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Sensory perception of bitterness and astringency in sorghum

by

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DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher learning.

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April 2008

ABSTRACT

Sensory perception of bitterness and astringency in sorghum

Rosemary Ikalafeng Kobue-Lekalake

Supervisor: Dr. H.L. de Kock
Co-Supervisor: Prof. J.R.N. Taylor

There is a conflict of interest between the sorghum producers, for whom condensed tannins in sorghum have agronomic advantages, and sorghum users for whom condensed tannins in sorghum are perceived as nutritionally harmful and unpalatable. However, in recent years there has been growing interest in food phenolics due to their antioxidant potential. Thus, enhancing the content of phenolic compounds in plant foods through selective breeding and/or genetic improvement is now being viewed as a potent dietary option for disease prevention and control. However, the objectionable sensory attributes (bitterness and astringency) of phenolic compounds, especially condensed tannins, have resulted in low consumption of foods rich in these compounds. This study investigated the sensory attributes of products of sorghums varying in total phenol and condensed tannin content as well as their acceptance.

A descriptive sensory panel described the sensory attributes including bitterness and astringency of two products, sorghum rice and bran infusions of six sorghum cultivars: three containing tannins and three with no detectable tannins. The products of all the sorghums (tannin and tannin-free) were perceived to different degrees as both bitter and astringent. The products of sorghums with the highest total phenol and tannin content were most bitter and astringent while those from tannin-free sorghums with the lowest total phenol content were least bitter and astringent. The products of NS 5511 (tannins - 1.8% catechin equivalents CE), were perceived similar in both bitterness and astringency to those of a tannin-free sorghum (PAN 8564). Using the Dual Attribute Time Intensity (DATI) sensory method the descriptive sensory panel determined the intensity and time course of bitterness and astringency of bran infusions of sorghums varying in total phenol and condensed tannin content. The infusion from the sorghum with the highest condensed tannin content (PAN 3860) was perceived as most bitter and most astringent and that from

the tannin-free sorghum with the least total phenol content (Phofu) was least bitter and astringent. Bitterness of the sorghum infusions developed and reached maximum intensity significantly faster than astringency. The total duration of the astringency sensation lasted significantly longer than bitterness. The more bitter and more astringent the sorghum was, the longer the persistence of the bitter and astringent after-taste. The infusion of NS 5511 was again perceived similar to tannin-free sorghums in both bitterness and astringency. These findings seem to suggest that there is a condensed tannin threshold level at which the tannins are not 'strongly' perceived and thus are not objectionable.

A consumer panel classified by 6-*n*-propylthiouracil (PROP) taster status assessed the colour, texture, flavour and overall liking of sorghum rice of two tannin-containing (tannin) sorghums and two tannin-free sorghums. The sorghum rice from PAN 3860, with the highest tannin content, received significantly lower acceptance ratings for all the sensory attributes than the other sorghums. With the exception of appearance, the acceptance of the sorghum rice from the tannin sorghum NS 5511 was not significantly different from that of the two tannin-free sorghums. The PROP tasters (medium and super) could distinguish differences among the sorghum cultivars varying in tannin content levels which presumably led to the significant difference in their acceptance ratings for the most bitter and astringent sorghum compared to others. On the other hand the non tasters preferred the cultivars equally, presumably because they could not detect taste differences (in bitterness and astringency) between the sorghum cultivars. The results of the consumer panel confirm the predictions made from the descriptive sensory panel results that not all the tannin sorghum products would be objectionable to consumers.

It is proposed that the condensed tannin threshold level is 2.0% CE inclusive of the tannin content level of NS 5511 (1.8% CE). It is recommended that future breeding programmes investigate production of sorghums like NS 5511 with condensed tannin levels that fall within this threshold limit. The level of condensed tannins in these sorghums would provide the agronomic advantages for the farmer by reducing pre-harvest and post-harvest losses as well as provide the antioxidant benefits associated with them without negatively affecting the nutritional value of the food/feed. Since the negative sensory properties of these sorghums are not strongly perceived they would not be objectionable to consumers, thus making them a promising health option for millions of people.

DEDICATION

This work is dedicated to my late father Arrat Esrom Kobue, who encouraged me to reach higher heights.

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I praise and thank my Lord and my God for giving me the wisdom, guidance, direction, endurance, good health and well being throughout my studies. To God be all the glory!

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1. INTRODUCTION

1.1. Statement of the problem

Polyphenols are ubiquitous in plants and are an integral part of both human and animal diets (Bravo, 1998). Polyphenols protect crops from pathogens and predators by acting as phytoalexins and by increasing the astringency of food to make it unpalatable. To reduce bird damage, farmers grow condensed tannin-containing (tannin) sorghums, which are astringent during the immature stages when bird damage is highest (Bullard, Garrison, Kilburn and York, 1980). However, these agronomic advantages of condensed tannins to the farmer are accompanied by nutritional disadvantages (Butler, 1982; Chung, Wong, Wei, Huang and Lin, 1998). Tannins form complexes with proteins, starch and digestive enzymes causing a reduction in the nutritional value of food (Butler, 1982; Chung *et al.*, 1998). Nonetheless, the agronomic advantages of tannin sorghums outweigh such negatives as reduced nutrient availability or astringency (Awika and Rooney, 2004).

Interest in food phenolics has increased over recent years owing to their antioxidant properties (Bravo, 1998). High-tannin sorghums were found to have higher antioxidant capacity than is commonly found in fruits (Awika, Rooney, Wu, Prior and Cisneros-Zevallos, 2003b). Consumption of fruits, vegetables and cereals has been associated with lower risks of coronary heart disease and certain forms of cancer, due to the antioxidant properties of phenolic compounds, vitamins and dietary fibre in these foods (Steinmetz and Potter, 1996; Ness and Powles, 1997; Hollman and Katan, 1999; Ross and Kasum, 2002; Kamatha, Chandrashekarb and Rajinia, 2004). Thus, enhancing the content of phenolic compounds in plant foods through selective breeding and/or genetic improvement is viewed as a potent dietary option for disease prevention and control (Drewnowsky and Gomez-Carneros, 2000). However, phenolic compounds such as 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). Some of these bitter compounds include phenols found in tea, citrus fruits, wine and soy; triterpenes found in citrus fruits, and organo-sulphur compounds found in cruciferous vegetables like broccoli and cabbage (Reed, Tanaka and McDaniel, 2006). The objectionable sensory attributes of phenolic compounds may be the cause of the low

consumption of foods rich in these compounds (Drewnowski and Gomez-Carneros, 2000; Kamatha *et al.*, 2004).

Therefore, as research efforts focus on enhancing the content of phytochemicals like phenolic compounds in plant foodstuffs for health, it is necessary to determine how the sensory properties of these compounds affect consumer acceptance (Drewnowsky and Gomez-Carneros, 2000). Several studies have been carried out to identify and quantify phenolic compounds in sorghum (Kaluza, McGrath, Roberts and Schroder, 1980; Hahn, Faubion and Rooney, 1983; Awika, Dykes, Gu, Rooney and Prior, 2003a; Awika *et al.*, 2003b; Dykes, Rooney, Waniska and Rooney, 2005; Awika, McDonough and Rooney, 2005; Dlamini, Taylor and Rooney, 2007) as well as determining their antioxidant activity (Awika *et al.*, 2003a; Awika *et al.*, 2003b; Awika *et al.*, 2005; Dykes *et al.*, 2005; Dlamini *et al.*, 2007). However, quantitative assessment of the sensory attributes of phenolic compounds as well as their effect on the acceptability of sorghum foods is limited (Subramanian, Murty, Jambunathan and House, 1982; Yetneberk, de Kock, Rooney and Taylor, 2004; Yetneberk, Rooney and Taylor, 2005).

In eastern and southern Africa, traditional sorghum cultivars of moderate tannin content are widely grown and used as staple food and for alcoholic beverages (Awika and Rooney, 2004). According to these authors, some African cultures 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. These authors attributed this property to the slow digestibility and nutrient release from the tannin-complexed food matrix.

The question is, are there tannin sorghums that can address the needs of the sorghum producers, for whom condensed tannins have agronomic advantages, and simultaneously benefit the sorghum end users for whom condensed tannins are potentially potent antioxidants without compromising on their palatability?

1.2. Literature review

1.2.1. Sorghum (*Sorghum bicolor* [L] Moench)

Sorghum ranks fifth among the most important cereal crops in the world following wheat, rice, maize and barley (FAOSTAT, 2006). In the semi-arid tropics worldwide, sorghum is generally cultivated at a subsistence level and consumed as food by humans (Cothren, Matocha and Clark, 2000). Thus, it contributes significantly to the nutritional livelihood of impoverished populations of the world. Sorghum is eaten as porridge, fermented and unfermented breads, leavened and unleavened bread, snacks, non-alcoholic beverages and sorghum beer and malt (Murty and Kumar, 1995). In Japan, white tan-plant sorghums are processed into flour and other products such as snacks, cookies and ethnic foods (Awika and Rooney, 2004). In the USA, such sorghums are also gaining popularity as a substitute for wheat for people allergic to wheat gluten (Awika and Rooney, 2004).

1.2.1.1. Sorghum anatomical structure

The sorghum kernel is composed of three main parts: the outer covering (pericarp), the storage tissue (endosperm) and the embryo (germ) (Rooney and Miller, 1982) (Fig. 1.1; Taylor, 2003). The pericarp makes up 3-6%, the endosperm 84-90% and the embryo 5-10% of the grain depending on the kernel size. The sorghum kernel is called a caryopsis because the ovary wall dries and adheres strongly to the mature ovule. The pericarp originates from the ovary wall and is divided into the epicarp, the mesocarp, the cross cell layer and the tube cell layer. The epicarp is the outermost layer of the kernel and is divided into the epidermis containing pigments, and the hypodermis. The mesocarp is the middle part of the pericarp and may vary in thickness from thin (translucent) without starch granules to thick (chalky) with starch granules. The endocarp is the innermost part of the pericarp containing the cross and tube cells.

The endosperm consists of the aleurone layer, peripheral, horny (corneous) and floury portions (Fig. 1.1) (Rooney and Miller, 1982). The peripheral endosperm has starch granules embedded in a dense matrix of protein bodies and matrix proteins making the starch poorly available for hydrolysis. The corneous endosperm is located beneath the peripheral endosperm and is often called hard, vitreous or horny.

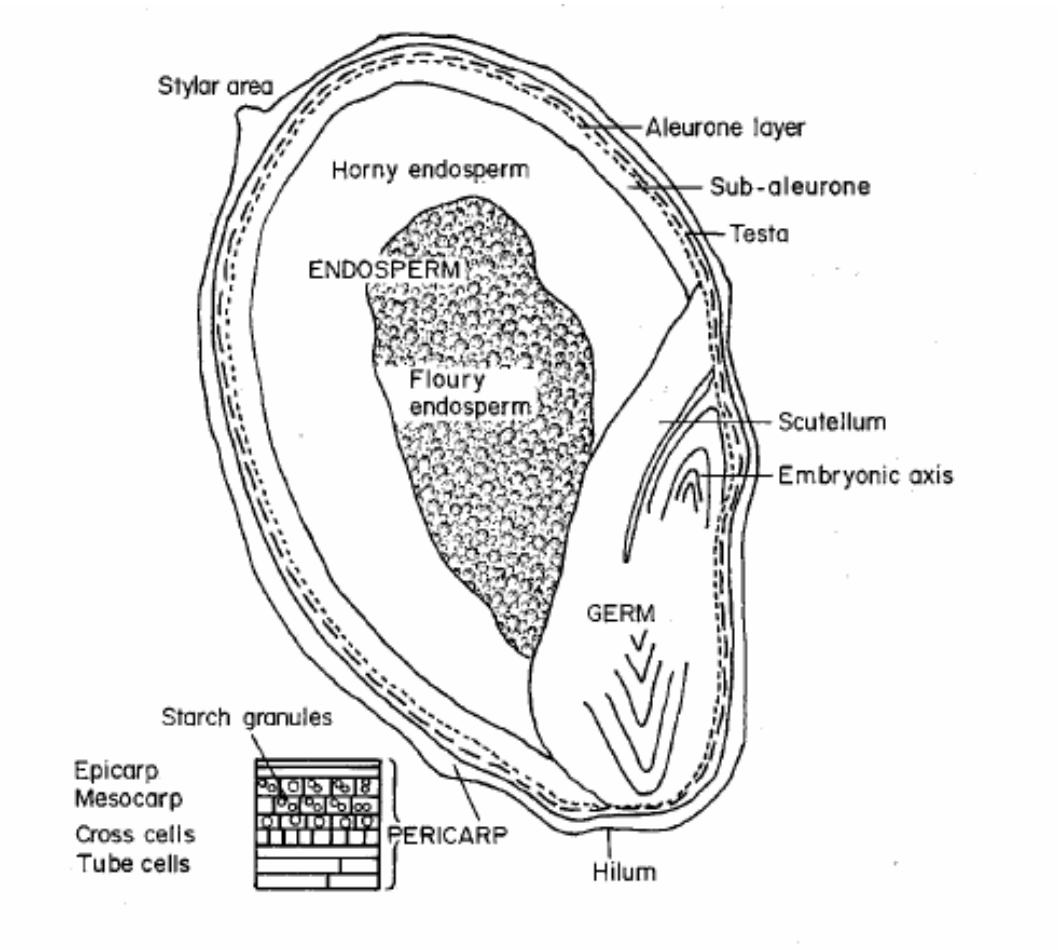


Figure 1.1. Cross-section of a sorghum kernel (Taylor, 2003).

The starch granules in this part of the endosperm are angular in shape with depressions where protein bodies were located. This part of the endosperm has strong starch-protein bonds and the starch granules often break easily rather than pull from the protein matrix. The floury endosperm is located in the inner most part of the kernel, and is composed mainly of starch with a smaller amount of protein bodies than found in the corneous endosperm. The relative proportion of corneous to floury endosperm within a sorghum kernel is often referred to as endosperm texture. The texture can be determined by visual examination of kernels cut longitudinally. A rating of 1 can be assigned to a kernel that contains very little floury endosperm and a rating of 5 can be assigned to a kernel which is predominantly floury (Rooney and Miller, 1982). Endosperm texture is important during processing. Sorghums with a corneous endosperm texture have higher milling yields

because the pericarp is more readily separated from the intact endosperm (Rooney and Miller, 1982). Sorghums with a corneous endosperm are also more resistant to insect attack during storage than those with a floury endosperm.

The embryo or germ is composed of two main parts, the embryonic axis and the scutellum (Fig. 1.1) (Rooney and Miller, 1982). The scutellum contains oil globules, protein bodies and a few starch granules. According to these authors, the embryo plays a major role in moisture uptake and mold susceptibility of the sorghum kernel.

Sorghum grain contains condensed tannins when there is the presence of a pigmented testa (Fig. 1.2) (Awika and Rooney, 2004). The pigmented testa is located just beneath the cross and tube cells (Rooney and Miller, 1982). The presence or absence of a pigmented testa is controlled by B_1 and B_2 genes and the testa is present when both B_1 and B_2 are dominant (B_1- B_2 -) (Rooney and Miller, 1982). These genes B_1- B_2 also affect pericarp colour when they are dominant in combination with the spreader gene (S-) and result with an intense pigment in the epicarp imparting a brown colour to the pericarp.

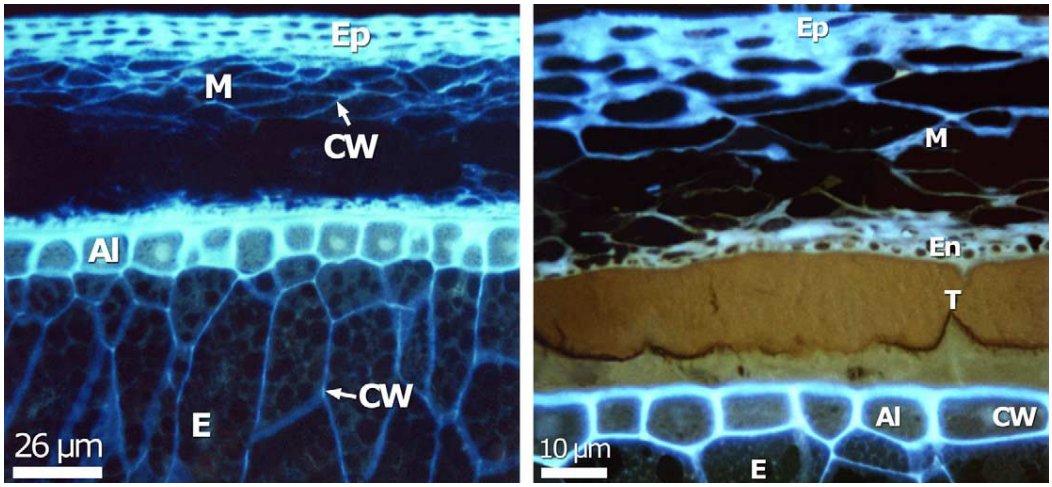


Figure 1.2. Fluorescence photomicrograph of sorghum bran cross-section, showing structural differences between a tannin-free sorghum (left) and a tannin sorghum with a pigmented testa (right). Al, aleurone layer; CW, cell wall; E, endosperm; En, endocarp; Ep, epicarp; M, mesocarp; T, pigmented testa (Awika and Rooney, 2004).

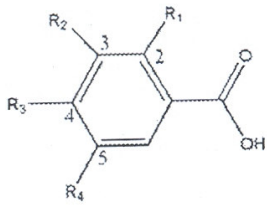
Sorghums have been classified into groups I, II and III based on the presence or absence of a pigmented testa (Rooney and Miller, 1982). Group I sorghums do not have a pigmented testa; group II have a testa (B_1- B_2- ss) and group III have a pigmented testa and a spreader gene (B_1- B_2- SS). According to Butler (1982) group I sorghums do not contain significant levels of tannins shown by low values of protein precipitation, Vanillin-HCl and anthocyanin production assays. Group II sorghum tannins are extractable in acidified methanol but not methanol alone. The typical high-tannin sorghums classified as group III sorghums, contain methanol-extractable tannins as well as group II type tannins extractable only in acidified methanol. Dicko, Hilhorst, Gruppen, Traore, Laane, Van Berkel and Voragen (2002) classified sorghums based on their whole grain tannin content as follows: low tannin sorghums $\leq$ 0.25%, medium tannin sorghums 0.26-0.5%, high tannin sorghums 0.51-0.75% and very high tannin sorghums $\geq$ 0.75% of tannin.

1.2.1.2. The chemistry of phenolic compounds of sorghum

Phenolic compounds are one of the most widely distributed groups of substances in the plant kingdom (Ross and Kasum, 2002). There are more than 8000 known phenolic compound structures, the common feature of which is an aromatic ring with at least one hydroxyl group (Ross and Kasum, 2002). There are more than 15 different classes of phenolic compounds in foods, ranging from simple phenolics with molecular weights of less than 500 to polymers of high (3000) molecular weight (Drewnowski and Gomez-Carneros, 2000).

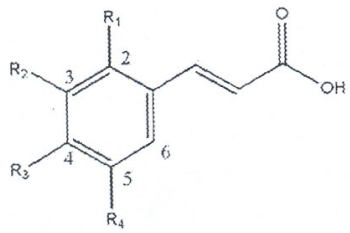
All sorghums contain phenolic compounds (Dykes *et al.*, 2005). Phenolic compounds are located mainly in the pericarp of the sorghum kernel (Awika and Rooney, 2004; Dykes and Rooney, 2006). Phenolic compounds identified in sorghum include phenolic acids, flavonoids and condensed tannins (Hahn *et al.*, 1983; Awika *et al.*, 2003a; Awika and Rooney, 2004). Phenolic acids in sorghum are mainly benzoic and cinnamic acid derivatives (Fig. 1.3) (Hahn *et al.*, 1983; Awika and Rooney, 2004). The benzoic derivatives have a C6-C1 structure and include gallic acid, *p*-hydroxybenzoic acid, vanillic, syringic and protocatechuic aids (Dykes and Rooney, 2006). Hydroxycinnamic acids have a C6-C3 structure and include coumaric, caffeic, ferulic and sinapic acids (Dykes and Rooney, 2006). Hahn *et al.* (1983) identified eight phenolic acids in sorghum namely: gallic, protocatechuic, p-hydrobenzoic, vanillic, caffeic, p-coumaric, ferulic and

cinnamic acids. According to these authors phenolic acids exist in sorghum as free forms mainly in the bran and bound forms esterified to cell wall polymers in the endosperm.



Benzoic acids

Gallic acid : $R_1 = H, R_2 = R_3 = R_4 = OH$
Gentisic acid : $R_1 = R_4 = OH, R_2 = R_3 = H$
Salicylic acid : $R_1 = OH, R_2 = R_3 = R_4 = H$
p-hydroxybenzoic acid : $R_1 = R_2 = R_4 = H, R_3 = OH$
Syringic : $R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH$
Protocatechuic : $R_1 = R_4 = H, R_2 = R_3 = OH$



Cinnamic acids

Caffeic acid : $R_1 = R_4 = H, R_2 = R_3 = OH$
Ferulic acid : $R_1 = R_4 = H, R_2 = OCH_3, R_3 = OH$
o-coumaric acid : $R_1 = OH, R_2 = R_3 = R_4 = H$
p-coumaric acid : $R_1 = R_2 = R_4 = H, R_3 = OH$
Sinapic : $R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH$

Figure 1.3. Basic structures of phenolic acids (benzoic and cinnamic acids) found in sorghum grain (Awika and Rooney, 2004).

Flavonoids are compounds with a C6-C3-C6 struture with two aromatic rings joined by a three carbon link (Fig. 1.4) (Dykes and Rooney, 2006). The basic structure of flavonoids allows a multitude of substitutions on the benzene rings A and B (Fig. 1.4.) (Hollman and Katan, 1999). There are two main subgroups of flavonoids namely, the 3-desoxyflavonoids (chalcones, flavanones, flavones) and the 3-hydroxyflavonoids (flavonols, anthocyanidins, leucoanthocyanidins and flavanols) (Brown, 1980). Flavanones, flavones, anthocyanins and flavanols have been identified in sorghum grain (Fig. 1.4) (Awika and Rooney, 2004). Flavanones identified in sorghum include naringenin and taxifolin and flavones include luteolin (Awika and Rooney, 2004).

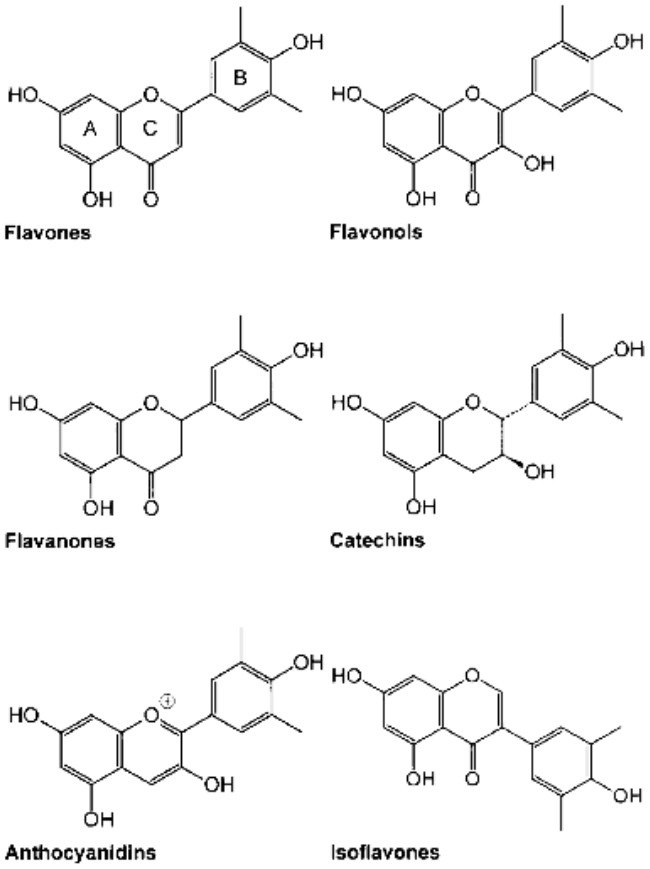


Figure 1.4. Structures of flavonoids (Hollman and Katan, 1999).

The most common anthocyanidins found in sorghum include 3-deoxyanthocyanidins: apigeninidin, luteolinidin and their derivatives (anthocyanins) (Fig. 1.5) (Awika and Rooney, 2004; Awika, Rooney and Waniska, 2004b).

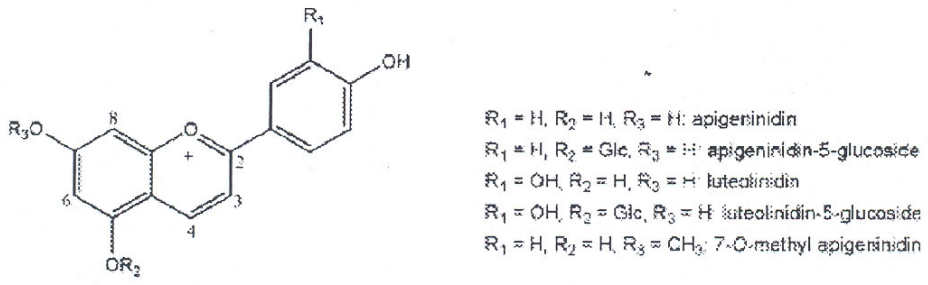


Figure 1.5. Basic structures of anthocyanidins (3-deoxycyanidins and their glucosides (anthocyanins)) found in sorghum grain (Awika and Rooney, 2004).

Monomeric flavan-4-ols such as luteoferol and apiforol may be present in either group I (tannin-free) or group III (tannin) sorghums (Fig. 1.6) (Butler, 1982). Luteoferol, with one OH-group, and apiferol, with two OH-groups, are flavan-4-ols of eriodictyl and naringenin respectively. A small amount of flavan-4-ols may be present in group II (tannin) sorghums. According to this author the presence of flavan-4-ols in sorghum grain is independent from that of tannins, but sorghums which contain flavan-4-ols but no tannin seem more abundant than those which contain tannin but not flavan-4-ols. Flavan-3-ol monomers identified in sorghum include catechin and epicatechin (Fig. 1.7) (Awika and Rooney, 2004).

