum-based food systems the presence of condensed tannins in sorghum may not necessarily impart the objectionable attributes associated with them.

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## **2.3. Consumer acceptability of sorghum rice from tannin and tannin-free sorghums and the influence of PROP taster status**

### **2.3.1. Abstract**

Condensed tannins in sorghum are potentially excellent antioxidants yet their sensory properties are believed to be objectionable to consumers. The objective of this study was to determine consumer acceptability of sorghum rice from sorghums containing different levels of condensed tannins and the influence of 6-*n*-propylthiouracil (PROP) taster status on acceptability. Consumers ( $n = 194$ ) evaluated the sensory attributes (appearance, flavour, texture and overall liking) of sorghum rice from tannin and tannin-free sorghums prior to the one-solution PROP test. The sorghum rice from cultivar PAN 3860, with the highest tannin content (8.2% catechin equivalent [CE]), received significantly lower ratings for all the sensory attributes than the other sorghums. With the exception of appearance, the rice from tannin sorghum NS 5511 (1.8% CE) was not significantly different from that of the two tannin-free sorghums. The findings suggest that not all tannin sorghum products are objectionable to consumers. The PROP tasters (medium and super) could presumably distinguish differences among the sorghum cultivars varying in tannin content levels which led to significant differences in their acceptance ratings for the sorghums. On the other hand, non tasters preferred the cultivars equally, presumably because they could not detect differences in bitterness and astringency between the cultivars. These results support the assertion that there may be a condensed tannin threshold level at which the tannins are not perceived as objectionable.

### 2.3.2. Introduction

Sorghum is a rich source of phytochemicals such as phenolic acids, anthocyanins and condensed tannins (Awika and Rooney, 2004). Condensed tannins are well-known for eliciting negative consumer response (especially at high intensity) because of their dominant sensory properties: namely, bitterness and astringency (Lesschaeve and Noble, 2005). Sensory attributes of whole grain sorghum rice and bran infusions of tannin and tannin-free sorghums were profiled using quantitative descriptive analysis (Chapter 2.1). The products of all the sorghum cultivars were, to different degrees, perceived as both bitter and astringent. The sorghum rice from PAN 3860 with the highest condensed tannin content (8.2% catechin equivalent [CE]) was most bitter and most astringent. Surprisingly, NS 5511 (tannins – 1.8% CE) was perceived similar in both bitterness and astringency to a tannin-free sorghum (PAN 8564). In a follow-up study to determine the temporal relationship between bitterness and astringency of bran infusions of tannin-free and tannin sorghums, it appeared that bitterness and astringency are generally, but not always, of the same strength in different sorghums (Chapter 2.2). The bitterness of the infusion from tannin sorghum Ex Nola 97 GH (tannin 5.7% CE) seemed more predominant than its astringency. This is because the infusion from Ex Nola 97 GH was significantly more bitter than that from a tannin-free sorghum (PAN 8564), whereas the astringency of these sorghums was not significantly different. The infusion of NS 5511 was again perceived similar in both bitterness and astringency to a tannin-free sorghum (PAN 8564). It seems that in sorghum-based foods the presence of condensed tannins may not necessarily impart the objectionable sensory attributes associated with them. There may be a condensed tannin threshold level at which the tannins are not ‘strongly’ perceived and thus are not objectionable.

According to Drewnowski and Rock (1995) the sense of taste is a powerful predictor of food selection. An individual’s sensitivity to taste has potential in influencing their ingestion of bitter foods and beverages (Mattes, 1994). Genetic variation in taste perceptions has been investigated by many researchers since Fox (1931) accidentally discovered that his colleague could taste the bitterness of phenylthiocarbamide (PTC), whilst he found it tasteless. PTC and 6-*n*-propylthiouracil (PROP) carry the chemical group H-N-C=S responsible for their characteristic bitter taste (Bartoshuk, 1993). Blakeslee and Fox (1932) investigated the genetics of taste acuity, and their results

demonstrated evidence of the inheritance of the taste capacity for PTC. Kalmus (1958) reported that sensitivity to the bitter taste of PTC is genetically linked to the dominant allele - 'T.' Non tasters of PTC being genotype – 'tt', and tasters being genotypes – 'Tt' and 'TT.' Greater sensitivity to the bitterness of PROP has been linked to reduced acceptability of foods such as dry milk products and cheese (Marino, Bartoshuk, Monaco, Anliker, Reed and Desnoyers, 1991), broccoli and cheese (Tepper, 1999; Keller, Steinmann, Nurse and Tepper, 2002), broccoli, spinach, Brussels sprouts, black coffee, soy milk and soybean tofu (Kaminski, Henderson and Drewnowski, 2000), grapefruit juice (Drewnowski, Henderson and Shore, 1997) and red wine (Pickering, Simunkova and DiBattista, 2004). Thus, it is possible that since PROP super tasters have greater sensitivity to bitterness, the acceptability of foods from sorghums differing in tannin levels may differ between super and non tasters.

