

Influence of waxy wheat flour blends on dough and bread baking quality as well as shelf life

by

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Abstract

Waxy wheats are a naturally occurring genetic mutation of the hexaploid bread wheat *Triticum aestivum*. They contain only amylopectin starch due to the absence of the protein responsible for producing amylose, called granule bound starch synthase (GBSS). The amylopectin content retards starch retrogradation as amylopectin retrogrades more slowly than amylose. This can be utilised to increase the shelf life of bread by slowing down the staling process in which starch retrogradation is involved. One hundred percent waxy wheat cannot be used to make bread because of a resulting undesirable loaf appearance. Blends of waxy wheat and non-waxy wheats were thus used to create a loaf of bread which not only had an extended shelf life but also a desirable appearance.

The starch granule morphology and percentage crystallinity of starch isolated from four waxy wheat lines (375, 376, 377 and 378), was determined using a scanning electron microscope and X-ray diffraction respectively. A non-waxy wheat control was used. No differences were seen in granule size and morphology between the lines and the control but more B-type granules were observed in control. The control was found to have an unusually high percentage crystallinity (36.5%) but was still lower than, or equal to, the waxy wheat lines (36.5 – 38%).

Flour of each line was blended with the control in ratios of 10, 15, 20 and 25% waxy wheat to non-waxy wheat. Pasting properties were determined by the Rapid Visco Analyser (RVA). Blends of lines 375, 376 and 377 were found to have a lower peak viscosity, a faster peak time and a lower final viscosity than the control, while line 378 was similar in values to the control. No significant differences were seen between the blends and the control for the arrival time, water absorption, and stability as determined by the Farinograph. Likewise, no significant differences were seen for the peak time, peak height and tail height determined by the Mixograph between all blends and the control. Biaxial extension of the dough from each blend using the Alveograph showed no significant differences from the control for the P, L, P/L and W parameters.

The blends were baked into loaves of bread to determine final loaf quality and shelf life. The C-Cell showed no significant differences for the cell and hole number, cell area and slice brightness between the blends and control. Lines 375 and 377 had the highest percentage concavity and therefore the worst appearance. Line 376 and 378 had the best appearance with the highest amount of waxy wheat. The texture analyser showed that waxy wheats create a softer initial loaf. On day six, only blends from line 376 successfully decreased the firmness compared to the control.

The addition of up to 25% waxy wheats to non-waxy wheats marginally affects the processing properties of dough but negatively affects the outward appearance of bread. Bread baked with blends of 20 – 25% of line 376 had an improved shelf life, whilst still being visually appealing.

Uittreksel

Wasagtige korings is 'n genetiese mutasie van die heksaploiede broodkoring, *Triticum aestivum*, wat natuurlik voorkom. Hierdie broodkorings bevat slegs een tipe stysel, amilopektien, omdat die proteïen wat verantwoordelik is daarvoor om amilose te vervaardig naamlik, granulêr gebonde stysel sintase (GBSS), nie teenwoordig is nie.

Aangesien amilopektien stadiger retrogradeer as amilose, word stysel retrogradasie vertraag in wasagtige korings. Stysel retrogradasie is betrokke by die verouderingsprosess van brood. Die hoë amilopektieninhoud van wasagtige korings kan dus gebruik word om die rakleef tyd van brood te verleng,

Die gebruik 100% wasagtige koring in die bak van brood is nie ideaal is nie, aangesien dit 'n ongewenste voorkoms aan die brood verleen. Mengsels van wasagtige en nie-wasagtige korings word gebruik om brood te bak wat 'n aanvaarbare voorkoms, sowel as verlengde rakleef tyd het.

Stysel van vier wasagtige koring lyne (375, 376, 377 en 378) is op grond van die stysel se granulêre morfologie en persentasie kristalliniteit, deur middel van 'n skanderings elektron mikroskoop en x-straal diffraksie onderskeidelik, geïsoleer en geklassifiseer. 'n Nie-wasagtige koring is as kontrole gebruik. Daar is geen verskille in die grootte van die granule, sowel as die morfologie tussen die toetslyne en die kontrole opgemerk nie. Die stysel van die kontrole-koring het meer B-tipe granules bevat, asook 'n uitsonderlike hoë persentasie kristalliniteit (36.5%) gehad. Hierdie persentasie was steeds laer of gelyk aan die wasagtige koringlyne, waarvan die persentasie kristalliniteit gewissel het van 36.5 tot 38%.

Meel van elkeen van die toetslyne koring is in verhoudings van 10, 15, 20 en 25% wasagtige koring tot nie-wasagtige koring van met die meel van die kontrole-koring gemeng. Gom-eienskappe van die mengsels is deur 'n *Rapid Visco Analyser* (RVA) bepaal. Die mengsels met koring lyne van 375, 376 en 377 het 'n laer piekviskositeit, 'n vinniger piektyd en 'n laer finale viskositeit as die kontrole getoon, terwyl lyn 378 soortgelyke waardes as die kontrole gehad het. Daar was geen beduidende verskille tussen enige van die mengsels en die kontrole, ten opsigte van die aankomstyd, waterabsorpsie en stabiliteit, soos gemeet deur 'n Farinograaf, nie. Eweneens was daar geen beduidende verskille tussen die piektyd, piekhoogte en sterthoogte, soos bepaal deur 'n Miksograaf, vir enige van die mengsels en die kontrole nie. Die tweeassige uitstrekking van die deeg is bepaal met behulp van 'n Alveograaf. Daar was geen beduidende verskille vir die P, L, P/L en W parameters tussen die toetslyne en die kontrole nie.

