QUALITY AND POTENTIAL HEALTH BENEFITS OF WRAPS MADE FROM WHEAT LOW GRADE FLOUR WITH ADDED XANTHAN GUM

by

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Declaration

I Naledi Botha declare that the thesis which I herby submit for the degree MSc Food Science at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Naledi Botha

March 2014

Dedication

To my two daughters, and my darling husband,

To my grandmother Alvina Ntseoane, thank you Father for letting her live to see this day

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My heavenly Father, thank you for enabling me to start this journey, and complete it, thank you for never leaving me nor forsaking me, and being my strength in times of weakness.

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Abstract

Quality and potential health benefits of wraps made from wheat low grade flour with added xanthan gum

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Wraps are an unleavened, flat, and circular bread that is 1- 2 mm thick. White bread flour made from wheat is extensively used in the manufacture of wraps in South Africa. Low grade flour is a by product of milling and can be used as a cheaper alternative to white bread flour. Its high phenolic content and associated antioxidant activity may offer potential health benefits such as reduction in the risk of lifestyle diseases related to oxidative stress. In addition, its relatively higher dietary fibre content offers potential anti-diabetic properties unlike white bread flour which has a high glycaemic index. However the weak gluten quality of low grade flour can compromise the dough rheological properties and quality of wraps. Xanthan gum can improve weak gluten quality by mimicking the viscoelastic properties of gluten. Xanthan is also known to improve the quality of baked products. The objective of this research was to produce a health beneficial wrap of good quality using low grade flour with added xanthan gum.

Low grade wheat flour was used to manufacture wraps. Xanthan gum (0.5% and 0.25% (w/w)) was added to low grade flour. The dough rheological properties of the flour, the quality, and shelf life of the wraps were determined. White bread flour was used as a reference sample in this research.

Water absorption, and Farinograph mixing stability of low grade flour increased with increasing concentration of xanthan gum. Low grade flour with 0.5% xanthan gum had similar mixing stability to white bread flour. Mixing tolerance index (MTI) of low grade flour decreased with addition of xanthan gum, and the MTI of low grade flour with 0.25% xanthan gum was similar to white bread flour. At 50% flour hydration (constant hydration), as well as

at flour hydration according to Farinograph water absorption (adapted hydration), the dough tenacity of low grade flour was increased by the addition of xanthan gum, while the dough extensibility was decreased by xanthan gum addition. At adapted hydration, the dough extensibility of low grade flour with 0.5% xanthan gum was similar to the dough extensibility of white bread flour. The P/L ratio expresses the relationship between the dough strength, and the dough extensibility. The P/L ratio of low grade flour with 0.5% xanthan gum had a value of 0.5 which makes it suitable for bread making. The pasting viscosities of low grade flour increased with the addition of xanthan gum.

Wraps made from low grade flour did not puff during baking. Xanthan gum addition however did improve puffing in wraps made from low grade flour. The puffing of wraps made from low grade flour with 0.5% xanthan gum was similar to the puffing of wraps made from white bread flour. Wraps could only be stored for three days before mould growth was visible. Wraps made from low grade flour with 0.25% xanthan gum had the lowest rollability score, a lower modulus and higher extensibility over time. On day 0, all wraps exhibited a B-type crystallinity as determined by X-Ray diffractometer. The % crystallinity of all wraps increased over storage time. Percent crystallinity increased from 5% to 11.6% for wraps made from white bread flour; for wraps made from low grade flour it increased from 7% to 11%; for wraps made from low grade flour; with added xanthan gum (0.25%) it increased from 7% to 12%, and for wraps made from low grade flour with added xanthan gum (0.5%) it increased from 5.1% to 13%. A thermogram peak on the DSC was only visible on day 3 of storage between 95 and 102°C for the wrap made from white bread flour.

The total phenolic content and antioxidant activity of wraps made from low grade flour were higher than those of wraps made from white bread flour. Simulated gastro-intestinal digests of the wraps had higher total phenolic content and antioxidant activity than the acidified methanol extract.

The relatively higher total phenolic content and antioxidant activity of wraps made from low grade flour indicates that they may offer potential health benefits. Low grade flour however

produces a poor quality wrap, with a reduced shelf life. Addition of xanthan gum to low grade flour improves dough rheology, as well as the quality of wraps. This is possibly due to the interactions which occur between xanthan gum and wheat proteins, as well as between xanthan gum with the amylose of the starch molecule. The wraps with 0.25% xanthan gum produce wraps with a better quality.

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Chapter 1: Introduction

Wraps are known in different parts of the world by different common names such as tortilla, wraps, rotti, and chapatti. Wraps can be defined as a flat circular unleavened bread that is 1- 2 mm thick and 15–33 cm in diameter (Wang and Flores, 1999). A good quality wrap will remain soft and pliable during storage (Ghodke Shalini and Laxmi, 2007) . Wraps can be prepared using different flours such as maize, sorghum, and teff, however white bread flour is the most commonly used flour in South Africa for the manufacturing of wraps (Nicolau, 2012).

The problem with white bread flour is, it elicits a high glycaemic response after the ingestion of products made from it (Anton, 2008). Some dietary fibre and important phytochemicals are lost during the milling process of white bread flour (Hu, 2003, Slavin, 2003). Phytochemicals such as phenolic compounds have important bioactive properties due to their antioxidant activity (Dykes and Rooney, 2007), and together with dietary fibre are thought to play a major role in the reduction of the risk of some life style diseases like cancer, and cardiovascular disease (Boudet, 2007) which plague our society (Slavin, 2004).

With the increase in popularity of wraps among consumers (Nicolau, 2012), they can be used as a vehicle to deliver antioxidants to consumers, in order to help reduce the risk of various diseases of lifestyle that are hypothesised to be caused by oxidative stress. This can be achieved by adding health-promoting compounds to the ingredients, as is commonly done in some foods (Laurikainen et al., 1999). Alternatively, highly refined flour ingredients within a food formulation such as white bread flour, may be substituted with antioxidant-rich low grade flour in the production of wraps.

Low grade wheat flour is a by-product of milling, and is produced at the tail end of the breaks reduction system. This grade of flour consists of the outer parts of the wheat kernel such as the aleurone layer, bran (rich in fibre), and the germ which gives it a higher phenolic content

than other flour grades (straight grade flour, and short patent flour) (Hoseney, 1994, Kent and Evers, 1994, Klepacka and Fornal, 2006, Šramková et al., 2009). Low grade flour however, possesses a weaker gluten quality than white bread flour (Šramková et al., 2009, Figoni, 2010), because it is predominantly made up of the aleurone layer, and particles of bran and germ which contain albumin and globulin proteins (Waggle et al., 1967, Caterall, and Cauvain, 2003, Pierucci, 2008).

The weaker gluten of low grade flour (Kent and Evers, 1994, Campbell et al, 2007) could produce a low quality wrap, with broken outer margins, bad shape and unacceptable rollability score (Arora, 2003). The high bran content in low grade flour could also cause rapid staling of wraps (Ghodke Shalini and Laxmi, 2007).

Hydrocolloids like xanthan gum have the ability to improve the rheological properties of dough by strengthening dough, and mimicking the viscoelastic properties of gluten in gluten-free dough. It can also improve the shelf life and quality of bread (Gujral et al., 2004, Ghodke Shalini and Laxmi, 2007, Banu et al., 2012). Xanthan gum can therefore be added to low grade flour to overcome its limitations.

The use of low grade flour with added xanthan gum could potentially produce good quality wraps, with high content of bioactive antioxidant phenolics, which could potentially lower the risk of lifestyle diseases related to oxidative stress in consumers.

Chapter 2: Literature Review

2.1 Flatbread

The origin of flatbread dates back to ancient times, and it is one of the oldest food types known to man (Qarooni, 1996). There are many different types of flatbread (pizza, pita, khobz, Battaw, paratha, lavash, wraps, and matzo bread) found in different parts of the world, and it is estimated that the number of people consuming various types of flatbread around the world is over 1.8 billion (Qarooni, 1996). There are two major types of flatbread; double layered flat bread which is always leavened, and single layered flatbread which is the more diverse type. Single layered flatbread can either be leavened or unleavened which can sometimes contain chemical leavening agents (Qarooni, 1996).

Wraps, are an unleavened type of flatbread, and they can sometimes contain chemical leavening agents (Qarooni, 1996). They can be defined as a flat circular unleavened bread that is 1- 2 mm thick and 15–33 cm in diameter (Wang and Flores, 1999). Wraps are a traditional food in many countries and usually eaten on their own, or with a filling (fig 2.1) (Gocmen et al., 2009).

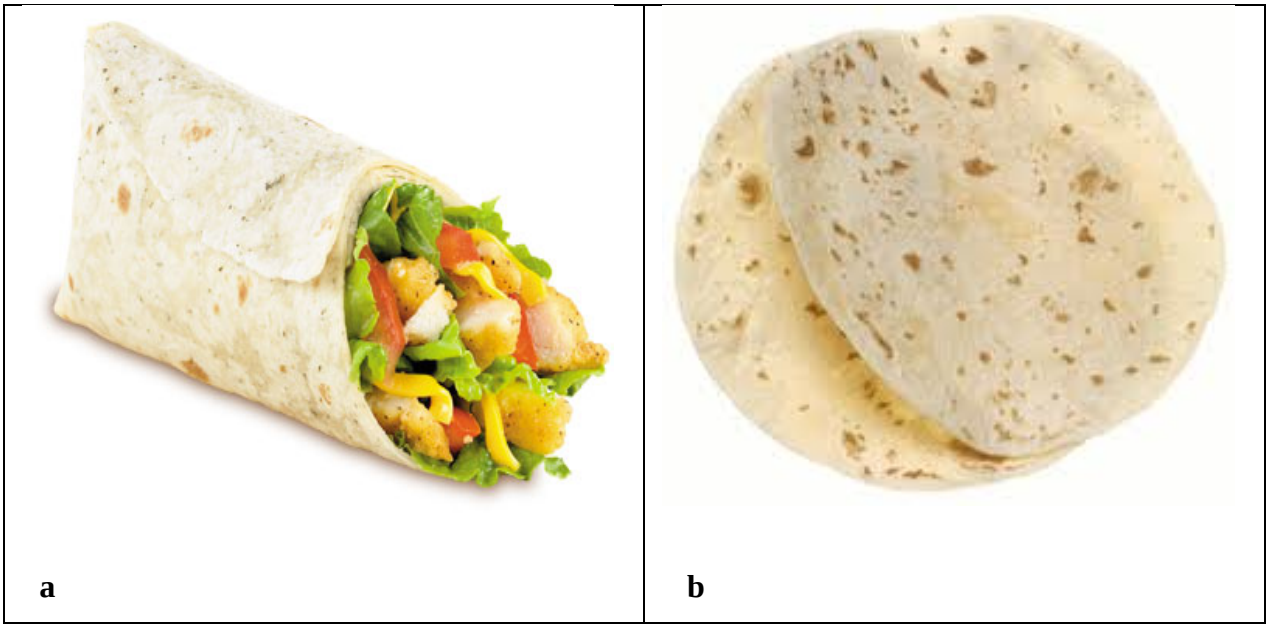


Figure 2. 1: a) Wrap with stuffing b) Wrap with no stuffing (Horner, 2000)

Wraps have seen a rapid increase in growth, and popularity over the years (Ghodke Shalini and Laxmi, 2007, Nicolau, 2012). In comparison to other countries, the growth of wraps in South Africa has been slow. However with more and more restaurants adding wraps to their menus, and supermarkets selling them to consumers, the growth is slowly catching up to the rest of the world (Nicolau, 2012).

A good quality wrap should be flexible without tearing and cracking when folded, soft without sticking together, light coloured, shelf-stable, and be able to retain flexibility for about two weeks (Wang and Flores, 1999).The quality of flatbread depends upon a number of variables such as flour quality, flour types, extraction rate, ingredients as well as processing methods (Mueen-Ud-Din et al., 2010)

2.1.1 Main ingredients of wraps

Traditionally wraps are made from flour, shortening, water, and salt (Gocmen et al., 2009). Using these ingredients gives wraps a shelf life of about 2-4 days (Pierucci, 2008). In order to preserve the quality over an extended time, and to add nutritional value to wraps, commercially made wraps can include additional ingredients such as anti-microbial agents, emulsifiers, acidulants, leavening agents, reducing agents and hydrocolloids (Friend et al., 1995).

2.1.1.1 Flour

Wraps can be prepared from a wide variety of cereal grains which are milled into flour (Qarooni, 1996, Wang and Flores, 1999). Wheat is extensively used for the production of wraps especially in South Africa (Mueen-Ud-Din et al., 2010, Nicolau, 2012). Wheat flour in the wrap ingredients accounts for 80-95% of the dry ingredients (Casso, 2003), and the gluten protein in the wheat flour plays a vital role in dough rheology, dough machinability, and end product quality of the flour (Rossell, 2011).

Gluten is made up of two subunits; gliadin (prolamin) and glutenin (glutelin), and is formed when water is added to the flour with mixing (Wieser, 2007, Rossell, 2011) . Gliadin proteins are small and have low molecular weight in the range of between 30000-100000 Da.

These proteins are single-chain proteins, made up of intra-molecular disulphide bonds. Glutenin has two major groups of subunits; the low molecular weight glutenin subunits (LMW-GS) which has a molecular weight of between 40000-55000 Da, and the high molecular weight glutenin subunits (HMW-GS) whose molecular range is between 80000-120000 Da (Staffer, 2003, Belton, 2007). The HMW-GS contain cysteine residues on both ends of the chain, with repeat regions, rich in glutamine and proline in-between. LMW-GS have the same concentration of cysteine as HMW-GS, but only on one end of the chain. The cysteine residues of both subunits (HMW-GS & LMW-GS) are involved in intra and intermolecular disulphide bonding which occur during dough formation (Schiraldi, and Fessas, 2007).

The formation of a gluten network involves the uncoiling of the glutenin polymer which is initially held together by bonds such as disulphide (-ss-) bonds. During dough development the disulphide bonds break, and reform at different positions within and between glutenin (Belton, 2007, Rossell, 2011). The loop and train model is used to explain the formation and breaking of bonds which occur during dough development. In the loop and train model, the glutenin subunits interact with each other via disulphide bonding at the ends of the subunits, and via hydrogen bonding along the repeat regions. A loop is formed at the repeat regions when water is bound, and this occurs when an extension force is applied to the dough during mixing. When the force is released, the loop disappears and the trains are formed again, which results in the development of a gluten network (Staffer, 2003, Belton, 2007).

The quality parameters of wraps such as diameter, and flexibility can be influenced by wheat gluten proteins (Suhendro et al., 1993, Qarooni et al., 1994). In addition to dough formation, glutenin and gliadin each give dough a different characteristic which contributes to the end wrap quality. Gliadin gives dough its viscosity, and extensibility, while glutenin contributes to the strength, and elasticity of the dough (Pierucci, 2008). Pascut et al. (2004) studied the effect of flour supplementation with glutenin and gliadin on the properties of wraps. Gliadin added to flour increased shelf stability of the wraps. Glutenin also increased shelf stability of wraps; however it also decreased the diameter of the wrap. Unlike bread which requires a dough with high extensibility and elasticity, wraps require a dough with high extensibility, low elasticity, and high dough strength (Pierucci, 2008). Wheat flour with weaker gluten

strength produces wraps with broken outer margins, bad shape and unacceptable rollability score. Stronger gluten flours on the other hand, produce wraps with larger diameter and tougher structure (Arora, 2003).

2.1.1.2 Water

Water is an essential ingredient, which plays the role of dispersing all the other ingredients that the wrap is made of. It is also responsible for the activation of the chemical leavening agents, (if any are included in the ingredients), as well as acting as a plasticizer. Water added to the ingredients is usually added at a temperature of about 30° C, which is optimum for dough resting (Arora, 2003, Casso, 2003). When water is added to wheat flour, it hydrates the gluten fragments (glutenin and gliadin). During kneading of the flour, the two protein fragments bond through chemical bonds such as hydrogen, hydrophobic, disulphide and di-tyrosine bonds forming a gluten network (Pierucci, 2008), which forms dough with viscoelastic properties (Tilley et al., 2001, Arora, 2003, Pierucci, 2008).

2.1.1.3 Salt

Sodium chloride is used to give flavour to the wraps. The salt enhances the desirable flavours of a food product by increasing the perception of sweetness and masking off-tastes, thereby positively impacting the taste of the wrap (Pierucci, 2008). Salt also causes a gluten toughening effect which influences dough properties. This occurs by electrostatic shielding of charged gluten protein amino acids by salt, allowing the amino acids to associate, thus forming stronger dough. Salt also increases the number of ionic bonds in proteins, thereby toughening the protein (Indrani and Venkateswara Rao, 2007).

2.1.1.4 Shortening

The main role of shortening in wraps is lubrication, and in addition, it facilitates dough expansion and improves dough handling by decreasing dough stickiness (Casso, 2003, Pierucci, 2008). Shortening added to wraps also impacts on the quality as well as the shelf life of the wraps (Pierucci, 2008). The shortening has the ability to interact with both the

protein, and starch of wheat flour (Serna-Saldivar et al., 1988). The lipids are thought to interact with gluten via the two gluten subunits glutenin and gliadin, either to both macromolecules simultaneously or one subunit at a time. They bind to gliadin by hydrophilic bonds, and to glutenin by hydrophobic bonds, and this interaction occurs via Van der Waals forces (Pomeranz and Chung, 1978). The binding of lipids to wheat flour, causes a reduction in gluten strength, and improves the rollability of the wrap (Serna-Saldivar et al., 1988). Shortening can also interact with the starch, by bonding to amylose via hydrogen bonding, which decreases staling (Arora, 2003, Pierucci, 2008).

2.1.2 Additional ingredients

2.1.2.1 Leavening agents

Chemical leavening agents can be used in the production of wraps, and they comprise of acid and base salts (Bejosano and Waniska, 2004, Pierucci, 2008). The mixture of sodium bicarbonate, mono-calcium phosphate and sodium aluminium sulphate are commonly used in the production of wheat wraps (Arora, 2003). In the presence of moisture and heat, the acid and base react to produce a neutral salt, CO_2, and water. The CO_2 produced fills the air pockets which were incorporated into the dough during mixing, resulting in extra puffing during baking, and a more tender final product with a decreased density (Pierucci, 2008).

2.1.2.2 Preservatives and acidulants

Preservatives commonly used in the production of wraps are sodium and calcium propionates and potassium sorbate, which are optimally effective at low pH dough. They are usually used in a combined formula, and are added to extend the shelf life of the wraps by inhibiting mould growth (Arora, 2003, Pierucci, 2008). Acidulants most commonly used in wrap production are fumaric, malic, phosphoric, acetic, and citric acids. These acidulants are added in order to lower the pH, and provide the most suitable environment to enhance the functionality of the preservatives (Pierucci, 2008).

2.1.2.3 Emulsifiers

Emulsifiers such as mono- and diglycerides, sodium stearoyl-2-lactylate (SSL), and lecithin are added to improve dough rheology, as well as final product quality (Casso, 2003).

Emulsifiers strengthen the dough and improve the dough machinability and gas retention by interacting with the gluten, gliadin and starch of the flour (Casso, 2003). The interaction of the gluten and emulsifiers is not fully understood, however it is thought that the emulsifier forms a complex with the various protein fractions of the gluten via hydrogen bonding, which results in strengthening of the dough (Van Haften, 1979). Emulsifiers improve the ability of gluten to form a film. This results in a larger puff of the wrap during baking. They also decrease the rate of staling and crumb firming in wraps, thereby producing wraps with a better quality (Arora, 2003, Casso, 2003, Pierucci, 2008).

2.1.3 Manufacturing process of wraps

Traditionally wheat wraps are prepared in the home by adding shortening and salt to flour, followed by water. The dough is then kneaded in order to develop it. The dough mixture is allowed to rest for a few minutes before dividing into small round balls. The balls are allowed to rest before sheeting into round flattened shapes and baking on a hot plate (Qarooni, 1996). Due to the increase in the popularity of wraps, they are no longer just prepared for home consumption, but are also sold at restaurants, super markets, and at wholesalers (Qarooni, 1996, Arora, 2003). This increase in growth of the market has caused a need for the development of large scale processing methods in order to accommodate the production capacity on a daily basis (Qarooni, 1996, Arora, 2003, Ghodke Shalini and Laxmi, 2007). There are three methods used commercially in the wrap industry (fig 2.2), the hand stretching method, the die cut method and the hot press method (Qarooni, 1996).

2.1.3.1 The hand stretching method

This method requires good sanitation, as well as a lot of time and labour (Qarooni, 1996, Arora, 2003). The dough balls are first sheeted into disks using a presser belt, the disks are then cross sheeted to form a circular shape, and then manoeuvred into a final circular shape by hand stretching on a hot plate (Pierucci, 2008). Wraps made using this method are usually not uniform in shape, and have an intermediate quality. They also have a powdery mouth feel due to powder dusting during sheeting (Qarooni, 1996, Arora, 2003, Pierucci, 2008).

2.1.3.2 The die-cut method

In this method, the extruder sheets the dough, which is then dusted with flour and rolled. The dough is rolled again by a cross roller to make it thinner, and cut into circular shapes using a cutter. Wraps made using this method have a low moisture content, low elasticity, and a floury mouth feel (Pierucci, 2008).

2.1.3.3 The hot press method

This is the most popular method used in industry. After proofing, the dough balls are pressed between two hot plates (200ºC) at a pressure of around 7584.2 KPa for 1.5 seconds forming circular disks. The hot plates cause a sealing effect on the wrap, which results in steam being trapped inside the two layers during baking, resulting in a greater puff. Wraps made using this method have a smooth surface texture and retain their flexibility longer during storage. (Qarooni, 1996, Srinivasan et al., 2000, Arora, 2003, Pierucci, 2008). Every step in the wrap manufacturing process is important, and affects the end product quality (Arora, 2003).

Wraps are usually baked in a commercial three-tier oven for between 18-40 seconds, at 250-270ºC. Once baked the wraps are then cooled and packaged. Before wraps can be packaged into polyethylene bags, they are first cooled to 25 ºC to avoid condensation and stickiness while in the packaging material (Pierucci, 2008).