The objective of this study was to determine consumer acceptability of whole grain rice from sorghums differing in condensed tannin levels and the influence of PROP taster status on acceptance.

### **2.3.3. Materials and methods**

#### **2.3.3.1. Sorghum**

Four sorghum cultivars were used. Two were tannin-free sorghums: PAN 8564 and Phofu; and two were tannin sorghums: PAN 3860 (8.2% CE) and NS 5511 (1.8% CE). Since the tannin sorghums had a red pericarp, a tannin-free sorghum with a red pericarp (PAN 8564) was used for comparison. The other tannin-free sorghum (Phofu) had a white pericarp (Chapter 2.1). Other details were given in Chapter 2.1.

#### **2.3.3.2. Consumer recruitment**

Consumers aged >18 years were recruited from the staff and students of the University of Pretoria. Two hundred consumers took part in the sensory evaluation but six of them did not do the PROP test and therefore their results for the hedonic rating test were excluded from the study (Table 2.16). Demographic data were obtained from the panellists including their age and gender. The final sample data set consisted of 194 subjects (55 men and 139 women), of whom 76% were between the ages of 18-25 years and the rest were older. Since consumers were recruited on a first come first serve basis, not according

to PROP taster status, age or gender, this resulted in an irregular distribution of the PROP taster, age and gender groups. The panellists signed a consent form prior to the assessment of the samples, informing them of the nature of the sorghum samples as well as PROP before they evaluated the samples.

#### 2.3.3.3. Sample preparation, presentation and assessment

Whole grain sorghum rice was prepared by washing sorghum grain (150 g) and soaking in boiled (96°C) deionised water (250 ml) in food grade polyethylene bags (150 mm x 200 mm) and left at room temperature for 1 h. The soaking water was then drained off. Boiling (96°C) deionised water (500 ml) was added to the soaked grain in the polyethylene bags and the grain cooked for 1h in boiling (96°C) water. The sorghum rice (15-20 g) was served warm (35 ± 5°C) in Styrofoam cups (100 ml) covered with a lid. To balance out any order effect, the sorghum rice sample presentation was randomized over the entire block and random three digit numbers were used to code the samples.

Four tasting sessions were undertaken per day and panellists were served in groups of sixteen per session. Each panellist assessed all four sorghum cultivars. The consumer tests (hedonic rating of sorghum rice and PROP status) were structured in such a way that the panellists assessed the sorghum rice first and after 4 min they continued with the one-solution PROP test developed by Tepper, Christensen and Cao (2001). The sensory evaluation software used was Compusense® Five release 4.6 [1986-2003] (Guelph, Ontario Canada).

Panellists sat in individual booths and evaluated the samples under white light. The panellists rated four sorghum rice attributes: appearance, flavour, texture and overall liking using a nine-point rating scale anchored 1 = 'dislike extremely', 5 = 'neither like nor dislike' and 9 = 'like extremely' according to Peryam and Pilgrim (1957). The panellists were also requested to make general comments on each of the samples. The panellists were given pieces of raw carrots and deionised water to cleanse their mouths thoroughly before tasting and in between samples.

#### 2.3.3.4. PROP classification

The one-solution test described by Tepper *et al.* (2001) was used to classify the consumers into non tasters, medium tasters and super tasters. The final cut-off scores based on the

PROP means ( $\pm 95\%$  confidence interval [CI]) were determined as follows: individuals who rated PROP  $\leq 7.2$  were classified as non tasters, those who rated PROP  $\geq 65.4$  were classified as super tasters and those who rated PROP between  $> 7.2$  and  $< 65.4$  were classified as medium tasters. The final groupings by taster status are tabulated in Table 2.16.

#### 2.3.3.5. Statistical analysis

The effect of sorghum cultivar and PROP status as main effects and first order interaction on the appearance, texture, flavour and overall liking of the sorghum rice were analysed using a two-way analysis of variance (ANOVA) with LSD. Separate analyses were done for the three taster groups with panellist and sample as main effects. Fischer's least significant difference test for sample mean differences ( $p \leq 0.05$ ) were applied where appropriate using STATISTICA (StatSoft, Inc. 2005 version 7.1 [www.statsoft.com](http://www.statsoft.com) Tulsa, OK, USA).

**Table 2.16.** Consumer (n = 194) classification by gender and PROP taster status for evaluation of sorghum rice: non, medium and super tasters (relative percentages in parentheses)

	<b>Men</b>	<b>Women</b>	<b>Total</b>
Non tasters	7 (12.7)	19 (13.7)	26 (13.4)
Medium tasters	36 (65.5)	80 (57.5)	116 (59.8)
Super tasters	12 (21.8)	40 (28.8)	52 (26.8)
Total	55	139	194 (100)