Om die finale brood kwaliteit en rakleef tyd te bepaal, is daar van elke een van die mengsels, sowel as die kontrole, brode gebak. Die *C-cell* het geen beduidende verskille gewys vir die selgrootte, die -hoeveelheid, -area en die sny helderheid tussen die verskillende mengsels en die kontrole nie. Lyne 375 en 377 het die hoogste persentasie konkaviteit en dus die swakste voorkoms gehad. Lyne 376 en 378 het die beste voorkoms getoon met die hoogste wasagtige koring inhoud. Die tekstuurontleder het aangedui dat wasagtige koring aanvanklik sagter brood maak, alhoewel

slegs mengsels van koringlyn 376 die fermheid van die brood op dag ses, suksesvol kon verlaag teenoor die kontrole.

Die prosesseringseienskappe van deeg word tot 'n geringe mate beïnvloed deur die toevoeging van tot 25% wasagtige koring by die nie-wasagtige koring. Dit lei egter tot negatiewe effekte op die uiterlike voorkoms van brood. Die brood wat met 20% tot 25% van koringlyn 276 gemaak is, het 'n verbeterde rakleefyd gehad, terwyl dit steeds 'n aanvaarbare voorkoms behou het.

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List of abbreviations

%	Percentage
°	Degree
°C	Degrees Celsius
a.u.	Arbitrary units
AACC	American Association of Cereal Chemistry
ANOVA	Analysis of variance
Be	Beryllium
BU	Brabender units
BVA	Brabender Visco Amylograph
Ca.	Circa
cm	Centimetre
cm ²	Centimetre squared
CO ₂	Carbon dioxide
cP	Centipoise
Cu	Copper
dH ₂ O	Deionised water
E	Extensibility
EU	Extensograph Units
g	Grams
g	Force
g/mL	Grams per millilitre
GBSS	Granule Bound Starch Synthase

GSP	Grain Softness Protein
h	Hour
J	Joules
kV	Kilovolts
L	Extensibility
mA	Milliampere
mb	Moisture Basis
min	Minutes
ML	Left of peak slope height
mL	Millilitre
mm	Millimetre
MP	Peak height
MR	Right of peak slope height
n	Number of samples
NaCl	Sodium chloride
NIR	Near Infrared Reflectance
P	Stability
P/L	Curve configuration ratio
PSI	Particle Size Index
R	Resistance
RVA	Rapid Visco Analyser
RVU	Rapid Visco Units
s	Seconds
SDS	Sodium Dodecyl Sulphate
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscope
SKCS	Single Kernel Characterization System
TP	Peak time
Tx	Width at 6 min
W	Deformation energy
X	Times
XRD	X-Ray Diffraction
λ	Lambda (Wavelength)
μ CT	Micro computed tomography

Chapter 1

Introduction

1. Introduction

Wheat is a popular and widely traded grain commodity and is a major part of many diets around the world (Maningat *et al.*, 2009). It is a highly adaptable grain which can successfully grow in a range of climates worldwide (Shewry, 2009). Approximately 750 million tons of wheat are grown a year worldwide (USDA, 2017), of which 67% is used as a food source, often in the form of bread (Maningat *et al.*, 2009; Shevkani *et al.*, 2017). With a shifting consumer focus from 'over processed' bread to 'artisanal' bread with a clean label, producers are finding it necessary to find cost effective ways to produce quality bread which is still within these trends (Best, 2016; Kenward, 2016). One such solution is finding replacements for additives and improvers which are effective, yet acceptable, to consumer demands. A possible way to achieve this is with the use of naturally occurring genetic mutation of bread wheat (*Triticum aestivum*), called waxy wheat. Waxy wheat can be used to replace expensive additives and improvers (Zhang *et al.*, 2014), as it has the ability to improve the shelf life of bread by retarding starch retrogradation (Graybosch, 1998; Shevkani *et al.*, 2017).

Waxy wheat is a hexaploid wheat cultivar where the endosperm of the wheat contains mainly amylopectin, with only trace amounts of amylose. This is due to the lack of the enzyme called granule bound starch synthase (GBSS) which is responsible for producing amylose (Nakamura *et al.*, 1995). GBSS is also called the Wx protein as it causes a waxy appearance in the endosperm of the wheat and is the reason for the name of this particular genetic mutation (Graybosch, 1998; Guzmán & Alvarez, 2016). As wheat has three genomes, the null allele of GBSS must be expressed in all three in order for the wheat to be considered a full waxy wheat (Graybosch, 1998). If it is only expressed in one or two of the genomes, it is considered a partial waxy wheat and will not be completely amylose-free. A full waxy wheat has not been found naturally and was first bred by Nakamura *et al.* (1995), using traditional breeding techniques.

Waxy wheats have commercial benefits and uses. They are most commonly utilised in eastern countries such as Japan, where the waxy wheat can greatly improve the sensory attributes of noodles. The unique starch properties of waxy wheat allow it to have a higher swelling potential due to the increase in amylopectin, which is the driving contributor to water absorption (Tester & Morrison, 1990). This results in a noodle with a smooth, clean and shiny surface (Wang & Seib, 1996) and a soft and elastic texture (Baik & Lee, 2003), which is more desirable to the consumer. Another benefit of waxy wheat is explored in the baking industry, particularly in bread baking. Due to the absence of amylose, starch from waxy wheats retrogrades more slowly than starch from non-waxy wheat and thus when used in bread can slow down staling and increase shelf life (Graybosch, 1998; Maningat *et al.*, 2009). This is beneficial as it could be used to replace expensive additives as well as create a loaf of bread which appeals to consumers who are concerned about what additives are in their food.