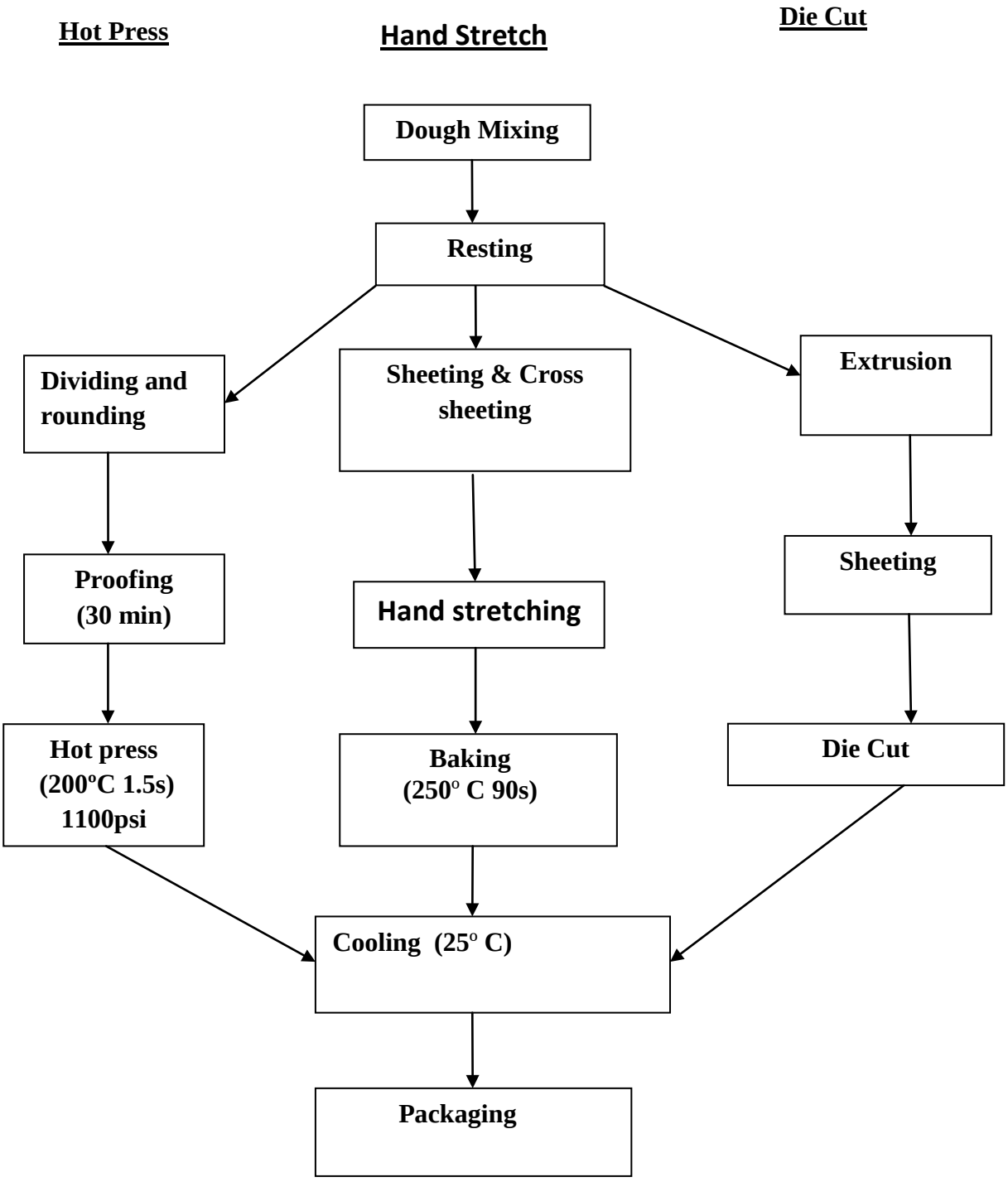


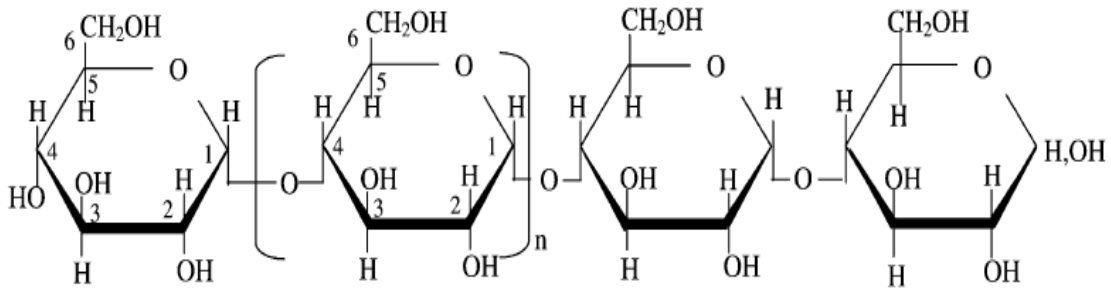
Figure 2. 2: Commercial processing methods for wheat flour wraps (Adapted from Qarooni, 1996)

2.2 Staling and quality of wraps during storage

Fresh wheat wraps are soft, pliable and elastic, but on staling they become hard and rigid (Ghodke Shalini and Laxmi, 2007). Staling is an undesirable phenomenon which occurs in starch-based foods such as wheat wraps. It causes the wraps to become more firm during storage, and occurs at both room, and refrigerated temperatures, but is accelerated more at refrigerated temperatures (Gray and Bemiller, 2006, Shaikh et al., 2008).

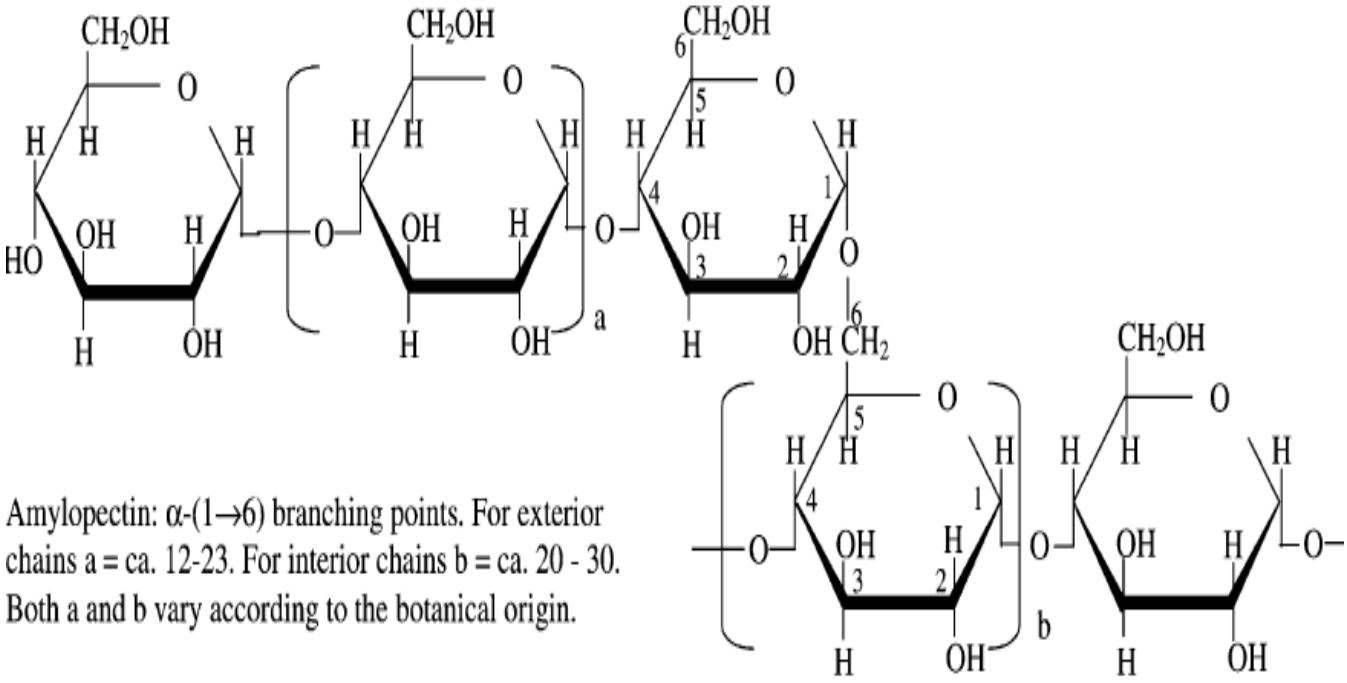
Wheat wraps without the addition of any additives, have a very short shelf life. The two major contributing factors to a reduced shelf life are thought to be microbial spoilage, and staling during storage (Khan et al., 2011). Although it has been studied for over 150 years, the exact mechanism of staling of bread and other baked products is not well understood. It is thought however that starch, water redistribution, and polymer plasticization changes play important roles in staling (Carini et al., 2010, Curti et al., 2011).

Starch which is a major component in wheat flour, is made up of two polysaccharides, amylose and amylopectin (Alhajji, 2011). Amylose is an essentially linear amorphous polymer made up of about 600-5000 α-(1, 4)-linked D-glucopyranosyl units (fig 2.3). It has been found that amylose is slightly branched by α-(1,6)-linkages (Goesaert et al., 2005a, Alhajji, 2011). Amylopectin on the other hand is a semi-crystalline multi-branched polymer made up of chains of α-(1,4)-linked D-glucopyranosyl residues which are interlinked by α-(1,6) glycosidic linkages (fig 2.4) (Goesaert et al., 2005a). The content of these two polymers play an important role in both gelatinisation and retrogradation of starch.



Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).

Figure 2. 3: Structure of Amylose (Tester et al., 2004)



Amylopectin: α-(1→6) branching points. For exterior chains a = ca. 12-23. For interior chains b = ca. 20 - 30. Both a and b vary according to the botanical origin.

Figure 2. 4: Structure of Amylopectin (Tester et al., 2004)

During the baking of wraps gelatinization of starch occurs. This involves the absorption of water by the starch granule which results in its swelling, and leaching out of amylose from the starch granule. As the temperature increases, starch polymers vibrate, which leads to the breakage of intermolecular bonds. This results in the exposure of hydrogen bonding sites which allow more water binding, and eventually the disruption of the crystalline structure in

the starch granule (Tester and Morrison, 1990, Goesaert et al., 2005a, Pateras, 2007, Shaikh et al., 2007). On cooling the disrupted crystalline structure attempts to return to its ordered semi-crystalline state, or recrystallise and this process which is referred to as retrogradation, is believed to be responsible for the staling of the wraps (Shaikh et al., 2007).

Retrogradation of starch is a term used to describe the changes which occur in gelatinized starch from an amorphous state, to a more crystalline order (Gudmundsson, 1994). Initially at the onset of retrogradation, amylose molecules which were solubilised during gelatinisation form a continuous network of double helices, a process referred to as gelation. After a few hours the helices form stable crystalline structures (Figure 2.5). Amylopectin recrystallisation occurs when the short lateral chains of amylopectin re-associate with one another by various types of intra molecular bonds (Pateras, 2007) to form a crystalline structure.

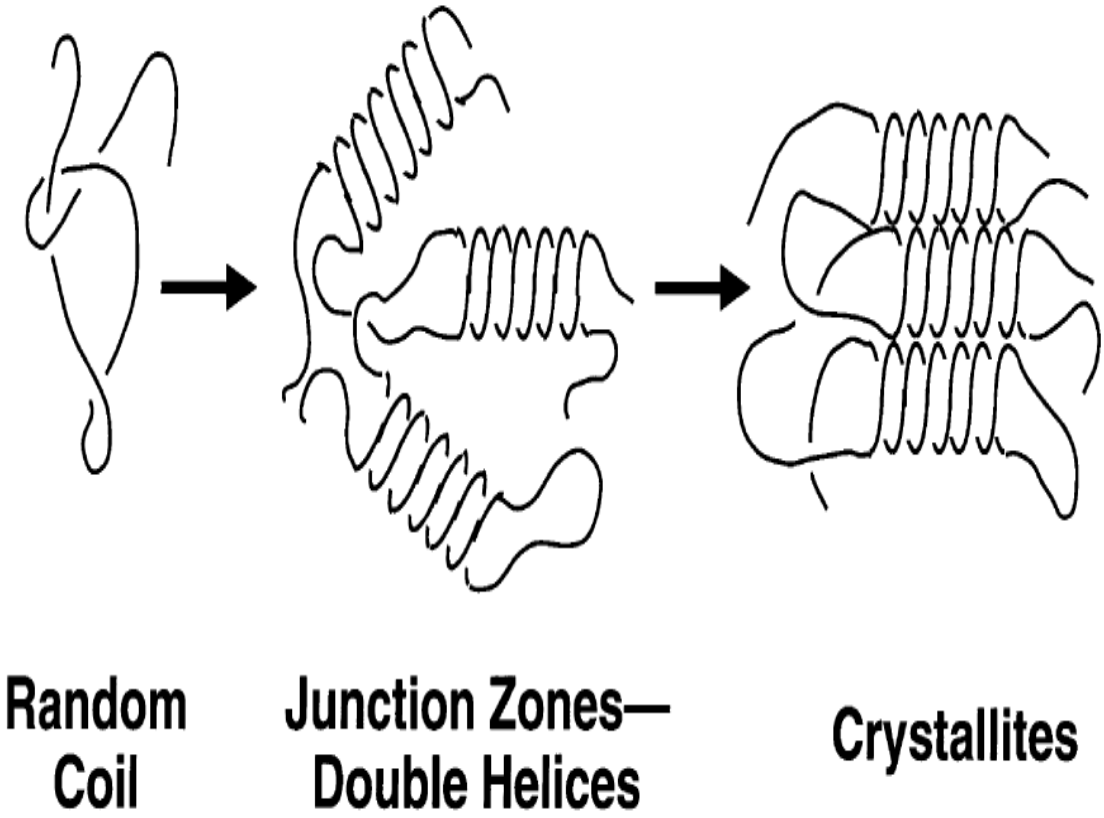


Figure 2. 5: Schematic of amylose retrogradation (Haralampu, 2000)

Both amylose and amylopectin are involved in starch recrystallisation, however the growth in crystallinity of amylose occurs a lot faster than that of amylopectin (Goesaert et al., 2005a). Amylose is responsible for short term retrogradation, which occurs when amylose forms double helices via hydrogen bonding (Pateras, 2007). Amylose recrystallisation does not continue increasing beyond 2 days, and is not reversible on heating < 100 ºC (Gudmundsson and Eliasson, 1990, Gudmundsson, 1994, Goesaert et al., 2005a). Long term retrogradation is responsible for the long term effects of staling seen in bakery products, and amylopectin is responsible for it (Gudmundsson and Eliasson, 1990). Unlike amylose, the recrystallisation of amylopectin reaches its crystallization limit between 30 and 40 days, and is thermally reversible (Gudmundsson and Eliasson, 1990, Gudmundsson, 1994, Goesaert et al., 2005a)). The staling of baked products after a maximum of two days would therefore be due to amylose, and amylopectin would be responsible for long term staling (Miles et al., 1985)

Studies have shown that moisture also plays an important role in the staling process. In order for retrogradation of amylopectin to occur, water distribution is needed to allow mobility of the polymer (Gray and Bemiller, 2006). During staling moisture migrates from the centre of the bread (crumb), to the outer margins (crust) and research has shown that this accelerates the firming of baked products (Piazza and Masi, 1995). It is not clearly understood exactly how moisture migration occurs, however it is clear that moisture in bread products does move from the crumb to the crust. The subject of moisture migration is a rather unclear one, because studies have shown that moisture moves from the starch to the gluten as shown by Senti and Dimler (1960). Willhoft (1971) on the other hand showed the inverse, that water migrates from the gluten to the starch. It is the unbound water which participates in moisture migration (Ruan et al., 1996). Water binding compounds such as sugars and hydrocolloids like xanthan gum can therefore compete with the starch and gluten polymers for water if added to the ingredients, and slow down the rate of staling (Xie, 2002, Gray and Bemiller, 2006).

2.3 Nutritional enhancement of wraps

Consumers today demand more nutritious and health beneficial food, which is also convenient (Hussain et al., 2012). This has opened a market in the food industry for the development of healthier alternatives to conventional foods (Anton, 2008, Hussain et al., 2012).

With the increase in popularity of wraps among consumers (Ghodke Shalini and Laxmi, 2007, Nicolau, 2012), they can be used as a vehicle to deliver nutrition to its consumer. Several studies have been done to improve the nutritional quality of wraps made from white bread flour (Suhendro et al., 1993, Anton, 2008, Yadav et al., 2010). Gonzalez-Agramon and Serna-Saldivar (1988) incorporated soybean into wheat flour tortilla ingredients, and found an improvement in the nutritional quality. The soya bean doubled the lysine content of wheat flour tortillas, and improved the protein quality of the product. In an attempt to improve the fibre content of wraps, fibre from different sources such as oat bran, wheat bran, and whole wheat flour have been incorporated into the ingredients of wraps. The fibre content of the wraps increased significantly, however the textural quality of the wraps was compromised. (Friend et al., 1992, Serna-Saldivar et al., 2004, Anton, 2008).

To increase the nutritional quality of wraps, white bread flour could be totally substituted with a more health beneficial, and cheaper flour like low grade wheat flour.

2.4 Low grade wheat flour and its potential health benefits

Low grade flour is a by product obtained from the milling of wheat (Neves et al., 2006). The wheat kernel is made up of three major milling components (fig 2.6) namely, the endosperm which comprises 80-85% of the kernel, and is made up mainly of starch (80%), and protein (13%); the germ, which is made up of lipids, small amounts of vitamins, phytosterols and some phenolic compounds; and the final milling component is the bran, which is made up of different layers: the aleurone layer, the hyaline layer (nucellar epidermis), the testa or seed coat, the inner pericarp (cross and tube cells), and the outer pericarp (Ansón, 2010). Bran has

high phenolic content, which is responsible for its high antioxidant activity (Slavin, 2004, Ansón, 2010)).

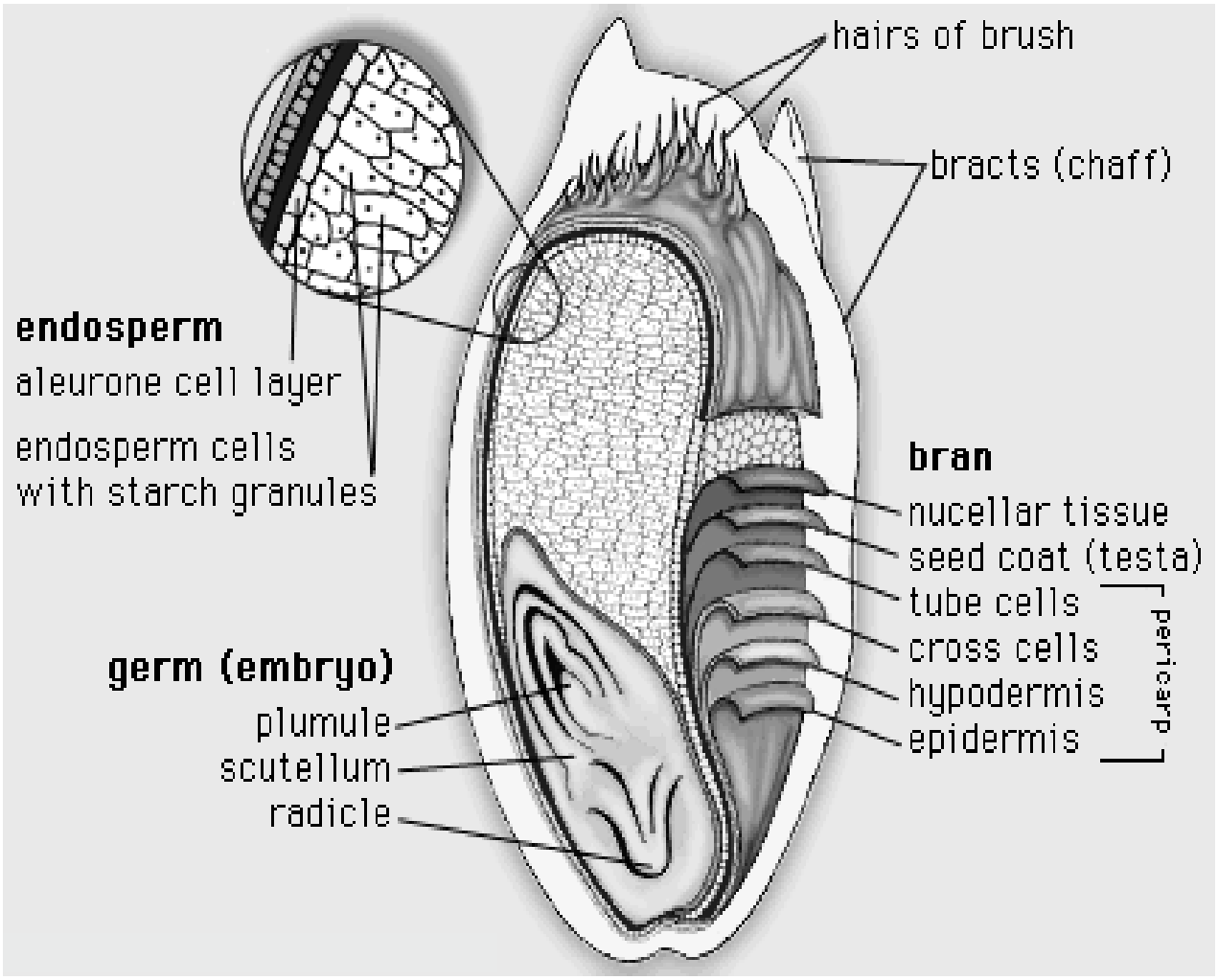


Figure 2. 6: Wheat grain anatomy (Šramková et al., 2009)

The breaks-reduction system is used for wheat milling, and different types of flour can be produced from this system (Kent and Evers, 1994). The breaks system consists of four or five breaks where the endosperm is separated from the bran and the germ. In each break, the grain is ground and separated into larger particles which move to the next break for further grinding. In the reduction system, the smaller endosperm particles are reduced to fine flour. At each grinding step different types of flours are produced such as straight grade flour, short patent flour, and low grade flour (Hoseney, 1994, Kent and Evers, 1994). Low grade flour (also known as clear flour or red dog) is a by-product of milling, and is produced at the tail end of the breaks reduction system (Hoseney, 1994, Atwell, 2001, Neves et al., 2006,

Campbell et al, 2007). It is darker in colour, and contains higher amounts of ash as well as gluten components than the other flour grades (Wihlfahrt and Brooks, 1953).

2.4.1 Properties of low grade flour

Low grade flour is made up of the aleurone layer, bran, germ, and a small amount of the endosperm (Waggle et al., 1967, Caterall, and Cauvain, 2003, Campbell et al, 2007). The aleurone layer is the part of the endosperm closest to the bran (fig 2.6). This layer is a concentrated source of vitamins, minerals and other nutrients. The aleurone layer also has a high protein level, however it does not provide the same proteins found in the endosperm (Šramková et al., 2009). The germ lies at one end of the wheat kernel, and is rich in proteins, lipid, and has a high vitamin and mineral content. The bran is the outer protective layer of the kernel surrounding the germ and endosperm. More than 50% of the bran comprises of fibre, which is made up of cellulose and pentosans, polymers based on xylose and arabinose, which are tightly bound to proteins (Šramková et al., 2009). The bran layer also contains a small amount of protein, a high mineral content, as well as phenolic compounds (Šramková et al., 2009).

Phenolic compounds are distributed throughout the endosperm, germ, and bran fractions of the wheat grain as free, bound, and soluble-conjugated forms. The germ and bran fractions of the wheat grain, which make up a big portion of the flour (Waggle et al., 1967, Caterall, and Cauvain, 2003), show considerably higher levels of phenolic compounds than the endosperm (fig 2.7) (Adom et al., 2005). Research has also shown that, the outer milled fraction of the grain, including the bran has the highest potential health benefit, due to its high antioxidant activity (Liyana-Pathirana and Shahidi, 2007). Low grade four therefore contains a higher mineral, and bran content than white bread flour (Table 2.1) which does not have high amounts of these outer milled fractions (Tang et al., 2013).

Table 2. 1: Chemical components of white bread flour and low grade flour

Flour	Protein content (%)	Ash content (%)	Bran content (%)
White bread flour	10.8	0.7	2.4
Low grade flour	13.1	1.6	5

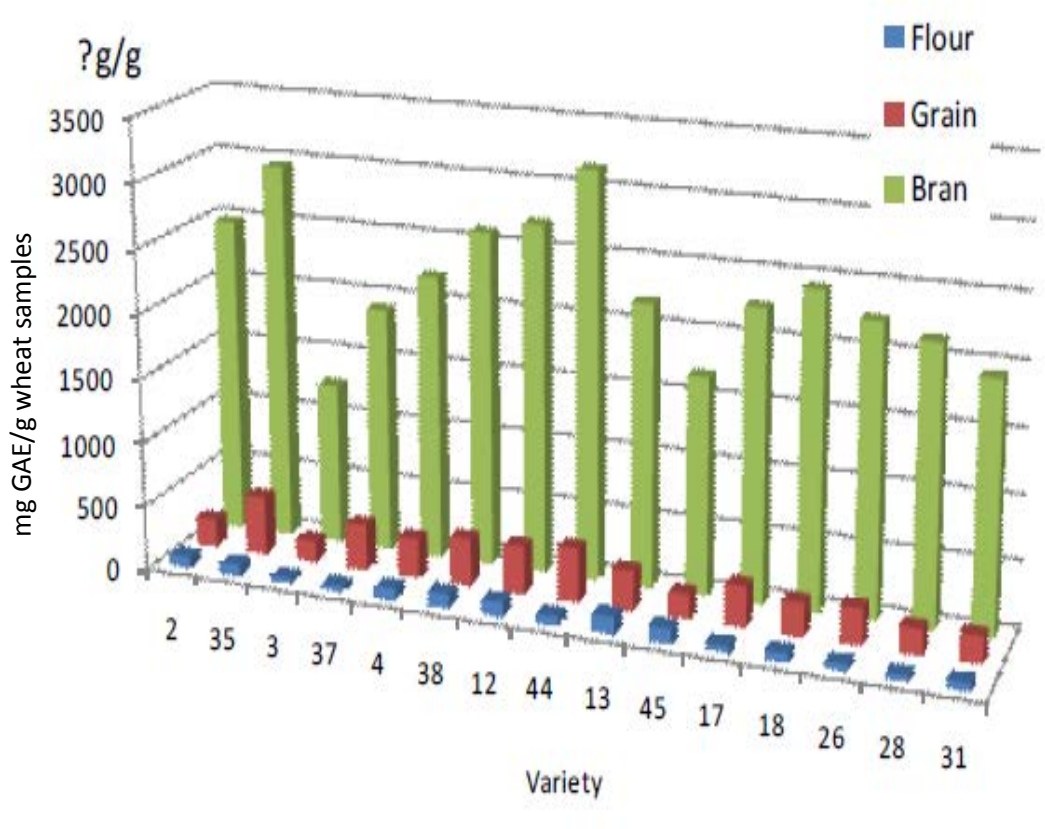


Figure 2.7: The total phenolic content of the bran layer, flour and whole grain of different spring and winter varieties determined by the Folin-Ciocalteau assay. The results are presented as microgram gallic acid equivalents (GAE) per gram of wheat samples (Vaher et al., 2010)

2.4.2 Phenolic compounds

Phenolic compounds can be defined as compounds which possess one or more aromatic rings, with one or more hydroxyl groups, and can be categorised as phenolic acids, flavonoids, stilbenes, coumarins, as well as tannins (Liu, 2007). Phenolic acids, and

flavonoids are two of the most common phenolic compounds found in wheat which exhibit a potent antioxidant activity (Liyana-Pathirana and Shahidi, 2007).