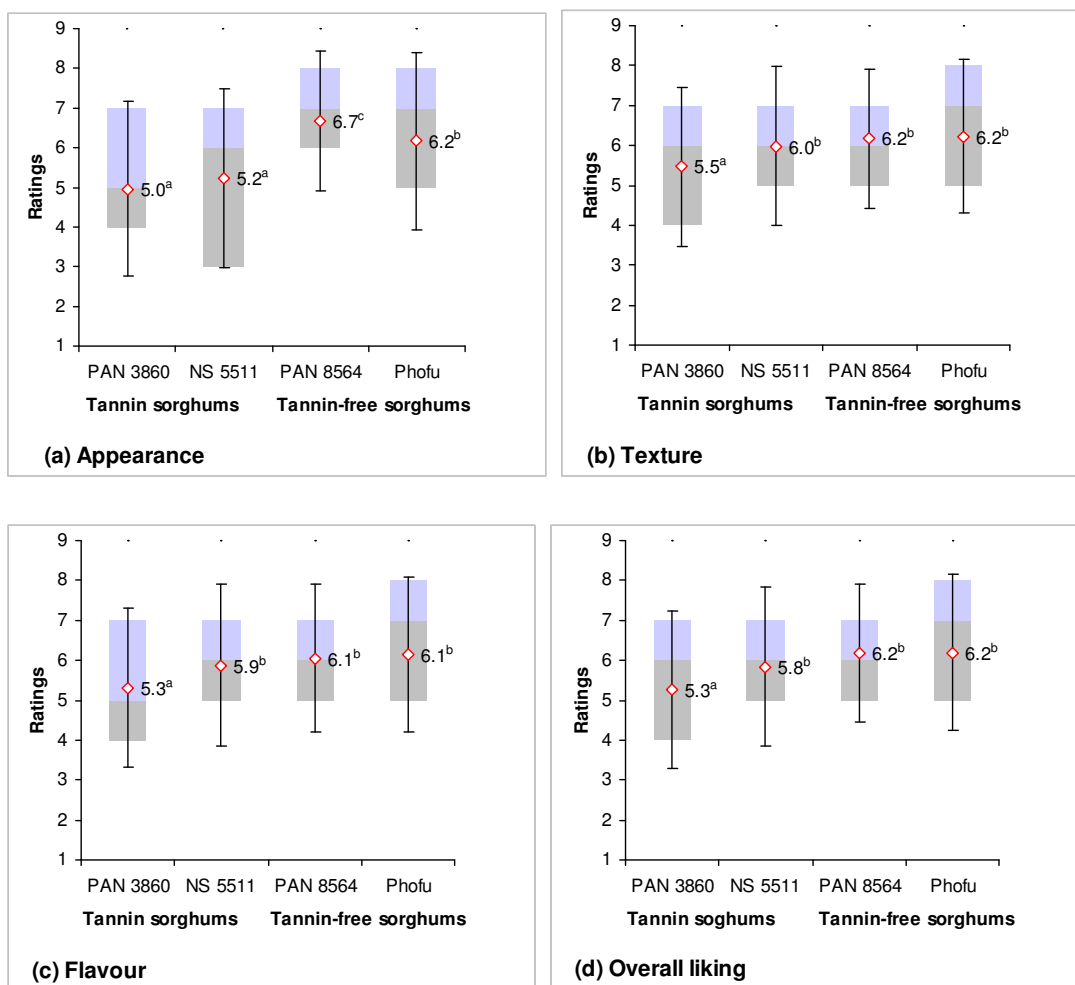
#### 2.3.4. Results and discussion

There was no significant cultivar x PROP taster status interaction effects (data not shown). There was a significant cultivar effect on the mean hedonic ratings of the sorghum rice for appearance, texture, flavour and overall liking (Fig. 2.14). Essentially, with the exception

of appearance, the acceptability of texture, flavour and overall liking for the different sorghums followed the same trend. There was more consensus among consumers on the acceptance of the texture, flavour and overall liking of NS 5511 and PAN 8564 than PAN 3860 and Phofu as demonstrated by the data spread (Fig. 2.14). Ratings distinguished between the sorghum products on the basis of condensed tannin content. The rice from the sorghum grain with the highest tannin content (PAN 3860 – 8.2% CE) was liked less than all the other sorghums, and had significantly lower scores for all the attributes. This was not surprising since the rice from this sorghum cultivar was described by the descriptive sensory panel as dark, hard and its flavour was more bitter and more astringent than all the other sorghums (Chapter 2.1). This finding was attributed to the high condensed tannin content of the sorghum grain (Chapter 2.1). Although NS 5511 also contained condensed tannins (1.8% CE), the consumer ratings for the texture, flavour and overall liking of its sorghum rice were not significantly different from those of the tannin-free sorghums (PAN 8564 and Phofu). NS 5511 was equally liked by the consumers as the tannin-free sorghums. The finding here is also consistent with the descriptive sensory panel results in that the sorghum rice from this cultivar (NS 5511) was perceived as similar in both bitterness and astringency to that of the tannin-free sorghums, PAN 8564 and Phofu (Chapter 2.1). These findings again indicate that there is a condensed tannin threshold level in sorghum at which the tannins do not impart objectionable sensory attributes associated with them.