Much research has already been done on waxy wheats where starch and dough rheology have been observed, as well as the shelf life and loaf quality of baked bread. The pasting properties of

waxy wheat, measured by the Rapid Visco Analyser (RVA), showed that the peak time occurs sooner than non-waxy wheat (Chakraborty *et al.*, 2004; Zhang *et al.*, 2013) and that the final viscosity is lower (Zhang *et al.*, 2013). The faster development of a dough from waxy wheats was confirmed by results obtained from both the Farinograph (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Zhang *et al.*, 2014; Blake *et al.*, 2015) and the Mixograph (Abdel-Aal *et al.*, 2002; Guo *et al.*, 2003; Takata *et al.*, 2005; Guan *et al.*, 2009; Jung *et al.*, 2015; Graybosch *et al.*, 2016). These results suggest that waxy wheat could develop into a dough more quickly and that it could retrograde more slowly than dough from non-waxy wheat. This has economic advantages; but the use of waxy wheats also decreased the stability of the dough and was thus more sensitive to overmixing (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Morita *et al.*, 2002a; Takata *et al.*, 2005; Zhang *et al.*, 2014; Blake *et al.*, 2015; Jung *et al.*, 2015; Graybosch *et al.*, 2016). Most shelf life extension research involved blending waxy wheat flour with that of non-waxy/normal wheat as 100% waxy wheat flour creates a loaf of bread with an undesirable appearance of collapsed sides (Ghiasi *et al.*, 1984; Graybosch, 2001; Garimella Purna *et al.*, 2011). Blends of 15 to 30% of waxy wheat with bread wheat were found to have the ability to increase the shelf life of bread, without detrimentally affecting the appearance (Bhattacharya *et al.*, 2002; Qin *et al.*, 2009).

The Scanning Electron Microscope (SEM) is used to determine the starch granule morphology of wheat samples. Little to no differences in shape and size of starch granules have been found between waxy and non-waxy wheat (Abdel-Aal *et al.*, 2002; Yoo & Jane, 2002; Kim *et al.*, 2003; Jung *et al.*, 2015) however Zhang *et al.* (2013) noted that the non-waxy wheats appeared to have more smaller starch granules than waxy wheat. Waxy wheats tend to have a higher percentage of crystallinity due to the double helical nature of amylopectin and due to the fact that there is more of this starch present than in the non-waxy wheats (Kim *et al.*, 2003; Zhang *et al.*, 2013; Wang *et al.*, 2015; Xurun *et al.*, 2015). Both waxy and non-waxy wheats displayed an A-type starch pattern on the resulting X-Ray Diffraction (XRD) diffractograms (Kim *et al.*, 2003; Zhang *et al.*, 2013; Wang *et al.*, 2015; Xurun *et al.*, 2015).

The aim of this study was to characterise the starch structure of four South African waxy wheat lines in terms of starch granule size and morphology, as well as the percentage crystallinity. The pasting properties of blends of the four waxy wheats with a non-waxy wheat control were also determined, in addition to their dough processing properties. The baking quality of the final loaf of these blends was also determined and each one's potential to extend the shelf life of bread, analysed.

Chapter 2

Literature review

2. Literature Review

2.1. Introduction

Wheat is one of the world's most produced and traded grains (Maningat *et al.*, 2009). Wheat has become popular around the world for many reasons, as it is a highly adaptable crop which can grow in a wide range of climates whilst simultaneously producing large yields (Shewry, 2009). Wheat is currently being grown across five continents and 180 countries and provides up to 20% of the world population's caloric intake (Maningat *et al.*, 2009). The unique properties which wheat dough possesses, allows staple foods such as bread to be produced (Shewry, 2009). As bread is considered one of the world's most consumed products, there is a good opportunity to explore other wheat cultivars which could aid in realising growth in the bread industry.

Waxy wheat is a wheat cultivar where the endosperm contains a high amylopectin starch content and only a trace amount of amylose. This change in starch content alters the starch and dough rheology of the waxy wheat and thus affects the final loaf quality and shelf life of baked bread (Graybosch, 1998). This literature review will discuss how waxy wheat is bred and its uses and purpose in the bread industry. The principles of dough rheology (Farinograph, Mixograph and Alveograph) and starch rheology (Rapid Visco Analyser) will also be discussed and how these results of the waxy wheats processing qualities differ from a non-waxy wheat. The C-Cell digital image analyser and the texture analyser will be discussed to illustrate their role in determining final bread loaf quality, as well as its shelf life. Furthermore, the Scanning Electron Microscope and X-Ray Diffraction and how they aid in the determination of starch granule morphology and crystallinity, will be discussed. This review will discuss and compare findings from previous research on waxy wheats.

2.2. Waxy wheat genetics

Waxy wheat is wheat which contains very low amounts of amylose starch. The lack of amylose starch is the result of a genetic mutation. The Granule Bound Starch Synthase (GBSS) enzyme in wheat is responsible for the production of amylose (Nakamura *et al.*, 1995). GBSS is also known as the *Wx*-protein, since the absence of this protein results in the waxy phenotype being expressed (Nakamura *et al.*, 1995; Guzmán & Alvarez, 2016). Waxy wheat acquires its name from amylose-free maize, whose endosperm has a waxy appearance, as opposed to a translucent or flinty typical of non-waxy maize (Graybosch, 1998).