2.4.2.1 Phenolic acids

Phenolic acids in wheat are mainly concentrated in the aleurone layer (Vaher et al., 2010), and they can be subdivided into two main groups, the hydroxybenzoic acid derivatives, as well as the hydroxycinamic acid derivatives. The basic skeletons of the two structures (Figure 2.8) are similar. The cinamic acid derivative contains a carboxylic acid substituent, while the benzoic acid derivative contains a carboxyl substituent. They also differ in the numbers and position of the hydroxyl groups on the aromatic ring, (Robbins, 2003).

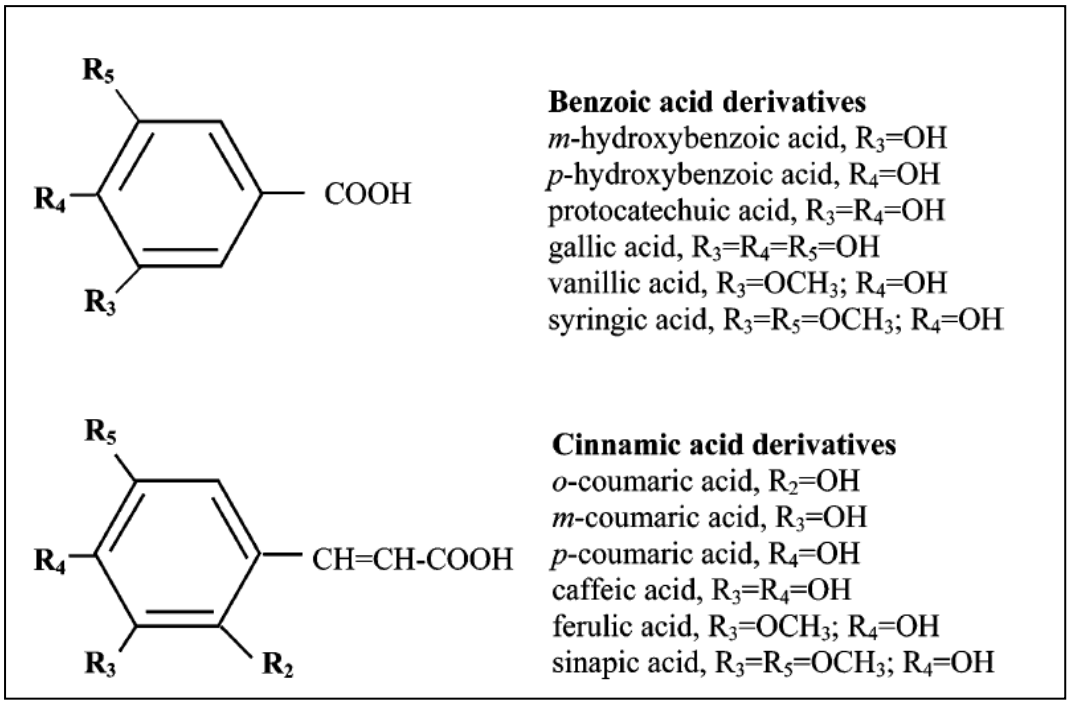


Figure 2.8: Phenolic acid derivatives (Mattila et al., 2005)

The hydroxyl (OH), and methoxy (OCH3) groups substituted at various places of the aromatic ring, are characteristic of all phenolic acids (Dykes and Rooney, 2007). The antioxidant activity of phenolic acids and their esters depends on the number and position of hydroxyl groups (Rice-Evans et al., 1997) and methoxy derivatives (Cai et al., 2006). After conducting an experiment involving the antioxidant activity of different phenolic acids, Cai et

al. (2006) found that phenolic acids which contained less OH- groups had lower antioxidant activity, and those with high numbers of OH- groups had the highest antioxidant activity. Phenolic acids may be found either in the free form, glycosylated, or esterified to other compounds (Manach et al., 2004). The free form of phenolic acids are mainly located in the outer layer of the pericarp, while the bound form can mainly be found bound to the pericarp cell wall (Gani et al, 2012).

The hydroxybenzoic acid derivatives include p-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic acids. Hydroxybenzoic acid derivatives are usually found in bound form to complex structures such as lignins and tannins (Liu, 2007), while the hydroxyl cinnamic acid derivatives include p-coumaric, caffeic, ferulic and sinapic acids. They are also usually found bound through ester bonds to lignins, cell wall structural components, and proteins (Liu, 2007).

Ferulic acid is one of the most abundant phenolic acids in wheat, and it is located in the wheat bran, aleurone layer and pericarp, esterified to the hemicelluloses of the cell wall (Klepacka and Fornal, 2006, Liu, 2007, Liyana-Pathirana and Shahidi, 2007, Gani et al, 2012). Ferulic acid is found in the free, soluble, conjugated and bound form in whole-grains (Gani et al, 2012). Although the ferulic acid is found mostly in the bound form, it is thought that it plays a vital role in reduction of the risk of colon cancer. The bound ferulate is thought to survive the gastrointestinal tract, and then released by the fermentative action of microorganisms of the colon where it may exert antioxidant effects (Slavin, 2004, Liu, 2007).

2.4.2.2 Flavonoids

The major flavonoids in wheat are the apigenin-C-diglycosides, cyanidin-3-glycosides, and the peonidin3-glycoside (Vaher et al., 2010)*).* Flavonoids are low molecular weight secondary metabolites characterised by a flavan nucleus (Heim et al., 2002). Flavonoids share a common diphenylpropane (C6-C3-C6) structure (Rice-Evans et al., 1997), which consists of two aromatic rings (A and B) that are bound together by three carbon atoms which form an oxygenated heterocycle ring (C). Depending on the heterocycle, the flavonoids can

be divided into 6 subclasses which are flavonols, flavones, isoflavones, flavanones, anthocyanidins, as well as flavanols (Manach et al., 2004). Flavonoids usually occur in plants as aglycones, although they are most commonly found as glycoside derivatives (Manach et al., 2004, Bravo, 2009).

Heim et al. (2002) reported that the antioxidant activity of flavonoids is dependent on the arrangements of the functional groups on the nuclear structure. Both the configuration, and the total number of hydroxyl groups influence the antioxidant activity of the flavonoid. The highly reactive OH- group scavenges the free radicals by donating an H atom. The hydroxyl groups on the B-ring of the flavonoid donate hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them (radicals) and giving rise to a relatively stable flavonoid radical (Heim et al., 2002). The antioxidant activity of flavonoids is increased with the presence of a 2, 3-double bond in the C ring (Figure 2.9). This is due to electron delocalization across the molecule for stabilization of the radical formed during electron donation to the free radical. The presence of the 4-oxo function on the C-ring is also important for antioxidant activity. The ortho 3'4'-dihydroxy moiety in the B-ring also increases the antioxidant activity (Rice-Evans et al., 1996).

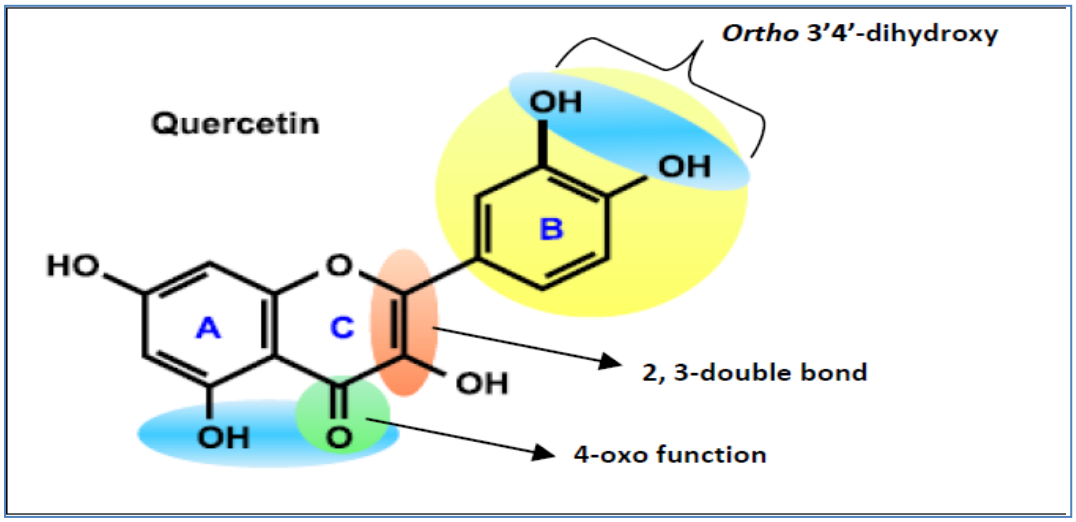


Figure 2. 9: Structure of quercetin highlighting antioxidant activity (Adapted from (William et al., 2004)

2.4.3 Potential health benefits of low grade flour: Antioxidant activity of phenolic compounds

Low grade flour is rich in phenolic compounds, because it contains large fragments of the outer wheat kernel (Waggle et al., 1967, Caterall, and Cauvain, 2003, Campbell et al, 2007). It is the antioxidant activity of phenolic compounds that gives them their potential health benefit (Rice-Evans et al., 1997, Heim et al., 2002, Slavin, 2004). This antioxidant activity has potential involvement in the reduction and prevention of some chronic diseases related to oxidative stress such as cancer, diabetes, and cardiovascular disease (Boudet, 2007).

Free radicals result in the body from normal metabolic activity, the diet, and the environment (Slavin, 2004). The body has defence mechanisms which prevent free radical damage, as well as repairs any free radical damage (Slavin, 2004). However when these mechanisms are insufficient disease may occur (Heim et al., 2002, Slavin, 2004). Reactive oxygen species (ROS) may occur in the form of free radicals and are capable of oxidising cellular proteins, DNA, and lipids, and by so doing contribute to cellular ageing, carcinogenesis, and cardiovascular disease (Heim et al., 2002). Dietary antioxidants such as phenolic compounds can help to reduce the activity of free radicals in the body thus reducing the risk of these diseases (Slavin, 2004).

For a polyphenol to be defined as an antioxidant it must satisfy two basic conditions: first when present in low concentration relative to the substrate to be oxidized it can delay, retard, or prevent the autoxidation or free radical-mediated oxidation; second the resulting radical formed after scavenging must be stable through intra-molecular hydrogen bonding or further oxidation (Rice-Evans et al., 1996). The formation of the stable free radical after scavenging is illustrated in Figure 2.10.

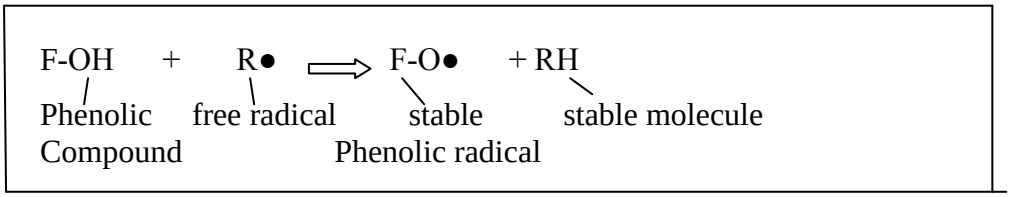


Figure 2. 10: Formation of a stable phenolic radical after reaction between a phenolic compound and free radical species (Rice-Evans et al., 1997)

Phenolic compounds are powerful antioxidants, and can reduce the risk of degenerative diseases or chronic diseases of lifestyle where reactive oxygen species are involved (Dykes and Rooney, 2007). In addition to the possibility of absorbed phenolics exerting bioactive and antioxidant effects in the body, recent research also suggests that, undigested phenolic compounds provide some protection in the large intestines (Gani et al, 2012).

2.5 Overcoming the limitations of low grade flour

The health benefits of low grade flour make it desirable; however it does have some undesirable characteristics. Low grade flour has a weaker gluten quality than white bread flour (Šramková et al., 2009, Figoni, 2010), which may compromise its quality and rheological properties (Waggle et al., 1967, Caterall, and Cauvain, 2003, Pierucci, 2008).

Hydrocolloids like xanthan gum have been used in the baking of gluten free bread, and found to mimic the viscoelastic properties of the dough (Lazaridou et al., 2007). Xanthan gum can thus be added to the low grade flour in order to supplement the weak gluten present in low grade flour.

2.5.1 Xanthan gum

Xanthan gum is an extracellular polysaccharide, which was discovered on cabbage bacteria in the late 1950's. After years of testing for its toxicity, it was deemed safe to use as a food additive by the Food and Drug Administration (FDA), about 10 years after its discovery (Katzbauer, 1998).

The structure of xanthan gum (figure 2.11) consists of a linear backbone of 1,4-linked β-D-glucose. At the C(3) position of every alternate glucose residue, there is a charged

trisaccharide side chain containing a glucuronic acid residue between two mannose units. The terminal β-D-mannose is linked (β-1,4) to the glucuronic acid which, in turn, is linked (α-1,2) to the α-D-mannose. On approximately one half of the terminal mannose residues a pyruvic acid moiety is joined by a ketal linkage to the O(4) and O(6) positions (Casas et al., 2000)

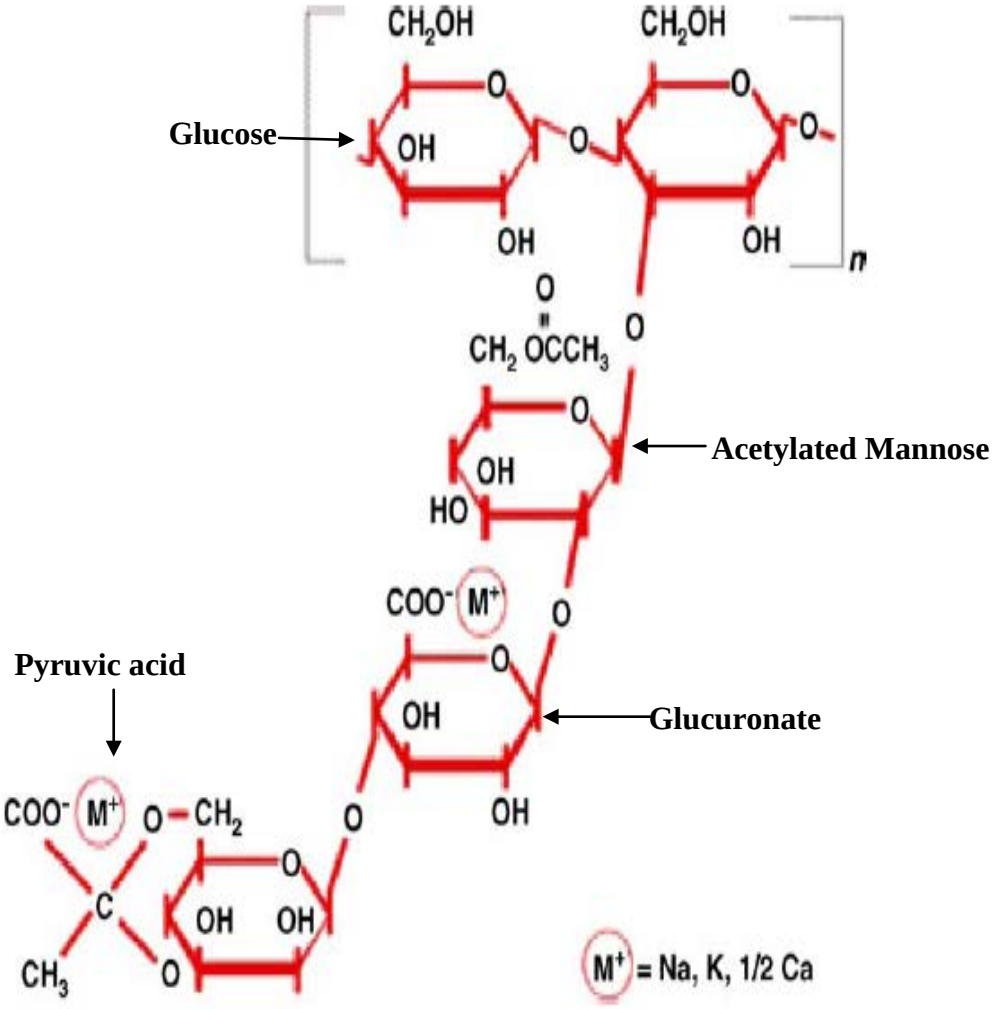


Figure 2. 11: Structural unit of xanthan gum (Rosalam and England, 2006)

Xanthan gum is produced by fermentation, whereby the bacteria *Xanthomonas campestris*is is cultured in a well aerated and well agitated fermenter. The medium which the culture is grown in contains glucose, a nitrogen source, as well as some nutrients. Once the fermentation process is complete, the broth is heated to kill the bacteria, and the xanthan gum

recovered by precipitation using isopropyl alcohol. The xanthan gum is then dried, milled, and packaged (Katzbauer, 1998).

Because it has excellent rheological properties, and is stable at a wide range of temperatures, salt concentrations and pH, xanthan gum is widely used in many industries including the food industry. Within the food industry it is used in a variety of products, from beverages, soups, salad dressings, to bakery products, and only a small amount is required for a desired effect, which does not affect the overall taste of the product (Katzbauer, 1998).

2.5.1.1 Effect of xanthan gum on dough rheology

Xanthan gum has the ability to alter the rheological properties of dough, such as dough strength and water absorption (Smitha et al., 2008a). The xanthan gum molecular structure contains numerous hydroxyl residues, which interact with water through hydrogen bonding, increasing water absorption of wheat flour (Wang et al., 2002, Anil, 2007, Smitha et al., 2008a). Addition of xanthan gum to wheat flour will also strengthen the dough, by forming strong interactions with the gluten protein of the flour (Smitha et al., 2008a). Xanthan gum forms an electrostatic complex with some fractions of gluten proteins. This occurs by formation of ionic pairs between ionised carboxyl groups of the hydrocolloid and ε-amino groups of the gluten (Ribotta et al., 2005). The hydroxyl sites of xanthan gum, are also involved in the xanthan-gluten interaction, by forming non-covalent bonds with the numerous amide groups on the gluten protein (Ribotta et al., 2005). Xanthan gum can also affect pasting properties. Tang et al. (2013) found that xanthan gum increased the peak and final viscosity of rice starch, while lowering the setback, and breakdown viscosity values. These changes were attributed to the inhibition effect of xanthan gum on the starch granule expansion. Xanthan gum inhibited the leaching out of amylose from the starch granule, preventing the starch from expanding during the heat process (Tang et al., 2013).

2.5.1.2 Effect of xanthan gum on wrap quality

Smitha et al. (2008a) reported that xanthan gum also improved overall product quality of baked products by improving product texture. This effect was shown by Smitha et al.

(2008a), where xanthan gum was added to the white bread flour flatbread formulation and the final product showed improved pliability as well as chewiness. Ghodke Shalini and Laxmi (2007) also found that hydrocolloids including xanthan gum improved the texture or softness of flatbread, when added to wheat flour flatbread ingredients. Although the mechanism by which this occurs is not completely understood, it is thought that xanthan gum, and other hydrocolloids have a weakening effect on the starch structure which promotes better water distribution and retention (Ghodke Shalini and Laxmi, 2007). Xanthan gum is also believed to cause a decrease in the crumb resistance resulting in a softer texture of wraps (Ghodke Shalini and Laxmi, 2007, Kohajdová and Karovičová, 2008).

Moisture retention of baked products also improves with the addition of xanthan gum; This occurs by the hydroxyl groups in the xanthan structure (which is able to hold water up to 6 times its own size) allowing more water interactions through hydrogen bonding in wheat dough (Tavakolipour and Kalbasi-Ashtari, 2007). This results in the retardation of moisture migration from the crumb to the crust, thereby slowing down the staling process of wraps during storage, as well as retarding the re-crystallization of starch polymers and improving the shelf life of the wraps (Kohajdová and Karovičová, 2008). Gujral et al. (2004) did a study on the effect of different hydrocolloids on the quality of chapatti, Indian unleavened bread, and found that xanthan gum, decreased the rate of staling of the bread. This was attributed to the interaction which occurs between starch and xanthan gum. In this interaction, the amylose of starch bonds with xanthan gum via hydrogen bonding (Tang et al., 2013). The interaction which forms between starch and xanthan gum is believed to cause a weakening in the starch structure, by inhibiting amylose re-associations thus preventing the formation of the amylose double helices which forms at the onset of starch retrogradation (Biliaderis et al., 1997).

2.6 Concluding Remarks

- Wraps are gaining popularity in South Africa as a convenience food.
- Wraps are commonly made using white bread flour.
- Wraps can be used as a vehicle to deliver health benefits to consumers.

- Functional ingredients are added during wrap manufacture to increase their nutritional quality.
- White bread flour can be substituted with low grade flour in the production of wraps.
- Low grade flour is more health beneficial than white bread flour.
- Low grade flour contains more of the outer fractions of the wheat grain known to be high in phenolic content, antioxidant activity, and potential health benefits.
- Gluten quality of low grade flour is poor, and may have adverse effects on the dough rheological properties and quality of the wraps.
- Hydrocolloids are used in the formulation of gluten free bread, to supplement gluten function in the bread.
- Xanthan gum is one such hydrocolloid, successfully used to improve dough rheological properties such as dough strength, water absorption, dough stability, and pasting properties.
- Xanthan gum has also been found to improve the quality and shelf life of baked products by means of, reducing the rate of retrogradation, increasing firmness during storage, and improving the overall tensile properties during storage.

Chapter 3: Hypothesis and objectives

3.1 HYPOTHESES

- Xanthan gum will improve the dough rheological properties of low grade flour as well as the quality of wraps made from low grade flour. Xanthan gum has the ability to alter the rheological properties of dough, such as dough strength and water absorption (Smitha et al., 2008a). Xanthan gum binds to flour proteins, causing strengthening of the dough (Kohajdova and Kaovicova, 2009). Xanthan gum forms an electrostatic complex with some fractions of gluten proteins. This occurs by formation of ionic pairs between ionised carboxyl groups of the hydrocolloid and ε-amino groups of the gluten (Ribotta et al., 2005).The hydroxyl groups in the xanthan gum structure allow more water interactions through hydrogen bonding thereby increasing water absorption (Guarda et al, 2004).

- Wraps made from low grade flour with added xanthan gum will be more stable against retrogradation than wraps made without added xanthan gum. Xanthan gum interacts with starch via hydrogen bonding (Tang et al., 2013). The interaction is believed to cause a weakening in the starch structure, by inhibiting amylose re-associations thus preventing the formation of the amylose double helices which forms at the onset of starch retrogradation, which results in the delay of staling in baked products (Biliaderis et al., 1997). The hydroxyl groups in the xanthan gum structure promote the binding of water via hydrogen bonding (Tavakolipour and Kalbasi-Ashtari, 2007). This results in the retardation of moisture migration from the crumb to the crust, thereby slowing down the staling process of wraps during storage, as well as retarding the re-crystallization of starch polymers and improving the shelf life of the wraps (Kohajdová and Karovičová, 2008).

- Wraps made from low grade flour, will have a higher total phenolic content and antioxidant activity than wraps made from white bread flour. Phenolic compounds in wheat are mainly located in the outer layers of the kernel (Liyana-Pathirana et al., 2006). Low grade flour is made up of the aleurone layer, and bran layer, which make up the outer layers of the wheat kernel (Waggle et al., 1967, Caterall, and Cauvain, 2003, Campbell et al, 2007). Phenolic compounds are known to have antioxidant properties due to their hydrogen-donating ability (Lule and Xia, 2005)

3.2 Objectives

- To determine the effect of xanthan gum, on the dough rheological properties (water absorption, extensibility, dough strength, pasting properties) of low grade flour.
- To determine the effect of xanthan gum on the quality (rollability, tensile strength, XRD, DSC, and moisture content), and shelf life of wraps made from low grade flour.
- To determine the total phenolic content and antioxidant activity of wraps made from low grade flour, and wraps made from white bread flour.

Chapter 4: Experimental

4.1 Experimental Design

The experimental design was divided into three sections.

The first section was the dough rheological properties which had one factor

Xanthan gum concentration ((0.25% (w/w) and 0.5% (w/w) xanthan gum of total low grade flour). Low grade flour with no xanthan gum was used as a control, and white bread flour was used as a reference.