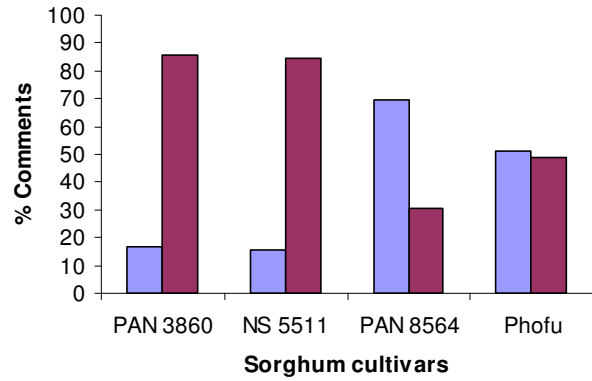
The results in this study are in general agreement with those of Subramanian, Murty, Jambunathan and House (1982). These authors determined characteristics of decorticated boiled sorghum (sorghum rice) using a panel of six women who regularly consumed sorghum. Colour, taste, texture and keeping quality were evaluated. The most preferred sorghums were tannin-free sorghum cultivars (S-29 and S-13) with white and pale yellow grain colour. Dobbs, with a pigmented testa and brown grain colour (tannin sorghum), was the least preferred and had poor ratings for colour, taste, texture and keeping quality, whereas IS-2317, also with condensed tannins, received good ratings for taste, texture and keeping quality, and was rated better than some tannin-free sorghums such as P-721, Patcha-Jonna, and IS-158. They did not determine condensed tannin content of the sorghums. However, it is probable that the condensed tannin content of Dobbs was significantly higher than IS-2317.

Concerning appearance, the sorghum rice from the tannin sorghums (PAN 3860 and NS 5511) received significantly lower ratings than the tannin-free sorghums (PAN 8564 and Phofu; Fig. 2.14). In this study more negative comments were received on the appearance of the condensed tannin containing sorghums (PAN 3860 and NS 5511) than positive comments and these negative comments had to do with the dark colour of these sorghums (Fig. 2.15). The finding here agrees with the quantitative descriptive analysis (Table 2.6, Chapter 2.1). The tannin sorghums were darker than the tannin-free sorghum, PAN 8564, which like the tannin sorghums, also had a red pericarp. The dark colour is due to the presence of a pigmented testa in these sorghums (Rooney and Miller, 1982). According to Awika, McDonough and Rooney (2005) the pigmented testa is typically darker than the pericarp. This study is in agreement with the findings of Subramanian *et al.* (1982) in that the colour of sorghums with a pigmented testa (condensed tannins; WS-1297, IS-2317, IS-7055 and Dobbs) were rated as unacceptable despite the fact that all the sorghums were decorticated before cooking. The darker colour was attributed to the leaching of the pigments into the endosperm. Regarding the texture of the sorghum rice, the sorghum cultivar with the highest condensed-tannin content (PAN 3860) was rated lowest (Fig. 2.14). The descriptive sensory panel described the texture of this sorghum (PAN 3860) as significantly harder than the other sorghums and they rated Phofu as the softest (Chapter 2.1). This is consistent with the general comments made by the consumer panel on the texture of the sorghum rice (Fig. 2.15). Generally, the negative comments received on the texture of the sorghum rice were that it was hard to chew and needed to be cooked a bit longer. The sorghum rice that received the most positive comments for texture was Phofu, which was described as having a smooth and soft texture. PAN 3860 received the lowest scores for flavour and overall liking (Fig. 2.14). The general comments made on the flavour of the sorghum rice from NS 5511 and PAN 8564 were more positive than negative (Fig. 2.15). However, the flavour of these sorghum rices was not significantly different from Phofu. Positive comments on PAN 3860 were that its rice had a strong, natural, nutty and healthy flavour, and the negative comments made about this sorghum rice included strong, bitter flavour and an astringent after-taste, while some panellists described it as bland and tasteless. Phofu sorghum rice was said to taste like maize and it was described as better than the others in that it did not taste bitter and astringent, whereas negative comments received for this sorghum rice were that it was tasteless, bland and needed some salt to give it flavour.

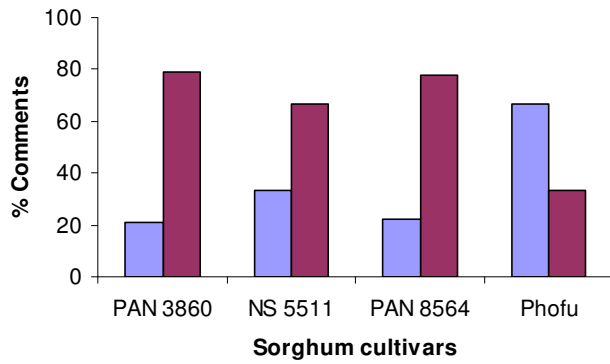


**Figure 2.14** Sorghum cultivar effect on consumer ratings of appearance (a), texture (b), flavour (c) and overall liking (d) of the sorghum rice. Data collapsed across all taster groups. Means and SD; means in a graph with different letter notations <sup>(a-c)</sup> are significantly different at  $p \leq 0.05$ . Dark shaded area is the lower percentile and represents the value above which 75% of the ratings fell. The light shaded area is the higher percentile and represents the value above which 25% of the ratings fell. The median is the value between the two shaded areas and 50% of the values fell above it and 50% fell below it. 1 = ‘dislike extremely’, 5 = ‘neither like nor dislike’ and 9 = ‘like extremely’

