Sorghum hot water extract: Influence of grain physico-chemical characteristics

by

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DECLARATION

I declare that this dissertation submitted at the University of Pretoria for the degree: MSc (Agric) Food Science & Technology has not been submitted by me at any other University or institution of higher education.

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ABSTRACT

Traditionally, barley is the preferred cereal for traditional lager brewing. The use of sorghum for the production of European-style lager beers has recently been recognised. The use of sorghum as brewing adjunct could be a major socio-economic advantage in the developing countries in Africa. Limitations for its use, however, include its low amylolytic potential, high gelatinization temperatures, and the presence of tannins.

Adjuncts are often combined with cereal malt during the brewing process to provide extra sources of fermentable carbohydrates. As with all cereals, the functional properties of sorghum grains are influenced by their physico-chemical characteristics. It is therefore critical to understand the structure, chemistry and functionality of the sorghum cultivar(s) considered for use as brewing adjuncts.

Hot water extract describes the quality of the wort of an adjunct and depicts the amount of starch that was solubilised during mashing. The determination of hot water extract is expensive, laborious and time-consuming. The provision of a possible predictive marker(s) for sorghum hot water extract that is less complicated to determine, could be of great economical value to the brewer. Hot water extract was determined for 43 sorghum cultivars and then compared to various physico-chemical characteristics.

Sorghum endosperm texture was visually determined. Suspensions of whole sorghum flour were pasted using a Rapid Visco Analyser (RVA) with an extended heating cycle. Significant negative correlations were obtained between extract content and pasting temperature and time in corneous endosperm samples. There was also a significant positive relationship between tannin-free sorghums and peak viscosity in pasted samples.

Protein contents of 10 different sorghums were compared to their hot water extracts, where there was a significant negative relationship between these characteristics. Protein content could be used successfully as a predictive marker for extract. No significant relationship could be established between sorghum hot water extract and starch content.

Tannin-containing sorghum cultivars gave significantly lower extracts and had higher malt diastatic power (DP) than non-tannin cultivars. There was no significant relationship between the DP and extract content of non-tannin sorghums. When only non-tannin sorghum cultivars that pasted were subjected to principal component analysis, it seemed that a positive relationship existed between peak viscosity and extract content.

Low protein sorghum cultivars with no tannins and corneous endosperm would be suitable for use as brewing adjuncts. Protein content, the presence of tannins, endosperm texture and peak viscosity could be used as predictive markers for sorghum hot water extract

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1 INTRODUCTION

Sorghum is a cereal indigenous to Africa and serves as a staple food for millions of people in developing countries. Sorghum can withstand harsh environmental conditions and is mainly cultivated in the semi-arid areas over the world (Taylor and Dewar, 2001). Other cereals such as barley, which is traditionally used for lager beer brewing, cannot be grown in these conditions (Agu and Palmer, 1998).

With regard to quantities produced, sorghum is one of the five major cereal grain crops grown for human consumption in the world (Axtell et al., 1981). World production of sorghum in 2007 was estimated at 64 million tons (FAO, 2009). In South Africa, approximately 166 000 tons were planted in the 2007/08 season (FAO, 2009). From 2003-2007, an average of 231 000 tons were produced annually in South Africa, which is approximately 2.6% and 12.3% of the average maize and wheat production in South Africa, respectively (FAO, 2009).

In South Africa, an area of only 69 000 ha was used in 2007 for the cultivation of sorghum (FAOSTAT, 2009). The possibility of sorghum being employed in the manufacture of lager beer will present local farmers with an excellent opportunity for an income source.

Traditionally, sorghum is used in Africa for the production of sorghum beer (a hazy or opaque beer) (Ziegler, 1999; Taylor et al., 2006). Sorghum has been introduced into the lager beer industry recently, either as sorghum malt or as a brewing adjunct, instead of the usual adjunct material for lager brewing, which is either maize or rice (Figueroa et al., 1995). There are, however, many limiting factors regarding the use of sorghum for lager beer brewing. These factors include low β-amylase activity (Dufour et al., 1992; Agu and Palmer, 1998), high gelatinisation temperatures (Dufour et al., 1992) and the presence of tannins that further inactivate already low levels of amylase enzymes (Taylor, 1992).

Several efforts have been made to enhance the use of sorghum for lager beer production. These include tannin inactivation through steeping in dilute alkali (Beta et al., 2000), optimisation of germination conditions for increased enzyme levels (Taylor and Robbins, 1993; Igyor et al., 2001) as well as optimisation of mashing procedures for more complete starch breakdown (Olatunji et al., 1993; Agu and Palmer, 1997; Ezeogu et al., 2005).

Sorghum proteins are less digestible than those from other cereals (Axtell et al., 1981). High digestibility sorghum cultivars do exist, and there has been some investigation concerning the viability of these cultivars (Axtell et al., 1981; Dowling et al., 2002; Tesso et al., 2006). As a result of increased protein digestibility, it is expected that these cultivars should be of interest to the brewing industry, as increased protein digestibility may lead to optimised starch gelatinisation and pasting.

Sorghum endosperm type may also have an influence on the starch properties and pasting behavior of sorghum flours. Grain strength is usually referred to and measured as ‘hardness’ (Chandrashekar and Mazhar, 1999). Although corneous (hard) sorghum endosperm types are preferable for milling (Anglani, 1997), they exhibit suppressed starch digestion compared to more floury sorghum endosperm types (Ezeogu et al., 2005). This may lead to less substrate available for yeast cells during the fermentation step in the lager brewing process.

To date, the possibility of using South African sorghum cultivars for the production of lager beer has not been investigated. When selecting the appropriate sorghum cultivar for brewing, it is essential to consider all the aspects of that cultivar that will influence its efficiency during the brewing process, such as enzymatic activity, starch content, gelatinisation temperature and pasting behaviour.

2 LITERATURE REVIEW

2.1 SORGHUM – A CEREAL WITH LAGER BREWING POTENTIAL

Due to sorghum originating in developing countries, the advantages of using sorghum for the processing of quality food products have only been recognised and explored in the last 30 years (Taylor and Dewar, 2001). With major advancements in sorghum processing technology, as well as its ability to withstand harsh environmental conditions that other cereals cannot (Odibo et al., 2002), sorghum may become one of the most important crop choices for the future (Taylor and Dewar, 2001).

Sorghum's potential for use as an industrial brewing material has long been recognised (Odibo et al., 2002). In African countries, the use of sorghum for the production of beer and other malt beverages is not a new phenomena (Omidiji and Okpuzor, 2002), where opaque sorghum beers are already popular alcoholic beverages (Kayodé et al., 2007).

Although sorghum has some limitations for use as brewing material due to its insufficient amylolytic potential (Dufour et al*.,* 1992; Agu and Palmer, 1998) and high gelatinisation temperatures (Dufour et al*.,* 1992), the addition of exogenous commercial enzyme in the mashing stage have been successfully applied for the improvement of wort extraction from sorghum grains (Agu and Obanu, 1991). The other limiting factor of sorghum for use as brewing material, the presence of tannins (Taylor et al*.,* 2006), can also be overcome by soaking the grain in dilute NaOH prior to use (Beta et al., 2000).

There has been a great deal of research regarding the production of a European-style lager beer with the use of cereal malt, sorghum, industrial enzyme or a combination of these (Agu, 2002; Odibo et al., 2002; Ogu et al., 2006). There

does, however, remain the fact that although raw sorghum is being used for brewing with commercial enzyme, no specific variety has been developed for this purpose, which may lead to changes in the efficiency of the brewing process (Agu et al., 1995).

2.1.1 The sorghum grain endosperm

Many of the functional properties of grains are influenced by their physical structure (Chandrashekar and Kirleis, 1988; Chandrashekar and Mazhar, 1999). In sorghum, the endosperm characteristics are of particular importance during the processing of this grain (Anglani, 1998). Sorghum endosperm type may, for example, influence the digestibility of the starches and proteins of sorghum flours (Chandrashekar and Kirleis, 1988; Duodu et al., 2002; Duodu et al., 2003).

Other factors unique to sorghum that may have an influence on its food and beverage end-use, include protein content and structure, the presence of tannins, non-starchy polysaccharide content, composition in the endosperm (Kavitha and Chandrashekar, 1997) and protein and starch digestibilities (Wong et al., 2009).

2.1.1.1 Sorghum endosperm structure

The sorghum endosperm (Fig. 2.1) contains both floury (starchy or soft) and corneous (hard or vitreous) regions. The relative proportions of the floury and corneous regions will determine its texture accordingly (Tesso et al., 2006). Corneous sorghum cultivars exhibit a larger peripheral corneous region which surrounds the smaller floury region in the centre. In floury sorghum cultivars, soft endosperm fills most of or the entire endosperm region.

Starch granules in the corneous endosperm are polygonal and covered with protein matrix (Hoseney et al., 1974). Embedded in the protein matrix are protein bodies composed of kafirin. Therefore these starch granules have limited access to water for absorption during cooking in the presence of water. Starch granules in the floury endosperm are round and loosely packed between protein bodies (Seckinger and Wolf, 1973). These starch granules are therefore more susceptible for water uptake, swelling and subsequent gelatinisation and pasting upon cooking in the presence of water.

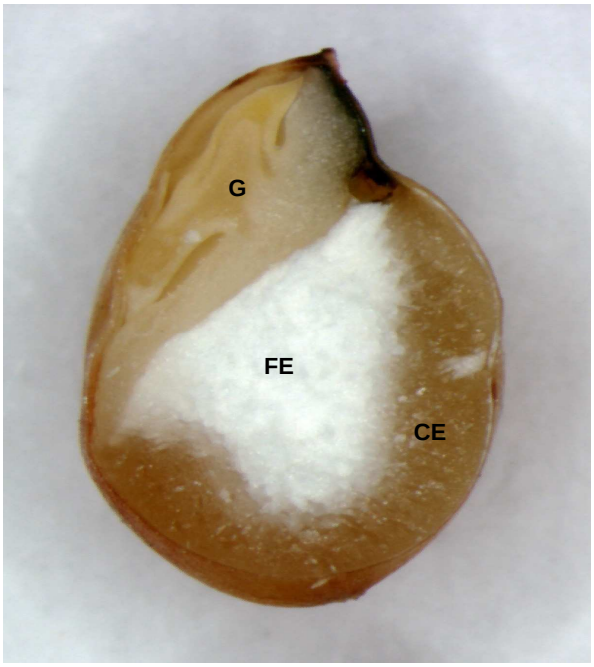


Figure 2.1 Photograph of a longitudinally dissected sorghum grain showing the germ (G), floury endosperm (FE) and corneous endosperm (CE).

Both scanning and transmission electron microscopy have shown that the corneous endosperm consists of polygonal starch granules that are tightly packed and surrounded by protein bodies embedded in the protein matrix (Figure

2.2a) and that the prolamin fraction was the major component of the protein bodies. In the floury region, the cells contain round starch granules that are loosely packed between surrounding protein bodies (Figure 2.2b) (Seckinger and Wolf, 1973; Chandrashekar and Mazhar, 1999; Duodu, et al., 2002).

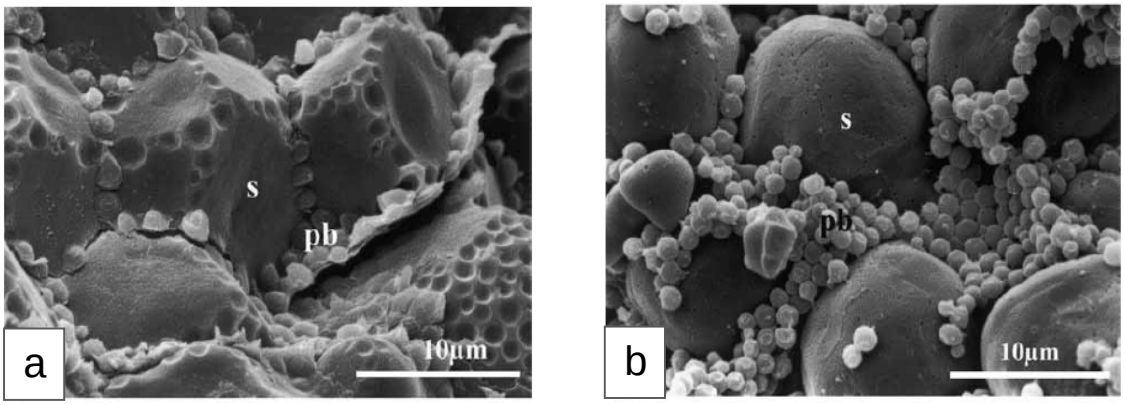


Figure 2.2 Scanning electron micrograph of the sorghum endosperm structure (Duodu et al., 2002).

(a) Corneous endosperm
(b) Floury endosperm
(s) Starch granules
(p) Protein bodies
(pb) Protein matrix

2.1.1.2 Sorghum endosperm proteins

Proteins from the corneous endosperm have a greater number of disulphide bonds between them and therefore exhibit a greater degree of cross-linking with the result of having a greater molecular weight than proteins from the floury endosperm (Ioerger et al., 2007.) Protein classification revealed that flour from the corneous endosperm of sorghum contained more kafirin proteins than flour from the floury endosperm (Chandrashekar and Kirleis, 1988; Ioerger et al., 2007**).**

Kafirins, the major prolamin storage proteins of sorghum, are located in the endosperm (Hamaker et al., 1995). The kafirins are found in the endosperm in the form of protein bodies (Seckinger and Wolf, 1973). The protein bodies that make up the structure of the protein matrix consist of both low and high molecular weight proteins (Hoseney, 1994). The former consists of a single polypeptide chain with intramolecular disulphide crosslinking, while the latter consists of many polypeptide chains that are linked to each other via intermolecular disulphide bonds. In normal sorghum endosperm, the prolamin storage protein accounts for 70-80-% of total endosperm protein (Hamaker et al*.,* 1995).

2.1.1.3 Sorghum tannins

Tannin-containing sorghum cultivars are of agronomic advantage to the farmer due to their resistance to bird damage. However, these tannins cause limitations during the mashing procedure due to their ability to inactivate amylase enzymes. De Jong et al. (1987) investigated the influence of sorghum tannins on enzymatic starch hydrolysis prior to fermentation. It was found that sugar accumulation was retarded in those cultivars containing tannins and that the same cultivars,

after treatment with NaOH, showed significantly increased sugar accumulation. Significant amylase activity was shown to be preserved by steeping the tannin-containing sorghum grains in a dilute solution of NaOH prior to germination (Beta et al., 2000). Steeping time was also found to be reduced when NaOH was used.

2.2 LAGER BREWING – A BRIEF OVERVIEW

Traditional to lager beer brewing, barley (*Hordeum vulgare*) is the preferred source for the production of malt (Briggs et al., 2004). However, malts used for lager brewing do not necessarily consist of only malted barley, and malted cereals such as wheat (*Triticum aestivum*) and sorghum may make up part of the malt mixture. It is therefore critical to properly understand the structure, chemistry and functionality of all cereal grains involved in lager beer brewing in order to maximise the efficiency and efficacy of the process. Apart from using only malts for the production of lager beers, adjuncts may be added in conjunction with the malt during the mashing procedure to provide extra sources of fermentable sugars (Briggs et al., 2004).