The second section was the quality of the wraps, and it had two factors

The xanthan gum concentration ((0.25% (w/w) and 0.5% (w/w) xanthan gum of total flour) used in the wraps. wraps made from low grade flour with no xanthan gum were used as a control, and wraps made from white bread flour were used as a reference.

The second factor was storage time (days 0, 1, 2, and 3).

The third section was the bioactive properties of the wraps

As can be seen in Figure 4.1, the following dough rheological analyses were done: Alveogram properties, farinogram properties, Mixolab properties, and pasting properties. Only the first factor (xanthan gum concentration) was used for all dough rheology experiments.

The following quality and shelf life analyses of wraps were done: rollability of wraps, modulus of wraps, stress and strain of wraps, X-ray diffraction, and DSC. The quality analyses were done over four days. Both factors (xanthan gum concentration, and storage time) were used for all quality and shelf life analysis.

The following analyses were done for bioactive properties: Total phenolic content, and antioxidant activity of the wraps made from low grade flour, and white bread flour. Two types of samples were studied: simulated *in vitro* gastro-intestinal tract (GIT) digests of the wraps and organic solvent (acidified methanol) extracts.

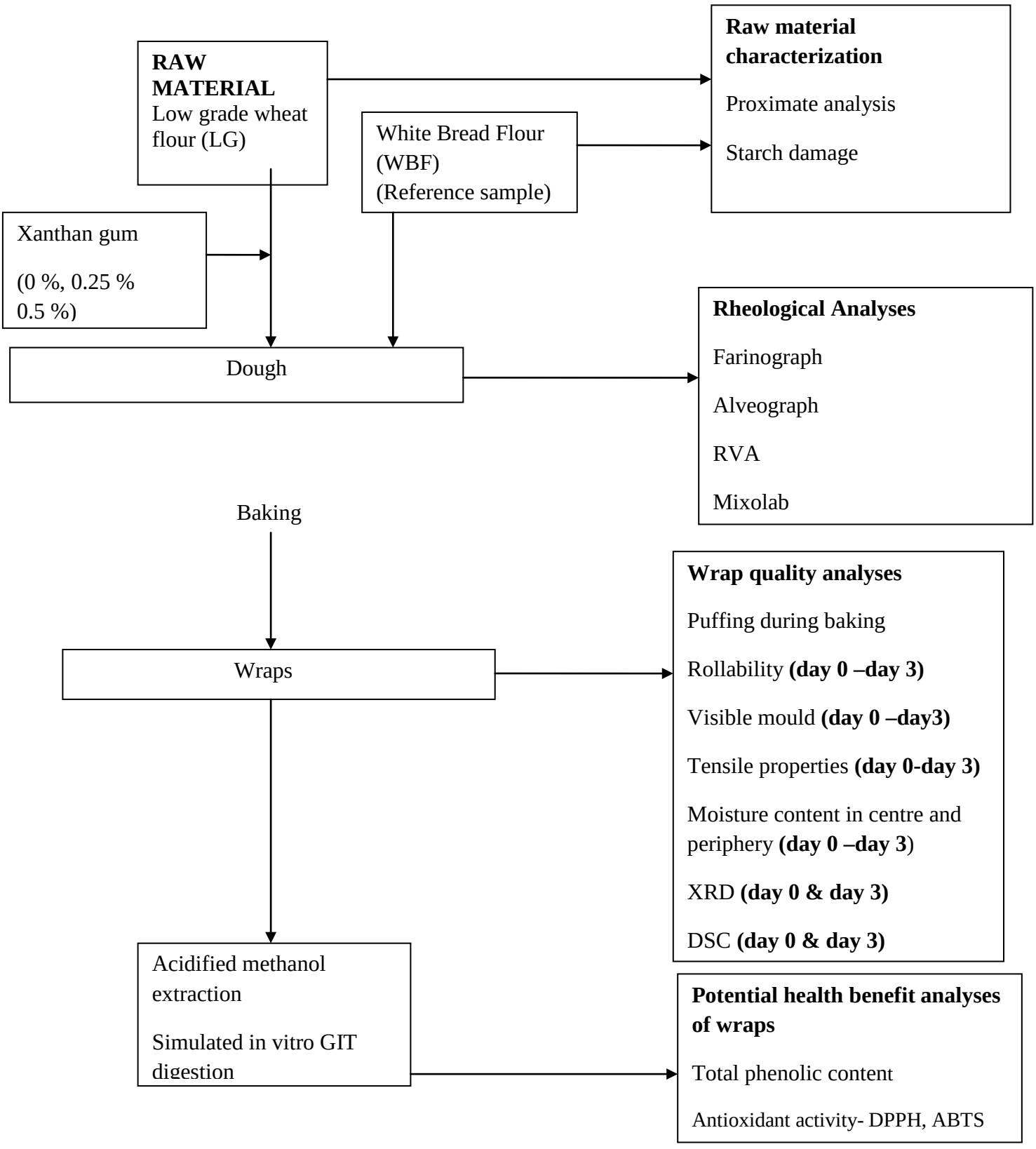


Figure 4. 1: Schematic representation of experimental design

4.2 Materials

Low grade wheat flour which was produced at the tail end of the milling process, and White bread flour were donated by Foodcorp (Pty) Ltd, (Johannesburg, South Africa). White bread flour was used as a reference. Xanthan gum was donated by A &D Food ingredients cc, (Johannesburg, South Africa).

4.2.1 Analyses of materials

4.2.1.1 Proximate analysis of flours

Moisture, ash, gluten, starch damage, fat, and protein (N x 5.7) of the white bread flour and low grade flour samples were determined according to the Approved Methods: (44–16 A), (08–07), (38-12), (76-33), (30–10), and (46-30) respectively of the American Association of Cereal Chemists (AACC 2000).

4.3 Preparation of flour samples

Four different flour samples were analysed and used to bake the wraps. (1) White bread flour with no additives (WBF), (2) Low grade flour with no additives (LG), (3) Low grade flour with 0.25 % (w/w) added xanthan gum (LG 0.25), and (4) Low grade flour with 0.5 % (w/w) added xanthan gum (LG 0.5). Low grade flour with xanthan gum was prepared by weighing out low grade flour into a ziplock bag, and adding 0.25% or 0.5% of xanthan gum to the bag. An air pocket was left in the bag before closing. The bag with the flour and xanthan gum were vigorously shaken to uniformly mix the flour and xanthan gum mixture. The flour-xanthan mixture was not prepared in advance, but prior to each analysis.

4.4 Analyses of flour samples

4.4.1 Farinograph

Flour samples (WBF, LG, LG 0.25, LG 0.5) were evaluated using a Brabender Farinograph (Brabender GmbH & Co., Duisburg, Germany) according to the AACC Approved method 54-21 (AACC, 2000). The parameters measured by the Farinograph included Water

absorption, dough development time, stability and mixing tolerance index. A typical farinogram is represented in Figure 4.2.

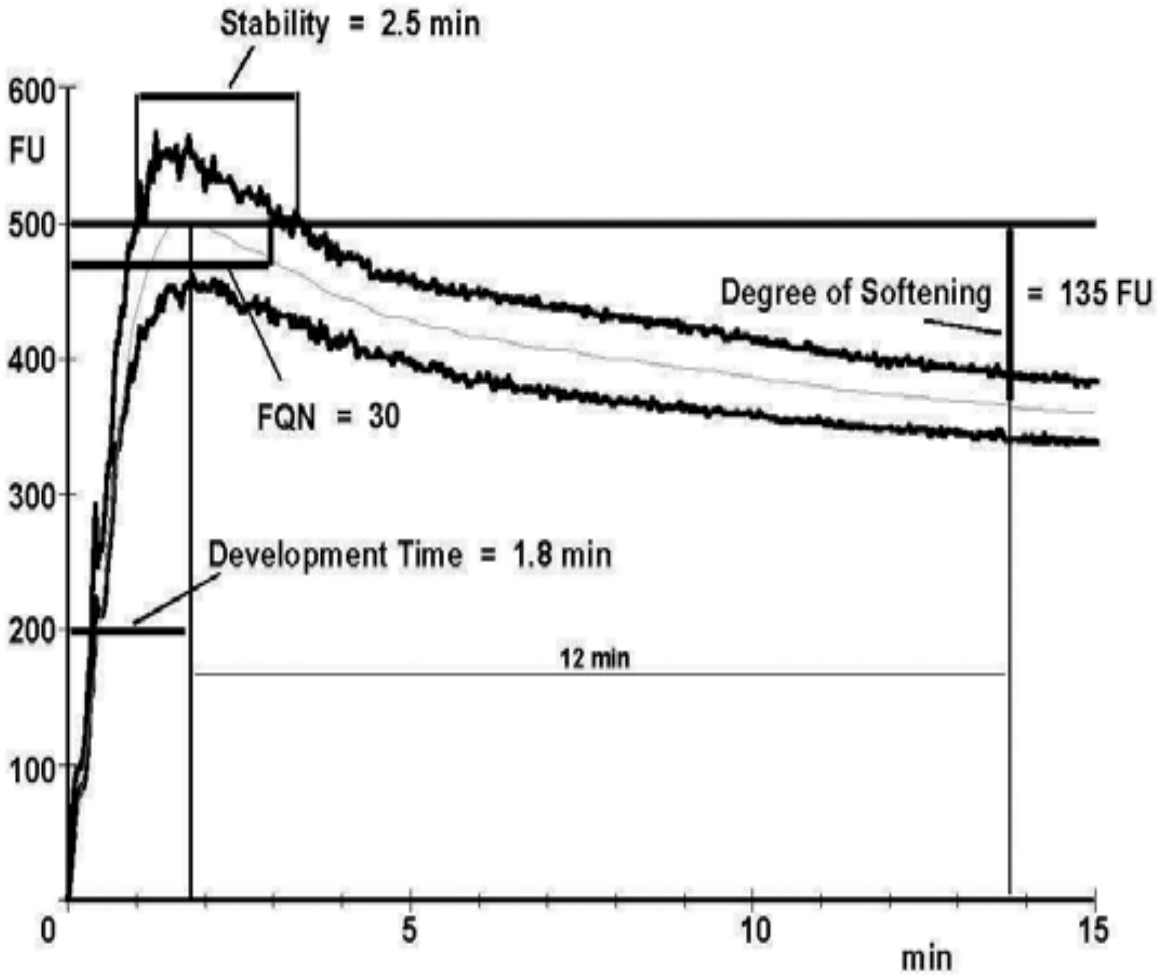


Figure 4. 2: Typical farinogram of white bread flour (Sluimer, 2005)

4.4.2 Alveograph

Dough behaviour of Flour samples (white bread flour, low grade flour, and low grade flour with added xanthan gum (0.25% and 0.5%) was evaluated using the Chopin Alveo-consistograph (Chopin Alveolink NG Consistograph; Villeneuve La Garenne, France) at constant and adapted hydration according to the AACC Approved method 54-21 (AACC, 2000). For adapted hydration, water was added according to the Farinograph water absorption of the flour, and not according to 50% hydration used for constant hydration.The following parameters from the graph were recorded and are illustrated in Figure 4.3:

P- The maximum overpressure (dough tenacity)

L- The average abscissa (dough extensibility)

W- The deformation of energy, derived from the area under curve (energy required to blow the bubble).

P/L ratio- The balance between dough tenacity, and extensibility

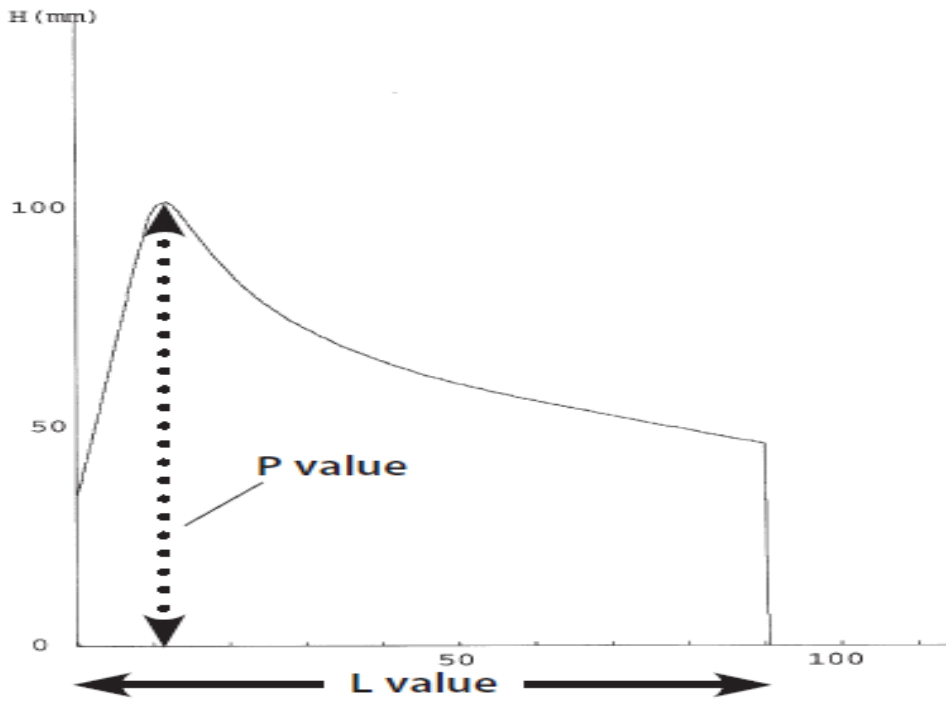


Figure 4. 3: Typical Alveograph of white bread flour (Sluimer, 2005)

4.4.3 RVA pasting

Pasting of the flour samples (white bread flour, low grade flour, and low grade flour with added xanthan gum (0.25% and 0.5%) was conducted using a Rapid Visco Analyzer Model 3D (Newport Scientific, Warriewood, Australia). Flour samples (3 g; 14% moisture basis) were suspended in distilled water and the weight adjusted to 28 g. The RVA was programmed to rapidly stir each sample mixture at 960 rpm for 10 s, then decrease and hold at 160 rpm for the duration of the test period. The temperature profile involved holding initially at 50° C for 2 min, then increase to 91°C over 4 min and holding at 91°C for 8 min before cooling to 50°C over a 4 min period, and keeping constant for 3 min. Peak viscosity and final viscosity were measured from the graph.

4.4.4 Dough torque measurements by Mixolab

The mixing and pasting behaviour of the different flour samples (white bread flour, low grade flour, and low grade flour with added xanthan gum (0.25% and 0.5%) were determined according to the ICC Standard 173 method using the Mixolab® system (Chopin Villeneuve La Garenne, France) which measures the torque (Nm/s) of dough between two kneading arms during real time. The standard ‘‘Chopin+’’ protocol was used (Table 4.1). Flour (75 g) was added to the Mixolab bowl, and water added according to the water absorption as measured by the Brabender Farinograph, and the protocol started to give a characteristic curve (Figure 4.4).

Table 4. 1:Chopin + standard Mixolab protocol (Rosell et al., 2010)

Chopin+ profile	
Mixing Speed	80 rpm
Dough weight	75g
Tank temperature	30°C
Temperature 1st Plateau	30°C
Duration 1st Plateau	8 min
Temperature 2nd Plateau	90 °C
Temperature gradient (15 min)	40°C
Duration 2nd Plateau	7 min
Temperature gradient (10 min)	40 °C
Temperature 3rd Plateau	50 °C
Duration 3rd Plateau	5 min
Total time	45 min

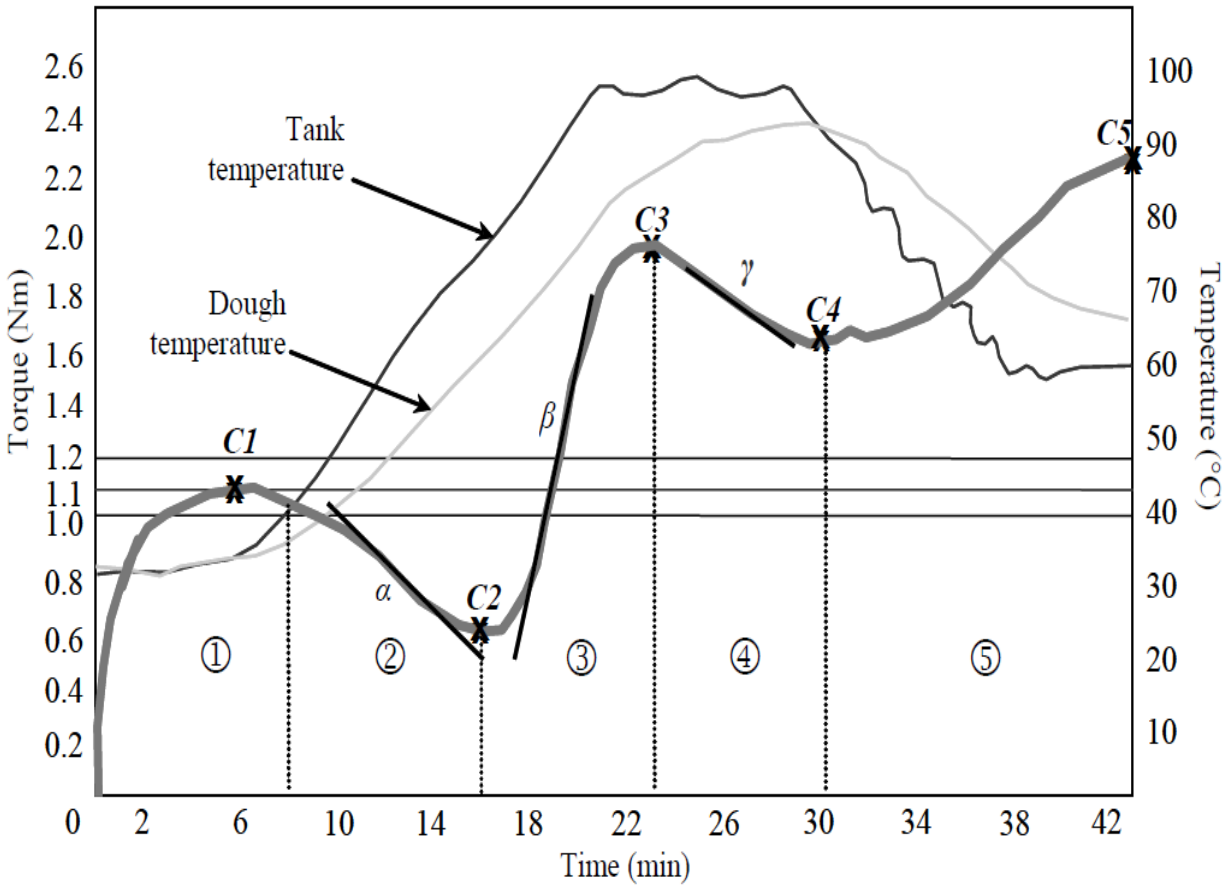


Figure **4. 4: Description of a typical curve obtained in the Mixolab (Rosell et al., 2010)**

The parameters expressed in the curve are:

C1 (Nm) – indicates the maximum torque during mixing;

C2 (Nm) – measures the protein weakening which is based on the mechanical work and temperature;

C3 (Nm) – expresses the uptake of water by the starch granule, and leaching out of amylose;

C4 (Nm) – indicates the stability of the starch gel formed;

C5 (Nm) – measures the starch retrogradation during the cooling stage;

α - represents the slope of the curve between the end of the period of 30ºC and C2, it gives an indication about the rate of the proteins thermal weakening;

β - Represents the slope of the curve between C2 and C3, and gives indications about the gelatinization rate;

γ - represents the slope of the curve between C3 and C4, and gives indications about the rate of enzymatic hydrolysis (Stoenescu et al., 2010).

4.5 Preparation of wheat wraps

The wraps were prepared as described by Shalimi and Laxmi (2007) and Anton et al (2009) with slight modifications. Each flour sample (1000 g) was added to a spiral mixer, and mixed for 60 seconds at low speed to uniformly mix the flour. Mixer was stopped and salt (15 g), vegetable shortening (60 g), and water (32 ^{o}C) according to the water absorption of the flour (as determined by the Brabender Farinograph) were added to the uniformly mixed flour. The amount of water added was as follows; LG (722g); LG 0.25 (733g); LG 0.5(743g); and WBF (626g).Mixer was turned on to low speed for the first minute, then to medium speed for a further 5 min to form the dough. Dough was then covered with a plastic lid and left to rest for 5 min. The dough was then divided into 35 g round dough balls using a dough divider. The dough balls were then proofed in a humidity controlled proofer for 30 min at 46 ^{o}C, and 89 % RH (relative humidity). Dough balls were then individually sheeted in four steps. First to 7 mm, then to a thickness of 4 mm, then to a thickness of 3 mm, and then to a final thickness of 2 mm (Dough was rotated during sheeting to form a circular shape). The sheeted dough was then baked on an electric hot plate preheated to 218 ^{o}C, for 30 s on the first side, flipped and baked for 50 s on the second side, and flipped and baked for another 10 s on first side The baked wrap was then put on a cooling rack, and cooled at room temperature (25 ^{o}C) for about 15 min before packing into sealable polyethylene zip lock bags.

4.5.1 Analyses of wrap quality

4.5.1.2 Visible mould growth

The wraps, were checked visually for any mould growth daily.

4.5.1.2 Rollability of Wraps

The rollability of wraps measures the cracking and breakage of a wrap. This was determined subjectively as described by Pascut et al. (2004). The wrap was rolled around a 1 cm dowel and the extent of cracking and breaking (rollability score) estimated using a subjective scale, defined as : 5 = no cracking 4= cracks on one side only, 3= breaks on one side , 2= breaks on one side and cracks on the other , 1= unrollable, breaks easily. Rollability was tested over a period of four days.

4.5.1.3 Tensile properties of wrap

The tensile properties of wraps were measured using the EZ-L Shimadzu texture analyser (KYOTO- Tokyo, Japan), according to the method of Gujral et al. (2004) with slight modifications. After 0, 1, 2, and 3 days respectively of wrap storage at 25° C, two rectangular strips from each wrap sized (20 mm x 100 mm) were cut. The wrap strip was held between two tensile grips, with one end attached to the analyzer platform and the other end attached to the analyzer arm. Both the tensile grips were properly aligned and set 7 mm apart, a load cell of 50 N was used at cross head speed of 1.0 mm/s to pull the wrap strip apart until it ruptured. The stress (MPa), and strain (%) values were recorded, and tensile modulus (MPa) calculated.

4.5.1.4 Moisture % of wraps

Moisture % of wraps was determined as described by Shaikh et al. (2008) with slight modification. 5 g pieces from the centre, and edge of the wrap sample were each placed in an air-oven at 103 °C for 3 h. Moisture content was determined according to AACC-approved method (44–16 A) (AACC, 2000)

4.5.1.5 Sample preparation for X-ray Diffraction, and DSC

The starch in the wraps was stabilized according to the method used by Alviola (2009) with slight modifications. Two wraps of each sample were mixed with 250 ml of methanol in a blender for 2 min, and vacuum-filtered. The sample was rinsed again with 200 ml methanol and filtered before drying at 50°C for 3 h in a forced-air oven, and grinding. Stabilized samples were stored in liquid nitrogen until further use in DSC, and X-ray diffraction analyses.

4.5.1.5a X-ray Diffraction

The X-ray diffraction analysis was performed according to the method described by Alviola (2009) with slight modifications. The diffractometer used was a PAnalytical X'Pert Pro Powder Diffractometer (Ostfildem, Germany), with an X'Celerator detector operating at 35 kV, 50 mA and Co-Kα, radiation (1.78901 Å) at a target voltage and current of 40 kV and

40mA. To adjust the moisture content, the destabilized wrap samples as prepared in 4.5.1.5, were milled into a fine powder using a pestle and mortar. They were then stored in a sealed vacuum chamber over a saturated NaCl solution (relative humidity 75% at 20–25 °C) for 5 days in order to standardise the water content of the samples to 13%. The samples were then scanned over an angular range 2 to 30° (2θ) with a scan step time of 14 s, step size of 0.017º and a divergence slit size of 1°. Diffractograms were interpreted using X'Pert High score Plus software.

4.5.1.5b Differential Scanning Calorimetry

DSC was determined according to the method described by Alviola (2009), using a high pressure differential scanning system with Stare® software (HP DSC 827e, Metler, Toledo Greifensee, Switzerland). Stabilized wrap samples (10 mg) were rehydrated with 20 mg of distilled water, and left to equilibrate at room temperature for 20 min. Scanning was done from 30 ^{o}C to120 ^{o}C at a rate of 10 ^{o}C /min. Indium (Tp = 156.61 ^{o}C, 28.45 J/g) was used as a standard, and an empty pan was used as a reference.