Wheat (*Triticum aestivum* L.) is considered a hexaploid as it contains three genomes and six sets of chromosomes (Graybosch, 1998). Waxy wheat has three homologous waxy genes named *WX-A1*, *WX-B1* and *WX-D1* and they are located on the 7A, 4A and 7D chromosomes respectively (Nakamura *et al.*, 1995; Graybosch, 1998). Each gene has its own isoform of the *Wx*-protein and they differ slightly in molecular mass and isoelectric points (Nakamura *et al.*, 1995). These three

proteins are named in accordance with the gene with which they are associated and hence are called *WX-A1* protein, *WX-B1* protein and *WX-D1* protein (Guzmán & Alvarez, 2016). If the null allele of the *Wx* protein is expressed in one or two of the genomes, the wheat is considered a partial waxy wheat (Graybosch, 1998). If all three null alleles of the GBSS protein are expressed, the wheat is considered a full waxy wheat which is often simply referred to as a 'waxy wheat' (Graybosch, 1998).

As the expression of the null alleles is a genetic mutation, a full waxy wheat has not been found to occur naturally (Nakamura *et al.*, 1995). This led to Nakamura *et al.* (1995) using traditional plant hybridisation methods to breed a full waxy wheat. This was done by using two partial waxy wheats as the parent plants, with each of the plants expressing the null alleles of the *Wx* protein in different genomes (Nakamura *et al.*, 1995). Nakamura *et al.* (1995) describes the specific breeding techniques and results of the trials in more detail.

There are currently many waxy wheat cultivars that are commercially grown around the world and new cultivars are continually being bred for different regions and climates (Jung *et al.*, 2015).

2.3. Starch microstructure

2.3.1. Starch structure

Starch comprises between 54 and 75% of the mass of a wheat kernel (Zhang *et al.*, 2013; Velisek, 2014; Xurun *et al.*, 2015). It provides energy for the plant and is found in granular form in the endosperm (Ottenhof & Farhat, 2004). The starch is found as two α -glucan polymers: namely amylose and amylopectin. In general, a kernel consists of 20 to 35% amylose (Morita *et al.*, 2002a; Zhang *et al.*, 2013; Velisek, 2014). In the case of waxy mutants, the kernel consists of 100% amylopectin.

2.3.1.1. Amylose

Amylose is a linear polysaccharide of glucose molecules which are connected via α -D-(1-4)-linkages (Figure 2.3.1) (Ottenhof & Farhat, 2004; Velisek, 2014). Amylose is a much smaller molecule than amylopectin and its degree of polymerisation - which indicates how many glucose units it consists of - is on average between 500 and 6000 (Zhang *et al.*, 2013). It has the ability to form complexes with other organic matter such as alcohols and with particular relevance to wheat, lipids (Jane, 2009). This affinity for other molecules is as a result of the formation of a single helical structure by the amylose which creates an inner space where hydrophobic molecules can be found (Ottenhof & Farhat, 2004). These hydrophobic molecules, such as lipids, then act as a ligand and bind with the starch (Ottenhof & Farhat, 2004). An amylose molecule has one monosaccharide-reducing end (Velisek, 2014).

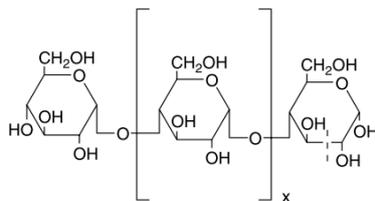


Figure 2.3.1 Structure of amylose molecule with α -(1-4) linkages (Pérez *et al.*, 2009).

2.3.1.2. Amylopectin

Unlike amylose, amylopectin is a branched polysaccharide which contains not only α -D-(1-4)-linkages but also α -(1-6)-glucan linkages (Fig. 2.3.2) (Zhang *et al.*, 2013; Velisek, 2014). This makes the amylopectin a much larger molecule, which has a degree of polymerisation between 50 000 and 1 000 000 (Ottenhof & Farhat, 2004; Zhang *et al.*, 2013).

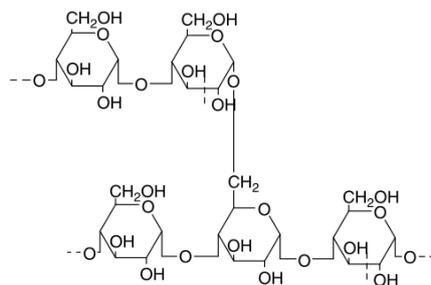


Figure 2.3.2 Structure of amylopectin molecule showing both α -(1-4) linkages and α -(1-6) linkages (Pérez *et al.*, 2009).

As a result of the branching which occurs, amylopectin consists of three different types of chains termed the A, B and C chains (Figure 2.3.3) (Ottenhof & Farhat, 2004; Jane, 2009). The A chains are considered the outer chains and attach to the B and C chains, but generally do not have any branches themselves (Jane, 2009). The B chains are the inner chains and are branched with either A or other B chains (Jane, 2009). Lastly the C chain is the backbone of the molecule and possesses the only reducing end on an amylopectin molecule (Jane, 2009). These clusters are in a double helix formation and contain alternating amorphous and crystalline sections (Figure 2.3.3) (Ottenhof & Farhat, 2004).