The major steps involved during the production of a lager beer, include malting, mashing, hop-boiling and fermentation (Briggs et al., 2004).

2.2.1 Malting

Malting can be defined as the controlled germination of grain in moist air and can be divided into three operations, i.e. steeping, germination and drying (kilning) (Dewar et al., 1997). During the malting process, endogenous enzymes are synthesised and mobilised in order to hydrolyse the grain's macromolecules

(such as starch and protein) during the next step – mashing (Briggs et al., 2004). Malting is terminated by kilning, which is drying of the grain by using warm to hot air (Briggs et al., 2004). The malted, kilned grain is then milled to form a product called malt.

2.2.1.1 Steeping

Steeping is the initiation process of malting, during which the grain is soaked in water. Steeping has been acknowledged as being the most important step during malting (French and McRuer, 1990). Germination should be initiated such that endosperm modification will progress at a rate that will produce malt of high quality (Dewar et al., 1997). Crucial for proper initiation of germination is sufficient moisture, steeping temperature and the presence of oxygen (French and McRuer, 1990).

Sorghum β-amylase production as affected by grain cultivar, steep regime and steep liquor composition was investigated by Okungbowa et al. (2002). Differences in varietal responses of sorghum grains to various steeping conditions were observed and the possibility that grains from different sorghum cultivars employ different β-amylase biosynthesis models was suggested. Steeping regimes that included air rest periods produced sorghum malts with significantly higher β-amylase activities, indicating the importance of aeration during steeping. Steeping of grains in KOH produced sorghum malts with significantly less β-amylase activity than those that were steeped in either NaOH or $Ca(OH)_2$.

Dewar et al. (1997) reported that steeping time and temperature had a highly positive, significant effect on the quality of sorghum malt (as measured by diastatic power, free amino nitrogen and hot water extract). Steeping of grains

for 40h at 30℃ with the use of air rests, produced malts with the highest diastatic power. This study also showed that the steep-out moisture of the grain is related to steeping time and temperature, and significantly increases the final malt quality in terms of diastatic power.

2.2.1.2 Germination

Although cereal β-amylases resemble each other closely regarding their enzymatic activity, the suitability of particular cereals as sources of this enzyme for specific purposes (such as brewing) depends largely on the initial levels of the β-amylase present in the grain as well as how much activity of the enzyme the grains can develop during the process of germination (Ziegler, 1999).

The β-amylase activity of malted sorghum is less than 25% than that of malted barley (Taylor and Robbins, 1993). In ungerminated sorghum grain, both α- and β-amylase display similar activities (Dicko et al., 2006). After germination, the α-amylase activity in sorghum grain increased several-fold, whereas β-amylase activity did not increase uniformly (Dicko et al., 2006). The production of improved malts – specifically with regards to increased β-amylase activity – has been a subject of continuous research (Dewar et al.,1997; Uriyo & Eigel, 1999; Igyor et al., 2001; Okungbowa et al., 2002; Balogun et al., 2005).

It has been determined that sorghum malt quality (measured as β-amylase activity and total diastatic power) can be heightened by manipulating the germination time and temperature, where the highest diastatic power and β-amylase activity was obtained at 32℃ over a period of 6.5 days (Taylor and Robbins, 1993). Balogun et al. (2005) reported a significant increase in the in vitro fermentability of starch and the production of volatile fatty acids (VFA) from sorghum grain after a germination time of 3 and 5 days. The in vitro

fermentability of the sorghum grain used in this study was significantly higher when temperatures of 25℃ and 32℃ were used compar ed to that of 18℃.

2.2.1.3 Drying (Kilning)

The duration of drying showed to significantly influence the production of α-amylase, β-amylase and endo-(1,3)(1,4)-β-D-glucanase in sorghum malts (Uriyo & Eigel, 1999). During this study, optimal enzymatic activity was achieved at a germination period of 48h, followed by a drying period of 5h at 60℃.

The combined effect of steeping liquor and kilning conditions was investigated by Okungbowa et al. (2002). Steeping of sorghum grain in $Ca(OH)_2$ produced malts with significantly higher β-amylase activity after kilning at 50℃ than at 40° C. This phenomenon was attributed to either enhancement of the rate of zymogen activation, the possible development of more thermostable forms of β-amylase or both.

2.2.2 Mashing

Mashing involves the mixing of malt and salts with water at a specific temperature and then heating the system for a specific period of time (Briggs et al., 2004). The science of mashing is complicated and many physical, chemical and biochemical reactions take place during the process (Briggs et al., 2004). Starch gelatinisation is crucial and has to be as complete as possible for enzymatic hydrolysis to be maximised during mashing. This will ensure the availability of sufficient fermentable yeast substrate.
A proper understanding of the chemistry and biochemistry of mashing is essential for selection of the correct grain cultivar and / brewing adjunct. From

an economical point of view, mashing should be carried out to provide wort of the highest possible yield in the shortest possible time (Briggs et al., 2004).

Complete starch gelatinisation of the malt and / or adjunct used during mashing is of extreme importance to the brewer, as it will have a direct influence on the quality of the wort that is produced. Insufficient starch gelatinisation will cause a decrease in the quantity of fermentable sugars for yeast cells during fermentation, leading to the production of a beer of inferior quality (Briggs et al., 2004).

The mashing method that will be followed depends on factors such as malt origin and composition, the type of adjunct (should an adjunct be used), the type of beer that will be produced as well as the quantities of beer that will be produced (Olantunji et al., 1993).

2.2.2.1 Starch hydrolysis during mashing

Starch consists of two main components, namely amylose and amylopectin. Amylose is an essentially linear molecule consisting of only α-(1,4)-glucosidic linkages, while amylopectin is a highly branched molecule, consisting of α-(1,4)-bound glucose chains that are linked together via α-(1,6)-glucosidic linkages at their non-reducing ends (Benmoussa et al., 2006). During mashing (cooking of the starch in the presence of water), the starch granules undergo gelatinisation. During this process, the granules swell due to water absorption, and lose their crystallinity (Arámbula et al., 1998).

Usually, the amylopectin can be found throughout the swollen starch granules, whereas the amylose tends to leach out of the granules and make up part of the continuous phase that surrounds the granules (Hermansson and Svegmark,

1996). Using a Rapid Visco Analyser (RVA) to analyse the pasting behaviour of amylose and amylopectin and their mixtures, Juhász and Salgó (2008) found that peak viscosity and their parameters were negatively correlated with amylose content. This indicated that amylopectin is mainly responsible for water uptake during gelatinisation.

In a study conducted by Beta et al. (2000) it was determined that amylose content was significantly negatively correlated to sorghum grain floury endosperm texture, indicating that the corneous endosperm in sorghum contains more amylose than the floury endosperm. In the same study it was found that starches containing higher amylose contents had higher pasting temperatures (the temperature at which the flour-water mixture starts to increase in viscosity upon heating), therefore requiring more energy for water absorption.

2.2.2.2 Enzymatic action during mashing

The three major enzymes responsible for starch hydrolysis during mashing are α-amylase, β-amylase and limit dextrinase (MacGregor et al., 1999). The α-amylase is responsible for randomly cleaving internal α-1,4-glucosidic linkages of solubilised starch molecules, producing a range of both linear and branched dextrins. The limit dextrinase (also known as debranching enzyme) cleaves the α-1,6-glucosidic linkages at the branching points of the amylopectin molecules, releasing linear chains for further breakdown by β-amylase which sequentially removes units of maltose from the non-reducing end of the larger dextrins.

After investigation of the contribution of α-amylase and β-amylase responsible for the breakdown of starch during the mashing procedure, MacGregor et al. (1999) concluded that a deficiency of either one of these enzymes can be compensated for by the presence of an excess of the other.

For sorghum malt starch, the gelatinisation temperature is between 64 and 68℃, which is about 10℃ higher than that of barley malt starch (Taylor, 1992). Figueroa et al. (1995) reported the gelatinisation temperature of sorghum starch to be 72℃, while Igyor et al. (2001) reported an even higher gelatinisation temperature of 80℃ for sorghum starch. This complicates the mashing procedure, as these elevated mashing temperatures cause inactivation of the amylase enzymes.

Odibo et al. (2002) experimented with the use of commercial enzymes and sorghum adjunct as mashing material. An extract of the wort produced was prepared, and it was found that sorghum varieties with higher carbohydrate content produced more alcohol, and sorghum varieties with higher nitrogen content fermented faster.

In a study done by Taylor (1992), different mashing procedures were carried out and it was concluded that decoction-type mashing showed to be the best way to carry out mashing of sorghum malt. During decoction mashing, malt is added to water and then heated to about 35℃, whereafter a part of the sorghum malt is cooked separately (about 1/3), added back to the initial malt mixture and the process is repeated several times (Briggs et al., 2004).

Similarly, Agu and Palmer (1998) reported that during the mashing of sorghum malt, a controlled mashing procedure, rather than a standard mashing procedure at 65℃ (as is usually the case during barley malt mashing) has to be carried out in order to obtain maximum extract yields. Olatunji et al. (1993) produced lager beer using only sorghum and exogenous enzyme and found that the highest quality beer was obtained when the sorghum starch was gelatinised prior to enzyme addition.

2.2.2.3 Wort composition

Wort, the product of mashing, is a complex solution, with carbohydrates making up approximately 92% of the solids in the mixture (Briggs et al., 2004). Of these carbohydrates, an average of 64-77% consists of fermentable carbohydrates (including mono-, di- and trisaccharides), while the rest (the unfermentable carbohydrates) includes dextrins, pentosans and β-glucans.

Of all the carbohydrates in the malt mixture, starch makes up the greatest proportion at about 58% (dry basis), which inevitably leads to starch hydrolysates making up the biggest proportion of fermentable substrate (Briggs et al., 2004). It has been reported that the glucose content in sorghum malt that has undergone mashing make up nearly 30% of the fermentable substrate for yeast cells, which is almost twice the value for barley malts (Dufour et al., 1992). Malt extract can be described as a measurement of the percentage of dry matter that was solubilised during the mashing procedure (a hot water extraction) (MacGregor et al., 1999). As mentioned above, starch makes up a big portion of the dry matter in malt, and therefore the extract composition can be used as an indicator of the amount of starch that can be solubilised during mashing. Hot water extract is used as the major determinant of the quality of the wort that was produced and depicts the amount of starch that was solubilised during the mashing process.

2.2.3 Hop-boiling

Hops are derived from the female cones of the hop plant (*Humulus lupulus)* and is essential during brewing not only to impart taste and flavour, but also to

stabilise beer foam (Vanhoecke et al., 2005). Hops can be used ground up, whole, as pellets or as extracts (Briggs et al., 2004).

During hop-boiling, the sweet wort is boiled together with hops (Briggs et al., 2004). Hop aroma is the major flavour contributor of beer (Igyor et al., 2001). This step is important as it adds not only the bitter taste characteristic of lager beers but also serves to sterilise the wort mixture (Briggs et al., 2004).

2.2.4 Fermentation

During fermentation, fermentable sugars that were formed during mashing (which mainly include maltose), are metabolised by yeasts (usually *Saccharomyces cerevisiae*) to form mainly carbon dioxide and ethanol.

The extent of fermentation by the yeast cells depends on the fermentable substrate present, as mentioned earlier. In a study conducted by Del Pozo-Insfran et al. (2004)**,** the effect of the addition of the glucose producing enzyme amyloglucosidase on wort composition and fermentable carbohydrate utilization during fermentation of lager beers from barley malts, sorghum malts and brewing adjuncts (maize and waxy sorghum grits) was investigated. It was found that the addition of this enzyme increased the amount of fermentable carbohydrate in sorghum malts by more than 20%, with a two-fold increase in glucose. The time it took to reach half of the initial amount of glucose was 79 and 76 hours less than for that of maltose and malto-triose, respectively, indicating that the preferred carbohydrate substrate for the fermenting yeast is glucose. Amyloglucosidase is a glucoamylase that cleaves both α, 1-4 and α, 1-6 glycosidic bonds of amylose and amylopectin molecules (Del Pozo-Insfran et al., 2004; Blazek and Copeland, 2010) to release glucose or maltose units from the non-reducing ends of starch and oligosaccharides (Ákerberg et al., 2000).

2.3 BREWING WITH ADJUNCTS

Adjuncts that are used during the mashing process are usually cereals that lack the required levels of cereal amylases and may include maize, rice and / or sorghum (Briggs et al., 2004). Additional industrial enzyme may be added during the mashing process in conjunction with the adjunct. The starch content, composition and availability as well as gelatinisation temperatures of the adjunct that will be used are of high importance for the production of wort with sufficient available fermentable substrate. Traditionally, maize and rice have been used as adjunct materials during brewing (Figueroa et al. 1995), but due to its high availability in Africa, sorghum has been introduced to this industry as an alternative adjunct.

2.4 SORGHUM LAGER BREWING

Lager beer brewing technology using sorghum can be divided into three different categories: namely sorghum grain adjunct with barley malt, sorghum grain adjunct with the addition of industrial enzymes, and malted sorghum (Taylor and Dewar, 2001).

In sorghum brewing, starch hydrolysis during mashing depends on many different factors, such as endosperm corneousness (Ezeogu et al., 2005), gelatinisation temperatures (Dufour et al., 1992), amylose/amylopectin ratio (Figueroa et al., 1995) and enzymatic activities of the specific sorghum grain.

2.4.1 The quality of sorghum as a brewing adjunct

2.4.1.1 Sorghum endosperm type

The influence that the protein matrix has on starch hydrolysis of various sorghum and maize endosperm flours during wet cooking was investigated by Ezeogu et al. (2008) using three-dimensional (3-D) fluorescence microscopy. Reconstructed 3-D images showed that for the corneous endosperm flours of both sorghum and maize the protein matrixes appeared thicker and denser than for the floury endosperm flours. Upon cooking there was an expansion of the protein matrix of the floury endosperm. This was attributed to the starch granules in the floury endosperm being loosely packed within the protein matrix, therefore being more susceptible to water absorption and swelling during cooking. Contrary to this, the honeycomb-like structure of the protein matrix in the corneous endosperm flours collapsed during cooking. This shows that the digestibility of starch in the corneous endosperm is decreased during cooking of these flours, as the protein network surrounding the starch granules renders the granules less susceptible for gelatinisation and digestion by amylase enzymes.

Figueroa et al. (1995) investigated the gelatinisation temperatures and peak viscosities of different cereals, including barley, rice, maize, normal sorghum and waxy (high amylopectin) sorghum. They found that gelatinised waxy sorghum starches swell and lose their shape much faster and at lower temperatures compared to gelatinised starches from normal sorghum varieties. They also found that the waxy starches reached a much higher peak viscosity than normal sorghum varieties.