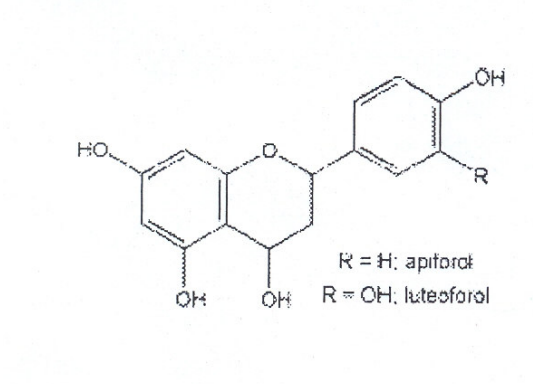


Figure 1.6. Basic structure of flavan-4-ols (apiforol and luteoforol) identified in sorghum (Awika and Rooney, 2004).

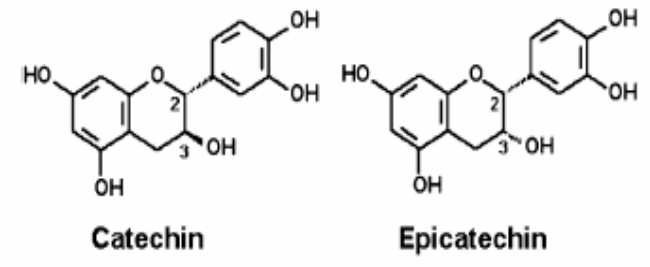


Figure 1.7. Basic structure of flavan-3-ols, (+)-catechin and (-)-epicatechin (Dixon, Xie and Sharma, 2005)

Plant tannins are defined as water-soluble phenolic compounds with molecular weights ranging from >500 to 3000 with the ability to precipitate gelatine and other proteins

(Swaim and Bate-Smith, 1962; Strumeyer and Malin, 1975). Structures of tannins vary in the nature of constitutive sub-units, degree of polymerization or chain length and linkage position (Vidal, Francis, Noble, Kwiatkowski, Cheynier and Waters, 2004). For instance major constituents of proanthocyanidins (condensed tannins) from grape seeds and skins include (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate and (-)-epigallocatechin (Souquet, Cheynier, Brossaud and Moutounet, 1996). Proanthocyanidins in sorghum are mainly composed of a series of condensed flavan-3-ols (Fig. 1.8) and flavan-3,4-diols molecules (Bullard *et al.*, 1980). According to Butler (1982) the condensed tannins in sorghum often exist as oligomers of five to seven flavan-3-ols which depolymerize into monomeric anthocyanidin pigments and thus are designated as proanthocyanidins.

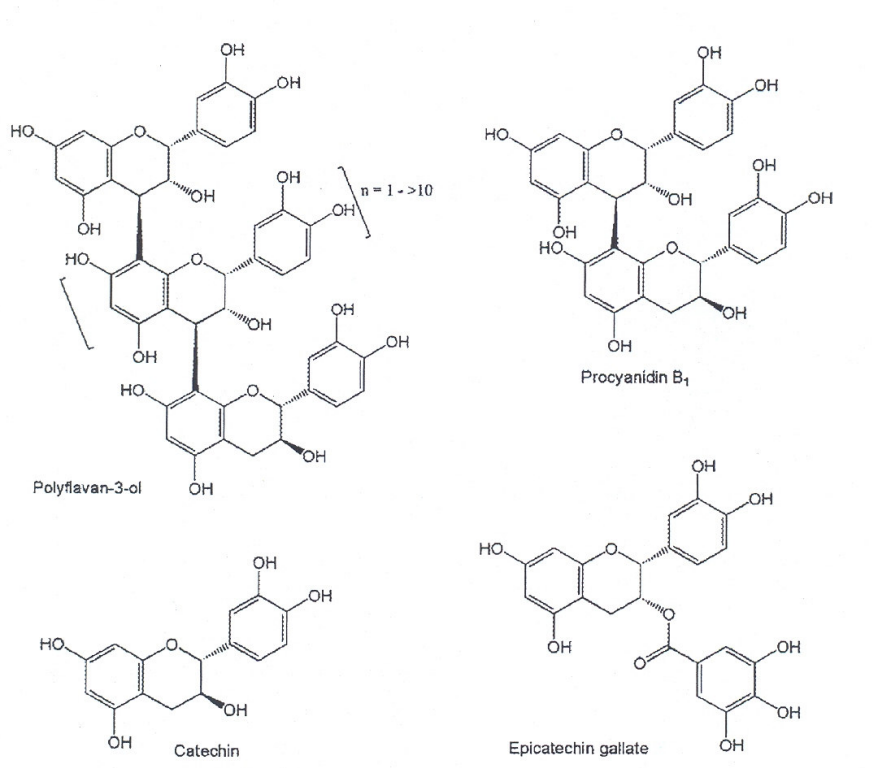


Figure 1.8. Structures of proanthocyanidins commonly found in sorghum grain (Awika and Rooney, 2004).

Tannins are a major phenolic component of sorghums with a pigmented testa (Awika *et al.*, 2003a). Some proanthocyanidins have been identified in sorghum with (-)-epicatechin chain extension units and a (+)-catechin chain termination units (Fig. 1.8) (Awika and

Rooney, 2004). Procyanidin B1 (epi (C4-C8) cat) is the most common dimer present in sorghum (Fig. 1.8) (Awika and Rooney, 2004).

1.2.1.3. Content of phenolic compounds in sorghum

The amount of phenolic compounds present in any particular sorghum cultivar is influenced by its genotype and the environment in which it is grown (Dykes *et al.*, 2005). These authors determined total phenol, condensed tannins, flavan-4-ols, anthocyanins and antioxidant activity of sorghum grain of clearly identified genotypes. The sorghum grain varied in pericarp colour, mesocarp thickness and the presence and intensity of the pigmented testa layer. Sorghum grains grown from purple/red coloured plants had higher total phenol content than tan plant types. Sorghums with a thick pericarp had higher total phenol content than sorghums with a thin pericarp. Sorghums with a pigmented testa gene B_1- B_2- and the spreader gene S had increased total phenol content, with B_1- B_2- S genes having the highest total phenol contents. Sorghums with a red pericarp contained flavan-4-ols such as luteoforol and apiforol, produced by flavanones, naringenin and eriodictyol. Consequently tan plant sorghums had the lowest content of flavan-4-ols, followed by purple/red plant sorghums with a thin pericarp. Purple/red plant sorghums with a thick pericarp had the highest content of flavan-4-ols. In tannin-free sorghums with a red pericarp, the total phenols were contributed mostly by the flavan-4-ols. Anthocyanin content followed the same trend as flavan-4-ols. Sorghums with a black pericarp contained the highest levels of anthocyanins. According to these authors, sorghums with a black pericarp are genetically red but turn black during maturation in the presence of sunlight.

Generally, cereals that contain condensed tannins (pigmented) like sorghum have higher levels of phenols than non-pigmented tannin-free sorghums and cereals like wheat and barley (Table 1.1 and Table 1.2) (Dykes and Rooney, 2007). Condensed tannin content levels reported in scientific literature using the Vanillin-HCl method range between 0.0-4.7 mg CE/g in tannin-free sorghums and 10-73 mg CE/g in tannin sorghums (Table 1.1). Phenol content levels reported in sorghum range from 0.8-5.6 mg gallic acid equivalents (GAE)/g in whole grain tannin-free sorghums and 11.7-22.5 mg GAE/g in whole grain tannin sorghums using the Folin-Ciocalteu method (Table 1.2). Phenolic content in the other cereals without a pigmented testa was comparable to that of the tannin-free sorghums (Table 1.2). Phenol content levels in bran are about four times the amount found in the

whole grain, supporting the view that phenolics are concentrated in the pericarp of sorghum (Table 1.2; Dykes and Rooney, 2007).

Table 1.1. Tannin content in different sorghum grains

Sorghum (whole grain)	Tannin content (mg CE/g dry wt) [1, 2]	Reference
Tannin-free sorghums	0.5-3.8	Awika and Rooney (2004)
Tannin-free sorghum (IS 2284)	4.7	Yetneberk *et al.* (2005)
Tannin sorghum (Sumac)	50.1	Awika *et al.* (2005)
Tannin sorghums	10.0-68.0	Awika and Rooney (2004)
Tannin sorghum (SC 103)	28.2	Awika *et al.* (2005)
Tannin sorghum (Seredo)	73	Yetneberk *et al.* (2005)
Tannin sorghum (Red Swazi)	33.6	Dlamini *et al.* (2007)
Tannin sorghum (NS 5511)	49.1	Dlamini *et al.* (2007)

[1] mg catechin equivalents (CE)/g (dry wt)

[2]Vanillin-HCl method

Processing procedures, such as decortication (Chibber, Mertz and Axtell, 1978; Beta, Rooney and Taylor, 2000; Awika *et al.*, 2005), fermentation (Towo, Mutuschek and Svanberg, 2006; Dlamini *et al.*, 2007), chemical treatment (Beta, Rooney, Marovatsanga and Taylor, 1999; Beta *et al.*, 2000), cooking (Butler, 1982; Towo *et al.*, 2006; Dlamini *et al.*, 2007) and extrusion cooking (Awika *et al.*, 2003a; Dlamini *et al.*, 2007) lower the total phenol and condensed tannin content of sorghum. On the other hand, germination (malting) increases the phenolic content of the sorghum (Butler 1982; Beta *et al.*, 1999). The apparent increase in phenolic content as germination proceeded is attributed to the production of non-tannin phenolic compounds by the developing roots and shoots (Beta *et al.*, 1999).

Table 1.2. Total phenolic content in the grain and bran of different sorghums and selected cereal grains

Type of Cereal	Total phenol content (mg GAE/g [1,2])		Reference
	Grain	Bran	
White sorghum (tannin-free)	0.8	4.8	Awika *et al.* (2003b)
White (Macia) (tannin-free)	2.7	N/D	Dlamini *et al.* (2007)
Red (Tx2911) (tannin-free)	4.8	19.5	Awika *et al.* (2004b)
Black (Tx430) (tannin-free)	5.6	26.1	Awika *et al.* (2004b)
Sorghum(tannin-free)	4.0	N/D	Ragaee, Abdel-Aal & Noaman (2006)
Tannin sorghum (NS 5511)	22.4	N/D	Dlamini *et al.* (2007)
Tannin sorghum (Red Swazi)	19.7	N/D	Dlamini *et al.* (2007)
Tannin sorghum (SC103)	11.7	48.7	Awika *et al.* (2004b)
Tannin sorghum (CSC3*R28)	12.9	56.6	Awika *et al.* (2004b)
Tannin sorghum (Sumac)	22.5	88.5	Awika *et al.* (2004b)
Pearl millet (standard)	3.4	N/D	El Hag, Tinay & Yosif (2002)
Pearl millet (Ugandi)	4.4	N/D	El Hag *et al.* (2002)
Rye	1.0	N/D	Ragaee *et al.* (2006)
Barley	0.9	N/D	Ragaee *et al.* (2006)
Hard wheat	0.6	N/D	Ragaee *et al.* (2006)

[1] mg Gallic Acid Equivalent (GAE)/g (Folin-Ciocalteu method)

[2] N/D – not determined

1.2.2. Harmful and beneficial effects of phenolic compounds

Phenolic compounds are thought to be both harmful and beneficial to the consumer (Chung *et al.*, 1998). Tannins appear to decrease the nutritive value of diets when added or when found naturally in high levels in certain foodstuffs (Strumeyer and Malin, 1975). This is because tannins are known to bind macromolecules such as proteins, starch and digestive enzymes (Butler, 1982; Haslam and Lilley, 1988). This tannin binding action causes a reduction in the nutritional and functional value of the bound constituents (Beta, 2003). According to Awika and Rooney (2004) the tannins in sorghum bind to and reduce digestibility of various food/feed nutrients, thus negatively affecting productivity of

livestock. According to Butler (1982), the tannin in high-tannin sorghums (2-3% dry weight) is sufficient under optimum conditions to bind considerably more protein than is present in the grain. Thus, it is likely that dietary tannins may be available to bind proteins of the digestive tract and thus interfere with digestion and absorption. This was demonstrated by Mamary, Habori, Aghbari and Obeidi (2001) who studied the extent of the *in vivo* inhibitory effects of two levels (1.4% and 3.5% catechin equivalent [CE]) of dietary sorghum tannins on rabbit digestive enzymes as well as mineral absorption. Addition of sorghum grain with 1.4% CE tannin content to the diet of rabbits did not significantly change the growth rate, food consumption or the feed conversion ratio. However, addition of sorghum grains with 3.5% CE tannin content significantly reduced the animals' body weight gain, feed conversion ratio, and slightly increased food consumption with respect to the control. Mamary *et al.* (2001) proposed that the lack of impact in the growth rates of animals fed the low-tannin (1.4% CE) sorghum grains may suggest the existence of a threshold-limit.

Since studies on the negative effects of phenolic compounds on sorghum foods have mostly focused on protein in general, Emmambux and Taylor (2003) investigated the interaction of sorghum-kafirin with phenolic compounds because like proline-rich saliva proteins, sorghum-kafirin is also rich in proline (Taylor, Von Benecke and Carlsson, 1989). Phenolic acids and flavonoid-type phenolics did not complex kafirin to form haze, whilst tannic acid and sorghum condensed tannins did. It was concluded that since condensed tannins in sorghum complex kafirin, this complexation might be involved in decreasing the protein digestibility of high-tannin sorghums. In contrast, the endogenous phenolic compounds found in tannin-free sorghums, such as flavonoids and phenolic acids, may not play a significant role in decreasing protein digestibility when such sorghums are wet cooked.

On the other hand, the phenolic compounds play an important agronomic role by reducing grain damage (pre-harvest and post-harvest losses) and bird predation (Strumeyer and Malin, 1975; Hahn *et al.*, 1983). The agronomic advantages such as resistance to bird predation are associated with high-tannin sorghums, which have low nutritional value for non-ruminants (Butler, Riedl, Lebryk and Blytt, 1984). Generally, higher concentrations of phenolic compounds are found in sprouts and seedlings than in the mature plant (Bravo, 1998; Chung *et al.*, 1998; Goldman, Kadar and Heintz, 1999). Mature grain of tannin

sorghums contains 2% condensed tannins or more while the immature grain has even higher tannin levels (Butler *et al.*, 1984).

Phenolic compounds are also thought to be harmful in that incidences of certain cancers, such as oesophageal cancer, have been associated with consumption of tannin-rich foods such as betel nuts and herbal tea (Chung *et al.*, 1998). Polyphenols in foodstuffs have been implicated in carcinogenesis (Lule and Xia, 2005). In the presence of oxygen, transition metal ions such as Cu and Fe catalyze the redox cycling of phenolics, leading to the formation of reactive oxygen species (ROS) and phenoxyl radicals that can damage DNA, lipids, and other biological molecules (Li and Trush, 1994; Lule and Xia, 2005). However, the dosage of tannins required to induce cancers probably far exceeds the level encountered during normal food intake; as such, tannins are not believed to be potent carcinogens (Chung *et al.*, 1998).

However, the role of phenolic compounds as antioxidants has been linked to low incidences of certain forms of cancer (Block, Patterson and Subar, 1992) and coronary heart diseases (Ness and Powles, 1997; Hollman and Katan, 1999). The cardio-protective effects of phenolic compounds stem from their ability to inhibit lipid peroxidation, chelation of redox-active metals and attenuation of other processes involving reactive oxygen species (Heim, Tagliferro and Babilya, 2002). According to Krishnaswamy and Polasa (2001) it has been established through epidemiological studies that vitamins A, C and E, β-carotene, selenium and calcium are micronutrients with cancer chemopreventive properties, while flavonoids, plant sterols, saponins, phytic acid, glucosinolates and terpenoids are non-nutritive cancer chemopreventers. The non-nutrient inhibitors of carcinogenesis have different modes of action (Krishnaswamy and Polasa, 2001). Some, like ferulic acid and ellagic acid, act as blocking agents, by inhibiting the activity of enzymes which convert pro-carcinogens to carcinogens. Others, like isoflavones and epigallocatechin gallate, act as suppressing agents, by restraining different steps in the metabolic pathways required in tumour development. Others like ellagic acid are trapping agents that physically react with carcinogens and detoxify them. Sources of these non-nutritive chemopreventers include cereal grains, vegetables, fruits and spices like turmeric, cloves, ginger, thyme, mustard, cinnamon and anise (Krishnaswamy and Polasa, 2001). This protective effect has been attributed to the antioxidant property of these compounds (Krishnaswamy and Polasa, 2001).

1.2.3. Sensory properties of phenolic compounds

Sensory attributes associated with smaller phenolic compounds like phenolic acids include sweet, sour, bitter and astringency (Peleg and Noble, 1995). Peleg and Noble (1995) investigated the sensory properties of phenolic acids (benzoic acid derivatives) commonly found in fruits, vegetables, grains and spices. These included salicylic acid (2-hydroxy benzoic acid), *m*-hydroxyl benzoic acid (3-hydroxy benzoic acid), gentisic acid (2,5-hydroxyl benzoic acid) protocatechuic acid (3,4-hydroxy benzoic acid) and gallic acid (3,4,5-trihydroxy benzoic acid) in water. Each of these compounds elicited multiple sensations including sweetness, sourness, astringency, bitterness and prickling. Although the compounds were structurally similar their sensory properties differed qualitatively and quantitatively. Gentisic acid was most sour, benzoic acid was highest in prickling sensation, salicylic acid was most astringent, *m*-hydroxyl benzoic acid was the sweetest and gentisic, benzoic and protocatechuic acids were most bitter.

Polyphenols of high molecular weight such as condensed tannins are predominantly bitter and astringent (Lesschaeve and Noble, 2005). Bitterness and astringency in some fruits and beverages, such as tea, cider and red wine, are elicited primarily by polyphenols (Lesschaeve and Noble, 2005). Flavan-3-ols, such as catechin, epicatechin and their oligomers and polymers (proanthocyanidins or condensed tannins) are abundant in tea and wine. Bitterness and astringency are sensory attributes mostly cited as the cause of condensed tannins in sorghum being objectionable (Bullard *et al.*, 1980; Hahn *et al.*, 1983; Asante, 1995; Mugula and Lyimo, 2000; Awika and Rooney, 2004; Yetneberk *et al.*, 2005). A bitter taste and after-taste has been reported in *injera* produced from tannin sorghum (Yetneberk *et al.*, 2004; Yetneberk *et al.*, 2005). As more of the pericarp was removed, the bitterness of the *injera* decreased and the overall rating improved.

Variation in phenol composition such as molecular size or chain length (monomer, dimer, trimer), extent of galloylation, small differences in configurations such as stereochemistry of the sub-units (catechin or epicatechin) and site of linkage between the sub-units (C4→C6 or C4→C8) produce significant differences in the intensity and duration of the bitterness and astringency of phenolic compounds (Arnold, Noble and Singleton, 1980; Peleg, Gacon, Schilch and Noble, 1999; Vidal, Francis, Guyot, Marnet, Kwiatkowski,

Gawel, Cheynier and Waters, 2003). Arnold *et al.* (1980) determined the bitterness and astringency of four grape seed phenolic fractions in wine: (I) catechin, (II) dimeric anthocyanogens, (III) trimeric and tetrameric anthocyanogens and (IV) condensed tannins in model wine. All the fractions were found to be bitter and astringent. All the fractions, including the most astringent fraction (IV), were more bitter than astringent. Astringency increased with increasing molecular weight from fraction I to IV ($p < 0.001$). The condensed tannin fraction (IV) was the most intensely bitter and astringent fraction. Peleg *et al.* (1999) also examined seven flavonoids (+)-catechin, (-)-epicatechin, dimer B3 (catechin (4→8) catechin), dimer B6 (catechin (4→6) catechin), dimer B4 (catechin (4→8) epicatechin), trimer C2 (cat (4→8) cat (4→8) cat) and trimer C (cat (4→8) cat (4→8) epi) in water (Fig. 1.9). (-)-Epicatechin was significantly more bitter and more astringent and had a longer duration than its chiral isomer (+)-catechin. Difference in molecular size was the major factor influencing the sensory properties of bitterness and astringency in the phenolic compounds investigated. As the degree of polymerization increased, maximum bitterness intensity and duration decreased, whereas astringency increased. The monomers were more bitter than they were astringent while the trimers were more astringent than they were bitter. The bond linking the monomeric units also influenced both bitterness and astringency. The catechin-catechin dimer linked by a 4→6 bond (B6) was more bitter than both catechin-catechin (4→8) (B3) and catechin-epicatechin (4→8) (B4) dimers. Catechin-catechin (4→8) (B3) was less astringent than both catechin-catechin (4→6) (B6) and catechin-epicatechin (4→8) (B4). In agreement with the findings of Arnold *et al.* (1980) relative astringency increased with increasing molecular weight. Contrary to the findings on Arnold *et al.* (1980), Peleg *et al.* (1999) reported that procyanidin fractions with higher degree of polymerization (DP) were less bitter than fractions with lower DP. Vidal *et al.* (2003) carried out a descriptive sensory analysis on a range of purified apple, grape seed and grape skin tannin fractions differing in chain length and degree of galloylation in a model wine. The degree of polymerization appeared to be the most discriminatory variable among the fractions. Overall astringency increased with increasing chain length. Increased degree of gallolylation of the fractions increased a rough sensation associated with coarseness, drying and chalkiness. Like Arnold *et al.* (1980), Vidal *et al.* (2003) also reported that chain length did not affect bitterness perception. The bitterness scores were very low for all the samples.

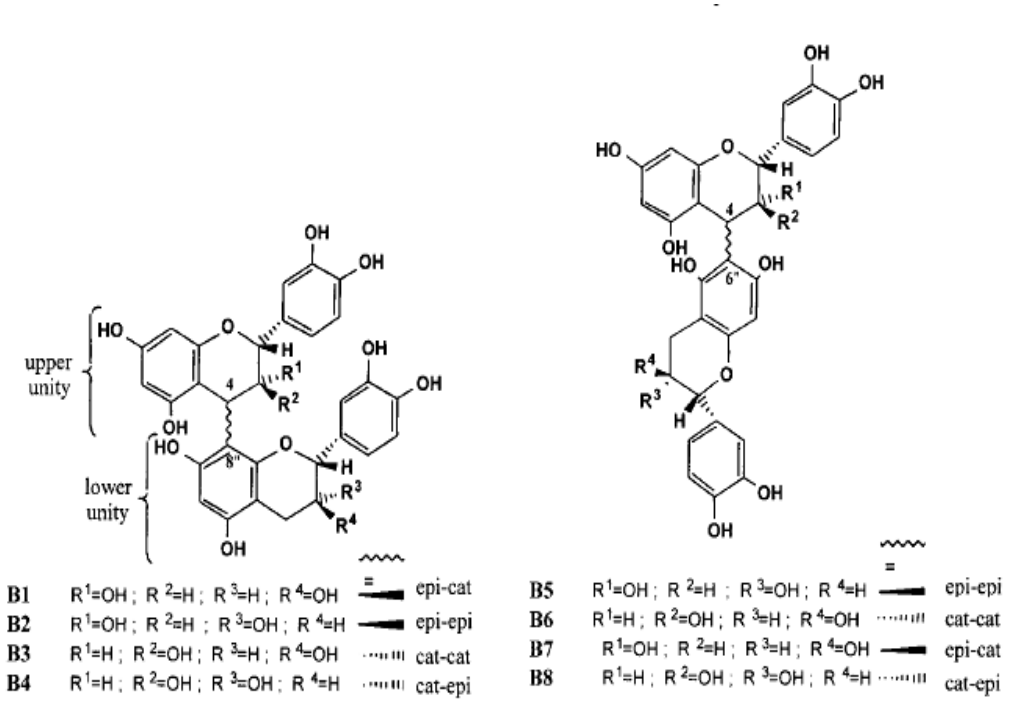


Figure 1.9. Molecular structures of procyanidin dimers with a C4-C8 linkage (B1-B4) and dimers with a C4-C6 linkage (B5-B6) (De Freitas and Mateus, 2001).

Since the astringency sensation is important in many beverages, Valentová, Skrovánková, Panovská and Pokorný (2002) compared the time dependence of astringency sensations in model aqueous solutions (tannic acid and (+)-catechin solutions) and different beverages (orange drink, model vermouths, red wines and Ceylon black tea), and investigated the interactions of astringency with other basic tastes and ethanol. Astringency was detected without difficulty in the presence of other tastes. The time dependence of the astringency in black tea was similar to that of (+)-catechin in aqueous solutions. The effect of astringent substances in wine was much more difficult to ascertain than in model solutions. Wine, particularly red wine, contains many phenolic substances that may taste either astringent or bitter or both (Lea and Arnold, 1978). Certain relations exist between the astringency and bitterness as most phenolic substances may taste both astringent and bitter (Lea and Arnold, 1978). Valentová *et al.* (2002) found that the time dependence of astringency in beverages was similar to that of bitterness. Differences among assessors were similar for the two sensations (bitterness and astringency) and dependent on saliva flow. Experienced assessors could distinguish both sensations (bitterness and astringency)

in the presence of each other. The development of astringency and its fading after swallowing followed an exponential course, but it was different for different beverages.

Bitterness and astringency contribute to the good taste of ciders and wines (Lule and Xia, 2005). Lea and Timberlake (1974) reported that as the bitterness and astringency in ciders increased, the concentrations of oligomeric and polymeric flavan-3-ols also increased. They concluded that the highly polymerized material was primarily responsible for both astringency and bitterness, while the isolated monomers, dimers and trimers contributed only slightly to these sensations. Lea and Timberlake (1974) and Lea and Arnold (1978) also reported that astringency and bitterness of cider procyanidins increased with increasing molecular weight. No specific polyphenol fraction was found to be exclusively responsible for bitterness and astringency (Lea and Arnold, 1978; Lea and Arnold, 1983). Kallithraka, Bakker and Clifford (1997c) reported (+)-catechin and (-)-epicatechin as bitter and astringent in a model wine and real wine. (-)-Epicatechin was found to be more astringent than (+)-catechin on an equal weight basis. According to Noble (1995) young wines with a high amount of smaller oligomers (dimers and trimers) are described as 'hard' (bitter and astringent), whilst older wines with more polymerized phenols (8-10 units) are described as 'soft' (less bitter and mainly astringent). Yamanishi (1990) reported that (+)-catechin and (-)-epicatechin in tea were exclusively bitter, while (-)-epicatechin gallate had an astringent threshold of 50 mg/l.