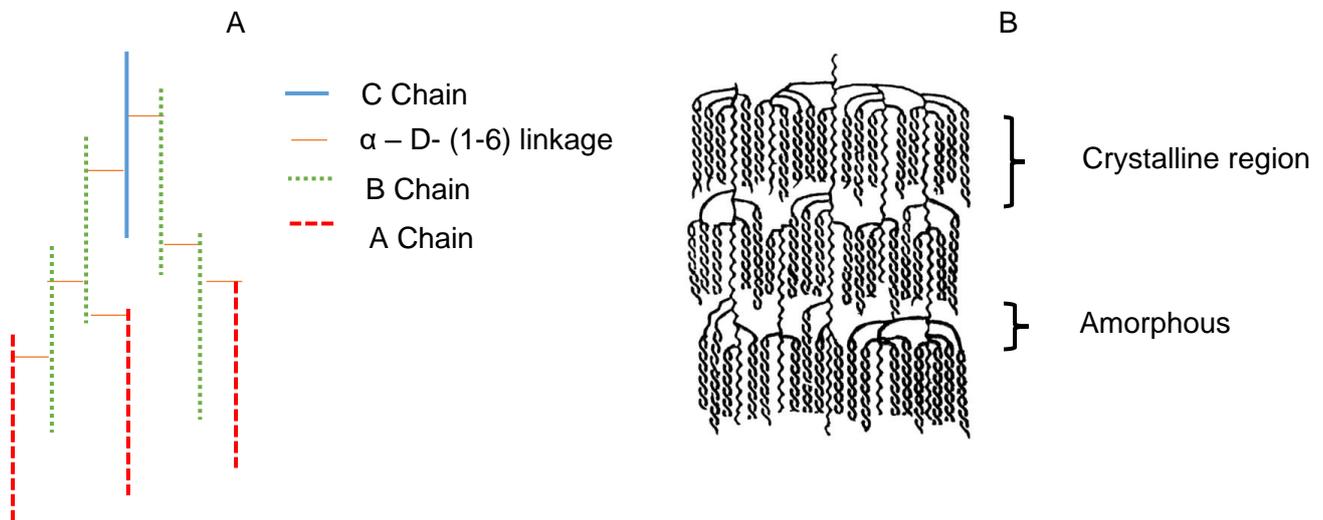


Figure 2.3.3 Cluster model of amylopectin illustrating the branching (A) and the crystalline and amorphous regions as well as the double helices (B) (Pérez *et al.*, 2009).

2.3.1.3. Starch granule

In a starch granule, the amylopectin clusters are positioned radially around a central point, with the non-reducing ends of the chains making up the surface of the granule (Figure 2.3.4) (Velisek, 2014). Many studies have been conducted to determine the role of amylose in the structure of the granule, as reviewed comprehensively by Jane (2009). The overall conclusions were that the amylose is situated in the amorphous regions of the amylopectin and that it co-crystallises with the amylopectin by intertwining with it (Takeda *et al.*, 1990; Jane *et al.*, 1992; Kasemsuwan & Jane, 1994). Other observations included that the amylose was concentrated more towards the surface of the granule than in the centre and that the amylopectin polymers were found closer to the centre and had longer branch chains (Jane & Shen, 1993; Pan & Jane, 2000; Li *et al.*, 2007).

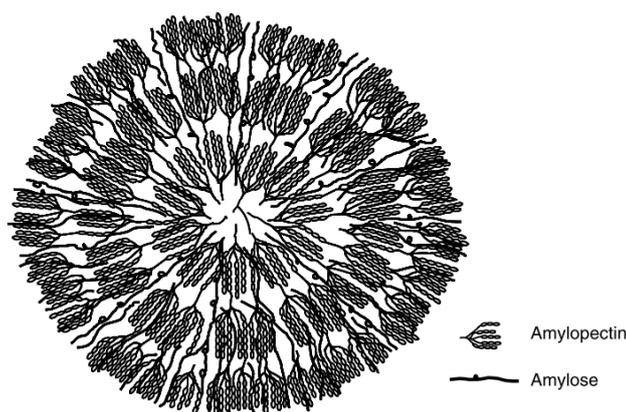


Figure 2.3.4 Illustration of the structure of a starch granule (Jane, 2009).

The degree to which the starch granule is crystallised separates starch into four polymorphic types. The A type is typically found in cereal grains and is the most stable of the four forms (Zhang *et al.*, 2013; Velisek, 2014). It is considered the most stable because a double helix fills the channel made

by another double helix (Velisek, 2014). The space in between these double helices is filled with bound water (Velisek, 2014). The B type starch is the least stable and is often found in high amylose grains and tubers (Zhang *et al.*, 2013; Velisek, 2014). B type starch is the least stable as a single double helix's channel is filled only with water molecules and not by another double helix (Velisek, 2014). C type starch has a mixture of both A and B types and is common to legumes, while the final V type is rarely found and generally applies to gelatinised starch which contains lipids (Zhang *et al.*, 2013; Velisek, 2014).

2.3.2. Starch granule morphology

2.3.2.1. Principles of the Scanning Electron Microscope

The Scanning Electron Microscope (SEM) is used to observe the microstructure of both biological and organic samples (Groves & Parker, 2013). It involves the electrons being charged from an electron gun and then being accelerated towards the sample (Clarke & Eberhardt, 2002). The electrons then scatter off the sample and detectors convert the information into a magnified image which allows for micro and even nano-structures to be observed (Figure 2.3.5). This method is considered to be non-destructive but has specific sample requirements. The instrument works under a high vacuum and thus the samples should be completely dry as well as stable to the electron beam (Clarke & Eberhardt, 2002; Groves & Parker, 2013).