Ezeogu et al. (2005) determined the influence of endosperm texture, cooking conditions and the possible influence of the endosperm proteins on in vitro starch hydrolysis in sorghum and maize flours. Corneous sorghum endosperm starch

showed to be less digestible than floury endosperm starch. This appeared to be caused by the limited availability of starch in the corneous endosperm for α-amylase digestion due to the presence of prolamin protein networks surrounding the starch granules. Cooking time and temperature also showed to be different for corneous and starchy endosperm sorghum types. Longer cooking times showed an increase in starch digestibility for both corneous and starchy endosperm flours. Cooking under pressure improved corneous endosperm starch digestibility and may be due to rupturing of the protein matrices that surround the starch granules, thereby making the starch more available for digestion.

Digestibility of cooked sorghum and maize flours has also investigated using α-amylase (Zhang and Hamaker, 1998). The starch digestibility of cooked sorghum flours was 15-25% less than that of maize flour, but there was no difference in the digestibility of the starches from sorghum and maize. Treatment of sorghum flours with the proteolytic enzyme pepsin and subsequent enzymatic digestion showed an increase in starch digestibility. These researchers suggested not only that endosperm protein restrict starch digestibility, but that there also could be the possibility of formation of starch-protein interactions that could negatively affect starch digestibility.

2.4.1.2 Sorghum endosperm proteins

In sorghum, the prolamin kafirin is the major component of protein bodies, while the protein matrix consists mostly of globular glutelin-type protein (Seckinger and Wolf, 1973). When sorghum is cooked and subsequently undergoes hydrolysis by protease, it has been shown that the protein matrix is digested prior to the protein bodies (Rom et al., 1992). Corneous endosperm contains much higher levels of kafirin than do floury endosperm (Chandrashekar and Kirleis, 1988).

This can suggest that there are more protein bodies present in the protein matrix in corneous endosperm than there are in floury endosperm, which may lead to the corneous endosperm being less digestible.

It has been determined that uncooked and cooked sorghum showed an increase in *in vitro* protein digestion as the grain was structurally reduced from whole grain flour to endosperm flour and then to protein body-enriched material (Duodu et al., 2002). This may indicate that there exist some exogenous factors which interfere with protein digestibility (Duodu et al., 2003). These may include polyphenols, phytates and non-starch polysaccharides in the pericarp, non-starch polysaccharides in the endosperm cell walls and starch in the endosperm.

The digestion of both protein and starch in sorghum has been linked to the structural features of the grain endosperm (Wong et al., 2009). A highly digestible sorghum cultivar was compared with a normal sorghum cultivar. It was found that the low digestibility of the normal cultivar was due to the greater number of disulphide cross-links in this cultivar as well as the different structure of the protein matrix that surround the starch granules.

A sorghum grain phenotype with a unique modified endosperm texture, high protein digestibility and high lysine content has been identified (Tesso et al., 2006). Although this sorghum phenotype exhibited near-normal endosperm hardness, the microstructure of the corneous portion of the endosperm is dramatically different than that of normal corneous endosperm. The starch granules remained polygonal, but the continuous protein matrix that surrounds the starch granules in normal corneous endosperm was absent. It was suggested that the lack of protein matrix around the starch granules in this phenotype will result in starch that is more available for amylase digestion.

The influence of protein on starch gelatinisation was investigated by Chandrashekar and Kirleis (1988). Sorghum flours from both corneous and

floury endosperm portions were cooked in the presence of water after which it was enzymatically hydrolysed to determine the degree of gelatinisation. The corneous cultivars, which contain more kafirin protein, produced pastes with much lower viscosities than those of the floury cultivars and also had a much lower degree of starch gelatinisation. When treated with 2-mercaptoethanol, a reducing agent, starch gels made from corneous endosperm showed an increase in the degree of gelatinisation due to disruption of the disulphide cross-links within the protein networks and protein bodies surrounding the starch granules.

2.5 CONCLUDING REMARKS

The physico-chemical characteristics of sorghum has a great influence on its processing behaviour. A proper understanding of the chemical, structural and functional properties of the sorghum grain constituents is crucial when selecting a cultivar for lager brewing.

The presence of tannins, endosperm texture and starch and protein digestibilities are some of these characteristics that determine sorghum's processing behaviour and may determine the quality of the wort that is produced. Cultivars should be chosen to produce a wort that can give the highest possible extract content.

3 HYPOTHESES AND OBJECTIVES

3.1 HYPOTHESES

1. The pasting properties of sorghum cultivars with a high proportion of corneous endosperm will differ from those of normal cultivars and are expected to have lower peak viscosities, higher peak temperatures and longer peak times. Starch present in the corneous portion of the sorghum endosperm has limited availability to water due to the presence of prolamin protein networks and protein bodies surrounding the starch granules and acting as a barrier (Hoseney et al., 1974). When treated with 2-mercaptoethanol, a reducing agent, starch granules within the vitreous endosperm show an increase in the degree of gelatinisation due to disruption of the disulphide cross-links within the protein networks and protein bodies surrounding the starch granules (Chandrashekar and Kirleis, 1988). Longer cooking times and cooking under pressure resulted in an increase in starch digestibility in corneous sorghum endosperm, due to the physical disruption of the protein networks that surround the starch granules (Ezeogu et al., 2005).

2. The worts from sorghum cultivars with a higher proportion of floury endosperm than normal cultivars will have higher extract content and will therefore be more effective for use as adjuncts in the lager beer brewing process. In the starchy regions of the sorghum endosperm, spherical starch granules are loosely packed within endosperm cells with numerous air spaces in between the granules (Duodu et al., 2002). This renders more starch available for water uptake and starch digestion during the mashing (cooking) process in lager beer brewing. Increased starch digestion will lead to more soluble solids present in the wort extract.

3. Sorghum cultivars with higher total starch contents than normal sorghum cultivars will have higher extract contents, higher peak viscosities and shorter peak times during pasting. Extract can be considered as the dissolved materials that formed during mashing and mainly consist of simple carbohydrates that were derived from starch (Briggs et al., 2004). Increased starch concentration in an adjunct will lead to increased starch derivatives present after completion of the mashing procedure. Increased amounts of starch available for solubilisation, pasting and subsequent saccharification, will lead to an increased amount of soluble sugars present in the extract after mashing (Dona et al., 2010).

3.2 OBJECTIVES

1. To relate the hot water extract content of South African and other sorghum cultivars with various grain physico-chemical characteristics, including endosperm texture, pasting properties, presence of tannins, protein content and starch content.

2. To determine a simple alternative marker to predict hot water extract in whole sorghum grain adjunct.

3. To determine the most suitable types of sorghum cultivars for maximum hot water extract.

4 RESEARCH

Effect of sorghum grain physico-chemical properties on hot water extract

Abstract

The high starch gelatinisation temperature and low amylase activity of sorghum malts result in sorghum being mainly used as an adjunct in lager brewing. Hot water extract is the major determinant of wort quality. Due to the time-consuming method for hot water extract determination, a faster predictive marker for sorghum extract could be a major advantage. Endosperm texture, protein content, starch content, the presence of tannins and pasting properties were determined and their relationships to extract content in whole sorghum grain were established. There were significant negative correlations at $p<0.05$ between extract content and pasting temperature and pasting time in corneous endosperm sorghums with r-values of -0.939 and -0.941, respectively. There was a significant negative relationship between grain protein content and extract, with an r-value of -0.831 at $p<0.001$. There was no significant relationship between extract and starch content. Tannin-containing sorghums gave significantly lower extract contents than non-tannin types. There was also a significant positive correlation between extract content and peak viscosity in non-tannin sorghums, with an r-value of 0.611 at $p < 0.005$. Sorghum grain protein content, endosperm texture and the presence of tannins could be used as predictive markers for hot water extract. Cultivars low in protein with corneous endosperm and no tannins would be the best choice as brewing adjuncts.

4.1.1 Introduction

The use of sorghum for the production of lager beer has been a field of continuous research with much emphasis on sorghum malt characteristics and how these can be improved (Uriyo and Eigel, 1999; Okungowa et al., 2002; Adewale et al., 2006; Ogu et al., 2006; Letsididi et al., 2008).

Adjuncts may be used during lager brewing and provide less expensive extract than malt and / or may impart additional desirable characteristics to the final beer (Briggs et al., 2004). The most important quality of a cereal when used as an adjunct in lager brewing it its extract yield, i.e. the extent to which the starch present in the endosperm can be enzymatically hydrolysed into soluble sugars. These sugars may range from simple sugars to oligosaccharides. When brewing with adjuncts, commercial enzymes may also be added to assist with the starch saccharification during mashing.

Africa represents 55% of the world's total sorghum cultivation area (Belton and Taylor, 2004). Most of this sorghum, however, is grown purely for subsistence (Mackintosh and Higgins, 2004). The successful use of sorghum for commercial lager beer has already provided some of these African farmers with a sustainable commercial market for their produce. Further insight on proper cultivar selection could be of major economical significance to both the farmer and the industry.

Many of the functional properties of sorghum, including its use as an adjunct, are influenced by grain physical structure (Chandrashekar and Kirleis, 1988; Chandrashekar and Mazhar, 1999). It has been shown that sorghum endosperm type influences the digestibility of the starches and proteins of sorghum flours (Chandrashekar and Kirleis, 1988; Duodu et al., 2002; Duodu et al., 2003). Although corneous (hard) sorghum endosperm types are preferable for milling (Anglani, 1998), they exhibit suppressed starch digestion compared to more floury sorghum endosperm types (Ezeogu et al., 2005) and may lead to lower extract in brewing.

Although much research has been done on starch gelatinisation as well as protein and starch digestibilities of sorghum cultivars varying in endosperm hardness (Chandrashekar and Kirleis, 1988; Rom et al., 1992; Zhang and Hamaker, 1998; Elkhalifa et al., 1999; Ezeogu et al., 2008), little information is available on the pasting properties and extract yields of different sorghum cultivars.

The determination of the extract content of cereals is a laborious, time-consuming and expensive procedure. The objective of this research was to determine whether correlations between sorghum extract content and its physico-chemical characteristics exist in order to provide a predictive marker for extract yield.

4.2 EXPERIMENTAL

4.2.1 Materials

Table 4.1 Sample names, descriptions, presence of tannins, protein digestibility, origin and diastatic power of all sorghum cultivars analysed

Name/Code[1]	Full Name / Description	TC[2]	PD[3]	Origin	DP[4]
BANJO	Red hybrid	No	ND[6]	South Africa	25
GP1	HDPGO4C (HD1), white tan-plant hybrid	No	High[5]	USA	ND
GP10	NK8828, white tan-plant hybrid	No	High	USA	ND
GP11	Sudan 96, white variety with purple glumes	Type II	Low	Sudan	ND
GP12	NK8828, white tan-plant hybrid	No	High	USA	ND
GP2	LDPBTX436 (LD5), parent of GP1), white tan-plant hybrid	No	Low	USA	ND
GP3	Macia; white tan-plant variety	No	High	Southern Africa	ND
GP4	Kari Mtama 1 (KAT369), white tan-plant variety	No	Medium	Kenya	ND
GP5	Seredo, red variety	Type III	Low	East Africa	ND
GP6	Town, red variety	No	Medium	Botswana	ND
GP7	3442-2-OP, red variety	No	Medium	Ethiopia	ND
GP8	PAN8564; red hybrid, GM malting type	No	High	South Africa	ND
GP9	Sima, white tan-plant variety	No	Medium	Southern Africa	ND
NS5511	Red hybrid, GH malting type	Type III	ND	South Africa	63
NS5655	Red hybrid, GM malting type	No	ND	South Africa	38
ORBIT	White tan-plant hybrid	No	ND	USA	ND
OVERFLOW	Red hybrid	No	ND	South Africa	27
PAN8017	Red hybrid	No	ND	South Africa	38
PAN8127	Red hybrid, GH malting type	Type III	ND	South Africa	64
PAN8229	Red hybrid, GH malting type	Type III	ND	South Africa	65
PAN8247	Red hybrid, GM malting type	No	ND	South Africa	41
PAN8249	Red hybrid, GM malting type	No	ND	South Africa	ND

Table 4.1 (continued)

Name/Code1	Full Name / Description	TC[2]	PD[3]	Origin	DP[4]
PAN8337	Red hybrid	No	ND	South Africa	35
PAN8358	Red hybrid, GM malting type	No	ND	South Africa	48
PAN8387	Red hybrid	No	ND	South Africa	30
PAN8389	Red hybrid, GH malting type	Type III	ND	South Africa	55
PAN8407	Red hybrid	No	ND	South Africa	24
PAN8420	Red hybrid, GM malting type	No	ND	South Africa	44
PAN8474	Red hybrid, GH malting type	Type III	ND	South Africa	44
PAN8507	Red hybrid, GH malting type	Type III	ND	South Africa	71
PAN8553W	White tan-plant hybrid	No	ND	South Africa	28
PAN8568	Red hybrid, GH malting type	Type III	ND	South Africa	53
PAN8609	Red hybrid, GM malting type	No	ND	South Africa	46
PAN8625	Red hybrid, GH malting type	Type III	ND	South Africa	65
PAN8648W	White tan-plant hybrid	No	ND	South Africa	26
PAN8657	Red hybrid, GM malting type	No	ND	South Africa	46
PAN8677	Red hybrid,	Type III	ND	South Africa	60
PAN8806	Red hybrid, GM malting type	No	ND	South Africa	42
PAN8816	Red hybrid, GM malting type	No	ND	South Africa	49
TX103	(BTx635*P850029)-CS9-CS1-CS1, white tan-plant hybrid	No	High	USA	ND
TX115	(96GCPOB124*P851171)-CS28-CS1-CS1-CS1, white tan-plant hybrid	No	High	USA	ND
TX2907	ATx2928/RTX436, white tan-plant hybrid	No	Low	USA	ND
TX436	ATxARG-1/RTx2907, waxy, white tan-plant hybrid	No	Low	USA	ND

[1] Name of sample used throughout the experimental and discussion

[2]Tannin containing

[3]Protein digestibility

[4]Diastatic Power (Sorghum Diastatic Units / g malt)

[5]Taylor (2008). Preparation, characterisation and functionality of kafirin microparticles. PhD thesis, University of Pretoria

[6]Not determined

4.2.2 Milling

Whole grain sorghum was ground using a hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 0.5 mm opening screen. Samples were stored in polythene zip lock-type bags at ≈5℃ until needed for analysis.

4.2.3 Estimation of sorghum grain endosperm texture

The endosperm texture of the sorghum grains was determined according to the following reference: ICC Standard 176 Estimation of Sorghum Grain Endosperm Texture (Revised January 2007). International Association for Cereal Science and Technology (ICC) (2008).

In this assay, sorghum grains were cut into halves longitudinally. One half was then inspected with the naked eye and the relative proportion of corneous endosperm to floury endosperm was determined with reference to a standard. The grains were then classified as being floury, intermediate or corneous on the basis of the relative proportion of corneous to floury endosperm, using illustrations for reference.

4.2.4 Determination of total starch

Starch content was determined using the Megazyme Total Starch Assay Procedure (Amyloglucosidase / α-Amylase Method) (Megazyme® International Ireland Limited). In this assay procedure, the starch in the flour is gelatinised and cooked and thereafter digested with amyloglucosidase into D-glucose. The D-glucose is then measured colorimetrically at 510 nm.