Delcour, Vandenberge, Corten and Dondeyne (1984) determined the taste detection thresholds of polyphenolics in deionised water. Phenolics evaluated were (+)-catechin (flavanol), procyanidin B3 (catechin-catechin (4→8) dimer), quercetin dehydrate (flavonol), tannic acid (hydrolysable tannin) and a mixture of trimeric and tetrameric procyanidins. The detection threshold depended on their degree of polymerization. The higher the molecular weight of these substances the lower their detection threshold values. For instance, the detection threshold levels were: 46.1 mg/l for (+)-catechin, 17.3 mg/l for procyanidin B3, 8.9 mg/l for quercetin dehydrate, 14.1 mg/l for tannic acid and 4.1 mg/l for a mixture of trimeric and tetrameric procyanidins.

1.2.4. Bitterness

There are five basic tastes: sweet, sour, salty, bitter and umami (Kim, Breslin, Reed and Drayna, 2004). According to these authors these tastes are mediated by taste receptor proteins residing on the surfaces of taste receptor cells (TRCs) within the taste buds of the tongue. At a molecular level, taste is a gustatory stimulus that stimulates a taste receptor cell (TRC), which in turn conveys the message to a sensory neuron (McLaughlin and Margolskee, 1994). A nerve impulse then relays the message to the gustatory centres of the brain where it registers as a taste. Stimuli for a single taste may come from several different types of chemicals; in the case of bitterness for example caffeine, a purine; morphine, an alkaloid; and potassium chloride, a simple salt are all bitter. The first step in taste recognition takes place in the taste pore, where molecules that are perceived to have taste (tastants) enter the taste pore and interact with receptor molecules and channels within the microvillar membrane of the TRCs (McLaughlin and Margolskee, 1994). Besides detecting taste stimuli, TRCs also convey taste information to the brain through a neuron (McLaughlin and Margolskee, 1994). The neurons make contact with taste cells at the synapse, a specialized region between the receiving end of the neuron, and the sending end of the taste cell. Information is then passed from the TRC to the neuron via chemical transmitters called neuro-transmitters secreted by the taste cell into the synapse. When the neurons detect these transmitters they react to them with a nerve impulse that is transmitted to the brain (McLaughlin and Margolskee, 1994). This process of receiving sensory information that is translated into a useful signal to the nervous system is called sensory transduction (McLaughlin and Margolskee, 1994).

1.2.4.1. Bitter taste transduction and other basic tastes

According to Kinnamon (1996), research data suggest that different mechanisms are utilized for the transduction of different taste stimuli. Ionic taste stimuli such as salts (Na^+), acids and some bitter compounds interact directly with apically located ion channels to depolarize taste cells. Amino acids, sweet stimuli and other bitter compounds bind to specific membrane receptors usually coupled to G-proteins and secondary messenger systems. According to Herness and Gilertson (1999) sour and salty tastes depolarize TRCs by directly interacting with ion channels. In contrast amino acids, sugars and other compounds perceived as sweet and most bitter compounds activate G-protein-coupled receptors (GPCRs) (Kim *et al.*, 2004). According to Kinnamon (1996) both H^+ and Na^+ use the same channel. However since salt (Na^+) can be distinguished from the taste of acids, other mechanisms must exist for acid transduction. Other mechanisms of acid

transduction include a proton-gated Ca^{++} channel and a proton transporter. Some bitter compounds are transducted by the same apical K^+ conductance involved in acid transduction (Kinnamon, 1996). Quinine and divalent salts like $CaCl_2$ directly block the K^+ conductance while K^+ salts permeate the conductance to depolarize taste cells. Specific membrane receptors appear to be required for the transductance of sugars, synthetic sweeteners, amino acids and most bitter compounds. Most of these receptors are coupled to G- proteins and second messengers. Thus, bitter stimuli interact with both apical ion channels and specific membrane receptors for transduction (Kinnamon, 1996). It is not clear whether the bitter taste of procyanidins (flavan-3-ols) is a result of receptor or surface membrane interaction (Peleg *et al.*, 1999). Regardless of whether bitterness of procyanidins is elicited by interaction with a specific bitter membrane-bound receptor or through surface membrane interactions, increasing the size of procyanidins decreased their bitterness. The monomers were perceived as more bitter than the dimers which were in turn more bitter than the trimers. These authors suggested that this could be a result of increased steric interference reducing the strength of interactions between the flavonoid and the receptor or the receptor membrane thus causing the trimers to be perceived as least bitter.

The bitter taste appears to be the most complex taste quality in humans, given the variety of chemically diverse structures that elicit bitterness on an apparently large number of gene encoding receptors (McLaughlin and Margolskee, 1994; Kim *et al.*, 2004). Bitter taste can be detected at very low concentrations (Glendinning, 1994). It is believed to have evolved to enable organisms to detect and avoid environmental toxins (Glendinning, 1994; Kim *et al.*, 2004). There are many compounds identified as tasting bitter including inorganic salts (KCl), amines (denatonium), amino acids (tryptophan), peptides, alkaloids (quinine and morphine), acetylated sugars (sucrose octa-acetate), flavanols/phenols (epicatechin), carbamates/thioureas (6-n-propylthiouracil [PROP] and phenylthiocarbamide [PTC]), to name a few (Keast and Breslin, 2002). In order to be able to taste such divergent structures, mammals have evolved multiple mechanisms which have an affinity for the divergent chemical structures. According to McLaughlin and Margolskee (1994), some bitter compounds have an affinity for fatty acid molecules in the cell membrane and are termed lipophilic, whilst other molecules are hydrophilic. Thus, it is assumed that bitter compounds share taste receptor sites and transduction mechanisms,

since it seems improbable that each of the thousands of bitter compounds would have its own unique transduction sequence (Keast and Breslin, 2002).

There are many substances for which different individuals show great differences in their taste thresholds. Yokomukai, Cowart and Beauchamp (1993) investigated individual differences among humans in their perception of different bitter tasting compounds. They found that sensitivity to quinine sulphate (QSO_4) and sensitivity to urea were unrelated. Subjects who were highly sensitive to one bitter compound could be insensitive to another. Out of the 52 subjects tested, 18 found these compounds to be equally bitter, 17 found QSO_4 to be more bitter than urea, and 17 found urea to be more bitter than QSO_4. QSO_4-sensitive subjects found the bitterness of caffeine and sucrose octa-acetate (SOA) to be more than that of magnesium sulphate; whereas the reverse was true for the urea-sensitive subjects. Thus, it was concluded that the results support the existence of multiple bitter transduction sequences, in that individual differences in response to various bitter compounds may reflect differences in the relative availability of specific transduction sequences. Delwich, Buletic and Breslin (2001) investigated whether classes of bitter transduction processes in the general population might be revealed by examining and correlating individual differences in sensitivities to bitter compounds namely: quinine HCl, caffeine, (-)-epicatechin, L-phenylalanine, L-tryptophan, tetralone, magnesium sulphate, urea, SOA, denatonium benzoate and PROP. It was assumed that bitter tasting compounds that cluster together as a function of the subject's perceptual sensitivities share some common physiological mechanism. The subjects rated the bitterness intensities of different compounds followed by ranking the intensity of bitterness of the compounds from the weakest to the strongest. By examining the subject's (n=26) individual differences in ratings and rankings of the bitter compounds, four clusters emerged. The first group included urea, phenylalanine, tryptophan and epicatechin, the second group included quinine, caffeine, SOA, denatonium benzoate and tetralone, the third group included magnesium sulphate and the fourth included PROP. From these results, it was concluded that bitterness appears to be transduced in humans via several different transduction mechanisms. A separation was found between those that contain at least one primary amine (group 1) and those that contain at least one methyl group (group 2). Magnesium sulphate does not contain methyl groups or amines and was thus seen as an isolated compound. The panellists differed by their sensitivity to PROP and also differed in their bitterness ratings; however they did not differ in rankings. Therefore it was

concluded that there are subjects who possess diminished absolute sensitivity to bitter stimuli but do not differ from other subjects in the relative sensitivity to these compounds.

1.2.4.2. Genetic variation

Genetic variation in taste perceptions has been investigated by different researchers since Fox (1931) accidentally discovered that his colleague could taste the bitterness of a chemical compound he was working with, phenylthiocarbamide (PTC), whilst he found it tasteless. Blakeslee and Fox (1932) solicited volunteers to taste PTC by posing the question: "What taste world do you live in?" Twenty eight percent of the people found it to be tasteless whilst the remainder described it as bitter to varying degrees. The responses essentially showed a bimodal distribution that distinguishing two phenotypes: tasters and non tasters. In subsequent studies, chemical compounds sharing the H-N-C=S chemical moiety like PROP also showed the same bimodal threshold distribution, leading to the designation of 'tasters' for the more sensitive and 'non tasters' for the less sensitive individuals (Hall, Bartoshuk, Cain and Stevens, 1975). The incidences of PROP taste 'blindness' varies around the world. In western Africa about 3% of the population are non tasters, > 40% in India, and about 30% of the adult Caucasian population in North America are non tasters (Tepper, 1998). However, it should be noted that so-called "non tasters" can taste PROP at high concentrations (Tepper, 1998).

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'. Some offspring of non taster parents have been found to be tasters (Olson, Boehnke, Neiswanger, Roche, and Siervogel, 1989). Sex and age have also been found to influence PTC sensitivity thresholds (Kalmus, 1958). Females were found to be more sensitive to PROP than males. Using threshold and supra-threshold tests, Bartoshuk, Fast, Karrer, Marino, Price and Reed (1992) demonstrated that there are three phenotypical groups and not two. The threshold is defined as the lowest concentration of a test solution that can be distinguished from plain water (Tepper, 1998). Tasters have very low thresholds ($< 1.0 \times 10^{-4}$ mol/l; high sensitivity to PROP at very low concentrations), whereas non tasters have higher thresholds ($> 2.0 \times 10^{-4}$ mol/l; poor sensitivity at low concentrations). Roughly one third of the taster population is homozygous (TT) tasters and is classified as super tasters and two thirds are heterozygous (Tt) tasters and classified as medium tasters. Bartoshuk *et al.* (1992) identified the three phenotypic groups using

supra-threshold taste scaling for NaCl (1.0×10^{-2} – 1.0 mol/l) and PROP (3.2×10^{-5} – 3.2×10^{-3} mol/l). The supra-threshold is a range above the threshold. Non tasters rated the intensity of PROP considerably lower than NaCl, super tasters rated the intensity of PROP considerably higher than NaCl, and the medium tasters' ratings for PROP and NaCl overlapped.

Sensitivity to PROP has been reported to be correlated with the density of both fungiform taste papillae and taste pores or buds (Miller and Reedy, 1990a; Miller and Reedy, 1990b; Bartoshuk, Duffy and Miller 1994; Duffy, Miller and Bartoshuk, 1994). In these studies, super tasters were found to have the highest densities followed by medium tasters followed by non tasters. This might explain the greater sensitivity of PROP tasters to basic tastes like bitterness and sweetness (Tepper, 1998).

1.2.4.3. Sensitivity to PROP and bitterness of other compounds

In addition to differing in the ability to perceive the bitterness of thioureas (PTC and PROP), tasters and non tasters have been reported to differ in their perception of the bitterness of other compounds (Mela, 1989). According to Drewnowski and Rock (1995) not all studies have found associations between the taste of PTC/PROP and other bitter compounds. Discrepancies have been found in studies investigating the relationship between PROP, urea, caffeine and quinine. Gent and Bartoshuk (1983) and Leach and Noble (1986) reported a significant positive relationship between PROP/PTC and quinine, whereas Kalmus (1958) and Mela (1989) did not find such a relationship. Gent and Bartoshuk (1983) reported that tasters found quinine hydrochloric acid (QHCl) significantly ($p < 0.02$) more bitter than non tasters. Leach and Noble (1986) compared the bitterness of PROP to quinine and caffeine using the time intensity (TI) sensory method. The tasters (n=8) rated the maximum intensity of quinine significantly ($p < 0.001$) higher than the non tasters (n=6). However, there was no significant difference between the two groups for their ratings of maximum intensity of caffeine. When comparing the intensity ratings for PTC and quinine by tasters and non tasters, Kalmus (1958) reported lower ratings (2.5) for intensity of PTC by non tasters than tasters (10.6), whereas the intensity ratings for quinine by the two groups (non tasters and tasters) were essentially similar (10.0 and 10.6, respectively). Mela (1989) assessed the perceived intensity of NaCl and five bitter compounds: caffeine, denatonium benzoate, QHCl, SOA and urea by tasters and non tasters. Mela (1989) reported that non tasters did not differ

significantly from the tasters in their bitterness ratings of both quinine and caffeine. However, significant group x concentration interactions were noted for urea, denatonium benzoate and SOA. Hall *et al.* (1975) studied thresholds of non tasters (n=10) and tasters (n=10) for PTC, caffeine, urea, QHCl and NaCl. A bimodal distribution was reported for caffeine at lower concentrations (albeit to a lesser degree) as noted for PTC. The caffeine thresholds were correlated with the PTC thresholds (Spearman rank correlation coefficient = 0.83, $p< 0.001$). Urea, like caffeine, was also perceived as slightly less bitter by non tasters than tasters at low concentrations; suggesting that although urea and caffeine do not possess a HNCS group they may stimulate the same receptor sites as PTC. Although QHCl also tastes bitter, it did not follow the same trend, and is seemingly coded by a different receptor site. In agreement with the findings of Kalmus (1958), Hall *et al.* (1975) reported that QHCl was equally bitter to tasters and non tasters. Contrary to the findings of Hall *et al.* (1975), but in agreement with the findings of Leach and Noble (1986), Mela (1989) reported that PROP tasters and non tasters did not differ in their ratings of the intensity of caffeine. While Hall *et al.* (1975) reported that urea was slightly less bitter to non tasters than tasters Mela (1989) did not find a relationship between PROP and urea. Delwich *et al.* (2001) reported significant differences between the ratings of the non tasters (n=4) and tasters (4 super tasters and 18 medium tasters) for the bitterness of QHCl, caffeine, (-) epicatechin, tetralone, L-phenylalanine, L-tryptophan, magnesium sulphate, urea, SOA and denatonium benzoate. However, such differences were not observed for the bitterness rankings of these compounds as a function of PROP taster status. It was concluded that a lack of significant difference in the compound rankings is an indication that the subjects in each group differ only quantitatively, not qualitatively. In other words, non tasters have lower system gains for bitterness due to their fewer taste buds and/or fewer taste pores compared to the medium and super tasters.

Frank and Korchmar (1985) studied the taster group's reaction time (RT) as well as the intensity ratings for different taste stimuli (sucrose, NaCl, QSO_4, HCl, PTC and water). The existence of two non taster sub-groups was reported. One group was insensitive to thiourea compounds only, whilst the other group was insensitive to thiourea and a number of the other compounds (sucrose, NaCl, QSO_4 and HCl). The second sub-group appeared to have a specific PTC sensitivity deficit that did not influence their processing of other taste stimuli. This finding is consistent with the view that insensitivity to PTC is a result of a lack of a PTC taste receptor (Frank and Korchmar, 1985). Delwich *et al.* (2001)

surmised that some of the discrepancies noted in the different studies (Kalmus, 1958; Hall *et al.*, 1975; Gent and Bartoshuk, 1983; Leach and Noble, 1986; Mela, 1989) on the bitterness of PROP, urea, quinine, caffeine and other bitter compounds may be due to the inclusion of differing proportions of the non taster sub-groups reported by Frank and Korchmar (1985). According to Delwich *et al.* (2001) studies with a high percentage of non tasters insensitive to PROP and other bitter compounds would be more likely to find a significant relationship between PROP and other compounds than study groups with a lower percentage this non tasters sub-group.

1.2.4.4. Sensitivity to PROP and phenolic compounds

Thorngate and Noble (1995) studied the time-course of bitterness and astringency of monomeric flavan-3-ols (-)-epicatechin and (+)-catechin in water, using the time intensity sensory method. Epicatechin was significantly more bitter and more astringent and had longer total duration than catechin. According to these authors, PROP status had no significant effect on any of the parameters: time to max (T_{max}), intensity at max (I_{max}) and total duration for both bitterness and astringency. Ishikawa and Noble (1995) investigated the interaction between astringency and sweetness of red wine using the time intensity methodology. As the level of tannic acid in the experimental wine was increased, all the astringency parameters increased. Maximum intensity and total duration of astringency were significantly reduced as the sucrose concentration increased. No differences were noted in the perception of astringency and sweetness between the PROP tasters (n=14) and non tasters (n=10). However, there was a significant difference in the intensity and persistence of astringency as a function of salivary flow rate. Low flow subjects rated the astringency higher and longer than high flow subjects. Smith, June and Noble (1996) examined the effects of viscosity and sweetness on astringency of aqueous solutions of grape seed tannin thickened with carboxymethyl cellulose or sweetened with aspartame. Maximum intensity and total duration of astringency decreased significantly as viscosity increased. Maximum intensity and total duration of bitterness were not significantly affected by increasing viscosity. Increasing sweetness had no effect on astringency parameters, but maximum intensity of bitterness was significantly decreased. PROP status and salivary flow rate had no effect on the perception of bitterness or astringency of the grape seed tannin aqueous solutions.

1.2.4.5. PROP sensitivity on acceptability of bitter foods

Greater sensitivity to the bitterness of PROP has been linked to reduced acceptability of bitter foods and beverages such as dry milk products and cheese (Marino, Bartoshuk, Monaco, Anliker, Reed and Desnoyers, 1991), brussels sprouts (Van Doorn, Van der Kruk, Van Holst, Raaijmakers-Ruijs, Postma, Groenweg and Jongen, 1998), 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, 2003). Marino *et al.* (1991) investigated how tasters and non tasters would rate the sensory attributes (bitterness, sweetness, saltiness, sourness and creaminess) of a variety of cheeses. Cheddar and Swiss cheese were reported to taste more bitter to tasters than non tasters; and American and cottage cheeses were saltier to tasters than non tasters. On the other hand, sweetness, sourness and creaminess showed no taster/non taster association. Dry milk powders were also perceived to be more bitter to some tasters than non tasters. Casein was found to be more bitter to some adult tasters than non tasters. Since protein molecules are too large to stimulate taste, the bitter taste was attributed to fragments of proteins (amino acids) resulting from processing (Marino *et al.*, 1991). According to Tepper (1998), PROP and PTC are chemically related to the isothiocyanates and goitrin, which are bitter compounds found in cruciferous vegetables such as cabbage, broccoli, brussels sprouts, turnips and kale. Kaminski *et al.* (2000) studied food preferences of young women for brussels sprouts, broccoli, spinach, black coffee, soy milk and soybean tofu. PROP super tasters rated brussels sprouts as significantly more bitter than non tasters. The subjects who rated the foods as more bitter also rated them as less pleasant and less palatable. Bitterness was most frequently responsible for decreased food preference. Thus, food preferences were linked to taste preferences. Tepper (1999) investigated the influence of PROP taster status on the acceptance of broccoli, cheese and whole milk. PROP taster children gave significantly lower hedonic ratings for raw broccoli, cheese and whole milk than non taster children. Keller *et al.* (2002) determined the acceptance of bitter and fatty foods by taster and non taster children. Taster children showed a significantly lower acceptance of raw broccoli and American cheese; and taster girls showed a significantly lower acceptance of full-fat milk than non taster girls. According to Drewnowski *et al.* (1997) increased taste acuity for both PROP and naringin was associated with greater dislike for each bitter compound. Naringin is the primary bitter compound in grapefruit juice. PROP super tasters disliked bitter naringin solutions significantly more than non tasters. PROP sensitivity was also

associated with reduced acceptability of grapefruit juice. Drewnowski *et al.* (1997) reported that increased taste acuity for naringin, the primary bitter compound in grapefruit juice, and PROP were associated with greater dislike for each of the compounds. PROP super tasters disliked bitter naringin solutions significantly more than non tasters. PROP sensitivity was also associated with reduced acceptability of grapefruit juice.

1.2.5. Astringency

In addition to the taste and smell systems, there is a chemical and tactile responsiveness mediated by trigeminal nerves (Lawless and Heymann, 1998). According to these authors a variety of everyday flavour experiences arising from trigeminal stimulation including the fizzy tingle of carbonated drinks, burn of capsaicin in hot peppers, and the pungency of spices such as ginger and cumin. The trigeminal nerves also signal tactile, thermal, and pain sensations. Unlike bitterness which is mediated through taste receptors, astringency is an oral sensation signalled by trigeminal nerves (Vidal *et al.,* 2003). Astringency is an important sensory attribute of foods and beverages that contain tannins such as coffee, tea, beer, wine, apples, ciders and many berry crops and nuts (Lee and Lawless, 1991). The word astringent is derived from the Latin word *ad* (to) and *stringere* (bind). Thus, astringency is defined as a binding reaction relating to the ability of astringent materials to bind and precipitate proteins (Haslam and Lilley, 1988; Lee and Lawless, 1991). Saliva contains a considerable quantity of proteins (proline-rich proteins and possibly mucins) that lubricate the mouth (Siebert and Chassy, 2003). When these salivary proteins (especially those rich in proline) bind preferentially with polyphenols in foods, they form insoluble complexes (Gawel, Iland and Francis, 2001; Siebert and Chassy, 2003). Like sorghum kafirin (Taylor *et al.*, 1989), saliva contains proline-rich proteins (PRP) that interact strongly with tannins (Muenzer, Bildstein, Gleason, and Carlson, 1979; Hagerman and Butler, 1981). This results in a decrease in salivary lubrication properties and thus elicits the astringency sensation (Gawel *et al.*, 2001; Siebert and Chassy, 2003). According to Mehansho, Hagerman, Clements, Butler, Rogler and Carlson (1983) rats fed with a diet containing 2% CE tannin had an increased secretion of proline-rich salivary proteins. The increase in the proline-rich protein fraction was attributed to the tannins in the diet. This is seen as a protective mechanism for other dietary and digestive proteins not to interact with tannin (Hagerman and Butler, 1981). According to Mehansho, Clements, Sheares, Smith and Carlson (1985) proline-rich proteins are very efficient in

selectively binding to tannins and removing them from ingested food, hence reducing their detrimental effects. Also unlike bitterness, astringency is a tactile sensation because it has to do with feeling and not taste (Breslin, Gilmore, Beauchamp and Green, 1993). 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). Astringency belongs to mouth-feel sensations, particularly important in beverages such as fruit juices, tea and wine (Valentová *et al.*, 2002).

1.2.5.1. Compounds that cause astringent sensations

There are four main groups of compounds that cause astringency: plant polyphenols, salts of multivalent metallic cations (Al, Cr, Zn, Pb, Ca) particularly aluminium salts such as alum, mineral and organic acids, and dehydrating agents such as alcohol (ethanol) (Haslam and Lilley, 1988; Siebert and Chassy, 2003). Tannins make the mouth feel rough and dry because they cause a drawing, puckering or tightening sensation in the cheeks and muscles of the face as a result of coagulating saliva and mouth proteins (Haslam and Lilley, 1988; Lawless and Hyman, 1998). Polyphenols such as tannins form complexes with mucoproteins of the saliva and by either precipitating them or causing sufficient conformational changes so that they lose their lubricating power (Bate-Smith, 1973). According to Bate-Smith (1973), a threshold exists for the subjective experience of astringency to be sensed in the mouth. At low concentrations, not eliciting the 'puckery' sensation, the sensation is described as 'body' or 'substance' in wine or fruit. According to Asano, Shinagawa and Hashimoto (1982), the proline-rich haze forming proteins in beer have unfolded molecular structures that facilitate the entry of polyphenols into them. Peptides that contain proline were found to combine with polyphenols to form complexes that scatter light (indicating the presence of colloidal or larger size particles) in proportion to their proline content (Asano *et al.*, 1982; Siebert, 1999).

Organic and inorganic acids have also been reported to be astringent even though they do not resemble plant tannins (Rubico and McDaniel, 1992; Corrigan Thomas and Lawless, 1995). Organic acids (malic, citric and quinic acid) have been found to be both astringent and sour in model solutions (Rubico and McDaniel, 1992). Corrigan Thomas and Lawless (1995) compared astringency, astringent sub-qualities (drying, roughing and puckering), and sourness of organic and inorganic acids. The astringency profile of the organic acids (lactic, citric, acetic, fumaric and malic) was similar but slightly different from the

inorganic acids (HCl and phosphoric). HCl and phosphoric acids were more astringent and less sour while the organic acids were more sour than astringent. The accepted astringency mechanism of salivary proteins binding with tannins, involves hydroxyl groups on the tannin molecule binding to an electronegative site like the keto-imide linkage on the protein forming a complementary hydrogen bond pair (McManus, Davis, Lilley and Haslam, 1981). This mechanism might explain why some acids like tartaric acid, which has adjacent hydroxyl (–OH) groups, are potent astringents (Corrigan Thomas and Lawless, 1995). For the inorganic acids used here, a mechanism such as denaturation of salivary proteins may be responsible for the astringent sensation (Corrigan Thomas and Lawless, 1995). Salts like potassium aluminium sulphate (alum) and alcohols (ethanol) have dehydrating properties, and the resulting removal of water is presumably the cause of their astringency (Haslam and Lilly, 1988; Siebert and Chassy, 2003). Small multiple charged cations such as aluminium bind water very tightly (Siebert and Chassy, 2003). Peleg, Bodine and Noble (1998) proposed that alum interacts with salivary proteins or epithelial proteins to elicit the astringency sensation.