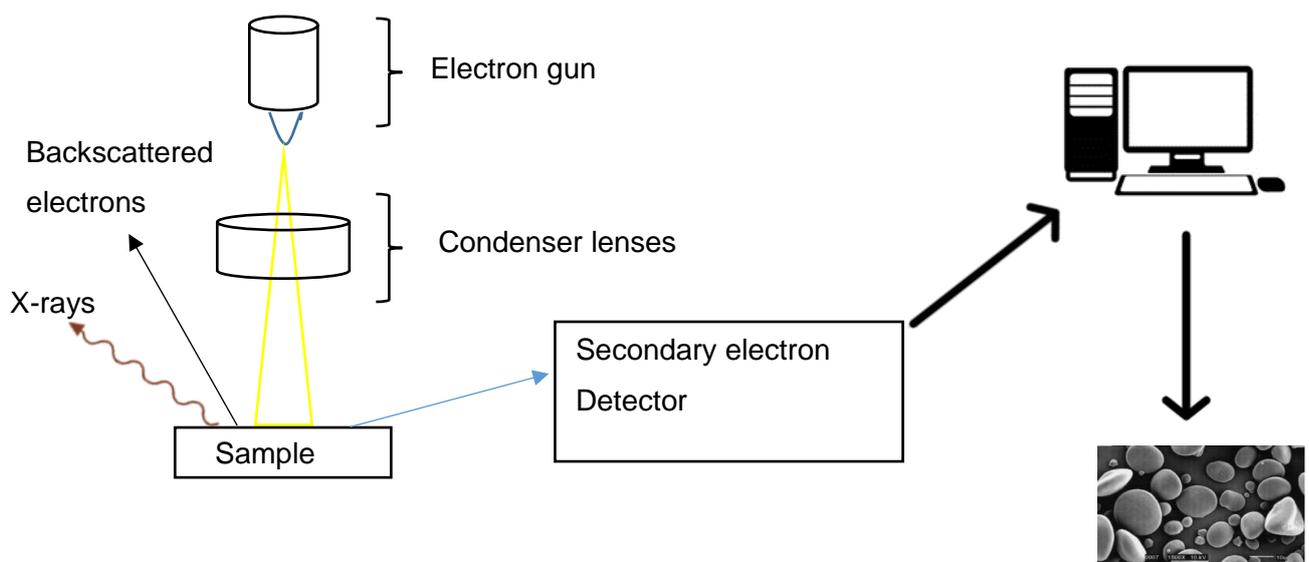


Figure 2.3.5 Basic outline on the principles of SEM where the yellow shape is the incident/electron beam and the blue arrow is the beam of secondary electrons.

The electron beam is generated from a glowing cathode filament, also referred to as the electron gun (Groves & Parker, 2013). The filament is normally tungsten and the electron beam is generated by a high voltage (5 Kv). The electrons are accelerated and focused towards the sample using kinetic energy via electrostatic and electromagnetic lenses (Groves & Parker, 2013). It is thus also important that the sample conducts electrons: this can be a problem for biological samples and they are therefore coated with platinum or gold-palladium (Clarke & Eberhardt, 2002).

Once the electron beam reaches the sample, it interacts with the elements in it and electrons are scattered or emitted in various ways. Inelastic interactions with the sample create secondary electrons, whereas elastic interactions create backscattered electrons (Clarke & Eberhardt, 2002; Groves & Parker, 2013). X-rays are also created and each of these outcomes can be used to create an image with a specific detector. The most common, however, is the use of the secondary electrons which are collected by a detector and accelerated towards a scintillator, which has been placed on a photomultiplier tube (Clarke & Eberhardt, 2002). The image produced is then displayed on a screen, where it can be viewed for analysis.

2.3.2.2. Scanning Electron Microscope and waxy wheats

The SEM is often used in studies on starch to observe the granule morphology of different cereal grains (Figure 2.3.6). It is an effective way to observe whether there is damage to the starch in the forms of pores, cracks and flakiness (Barrera *et al.*, 2013). Starch damage affects the way starch behaves and it is therefore important to know the extent of this when using the flour/starch for commercial purposes. SEM can also be used to see how two different starch samples differ from each other in terms of the size and the shape of granules. It is the latter which is most often utilised in studies of waxy wheat. Research shows that both A-type (large and disc-shaped) and B-type (small and spherical) starch granules were present in both waxy and non-waxy wheats (Figure 2.3.6) (Abdel-Aal *et al.*, 2002; Yoo & Jane, 2002; Kim *et al.*, 2003; Jane, 2009; Zhang *et al.*, 2013; Jung *et al.*, 2015; Wang *et al.*, 2015). The A-type granules were found to be between 17-33 μm in diameter whereas the B-type were between 2-8 μm (Yoo & Jane, 2002; Kim *et al.*, 2003). Some pores and indentations were observed but no cracks or fissures as a result of starch damage (Wang *et al.*, 2015). The grooves and indentations most likely occurred during the development of the starch and are impressions of proteins or other starch granules (Wang *et al.*, 2015). It was noted that there were little to no morphological differences between the waxy wheat and the non-waxy wheat (Abdel-Aal *et al.*, 2002; Yoo & Jane, 2002; Kim *et al.*, 2003; Jung *et al.*, 2015). However, Zhang *et al.* (2013) found that the non-waxy wheat appeared to contain more B-type granules than the waxy wheats.