4.2.5 Determination of moisture content

For the determination of moisture content, the following method was used: ICC Method No.109/1: Determination of Moisture Content of Cereals and Cereal Products (ICC, 1976). Due to small amount of sample, 1 g was dried at 103℃ for 3 hours (all analyses were done in duplicate).

4.2.6 Determination of pasting properties

Pasting properties of the sorghums were measured using a Rapid Visco Analyser (RVA Model 3D) (Newport Scientific, Warriewood, Australia). Whole sorghum flour (3 g corrected to 14% moisture) was suspended in distilled water and the weight adjusted to 28 g. The pasting profiles for both cycles are described below. The standard RVA profile was adjusted to have a slower heating rate. This was done to clearly observe differences in the pasting profiles of sorghum cultivars differing in endosperm corneousness.

Adjusted Pasting Cycle: The adjusted cycle began with an initial stirring of 960 rpm at 50℃ for 10 s. The stirring speed then decreased to 160 rpm and the sample was held at this speed at 50℃ for 60 s. The speed remained at 160 rpm for the rest of the cycle. The temperature was increased to 91℃ at a rate of 2.0℃/min and held at this temperature for 5 min. The pastes were then cooled to 50℃ at a rate of 6.3℃/min.

4.2.7 Determination of extract content

For the determination of extract content of the sorghums, the following two methods were combined:

[1]Method 4.5.1: Extract of Malt: Congress Mash;
[1]Method 6.6: Extract Content of Maize: Enzymatic Method
(European Brewery Convention (EBC) 1998. Analytica EBC, 5th Edition, Fachverlag Hans Carl, Nurenberg, Germany.

Only limited quantities of sorghum flour from some cultivars were available and these methods were therefore adjusted in order to analyse small sample sizes. The adjusted method was tested for accuracy and repeatability. Two sorghum flour samples and one maize flour sample (obtained from Nile Breweries, Uganda) were analysed according to the combined methods described above. These samples were then analysed again according to the adjusted method and the results were reproduced (Table 4.2).

Table 4.2 Extract content in maize and sorghum as determined by both original and adjusted methods

Sample	Extract (% db)[1]	Extract (% db)[2]
Maize	84.7 (0.1)	85.5 (0.6)
Sorghum 1	72.1 (0.7)	69.6 (0.1)
Sorghum 2	74.5 (1.1)	74.5 (0.6)

[1]Original, combined method
[2]Adjusted method for small sample sizes
[3]Standard deviation

The adjusted mashing procedure was as follows:

Ten g whole sorghum flour was mixed with 58 ml of distilled water and 2 ml $CaCl_2$ solution. Termamyl® SC* (0.02 ml, 120 KNU-S / g) was added, after which the mixture was brought to the boil within 15 minutes. The mixture was then boiled for 15 minutes, after which it was cooled down to 45℃ in a pre-heated water bath. When a temperature of 45℃ was reached, a further 0.1 ml Termamyl® and 50 ml of distilled water was added to the mash. The temperature in the mash was then maintained at 45℃ for exactly 30 minutes with continuous stirring. The temperature of the mash was then raised by 1℃ a minute for 25 minutes to a temperature of 70℃. This temperature was then maintained for a period of 1 h with continuous stirring. The mash was then cooled to 22℃ within 10–15 minutes. The contents of the beaker was then adjusted to exactly 90 g by the addition of distilled water. The contents of the beaker was mixed very well and then transferred to an appropriate sized centrifuge tube. The tube was capped and then centrifuged at 22℃ at 10,000 g for 10 minutes. After centrifugation, the supernatant was decanted very carefully into a clean, dry glass beaker and used to determine the specific gravity.

* *Thermostable α-amylase, kindly donated by Novozymes SA, Marlboro, South Africa.*

4.2.7.1 Determination of specific gravity

A thoroughly dry and clean pycnometer with a volume of 50 ml was weighed to four decimal places. The pycnometer was first washed and then filled with the supernatant, after which it was capped and dried thoroughly. The filled pycnometer was then weighed to four decimal places. This procedure was carried out at 22℃. The following calculation was used to determine the specific gravity:

$$\text{Specific Gravity} = \frac{\text{weight of volume of supernatant in filled pycnometer}}{\text{weight of volume of water in filled pycnometer}}$$

Extract (g extract in 100 g of wort) was calculated using Table 3: Extract in Wort and Beer (American Society of Brewing Chemists (ASBC)) 1976, St Paul, MN).

4.2.8 Statistical Analysis

All sample means refer to the use of two closely agreeing repeated analyses. Sample means were compared using analysis of variance using Fisher's Least Significant Difference Test (LSD).

Correlation Coefficients as well as Principal Component Analyses (PCA) were used to determine the effect of endosperm texture, RVA variables, starch content, presence of tannins, protein content and diastatic power on extract content.

4.3 RESULTS AND DISCUSSION

4.3.1 Endosperm Texture

Of the 43 cultivars analysed, 12 cultivars had floury endosperm texture, 22 had intermediate endosperm texture and 8 had corneous endosperm texture. GP2 had the most corneous endosperm, followed by TX103 and TX115. PAN8568 had the most floury endosperm, followed by PAN8625 and PAN8337 (Table 4.3). Due to TX2907 being a waxy sorghum type, it was not included in Table 4.3 nor anywhere else where endosperm texture was compared to other sorghum characteristics.

Table 4.3 Endosperm textures of the 43 different sorghum cultivars

Cultivar	Endosperm Texture[1]	Cultivar	Endosperm Texture[1]
BANJO	2	PAN8337	1
GP1	1	PAN8358	2
GP2	3	PAN8387	2
GP3	2	PAN8289	1
GP4	2	PAN8407	3
GP5	2	PAN8420	2
GP6	3	PAN8474	1
GP7	2	PAN8507	2
GP8	2	PAN8553(W)	2
GP9	2	PAN8568	1
GP10	3	PAN8609	2
GP11	1	PAN8625	1
GP12	3	PAN8648(W)	2
NS5511	1	PAN8657	2
NS5655	2	PAN8677	1
OVERFLOW	2	PAN8806	2
ORBIT	2	PAN8816	1
PAN8017	2	TX103	3
PAN8127	1	TX115	3
PAN8229	1	TX2907	3
PAN8247	2	TX436	3
PAN8294	2		

[1] Endosperm txture is depicted as being floury (1), intermediate (2) or corneous (3)

4.1.1) Banjo (Intermediate)

4.1.2) GP1 (Floury)

4.1.3) GP2 (Corneous)

4.1.4) GP3 (Intermediate)

Figure 4.1 Photographs of the 43 different longitudinally dissected sorghum cultivars, showing the proportions of corneous and floury endosperm

4.1.5) GP4 (Intermediate)

4.1.6) GP5 (Intermediate)

4.1.7) GP6 (Corneous)

4.1.8) GP7 (Intermediate)

Figure 4.1 (Continued)

4.1.9) GP8 (Intermediate)

4.1.1.10) GP9 (Intermediate)

4.1.11) GP10 (Corneous)

4.1.12) GP11 (Floury)

Figure 4.1 (Continued)

4.1.13) GP12 (Corneous)

4.1.14) NS5511 (Floury)

4.1.15) NS5655 (Intermediate)

4.1.16) Orbit (Intermediate)

Figure 4.1 (Continued)

4.1.17) Overflow (Intermediate)

4.1.18) PAN8017 (Intermediate)

4.1.19) PAN8127 (Floury)

4.1.20) PAN8229 (Floury)

Figure 4.1 (Continued)

4.1.21) PAN8247 (Intermediate)

4.1.1.22) PAN8294 (Intermediate)

4.1.23) PAN8337 (Floury)

4.1.1.24) PAN8358 (Intermediate)

Figure 4.1 (Continued)

4.1.25) PAN8387 (Intermediate)

4.1.26) PAN8389 (Floury)

4.1.27) PAN8407 (Corneous)

4.1.1.28) PAN8420 (Intermediate)

Figure 4.1 (Continued)

4.1.29) PAN8474 (Floury)

4.1.30) PAN8507 (Intermediate)

4.1.31) PAN8553W (Intermediate)

4.1.32) PAN8568 (Floury)

Figure 4.1 (Continued)

4.1.33) PAN8609 (Intermediate)

4.1.34) PAN8625 (Floury)

4.1.35) PAN8648W (Intermediate)

4.1.36) PAN8657 (Intermediate)

Figure 4.1 (Continued)

4.1.37) PAN8677 (Floury)

4.1.38) PAN8806 (Intermediate)

4.1.39) PAN 8816 (Floury)

4.1.40) TX103 (Corneous)

Figure 4.1 (Continued)

4.1.41) TX115 (Corneous)

4.1.42) TX436 (Corneous)

4.1.43) TX2907 (Waxy Sorghum)

Figure 4.1 (Continued)

4.3.2 Extract Content

TX436, GP10 and GP12 had the highest extract contents, within a range of 77.3-78.1% (Table 4.4). PAN8625, PAN8229 and PAN8568 had the lowest extract contents within a range of 46.4-61.0%. All sorghum cultivars with an extract content of 68.0% and lower were tannin-containing cultivars. The low extract content of the tannin sorghums was presumably due to the fact that tannins reduce the digestibility of proteins and carbohydrates (Dykes and Rooney, 2006). Importantly, it has been shown that polyphenols (tannins) present in the testa and nucellar layer of the grain from malt produced from birdproof (tannin) sorghum cultivars had no effect on enzyme production and activity (Daiber, 1975). However, during milling, as the grain is physically disrupted, tannins combine with cellular substances and inactivate endogenous enzymes.

Of the South African cultivars analysed, Orbit, PAN8648W and Banjo had the three highest extract contents at 76.2%, 75.3% and 75.0%, respectively. These cultivars all had intermediate endosperm textures and did not contain tannins.
It can therefore be said that the tannins in the tannin-containing sorghum cultivars bound with the added amylase enzyme, required for starch hydrolysis, rendering it less active and leading to lower amounts of soluble sugars and hence the lower extracts of these cultivars.

Table 4.4 Extract content of the 43 different sorghum cultivars

Cultivar	Extract Content (% db)	Sample	Extract Content (% db)
Banjo	75.0^{o}(0.5)	PAN8337	73.6^{jklmn}(0.3)
GP1	72.5^{j}(0.1)	PAN8358	73.3^{jklmn}(2.0)
GP2	74.1^{klmno}(0.2)	PAN8387	70.4^{hi}(0.1)
GP3	74.3^{mno}(0.4)	PAN8389	69.7^{h}(0.8))
GP4	72.9^{j}(0.3)	PAN8407	72.7^{j}(0.1)
GP5	65.4^{e}(0.8)	PAN8420	74.5^{no}(0.6)
GP6	69.5^{h}(0.9)	PAN8474	63.6^{d}(0.5)
GP7	70.1^{hi}(0.9)	PAN8507	63.4^{d}(0.7)
GP8	76.7^{q}(0.3)	PAN8553W	74.5^{no}(0.2)
GP9	73.0^{jk}(0.2)	PAN8568	61.0^{c}(0.8)
GP10	77.3^{qr}(0.1)	PAN8609	73.1^{jklm}(0.0)
GP11	68.0^{fg}(0.2)	PAN8625	46.4^{a}(2.2)
GP12	77.3^{qr}(0.1)	PAN8648W	75.3^{op}(0.2)
NS5511	70.7^{hi}(0.3)	PAN8657	74.2^{klmno}(0.1)
NS5655	74.5^{no}(0.1)	PAN8677	67.8^{fg}(0.1)
Overflow	74.3^{mno}(0.3)	PAN8806	70.7^{hi}(0.1)
Orbit	76.2^{pq}(0.6)	PAN8816	74.2^{lmno}(0.1)
PAN8017	72.6^{j}(0.1)	TX103	71.2^{i}(0.3)
PAN8127	67.1^{f}(0.1)	TX115	68.3^{g}(0.3)
PAN8229	57.9^{b}(0.9)	TX2907	73.1^{jkl}(0.2)
PAN8247	74.3^{mno}(0.3)	TX436	78.1^{r}(0.2)
PAN8294	72.9^{j}(0.2)		
Mean	71.1		
Minimum	46.4		
Maximum	78.1		

Values given are means and standard deviations of two closely repeatable analyses.

Values followed by different superscript letters are significantly different at p≤0.05

4.3.3 Starch Content

Due to time constraints, only 10 sorghum cultivars were selected for starch content determination. Of the 10 sorghum samples that were analysed, TX436 had the highest starch content at 77.6 g/100 g, while PAN8247 had the lowest starch content at 70.2 g/100 g (Table 4.5). None of the sorghums analysed were tannin-containing.

When extract content and total starch were compared by means of linear correlation, the r-value was 0.478 and not significant ($p > 0.05$). However, TX436 had the highest starch content as well as the highest extract content, which suggests that some relationship may exist between these two variables. If more starch is available for gelatinisation, pasting and subsequent saccharification, more soluble sugars will be present in the extract after mashing. The determination of a relationship by means of a correlation coefficient is a very strong analytical tool, and there may have been a significant correlation between total starch and extract content if time had allowed for more sorghum samples to undergo starch analysis. The range of significant difference in starch content between the sorghums was too small and starch content will therefore not be a good marker for extract.

Table 4.5 Starch content of 10 different non-tannin sorghum cultivars

Cultivar	Starch (g/100g) DB
PAN8247	70.2^{a}(0.8)
GP3	70.8^{a}(1.0)
TX103	71.1^{a}(0.4)
PAN8816	72.8^{ab}(0.9)
TX115	72.8^{ab}(0.6)
GP1	73.2^{ab}(1.2)
TX2907	74.0^{abc}(1.3)
GP2	74.2^{abc}(0.6)
PAN8420	75.5^{abc}(3.0)
TX436	77.6^{c}(4.3)
Mean	73.2
Minimum	70.2
Maximum	77.6

Values given are means and standard deviations of two closely repeatable analyses
Values followed by different superscript letters are significantly different at P≤0.05

4.3.4 Protein Content

GP11 had the highest protein content at 14%, while GP8 had the lowest protein content at 6.3% (Table 4.6). When the correlation coefficient was determined between protein content and extract content, there was a significant negative correlation with an r-value of -0.831 at $p < 0.001$. This correlation indicates that as the protein content of sorghum grain increases, the extract content decreases. It could be argued that as the protein content increased, the total starch available for gelatinisation and subsequent enzymatic hydrolysis decreased, which led to a decrease in the amount of sugars present in the extract after mashing.

Also, because of the strong association between the protein matrix and starch granules in the corneous sorghum endosperm, an increase in protein content could have led to more protein adhering to these starch granules, making them less susceptible for water absorption, gelatinisation, pasting and subsequent enzymatic hydrolysis (Chandrashekar and Kirleis, 1988; Ezeogu et al., 2005). This could then have led to the lower extract content in sorghums with higher protein contents.

