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).

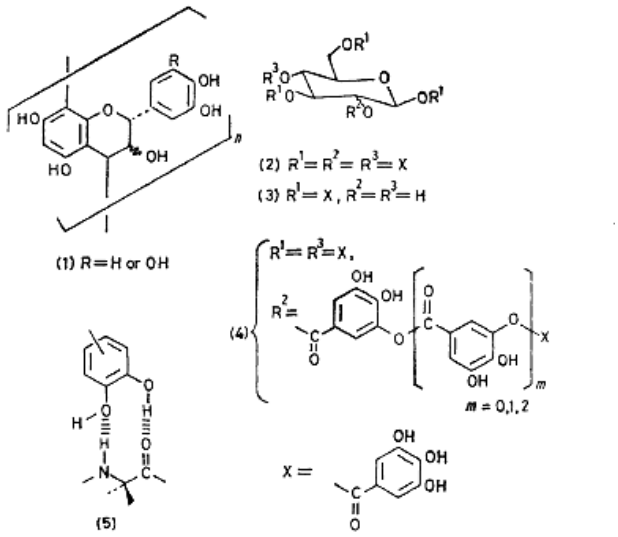

Figure 3.1. Proanthocyanidins (1) and the esters of gallic acid (2, 3 and 4); protein-polyphenol complexation occurs primarily via a bidentate hydrogen bond formation with the keto-imide groups on the protein (5) (McManus *et al.,* 1981).

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)).

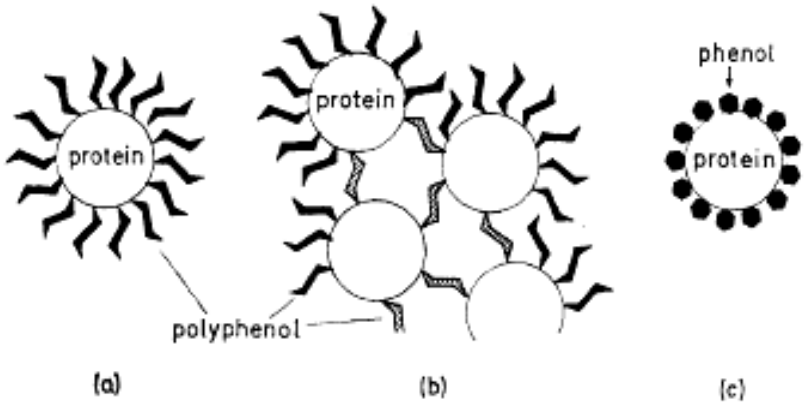

Figure 3.2. Proposed mechanisms for protein precipitation by phenols: (a) polyphenols and low protein concentrations; (b) polyphenols and high protein concentrations; (c) simple phenols (McManus *et al.*, 1981).

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.

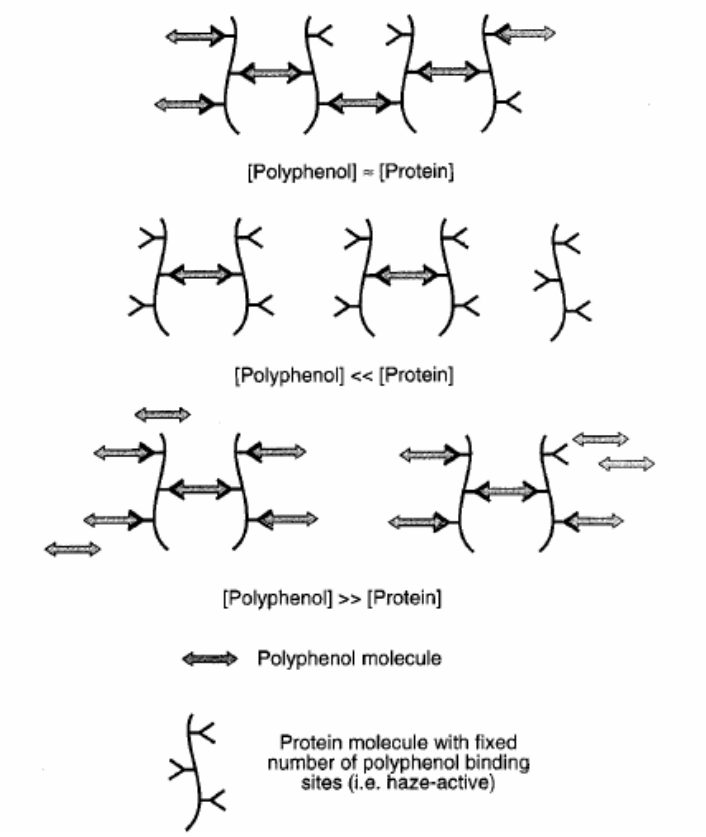

Figure 3.3. The concept of protein-polyphenol interactions leading to haze formation (Siebert and Lynn, 1998).

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.