1.2.5.2. Sensory perception of astringency

Astringency, unlike true tastes, is a complex and persistent sensation that does not demonstrate adaptation (Lyman and Green 1990; Ishikawa and Noble, 1995). The intensity and duration of the astringency sensation has been found to increase with repeated ingestion (Guinard, Pangborn and Lewis, 1986a; Guinard *et al.*, 1986b; Lyman and Green 1990; Lee and Lawless 1991). Lyman and Green (1990) found that the astringent sensation can be altered by the presence of other compounds. Sweeteners in particular, reduced the astringency sensation. Sucrose was found to suppress the astringency of vermouths. The authors presumed it was probably due to an increase in the salivary flow rate. The salivary flow rate was measured as a function of pre-exposure to water, tannic acid, a mixture of tannic acid and sucrose (tannic acid+sucrose), and sucrose. Sucrose and a mixture of tannic acid+sucrose increased the salivary flow rate significantly more than water and tannic acid. However, although salivary flow rate was highest when subjects sipped sucrose alone, there was no significant difference between the salivary flow rates of sucrose and tannic acid+sucrose. Different theories were proposed as to why sucrose decreased the astringency sensation. It was postulated that by sucrose stimulating more saliva production, additional proteins in the saliva reversed the phenol/protein complexes causing the astringent sensation in the mouth as proposed by Haslam and Lilley

(1988). Alternatively, it was proposed that the increased salivation simply helped clear out the phenols from the mouth, as has been proposed by Lagerlof and Dawes (1985), and/or provided new proteins to replace the precipitated proteins as has been proposed by Joslyn and Goldstein (1964). Another theory was that the viscosity of the sucrose solution provided lubrication that helped mask the astringency sensation. Peleg and Noble (1999) reported that increasing the viscosity of cranberry juice using carboxymethylcellulose lowered its perceived astringency. Smith *et al.* (1996) also reported that increased viscosity caused by carboxymethylcellulose lowered the perceived astringency of aqueous solutions of grape seed tannin.

Astringency of phenolic compounds has been reported to increase in the presence of added acid (to lower pH) (Fischer, Boulton and Noble, 1994; Peleg *et al.*, 1998). Astringency of aqueous solutions of phenolic compounds (grape seed tannin, tannic acid, catechin and gallic acid) increased upon addition of citric acid, whereas the astringency of alum was reduced (Peleg *et al.*, 1998). The difference noted between the phenolic compounds and alum was attributed to the chemical modifications affecting the binding capacity of the different astringents to salivary proteins. Chelation of the aluminium ion in alum by acids reduced its availability to bind the salivary proteins. On the other hand, the increased astringency noted for the phenolic compounds upon acidification was speculated to result from the pH driven increase in affinity of the phenols for binding with proteins. Kallithraka, Bakker and Clifford (1997a) assessed how addition of malic acid and lactic acid affected the bitterness, astringency and sourness of red wines and model solutions. The intensity and duration of astringency and sourness increased with decreasing pH in both the model solutions and red wine. Bitterness was not affected by the addition of either acid. Peleg and Noble (1999) also reported that the astringency of cranberry juice could be modified by altering the pH.

Kielhorn and Thorngate (1999) used a multidimensional scaling (MDS) study of ten diverse compounds: (+)-catechin, (-)-epicatechin, caffeine (bitter), citric acid (acid), alum, tannic acid, grape seed tannin, gallic acid, ethanol and capsaicin. Three recognizable groupings emerged: a bitter neighbourhood comprising of (+)-catechin, (-)-epicatechin, caffeine and ethanol; an acid neighbourhood comprised of citric acid and gallic acid; and an astringent neighbourhood comprised of tannic acid and grape seed tannin. Thus, although (+)-catechin and (-)-epicatechin are described as astringent, they were more

closely associated with caffeine and ethanol than the traditional astringents (tannic acid and grape seed tannin) in the MDS plot. Dimension 2 was defined by capsaicin, indicating that it was unique to the perceptual space. According to Kielhorn and Thorngate (1999) as aspartame and sucrose share 'sweetness' even though the quality of the sweetness is different, so may the monomeric flavan-3-ols and their polymeric counterparts share 'astringency' although the true quality of the sensation is different. Although small chemically, benzoic acid derivatives: salicylic acid (2-hydroxy benzoic acid), *m*-hydroxyl benzoic acid (3-hydroxy benzoic acid), gentisic acid (2,5-hydroxyl benzoic acid) protocatechuic acid (3,4-hydroxy benzoic acid) and gallic acid (3,4,5-trihydroxy benzoic acid) in water, are also reported to elicit astringency (Peleg and Noble, 1995). McManus *et al.* (1981) determined the association of small phenols: resorcinol (1,3-dihydroxybenzene), catechol, and pyrogallol with bovine serum albumin (BSA) (Fig. 1.10). The astringency of these small phenols was attributed to precipitation of or strong binding with proteins due to the presence of 1,2-dihydroxy or 1,2,3-trihydroxy groups. The affinity of resorcinol for BSA was weaker than that of catechol and pyrogallol, which have two and three *ortho*-disposed phenolic groups respectively, to more strongly bind the protein.

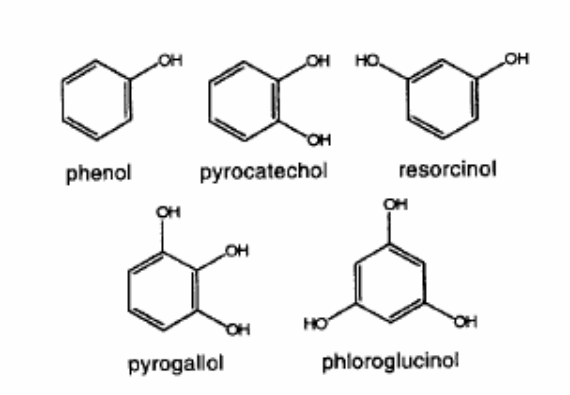


Figure 1.10. Basic structures of small phenolic compounds (Kennedy, Saucier and Glories, 2006).

One difficulty in studying astringency is that many untrained observers confuse astringency with bitterness (Lee and Lawless, 1991). Lea and Arnold (1978) classified bitterness and mouth drying as 'twin sensations' because nearly all astringents are also bitter, and untrained panellists sometimes confuse the two qualities. In addition to being

bitter, many astringent materials (particularly organic acids) also have a sour side-taste. Lee and Lawless (1991) examined quantitative and qualitative perceptual reactions to astringent materials for three diverse chemical substances (alum, tannic acid and tartaric acid) at several concentrations producing moderate to strong levels of perceived sensation. A trained sensory panel developed six descriptors for the sensory sensations elicited by the astringent substances as follows: astringency, mouth-drying, puckery feeling, mouth-roughing, bitterness and sourness. The time intensity ratings for each attribute were found to depend on both the particular astringent substance and concentration tested. These authors recommended the use of alum as a standard in future structure activity studies using time intensity procedures because it was relatively low in perceived bitterness and sourness, but produced pronounced drying, roughing, puckering/drawing sensations.

1.2.5.3. Acceptability of astringency in food

Several predominantly astringent and bitter beverages such as tea, wine, beer and coffee are widely consumed (Guinard *et al.*, 1986a; Mattes, 1994; Drewnowsky and Gomez-Carneros, 2000; François, Guyot-Declerck, Hug, Callemien, Govaerts and Collin, 2006). Astringency is an essential characteristic of wine caused by procyanidins, affecting perceived 'mouth-feel' that is informally described as 'soft', 'hard' or 'rough' especially when referring to red wine (Guinard *et al.*, 1986a; Ishikawa and Noble, 1995). Pickering *et al.* (2003) examined the relationship between taste and astringency perception elicited by red wines and sensitivity to PROP. Bitterness, astringency and acidity intensities were all correlated with PROP taster status. PROP non tasters gave significantly lower intensity ratings for astringency, bitterness and acidity of the red wines than did PROP tasters. However, Ishikawa and Noble (1995) investigating the astringency and sweetness of Carnelian red wine using the time intensity methodology and found no relationship between PROP taster status and the astringency perception of the wine. According to these authors the magnitude of astringency and sweetness of wine did not differ between PROP tasters (n=14) and non tasters (n=10).

1.2.6. Time intensity sensory evaluation procedure

The time intensity sensory evaluation method is used to continuously capture, in great detail, nuances of flavour growth, decay and disappearance (Lawless and Heymann, 1998). Over the years, the time intensity evaluation method has been used in tracking the changes

in the sensory properties (flavour and texture) of different foods and beverages. Foods possess a composite of many taste attributes, but in most cases the evaluation of these attributes is typically made for each individual attribute (Duizer, Bloom and Findlay, 1997). Furthermore, many sensory evaluation methods, such as quantitative descriptive analysis and difference tests, measure the perception of food flavour and texture as static events even though the intensity perception does not occur at a single point in time (Bloom, Duizer and Findlay, 1994; Dijksterhuis and Piggott, 2001). Processes involved in eating, such as mastication and salivation are dynamic (Dijksterhuis and Piggott, 2001); both flavour and texture intensities change as the food moves through the mouth and is prepared for swallowing (Bloom *et al.*, 1994). Therefore methods taking these dynamic processes into consideration are likely to produce more valid results than static methods (Dijksterhuis and Piggott, 2001). This is why the time intensity method is gaining wide application because it measures changes in the perception of product attributes over time (Bloom *et al.*, 1994).

1.2.6.1. Single attribute time intensity (SATI) and dual attribute time intensity (DATI) sensory methods

According to Leach (1984), bitterness and astringency are characterized by a persistent after-taste and thus cannot be estimated solely by scalar intensity procedures. 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. Thus, to fully characterize the differences in their sensory properties requires analysis of the time-course of perceived intensity. Time intensity sensory evaluation has mostly been used to measure single attributes.

The time intensity method has been used in the study of the bitterness and/or astringency of wine (Boulton and Noble, 1994; Valentová *et al.*, 1997; Kallithraka, Clifford and Bakker, 1997b), beer (François *et al.*, 2006; King and Duineveld, 1999) and soymilk (Courregelongue, Schlich and Noble, 1999). Zimoch and Findlay (1998) used the time intensity sensory method to study the juiciness and toughness of beef samples. McGowan and Lee (2006) also used it in a study of artificial sweeteners in chewing gums. Most of these studies used the single attribute time intensity (SATI) method. However, in recent years time intensity is gaining popularity measuring dual attributes simultaneously.

Duizer *et al.* (1997) compared the SATI method to the dual attribute time intensity (DATI) method in investigating dual attributes of sweetness and peppermint flavour of four samples of chewing gum with varying rates of sweetness and peppermint flavour release. They observed that in general, the DATI method was as sensitive as the SATI method in distinguishing sweetness and peppermint flavour of the chewing gum.

According to Duizer *et al.* (1997), advantages inherent with using the DATI method include collection of sensory data that more accurately reflects what is taking place in the mouth during consumption of a food; using the DATI method means that only half the time was required to collect the same information by the SATI method since both attributes were measured simultaneously; the DATI method can also possibly provide solutions to two known methodological problems: dumping and inter-sample variability. Dumping is a problem that occurs in single attribute measurements when a single attribute is rated more intense when evaluated alone than when evaluated with other attributes (Duizer *et al.*, 1997; Zimoch and Findlay, 1998).

1.2.6.2.‘Panellist’s signature’

According to Boulton and Noble (1994), the human judge is a multi-purpose instrument who can be trained to measure many attributes. However, despite extensive training of judges to calibrate their use of descriptive terms and rating scales, individual physiological and psychological differences affect perception of sensory properties. Time intensity studies are subject to different biases, one of which is panellist variation (Valentová *et al.*, 2002). Valentová *et al.* (2002) paid particular attention to the variability of different judge’s responses. They reported that there were slow, medium and rapidly reacting subjects. Differences in the salivary flow rates of judges have been attributed to noted differences among judges (Boulton and Noble, 1994; Fischer *et al.,* 1994; Ishikawa and Noble, 1995; Kallithraka, Bakker, Clifford and Vallis, 2001). Consequently, individual curve shapes showed a high variance among judges (Pangborn, Lewis and Yamashita, 1983).

Fischer *et al.* (1994) studied the physiological factors contributing to the variability of sensory assessments, i.e. the relationship between salivary flow rate and temporal perception of gustatory stimuli using wines varying in ethanol, pH and phenolic composition. They reported that the perceived intensity and duration of bitterness and

astringency were affected by salivary flow rate, possibly due to salivary volume, salivary pH and protein composition. Subjects with low saliva flow rates took longer to reach maximum intensity (T_{max}) and had a longer duration (D_{tot}; persistence) of bitterness and astringency than subjects with high flow rates. Low flow subjects also perceived the intensity (I_{max}) of bitterness and astringency higher than subjects with high flow rates. Ishikawa and Noble (1995) studied the temporal perception of astringency and sweetness in red wine using the time intensity methodology. They found a significant difference for both intensity (I_{max}) and duration (D_{tot}) of astringency of red wine between the low and high saliva flow subjects. The low-flow subjects rated astringency higher and longer than the high-flow subjects.

1.3. Conclusions

A lot of research has been done to determine the sensory properties of phenolic compounds in fruits, tea, wine, beer and other foods but information on the sensory properties of phenolic compounds in sorghum is limited. Condensed tannins are potentially important antioxidants, but consumption of tannin-containing (tannin) sorghums is hampered by the general belief that tannins confer objectionable sensory attributes to this food. Therefore it is necessary to determine the sensory attributes of sorghums containing varying amounts of phenolic compounds, especially condensed tannins and to determine their acceptability to consumers. Bitter taste perception has been genetically linked to sensitivity to PROP, in that some people can taste its bitterness (tasters) whilst others cannot (non tasters). Since condensed tannins are bitter and astringent, preference ratings of sorghum sensory attributes may be influenced by genetic sensitivity to PROP.

1.4. Hypotheses

Tannin sorghums will be more bitter and more astringent than tannin-free sorghums because the total phenol content (including tannins) of these sorghums exceeds that of the tannin-free sorghums. It has been found that phenolic compounds in sorghum contribute significantly to the perceived bitterness and astringency of sorghum products (Asante, 1995; Yetneberk *et al.*, 2004).

High molecular weight phenolic compounds are known to be predominantly astringent; while the low molecular weight compounds are known to be predominantly bitter (Leach, 1984; Peleg *et al.*, 1999). Therefore, astringency will predominate in tannin sorghums, while bitterness will predominate over astringency in the tannin-free sorghums.

Condensed tannins in foods are well-known for eliciting negative consumer response at high intensity because of their dominant sensory attributes: bitterness and astringency (Cheynier, 2005; Lesschaeve and Noble, 2005). Therefore the bitterness and astringency of tannin sorghums will be more intense than tannin-free sorghums and as a result these sorghums and will be less acceptable to consumers.

Bitter taste perception has been genetically linked to sensitivity to 6-*n*-propylthiouracil (PROP), in that some people can taste its bitterness (tasters) whilst others cannot (non tasters; Bartoshuk, 1993). Since condensed tannins are bitter and astringent, acceptance ratings of these sorghums will be influenced by genetic sensitivity to PROP. Therefore, the bitterness of the tannin sorghums will have a negative influence on the acceptability of these sorghums to PROP tasters, while non tasters will find these sorghums equally acceptable.

1.5. Objectives

The objectives of the study were:

1. To determine the bitterness, astringency and other sensory attributes of bran infusions and sorghum rice of sorghums containing different levels of phenolic compounds using a trained sensory panel.

2. To determine the intensity and time course of bitterness and astringency of bran infusions of sorghums varying in condensed tannin content using the time intensity sensory method for dual attributes.

3. To determine which sensory attribute (bitterness or astringency) predominates in the bran infusions from tannin and tannin-free sorghums.

4. To determine consumer acceptability of the rice of sorghums containing different levels of condensed tannins.

5. To determine whether PROP taster status influences the acceptability of the rice of sorghums containing different levels of condensed tannins.

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, Segaolane 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[1] = 1 and Many[2] = 9
Split kernels	Number of split kernels	Few[1] = 1 and Many[2] = 9
Lumpy	Number of swollen and clustered/clumped together kernels	Few[1] = 1 and Many[2] = 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[1] - barely detectable/noticeable

Many[2] – 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 ± 5°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 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.

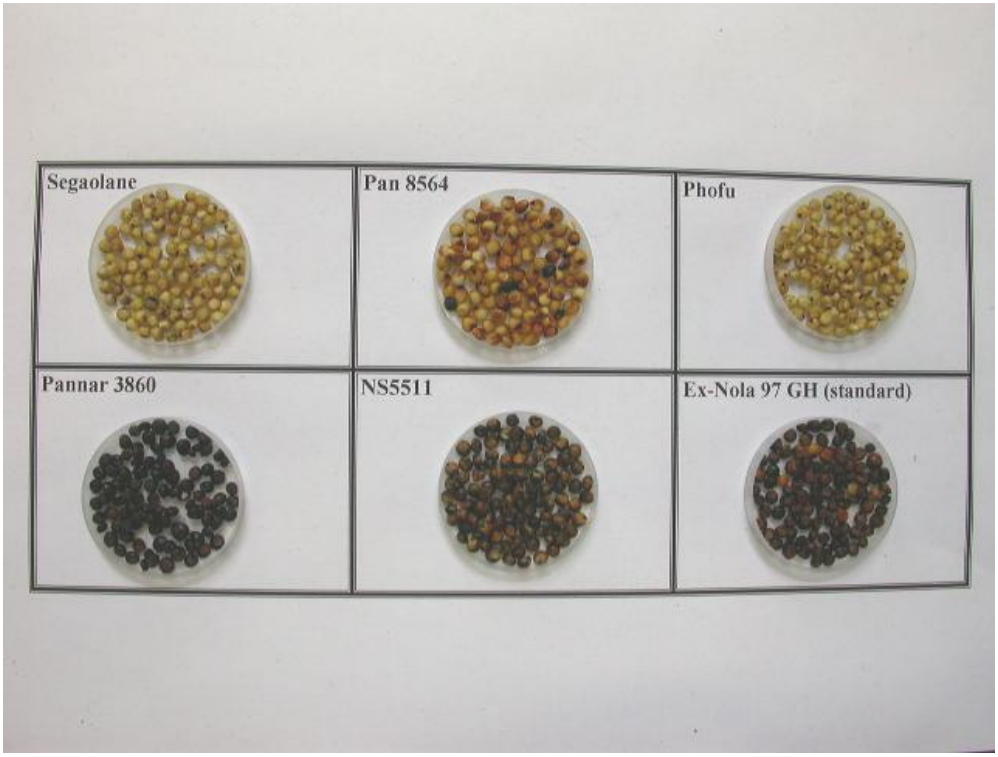

Figure 2.3. Determination of pigmented testa presence in the sorghum grain using the bleach test (Taylor, 2001). Top row sorghums (Segaolane, PAN 8564 and Phofu) have no pigmented testa (no detectable tannins). Bottom row sorghums (PAN 3860, NS 5511 and Ex Nola 97 GH) have a pigmented testa (condensed 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[1]	Tannin Content (% CE db) [2]	Endosperm Texture[3]	
	Colour	Thickness				Visual Hardness Score[4]	Decortication Yield (TADD) (%)[5]
PAN 3860	Red	Medium	Red	Yes	8.2^{c} (0.1)	3.62^{d} (0.49)	81.6^{a} (1.8)
Ex Nola 97 GH	Red	Thick	Red	Yes	5.7^{b} (0.3)	3.85^{e} (0.78)	80.5^{a} (1.3)
NS 5511	Red	Medium	Red	Yes	1.8^{a} (0.2)	3.33^{c} (0.48)	86.8^{b} (0.1)
PAN 8564	Red	Medium	Red	No	ND	3.17^{c} (0.46)	86.9^{b} (1.0)
Segaolane	White	Thin	Purple	No	ND	2.50^{b} (0.60)	88.5^{c} (0.8)
Phofu	White	Medium	Red	No	ND	2.23^{a} (0.43)	88.4^{c} (0.4)

[1]Yes = Pigmented testa present, No = Pigmented testa not present.

[2]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 $p \leq 0.05$.

[3]Means plus standard deviation; means in columns with different letter notations $^{(a-c)}$ are significantly different at $p \leq 0.05$.

[4]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).

[5]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 Kozlowska (2000) also reported methanol (80%) as a better extraction solvent for total phenols in cereals (wheat, barley, rye and oat) than water. Zielinski and Kozlowska (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 (Naczk 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^{-1} tannic acid equivalents db)

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

Means of six replicate experiments and standard deviations.

Means in rows with different letter notations $^{(a\text{-}n)}$ are significantly different at $p \leq 0.05$.

[1]Extraction solvent – 75% aqueous acetone.

[2]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 Segaolane (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 Segaolane 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[1] of bran infusions of different sorghum cultivars as evaluated by a trained descriptive sensory panel (n=12)

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

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

Means in rows with different letter notations $^{(a-e)}$ are significantly different at $p \leq 0.05$

[1]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 sendimented out of solution as described by Siebert *et al.* (1996), and since they are insoluble (Naczk 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 Segaolane).

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 - Segaolane). Although Segaolane 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, Segaolane 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.

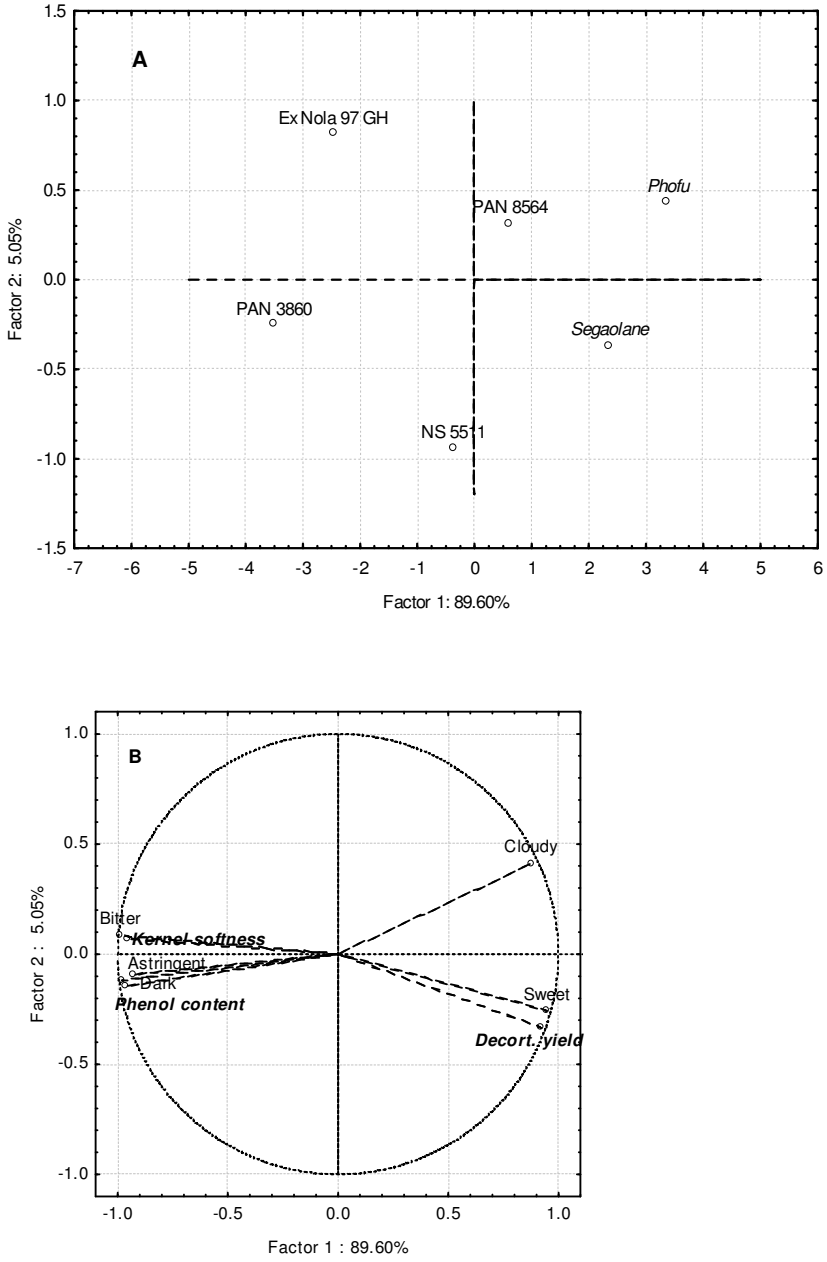

Figure 2.4. Principal component analysis (correlation matrix) of phenol content, endosperm texture and descriptive sensory evaluation of sorghum bran infusions of six sorghum cultivars. (**A**) Plot of the first two principal component scores of the sorghum cultivars. (**B**) Plot of the first two principal 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[1] of sorghum rice of different sorghum cultivars as evaluated by a trained descriptive sensory panel (n=10)

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

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

[1]Refer to Table 2.2 for sorghum rice sensory properties definitions and rating scale.