Table 4.6 Protein content of 14 different sorghum cultivars

Cultivar	Protein (N x 0.25) (% db)
GP1	10.9(0.3)
GP2	10.0(0.5)
GP3	9.20(0.3)
GP4	7.90(0.1)
GP5	11.8(0.0)
GP6	10.3(0.1)
GP7	11.4(0.5)
GP8	6.50(0.2)
GP9	10.6(0.1)
GP10	6.90(0.3)
GP11	14.4(0.5)
GP12	7.00(0.3)
TX103	11.7(0.1)
TX436	7.70(0.1)
Min	6.5
Max	14.4
Average	9.7

Data courtesy of Dr Janet Taylor of the University of Pretoria

4.3.5 Pasting Properties

The data in Table 4.7 represent the pasting properties of all the sorghum samples that reached a pasting peak (reached a peak viscosity during the heating cycle). There were 10 cultivars of the total of 43 cultivars analysed that did not reach a pasting peak and therefore it was not of any value to include them in the Table as they did not have true peak viscosities and peak temperatures.

TX2907 reached the highest peak viscosity of all the cultivars and was also the only waxy sorghum sample analysed. When TX2907 was excluded from the data range, PAN8625 reached the highest peak viscosity at 104 RVU, followed by PAN8568 at 103 RVU and NS5511 at 100 RVU. PAN8806 had the lowest peak viscosity at 58.3 RVU, followed by PAN8387 at 59.1 RVU and PAN8358 at 61.1 RVU.

Of the three sorghum cultivars that reached the highest peak viscosity of all the samples that pasted, all three were of floury endosperm type. The three sorghum cultivars that reached the lowest peak viscosities of all the samples that pasted were all of intermediate endosperm type. Although a correlation could not be established between peak viscosity and endosperm type, these results suggest that floury endosperm type sorghum samples may have higher peak viscosities than those of intermediate and corneous types. There are, however, many other factors that may influence the pasting behaviour of whole sorghum flour, such as fibre content, starch content, the presence of tannins and the formation of starch-lipid complexes during cooking of starch in water (Morrison et al., 1993; Gelders et al., 2006; Tang and Copeland, 2006; Putseys et al., 2010).

It was expected that sorghum cultivars of floury endosperm type would reach higher peak viscosities than those of intermediate endosperm types and that cultivars of corneous endosperm type would have the lowest peak viscosities. In

the floury endosperm of sorghum, the starch granules are loosely packed with air spaces between them (Seckinger and Wolf, 1973; Chandrashekar and Mazhar, 1999; Duodu, et al., 2002), and therefore more susceptible for water uptake, starch gelatinisation and subsequent pasting. However, in the corneous endosperm of sorghum, the protein matrix form a tight and intricate network around the starch granules present, resulting in less space for water uptake, granule expansion. This, in turn, may lead to a lower level of starch gelatinisation and pasting.

Table 4.7 Peak viscosities, peak times, peak temperatures, pasting times, pasting temperatures and increase times[1] of 31 sorghum cultivars

Sample	Peak Viscosity (RVU)	Peak Time (min)	Peak Temp (℃)	Pasting Temp (ºC)	Pasting Time (min)	Increase Time (min)[1]
PAN8806	58.3^{a} (0.8)	22.0^{nop} (0.1)	91.2^{ghi} (0.1)	76.3^{g} (0.5)	14.2^{f} (0.2)	7.8^{d} (0.3)
PAN8387	59.1^{a} (0.2)	22.5^{ghi} (0.3)	91.0^{fghi} (0.1)	74.0^{e} (0.4)	13.0^{d} (0.1)	9.5^{hi} (0.2)
PAN8358	66.1^{b} (1.1)	21.7^{fghi} (0.0)	91.4^{i} (0.0)	71.0^{a} (0.1)	11.5^{a} (0.0)	10.2^{lmno} (0.0)
PAN8017	70.5^{c} (0.8)	20.8^{de} (0.1)	89.5^{c} (0.3)	75.1^{f} (0.0)	13.5^{e} (0.0)	7.2^{c} (0.1)
PAN8407	70.5^{c} (1.4)	22.5^{ghijk} (0.1)	91.2^{ghi} (0.0)	71.9^{c} (0.5)	12.1^{b} (0.4)	10.4^{nop} (0.3)
PAN8294	72.2^{d} (0.6)	21.0^{fgh} (0.0)	90.1^{d} (0.0)	71.0^{a} (0.1)	11.5^{a} (0.1)	9.5^{gh} (0.1)
TX115	72.8^{d} (0.8)	21.9^{q} (0.0)	91.3^{hi} (0.0)	79.0^{h} (0.0)	15.5^{g} (0.0)	6.4^{b} (0.0)
PAN8677	74.8^{e} (0.9)	21.6^{mnop} (0.1)	91.3^{ghi} (0.1)	71.0^{a} (0.0)	11.5^{a} (0.0)	10.1^{klmn} (0.1)
PAN8657	75.8^{e} (0.4)	21.3^{lmn}o (0.2)	90.4^{de} (0.5)	71.1^{a} (0.1)	11.5^{a} (0.0)	9.7^{hij} (0.2)
PAN8337	76.2^{e} (0.3)	21.8^{fghi} (0.1)	91.3^{hi} (0.0)	71.1^{a} (0.0)	11.6^{a} (0.1)	10.3^{mnop} (0.2)
PAN8420	79.6^{f} (0.2)	22.4^{hijk} (0.2)	91.1^{fghi} (0.1)	71.1^{a} (0.1)	11.5^{a} (0.0)	10.9^{qr} (0.2)
ORBIT	80.5^{f} (0.3)	22.2^{cd} (0.2)	91.2^{ghi} (0.0)	71.6^{bc} (0.7)	11.6^{a} (0.0)	10.6^{pq} (0.2)
PAN8609	82.3^{g} (0.5)	22.1^{jkl} (0.1)	91.3^{ghi} (0.0)	74.1^{e} (0.0)	13.1^{d} (0.0)	9.1^{f} (0.1)
PAN8507	84.0^{h} (1.4)	20.1^{ijk} (0.1)	88.3^{b} (0.2)	71.0^{a} (0.1)	11.5^{a} (0.0)	8.6^{e} (0.1)
PAN8648(W)	85.4^{hi} (0.1)	20.7^{lmn} (0.3)	89.5^{c} (0.6)	71.0^{a} (0.0)	11.5^{a} (0.0)	9.2^{fg} (0.3)
PAN8127	85.7^{i} (1.0)	21.9^{de} (0.0)	91.4^{hi} (0.0)	75.0^{f} (0.0)	13.5^{e} (0.0)	8.4^{e} (0.0)
PAN8553(W)	86.5^{ij} (0.3)	22.7^{ijk} (0.3)	90.9^{efgh} (0.0)	71.1^{a} (0.1)	11.5^{a} (0.0)	11.2^{r} (0.3)
TX103	87.7^{j} (0.3)	21.3^{pq} (0.0)	90.6^{de} (0.0)	75.1^{f} (0.0)	13.6^{e} (0.1)	7.7^{d} (0.1)
PAN8816	89.8^{k} (0.2)	21.9^{opq} (0.1)	90.9^{efgh} (0.1)	71.3^{ab} (0.3)	11.7^{a} (0.1)	10.2^{lmno} (0.2)
TX436	91.3^{kl} (0.5)	23.7^{s} (0.2)	91.2^{ghi} (0.0)	75.1^{f} (0.01)	13.5^{e} (0.0)	10.2^{lmno} (0.2)
O/FLOW	91.7^{l} (1.7)	21.8^{c} (0.1)	91.4^{hi} (0.0)	79.0^{h} (0.0)	15.5^{g} (0.0)	6.2^{b} (0.0)
NS5655	94.3^{m} (0.6)	23.3^{c} (0.0)	91.0^{fghi} (0.1)	79.0^{h} (0.0)	15.5^{g} (0.0)	7.8^{d} (0.0)
PAN8229	94.8^{m} (0.4)	21.7^{ef} (0.0)	91.4^{i} (0.0)	71.0^{a} (0.0)	11.5^{a} (0.0)	10.2^{lmno} (0.0)
BANJO	95.3^{m} (0.8)	22.9^{a} (0.2)	91.3^{ghi} (0.4)	74.1^{e} (0.1)	13.1^{d} (0.3)	9.8^{ijk} (0.1)
PAN8247	95.6^{m} (0.1)	22.0^{fg} (0.2)	91.1^{fghi} (0.4)	72.7^{d} (0.4)	12.4^{c} (0.3)	9.6^{hi} (0.1)
PAN8474	97.6^{n} (0.8)	22.0^{hijk} (0.1)	90.8^{efg} (0.7)	71.1^{a} (0.1)	11.5^{a} (0.0)	10.4^{op} (0.1)
PAN8389	98.4^{no} (0.2)	21.6^{ghij} (0.3)	91.1^{fghi} (0.4)	71.1^{a} (0.0)	11.6^{a} (0.0)	10.0^{jklm} (0.3)
NS5511	99.6^{o} (0.5)	22.8^{b} (0.1)	91.0^{fghi} (0.1)	79.1^{h} (0.1)	15.5^{g} (0.0)	7.2^{c} (0.1)
PAN8568	103^{p} (1.7)	21.4^{ijk} (0.1)	90.8^{efg} (0.3)	71.1^{a} (0.0)	11.5^{a} (0.0)	9.9^{jkl} (0.1)
PAN8625	104^{p} (0.3)	22.4^{klm} (0.1)	91.0^{fghi} (0.0)	71.0^{a} (0.0)	11.5^{a} (0.0)	10.8^{q} (0.1)
TX2907	135^{q} (0.6)	18.8^{r} (0.10)	85.6^{a} (0.3)	75.0^{f} (0.0)	13.5^{e} (0.0)	5.3^{a} (0.1)
Mean	85.7	21.8	90.7	73.28	12.7	9.2
Minimum	58.3	18.8	85.6	71	11.5	5.3
Maximum	135	23.7	91.4	79.1	15.5	11.2

Values given are means and standard deviations of two closely repeatable analyses
Values followed by different superscript letters are significantly different at P≤0.05.
[1]Increase time refers to the time it took for the sample to go from its viscosity increase point to its peak viscosity (i.e. Increase Time = Peak Time – Pasting Time)

Sorghum samples that did not reach pasting peaks were excluded from statistical analysis. TX2907 reached a viscosity of 135 RVU (statistically the highest), whereas PAN8806 reached a viscosity of 58.3 RVU (statistically the lowest) (Table 4.7). TX2907 had the shortest peak time (time it took for sample to reach its peak viscosity), whereas PAN8387 had the longest peak time (22.5 minutes) with a peak viscosity of 59.1 RVU (second lowest, but statistically the same as that of PAN8806) (Figure 4.2).

As stated, TX2907 was the only waxy sorghum in the whole collection. It reached by far the highest peak viscosity within the shortest time. Many important physicochemical, thermal and rheological properties of starch are influenced by its amylose / amylopectin ratio (Fredriksson et al., 1997; Sang et al., 2008) as well as its amylopectin molecular structure (Sang et al., 2008) During gelatinisation, when the starch granules absorb water and swell, the swollen granules are enriched with amylopectin while the linear amylose molecules tend to diffuse out of the granules (Hermansson and Svegmark, 1996). The starch of waxy sorghum consists essentially of amylopectin and therefore the high peak viscosity reached by TX2907 is probably due to its high amylopectin content. Amylopectin also gelatinises faster and at lower temperatures than does amylose (Figueroa et al., 1995), and the short pasting time of TX2907 could therefore also be attributed by its high amylopectin content.

In a study conducted by Sang et al. (2008) where sorghum starch of different amylose contents were isolated and the pasting curves determined by use of an RVA, similar results were found. The waxy and heterowaxy sorghum starches had higher peak viscosities (as well as lower pasting temperatures) than the starch from the normal sorghum cultivar.

Although the pasting temperature (the temperature where the initial increase in viscosity is detected within the sample) is not the same as the gelatinisation temperature, it can be used as an indication of the temperature range in which

the starch granules start to absorb water and swell. In the case of the waxy sorghum (TX2907), the pasting temperature was 75℃. This information is extremely important to the brewer, as lower gelatinisation temperatures (and subsequent pasting temperatures) will result in lower energy inputs and therefore will be more economical. However, other attributes such as peak viscosity, peak time, peak temperature and increase time are also important, and a combination of the best of these will be of significance for use as a brewing adjunct. For example, a cultivar with a high peak viscosity, a short peak time, low peak temperature but a very long increase time will be less valuable than a cultivar with a slightly lower peak viscosity and a higher peak time but with a very short increase time.

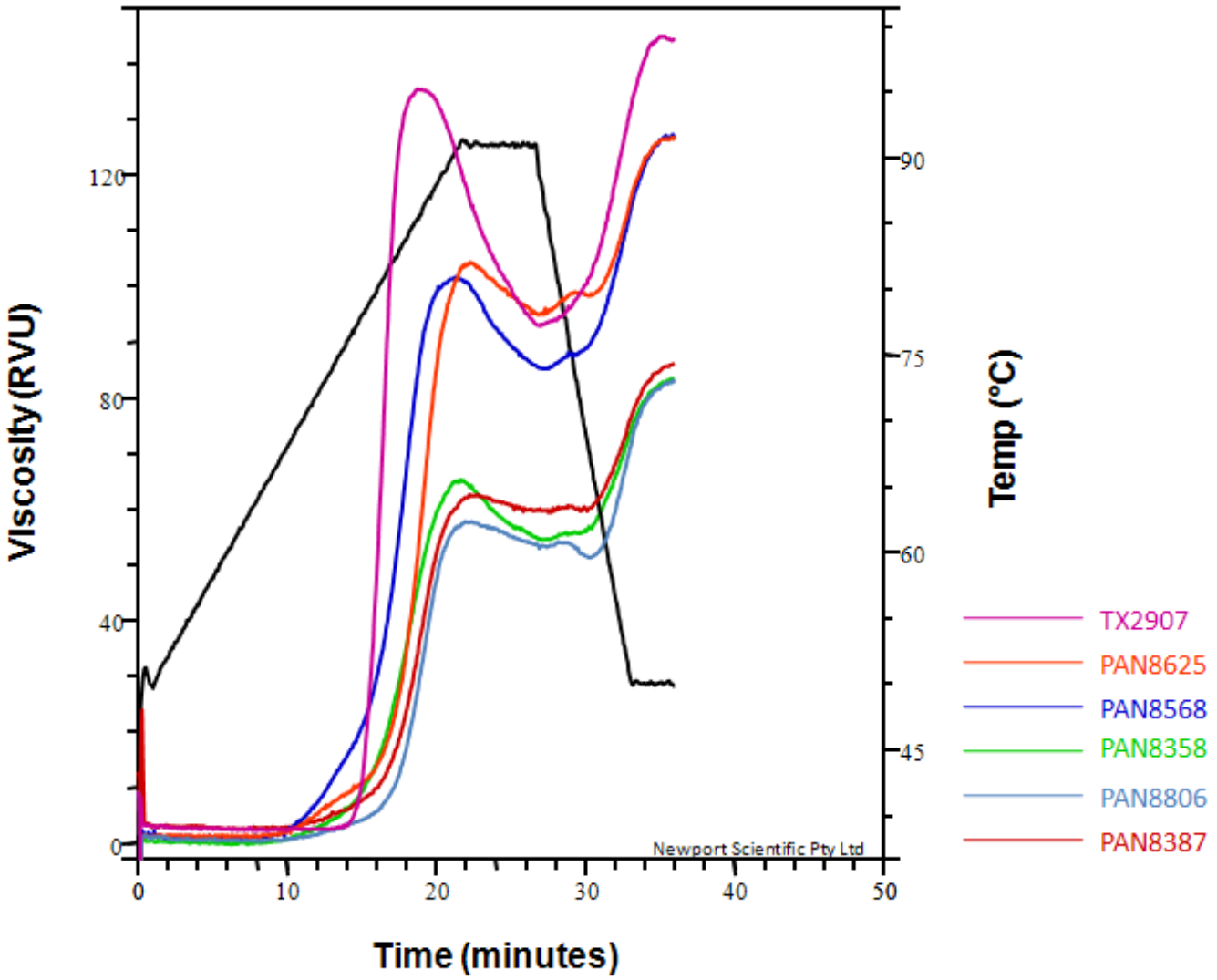


Figure 4.2 **RVA pasting profiles of the three sorghum cultivars that reached the highest peak viscosity and the three sorghum cultivars that reached the lowest peak viscosity of all the samples that pasted**

All the GP samples (Figure 4.3) did not reach a peak viscosity during the heating cycle, which indicates that there was limited starch pasting concerning these samples. In Figure 4.3, the curve of PAN8625 (with floury endosperm) represents the overall behaviour of sorghum samples that pasted. GP1, which had the highest peak viscosity of all the GP samples, was the only cultivar of floury endosperm type. All the other GP samples had either an intermediate or corneous endosperm texture. Further, all the GP samples depicted in Figure 4.3 had very long pasting times and very high pasting temperatures. This indicates that starch gelatinisation and subsequent pasting was delayed. Since the GP samples represent floury, intermediate and corneous endosperm textures, it is not likely that the corneousness of the endosperm is the reason for late and incomplete pasting as initially expected. GP11, which reached the third lowest viscosity, was the only sample represented in Figure 4.3 that was tannin-containing.