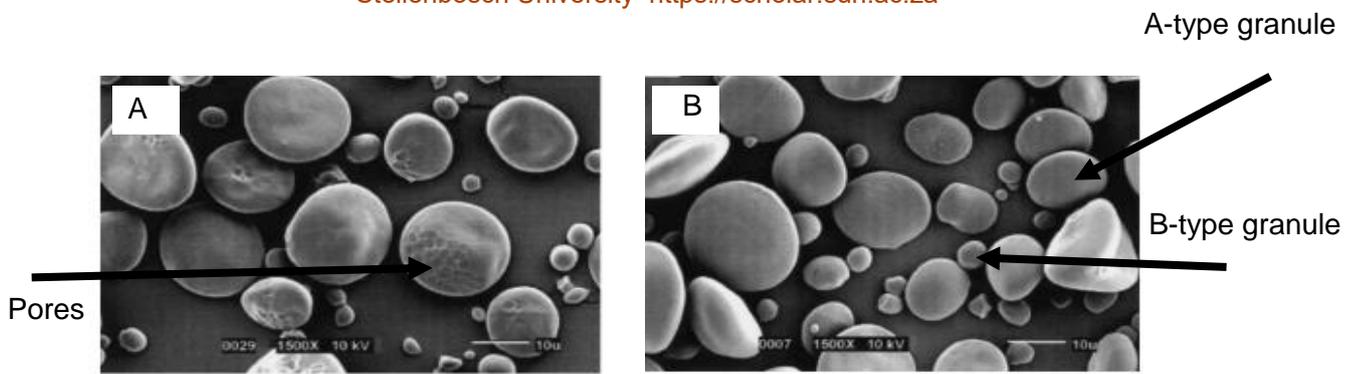


Figure 2.3.6 Example of SEM images where A is a waxy wheat and B is a non-waxy wheat (Yoo & Jane, 2002).

2.3.3. Relative percentage crystallinity

2.3.3.1. The principles of X-Ray Diffraction

X-Ray Diffraction (XRD) is used across many disciplines to characterise the structure of materials, in particular the crystalline structure (Kvick, 1999). It is a non-destructive, semi-quantitative method and makes use of radiation scattered by the atoms in a material to determine its percentage crystallinity (Kvick, 1999; Chakraborty *et al.*, 2004). This is a method that can only be applied to materials with long range order, or in other words, crystalline solids (Chakraborty *et al.*, 2004; Pecharsky & Zavalij, 2005a). The process begins when X-rays penetrate through a material and the resulting scattered radiation is transformed by a detector into a digital diffractogram. The ratios of the various peaks created, are then used to determine the structural characteristics of the material (Figure 2.3.7).

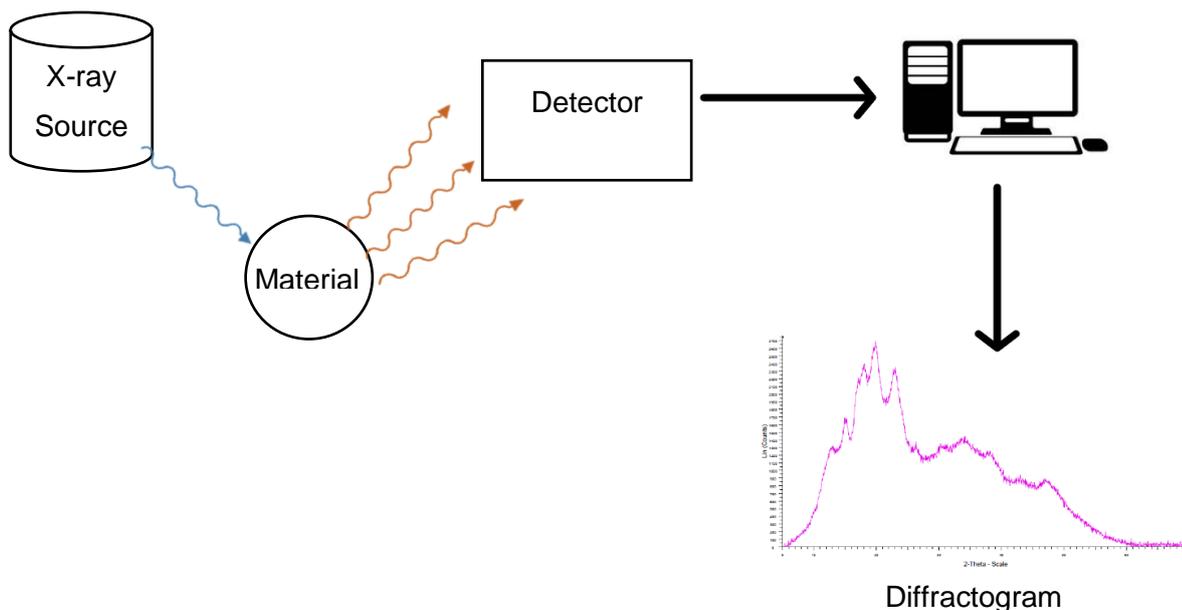


Figure 2.3.7 Basic overview of how XRD works, where the blue ray is the incident ray and the red rays are the scattered/reflected rays.

The most commonly used source of X-ray radiation is called the X-ray tube. It is also referred to as the laboratory, or conventional X-ray source, due to its prevalence (Pecharsky & Zavalij, 2005b). The X-rays, which are electromagnetic waves, are created by bombarding a metal anode with high energy electrons. The electrons are released from a cathode which is electrically heated and which is typically a tungsten filament; then accelerated by a high electrostatic potential towards the anode (Pecharsky & Zavalij, 2005b). The electrostatic potential between the anode and cathode is maintained between 30 and 60 kV (Pecharsky & Zavalij, 2005b). All this is sealed inside a tube which is under a high vacuum and has a current of 10 – 50 mA running through it (Pecharsky & Zavalij, 2005b). The anode is constantly cooled, as it produces large amounts of energy in the form of heat during the electron bombardment. The X-rays produced then leave the tubes via four beryllium (Be) windows which are placed at 90° intervals around the tube (Figure 2.3.8).