4.6 Preparation of wrap extracts for bioactive properties analyses

4.6.1 Acidified methanol extraction

Acidified methanol (1% HCl (v/v) in methanol) was used as the extracting solvent, for the determination of total phenolic content and antioxidant activity of the LG and WBF wrap samples. Duplicate samples were extracted in 30 ml solvent in three phases as follows: each wrap sample was dried in a forced-air oven (50°C for 3 h), and blended using a Waring blender to obtain a fine powder. 10 ml solvent was added to 0.5 g of the sample in a conical flask, and completely covered with aluminium foil, stirred for 2 h with a magnetic stirrer, transferred to 40 ml plastic centrifuge tubes, centrifuged at 3500 rpm for 10 min at 25°C, and decanted keeping the supernatant. The sample residue was rinsed again with 10 ml of the solvent, stirred for 20 min, centrifuged again as above, and decanted keeping the supernatant. This step was repeated as in the second time, and the supernatants were combined and stored in a glass bottle covered with aluminium foil, and kept in cold storage (5ºC) until further analyses (Kayitesi, 2009).

4.6.2 Simulated in vitro GIT digestion

A simulated in vitro GIT digestion procedure was used as described by Gil-Izquierdo et al. (2002), with slight modification. Each wrap sample (WBF & LG) was blended with water to produce a slurry with porridge like consistency. For each sample (in duplicate) the slurry (5 g) was added to a flask, and pH adjusted to 2 by adding 10 M HCl. 5 ml of P7000-100G Pepsin, activity 863 units/mg protein (Sigma-Aldrich, St Louise, MO) was then added to the slurry, and the slurry incubated in a shaking water bath (130 rpm; 37 ^{o} C) for 2 h. 1 M $NaHCO_3$ was then added to the slurry to adjust the pH to 7, then pancreatin-bile salts mixture (7.5 ml) was added and slurry incubated again in a shaking water bath (130 rpm; 37 ^{o} C), for a further 2 h. The slurry was then centrifuged (3000 g, 25 °C, 15 min) and decanted, keeping the supernatant. The residue was rinsed using distilled water (50 ml), and centrifuged and decanted as before. This step was repeated a second time, and supernatants combined. The combined supernatants were heated to 75 ^{o} C and kept at that temperature for 15 min to denature the enzymes, before cooling down to room temperature (25 ^{o} C). The cooled supernatants were then filtered under vacuum, freeze dried, and stored at -20 ^{o} C (in air tight poly-ethylene bags covered with aluminium foil) until further analysis.

Freeze dried sample (0.1 g) was rehydrated with 10 ml distilled water for the total phenolic content, and antioxidant activity analyses.

4.7 Bioactive properties analyses

4.7.1 Determination of total phenolic content using the Folin-Ciocalteu method

The total phenolic content was determined using a modified Folin-Ciocalteu assay (Waterman and Mole, 1994). Folin-Ciocalteu reagent (1.25 ml) was added to phenolic extracts (0.25 ml) in 25 ml volumetric flasks and mixed. Within 8 min, 20% (w/v) sodium carbonate solution (3.75 ml) was added to the volumetric flasks, and content made up to volume with distilled water. Volumetric flasks were closed with stoppers, and thoroughly mixed. After two hours, the absorbance of the solutions was measured at 760 nm using a

Lambda EZ150 spectrophotometer (Perkin Elmer Corporation, USA). Catechin was used as a standard.

4.7.2 Determination of the antioxidant activity using the ABTS radical scavenging assay

The ABTS antiradical assay was used to measure antioxidant activity as described by Awika et al. (2003). To produce the ABTS radical, equal volumes of 8 mM ABTS and 3 mM potassium persulphate (prepared using distilled water) were mixed in a volumetric flask, and stored in the dark for at least 12 h for reaction to take place. 2.5ml of the ABTS radical solution was mixed with 72.5ml of a phosphate buffer solution (pH 7.4) (to form a working solution. The working solution (2.9 ml) was added to 1ml phenolic extracts as prepared above, as well as to 1ml Trolox standards at the following concentrations; 0, 200 µM, 400 µM, 600 µM, 800 µM, and 1000µM, and left to react for 30 min. Trolox standard were prepared in 1% acidified methanol. The absorbance measurements were then taken at 734 nm, and antioxidant activity determined as trolox equivalents (µmol TE/g sample, dry weight basis)

4.7.3 Determination of the antioxidant activity using the DPPH radical scavenging assay

The DPPH antiradical assay was used to determine the antioxidant activity as described by Awika et al. (2003). A mother solution was prepared by dissolving 24 mg DPPH in 100 ml methanol, and stored in a foil-covered glass bottle at -20 ^{o}C. 10 ml of the mother solution was added to 50 ml methanol to make a working solution. The working solution (2.85 ml) was added to phenolic extracts (0.15 ml) in tightly sealable test tubes, and left to react in a shaker for 6 h. The absorbance measurements were then taken at 515 nm. Trolox was used as a standard, and antioxidant activity was determined as trolox trolox equivalents (µmol TE/g sample, dry weight basis)

4.8 Statistical analysis

All analyses were done in triplicate . The results were subjected to one-way analysis of variance (ANOVA) with the exception of rollability score, and tensile modulus where two-way ANOVA was also used, because time was also a factor in those experiments. The flour samples, and wrap samples with added xanthan gum were the independent variables. The

dough rheology parameters, time, and wrap quality parameters were the independent variables. The significant differences between the sample means were determined using Fisher's least significance difference (LSD) test at the 95% significance level. STATISTICA® (version 9 StatSoft, Tulsa, OK, USA) was used for data analysis.

Chapter 5: Results

5.1 Proximate composition, gluten content and starch damage

The protein content, starch damage, fat percentage, gluten percentage, and ash percentage of low grade flour (Table 5.1) were significantly ($P< 0.05$) higher than white bread flour. The moisture content of low grade flour was however significantly ($P< 0.05$) lower than white bread flour.

Table 5.1: Proximate composition and starch damage of low-grade wheat flour and white bread flour (Dry basis)

Flour	Protein (%)	Fat (%)	Wet Gluten (%)	Starch damage (%)	Ash (%)
White bread flour	15.5a (0.1)	2.3a (0.8)	35.6a (0.2)	8.7a (0.1)	0.8a (0.2)
Low grade flour	18.6 b (0.1)	3.9b (0.9)	38.6b (0.3)	10.8b (0.2)	1.8b (0.3)

Values are means and standard deviations (in parentheses) of three determinations (n =3)
Data followed by the same character in the same column, are not significantly different ($P> 0.05$)
White bread flour (Used as a reference)

5.2 Dough rheology

5.2.1 Farinograph characteristics

The farinogram of white bread flour, low grade flour, and low grade flour with added xanthan gum are shown in Figure 5.1; the data derived from the farinograms are shown on Table 5.2. The farinogram data showed that the water absorption as well as the mixing tolerance index of low grade flour was significantly ($P< 0.05$) higher than white bread flour. The water absorption of low grade flour increased as the xanthan gum concentration added to low grade flour increased. The mixing tolerance index on the other hand significantly decreased with increasing xanthan gum concentration added to low grade flour. The mixing stability of low grade flour was also significantly ($P< 0.05$) increased by increasing concentrations of xanthan gum addition.

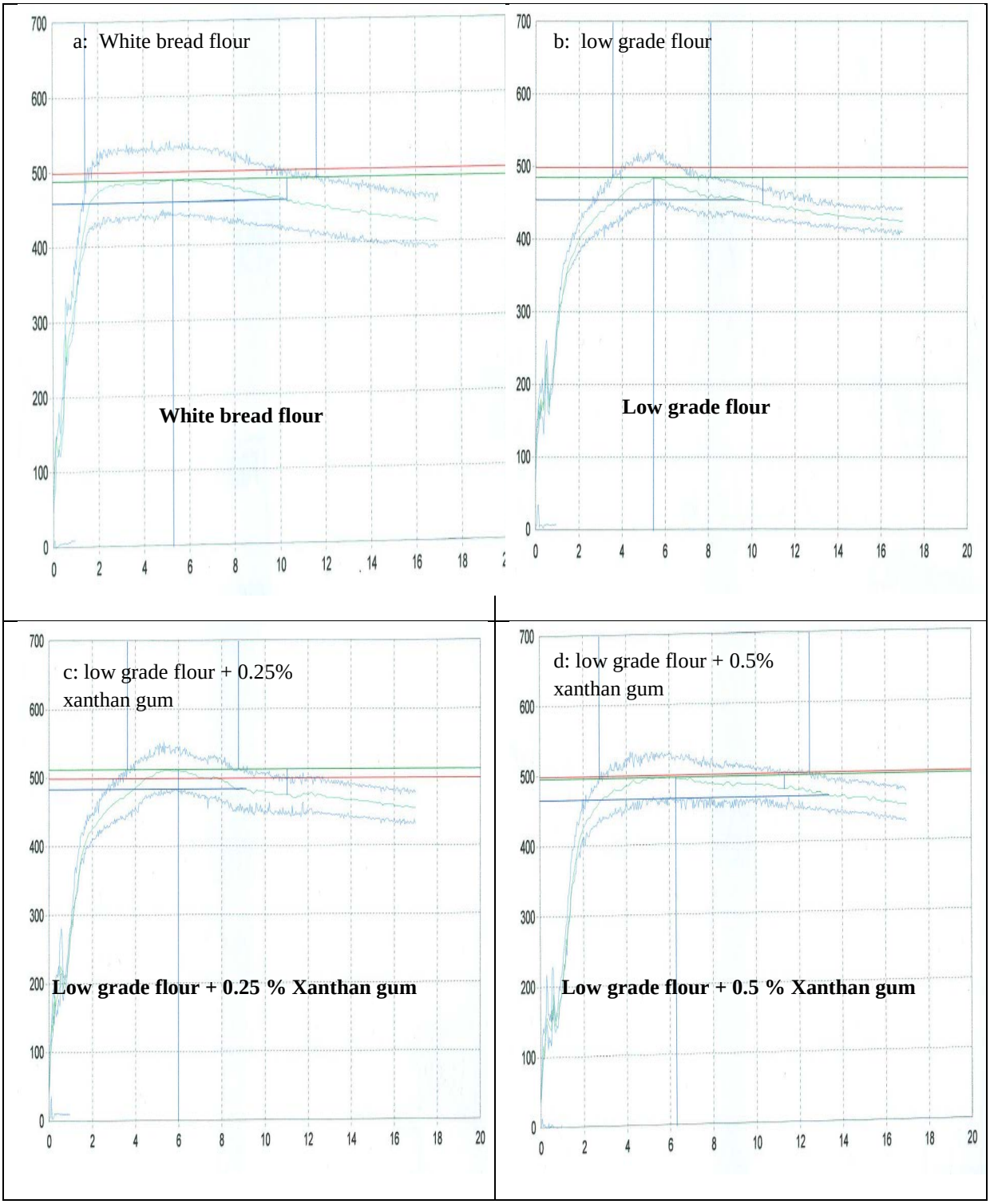


Figure 5.1: Effect of xanthan gum on farinogram of low grade flour

a-d: farinograms of flours

White bread flour (used as reference)

Table 5. 2: Effect of xanthan gum on farinogram values of low grade wheat flour

Flour	Water Absorption (%)	Dough Development Time (min)	Stability (min)	Mixing Tolerance Index (BU)
White bread flour	62.6a (0.1)	5.5a (0.3)	10.1a (0.1)	32.3a (3.2)
Low grade flour	72.2b (0.2)	5.6a (0.1)	4.1b (0.5)	44.0b (6.2)
Low grade flour + 0.25% Xanthan gum	73.3c (0.1)	6.0b (0.1)	5.9c (1.4)	34.7a (4.9)
Low grade flour + 0.5% Xanthan gum	74.3d (0.1)	5.5a (0.3)	8.5a (0.4)	21.7c (2.9)

Values are means and standard deviations (in parentheses) of three determinations (n =3)
Data followed by the same character in the same column, are not significantly different (*P*> 0.05)
Water absorption: Amount of water in percentage (v/m of solids) that results in a dough consistency of 500 BU (Brabender Units)
Dough development time: Time in minutes until the curve has reached is maximum dough consistency (500BU)
Mixing tolerance index: The difference in Brabender units (BU) from the top of the curve at the peak to the top of the curve measured 5 minutes after the peak is reached.
Stability: The difference in time (to the nearest half minute) between the point when the top of the curve intercepts the 500BU and the point where the top of the curve leaves the 500BU line
White bread flour (used as reference)

5.2.2 Alveograph characteristics

The Alveograph characteristics of low grade flour at constant hydration are shown in Table 5.3 and Figure 5.2. The dough tenacity (P) of low grade flour (84.3 mm) was significantly higher than white bread flour (71.3 mm), while the extensibility (L) of low grade flour (46.0 mm) was significantly lower than white bread flour (90.0 mm). The dough tenacity (P) and the deformation energy (W) of low grade flour were increased as the xanthan gum concentration added to low grade flour increased. The extensibility (L) on the other hand was found to decrease with increasing xanthan gum concentration added to low grade flour.

Adapted hydration was also used in determining the Alveograph characteristics of the flours. The reason for this was because there is a difference in Alveograph properties at constant hydration and adapted hydration (Preston et al., 1987). At constant hydration there is only 50% moisture, and the flour proteins can be under-hydrated which would alter the results (Preston et al., 1987). At constant hydration, water is added according to the Farinograph water absorption of the flour, and the flour proteins are well hydrated (Preston et al., 1987). The Alveograph characteristics of low grade flour at adapted hydration are shown in Table 5.3 and Figure 5.3. Dough tenacity (P) of low grade flour (26.3 mm) was significantly ($P< 0.05$) lower than that of white bread flour (60.7 mm), while the dough extensibility of low grade flour (123.3 mm) was significantly ($P< 0.05$) higher than that of white bread flour (85.0 mm). The dough tenacity (P) and deformation energy (W) of low grade flour increased with increasing xanthan gum concentration added to low grade flour. At adapted hydration, the addition of xanthan gum to low grade flour produced a more viscoelastic dough. The higher concentration of xanthan gum (0.5%) added to low grade flour gave dough with viscoelastic properties close to white bread flour.

Although the actual extensibility and dough tenacity values of low grade flour were different at the two different hydrations, it is clear that xanthan gum decreased dough extensibility but increased dough tenacity of low grade flour.

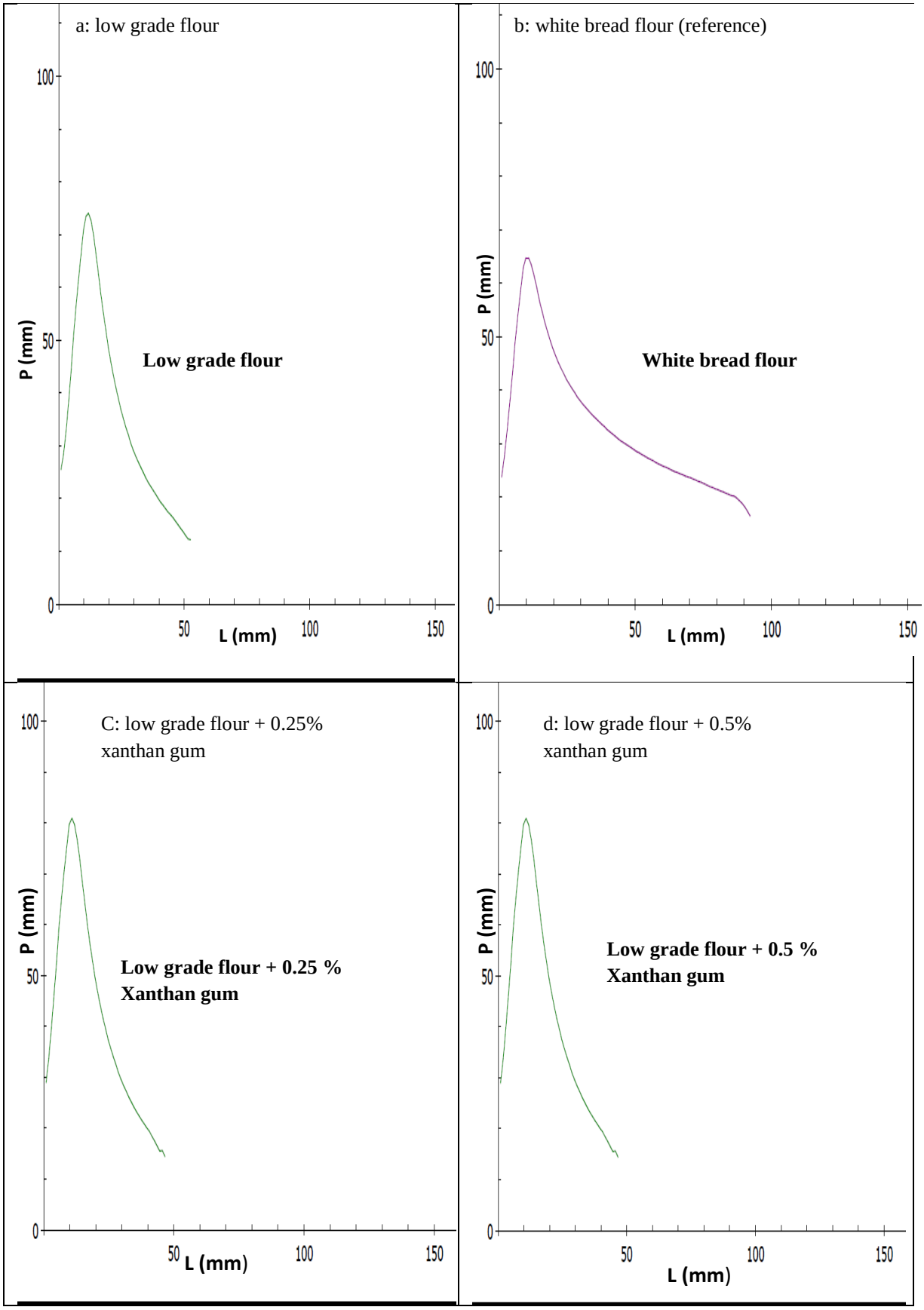


Figure 5. 2: Effect of xanthan gum on alveogram parameters of low grade flour at constant hydration
P-dough strength, L- dough extensibility; Constant hydration: 50% hydration, a-d: alveograms of flours at constant hydration

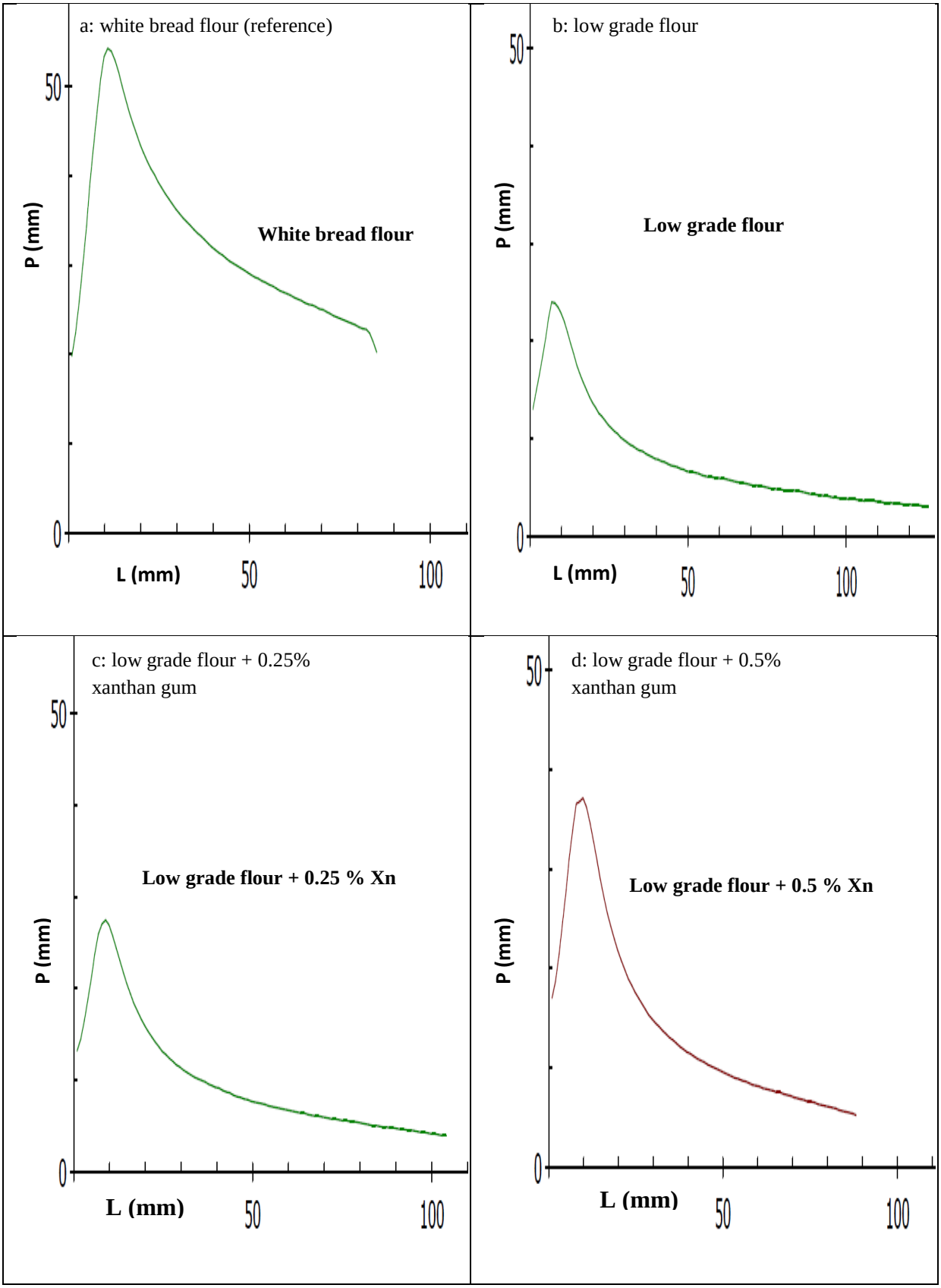


Figure 5. 3: Effect of xanthan gum on alveogram parameters of low grade flour at adapted hydration
P-dough strength, L- dough extensibility; Adapted hydration: Hydration according to Farinograph water absorption, a-d: alveograms of flours at adapted hydration

Table 5. 3: Effect of xanthan gum on hydration adapted alveogram values of low grade flour at constant hydration and adapted hydration

SAMPLE	P (mm)	L (mm)	G	W (x10^{-4}J)	P/L
a) Constant Hydration					
White bread flour	71.3a (1.5)	90.0a (4.0)	21.1a (0.5)	205.0a(10.0)	0.8a (0.1)
Low grade flour	84.3b (0.6)	46.0b (1.0)	15.1b (0.2)	118.3b (1.1)	1.8b (0.1)
Low grade flour + 0.25% Xn	89.3c (0.6)	45.7b (0.6)	15.0b (0.1)	124.3b (5.5)	2.0b (0.1)
Low grade flour + 0.5% Xn	116.3d (0.6)	33.7c (2.5)	12.9c (0.5)	141.0c (8.1)	3.47c (0.2)
b) Adapted Hydration					
White bread flour	60.7a (2.0)	85.0a (2.6)	20.5a (0.3)	187.7a (9.5)	0.7a (0.03)
Low grade flour	26.3b (0.6)	123.3b (2.5)	24.7b (0.3)	64.0b (3.6)	0.2b (0.01)
Low grade flour + 0.5 % Xn	31.0c (1.7)	106.6c (2.3)	22.9c (0.2)	69.7bc (4.7)	0.3c (0.02)
Low grade flour + 0.5 % Xn	42.7d (3.8)	89.3a (3.2)	21.0a (0.4)	86.7c (14.3)	0.5d (0.02)

Values are means and standard deviations (in parentheses) of three determinations (n =3)
Data followed by the same character in the same column under the same hydration are not significantly different (*P*> 0.05)
Adapted hydration: Hydration according to Farinograph water absorption
Constant hydration: 50% hydration
P -dough strength, L - dough extensibility, G - index of swelling, W- deformation energy,
P/L- configuration ratio; white bread flour (used as reference); Xn: xanthan gum

5.2.3 RVA pasting

The RVA pasting viscosity curves of white bread flour, low grade flour, and low grade flour with added xanthan gum are shown in Figure 5.4. White bread flour had significantly higher peak viscosity, setback, and final viscosities than low grade flour. The peak viscosities of low grade flour increased significantly ($P< 0.05$) with increasing concentrations of xanthan gum added to low grade flour. The peak viscosity of low grade flour with 0.5% added xanthan gum was significantly higher than the peak viscosity of white bread flour.