All of the GP samples analysed in this research behaved in the same way in that they did not paste, i.e. they did not reach a peak viscosity during the heating cycle. All of the GP samples were received pre-milled and had been stored air-tight at 5℃ for 4-6 months, whereas all the other sorghums analysed were milled and stored at 5℃ for only days. The only attribute that the GP samples had in common was the fact that they were pre-milled and had been stored for some time, therefore it can be possible that during storage the flour may have undergone some chemical changes, which changed the nature of the behaviour of the flours of these sorghums during the pasting cycle.

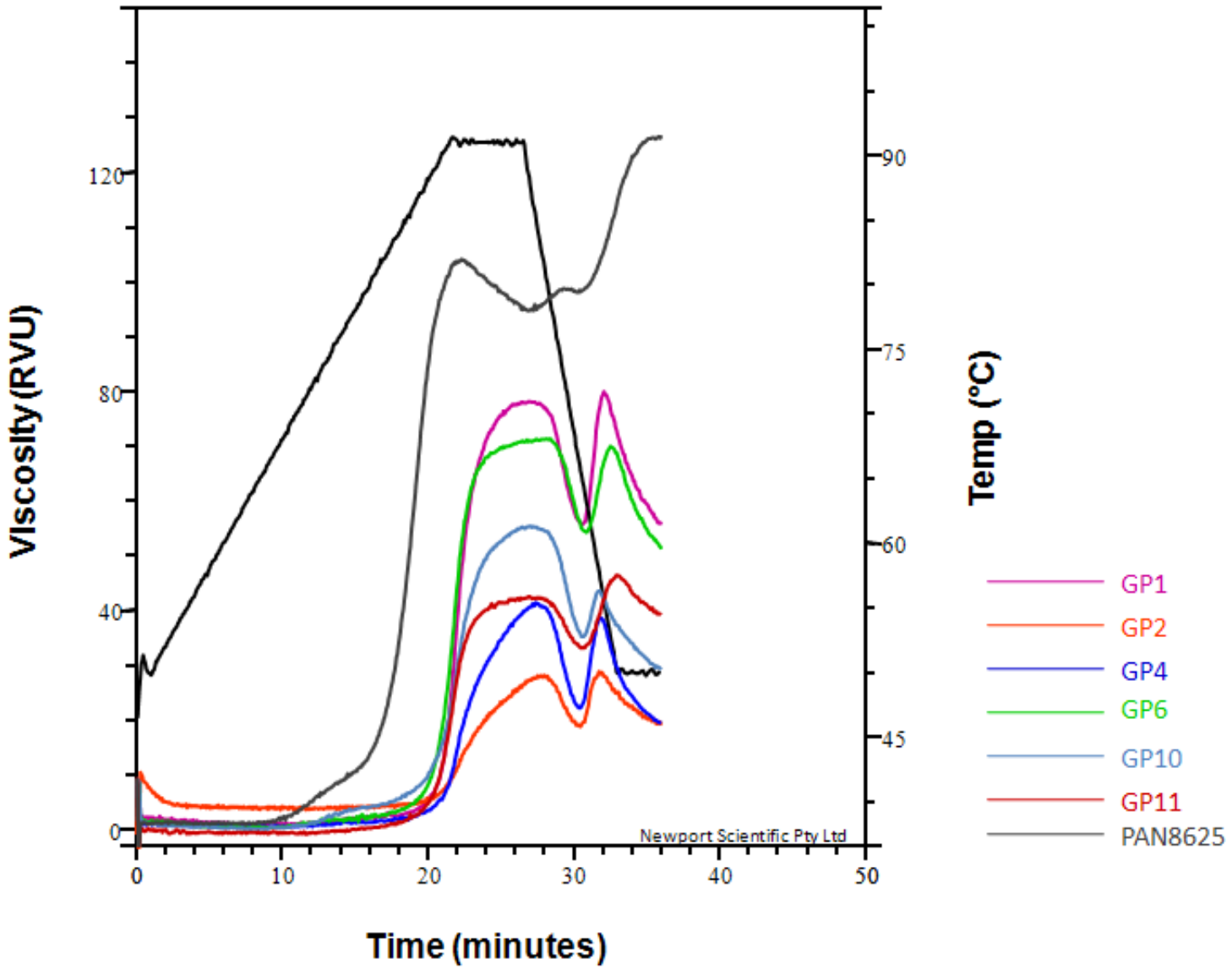


Figure 4.3 RVA pasting profiles of some GP sorghum cultivars and PAN8625 (a floury sample)

The four sorghum cultivars received from Texas A&M University were compared in one group (Figure 4.4) as none contained tannins, all had corneous endosperm textures (except the waxy sorghum), one was a waxy sorghum with low protein digestibility (TX2907), two had high protein digestibilities (TX103 and TX115) and one had low protein digestibility (TX436).

As stated, the reason for the high peak viscosity and short pasting time of TX2907 was that it is a waxy sorghum, therefore its starch consisted essentially of amylopectin. TX103 and TX115 which had the two lowest peak viscosities of the 4 Texas A&M sorghums both had high protein digestibilities, whereas TX2907 and TX436 had low protein digestibilities. In sorghum, protein has an influence on both starch gelatinisation and digestibility (reviewed by Duodu et al., 2003). Starch granules in sorghum corneous endosperm are polygonal and covered with protein matrix (Hoseney et al., 1974). The tight adherence of the protein network to the starch granules causes limited water absorption, gelatinisation and subsequent pasting of the starch granules (reviewed by Duodu et al., 2003).

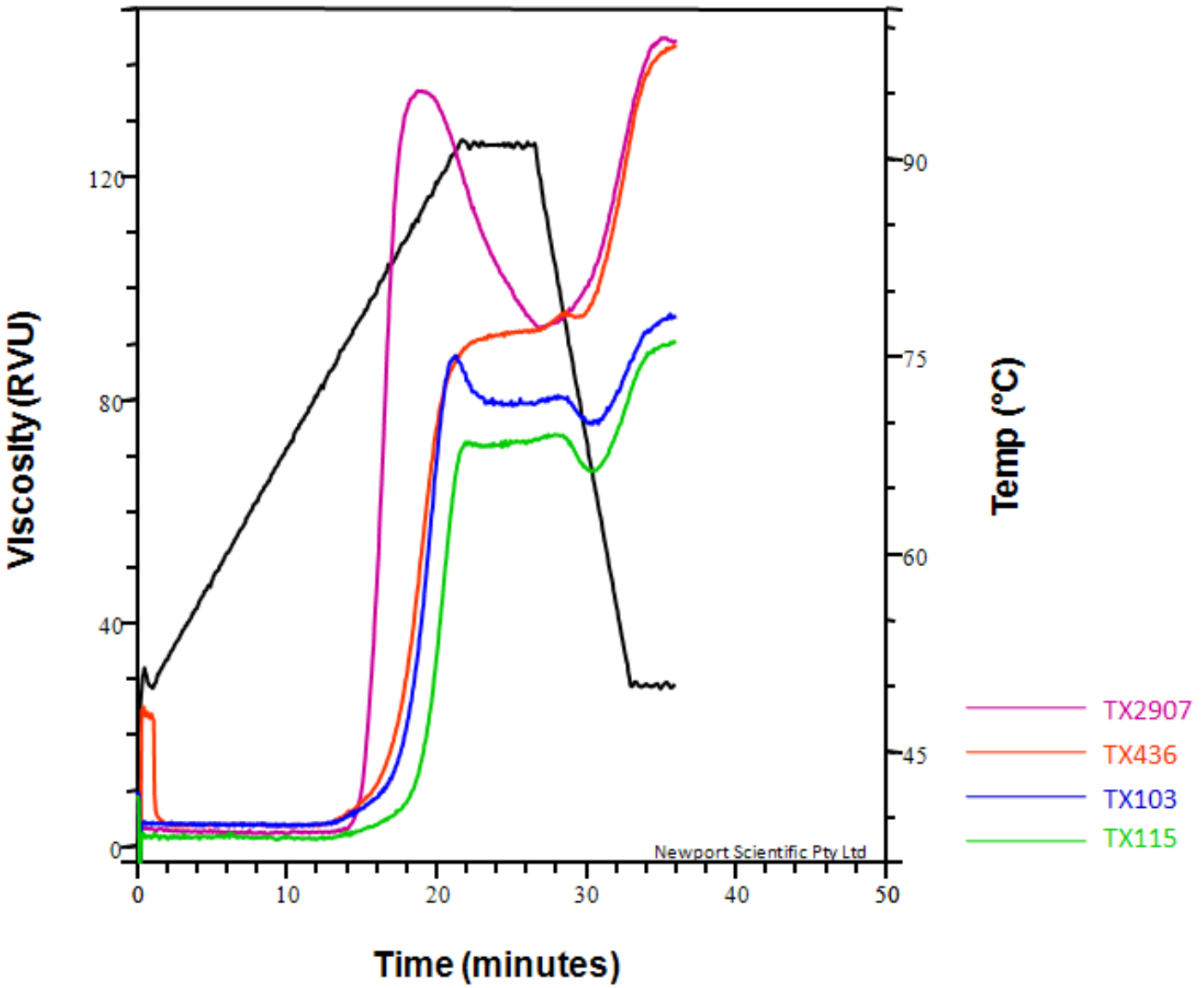


Figure 4.4 RVA pasting profiles of the four Texas A&M samples

Of the four sorghum types represented in Figure 4.5, TX436 reached the highest peak viscosity, followed by TX115, GP6 and GP10. GP6 and GP10 did not reach their peak viscosities during the heating cycle and therefore did not paste. TX436 and GP10 had the two highest extract contents of all 43 sorghum cultivars analysed, whereas TX115 and GP6 had the two lowest extract content.

Sorghums that contained tannins were excluded from Figure 4.5. This is because tannins bind to protein, causing the protein to alter shape and subsequently the protein cannot carry out its function (Emmambux & Taylor, 2003). In the case of tannin presence during mashing, the tannins inactivate the amylase enzymes (Daiber, 1975) which are crucial for the hydrolysis of starch into sugars, leading to incomplete saccharification and therefore a wort of poor quality.

It was expected that sorghum that reached higher peak viscosities would have higher extract contents, as more starch would have been available after more complete pasting for saccharification during the mashing procedure. On the contrary, the cultivars that reached the highest extract content did not have the highest peak viscosities and vice versa.

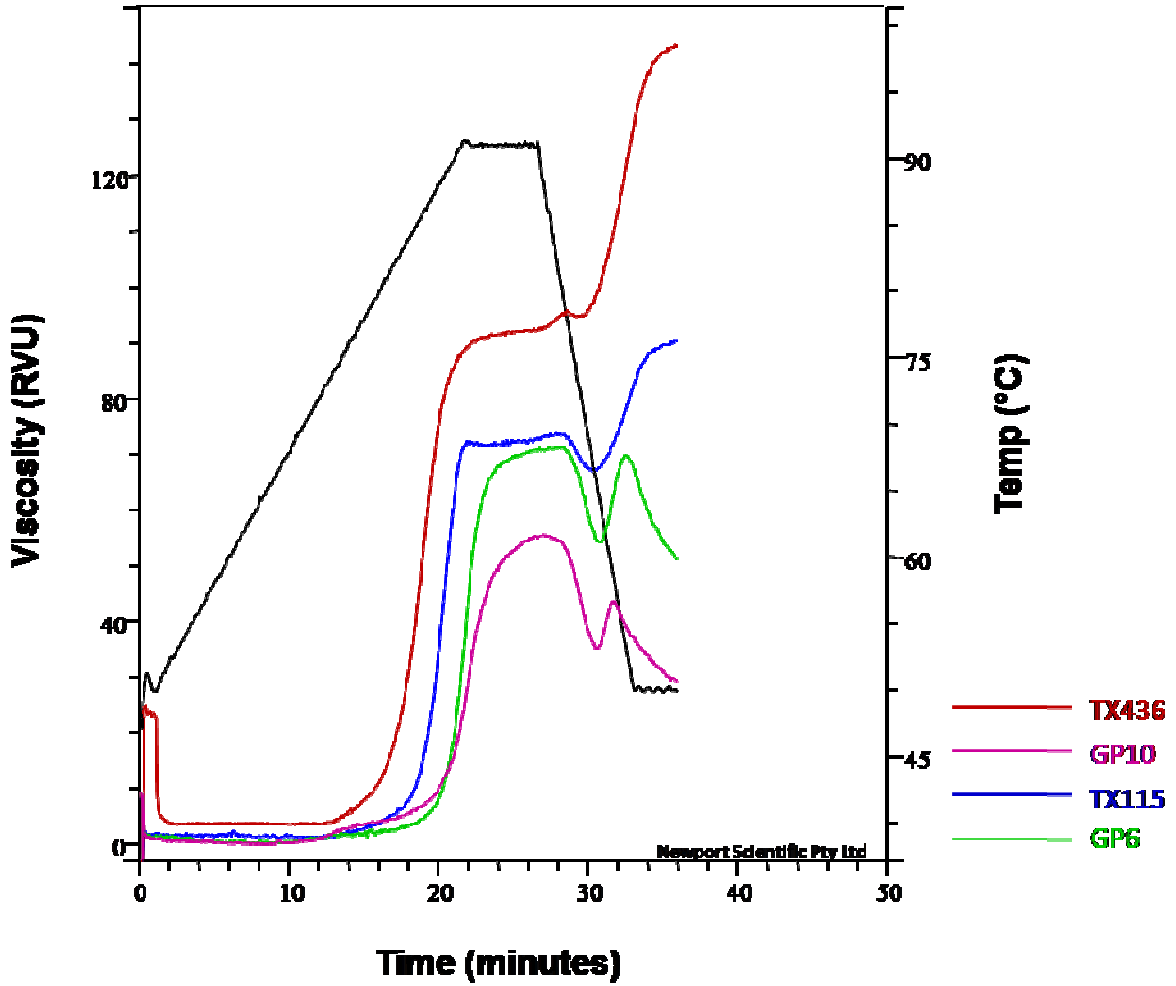


Figure 4.5 **RVA pasting profiles of the two sorghum cultivars that had the highest extract contents (TX436 & GP 10) and the two sorghum cultivars that had the lowest extract contents (TX115 & GP6) of all the non-tannin cultivars**

4.3.6 Tannins

Tannin sorghums gave substantially lower extracts than non-tannin sorghums (See Table 4.4). Tannins react with proteins to form insoluble complexes (Ali et al., 2008). This results in the protein losing its functional property, and therefore the amylase enzymes, which are proteins, are rendered inactive and cannot hydrolyse starch molecules into soluble sugars (Nguz et al., 1998).