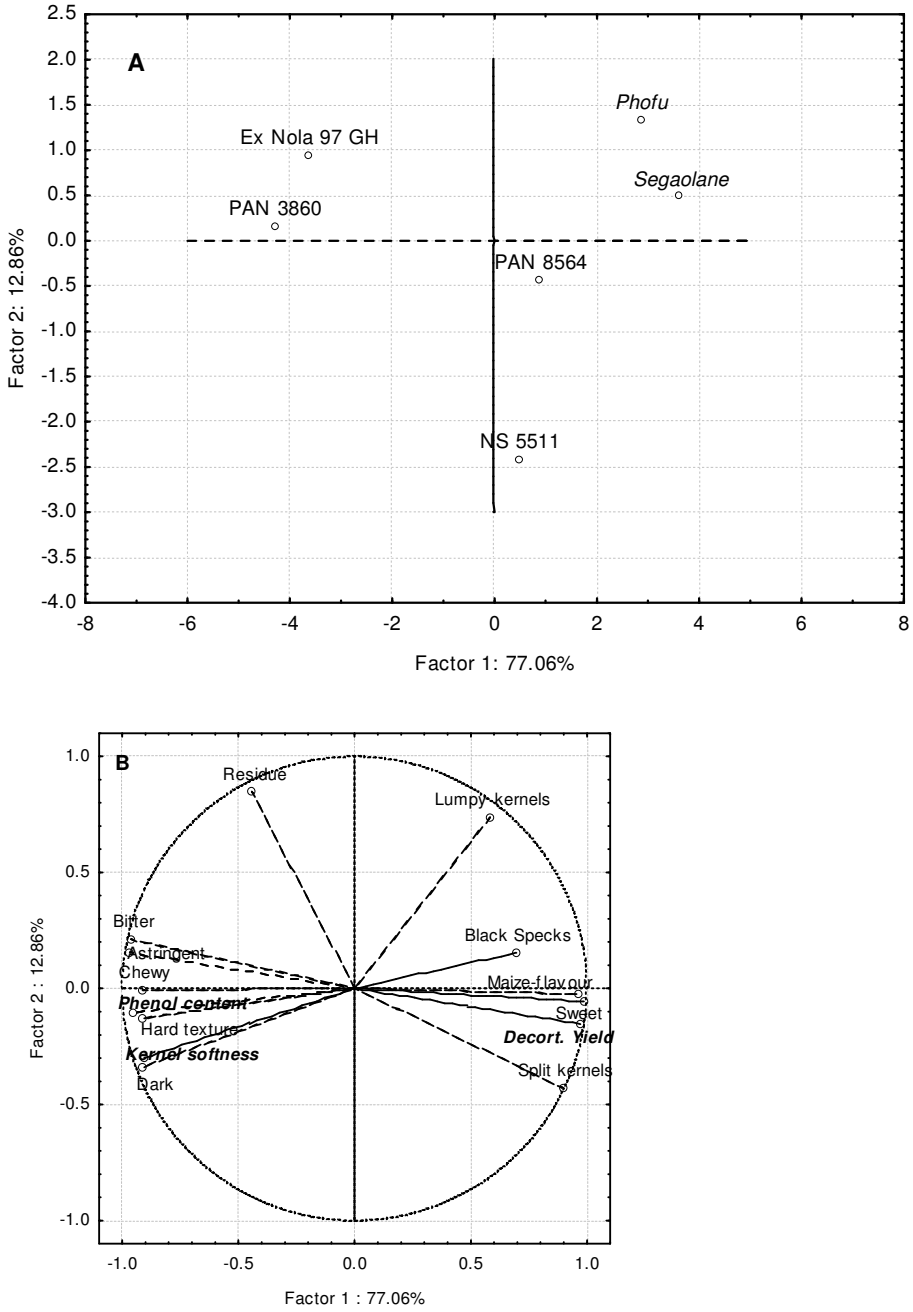

Figure 2.5. Principal component analysis (correlation matrix) of phenol content, endosperm texture and descriptive sensory evaluation of sorghum rice of six cultivars. (**A**) Plot of the first two principal component scores of the sorghum cultivars. (**B**) Plot of the first two principal component 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_{tot} 69.9 s) sensation lasted significantly longer than bitterness (D_{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, Segaolane 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^{o}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 vaccum 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® version 8.2 (SAS Institute Cary, NC).

GLM model:

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

Where:

μ - mean; α_i - session; β_j - panellist; γ_k - replicate; δ_l - sample order; ε_m - cultivar; $(\alpha\beta)i_j$ - session and panellist; $(\beta\gamma)_{jk}$ - panellist and replicate; $(\beta\delta)_{jl}$ - panellist and sample order; $(\beta\varepsilon)_{jm}$ - panellist and cultivar; ξ - 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_{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_{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 follwed 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		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
	df	F	F	F	F
Main effects					
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
Interaction effects					
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		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
	df	F	F	F	F
Main effects					
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
Interaction effects					
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 (±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_{max} (s)	27.2^{b} (1.2)	28.2^{b} (1.2)	20.9^{a} (1.3)	20.1^{a} (1.2)	18.3^{a} (1.3)	20.4^{a} (1.3)
I_{max}	56.9^{e} (1.9)	49.1^{d} (1.9)	36.4^{bc} (2.2)	37.8^{c} (1.9)	31.4^{b} (2.2)	24.3^{a} (2.1)
D_{tot} (s)	75.1^{c} (2.7)	74.1^{bc} (2.7)	63.3^{a} (3.0)	64.9^{ab} (2.7)	60.9^{a} (3.0)	59.6^{a} (2.9)
AUC	2673.7^{d} (123.5)	2381.1^{d} (123.6)	1338.6^{bc} (137.4)	1512.2^{c} (123.6)	1132.5^{ab} (137.4)	845.9^{a} (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 $^{(a\text{-}e)}$ are significantly different at $p \leq 0.05$.

T_{max} – time to maximum intensity, I_{max} – maximum intensity, D_{tot} – 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_{max}	I_{max}	D_{tot}	AUC	T_{max}	I_{max}	D_{tot}	AUC
Bitterness	T_{max}	1							
	I_{max}	0.37***	1						
	D_{tot}	0.29***	0.37***	1					
	AUC	0.43**	0.88***	0.50***	1				
Astringency	T_{max}	0.59***	0.24***	0.23***	0.23***	1			
	I_{max}	0.17***	0.70***	0.28***	0.66***	0.18***	1		
	D_{tot}	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_{max}) 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_{max}) 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_{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, Segaolane and Phofu). With the exception of NS 5511, the total duration (D_{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 (±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^{c} (1.4)	30.9^{c} (1.4)	30.3^{c} (1.6)	25.7^{ab} (1.4)	26.9^{abc} (1.6)	23.0^{a} (1.5)
I_{max}	55.1^{d} (2.1)	42.0^{c} (2.1)	33.9^{ab} (2.3)	36.6^{bc} (2.1)	31.4^{ab} (2.3)	28.9^{a} (2.2)
D_{tot} (s)	76.2^{c} (2.2)	74.2^{c} (2.2)	72.5^{bc} (2.4)	67.2^{ab} (2.2)	65.3^{ab} (2.4)	64.1^{a} (2.4)
AUC	2639.4^{d} (115.5)	1853.1^{c} (115.5)	1436.7^{ab} (128.5)	1562.6^{bc} (115.5)	1319.1^{ab} (128.5)	1102.1^{a} (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 $^{(a\text{-}e)}$ are significantly different at $p \leq 0.05$.

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

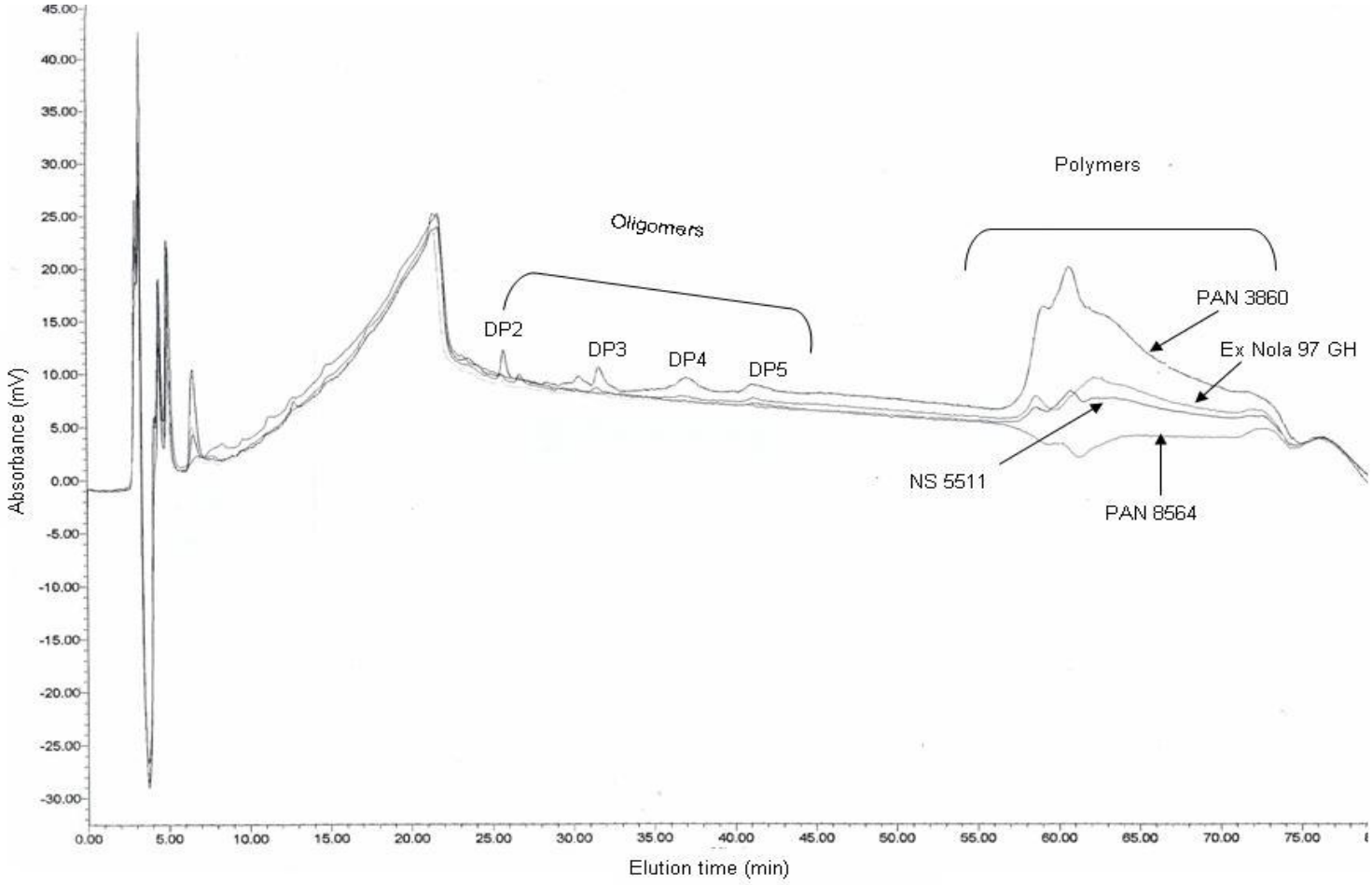

Figure 2.6. Normal phase HPLC procyanidin profiles of PAN 3860, Ex Nola 97 GH, NS 5511 and PAN 8564. Numbers (2, 3, 4, 5) denote degree of polymerization, P = mixed polymers (DP > 5).

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_{tot}^{*} (s)	AUC
Bitterness	22.5[a]	39.3[a]	66.3[a]	1647.3[a]
Astringency	27.9[b]	38.0[a]	69.9[b]	1632.2[a]

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

T_{max} – time to maximum intensity, I_{max} – maximum intensity, D_{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

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

Least Square Means of four replicate experiments.

Least Square Means in columns with different letter notations $^{(a\text{-}e)}$ are significantly different at $p \leq 0.01$

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

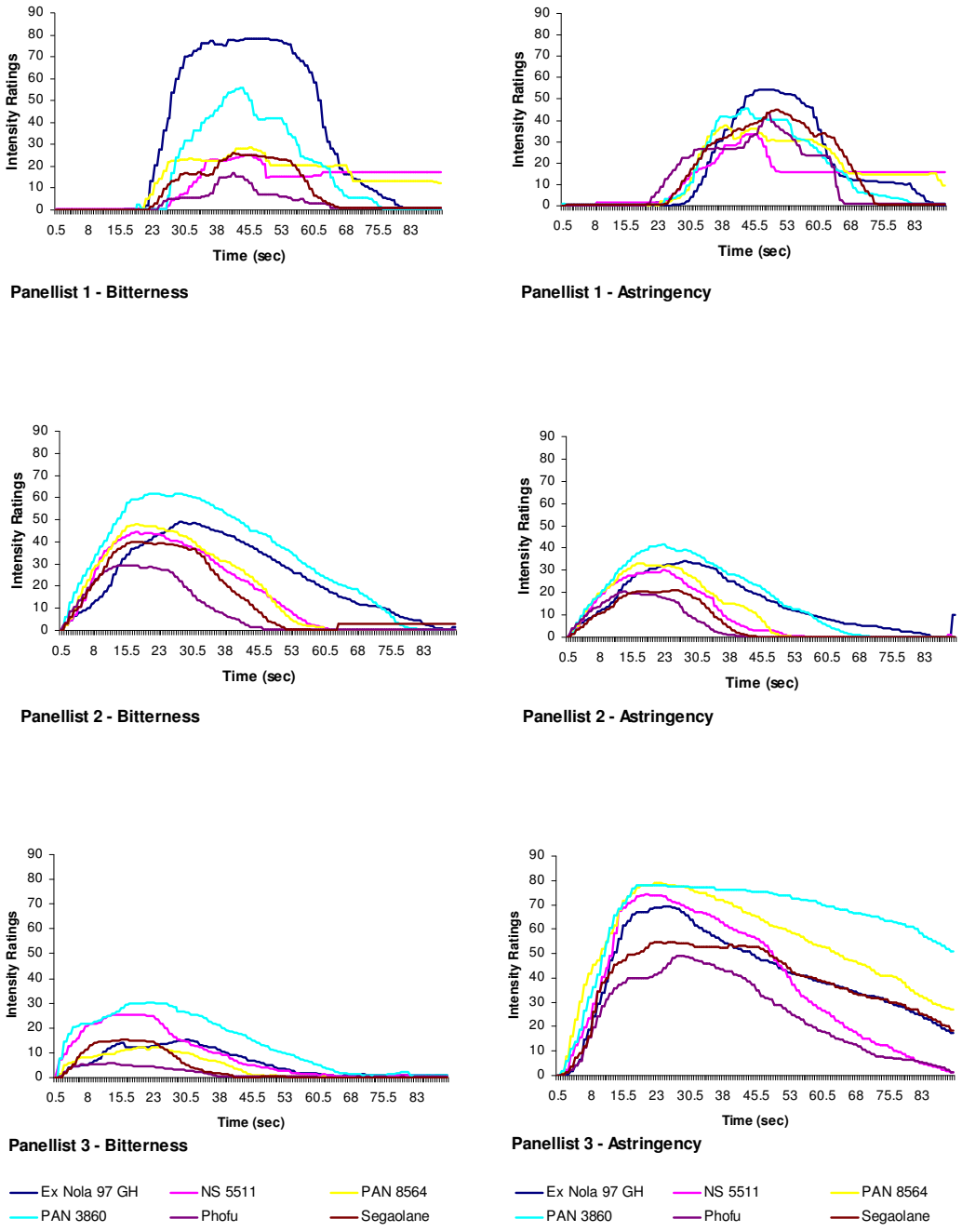

Figure 2.7. Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 1 – 3.

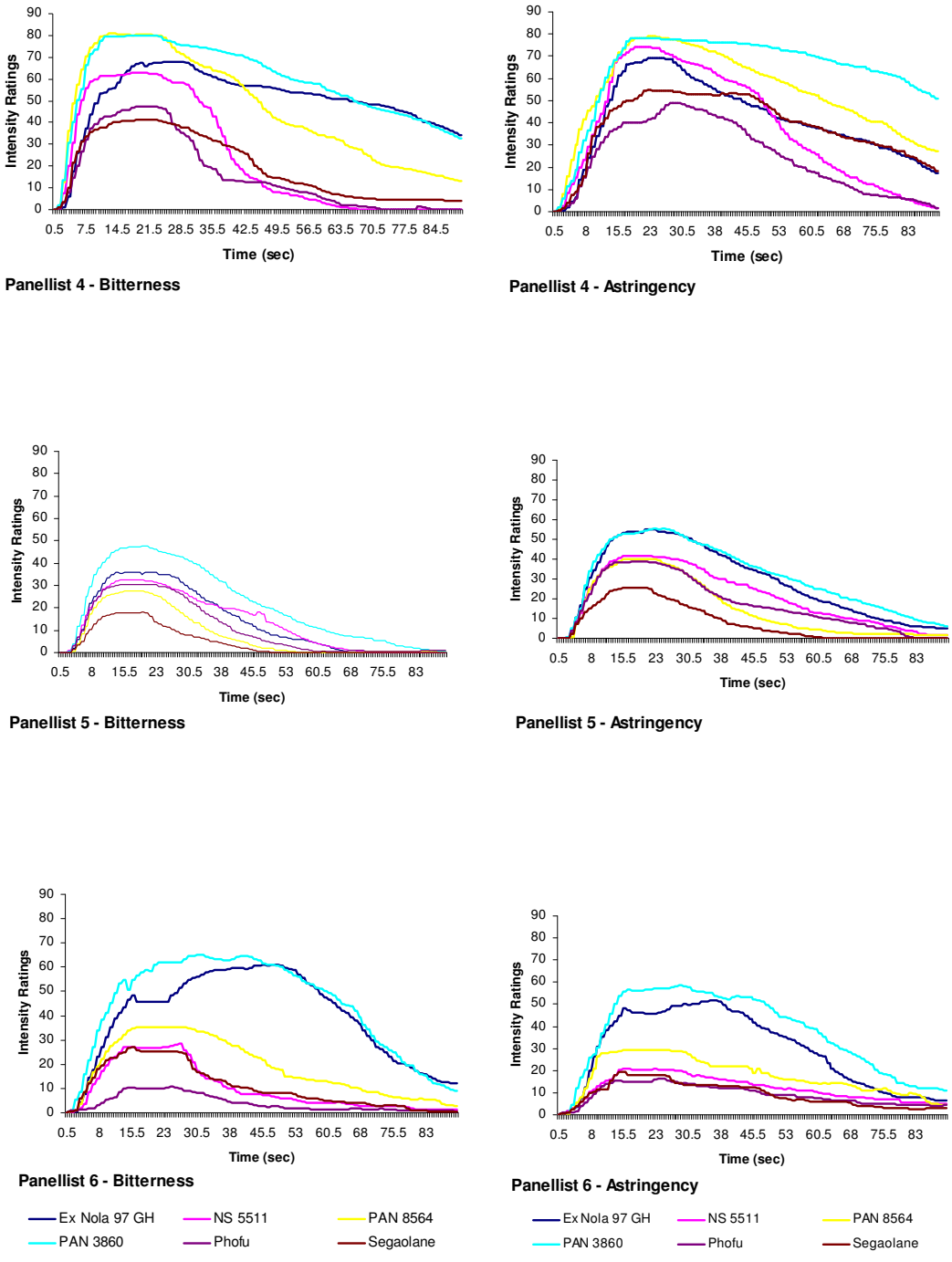

Figure 2.7. Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 4 – 6 (continued).

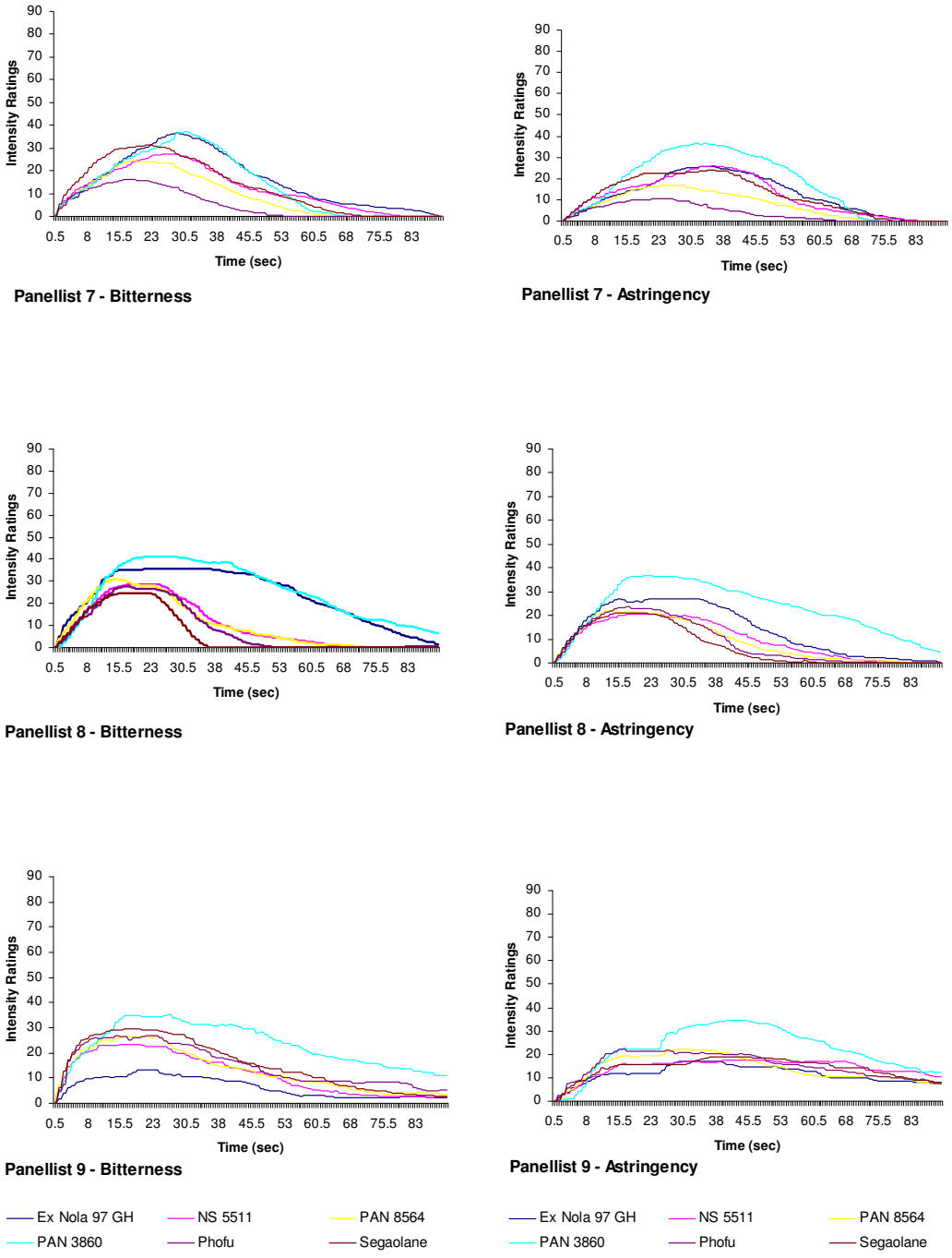

Figure 2.7. Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 7 – 9 (continued).

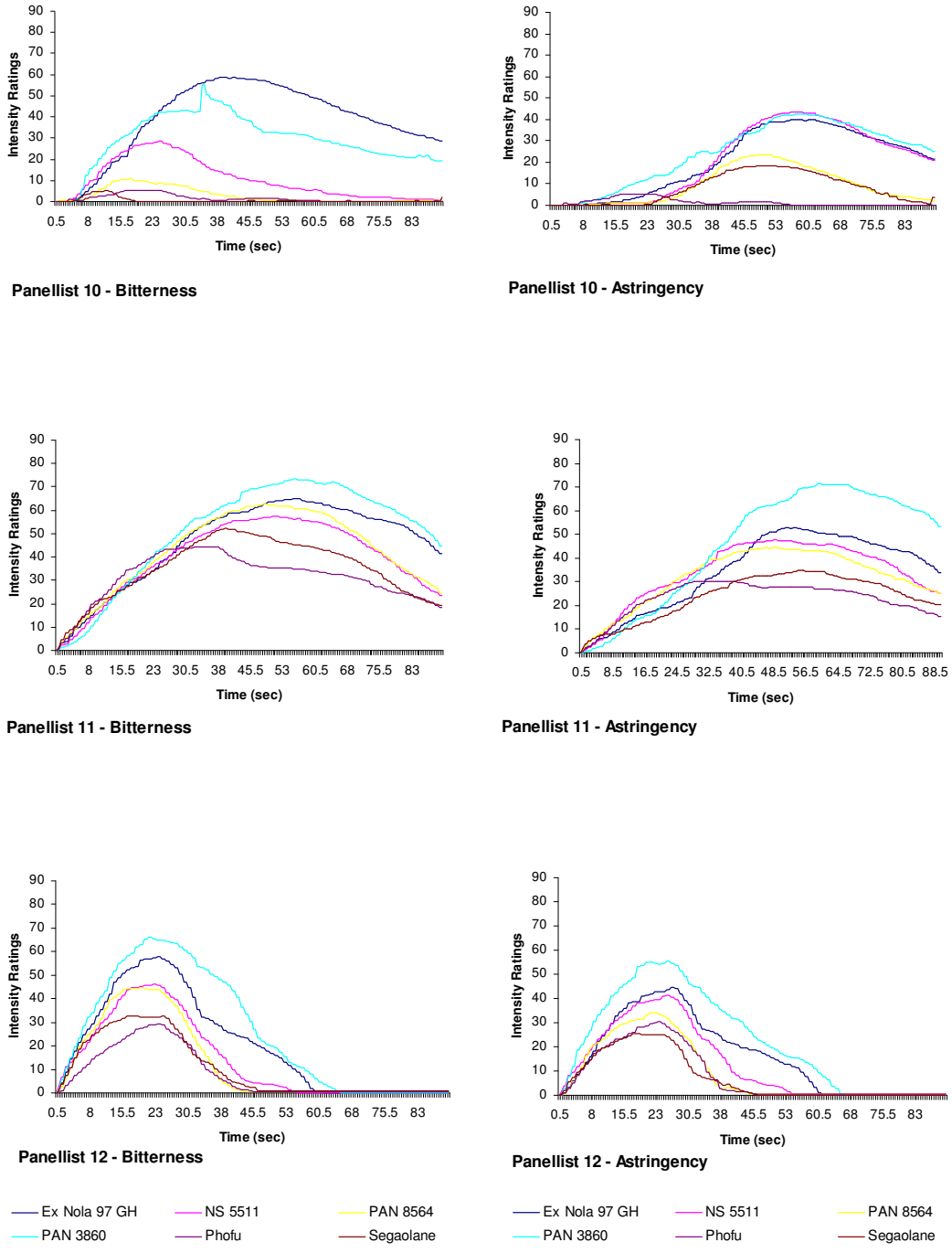

Figure 2.7. Time intensity curves for bitterness and astringency of different sorghum cultivars for panellists 10 – 12 (continued).