5.2.4 Mixolab

The Mixolab parameters of the flours are shown in Table 5.4 and Figure 5.5. The C1 (dough development), C2 (protein weakening), C3 (starch gelatinization), C4 (amylase activity), and C5 (starch gelling) values of white bread flour, were significantly ($P<0.05$) higher than corresponding values for low grade flour. The addition of xanthan gum to low grade flour did not significantly ($P> 0.05$) alter C1 to C4 values of the Mixolab values. Xanthan gum addition to low grade flour did however significantly ($P< 0.05$) decrease the C5 mixolab value.

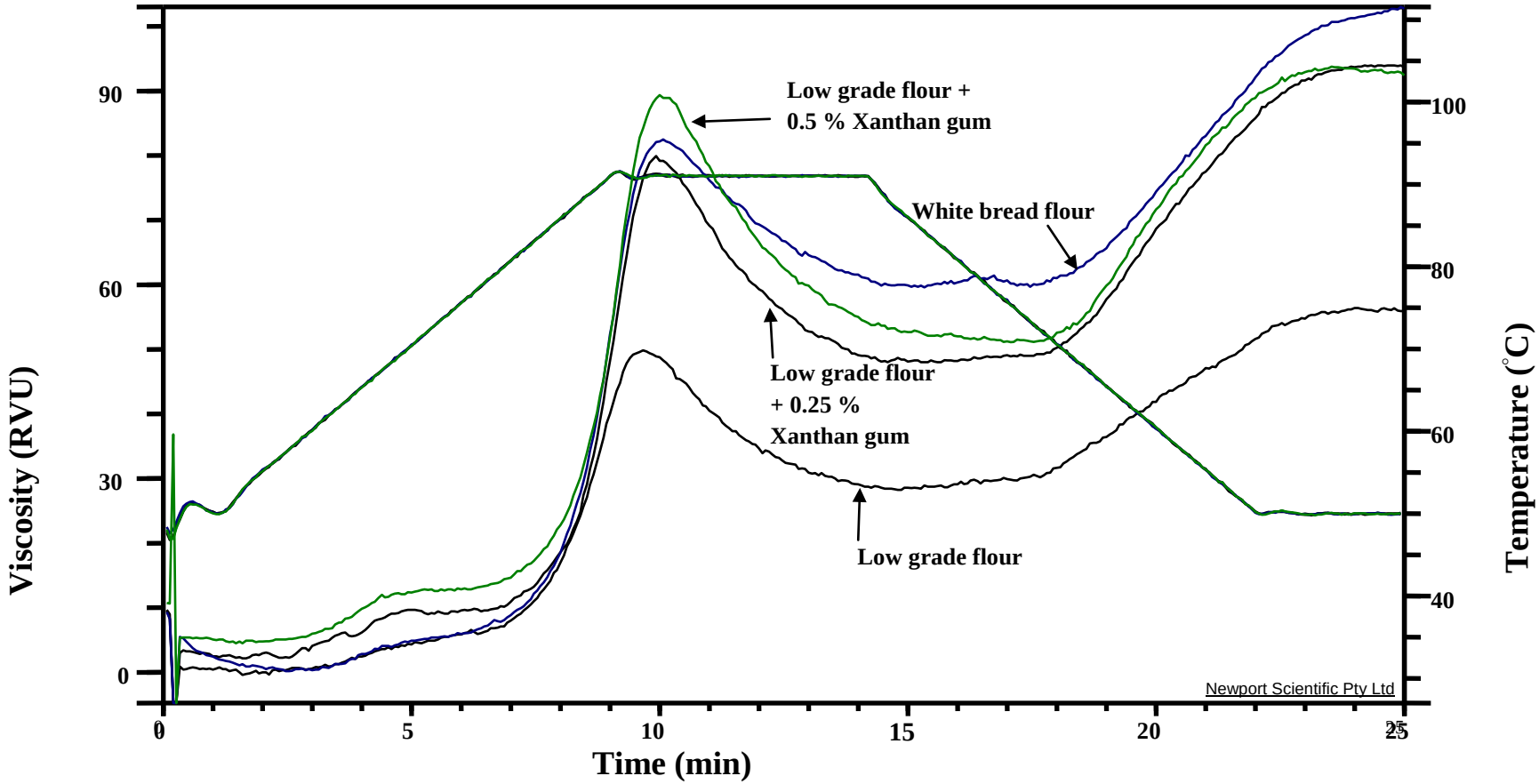


Figure 5. 4: Effect of xanthan gum on pasting properties of low grade flour

White bread flour (used as reference)

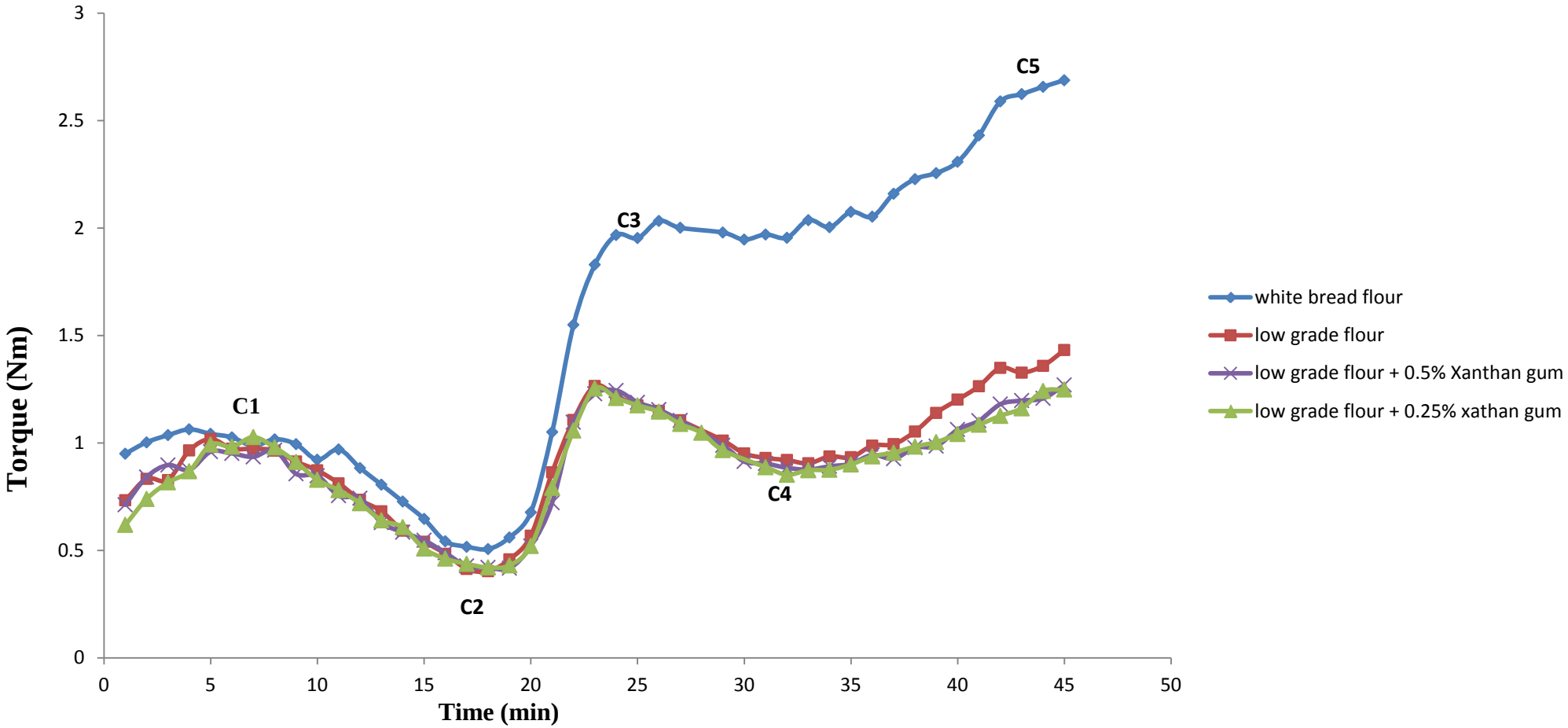


Figure 5. 5: Effects of xanthan gum on the Mixolab curve of low grade flour

White bread flour (used as reference)

Table 5. 4: Effect of xanthan gum on Mixolab parameters of low grade flour

flour	C1	C2	C3	C4	C5
White bread flour	1.06a (0.10)	0.52a (0.10)	2.04a (0.20)	1.96a (0.10)	2.78a (0.60)
Low grade flour	0.99b (0.10)	0.40b (0.10)	1.25b (0.20)	0.90b (0.20)	1.36b (0.50)
Low grade flour + 0.25% xn	0.99b (0.10)	0.40b (0.10)	1.26b (0.10)	0.87b (0.10)	1.25c (0.10)
Low grade flour + 0.5% Xn	0.97c (0.10)	0.41b (0.01)	1.27b (0.20)	0.84b (0.20)	1.22c (0.30)

Values are means and standard deviations (in parentheses) of three determinations (n =3)
Data followed by the same character in the same column, are not significantly different (P> 0.05)
C1: maximum point of the first mixing stage; C2-C5: maximum points of the corresponding stages
White bread flour (used as reference)
Xn: xanthan gum

5.3 Quality of wraps

5.3.1 Puffing of wraps

Figure 5.6 illustrates the puffing of wraps made from white bread flour, low grade flour, and low grade flour with added xanthan gum during baking. Puffing of wraps refers to the air build up which occurs during baking of wraps as a result of steam pressure build up, resulting in the separation of the two layers to form an aerated product like ball (Ram and Nigam, 1982).

No puffing was observed for wraps made from white bread flour, wraps made from low grade flour, and wraps made from low grade flour with added xanthan gum (0.25% and 0.5%) at 30 seconds. At 60 seconds however, bubbles forming at different sites of the wraps could be observed on wraps made from white bread flour, and low grade flour with added xanthan gum (0.25% and 0.5%). The wraps made from low grade flour with 0.25% xanthan gum continued to have the bubble formation sites even at 90 seconds. The bubbles did not coalesce to form one big puff. The bubbles which began to form on wraps made from white bread flour, and low grade flour with 0.5% added xanthan gum however did coalesce to form one large puff at 90 seconds.

5.3.2 Rollability score

The rollability scores of wraps made from white bread flour, low grade flour, and low grade flour with xanthan gum are shown in Table 5.5. On day 0 of storage, rollability score of wraps made from white bread flour, low grade flour, and low grade flour with added xanthan gum was 5. There was no significant (P<0.05) difference between the rollability scores of the four wraps. This suggests that wraps were still fresh and rollable on day 0. From day 0, the rollability score of the wraps (low grade flour, white bread flour, low grade flour with xanthan gum) decreased each day. On day 0 and day 1, there was no significant difference between the rollability scores of the different wraps. However, from day 2 the rollability score of wraps made from low grade flour increased with addition of xanthan gum, and the 0.5% treatment of xanthan gum increased rollability score of wraps made from low grade flour the most.

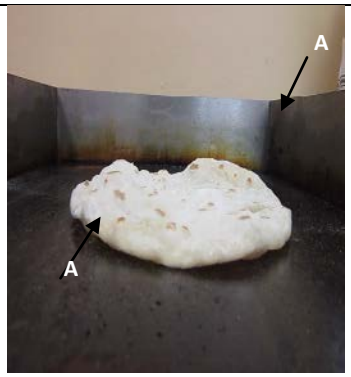
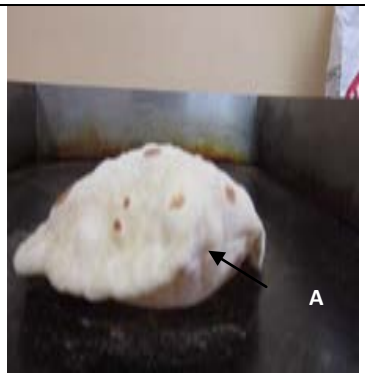
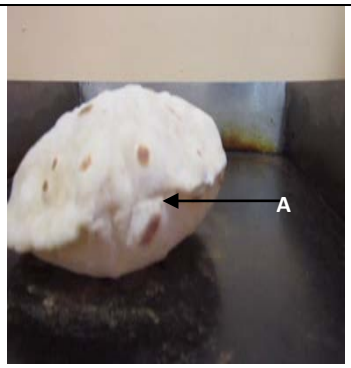
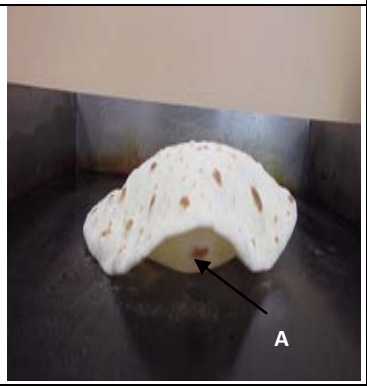
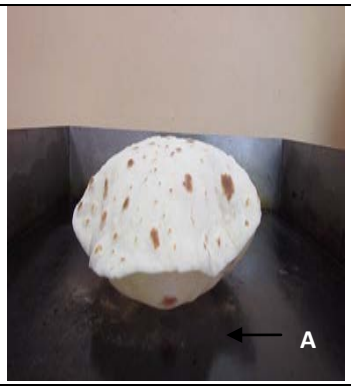
sample	30 seconds	60 seconds	90seconts
Low grade flour			
Low grade flour + 0.25 % Xanthan gum			
Low grade flour + 0.5 % Xanthan gum			
White bread flour			

Figure 5. 6: Puffing behaviour of wraps during baking

A: Puffing sites of the different wraps during baking White bread flour (used as reference)

Table 5. 5: Effect of xanthan gum on rollability scores of wraps over time

Wraps	DAY 0	DAY 1	DAY 2	DAY 3
White bread flour	5.0 A^{a} (0.0)	4.0 B^{b} (0.0)	3.0 C^{d} (0.0)	1.7 C^{c} (0.6)
Low grade flour	5.0 A^{a} (0.0)	4.0 B^{b} (0.0)	2.0 C^{e} (0.0)	1.0 D^{f} (0.0)
Low grade flour + 0.25 % Xanthan gum	5.0 A^{a} (0.0)	4.0 B^{b} (0.0)	2.7 C^{d} (0.6)	1.7 D^{e} (0.6)
Low grade flour + 0.5 % Xanthan gum	5.0 A^{a} (0.0)	4.0 B^{b} (0.0)	3.3 C^{c} (0.6)	1.8 D^{e} (03)

Values are means and standard deviations (in parentheses)
Data followed by the same lower case character in the same column, are not significantly different ($P > 0.05$).

Data followed by the same upper case character in the same row, are not significantly different ($P > 0.05$).White bread flour (used as reference)

Rollability was rated on a scale of 1–5, where 1 = wraps are unrollable; 2= wraps have cracks on both sides when rolled and down the middle; 3 wraps crack on one side only when rolled; 4= the two crusts of wrap separate and 5= wraps are flexible and rollable with no cracks or separation of the crusts

5.3.3 Tensile properties

The tensile stress and strain curves of wraps made from white bread flour, low grade flour and low grade flour with added xanthan gum are shown in Figure 5.7. On day 0, the tensile stress of the wraps was low. Over time the tensile stress of the wraps increased, while the strain decreased.

The tensile stress of wraps increased between 0.04 MPa on day 0, and 0.13 MPa on day 3. On each day, the wraps made from low grade flour with added xanthan gum had the lowest tensile stress. The wraps made from low grade flour with 0.25% added xanthan gum had the lowest tensile stress on each day. The strain of the wraps decreased over time, from around 30% to 9%. On the first two days (day 0 and day 1), wraps made from white bread flour and low grade flour with 0.25% added xanthan gum had the highest strain. On day 2 and day 3, the strain of the wraps made from low grade flour with 0.25% xanthan gum was higher than those of the other wraps. The 0.25% addition of xanthan gum improves the softness of wraps made from low grade flour the most.

The modulus of wraps made from white bread flour, low grade flour, and low grade flour with added xanthan gum (Table 5.6) was low on day 0 ranging from 0.27 MPa to 0.5 MPa. The modulus of all the wraps (white bread flour, low grade flour, low grade flour with added xanthan gum) seemed to increase over time. From day 2 of storage, the modulus of wraps made from low grade flour was higher than wraps made from white bread flour. The addition of xanthan gum to low grade flour significantly ($P<0.05$) decreased the modulus of wraps made from low grade flour. The modulus of wraps made from low grade flour with 0.25 % added xanthan gum increased at a slower rate, and was lower than the modulus of wraps made from white bread flour, low grade flour, and low grade flour with 0.5 % added xanthan gum.

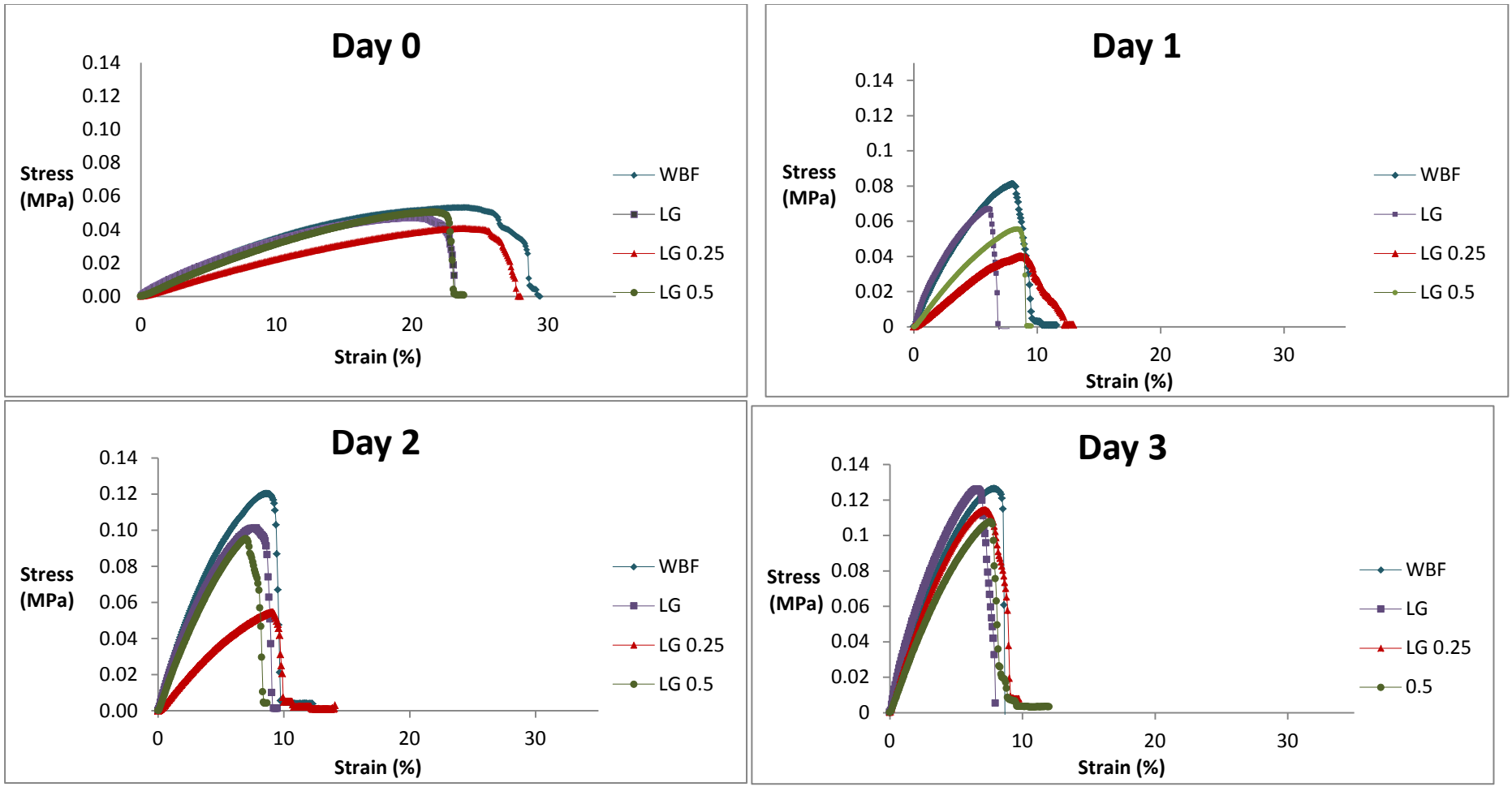


Figure 5. 7: Effect of xanthan gum on tensile properties of low grade flour wraps over time

WBF: wraps made from white bread flour (used as reference); LG: wraps made from low grade flour; LG 0.25: wraps made from low grade flour with 0.25% xanthan gum; LG 0.5: wraps made from low grade flour with 0.5% xanthan gum

Table 5. 6: Effect of xanthan gum on tensile modulus of wraps over time

Wraps	DAY 0 Modulus (MPa)	DAY 1 Modulus (MPa)	DAY2 Modulus (MPa)	DAY 3 Modulus (MPa)
White bread flour	0.3 A^{a} (0.0)	1.3 B^{a} (0.1)	2.2 C^{a} (0.1)	2.9 D^{ab} (0.6)
Low grade flour	0.5 A^{b} (0.1)	1.3 B^{a} (0.1)	1.8 B^{b} (0.1)	3.2 C^{b} (0.4)
Low grade flour + 0.25 % Xanthan gum	0.4 A^{c} (0.0)	0.8 B^{b} (0.1)	0.7 B^{c} (0.1)	1.2 C^{c} (0.1)
Low grade flour + 0.5 % Xanthan gum	0.4 A^{c} (0.0)	0.3 A^{c} (0.1)	1.7 B^{b} (0.2)	2.1 C^{a} (0.3)

Values are means and standard deviations (in parentheses) of three determinations (n =3)

Data followed by the same lower case character in the same column, are not significantly different (P> 0.05).

Data followed by the same upper case character in the same row, are not significantly different (P> 0.05)

White bread flour (used as reference)

5.3.4 Moisture content

The moisture content of wraps made from white bread flour, low grade flour, and low grade flour with added xanthan gum is shown in Table 5.7. The moisture content of the wraps ranged from about 30-34%. The moisture content of wraps in the centre and the periphery area of the wraps showed no significant difference (P>0.05). Moisture content of wraps made from low grade flour increased with increasing xanthan gum concentration. The moisture content of wraps was not significantly (P<0.05) altered during storage.

5.3.5 Relative crystallinity and DSC of wraps

Figure 5.8 shows the diffractograms of wraps made from white bread flour, low grade flour, and low grade flour with added xanthan gum (0.25% and 0.5%) on day 0 and day 3 of storage. Fresh wraps (the wraps on day 0 of storage), had a low degree of relative crystallinity and a large amorphous region. Only one peak at 2 theta= 22.5° was observed for all day 0 samples. More peaks were detected on day 3. On day 3, the wraps made from white bread flour had peaks detected at 2theta = (18°, 20.°, 23°, and 26.°); for wraps made from low grade flour, peaks were detected at 2 theta- (17.5°, 20°, 22.5°,and 26°); for wraps made from low grade flour with 0.25% added xanthan gum peaks were detected at 2 theta- (17.5°, 20°, 24°, and 27°); for wraps made from low grade flour with 0.5% added xanthan gum peaks were detected at 2 theta –(17.5°, 19.8°, and 26.9°). The relative crystallinity percentage of wraps was also calculated, and it ranged from 3% to 13%. A similar trend was observed for all wraps, the relative crystallinity of wraps on day 3 was higher than the relative crystallinity of fresh wraps (day 0).

Figures 5.9 shows the DSC thermograms of wraps made from white bread flour on day 0 and day 3 of storage. On day 3 of storage, an endotherm was detected between 93°C and 102°C. Resistant starch which is reflected on the XRD was not detected on the DSC.