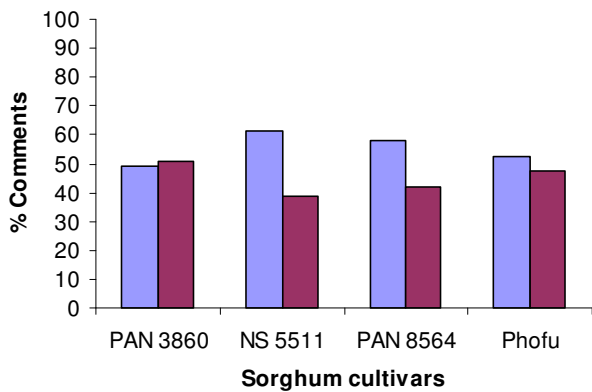




**Appearance**



**Texture**



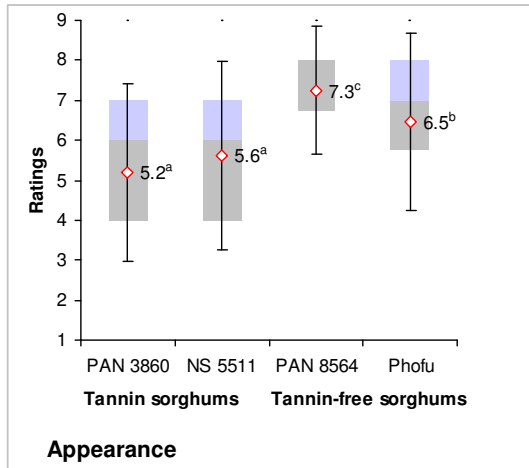
**Flavour**

■ Good/positive ■ Bad/negative

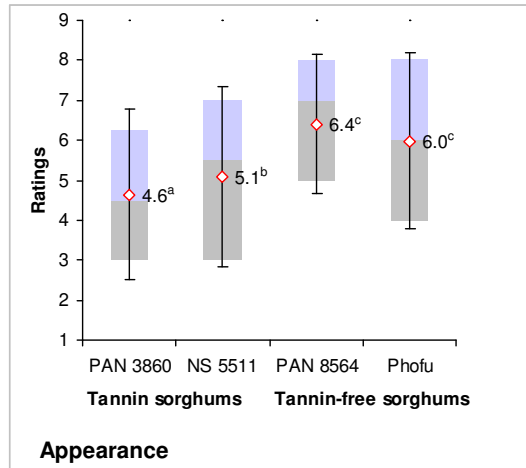
**Figure 2.15.** The relative percentage of good/positive and bad/negative comments made by consumers on the appearance, texture and flavour of the sorghum rice.

The data were analyzed separately by taster group. All the taster groups generally rated the appearance of the rice from the tannin sorghums lower than the rice from the tannin-free sorghums (Fig. 2.16). With the exception of appearance, the PROP non tasters' acceptance ratings for the sensory attributes: texture (Fig. 2.17), flavour (Fig. 2.18) and overall liking (Fig. 2.19) of the rice from different cultivars were not significantly different. In other words, the sorghum cultivars were equally preferred. This presumably was because the non tasters could not distinguish differences in the bitterness and astringency between the cultivars. This is probably related to PROP non tasters being reported to have fewer taste bud and taste pore densities than medium and super tasters (Miller and Reedy, 1990a; Miller and Reedy, 1990b; Bartoshuk, Duffy and Miller, 1994; Yackinous and Guinard, 2002). This finding is in agreement with the PROP taster status theory that non tasters have lower taste sensitivity to bitterness than the other PROP taster groups (Hall, Bartoshuk, Cain and Stevens, 1975; Bartoshuk, Fast, Karrer, Marino, Price and Reed, 1992; Tepper, 1998).