Malt of PAN8507 had the highest diastatic power (DP) at 71 SDU/g, followed by PAN8625 and PAN8229 at 65 SDU/g (Table 4.8). All three these sorghums were tannin-containing. PAN8407 had the lowest diastatic power at 24 SDU/g, followed by Banjo at 25 SDU/g and PAN8648(W) at 26 SDU/g. All three of these sorghums did not contain tannins.

During analysis of sorghum malt for diastatic power, the sorghum flour is extracted with a solution of peptide (hydrolysed protein) which bind to the tannins in the sorghum and renders them inactive (SABS 235, 1970).

When a correlation coefficient was determined between extract content and DP of non-tannin sorghums, there was no relationship at the 0.500, 0.050 or 0.001 level of significance. However, when looking at the data represented in Table 4.8 it was clear that sorghum malts that contain tannins gave higher DP than sorghum malts without tannins – provided that the tannins are inactivated.

Table 4.8 Malt diastatic power, extract content and presence of tannins of 25 different sorghum cultivars

Sample	Malt Diastatic Power (SDU / g malt)[1]	Grain Extract Content (% db)	Tannins
PAN8407	24	72.7^{j}(0.1)	N
Banjo	25	75.0^{o}(0.5)	N
PAN8648W	26	75.3^{op}(0.2)	N
Overflow	27	74.3^{mno}(0.3)	N
PAN8553W	28	74.5^{no}(0.2)	N
PAN8387	30	70.4^{hi}(0.1)	N
PAN8337	35	73.6^{jklmn}(0.3)	N
NS5655	38	74.5^{no}(0.1)	N
PAN8017	38	72.6^{j}(0.1)	N
PAN8247	41	74.3^{mno}(0.3)	N
PAN8806	42	70.7^{hi}(0.1)	N
PAN8420	44	74.5^{no}(0.6)	N
PAN8474	44	63.6^{d}(0.5)	Y
PAN8609	46	73.1^{jklm}(0.0)	N
PAN8657	46	74.2^{klmno}(0.1)	N
PAN8358	48	73.3^{jklmn}(0.5)	N
PAN8816	49	74.2^{lmno}(0.1)	N
PAN8568	53	61.0^{c}(0.8)	Y
PAN8389	55	69.7^{h}(0.8)	Y
PAN8677	60	67.8^{fg}(0.1)	Y
NS5511	63	70.7^{hi}(0.3)	Y
PAN8127	64	67.1^{f}(0.1)	Y
PAN8229	65	57.9^{b}(1.0)	Y
PAN8625	65	46.4^{a}(2.1)	Y
PAN8507	71	63.4^{d}(0.7)	Y
Mean	45	69.8	
Minimum	24	46.4	
Maximum	71	75.3	

[1]Data courtesy of Ms C Chiremba (Research Scientist, Agricultural Research Council, Grain Crops Institute, Potchefstroom)
Values given are means and standard deviations of two closely repeatable analyses.
Values followed by different superscript letters are significantly different at P≤0.05

4.3.7 Influence of endosperm texture and pasting properties on sorghum hot water extract

When extract content and the RVA variables were compared by means of correlation coefficients between only the samples that pasted (reached peak viscosity during the heating cycle) some significant correlations were found. There were significant negative correlations at $p<0.05$ between extract content and pasting temperature and time in corneous endosperm samples with r-values of -0.939 and -0.941, respectively (Table 4.9). These correlations indicate that in sorghums with corneous endosperm that paste, the pasting time and temperature should increase as the extract content decreases. This correlation indicates that more time and energy is needed for these sorghums to paste, and the more time and energy is needed, the lower the extract is expected to be. In sorghum cultivars with corneous endosperm, there is more interaction between protein and starch because of the tight protein matrix that surrounds the starch granules in the corneous part of the endosperm (Duodu et al., 2003). The starch granules in these cultivars may therefore be less susceptible for water uptake and gelatinisation, leading to less cooked starch available for enzymatic hydrolysis during mashing and subsequently lower extract contents.

The correlation between pasting time and pasting temperature was positive and highly significant at $p<0.001$ when sorghums of all endosperm types were considered. It can therefore be assumed that the longer it takes for any sorghum sample to reach an increase in viscosity, the higher the temperature will be at which this happens. Zhao et al., (2008) also used an RVA for the determination of bioethanol producing quality of sorghum cultivars. They similarly found that peak time, peak viscosity and final viscosity were highly correlated with each other

Table 4.9 Significant correlation coefficients between RVA variables and extract content of 31 different sorghum cultivars that pasted (reached a peak viscosity during the heating cycle)

	Endosperm texture	Extract content (% db)	Peak viscosity (RVU)	Peak time (min)	Pasting temp (℃)	Pasting time (min)
Peak temp (℃)	Floury		-0.632*			
	Intermediate			0.805***		
	Corneous		-0.953*	0.893*		
Pasting temp (℃)	Floury			0.723*		
	Intermediate					
	Corneous	-0.939*				
Pasting time (min)	Floury			0.724*	1.000***	
	Intermediate				0.999***	
	Corneous	-0.941*			1.000***	
Increase time (min)	Floury				-0.968***	-0.968***
	Intermediate				-0.795***	-0.800***
	Corneous					

*p<0.05, **p<0.005, ***p<0.001

[1]Increase Time: time (min) for the sample to go from its initial increase in viscosity to its final increase in viscosity, i.e. Increase Time = Peak Time – Pasting Time

4.3.8 Principal component analysis of different sorghum physico-chemical characteristics and hot water extract

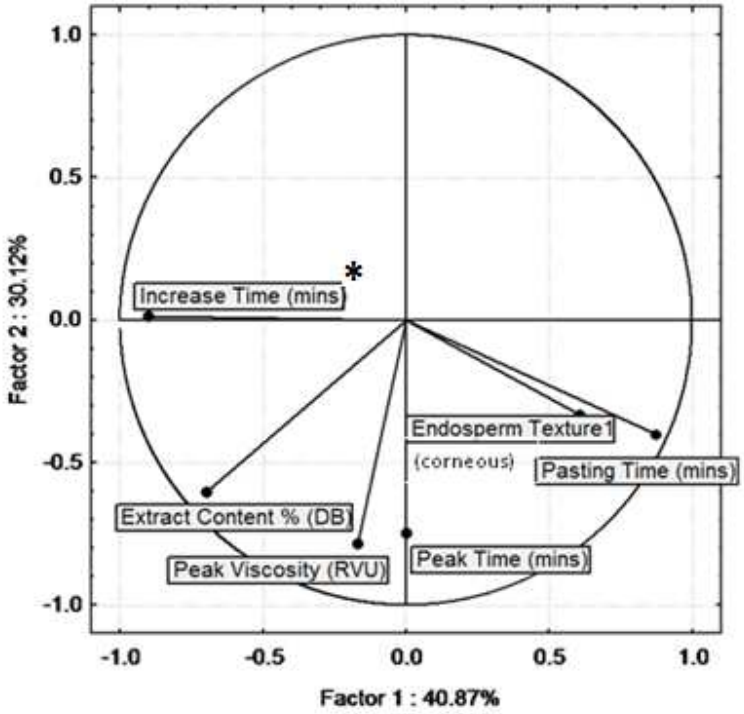
Sorghums that were tannin-containing were excluded from this analysis as the relationship between tannins and extract had already been established and discussed under heading 4.3.6. Sorghums that did not paste were also excluded from this analysis. TX2907 was also excluded from this analysis as it is a waxy sorghum.

Figure 4.6 shows the variable factor projections of the 20 sorghum cultivars that were included in this analysis. It seems that a relationship existed between peak viscosity and extract content, where samples that reached higher peak viscosities can be expected to yield higher extract. When looking at the corresponding data range in Figure 4.6, where the cases (samples) are represented on axes, there are 8 cases (cultivars) that are situated in the top left quadrant (Group 1), 5 cases in the bottom left quadrant (Group 2), 3 in the bottom right quadrant (Group 3) and 5 in the top right quadrant (Group 4) (Table 4.10).

The cases in each specific quadrant (Figure 4.6) contributed in similar ways to the variable that is projected on that specific quadrant in Figure 4.7. This indicates that in Group 2 all sorghums contributed to extract content and peak viscosity. TX436 is situated the furthest from the central line of the horizontal axis in Figure 4.7, and therefore had the greatest contribution to these variables, followed by BANJO, PAN8624, PAN8553W and PAN8420. TX436 (Group 2) had the highest extract content of all the samples in this group at 78.1%, followed by Banjo at 75.0%. PAN8247 had the highest peak viscosity at 96 RVU, followed by Banjo at 95 RVU. When the correlation coefficient was determined between extract content and peak viscosity within the whole data set (20 cases), there was a significant positive correlation with an r-value of 0.611 at $p < 0.005$. A higher peak viscosity indicates a higher degree of gelatinisation and pasting, i.e.

more starch granules that could be solubilised (Puspitowati and Driscoll, 2007). Increased starch solubilisation will result in an increase in the amount of solubilised starch available for hydrolysis by amylase enzymes during mashing. This could, in turn, lead to an increase in the amount of soluble sugar present in the wort and the hot water extract should therefore be higher.

These results suggest that non-tannin sorghums that gave high peak viscosity during pasting gave high extract. Therefore peak viscosity in non-tannin sorghums could possibly be used as a predictive marker for extract.



* Increase Time refers to the time it took for the sample to go from its initial increase in viscosity to its peak viscosity during the heating cycle.

Figure 4.6 Variable projections after PCA on the samples that pasted (reached a pasting peak during the heating cycle)

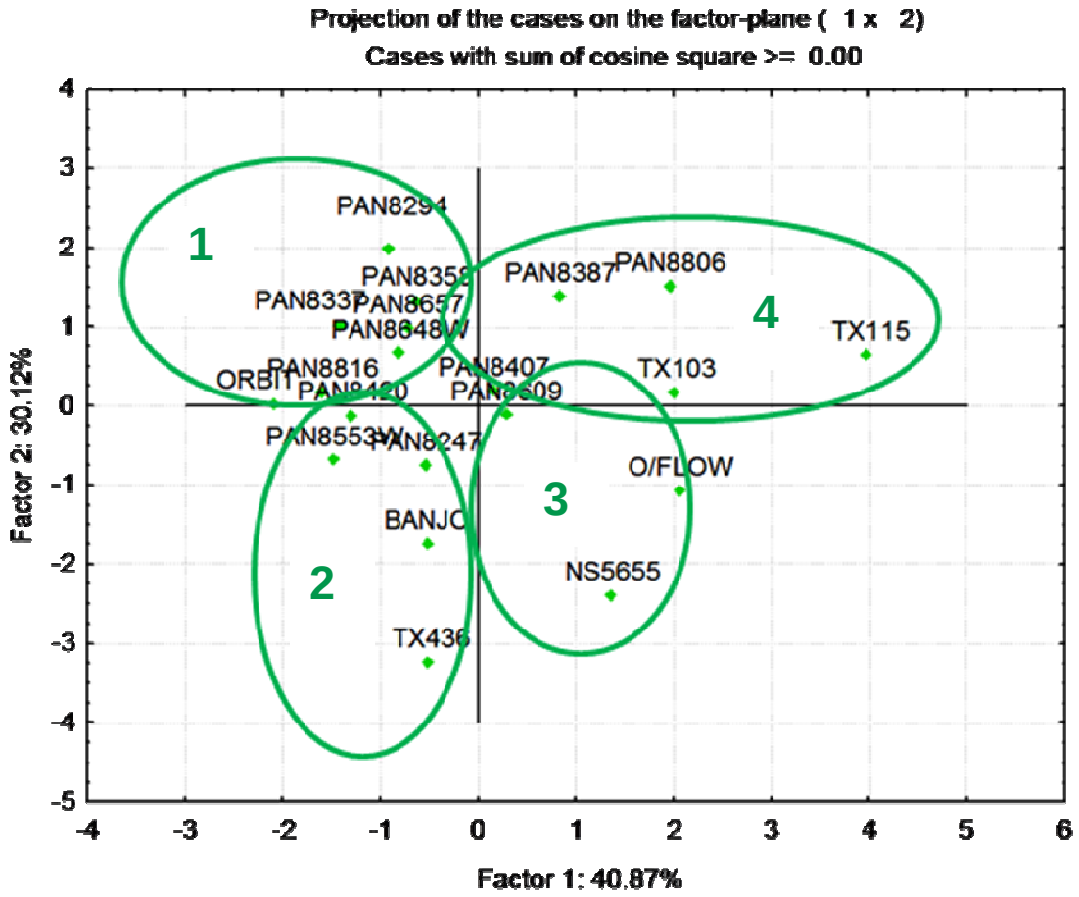


Figure 4.7 Sample (case) projections after PCA on the samples that pasted (reached peak viscosity during heating cycle

Table 4.10 Groups corresponding to the data in Figure 4.7 as per each quadrant

	Cultivar	Extract content % (DB)	Peak viscosity (RVU)	Peak time (min)	Pasting time (min)	Increase time (min)	Endosperm texture[1]
Group 1	Orbit	76.2	80.46	22.17	11.6	10.6	1
	PAN8294	72.9	72.22	21.00	11.5	9.5	1
	PAN8337	73.6	76.21	21.83	11.6	10.3	1
	PAN8358	73.3	66.13	21.70	11.5	10.2	2
	PAN8648W	75.3	85.42	20.70	11.5	9.2	2
	PAN8657	74.2	75.79	21.27	11.5	9.7	2
	PAN8816	74.2	89.75	21.87	11.7	10.2	1
Group 2	Banjo	75.0	95.29	22.94	13.1	9.8	2
	PAN8247	74.3	95.63	21.97	12.4	9.6	2
	PAN8420	74.5	79.63	22.40	11.5	10.9	2
	PAN8553W	74.5	86.46	22.70	11.5	11.2	2
	TX436	78.1	91.30	23.74	13.5	10.2	3
Group 3	NS5655	74.5	94.25	23.33	15.5	7.8	2
	Overflow	74.3	91.67	21.77	15.5	6.2	2
	PAN8609	73.2	82.34	22.14	13.1	9.1	2
Group 4	PAN8387	70.4	59.09	22.50	13.0	9.5	2
	PAN8407	72.7	70.54	22.47	12.1	10.4	3
	PAN8806	70.7	58.34	21.97	14.2	7.8	2
	TX103	71.2	87.71	21.27	13.6	7.7	3
	TX115	68.3	72.84	21.93	15.5	6.4	3

[1]Endosperm Texture: 1=Floury, 2=Intermediate, 3=Corneous

4.3.9 Conclusions

Protein content is an excellent predictive marker for extract in whole sorghum flours. Sorghums with corneous endosperm that reach low peak viscosity are expected to have lower extract contents than those that reach higher peak viscosity. In tannin-free sorghums, peak viscosity could also be used as a predictive marker for extract as higher peak viscosities in these sorghums are expected to produce higher extracts. Tannins inactivate sorghum amylases important for saccharification of the starch during mashing, and should be inactivated with a pre-treatment should these be considered for use as brewing adjuncts.