Table 5. 7: Effect of xanthan gum on moisture content (%) of wraps from low grade flour

Wraps	Position on wrap	Moisture (%) Day 0	Moisture (%) Day 1	Moisture (%) Day 2	Moisture (%) Day 3
White bread flour	centre	30.1 A^{ab} (1.4)	31.0 AB^{abcd} (1.0)	30.0 A^{a} (0.0)	31.0 AB^{abcde} (0.0)
White bread flour	periphery	30.5 AB^{abc} (1.0)	31.7 ABC^{abcde} (1.2)	30.7AB^{abc} (0.6)	32.3 AB^{bcdef} (0.6)
Low grade flour	centre	31.5 ABC^{abcde} (0.7)	30.5 AB^{ab} (1.3)	32.0 BC^{abcdef} (0.0)	31.5 AB^{abcdef} (0.3)
Low grade flour	periphery	32.0 ABC^{abcdef} (0.0)	32.7 A^{a} (0.6)	32.0 C^{cdef} (0.0)	30.5 A^{abc} (0.7)
Low grade flour + 0.25% xanthan gum	centre	32.5 BCD^{ef} (0.7)	32.7 BCD^{cde}f (0.6)	32.0 C^{abcdef} (0.0)	31.0 AB^{abcde} (0.0)
Low grade flour + 0.25% xanthan gum	periphery	33.0 (1.4)CD^{bcdef}	33.0 CD^{def} (0.0)	32.3 C^{bcdef} (0.6)	32.0 AB^{abc} (0.0)
Low grade flour + 0.5% xanthan gum	centre	33.5 CD^{ef} (0.7)	34.0 D^{f} (1.0)	33.0 AB^{abc} (1.0)	32.7 AB^{cdef} (1.2)
Low grade flour + 0.5% xanthan gum	periphery	34.5 D^{f} (0.7)	34.3 D^{f} (0.6)	33.3 C^{ef} (0.6)	33.3 B^{ef} (0.6)

Position refers to the position of the sample taken on the wrap. in: centre of wrap, out: outer margin of wrap

Values are means and standard deviations (in parentheses) of three determinations (n =3)

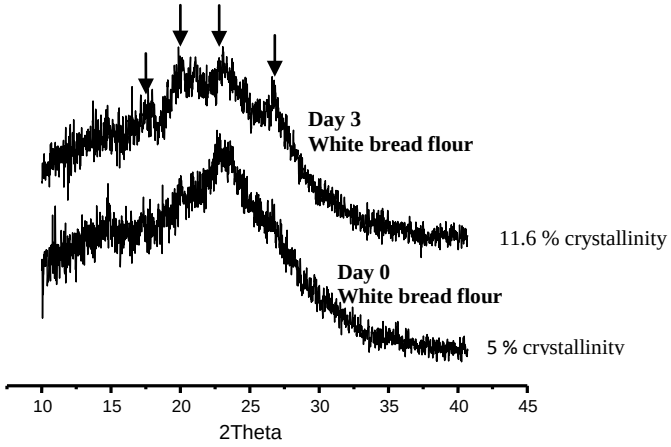
Data followed by the same lower case character in the same row, are not significantly different (P> 0.05)

Data followed by the same upper case character in the same column and on the same position on the wrap, are not significantly different (P> 0.05)

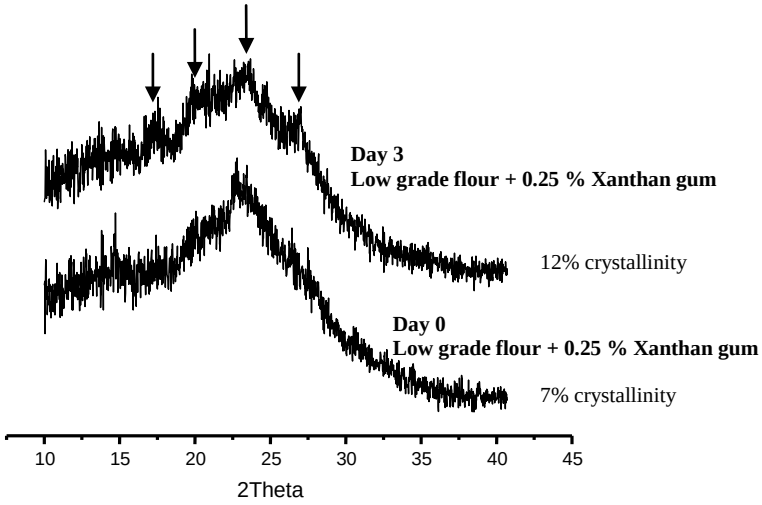
Centre: sample from the centre of the wrap; periphery: sample from the edge of the wrap

White bread flour (used as reference)

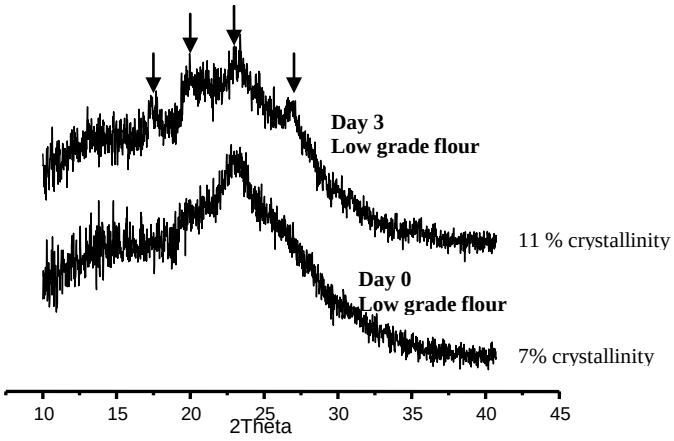
a: wraps made from white bread flour



b: wraps made from low grade flour with 0.25% xanthan gum



c: wraps made from low grade flour



d: wraps made from low grade flour with 0.5% xanthan gum

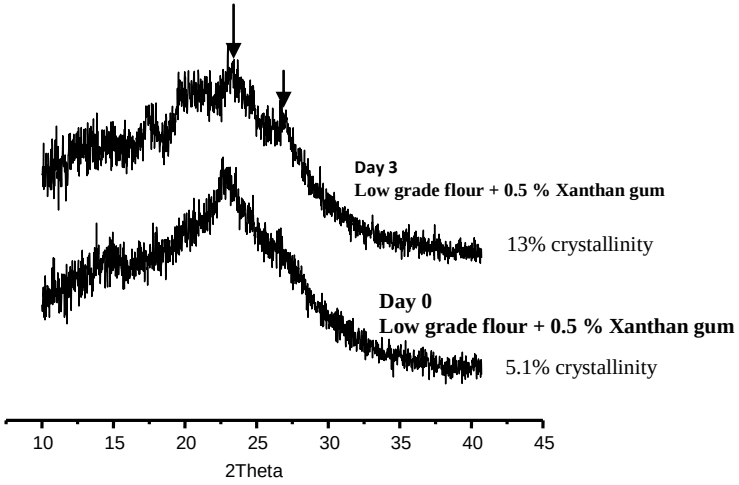


Figure 5. 8: Relative crystallinity of wraps

a-d: XRD diffractograms of wrap

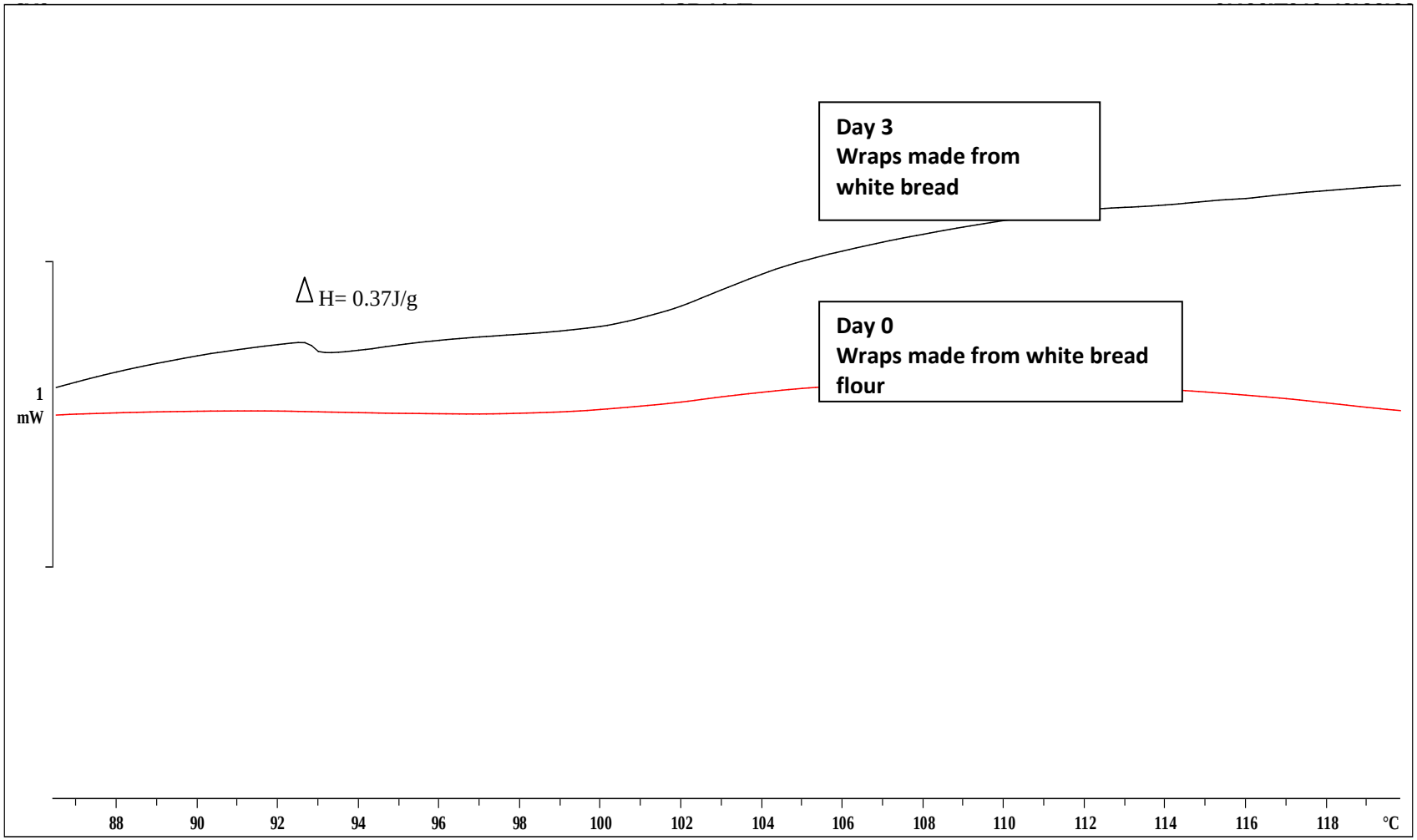


Figure 5. 9: DSC thermograms of white bread flour wraps after day 0 and day 3 of storage

Wraps made from white bread flour (used as a reference)

5.4 Bioactive properties of wraps

5.4.1 Antioxidant activity and total phenolic content of wraps

Table 5.8 shows the total phenolic content and antioxidant activity of wraps made from low grade flour, and white bread flour. The total phenolic content and antioxidant activity of wraps made from white bread flour were significantly lower than those of wraps made from low grade flour. This was the case for both acidified methanol extracts and simulated in vitro GIT digests. The total phenolic content and antioxidant activity of the simulated in vitro GIT digests were however higher than those of the acidified methanol extracts. Two different methods were used to determine antioxidant activity, and they both showed the same trend. Wraps made from low grade flour had higher antioxidant activity than wraps made from white bread flour.

Table 5. 8: Total phenolic content and antioxidant activity of wraps made from low grade flour and wraps made from white bread flour of enzyme digest extract and acidified methanol extract

Extract	Antioxidant Activity umol TE/100 mg wrap (DPPH)	Antioxidant Activity umol TE/100 mg wrap (ABTS)	Total phenolic content CE/mg wrap
Enzyme digest extract			
White bread flour wraps	2.1a (0.0)	9.0a (0.0)	0.6a (0.0)
Low grade flour wraps	3.8b (0.0)	10.5b (0.0)	0.7b (0.3)
Acidified Methanol extract			
White bread flour wraps	6.2a (0.0)	5.5a (0.2)	0.1a (0.0)
Low grade flour wraps	7.3b (0.1)	8.2b (0.5)	0.2b (0.0)

Values are means and standard deviations (in parentheses) of three determinations (n =3)
Data followed by the same lower case character in the same column of the same extract, are not significantly different ($P > 0.05$)

Chapter 6: Discussion

6.1 Critical review of methodology

The Alveograph, Farinograph, and Mixolab were used to determine the rheological properties of white bread flour, low grade flour, and low grade flour with added xanthan gum. These instruments are well suited to give the characteristics of flour without baking. The Mixolab even measures rheological parameters with added heat, which simulates baking (Chopin Mixolab Handbook, 2009). To determine the rheological properties of the flours, only flour and water were added to the Mixolab and Farinograph. In determining the rheological properties of flour using the Alveograph, a salt water solution was used in addition to the flour. Other ingredients used in the production of bread are known to also affect dough rheological properties (Gujral and Singh, 1999). The limitation in the determination of rheological properties is that the other ingredients used in the production of wraps were not incorporated into the flour-water mixture to evaluate the characteristics of the dough when all the ingredients are present. There is a need to evaluate dough characteristics when all ingredients are incorporated.

The Rapid Visco Analyser (RVA) is widely used to determine the pasting behaviour of starch-water suspensions (Booth and Bason, 2007). This instrument measures the changes in paste viscosity while heating and stirring is applied (Booth and Bason, 2007). To avoid formation of lumps, and settling of the starch on the walls of the canister, water was first measured into the canister before adding the flour-xanthan or flour only mixtures. The starch suspension was then stirred manually for 20-30 seconds using the plastic paddle, before inserting into the RVA. The mixing step was important in order to prevent lumping, which may cause a high variability among repetitions. Although all precaution was taken to prevent lumping and settling of the starch, it is difficult to completely eliminate this problem. This can negatively affect pasting curves, and so only repeatable measurements were taken as repeatable data.

The hot press method of producing wraps is considered to be the best method of preparation (Arora, 2003). In the hot press method dough balls are pressed between two hot plates

(200ºC) at a pressure of around 7584.2 KPa for 1.5 seconds forming circular disks (Pierucci, 2008). The pressing forces the gluten network to stretch in all directions, as opposed to uni-directionally as with more traditional methods of sheeting (Pierucci, 2008). During pressing, a thin skin is formed which helps to seal wraps and limit the release of steam which is generated during baking (Martínez-Bustos et al., 1999, Anton, 2008). The hot press produces wraps which are more elastic, smoother, resistant to tearing and cracking, and that can retain flexibility during storage (Arora, 2003, Anton, 2008). In the current study there was no hot press available for use, so dough balls were sheeted using a sheeter, and baked on a hot plate. In this method wraps were not well sealed, and therefore could have easily formed cracks during baking. The formation of cracks resulted in inadequate puffing of the wraps (Ram and Nigam, 1982), which compromised the texture of wraps. The characteristic quality and texture of wraps is dependent on the puffing which occurs during baking (Mcdonough et al., 1996).

After baking, wraps were placed on a cooling rack and allowed to cool in the test baking laboratory.

The laboratory is used to test the baking quality of the flour which is milled in the factory, and the level of cleanliness of the laboratory is not monitored. This could be a reason for mould growth observed on the wraps so soon after storage (Day 4). Sanitation can play a major role in the growth of moulds on baked products (Pierucci, 2008), which was the possible reason for observed mould growth in the current study. During the baking of certain products in the test baking laboratory, the air conditioner was switched on to aid in the cooling of the products. The air conditioner can cause excessive moisture loss, which can accelerate the staling process of baked products (Alhajji, 2011) .

The EZ Test was used to measure the tensile properties of the wraps. Strips were manually cut from the wraps using a blade, and a ruler to measure out the correct dimensions. The strips could have not been uniform due to human error. This could have affected the accuracy of the results, and so more than three analyses were done, and only the repeatable measurements were selected as reliable data. During the baking of wraps some toasted spots formed on the wrap. These toasted spots had a darker colour and had a harder texture than the rest of the wrap, which could have negatively affected the tensile property results obtained from the EZ Test. To eliminate this, the toasted spots were avoided when cutting strips for the

measurement of the tensile properties of the wrap. The relative humidity of the room where strips were cut, and tensile property tests were conducted was not controlled or maintained. The wraps could have dried out during the cutting of the strips which also could have affected the tensile property results of the wraps.

Organic solvents are often used to extract phenolic compounds (Awika et al., 2003, Dykes and Rooney, 2007, Menga et al., 2010). However, no single organic solvent is able to extract all phenolics from a sample (Pérez-Jiménez and Saura-Calixto, 2005). Non-extractable phenolic compounds may be excluded from the extract which will affect the results of total phenolic content and antioxidant activity (Pérez-Jiménez and Saura-Calixto, 2005). By using the simulated gastrointestinal tract (GIT) in vitro digestion method, it is possible that more phenolics could be extracted from a sample (Pérez-Jiménez and Saura-Calixto, 2005). This method simulates digestion occurring in the gastrointestinal tract, and is believed to give a better extraction than the organic solvent extraction method (Pérez-Jiménez and Saura-Calixto, 2005). It involves the use of enzymes such as pepsin to hydrolyse proteins to which phenolic compounds may be bound. This may favour the release of phenolic compounds, giving better yield of these bioactive compounds (Pérez-Jiménez and Saura-Calixto, 2005). Although the simulated GIT enzyme digests had higher total phenolic content and antioxidant activity than the organic solvent extracts, they do not represent any phenolics which may be detected beyond the small intestines. In the colon, fermentation by micro flora may yield a further release in antioxidants, and unfortunately this method cannot determine that (Pérez-Jiménez and Saura-Calixto, 2005). Another limitation of this method is that because it simulates digestion, it takes a long time to complete. A repetition of the same experiment can therefore not be completed on a single day.

The Folin-Ciocalteu method was used to determine the total phenolic content of wraps (Waterman and Mole, 1994). The Folin-Ciocalteu assay is widely used to determine total phenolic content in food products (Awika et al., 2003, Dykes and Rooney, 2007). This method is simple and does not require expensive equipment, however it has disadvantages. Although the assay gives reliable estimates of the levels of reducing phenolic groups present, it is not specific for only phenolic compounds. This method detects all phenolic hydroxyl groups in the extract. In using the Folin method for phenolic compound determination, it is possible for other chemical compounds such as sugars, tocols and carotenoids to be measured

as phenolic compounds, and therefore contribute to the total phenolic contents detected (Menga et al., 2010). Phenolic groups from extractable proteins like tyrosine (Naczk and Shahidi, 2004) can also react with the Folin-Ciocalteu reagent. This can lead to interference with the phenolic absorbance readings and increase the measurable phenols. The Folin-Ciocalteu assay however is extensively used for the determination of total phenolic content, and provides reasonable trends between the different samples. Other methods such as High Performance liquid chromatography that identifies and quantifies specific phenolic compounds can also be used.

The antioxidant activity was determined using the ABTS radical assay as well as the DPPH assay (Awika et al., 2003). The ABTS assay takes 12 hours for the generation of the ABTS free radical, and it is only stable for 16 hours. This makes the assay long, and it cannot be repeated on a single day, which is a major limitation in the experiment (Awika et al., 2003). The DPPH reacts very slowly with phenolic compounds, and takes 6 hours, as compared to the 20 minutes taken by the ABTS radical. Another drawback in this assay is that DPPH is prone to interference by colour in samples which contain pigments absorbing at the same wavelengths (515 nm). This can lead to an underestimation of the antioxidant activity (Arnao, 2000).

6.2 Discussion of results

The higher ash content of low grade flour than white bread flour is most likely due to the composition of the low grade flour. Low grade flour is made up of the outer most parts of the wheat kernel (Waggle et al., 1967, Caterall, and Cauvain, 2003, Campbell et al, 2007), including the bran which has a high mineral content (Šramková et al., 2009). The wet gluten of low grade was also found to be higher than the wet gluten of white bread flour. Flour produced at the tail end of milling such as low grade flour has higher amounts of gluten components, but poor gluten quality (Wihlfahrt and Brooks, 1953). This is a possible reason for the higher gluten content in low grade flour. The dough rheological properties of low grade flour however show that it is poorer in quality than white bread flour.

6.2.1 Dough rheology

Water absorption increased with the addition of xanthan gum (Table 5.2) because xanthan gum has a high water binding capacity (Sidhu and Bawa, 2000). This increased water

binding capacity is due to the many OH molecules found on the xanthan gum structure, which have a high affinity for water. The increased water binding capacity exerted by xanthan gum could have also been responsible for the slight decrease in modulus, and increase in rollability observed in wraps made from low grade flour with xanthan gum over time. The water binding capacity may also cause an increase in moisture content of wraps, which could reduce retrogradation (Román-Brito et al., 2007) , as will be discussed in a later section..

The Farinograph stability, Farinograph mixing tolerance index, and Alveograph p-value at adapted hydration, all show that low grade flour had lower dough strength than white bread flour. Low grade flour is a source of dietary fibre because it contains the outer bran fraction of the wheat kernel (Waggle et al., 1967, Caterall, and Cauvain, 2003, Campbell et al, 2007), thisis most likely responsible for its decreased dough strength as compared to white bread flour. Research has shown contrasting reports on the effect of fibre on the rheology of wheat flour. In some reports the fibre has been found to have a weakening effect on wheat dough strength (Zhang and Moore, 1999, Rosell et al., 2006, Rosell et al., 2010), while in other reports it is believed to strengthen the dough (Gómez et al., 2011, Banu et al., 2012). Two types of dietary fibre exist, soluble dietary fibre and insoluble dietary fibre (Noort et al., 2010). Insoluble dietary fibre is fairly rigid, does not hydrate easily, and has been linked to the weakening of dough (Goldstein et al., 2010, Awika, 2011). Soluble dietary fibre on the other hand is soluble in water (Awika, 2011), and has been linked to the strengthening of dough (Wang et al., 2002). The weakening effect of bran has been attributed to it disrupting the gluten network, resulting in decreased dough strength (Zhang and Moore, 1999). Contrary to this, Gómez et al. (2011) and Banu et al. (2012) found that wheat bran caused an increase in the dough strength of wheat flour, and attributed this to the interactions which occur between the wheat proteins and fibre. These interactions between protein and fibre are thought to occur via hydrogen bonding between the reactive components which are liberated during milling such as ferulic acid, and the gluten protein (Van Der Borght et al., 2005).

At adapted hydration, the dough strength of low grade flour was lower than that of white bread flour. The dough of low grade flour at adapted hydration was more viscoelastic and

produced a large bubble. At constant hydration however, the dough strength of low grade flour was higher than that of white bread flour. The viscoelasticity of low grade flour was reduced considerably at constant hydration; the dough lost extensibility and was too tough. Hruškova and Faměra (2003) also found the dough strength to be reduced and extensibility increased at adapted hydration compared to constant hydration. At constant hydration only 50% water is added (on a 14% moisture basis) regardless of the water absorption of the particular flour being analysed (Vinci et al., 2013). Constant hydration could thus have resulted in under-hydrated flour during mixing (Codinä, 2008, Dubois et al., 2008, Vinci et al., 2013), which could explain the vast differences between the results of the Alveograph at the two hydrations (adapted hydration and constant hydration), especially between low grade flour and white bread flour..

The dough strength and dough extensibility of low grade flour determined at adapted hydration was very different to the results obtained at constant hydration. Low grade flour had higher starch damage than white bread flour, which could be responsible for these results. Starch damage increases water absorption, and could therefore affect the viscosity of the dough (Preston et al., 1987). Apart from starch damage, the high fibre content in low grade flour (Campbell et al, 2007) could have also influenced water absorption (Wang et al., 2002, Anil, 2007), which in turn affected the Alveograph results (Preston et al., 1987). In a study done by Preston et al. (1987), it was found that dough prepared at 50% hydration was under-hydrated, stiff, and inextensible. They attributed this dough behaviour to the competition for water by the damaged starch, fibre, and gluten proteins (Preston et al., 1987). They concluded that the gluten proteins received the least amount of water, which resulted in a stiff dough with less extensibility (Preston et al., 1987). The higher starch damage, and fibre content of low grade flour could likewise be responsible for the difference in Alveograph results at constant hydration.

The increase in dough stability, increase in dough tenacity and deformation energy, and decrease in mixing tolerance index and dough extensibility of low grade flour when xanthan gum was added to it, reflects the strengthening effect of xanthan gum on low grade flour dough (Rosell et al., 2001, Indrani et al., 2007, Sudha et al., 2007). Researchers have found

that hydrocolloids like xanthan gum increase dough strength of wheat flour (Rosell et al., 2001, Smitha et al., 2008b). They attributed this to possible interactions which may occur between wheat proteins and xanthan gum (Ribotta et al., 2005, Rosell et al., 2007). These interactions are believed to occur via hydrogen bonding, or by electrostatic interactions (fig: 6.1) (Ribotta et al., 2005). Electrostatic interactions between xanthan gum, and gluten proteins possibly occur via bonding of the negatively charged carboxyl group of the xanthan gum, and the positively charged amino group in the gluten protein (Schmitt et al., 1998, Ribotta et al., 2005).