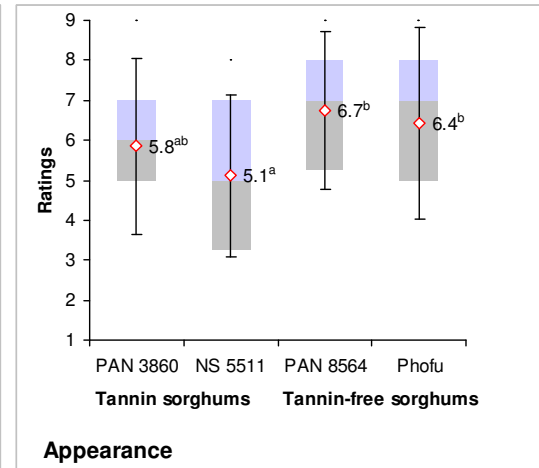
The super and medium tasters could distinguish differences between the rice from different sorghum cultivars for all the sensory attributes in accordance with the presence or absence of condensed tannins in the sorghums. Rice from the sorghum cultivar with the highest condensed tannin content, PAN 3860 (8.2% CE) was rated significantly lower for all the sensory attributes than the other sorghums presumably because it was dark, significantly more bitter and more astringent than the other sorghums (Chapter 2.1). Although the super and medium tasters are more sensitive to bitterness, they rated flavour (Fig. 2.18) and overall liking (Fig. 2.19) of NS 5511 rice similar to the products from the tannin-free sorghums. This is in agreement with the results of the descriptive sensory panel that the products (infusions and rice) from this sorghum cultivar were not significantly different in bitterness and astringency from those of the tannin-free sorghums PAN 8564 and Phofu (Chapter 2.1).



(a) Super tasters

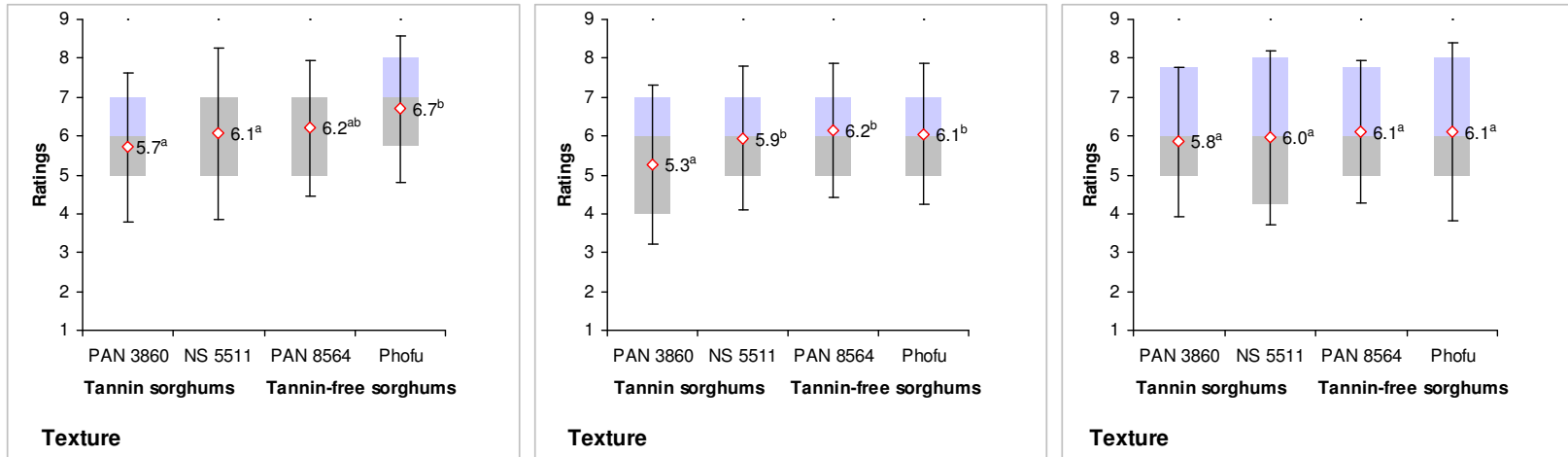


(b) Medium tasters



(c) Non tasters

**Figure 2.16.** Consumer ratings for appearance of sorghum rice from different sorghums by PROP taster status. Means and SD; means in a graph with different letter notations <sup>(a-c)</sup> are significantly different at  $p \leq 0.05$ . Dark shaded area is the lower percentile and represents the value above which 75% of the ratings fell. The light shaded area is the higher percentile and represents the value above which 25% of the ratings fell. The median is the value between the two shaded areas and 50% of the values fell above it and 50% fell below it.