5 DISCUSSION

This chapter provides a critical review of the methodology as applied during this research. It discusses the relationship between extract content and various physico-chemical characteristics of the 43 different sorghum cultivars. It also proposes how these physico-chemical characteristics may be used as predictive markers for sorghum extract content in the beer brewing industry

5.1 REVIEW OF METHODOLOGY

The methods followed during research in this project are critically discussed below. Any impact(s) these methods may have had on the results are also discussed.

During the analysis of sorghum grain endosperm texture, the sorghum grains were cut longitudinally, and the proportion of corneous to floury endosperm was estimated visually. Therefore each sorghum cultivar could only belong in one of three classes, namely floury, intermediate and corneous. Methods for the determination of the endosperm corneousness of cereals quantitatively are available, for example Digital Image Analysis. This method has been applied elsewhere for the determination of the endosperm corneousness of maize (Erasmus and Taylor, 2004). If such a technique could have been applied in this research, there would have been a definite value for the corneousness of each sorghum cultivar instead of the sorghum cultivars belonging to only one of three classes. These values could have provided the researcher with the possibility of doing correlation coefficients between endosperm texture and hot water extract with other physico-chemical properties which, in turn, could have provided for improved statistical data.

The RVA was used to determine the pasting curves and all data associated with the pasting of the whole sorghum flours. During the lager brewing process, information such as gelatinisation and pasting temperatures is essential for the selection of appropriate brewing adjuncts (Briggs et al., 2004). Usually adjuncts used in simple infusion mashing processes have low gelatinisation temperatures or have been pre-cooked in order to pre-gelatinise some of the starch. The pasting curve and the information obtained from it may therefore be an indicator as to how an adjunct (in this research whole sorghum flour) will behave during mashing. The normal RVA pasting cycle, which is usually used for starch or flour suspensions, was adapted in order to obtain pasting information critical to the brewer. The RVA has been used recently elsewhere (Zhao et al., 2008) to establish a relationship between RVA data and ethanol yield of grain sorghum for fuel ethanol production. As stated, these researchers found a strong linear relationship between ethanol yield and final viscosity as well as setback.

In this research, only RVA data contributing to the initial heating cycle (up to pasting time and temperature) was considered to establish a possible relationship with regards to hot water extract. It was hypothesised that sorghum flours that reached higher peak viscosities would have higher hot water extracts. However, statistically valid relationships could only be established between hot water extract and peak viscosity in non-tannin sorghum cultivars.

The samples were heated to 91℃ as the samples would boil at 95℃ as a result of the low atmospheric pressure where the research was carried out (at a level of approximately 1 400 m above sea level). The pasting cycle was adjusted to reduce the heating rate from the normal 6℃/min to 2℃/min until a temperature of 91℃ was reached (AACC 76-21, 2000). This was done in order to observe the behaviour of gelatinisation and initial pasting of the flours more closely. This information is crucial, as it could predict the quality of the extract that will be obtained after mashing, as well as the economical impact on the process. Although the values obtained from the RVA curves were repeatable, 12 of the 43

different sorghum cultivars did not paste during the heating cycle. The question lies here as to whether the pasting cycle should be adjusted again to include an even slower heating rate and to possibly extend the holding cycle. This extra time could allow for improved starch granule expansion and complete pasting.

Concerning the determination of extract content, this involves the gelatinisation of starch present in the starch granules, followed by saccharification of the starch into soluble sugars (Briggs et al., 2004). Some literature is available on the extract content of sorghum grain, sorghum malts and triticale as these cereals are becoming more popular for use as brewing adjuncts (Agu, 2002; Odibo et al., 2002; Del Pozo-Insfran et al., 2004; Glatthar et al., 2005; Ogu et al., 2006).

The use of a small-scale method for extract determination could prove to be very useful in the beer brewing industry, as well as where research in this area is conducted. Apart from the method using less energy, there are other factors to consider. The expenses involved in breeding programmes are enormous, and therefore the research that has to be conducted on such samples are extremely carefully considered and executed. Such a small-scale method for the determination of hot water extract will provide the researcher with the opportunity to use much less sample than usually required. In the standard method, sample sizes of 55 g are required, compared to the 10 g required for the small-scale method.

Concerning the use of pycnometry, this procedure is extremely sensitive and must be carried out with the outmost precision. Should the small-scale method described here be implemented for use in the industry, an alternative method could be considered for the determination of the extract yield. A proposed method is refractometry, which is a fast and non-destructive refractive index measurement where the sugar concentration in a sample can be easily calculated and only a small amount of sample is needed.

In this research, sorghum flour was mixed with distilled water after which a calcium chloride solution was added to stabilise the amylase. The enzyme solution was then added (Termamyl® SC) and the mixture was cooked for 15 minutes to gelatinise the starch. Some saccharification took place at this stage, as the viscosity of the cooking porridge decreased drastically after approximately 5 minutes of cooking in the presence of the amylase enzyme. The porridge was cooled down to 45℃, more Termamyl ® SC was added, and the temperature was then raised to carry out the rest of the mashing procedure at 70℃ for 1 h.

When cereal adjuncts such as sorghum flour are used for lager beer brewing, little or no endogenous enzymes are available in the grain for starch saccharification (Taylor & Robbins, 1993). Therefore exogenous amylase enzymes such as Termamyl® SC are added to enable starch hydrolysis into fermentable sugars. Enzyme concentration used in this research was 120 KNU-s (α-amylase units) / gram of Termamyl® SC. The extent of starch solubilisation and hydrolysis during this research by the added amylase enzymes was not determined. However, if some starch did not solubilise and hydrolyse during the process, the enzyme concentration could possibly be increased. It is, however, unlikely that this was the case during this research, as the amount of enzyme used was standard (EBC 5.5.1; 6.6, 1998).

There are other limiting factors when using whole sorghum flour as a brewing adjunct, for example its high gelatinisation temperature. Temperatures that are needed for starch gelatinisation in sorghum flours may be as high as 70℃ (Taylor et al., 1997). Therefore heat-stable amylases are an absolute necessity during mashing. Complete starch gelatinisation and pasting is essential during adjunct mashing (Briggs et al., 2004) in order to provide sufficient substrate for subsequent fermentation. Should all the starch present in the flour not have solubilised and pasted, increased quantities of enzyme will not increase extract content. Some of the sorghum samples in this research had a very high viscosity during the cooking stage, just before the first addition of exogenous enzyme,

which may also contribute to incomplete starch solubilisation because of the lower heat transfer efficiency of viscous solutions(Zhao et al., 2008). Another factor that may limit the extent of starch solubilisation and pasting is the physical structure of the sorghum grain endosperm. The configuration of the sorghum endosperm differs from that of other cereals in that the starch granules are surrounded by a strongly disulphide bonded protein matrix (Duodu et al., 2003) with strong interactions that intensify upon cooking (Hamaker et al., 1994). When the protein matrices intensify upon cooking, there is physically less space for the starch granules to absorb water and swell, and subsequently less starch leaches out of the granules for enzymatic hydrolysis. This may result in such sorghum samples providing less fermentable sugars during brewing.

Odibo et al. (2002) described a procedure for the mashing of sorghum with a combination of different enzymes, including Termamyl 120L (thermostable α-amylase), AMG 200L (Amyloglucosidase), neutral protease and Cereflo (β-glucanase). In their research, the sorghum was milled and mixed with distilled water and calcium chloride (0.1 g/L). This was followed by protein digestion and β-glucan degradation before starch saccharification was carried out. Although not the same cultivars were analysed as in this research, the sorghums apparently reached slightly higher extracts. This may be due to the proteolytic enzymes that were added, which may have hydrolysed the protein matrices that surround the starch granules, rendering more starch available for solubilisation and subsequent hydrolysis by the amylase enzymes.

5.2 DISCUSSION OF FINDINGS

As stated, the main criterion for the quality of an adjunct for its use during brewing is its hot water extract, or the amount of fermentable sugars that can be obtained from it during mashing (Letsididi et al., 2008). Research has shown that the tannins in tannin-containing sorghum cultivars act as inhibitors of amylases (Daiber, 1975). It has been established in this research that the tannins in tannin-containing sorghum cultivars result in those cultivars giving a substantially lower hot water extract due to the tannins binding to the amylolytic enzymes. Inactivation of tannins is possible by steeping the grain in diluted solutions of formaldehyde or NaOH (Beta et al., 2000).

In this research, no significant correlations could be found between hot water extract and sorghum starch content. However, only ten of the 43 sorghum cultivars were analysed for the determination of a relationship between total starch and extract due to time constraints. Notably, the cultivar that yielded the highest extract content also had the highest starch content, which suggests that some relationship may exist between total starch and extract.

A negative correlation coefficient with a highly significant r-value of -0.831 at $p < 0.001$ was obtained between grain protein content and sorghum hot water extract. Sorghum cultivars with higher protein contents could exhibit a higher degree of matrix formation around starch granules in the endosperm (Hoseney et al., 1974). This interaction would suppress starch solubilisation and hydrolysation, resulting in a lower content of solubilised sugars available for fermentation. No literature could be found on the relationship between protein content and hot water extract.

There were also significant negative correlations at $p<0.05$ between extract content and pasting temperature and time in corneous endosperm samples with r-values of -0.939 and -0.941, respectively, suggesting that the more energy is

needed to paste a given sorghum sample of corneous endosperm, the lower its extract would be. Related to this, there was a significant positive correlation with an r-value of 0.999 at $p < 0.001$, between pasting time and pasting temperature in sorghums of all endosperm types. This suggests that when more energy is needed for the starch granules to expand and for the starch molecules to solubilise, the longer this process will take. As more and more energy is applied during the heating cycle to initiate pasting, the temperature at which the starch starts to paste should increase.

Comparing all the non-tannin sorghum cultivars with the use of PCA, suggested that a relationship existed between extract and pasting peak viscosity. A significant positive correlation with an r-value of 0.611 at $p < 0.05$ was obtained. These data suggest that non-tannin sorghums that paste and give higher peak viscosities also give high extract contents. A higher peak viscosity could be an indication of more complete starch solubilisation, as more starch granules may have been present, which could result in an increase in the starch molecules available for solubilisation (Svihus et al., 2005). When more starch is solubilised, an increased amount of substrate will be present for hydrolysis by amylolytic enzymes. This will result in more soluble sugars available for fermentation, and therefore a higher extract content.

When selecting a suitable cultivar for use as a brewing adjunct, the data obtained from this research indicate that cultivars with low protein contents and those that have high peak viscosities should be considered. Also of importance is that the cultivar should either be a non-tannin sorghum, or the tannins should be inactivated prior to mashing by means of a pre-treatment with either formaldehyde or NaOH (Beta et al., 2000). Suitable cultivars analysed in this research include TX2907 (waxy endosperm, high peak viscosity) and GP10 (NK8828, a white tan-plant hybrid sorghum from the USA, with no tannins, high protein digestibility and low protein content).

5.3 FUTURE RESEARCH

As stated, the tannins in tannin sorghum cultivars have a significant impact on the quality of hot water extract. In an attempt to get more information regarding the selection of specific sorghum cultivars for use as brewing adjuncts, tannin sorghums could be pre-treated to inactivate the tannins prior to mashing.

Due to the close interaction between starch and protein in the sorghum endosperm (Duodu et al., 2003), proteolytic enzymes could be used in conjunction with amylolytic enzymes in an attempt to increase sorghum hot water extract.

6 CONCLUSIONS AND RECOMMENDATIONS

The determination of sorghum endosperm texture is fast and can provide immediate information related the behaviour of sorghum during brewing. Similarly, the RVA pasting cycle followed in this research is much less time-consuming than extract content determination.

Sorghum cultivars with corneous endosperm are more suitable for cultivation and milling, as the grain is less susceptible to insect and mould damage; the contrary applies to floury endosperm sorghum cultivars. When only corneous sorghums are being considered for the use of a brewing adjunct, those with a lower pasting temperature can be expected to give a higher extract yield. Lower pasting temperatures indicate that less energy is needed for starch solubilisation, which could also be economically advantageous.

Whole sorghum flours with higher pasting temperatures generally have higher peak temperatures. Such sorghum flours would then need more energy to reach peak viscosity and are therefore not of economic value. Should only peak viscosity be used as a criterion for the selection of a sorghum adjunct, those with lower pasting temperatures could be considered.

When only non-tannin sorghum cultivars are being considered for use as brewing adjuncts, those with higher peak viscosities will generally have higher extract yields. Higher peak viscosities indicates more pasted starch molecules being available for enzymatic hydrolysis, leading to increased amounts of sugars in the wort and therefore higher extract yields.

As the tannins in tannin-containing sorghum cultivars inactivate amylase enzymes, this leads to these sorghums giving substantially lower hot water extract values. Therefore, when selecting a sorghum cultivar for use as a brewing adjunct, tannin-containing sorghums should either be excluded, or the

tannins should be inactivated prior to the mashing process. This can be achieved by steeping the grain in a dilute solution of formaldehyde or NaOH.

Grain protein content has considerable value as a predictive marker for the selection of a sorghum cultivar for use as a brewing adjunct. Sorghums with lower protein content can be expected to yield higher hot water extract.

Starch content seemed not to be of significance when compared to sorghum extract yield. There was, however, no indication whether all of the starch present in the mash was completely solubilised during the mashing process. Determination of the starch left in the wort may indicate the need to add proteolytic enzymes for the hydrolysation of the protein matrices that surround the starch granules in the sorghum endosperm. If this could be achieved, more starch should be rendered available for saccharification, which would lead to an increase in extract yield.

A related factor which may influence extract yield is the level of starch saccharification during the mashing process. Should all the gelatinised starch not have been hydrolysed by the added amylolytic enzymes, an increase in the concentration of enzyme used could be considered.

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