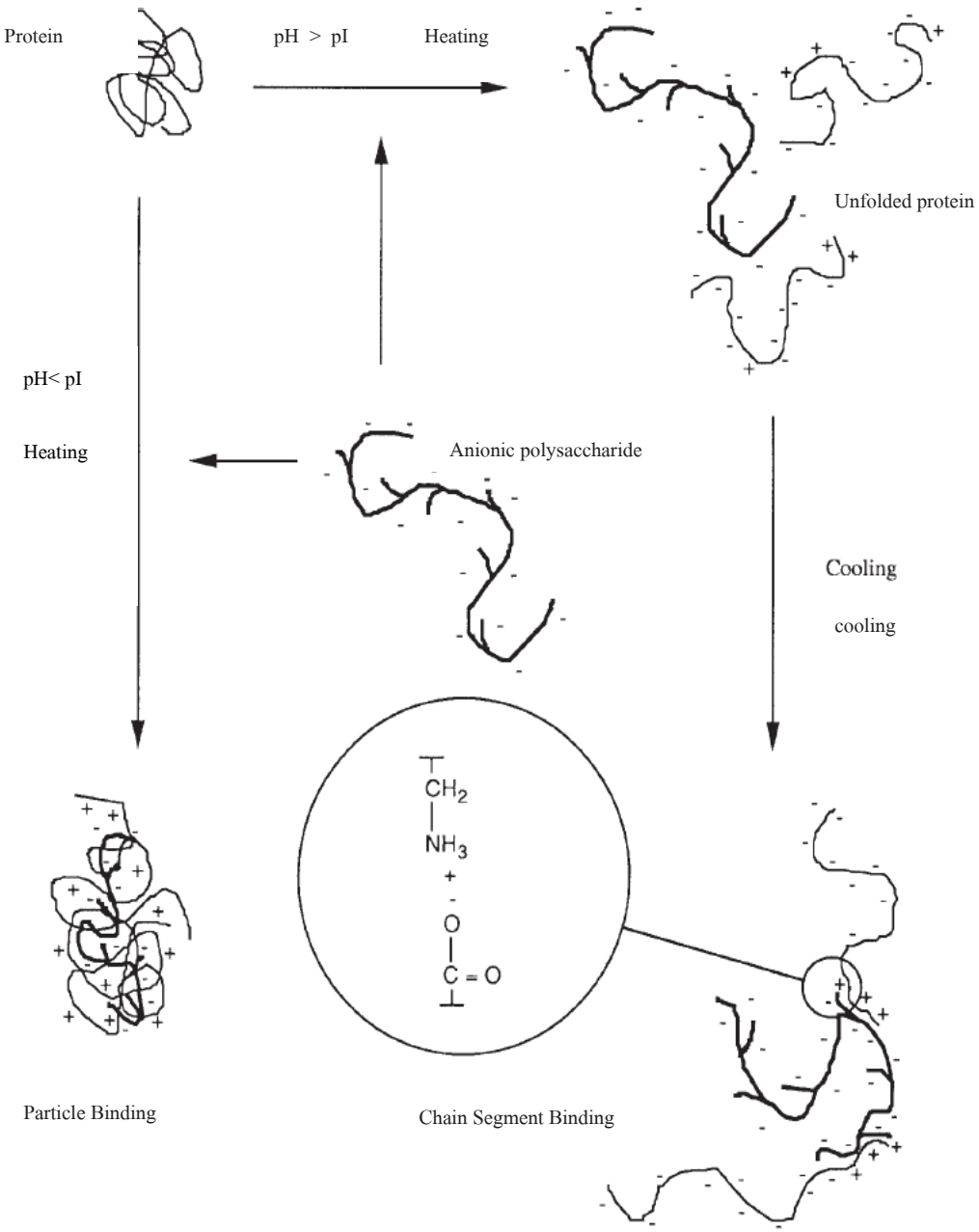


Figure 6. 1: interactions between a protein and an anionic polysaccharides (Schmitt et al., 1998)

The Mixolab was used to analyse the mixing and pasting behaviour of flour dough at adapted hydration. Five parameters can be derived from the Mixolab (Collar et al., 2007). C1 represents hydration of the flour compounds and formation of a three dimensional viscoelastic network which is responsible for the increase in torque (Rosell et al., 2007). C2 represents protein weakening and denaturation which occurs as mixing progresses and results

in a decreased torque (Rosell et al., 2007). The next three parameters (C3-C5) refer to the starch properties of the dough (Sedej et al., 2011). C3 represents the absorption of water by the starch granules, as well as the leaching out of amylose from the granules into the aqueous granular phase, and results in an increased torque (Rosell et al., 2007). C4 represents the disruption of the starch granules caused by mixing and high temperature, and results in a decreased torque (Rosell et al., 2007). C5 represents the re-annealing of the leached starch polymers, which occurs during the cooling of the dough. It occurs as a result of intermolecular associations in the molecular network, by means of secondary forces and sterical hindrances (Rosell et al., 2010), and causes an increase in the torque.

The Mixolab parameters of white bread flour were significantly higher than the Mixolab parameters for low grade flour. The higher fibre content of low grade flour (Campbell et al, 2007) could be responsible for its lower Mixolab parameters. Fibre in wheat dough is presumed to interfere with protein association and its further aggregation during heating, thereby affecting the dough torque during mixing (Rosell et al., 2010). During the protein weakening in the Mixolab, the fibre may interfere with the protein unfolding, which is probably the cause of the lower C2 value of low grade flour than white bread flour (Rosell et al., 2010). The lower C3 could indicate a reduced degree of starch granule swelling, which could be due to competition of the fibre OH molecules, and amylose for water (Rosell et al., 2010, Hadnađev et al., 2011). Fibre is thought to also interfere with the intermolecular association that takes place in the macromolecular network upon cooling of the dough, and thus caused a decreased C5 value (Rosell et al., 2010).

Addition of xanthan gum to low grade flour did not cause any change in the Mixolab parameters apart from the C5 parameter. C5 was reduced when xanthan gum was added to the flour, this was also observed by Rosell et al. (2007) and suggests a decrease in the rate of retrogradation (Rosell et al., 2007).

The RVA was also used to measure the pasting properties of flour. The viscosities of white bread flour were higher than the viscosities of low grade flour. This could be due to the high presence of fibre in low grade flour (Campbell et al, 2007), as was discussed earlier.

The addition of xanthan gum increased the viscosity of low grade flour. Xanthan gum has been found to increase the pasting viscosities of various flours (Mandala and Bayas, 2004, Chaisawang and Suphantharika, 2005). Two possible explanations exist for the increasing effect of xanthan gum on the flour viscosities: firstly the starch-hydrocolloid system is assumed to be in two phase systems, with xanthan gum located in the continuous phase. As the volume of the phase accessible to the xanthan gum is reduced due to the starch granule swelling during gelatinization, the xanthan gum concentration in the continuous phase increases resulting in increased viscosity (Chaisawang and Suphantharika, 2006). Secondly xanthan gum is believed to synergistically interact with starch polymers which have leached out from the starch granule causing an increase in viscosity (Mandala and Bayas, 2004).

The pasting results obtained from the Mixolab were different to the pasting results obtained on the RVA. In the RVA there was a significant difference between low grade flour and white bread flour, as was also observed in the Mixolab. Addition of xanthan gum to low grade flour had a significant effect on the pasting properties determined by the RVA. Apart from the C5 value, which represents the final viscosity, the Mixolab did not show any significant difference between the pasting properties of low grade flour and low grade flour with added xanthan gum. The Mixolab handbook states that xanthan gum has no effect on the Mixolab curve apart from the decrease in C5 (Chopin Mixolab Handbook, 2009). The Mixolab results are different from the RVA results. This difference could possibly be attributed to the amount of water used in each experiment, which influences the gelatinization behaviour of the starch in the samples (León et al., 1997). The swelling of the starch granule is greatly dependent on the water available in the medium which controls the gelatinization behaviour (León et al., 1997). RVA wheat flour slurries behave differently to the Mixolab wheat dough systems, where the amount of water is a limiting factor during starch swelling, even more noticeable when hydrocolloids are present due to their ability for holding water (Rosell et al., 2007).

6.2.2 Quality of wraps

During the baking of wraps puffing occurs due to the generation, and accumulation of steam between the two layers of the wrap (Ram and Nigam, 1982). Initially when puffing occurs, the separation of the two layers begins at certain points of the wrap, which eventually coalesce to form one large bubble. The large bubble however only occurs if no cracks form on the crust which will allow the steam to escape and cause the bubble to collapse (Ram and Nigam, 1982).

Wraps made from low grade flour did not puff at all during baking. This was probably due to the weak dough strength observed in the Alveograph results at adapted hydration. Ram and Nigam (1982) also found that flour with weaker dough strength produced wraps which did not puff. They attributed this lack of puffing to the formation of cracks in the crust during baking.

The addition of xanthan gum to low grade flour increased the dough strength of low grade flour. This increase in dough strength prevented crack formation in the wraps when the steam pressure increased during baking. Xanthan gum also improved the puffing behaviour of low grade flour wraps during baking. The increase of the dough strength when xanthan gum was added to low grade flour, was probably due to the interactions which occur between the flour proteins with xanthan gum (Ribotta et al., 2005) as explained earlier. The puffing of low grade flour with 0.5% xanthan gum in particular was comparable to white bread flour (reference sample); possibly because a higher concentration of xanthan gum gave low grade flour higher dough strength.

The rollability score, and tensile properties of wraps showed that wraps become more firm over time, which was an indication of staling of the wraps (Mao and Flores, 2001). These results are consistent with results obtained by other researchers (Mao and Flores, 2001, Gujral and Pathak, 2002, Gujral et al., 2004, Ghodke Shalini and Laxmi, 2007, Alviola,

2009). Xanthan gum lowered the rollability score of wraps made from low grade flour, which suggests that xanthan gum improves the softness of wraps during storage.

The tensile stress and strain curves as well as the modulus showed that wraps made from low grade flour with xanthan gum had a decreased rate of firming as compared to wraps made from low grade flour alone, and wraps made from white bread flour. The delayed rate of firming suggests that the staling of wraps is also delayed (Arora, 2003). This could be linked to the higher moisture content (Table 5.7) observed in the wraps made from low grade flour with added xanthan gum. The high water binding capacity of xanthan gum prevents the movement of moisture from gluten to starch by diffusion (Sidhu and Bawa, 2000). Sidhu and Bawa (2000) also reported this in their study of the effect of carboxyl methyl cellulose on bread firming. The process of movement of moisture from gluten to starch is referred to as moisture migration and is thought to play a role in bread staling (Bemiller, 2011). The lack of significant change in moisture from the centre of the wrap to the periphery supports the hypothesis that moisture migration did not occur in the wraps, and was therefore not a factor in the staling of the wraps.

Recrystallization and retrogradation of starch polymers (Karim et al., 2000, Gujral and Pathak, 2002, Hug-Iten et al., 2003) as well as loss of moisture from bread (Hallberg and Chinachoti, 2002) are also responsible for staling of bread (Karim et al., 2000, Gujral and Pathak, 2002, Hallberg and Chinachoti, 2002, Hug-Iten et al., 2003). The moisture content of wraps did not change significantly over storage time. It can therefore be deduced that moisture loss was not a reason for staling of the wraps. The stability of the moisture content of the wraps was most likely due to the fact that wraps were stored in sealed polyethylene bags which probably prevented the escape of moisture (Alhajji, 2011). Retrogradation is thus the most likely cause of wrap staling, as was also found by Gujral and Pathak (2002).

Retrogradation describes the changes which occur in gelatinized starch from an amorphous state to a more crystalline order (Gudmundsson and Eliasson, 1990). Retrogradation is believed to be one of the major causes of bread staling (Gray and Bemiller, 2006). In starch

based food, the retrogradation of amylose takes place at a much faster rate than the retrogradation of amylopectin (Goesaert et al., 2005b, Sajilata et al., 2006).

Staling in wraps made from white bread flour, was more than in wraps made from low grade flour. A high presence of moisture in baked products, is believed to play a major role in the reduction of the rate of retrogradation (Román-Brito et al., 2007).The high fibre content of low grade flour (Campbell et al, 2007), gave it a higher water absorption capacity compared to white bread flour (Wang et al., 2002). Wraps made from low grade flour therefore had higher moisture content than the wraps made from white bread flour. The increased moisture content of wraps made from low grade flour was the probable reason for a decreased rate of staling in these wraps, than in wraps made from white bread flour.

The addition of xanthan gum to low grade flour lowered the staling of wraps, especially addition of the lower concentration (0.25%) of xanthan gum. Gujral et al. (2004) studied the effect of hydrocolloids on the texture and staling of rice flour chapattis, and also found that the hydrocolloids decreased staling in the chapatti. They found that the interaction between hydrocolloids and starch chains reduced the rate of retrogradation. They also found that retrogradation was better reduced when the hydrocolloids were at a lower concentration, although no explanation was given for this. Xanthan gum is thought to retard retrogradation because in addition to interacting with starch (Gujral et al., 2004), it also causes an increase in moisture content (Román-Brito et al., 2007). The increase in moisture decreases water loss by retarding moisture migration, and increases starch gelatinization, thereby avoiding the retention of the starch crystal nuclei which facilitate starch associations during retrogradation (Román-Brito et al., 2007). In the current study, wraps with added xanthan gum had higher moisture content than both the control and the reference sample. This was as a result of the many OH groups in the structure of xanthan gum (Rosell et al., 2007, Smitha et al., 2008b). The high moisture content caused a reduction in the rate of retrogradation.

The increase in crystallites on day 3 shown on the XRD was an indication of higher relative crystallinity in the wraps over storage time, and was probably due to the re-association of the starch polymers (retrogradation), as suggested by Shaikh et al. (2008). On day 0, the XRD

diffractograms showed the presence of amylose lipid complexes (Figure 5.8). It is believed that amylose-lipid complexes retard retrogradation (Hizukuri et al, 2006). This occurs because the amylose lipid complex prevents amylose re-crystallization (Hizukuri et al, 2006). The amylose-lipid complex also acts as a barrier against water migration, which will retard retrogradation (Hizukuri et al, 2006). With time however, the B-type pattern emerges, and intensifies (Hizukuri et al, 2006), which is what was observed on the XRD diffractograms on day 3. The wraps on day 3 had XRD diffractogram peaks with a B-type crystallinity (2 theta = 17° and 2 theta = 23°) (Shamai et al., 2003), this B-type crystallinity can be attributed to the retrogradation of starch (Shamai et al., 2003).

Starch retrogradation in stored wraps can result in the formation of resistant starch (Rendón-Villalobos et al., 2006), which gives a B-type XRD crystallinity pattern (Shamai et al., 2003). A B-type crystallinity pattern was detected in all wraps on day 3.

Three types of resistant starches exist, but only one type forms as a result of retrogradation and that is RS3 (Sajilata et al., 2006). RS3 is made up mainly of retrograded amylose, which is formed during the cooling of gelatinized starch (Sajilata et al., 2006). Rendón-Villalobos et al. (2006) did a study on the digestibility of stored tortillas with hydrocolloids, and found that resistant starch increased during storage, however when hydrocolloids were present the resistant starch was reduced. In the current study, wraps were not stored long enough to observe this effect that hydrocolloids can have on resistant starch, thus no difference was visible on the XRD curves of wraps with xanthan gum.

Amylopectin retrogradation is believed to occur at a slower rate than amylose retrogradation (Goesaert et al., 2005b), probably because of its branched nature, which to some extent inhibits re-crystallization (Goesaert et al., 2005b, Sajilata et al., 2006). This is the most likely reason why no amylopectin retrogradation was detected on the XRD.

An endotherm on the DSC is observed when there is a crystallite present in the sample which requires melting (Gavilighi et al., 2006). On day 0 of storage the absence of any endotherms was an indication of a lack of crystallites in the wraps, because the wraps were still fresh. On day 3 however an endotherm was observed between 92 and 103°C which can either be due to amylose retrogradation (Shaikh et al., 2008), or can be due to amylose-lipid complexing,

which shows an endotherm peak on the DSC between 95 -110 °C (Sajilata et al., 2006). No RS3 crystallites were observed on the DSC, although they did appear on the XRD. The melting of the amylose crystallites representing amylose retrogradation, would occur at 158°C (Sajilata et al., 2006), and the scanning was only done from 30°C-120°C. Endotherms representing amylopectin retrogradation were not observed on the DSC. This was possibly because the wraps were only stored for three days before mould growth was visible, which was not enough time for amylopectin retrogradation (Goesaert et al., 2005b). Amylopectin retrogradation sets in later on during storage unlike amylose retrogradation which occurs soon after baking (Goesaert et al., 2005b).

6.2.3 Bioactive properties

Both the total phenolic content as well as the antioxidant activity of low grade flour wraps was higher than those of white bread flour wraps. Other researchers (Esposito et al., 2005, Liyana-Pathirana and Shahidi, 2005, Liyana-Pathirana and Shahidi, 2007), have found the total phenolic content and antioxidant activity of the germ, the bran, and aleurone layer of wheat milled fractions to be higher than the endosperm of the wheat grain **(Vaher et al., 2010)**. Most phenolic compounds present in wheat are found in the outer bran layer, as well as the aleurone layer of the grain (Esposito et al., 2005), which explains the high phenolic content, and antioxidant activity in the wraps made from the flour with high concentrations of these layers (low grade flour). During milling a lot of the bran fraction is removed from the millstreams used for white bread flour, which is why the antioxidant activity of white bread flour is lower than that of low grade flour (Tang et al., 2013, Liyana-Pathirana and Shahidi, 2006).

The total phenolic content and antioxidant activity of the enzyme digests were higher than that of acidified methanol extracts. In their study, Liyana-Pathirana and Shahidi (2005) found that the values of total phenolic content and antioxidant activity were higher when extracts were prepared under gastric pH conditions (Liyanapathirana and Shahidi, 2004, Liyana-Pathirana and Shahidi, 2005), and this was also observed by (Baublis et al., 2000). It has been suggested that the pH in the gastrointestinal tract, can result in some hydrolysis reactions which can lead to the release of some of the bound phenolics which are present in wheat (Liyanapathirana and Shahidi, 2004, Liyana-Pathirana and Shahidi, 2005). The higher

antioxidant activity of the simulated in vitro GIT digests are thus most likely due to the free phenolics and soluble phenolic esters, which may have been released because of the pH conditions of the gastrointestinal simulation (Liyana-Pathirana and Shahidi, 2005).

The ABTS and DPPH radical scavenging assays were used to determine antioxidant activity. These two assays employ the same principle: a synthetic coloured radical (ABTS$^{\cdot}$ or DPPH) is generated; and the assays measure the ability of a biological sample to scavenge the radicals in comparison with a standard amount of Trolox (a water soluble analogue of vitamin E) (Liyanapathirana and Shahidi, 2004, Liyana-Pathirana and Shahidi, 2005, Floegel et al., 2011). The ABTS assay gave higher antioxidant activity than the DPPH assay, especially when assessing the enzyme digests. This was also observed by Floegel et al. (2011) who attributed the higher antioxidant activity of the ABTS assay to the fact that the ABTS radical generated in this assay, is applicable to both hydrophilic and lipophilic antioxidant systems; whereas the DPPH assay uses a radical dissolved in organic media and is therefore applicable to hydrophobic systems (Floegel et al., 2011), giving the ABTS method a wider range of antioxidant detection than the DPPH method.

6.4 Future research and industrial application

Low grade flour with added xanthan gum can potentially be used to manufacture wraps with acceptable quality. Producing wraps using low grade flour will provide consumers with a quality product with potential health benefits. The use of low grade flour in the manufacture of wraps opens a market in the food industry for sale of low grade flour. Low grade flour is a by-product of milling (Klepacka and Fornal, 2006), and if there is more use for it within the food industry, it means millers can generate more of a profit from its sales. As was observed from the current study, low grade flour does not produce a good quality baked product, and has poor dough rheological properties. The low grade flour would therefore have to be sold, premixed with xanthan gum, similar to how leavening agents are premixed in self-raising flour.

The wraps made from low grade flour have a high phenolic content and antioxidant activity as the study has shown. This wrap could potentially contribute in the fight against chronic

diseases of lifestyle related to oxidative stress, and therefore contribute to the market of health beneficial foods already existing in South Africa.

In the current study, no preservatives were used in the manufacturing of wraps. In order to extend the shelf life of the wraps to longer than four days, preservatives should be added to the ingredients of the wraps. Preservatives can extend the shelf life of wraps by over twelve days (Friend et al., 1995). The aim of the addition to wrap formulation is to inhibit the microbial growth, and therefore extend the shelf life of wraps (Arora, 2003). Sodium and calcium propionates, as well as potassium sorbate are commonly used in the manufacture of wraps and may be combined (Pierucci, 2008). The pH of the wraps is however important for optimal functionality of the preservatives. Propionates require a pH of 5.5, while potassium sorbate requires a pH of 6.5. In order to achieve a low pH, acidulants are used together with the preservatives. The most common acidulant used in the manufacturing of wraps is fumaric acid. Unlike other acidulants, fumaric acid does not interfere with the leavening system of the wraps while lowering the pH (Pierucci, 2008). Once preservatives are added it can then be investigated whether or not they (preservatives) will have an effect on the quality of the wraps.

One objective of the current research was to produce a good quality wrap using low grade flour. The taste and how people perceive the wrap forms a part of the quality. In fact sensory quality is the ultimate measure of product quality and success (Drake, 2007). This objective cannot be completed without doing a sensory evaluation of the wraps in future. . A descriptive sensory evaluation of the wraps would be recommended. . Descriptive sensory evaluation can provide information that cannot be obtained from using analytical instruments. For example subtle changes in shelf life stability such as taste, cannot be monitored with instruments (Meilgaard et al., 2006). Descriptive sensory evaluation involves the training of a group of individuals to identify and quantify specific or sensory attributes of a food product such as texture, mouth feel, or aftertaste (Drake, 2007). The training of the panel is to ensure that the panel operates in unison as an instrument. A descriptive sensory analysis will give a detailed description of the product in comparison to other similar successful products already in the market (Meilgaard et al., 2006). A consumer sensory evaluation of the wraps would

also be recommended in addition to the descriptive evaluation. This method of sensory evaluation would not require a trained sensory panel because consumers are not trained to identify sensory attributes (Drake, 2007). The consumer sensory evaluation will provide insight on whether or not consumers like the wraps made from low grade flour, and may also indicate which wraps consumers prefer. In the future some sensory analysis should therefore be done on wraps made from low grade flour to determine their potential success in the market as well as to determine if there is a need to improve on their formulation according to consumer feedback.

The antioxidant activity and total phenolic contents of wraps made from low grade flour were found to be higher than those of wraps made from white bread flour. Further investigations should be done on the effect of the low grade flour on LDL oxidation, as well as on oxidative DNA damage. This will give a clear indication of whether or not wraps made from low grade flour are suitable for the reduction of some life style diseases such as atherosclerosis and cancer. An additional study on the effects of wraps made from low grade flour on diabetes can also be done in future work.

The current study has shown that low grade flour can be used to produce wraps. Future research could include the development of other bakery products such as biscuits, crackers, bread rolls, pizza, and bread. Low grade flour can also be composited with white bread flour to improve the nutritional content of white bread flour baked products. Some investigation would first have to be done to determine what proportion of the different flours (low grade flour and white bread flour) would be suitable to produce the best quality product

Chapter 7: Conclusion

Xanthan gum improves the dough rheological properties of low grade flour. Xanthan gum causes a decrease in the Mixing tolerance index, and an increase in the mixing stability, dough tenacity and deformation energy. This shows the strengthening effect of xanthan gum on low grade flour, which is a effect of the interactions which occur between xanthan gum and the flour proteins. At adapted hydration xanthan gum produces a more viscoelastic low grade flour dough because all the flour proteins are properly hydrated.

Xanthan gum also improves the quality of wraps made from low grade flour. The dough strengthening effect of xanthan gum gives wraps a good puff, which indicates the quality of wraps. Xanthan gum lowers the rate at which staling occurs in wraps made from low grade flour which was seen in the lower modulus values, and rollability scores of wraps with time. The interaction of xanthan gum with starch in the flour, and its affinity to bind with water causes a reduction in retrogradation. The many OH groups in the xanthan gum bind water and prevent moisture migration which in turn retards retrogradation. The lower xanthan gum concentration (0.25%) gives better quality of wraps made from low grade flour than the higher concentration (0.5%) of xanthan gum.

The high total phenolic content, and antioxidant activity of wraps made from low grade flour gives them potential health benefits. This is because low grade flour is made up of the outer fractions of the grain which are rich in phenolic compounds and antioxidants.

Low grade flour does produce wraps with a higher antioxidant activity than wraps produced from white bread flour. These wraps made from low grade flour have potential health benefits, but have a poor quality. Xanthan gum improves the dough rheological properties of low grade flour. It also improves the quality of wraps made from low grade flour, and a low concentration of xanthan gum is required to improve the quality. However further research is

required in order to determine consumer perception of the wraps. Further research is also required to determine the extent of the potential health benefit of the wraps to consumers.

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