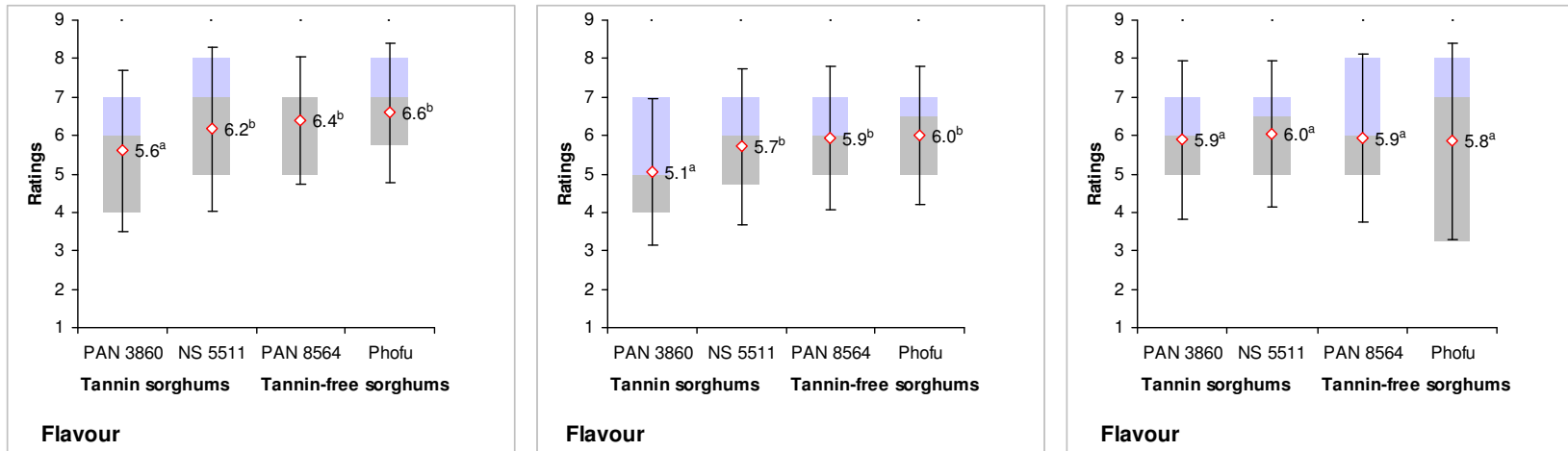


(a) Super tasters

(b) Medium tasters

(c) Non tasters

**Figure 2.17.** Consumer ratings for texture of sorghum rice from different sorghums by PROP taster status. Means and SD; means in a graph with different letter notations <sup>(a-b)</sup> are significantly different at  $p \leq 0.05$ . Dark shaded area is the lower percentile and represents the value above which 75% of the ratings fell. The light shaded area is the higher percentile and represents the value above which 25% of the ratings fell. The median is the value between the two shaded areas and 50% of the values fell above it and 50% fell below it.

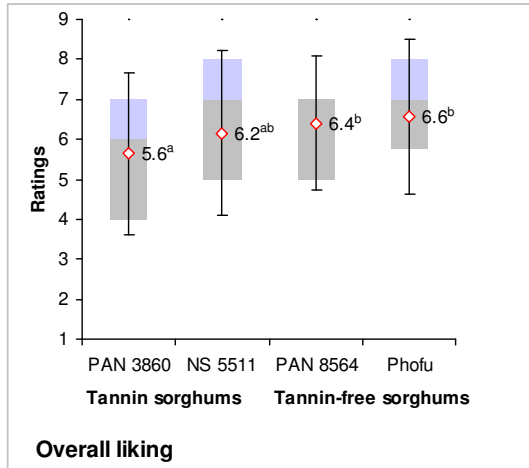


(a) Super tasters

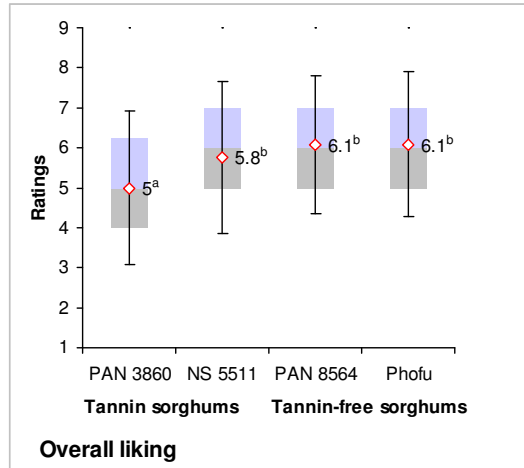
(b) Medium tasters

(c) Non tasters

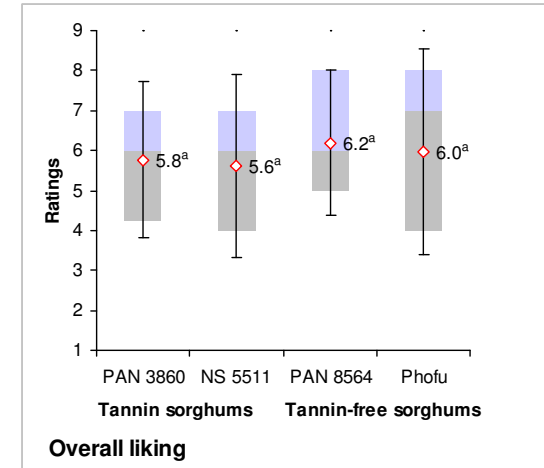
**Figure 2.18.** Consumer ratings for flavour of sorghum rice from different sorghums by PROP taster status. Means and SD; means in a graph with different letter notations <sup>(a-b)</sup> are significantly different at  $p \leq 0.05$ . Dark shaded area is the lower percentile and represents the value above which 75% of the ratings fell. The light shaded area is the higher percentile and represents the value above which 25% of the ratings fell. The median is the value between the two shaded areas and 50% of the values fell above it and 50% fell below it.