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.

	Bitterness				Astringency			
Session	T_{max} (s)	I_{max}	D_{tot} (s)	AUC	T_{max} (s)	I_{max}	D_{tot} (s)	AUC
1	22.0[a]	37.5[a]	66.8[a]	1555.9[a]	27.0[a]	33.7[a]	70.0[a]	1428.9[a]
2	23.0[a]	41.1[a]	65.9[a]	1738.8[a]	28.9[a]	42.3[b]	69.8[a]	1875.4[b]

LS Means of four replicate experiments averaged across two sessions.

LS Means in columns with different letter notations [(a-b)] are significantly different at $p \leq 0.001$.

T_{max} – time to maximum intensity, I_{max} – maximum intensity, D_{tot} – 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_{max}, I_{max} and AUC (Table 2.7). For astringency, the only significant ($p < 0.05$) replicate effect was for T_{max} (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.

	Bitterness				Astringency			
Replicates	T_{max} (s)	I_{max}	D_{tot} (s)	AUC	T_{max} (s)	I_{max}	D_{tot} (s)	AUC
1	24.8^{b}	37.5^{ab}	68.0^{a}	1592.3^{ab}	30.6^{b}	35.9^{a}	72.1^{a}	1576.8^{a}
2	22.8^{ab}	36.2^{a}	67.2^{a}	1523.6^{a}	27.7^{ab}	36.8^{a}	68.1^{a}	1625.5^{a}
3	21.1^{a}	40.6^{ab}	64.0^{a}	1562.0^{ab}	27.6^{ab}	38.0^{a}	67.9^{a}	1580.2^{a}
4	21.3^{ab}	43.0^{b}	66.1^{a}	1911.5^{b}	25.9^{a}	41.2^{a}	71.5^{a}	1826.2^{a}

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

Least Square Means in columns with different letter notations $^{(a-b)}$ are significantly different at $p \leq 0.01$.

T_{max} – time to maximum intensity, I_{max} – maximum intensity, D_{tot} – 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_{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_{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.

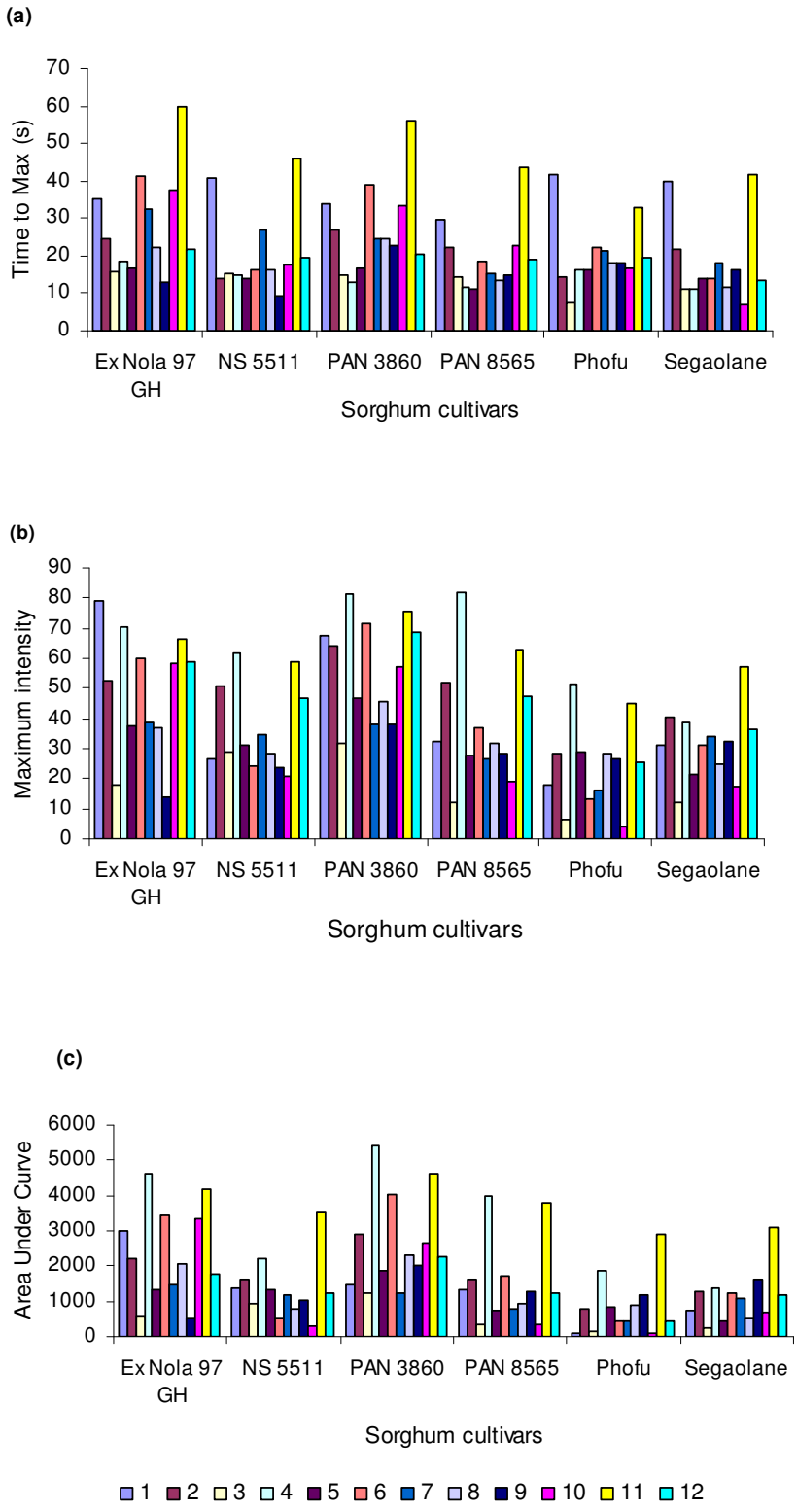

Figure 2.8. Least square means of panellist x cultivar interaction effect on (a) T_{max}, (b) I_{max} and (c) AUC for bitterness. Numbers 1 to 12 refer to individual panellists.

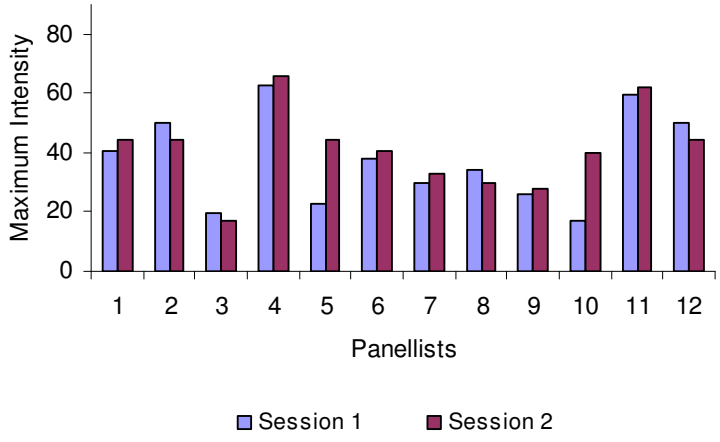

Figure 2.9. Least square means of panellist x session interaction effect for I_{max} for bitterness.

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.

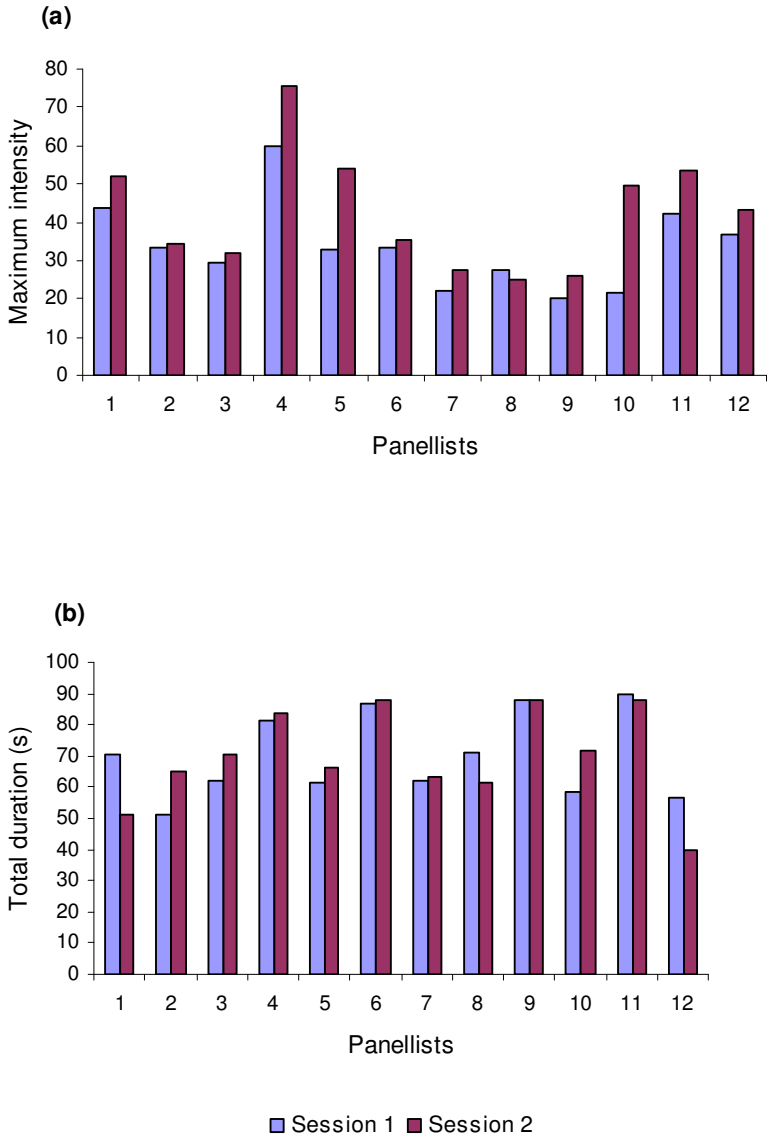

Figure 2.10. Least square means of panellist x session interaction effects for (a) I_{max} astringency and (b) D_{tot}.

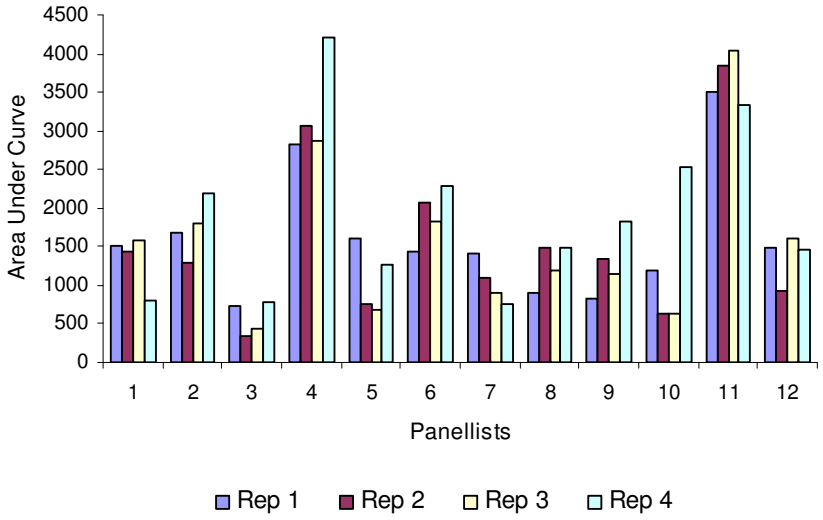

Figure 2.11. Least square means of panellist x replicate interaction effects for AUC for bitterness.

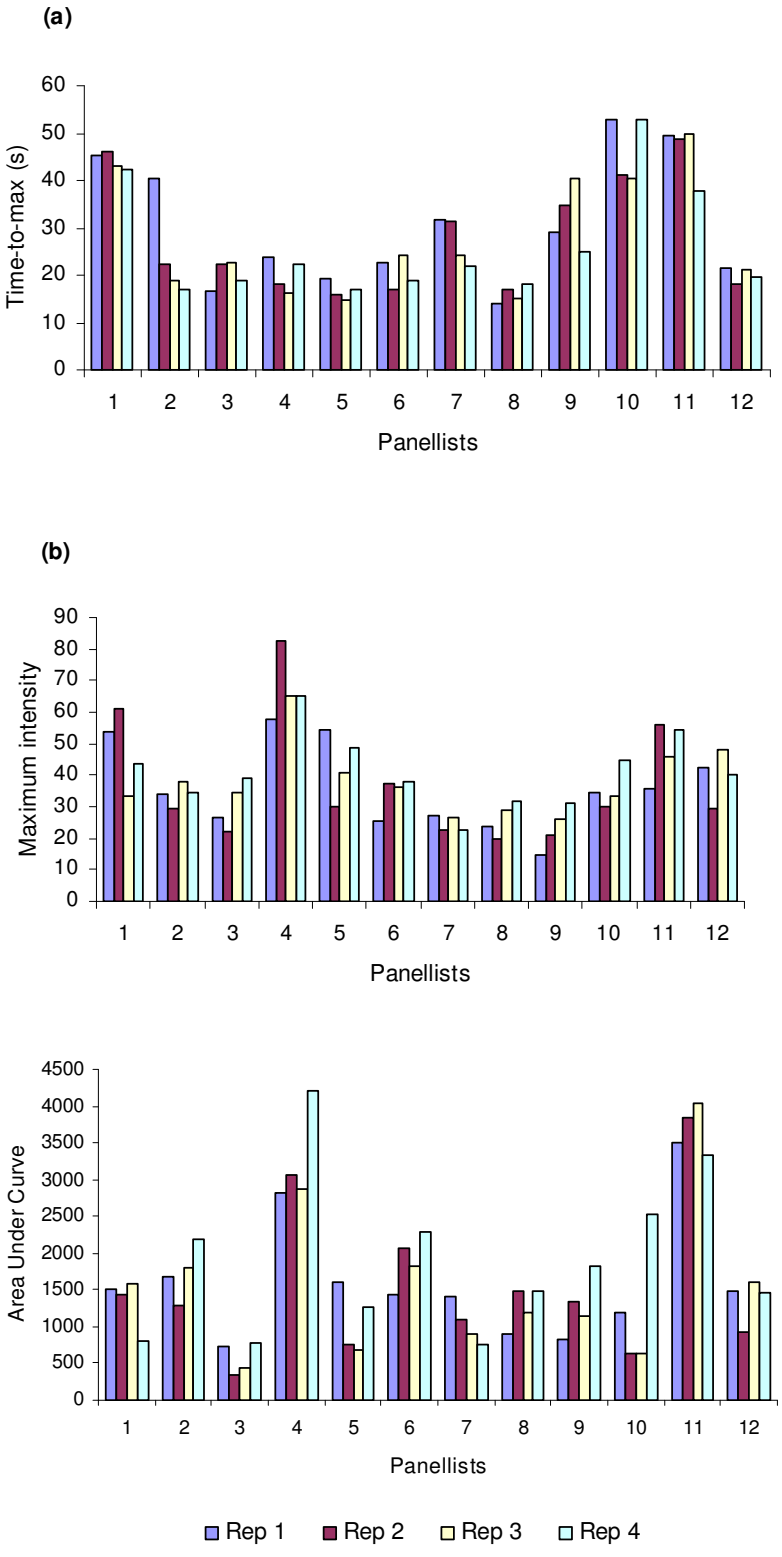

Figure 2.12. Least square means of panellist x replicate interaction effects for (a) T_{max}, (b) I_{max} and (c) AUC for astringency.

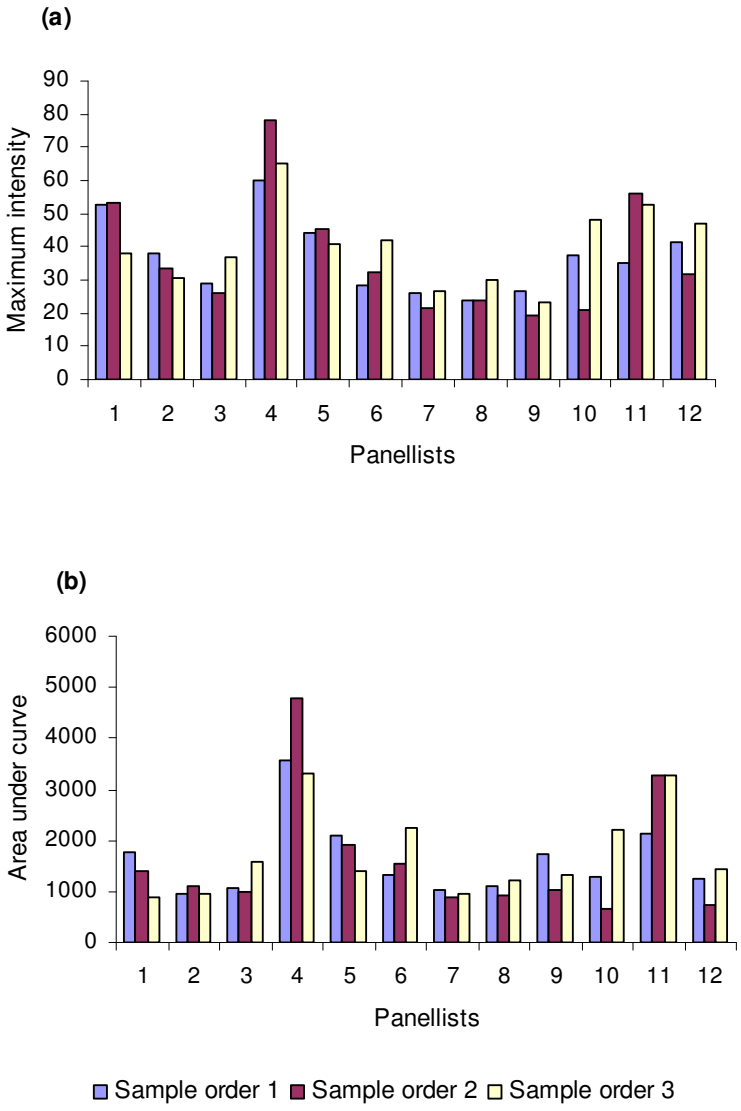

Figure 2.13. Least square means of panellist x sample order interaction effects on (a) I_{max} and (b) AUC for astringency.

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 srghums, 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 (±95% confidence interval [CI]) were determined as follows: individuals who rated PROP ≤ 7.2 were classified as non tasters, those who rated PROP ≥ 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 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)

	Men	**Women**	**Total**
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.

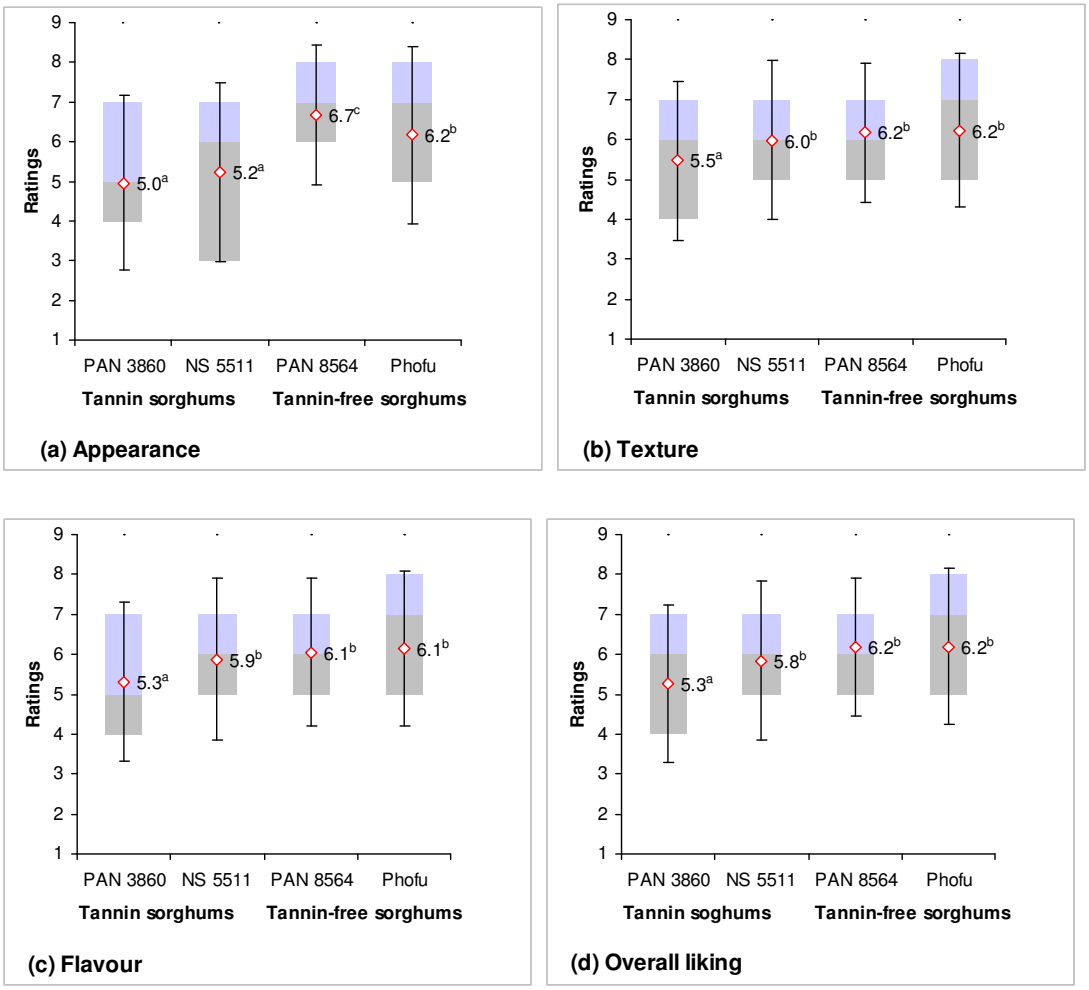


Figure 2.14 Sorghum cultivar effect on consumer ratings of appearance (a), texture (b), flavour (c) and overall liking (d) of the sorghum rice. Data collapsed across all taster groups. Means and SD; means in a graph with different letter notations $^{(a-c)}$ are significantly different at $p \leq 0.05$. 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'

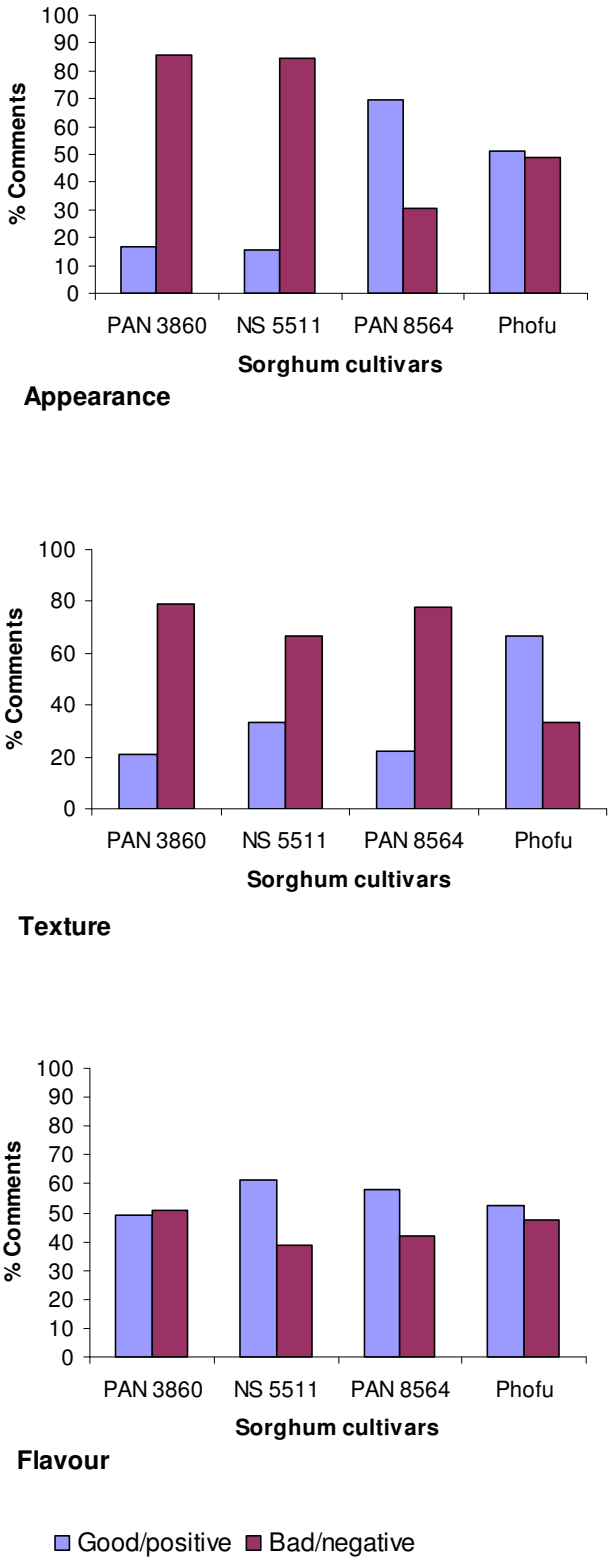
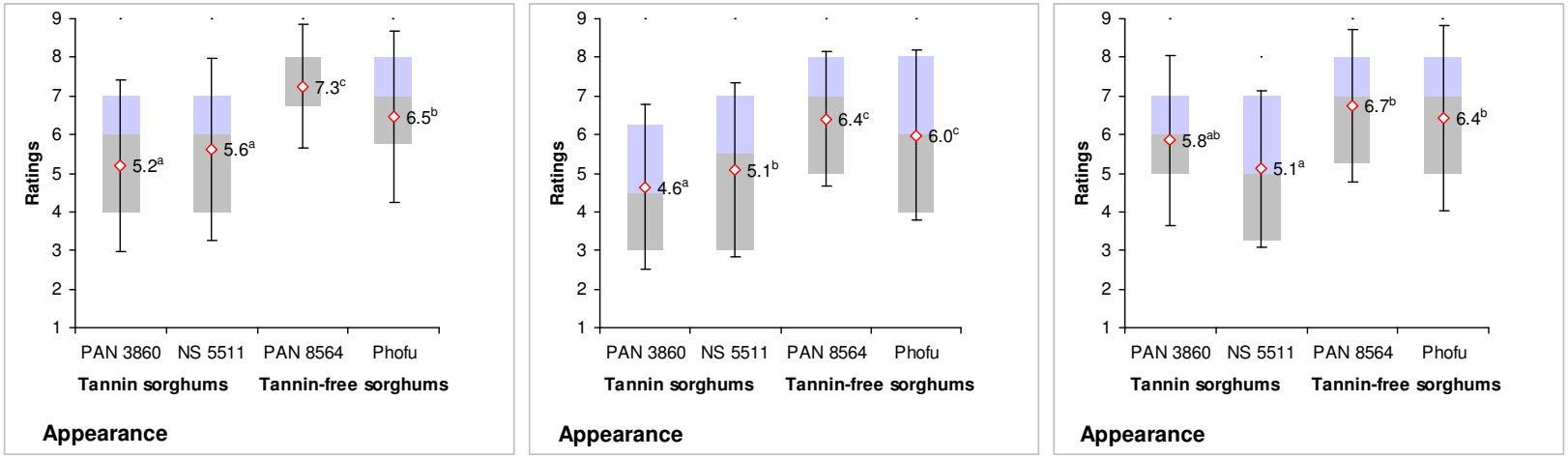


Figure 2.15. The relative percentage of good/positive and bad/negative comments made by consumers on the appearance, texture and flavour of the sorghum rice.