(a) Super tasters



(b) Medium tasters



(c) Non tasters

**Figure 2.19.** Consumer ratings for overall liking of sorghum rice from different sorghums by PROP taster status. Means and SD; means in a graph with different letter notations <sup>(a-b)</sup> are significantly different at  $p \leq 0.05$ . Dark shaded area is the lower percentile and represents the value above which 75% of the ratings fell. The light shaded area is the higher percentile and represents the value above which 25% of the ratings fell. The median is the value between the two shaded areas and 50% of the values fell above it and 50% fell below it.

For texture, differences were detected (except by non tasters) between the sorghum rices in accordance with condensed tannin content (Fig. 2.17). Rice from PAN 3860 (8.2% CE) was rated significantly lower for texture than the rice from the tannin-free sorghums. Texture sensations are due to mouth-feel characteristics such as the presence of moistness or particles and to mechanical characteristics that are associated with resistance to applied forces in the mouth (Tepper, 1998). The force required to chew a food such as peanut brittle is defined as a primary texture characteristic (hardness), whereas sauces and gravies that lack particles are perceived as smooth and creamy (Tepper, 1998). The presence of more trigeminal fibres on the surface of the tongue might give PROP super tasters an advantage in perceiving texture better than non tasters (Tepper, 1998; Tepper, 1999). This is probably why PROP tasters could distinguish texture differences of the rice from different cultivars while non tasters could not.

For flavour (Fig. 2.18) and overall liking (Fig. 2.19), the super and medium tasters rated rice from PAN 3860 which had the highest condensed tannin content (8.2% CE) significantly lower than the rice from other sorghums (NS 5511, PAN 8564 and Phofu). However, it is noteworthy that although the rice from PAN 3860 was the most bitter and astringent due to its high tannin content (8.2% CE), 50% of the consumers gave it positive ratings ( $\geq 5$ ) for flavour and overall liking. Furthermore, the mean ratings for flavour and overall liking of rice from NS 5511 (tannin content - 1.8% CE) by both super and medium tasters were not significantly different from the rice from tannin-free sorghums (PAN 8564 and Phofu).

The data was collapsed across all sorghum cultivars for PROP taster status main effects. With the exception of texture, there was a significant PROP taster status effect on the mean hedonic ratings of the sorghum rice for appearance, flavour and overall liking (Table 2.17). The super tasters rated the appearance, flavour and overall liking significantly higher than the medium tasters and their ratings were not significantly different from those of the non tasters.

**Table 2.17.** PROP taster status effect on consumer ratings of appearance, texture, flavour and overall liking of sorghum rice<sup>1,2</sup>

	Appearance	Texture	Flavour	Overall liking
Super tasters	6.1 <sup>b</sup> (0.2)	6.1 <sup>a</sup> (0.1)	6.2 <sup>b</sup> (0.1)	6.2 <sup>b</sup> (0.1)
Medium tasters	5.5 <sup>a</sup> (0.1)	5.9 <sup>a</sup> (0.1)	5.7 <sup>a</sup> (0.1)	5.7 <sup>a</sup> (0.1)
Non tasters	6.0 <sup>b</sup> (0.2)	6.0 <sup>a</sup> (0.2)	5.0 <sup>ab</sup> (0.2)	5.9 <sup>ab</sup> (0.2)

<sup>1</sup>Data collapsed across all sorghums

<sup>2</sup>Means and SEM; means in columns with different letter notations <sup>(a-b)</sup> are significantly different at p 0.01

According to Bartoshuk *et al.* (1994) age and gender have been implicated in food perceptions and acceptability. In the current study, the age and gender of the consumers generally did not influence the acceptability of the different sorghums. Age group main effects were only noted for the appearance ( $F = 3.138$ ,  $p \leq 0.02$ ) of the sorghum rice (data not shown). The 18-24 and 25-34 yrs age groups rated the appearance of the sorghum rice significantly lower than the older age groups (35-44 and >45 yrs). There was no significant difference among the different age groups (18-24, 25-34, 35-44 and >45 yrs) for flavour, texture and overall liking of the sorghum rice. Generally, gender did not have an effect on the ratings of the sorghum rice attributes except for flavour ( $F = 6.346$ ,  $p \leq 0.01$ ). The males' mean rating for the flavour of the sorghum rice was significantly higher than the females 6.0 and 5.7, respectively. According to Bartoshuk *et al.* (1994) women are more frequently super tasters than men and have more fungiform papillae and taste buds than men. This was observed in this study, in that 28.8% of the women were super tasters while for men 21.8% were super tasters (Table 2.16). It is noteworthy that in this study the super tasters' ratings were not significantly different from those of the non tasters and were generally significantly higher than those of medium tasters (Table 2.17).



### **2.3.5. Conclusions**

The findings of this study indicate that food products from tannin sorghums are not necessarily objectionable to consumers. Also the findings indicate that there is a condensed tannin threshold level at which the tannins do not impart objectionable sensory attributes associated with them. PROP tasters can presumably distinguish bitterness and astringency differences among the sorghum cultivars varying in tannin content levels, whereas the PROP non tasters cannot.

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