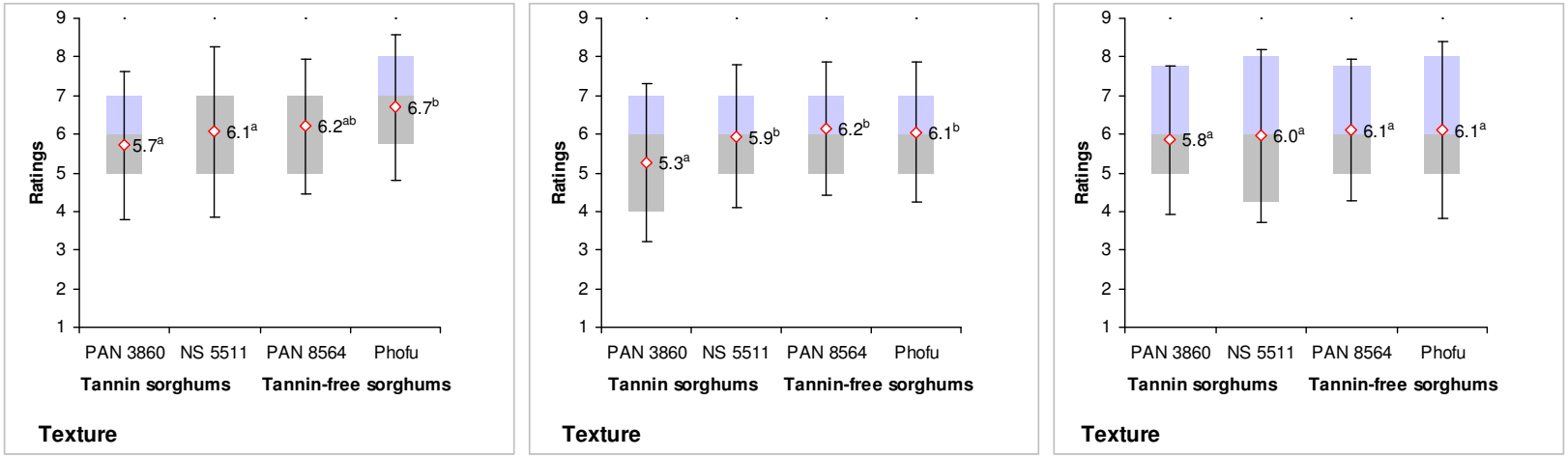
The data were analyzed separately by taster group. All the taster groups generally rated the appearance of the rice from the tannin sorghums lower than the rice from the tannin-free sorghums (Fig. 2.16). With the exception of appearance, the PROP non tasters' acceptance ratings for the sensory attributes: 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).



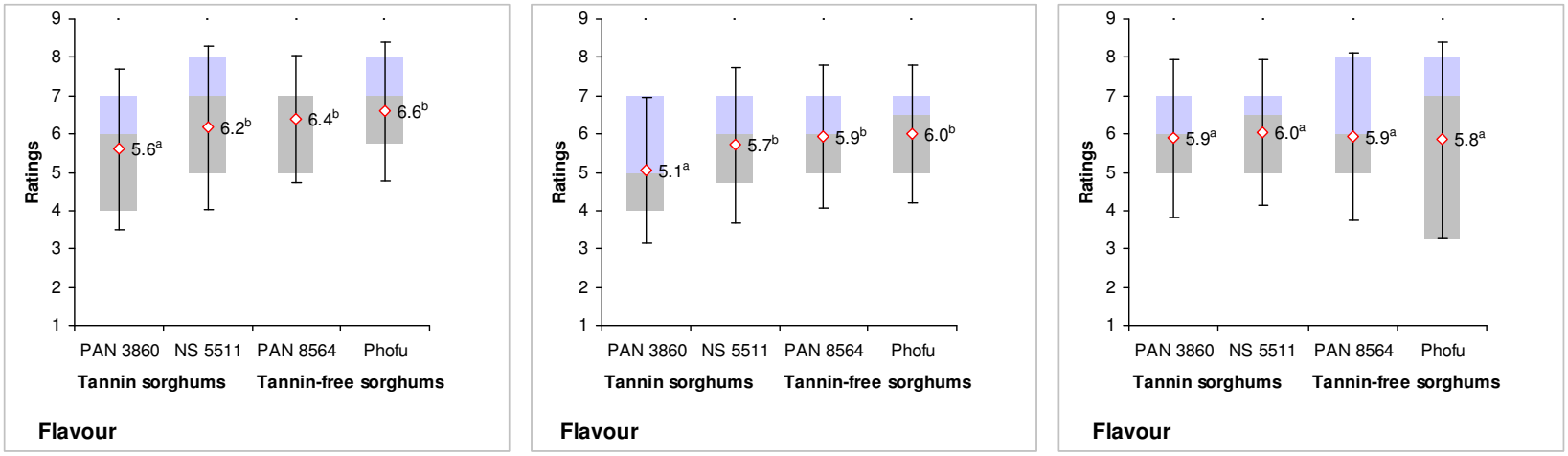
(a) Super tasters (b) Medium tasters (c) Non tasters

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 $^{(a-c)}$ 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.



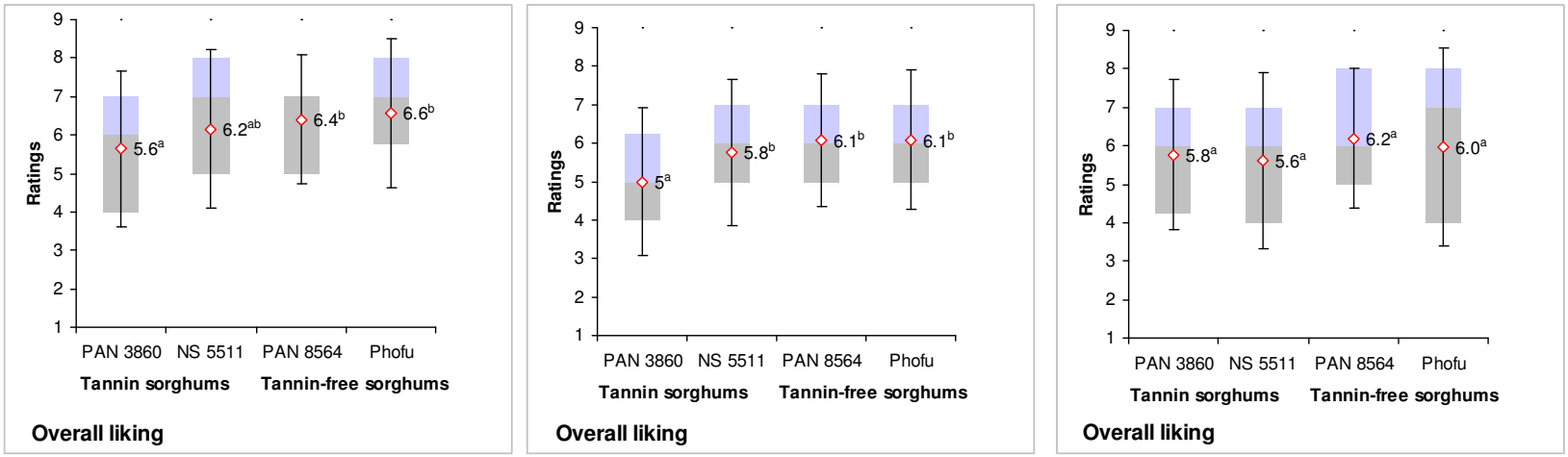
(a) Super tasters (b) Medium tasters (c) Non tasters

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 $^{(a-b)}$ 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 $^{(a-b)}$ 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 $^{(a-b)}$ 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 (≥ 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[1, 2]

	Appearance	Texture	Flavour	Overall liking
Super tasters	6.1^{b} (0.2)	6.1^{a} (0.1)	6.2^{b} (0.1)	6.2^{b} (0.1)
Medium tasters	5.5^{a} (0.1)	5.9^{a} (0.1)	5.7^{a} (0.1)	5.7^{a} (0.1)
Non tasters	6.0^{b} (0.2)	6.0^{a} (0.2)	5.0^{ab} (0.2)	5.9^{ab} (0.2)

[1]Data collapsed across all sorghums

[2]Means and SEM; means in columns with different letter notations $^{(a-b)}$ 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.

2.3.6. References

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3. GENERAL DISCUSSION

This chapter will first critically evaluate the methods used in this study. The next section will examine the main findings of the study, which is that although products from tannin sorghums were found to be bitter and astringent, not all tannin sorghum products were objectionable to the consumers. The last section will propose a theory to account for this finding, that is, there exists a condensed tannin threshold level at which the sensory attributes (bitterness and astringency) are not 'strongly' perceived.

3.1. Methodologies

Different methods were used to determine the presence and content of phenolic compounds in tannin and tannin-free sorghums, as well as to measure their bitterness and astringency. These methods are discussed, as well as what could have been done differently.

Three sorghums containing varying amounts of condensed tannins and three tannin-free sorghums with varying amounts of total phenolic content were used in the study. All the sorghums containing condensed tannins had a red pericarp. Therefore a tannin-free sorghum (PAN 8564) with a red pericarp was included as a reference standard to eliminate any colour bias, especially since the other two tannin-free sorghums had a white pericarp. Presence of condensed tannins in the grain was initially determined through the bleach test as described by Taylor (2001). However, since this method is not always reliable in that sorghum cultivars can give 'false positives' depending on maturation conditions (weathering, insect bites, etc), glume colour, and degree of pericarp pigmentation (Dykes, Awika, McDonough, Rooney and Waniska, 2002), the presence of tannins was also determined by chemical analysis using the modified Vanillin-HCl method as described by Price, Van Scoyoc and Butler (1978) (Chapter 2.1). The Vanillin-HCl method is one of the most commonly used methods to determine presence of condensed tannin content in sorghum. This method has been used by Bullard *et al.* (1980); Dykes *et al.* (2005); and Awika *et al.* (2005). This method is based on the ability of flavanols to react with vanillin in the presence of mineral acid to produce a red pigment (Awika *et al.*, 2005). The modified Vanillin-HCl method is considered more appropriate for tannin estimation than redox-based colorimetric assays like the Folin-Denis and the Prussian blue methods,

because the latter methods are less specific (Price *et al.*, 1978). Price and Butler (1977) cited the main disadvantage of the Prussian blue test or any other redox-method as the lack of distinction between tannins and other phenols. Another commonly used technique of tannin determination, developed by Hagerman and Butler (1978), is based on the ability of tannins to bind proteins. However, like the colorimetric methods it is said to also suffer from lack of specificity (Awika and Rooney, 2004). Other factors reported to influence the accuracy of these tannin assays include material particle size, type of solvent and the standard used (Awika and Rooney, 2004). Notwithstanding these methodological constraints, the modified Vanillin-HCl method of Price *et al.* (1978) was deemed adequate for the purposes of this study. The modified Vanillin-HCl method of Price *et al.* (1978) is generally considered more appropriate for tannin determination than colorimetric assays enumerated above (Awika and Rooney, 2004). According to these authors, high performance liquid chromatography (HPLC) based assays are promising for more accuracy.

An HPLC-based assay was used in this study to identify and quantify condensed tannins present in the tannin sorghums (PAN 3860, Ex Nola 97 GH and NS 5511) in order to correlate the data to the sensory results. Products of a tannin-free sorghum, PAN 8564 was consistently perceived similar to products of NS 5511 (tannin sorghum) for all the sensory attributes and were similar to Ex Nola 97 GH in astringency. Therefore, PAN 8564 was also analysed using HPLC to confirm the results of the modified Vanillin-HCl method of Price *et al.* (1978) which had not detected tannins in this sorghum. Initial sample preparation for analysis by HPLC involved phenolic compound extraction by boiling the bran in water, freeze drying the extracts, and dissolving the dried extract in 70% aqueous acetone before HPLC analysis. This was because only the water soluble phenolic compounds in the bran infusions elicited the bitterness and astringency in the infusions. However, this method did not work effectively because the freeze-dried residue did not dissolve in 70% aqueous acetone for injection into the HPLC for analysis. Therefore, a second extraction method involved extracting phenolics in milled whole grain sorghum samples as described by Awika, Dykes, Rooney and Prior (2003a). Samples were extracted, freeze-dried, and dissolved in 70% aqueous acetone before being injected in the HPLC for analysis. However, the extracted residue did not contain adequate material to enable identification and quantification of the condensed tannins in the different sorghum samples (Fig. 2.6). This was probably due to losses of some material in

the process of freeze-drying the extracts for shipment to USA for analysis. Nonetheless the HPLC chromatogram data corresponded to the Vanillin-HCl results in that PAN 3860 had the highest content followed by Ex Nola 97 GH and NS 5511, respectively. HPLC also confirmed that PAN 8564 did not contain tannins. Obviously, the procedure of freeze-drying the extracted sample was not appropriate because sample losses were incurred at this stage. Ideally, the sample should be injected soon after extraction to prevent sample losses. It would also have been useful to use HPLC to identify and quantify phenolic acids, anthocyanins as well as other flavonoids present in these sorghums to better understand the phenolic compounds eliciting the bitterness and astringency perceived in these sorghums.

Sorghum samples were presented to the panellists for analysis in the form of bran infusions and cooked whole grain rice. Although sorghum bran is not generally consumed in the form of infusions, some communities in Kenya do similar. From a focus-group study carried out in Kenya, it was recently learnt that some communities prepare 'tea' (infusions) for consumption from the glumes of red sorghums (Ms. N. Vilakati, University of Pretoria MSc Nutrition student; personal communication). Bran was used to make infusions because in sorghum, phenolic compounds are concentrated in the pericarp of the sorghum grain (Awika *et al.*, 2005). Preliminary trials in this study involved presenting the bran to panellists 'as is' (dry) for tasting. However, this did not work effectively because it was problematic to effectively clean out the bran residue from the mouth after tasting the different samples. Thus the bran was boiled in water to extract phenolic compounds in the sorghum. Infusions were easier to clean out by drinking water. Water was used to prepare the infusions because normally sorghum is cooked in water for human consumption. Thus, in sorghum food systems only those phenolic compounds soluble in water are tasted. Whole grain sorghum was also cooked and served to the panellists for analysis. As stated previously, the sorghum grain was not decorticated in order not to lose any phenolic compounds in the pericarp (bran). Sorghum rice can be prepared from decorticated grain or whole grain. For example, in Botswana, sorghum rice (*lehata*) is prepared from decorticated grain (Subramanian *et al.*, 1982) and it is eaten with milk or it is cooked whole (not decorticated) when prepared with beans or cowpeas (personal observation). Whole grain sorghum was used to prepare the sorghum rice because decorticating the grain would have resulted with major losses of phenolic compounds in the grain since as stated they are concentrated in the bran (Awika *et al.*, 2005). The

advantage of using whole grain sorghum rice as opposed to milled whole grain sorghum is that because the grain was cooked 'intact' the tannins and proteins were not 'free' to easily move within the grain to form complexes as would have been the case in porridges prepared from sorghum flour (Daiber, 1975). According to this author, the concept of rigid compartmentalisation of tissues and substances in the seed proposed by Loomis and Battaile (1966) was demonstrated in the non-inhibition of enzymes by polyphenols (tannins) of sorghum grain during malting. The complete separation of the tannin containing tissue from the embryo and endosperm ensured uninhibited metabolic activity of the enzymes within the germinating grain. However, when the malt was milled and mashed the previously separated compounds (tannins and enzymes) were mixed and the tannins reacted with the enzymes to form insoluble complexes. The tannin-protein complexes are not soluble (Emmambux and Taylor, 2003) and may not contribute to the bitterness and astringency of the sorghum. Thus it was important to minimize tannins binding to proteins as much as possible.

To obtain bran for use in sensory analysis, it had to be isolated from the grain. A Prairie Research Laboratory (PRL; Rural Industries Innovation Centre, Kanye, Botswana) type dehuller (decorticator) was used to isolate the bran since it progressively abrades off (Kebakile, Rooney and Taylor, 2007) the pericarp while not breaking the kernels to a substantial extent, thus endosperm 'contamination' (Awika *et al.*, 2005) is minimized. However, the total phenol content of Ex Nola 97 GH bran isolated using the PRL dehuller was consistently less than expected (Chapter 2.1, Table 2.4). The fact that the total phenol content (whole grain) of this sorghum (Ex Nola 97 GH) was similar to that of PAN 3860, the bran of these sorghums were expected to contain similar amounts; yet the total phenol content of PAN 3860 bran was significantly higher than that of Ex Nola 97 GH. This was probably due to the fact that Ex Nola 97GH had a softer endosperm (visual hardness score; Chapter 2.1, Table 2.3) that was more friable and thus the endosperm 'contaminated' the bran as described by Awika *et al.* (2005).

Prior to bran isolation, the sorghum grain was washed several times with tap water to remove dust, dirt and debris and dried before milling. Washing the grain was necessary because some of the grain was dusty and dirty. All sorghum grain samples were washed, including grain that was relatively clean, to ensure the samples received the same treatment. This was to reduce microbial contamination and growth during storage. The

effect of washing the sorghum grain on the total phenol content was determined using the Folin-Ciocalteu method of Waterman and Mole (1994). The Folin-Ciocalteu is the most commonly used method to determine total phenol content in sorghum (Kaluza *et al.*, 1980; Awika *et al.*, 2004a; Dykes *et al.*, 2005; Awika *et al.,* 2005). This method measures the redox potential of phenolic compounds (Awika *et al.,* 2005). However, Zielinski and Kozlowska (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 extraction solvent used was 75% aqueous acetone. Kaluza *et al.* (1980) found 75% aqueous acetone the best extraction solvent for phenolics compared to other solvents. Washing the grain reduced the total phenol content of the tannin sorghums significantly ($p \leq 0.05$) but the reduction was slight (2.2%, 9.4% and 13% for Ex Nola 97 GH, PAN 3860 and NS 5511, respectively), and deemed not detrimental to the sensory results expected while washing the tannin-free sorghums did not significantly reduce their total phenol content.

Phenolic compounds in the bran were extracted with deionised water and served to panellists as infusions. To determine the most effective method of extraction, trials involved steeping and boiling the bran. Total phenol content of infusions prepared by the two methods at different times was determined using the Folin-Ciocalteu method of Waterman and Mole (1994). Boiling the bran for 20 min was more effective in extracting total phenols than steeping or boiling for shorter periods but was not significantly different from boiling for 25 min.

From the results of the total phenol content in the different sorghums (Chapter 2.1; Table 2.4) this method of extraction (boiling bran in water) seemed relatively more effective in extracting phenolics in tannin-free sorghums than in tannin sorghums. However, the lower extractability percentages noted for the tannin sorghums might be in part attributable to the tannins forming complexes with the protein in the germ during boiling. Some sorghum protein is located in the germ of the sorghum caryopsis (Taylor and Schussler, 1986). Tannin-protein complexes are insoluble (Daiber, 1975; Emmambux and Taylor, 2003; Naczk and Shahidi, 2004) and difficult to extract (Awika *et al.*, 2003a).

Furthermore, because the tannin-protein complexes are insoluble (Daiber, 1975; Emmambux and Taylor, 2003; Naczk and Shahidi, 2004) it is possible they did not

contribute to the bitterness and astringency of the infusions from the tannin sorghums. Freshly prepared bran infusions from all the sorghums (tannin and tannin-free sorghums) became cloudy, formed haze, after being left to stand for about an hour. However, after several hours, the infusions made from the tannin sorghums became clear, while those prepared from the tannin-free sorghums remained cloudy. According to Siebert, Troukanova and Lynn (1996) proteins and polyphenols bind to form soluble colloidal size complexes, and when these complexes grow, they sediment out of solutions. The tannin-protein complex precipitation caused a significant reduction in the amount of tannins in the infusions to bind to salivary proteins and elicit the astringent sensation. The total phenol content of the water extracts were 25%, 26.8% and 35.6% less than those of the acetone extracts for PAN 3860, Ex Nola 97 GH and NS 5511, respectively (Chapter 2.1, Table 2.4). It is possible that astringency was more affected than bitterness because the bitterness of the tannin sorghums was consistently rated slightly higher than astringency in these sorghums, whereas in the case of the tannin-free sorghums this trend was not observed (Tables 2.5, 2.9 and 2.11). The tannins that bind and precipitate proteins were not present in the tannin-free sorghums, thus the bitterness and astringency in these sorghums was elicited by smaller non-tannin polyphenols. Notwithstanding these methodological constraints, bran infusions were still deemed the best method to use in assessing the sensory properties of phenolics in sorghum. This method effectively identified the sensory properties (Chapter 2.1) as well as the differences between the tannin and tannin-free sorghums. However, this method could have been improved by serving the infusions directly after preparation, to minimize the formation of the protein-tannin complexes that resulted in the apparent 'reduction' of the tannins available for tasting.

The sensory methods used in the study included quantitative descriptive sensory analysis to profile the sensory attributes of products (infusions and sorghum rice) from tannin-free and tannin sorghums. The dual attribute time intensity (DATI) sensory method was used to determine the time-course of bitterness and astringency of sorghums varying in condensed tannin content. A consumer test was carried out to determine the acceptability of whole grain rice from these sorghums.

Despite the 10 h training in the use of the time intensity sensory method, there were panellist variations (Chapter 2.2). According to Valentová *et al.* (2002) time intensity

studies are subject to different biases one of which is panellist variation. Despite extensive training of judges to calibrate their use of descriptive terms and rating scales, individual physiological and psychological differences affect perception of sensory properties (Boulton and Noble, 1994). The quantitative descriptive sensory analysis data was also mean values of varying responses from the panellists (Chapter 2.1). In this research, in addition to the physiological and psychological differences affecting perception of sensory properties, the additional challenge to the panellists was the ability to distinguish differences between bitterness and astringency by measuring them simultaneously using the DATI method. Nonetheless in this study the panellist variations did not compromise the overall time intensity sensory data in that the ratings for bitterness and astringency of the different cultivars using this method followed the same trends found in the quantitative descriptive sensory analysis data (Chapter 2.1). Furthermore the panellists could distinguish bitterness from astringency because the time intensity results revealed that bitterness develops faster than astringency and astringency persists longer than bitterness. These findings were made possible by measuring the attributes simultaneously.

In this study salivary flow rates of panellists were not determined. However, differences in salivary flow rates might have explained some of the panellist variations noted. Panellists' salivary flow rates have also been implicated in panellist variations in astringency assessments using the time intensity sensory method (Fischer *et al.*, 1994; Kallithraka *et al.,* 2001; François *et al.*, 2006) because individuals differ in their salivary flow rates and in the degree of salivary response to oral stimuli (Boulton and Noble, 1994). Subjects with low saliva flow rates have been reported to take longer to reach maximum intensity (T_{max}) and had a longer duration (D_{tot}) of bitterness and astringency than subjects with high flow rates (Fischer *et al.*, 1994). Low flow subjects also perceived the intensity (I_{max}) of bitterness and astringency higher than subjects with high flow rates.

Sample presentation to the panellists was in clear colourless glass tubes and the samples were served under white light. For the descriptive sensory analysis it was important to get a full description of all the sensory properties of the sorghum products as well as to quantify them. However, it might have been useful to use stained glass tubes and/or red light to camouflage the colour of the samples to minimize colour bias.

To assess the bitterness and astringency of bran infusions, a 10-point rating scale was used for the descriptive sensory method. The time intensity linear scale also had 10 markings and was anchored from 0 (barely detectable) at the start of the line to 100 (strongest imaginable) at the extreme end of the line. Although the observed trends were similar, in that the sorghums with the highest total phenol and condensed tannin content were most bitter and astringent, while those with the lowest phenol content levels were least bitter and astringent, the descriptive ratings were consistently and slightly higher than those from the DATI sensory data (Chapters 2.1 and 2.2). A 9-point rating scale was used to assess the acceptability of the sorghum samples by consumers and the descriptive sensory panel also used a 9-point rating scale to assess the intensity of the sensory attributes. In retrospect rather than using a 10-point scale for the infusions and a 9-point scale for the sorghum rice, it would have been more ideal to use the same rating scale (9-point rating scale) for all the sensory tests to facilitate comparison of results. Nonetheless, using the different rating scales (9-point and 10-point) did not detrimentally affect the findings of this study in that the trends were the same and it was clear from the results that not all tannin sorghums have objectionable sensory attributes and are not aversive to the consumers.

After recruitment of consumers to participate in a sorghum taste session, selection criteria of the consumer panel (n=194) was on a first come first serve basis because the objective was random selection. Panellists were not screened on the basis of PROP taster status prior to selection to ensure a representative distribution of PROP taster groups (super, medium and non) in the population. Ideally, the consumer panel selection criteria should have been on the basis of regular consumption of sorghum rice. However, panellists were simply asked whether they are consumers of sorghum and willing to taste sorghum products. Although sorghum rice is commonly consumed in Botswana this is not the case in South Africa (personal observation). Most of those who were familiar with sorghum consumed it as porridge. Notwithstanding the fact that most of the consumers were encountering sorghum rice for the first time, the results of the panel effectively demonstrated that the PROP tasters and super tasters could distinguish differences among the sorghum containing varying amounts of total phenols and condensed tannins while the non tasters could not.

In this study, beause a large number of (n = 200) consumers was used, it was more practical to use the one-solution PROP test developed by Tepper, Christensen and Cao (2001) to classify subjects by taste sensitivity to PROP than the three solution test because it uses fewer solutions. Different psychophysical procedures are available to classify individuals by PROP taster status namely, threshold tests (Bartoshuk *et al.*, 1994; Tepper *et al.*, 2001) and a paper screening test (Zhao, Kirkmeyer and Tepper, 2003). However, the threshold tests are laborious and require individuals to taste a considerable number of NaCl and PROP samples. This is not practical when dealing with consumer panels involving large numbers of people (Tepper *et al.*, 2001). These authors compared two methods: a three solution test and a one solution test to classify 89 adults for genetic sensitivity to PROP. The authors concluded that both methods can be used reliably to classify subjects by taster sensitivity to PROP.

3.2. Effects of total phenol and condensed tannin content on the sensory properties, bitterness and astringency, and acceptability of products from different sorghums

This section will discuss the bitterness and astringency of products from sorghums varying in total phenol and condensed tannin content, the possible mechanisms that elicited these sensations and how they influence consumer acceptance.

The products from all the sorghum cultivars were perceived to different degrees as both bitter and astringent (Chapters 2.1 and 2.2). The products from the sorghum with the highest total phenol and condensed tannin content (PAN 3860) were most bitter and most astringent, whilst the least bitter and least astringent products were of a tannin-free sorghum (Phofu) with the lowest total phenol content. These findings agree with the literature describing phenolic compounds, ranging from small to highly polymerized compounds, as both bitter and astringent. Phenolic fractions in wine (Arnold *et al.,* 1980; Kallithraka *et al.*, 1997b) and cider (Lea and Timberlake, 1974; Lea and Arnold, 1978) were evaluated for bitterness and astringency, and the isolated trimers, dimers and monomers contributed only slightly to these sensations while the highly polymerized material was primarily responsible for both bitterness and astringency. In addition to the total phenol and condensed tannin content, the fact that the tannin sorghums were perceived as more bitter and more astringent than the tannin-free sorghums may also be

due to the condensed tannins in these sorghums having lower detection thresholds than the phenolics in the tannin-free sorghums.

Generally, the higher the total phenol and condensed tannin content the more bitter and more astringent the sorghum products (Chapters 2.1 and 2.2) and the longer and more persistent the bitterness and astringency sensations (Chapter 2.2). For sorghum rice, Ex Nola 97 GH and PAN 3860 were equally bitter and astringent (Table 2.6, Chapter 2.1). However, the bitterness of the infusion from Ex Nola 97 GH, seemed more predominant than its astringency (Chapter 2.1; Chapter 2.2). The infusion from Ex Nola 97 GH was more bitter than that from PAN 8564 (tannin free), but the astringencies of these sorghums were not significantly different. As stated, the total phenol content of whole grain Ex Nola 97 GH was similar to that of PAN 3860 (Chapter 2.1), whereas the total phenol content of the bran and infusion of Ex Nola 97 GH were consistently less than expected, in that they were below that of PAN 3860 (Table 2.4, Chapter 2.1). Therefore the apparent 'reduction' in astringency of Ex Nola 97 GH compared to its bitterness was probably due to endosperm 'contamination' of its bran because of the softness of its endosperm. The endosperm contains starch and protein and these macromolecules could have bound some of the condensed tannins in the bran, thus reducing its potential to elicit astringency.

According to Delcour *et al.* (1984) the flavour detection threshold of phenolics depend on their degree of polymerization. A much higher flavour detection threshold level was reported for (+)-catechin (46.1 mg/l) compared to the highly polymerized mixture of trimeric and tetrameric procyanidins (4.1 mg/l) i.e. the concentration required for the detection of (+)-catechin was more than ten times (> 10) the amount required to detect the highly polymerized mixture of the trimeric and tetrameric polyphenols. Therefore it is possible that lower concentrations of the highly polymerized tannins in the tannin sorghums (PAN 3860, Ex Nola 97 GH) were required to elicit bitterness and astringency of similar strength to higher concentrations of the non-tannin phenolic compounds in the tannin-free sorghums. The mechanisms that elicited bitterness and astringency in the different sorghums are proposed below.

Bitterness and astringency are elicited by different mechanisms. As stated, bitterness is a taste mediated by sensory receptors (Kinnamon, 1996), while astringency is a tactile sensation signalled by trigeminal nerves (Vidal *et al.,* 2003). It is not clear what

transduction mechanism elicited the bitterness of the phenolic compounds in the different sorghums. Different mechanisms are utilized for the transduction of different taste stimuli. Salts, acids and some bitter compounds depolarize taste receptor cells (TRCs) by directly interacting with apical ion channels. Whereas amino acids, sugars and most bitter compounds activate G-protein cell receptors (GPCRs) (Kinnamon, 1996; Kim *et al.*, 2004). Thus, the bitter stimuli interact with apical ion channels or specific membrane receptors for transduction (Kinnamon, 1996). However, it is not clear whether the bitter taste of flavanols is a result of taste receptor or surface membrane interactions (Peleg and Noble, 1995).

As explained, astringency is a tactile sensation usually associated with the loss of mouth lubrication caused by the precipitation of salivary proteins by an astringent compound (Gawel *et al.*, 2001; Siebert and Chassy, 2003). An astringent is chemically defined as having the ability to precipitate proteins. However, many other compounds elicit an astringent sensation even though they do not precipitate protein (Peleg *et al.*, 1999). It is noteworthy that all the sorghums, including those without tannins were perceived as astringent. According to McManus *et al.* (1981) there are two classes of polyphenols (Fig 3.1) that have the unique property of precipitating macromolecules such as mucopolysaccharides and protein out of solution. These are (1) proanthocyanidins (condensed tannins) and esters of gallic acid (hydrolysable tannins; 2, 3 and 4). According to these authors, *ortho*-dihydroxyphenolic groups in natural polyphenols are the primary points for the association with protein; and the complexation occurring primarily *via* a bidentate hydrogen bond with the keto-imide groups on the protein (Fig. 3.1; (5)).

Sorghums contain condensed tannins (proanthocyanidins) not hydrolysable tannins (Awika and Rooney, 2004). The binding capacity of tannins for salivary proteins depend on their molecular size, number of binding sites in the molecule to bind protein, pH value and the relative concentration of both tannins and proteins (Hagerman and Butler, 1981; Siebert *et al.*, 1996; De Freitas and Mateus, 2001). Protein-tannin-complexes result in the precipitation and/or aggregation of salivary proteins causing them to lose their lubricating properties (Horne, Hayes and Lawless, 2002).

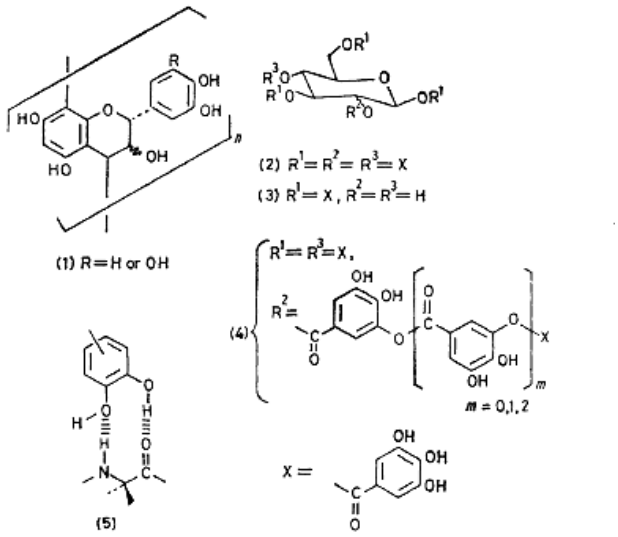

Figure 3.1. Proanthocyanidins (1) and the esters of gallic acid (2, 3 and 4); protein-polyphenol complexation occurs primarily via a bidentate hydrogen bond formation with the keto-imide groups on the protein (5) (McManus *et al.,* 1981).

According to Simon, Barathieu, Laguerre, Schmitter, Fouquet, Pianet and Dufourc (2003) all tannins bind the hydrophilic side of the saliva peptide, thus suggesting that the major interaction forces are governed by hydrogen bonds. Salivary proteins involved in polyphenol complexation are primarily proline-rich proteins, which make up about 70% of the whole human salivary protein content (De Freitas and Mateus, 2001). However, salivary histatins (histidine-rich proteins) 1, 3 and 5 (Naurato, Wong, Lu, Wroblewski and Bennick, 1999) and salivary α-amylase (Mateus, Pinto, Ruao and De Freitas, 2004) have also been reported to form complexes with polyphenols.

As stated, PAN 8564, Segaolane and Phofu were also perceived as astringent even though there were no detectable tannins in these sorghums (Table 2.4, Chapter 2.1). Since these sorghums had no detectable tannins, it is possible that phenolic acids and flavonoid monomers bound proteins to elicit astringency. Peleg and Noble (1995) reported bitterness and astringency in gallic acid, salicylic acid, *m*-hydroxyl benzoic acid (3-hydroxy benzoic acid), gentisic acid and protocatechuic acid dissolved in water. Eight phenolic acids including gallic, protocatechuic, p-hydrobenzoic, vanillic, caffeic, p-

coumaric, ferulic and cinnamic acids have been identified in sorghum (Hahn *et al.*, 1983). Monomeric flavan-3-ols (catechin and epicatechin) have also been reported as bitter and astringent in other studies (Kielhorn and Thorngate, 1999; Peleg *et al.*, 1999). In sorghum, catechin is the most commonly reported monomer (Awika and Rooney, 2004). McManus *et al.* (1981) proposed a mechanism for protein-polyphenol complexation mechanism that could explain how phenolic acids and flavanol monomers like catechin elicited astringency in the tannin-free sorghums (PAN 8564, Segaolane and Phofu). These authors proposed two mechanisms for polyphenol-protein complexation (Fig. 3.2). They proposed that at low protein concentrations the polyphenol associates at one of more sites on the protein surface to give a mono-layer which is less hydrophilic than the protein itself (Fig. 3.2 [a]). Protein-polyphenol aggregation and precipitation then takes place. When there is a high concentration of protein it is proposed that a relatively hydrophobic surface layer is formed by cross-linking of different protein molecules by the multi-dentate polyphenols (Fig. 3.2 (b)) followed by the protein-polyphenol complex precipitation. These authors also suggested that simple phenols such as resorcinol, catechol and pyrogallol should also be capable of precipitating protein from solution if they can be maintained in solution at concentrations sufficient enough to push the equilibrium, in favour of the protein-polyphenol complexes and thus form a hydrophobic layer of simple phenols on the protein surface (Fig. 3.2 (c)).

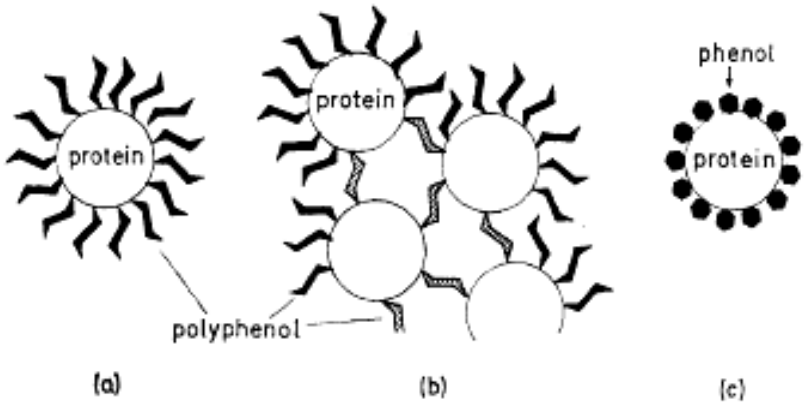

Figure 3.2. Proposed mechanisms for protein precipitation by phenols: (a) polyphenols and low protein concentrations; (b) polyphenols and high protein concentrations; (c) simple phenols (McManus *et al.*, 1981).

These simple phenols (resorcinol, catechol and pyrogallol) have also been perceived as astringent (McManus *et al.*, 1981). The astringency of these small phenols was attributed to the precipitation or strong binding of proteins due to their 1,2-dihydroxy or 1,2,3-trihydroxy groups. The affinity of resorcinol for bovine serum albumin (BSA) was reported to be weaker than that of catechol and pyrogallol, which had two and three *ortho* disposed phenolic groups respectively to more strongly bind the protein (McManus *et al.*, 1981). Peleg *et al.* (1999) postulated that the mechanism proposed by McManus *et al.* (1981) might explain the astringency of monomeric flavanols. Emmambux and Taylor (2003) reported that catechin and the sorghum flavonoids (mostly anthocyanins) from tannin-free sorghums did not form significant haze or bind kafirin. However, at high concentrations there was a slight increase in haze as these phenolic compounds bound BSA to form haze. According to these authors, BSA had more affinity for these phenolic compounds because it has more of an open structure than kafirin. The significant increase in haze formation observed when the concentration of these phenolic compounds was increased is probably due to the mechanism proposed by McManus *et al.* (1981) in Fig. 3.2 [a & c], thus explaining why the tannin-free sorghums were also perceived as astringent. Since salivary proline-rich proteins (PRPs) have an even more open structure than BSA (De Freitas and Mateus, 2001) they have even more affinity for the catechin and sorghum flavonoids.

The infusions of these tannin-free sorghums developed cloudiness (haze) (Table 2.5, Chapter 2.1). Haze formation is attributable to tannin-protein complexation. According to Emmambux and Taylor (2003) condensed tannins form irreversible complexes with kafirin, the prolamin protein of sorghum, to form haze. Siebert and Lynn (1998) proposed a mechanism of protein-polyphenol interaction leading to haze formation (Fig. 3.3). According to these authors, only a fixed number of sites in the haze-active protein serve as attachment points for haze active phenolic compounds. Small phenols like gallic acid are 'single-ended' because they can bind to one haze-active protein molecule. However, these 'single-ended' phenolic compounds cannot cross-link to one another to form haze. This protein binding capacity of gallic acid probably led to its astringency. Siebert and Lynn (1998) described flavonoid type polyphenols (like catechin) as 'double-ended' and the condensed tannins as 'multi-ended' because they have more protein binding sites. Haze

active polyphenols have two or more 'ends' that can bind to haze active proteins to form a bridge between two protein molecules as illustrated in Fig. 3.3.

According to De Freitas and Mateus (2001) flavonoid monomers (catechin and epicatechin), dimers and trimers have a higher affinity for PRPs than proteins such as α-amylase and BSA. The affinity of these phenolic compounds for PRPs was attributed to the randomly coiled structure of PRPs with more active binding sites as compared to the globular conformations of α-amylase and BSA. Thus, the binding action of the phenolic compound (whether 'single-ended,' 'double-ended' or 'multi-ended') to the protein must have elicited astringency as stated. In sorghum, the phenolic acids would elicit astringency significantly less than the flavonoid monomers (catechin), which would in turn elicit less astringency than the condensed tannins, thus explaining why the tannin-free sorghums would be less astringent than the tannin sorghums.

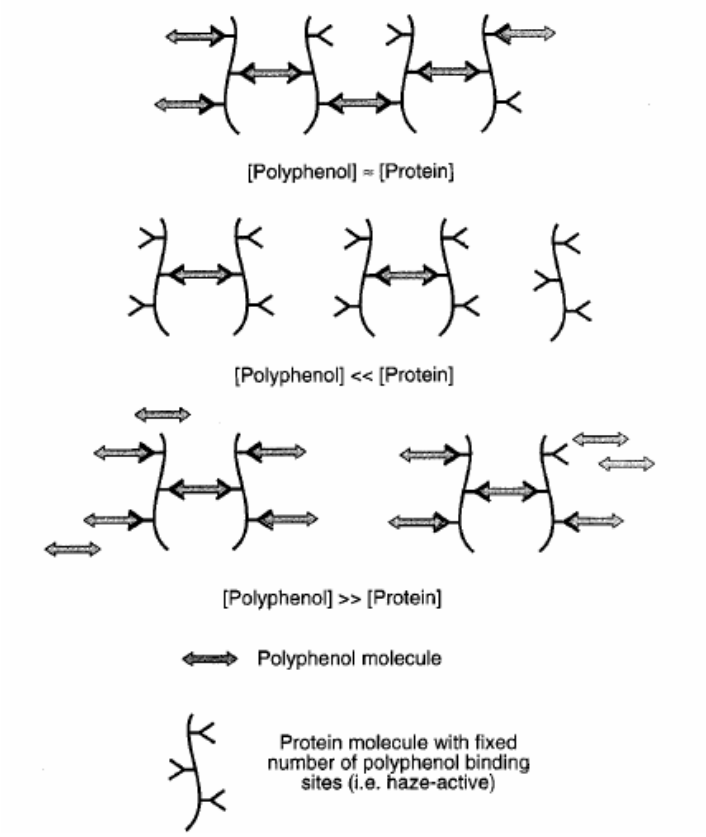

Figure 3.3. The concept of protein-polyphenol interactions leading to haze formation (Siebert and Lynn, 1998).

The increase in perceived astringency with the degree of polyphenol polymerization has been attributed to more extensive formation of phenol-protein complexes via hydrogen bonds between hydroxyl groups of the phenolic compounds and the carbonyl groups of the peptide linkages of the protein due to the presence of more hydroxyl groups in the highly polymerized material (Peleg *et al.*, 1999). This could possibly explain why the sorghum rice from Ex Nola 97 GH (5.7% CE) and PAN 3860 (8.2% CE) were perceived as most astringent (Tables 2.4 and 2.6, Chapter 2.1). It is highly likely these sorghums contained highly polymerized products of flavan-3-ols.

Concerning the consumer acceptability test results (Chapter 2.3), they followed the predicted trend in that the sorghum rice from PAN 3860, with the highest tannin content (8.2% CE), was least preferred. It is noteworthy, however, that although PAN 3860 was the most bitter and astringent sorghum due to its high condensed tannin content (8.2% CE) 50% of the consumers gave it a positive rating (Chapter 2.3). It is possible that the dark colour of the tannin sorghums affected the overall acceptability of these sorghums by consumers. If the colour bias had been removed by using red light, the acceptability of the tannin sorghums might have been slightly higher. PROP super tasters were expected to give significantly lower ratings for acceptability of this sorghum than the non tasters because super tasters have been reported to rate acceptability of bitter foods significantly lower than the non tasters (Marino *et al.*, 1991; Drewnowski *et al.,* 1997; Tepper, 1999; Kaminski *et al.*, 2000; Keller *et al.*, 2002; Pickering *et al.*, 2003). However, the PROP super tasters' ratings for flavour and overall liking of this sorghum were not significantly different from those of the non tasters. Given the fact that most of the consumers were encountering sorghum rice for the first time, the acceptability ratings of sorghum are promising for the promotion of whole grain sorghum consumption, especially from tannin containing sorghums. In the long run, repeated consumption of whole grain sorghum rice would probably improve acceptability ratings.

Whilst attempts have been made to increase consumption of whole grains, these efforts have been far lower than the recommendations and this was attributed to the sensory properties associated with these foods (Heinio, Liukkonen, Katina, Myllymaki and Poutanen, 2003). For instance rye is the second most commonly used cereal grain in the

production of bread but its use is mainly limited by its flavour, which is perceived as bitter and intense. According to Lesschaeve and Noble (2005) acquisition of liking for innately disliked products is possible. It has been found that repeated exposure (7 days) to a bitter beverage was reported to enhance hedonic ratings for the beverage by 68% (Stein, Nagai, Nakagawa and Beauchamp, 2003). Health related information about the beverage had no effect on perceptual changes that accompanied exposure. However, it did tend to increase a behavioural measure of acceptability, suggesting that health information may have a greater effect on behaviour than on hedonics. Furthermore, the bitter taste in foods is often masked or modified by presence of fat, sugar or salts (Drewnowski, 2004). In Botswana, sorghum rice from decorticated sorghum grain is usually consumed with milk; when the grain is not decorticated it is salted and consumed with pulses or meat (personal observation). Thus, any bitter taste in the sorghum rice prepared from high condensed tannin sorghums like PAN 3860 could be masked by other ingredients and/or other foods.

3.3. Condensed tannin threshold limit

The sensory data findings seem to confirm the suggestion that there may be a condensed tannin threshold level at which the tannins are not 'strongly' perceived and thus are not objectionable to consumers. There is a low consumption of foods rich in phenolic compounds (especially condensed tannins) due to their objectionable (unpalatable) sensory attributes (Drewnowski and Gomez-Carneros, 2000). Low consumption implies low nutritional potential because if a food is not consumed its nutritional value goes to waste. Thus, the identification of a condensed tannin threshold level would address the dilemma facing the sorghum farmers for whom tannins impart agronomic advantages, and to the sorghum users for whom optimal nutritional value and palatability are of great concern.

Tannins in sorghum are known to bind and reduce the digestibility of various macronutrients, thus negatively affecting productivity of livestock. However, a tannin threshold limit has been suggested at which animals fed low-tannin sorghums were reported to thrive (Mamary *et al.,* 2001). Mamary *et al.* (2001) investigated the extent of the *in vivo* inhibitory effects of two levels (1.4% and 3.5% CE) of dietary sorghum tannins on rabbit digestive enzymes as well as mineral absorption. Addition of sorghum grain with 1.4% CE tannin content to the diet of rabbits did not significantly change the growth rate, food consumption or the feed conversion ratio. While addition of sorghum grains

with 3.5% CE tannin content significantly reduced the animal's live body weight gain, feed conversion ratio, and slightly increased food consumption with respect to the control. This finding implies that such a threshold limit possibly exists for humans as well, whereby the tannins do not reduce the nutritional quality of sorghum for food. Thus, suggesting that not all tannin sorghums have nutritional disadvantages associated with them.

Palatability is also of great concern to the consumers of sorghum. Therefore identification of a palatability threshold for condensed tannins would be useful to identify in order to serve as a guide to the sorghum producers to know which tannin sorghums to produce for human food (due to their palatability) and which to produce for animal feed due to their feed value (feed conversion ratio).

It is proposed that the condensed tannin threshold limit in the sorghum grain at which sorghum food products are palatable is 2.0% CE, inclusive of the tannin content of NS 5511 (1.8 ± 0.2% CE) (Table 2.3, Chapter 2.1). In this study, the sensory attributes of products from NS 5511 were perceived similar to those from the tannin-free sorghums by the descriptive sensory panel and were equally preferred to the tannin-free sorghums by consumers. The palatability condensed tannin threshold limit being proposed here (2.0% CE), could result in improved consumption potential for tannin sorghums. Not only is NS 5511 palatable but its antioxidant potential was demonstrated by Dlamini *et al.* (2007). Furthermore, the fact that NS 5511 has condensed tannins addresses the agronomic advantages to the farmer as well.

Therefore, future breeding programmes should pursue breeding sorghums that fall within this condensed tannin threshold limit. In this study although PAN 3860 grain had 8.2% CE tannin content, 50% of the consumers gave it positive ratings. Promotion strategies for this sorghum would target the market of consumers for whom health is a high priority. Whereas sorghums like NS 5511, perceived as similar and equally preffered to the tannin-free sorghums, would satisfy a wider market because not only do they provide the health factor associated with condensed tannins they are palatable as well.

4. CONCLUSIONS AND RECOMMENDATIONS

As NS 5511 (tannin – 1.8% CE) was equally preferred by the consumers and its sensory attributes (except appearance) found to be similar to those of the tannin-free sorghums (PAN 8564 and Phofu), it appears that for sorghum-based food systems, there is a condensed tannin threshold level at which the tannins are not 'strongly' perceived and thus do not impart the objectionable sensory attributes (bitterness and astringency) associated with them. It is proposed that the condensed tannin threshold level in the sorghum grain at which its food products are palatable is 2.0% CE, inclusive of the tannin content of NS 5511 (1.8 ± 0.2% CE).

Tannin sorghums like NS 5511 would address the dilemma facing the sorghum farmers, for whom tannins impart agronomic advantages by reducing pre-harvest and post-harvest losses, without compromising on palatability, and due to their antioxidant potential, they are a promising health option for millions of people. Thus, it is recommended that future sorghum breeding programmes focus on producing sorghums with condensed tannin levels that fall within this tannin threshold limit (2.0% CE).

It is recommended that future sensory studies investigate the sensory attributes and acceptance of food products from other sorghum cultivars with tannin content levels between 2.0-2.5% CE to determine whether the tannin threshold limit exceeds 2.0% CE.

It is further recommended that sensory studies investigate the tannin threshold limit suitable for different food processing methods for products such as porridge and sorghum snacks among others. The research data would guide strategies to ensure that the right sorghums are produced and marketed for the right end-use. These strategies could improve sorghum consumption levels considerably and consequently improve sorghum production levels.

Finally, it is recommended that future sensory studies use HPLC to identify and quantify phenolic acids, anthocyanins as well as other flavonoids and condensed tannins present in different sorghum cultivars to better understand the compounds eliciting the bitterness and astringency perceived in these sorghums. Using the proposed protein-polyphenol interaction, the 'single-ended' phenolic acids would elicit astringency significantly less

than the ‘double-ended’ flavonoid monomers (catechin), which in turn would elicit less astringency than the ‘multi-ended’ condensed tannins, thus explaining why the tannin-free sorghums would be less astringent than the tannin sorghums. A wide array of sorghums including type I, type II and type III sorghums would need to be used.

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6. APPENDIX

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