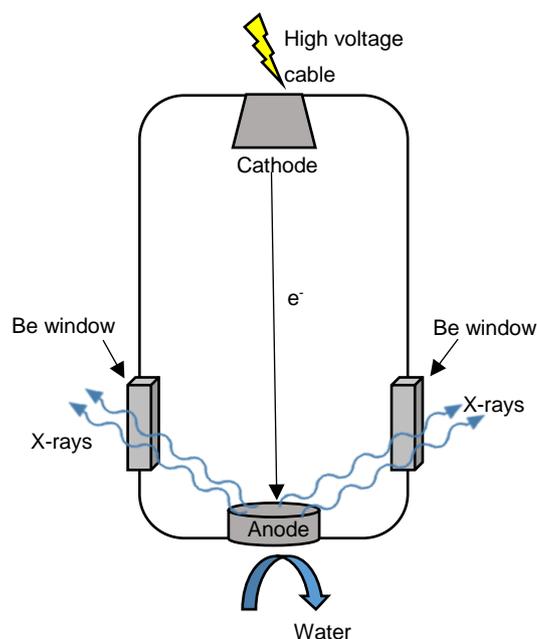


Figure 2.3.8 Example of a tube X-ray source (x-ray tube).

The X-ray spectrum which exits the tube is normally characterised by three different wavelengths namely $K\alpha_1$, $K\alpha_2$ and $K\beta$ (Pecharsky & Zavalij, 2005b). For successful diffraction, only one wavelength is required and thus monochromatisation methods are employed to reduce the multiple wavelengths to just one. Collimation is also done to reduce the variation of angles of the rays. These are both done before the X-rays reach the sample. Collimation is done by placing a divergence slit between the source and the sample and monochromatisation is done using four methods: β -filter, diffraction from a crystal monochromator, pulse height selection using a proportional counter and energy resolution using a solid state detector (Figure 2.3.9).

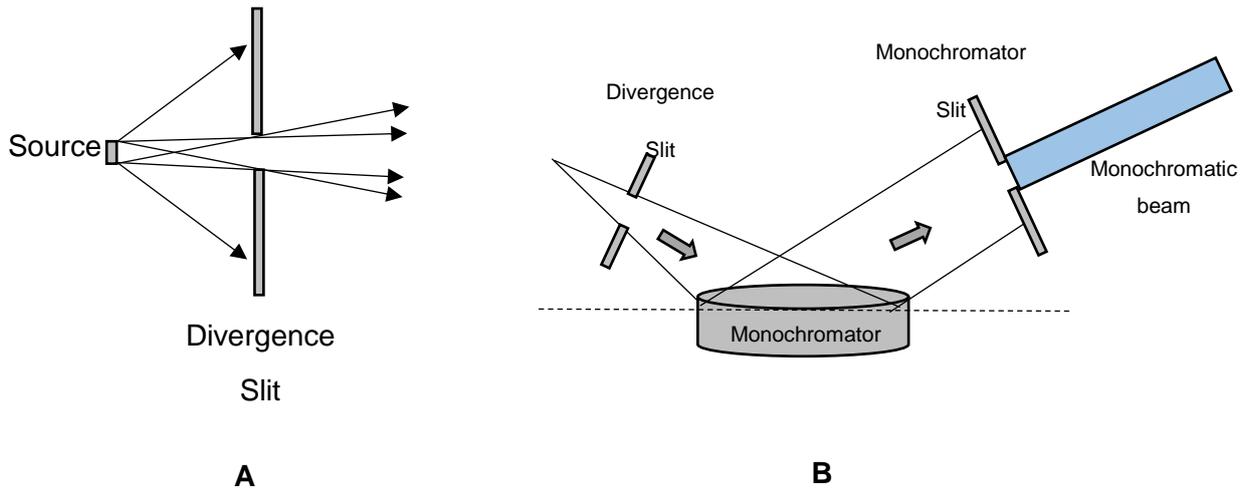


Figure 2.3.9 Examples of a) Collimation with a single divergence slit and b) monochromatisation using a crystal monochromator.

Once the X-rays have gone through these corrections, they finally hit the sample. The rays penetrate the sample as a plane wave of radiation: which interacts and excites electrons (Kvick, 1999). The radiation is both scattered and absorbed by the material but the absorption is often not significant in diffraction (Pecharsky & Zavalij, 2005b). The scattered radiation could be imagined as spheres of radiation emanating from the atoms in the material. These spheres of radiation interact with one another constructively and destructively and create Bragg reflections which are distinct spots in certain directions (Kvick, 1999). The angle at which these rays reflect (θ_{hkl}) gives information on the ordering dimensions of the material and the intensities of the ray give an indication of the location of electrons within the order (Kvick, 1999). The basis for this - and all diffraction studies - is Bragg's law (Equation 2.3.1):

$$\lambda = 2d_{hkl} \sin \theta_{hkl}$$

Equation 2.3.1

Where λ is the wavelength, d_{hkl} is the spacing of the atomic plane and θ_{hkl} is the angle of the diffracting plane where constructive interference occurs (Figure 2.3.10).

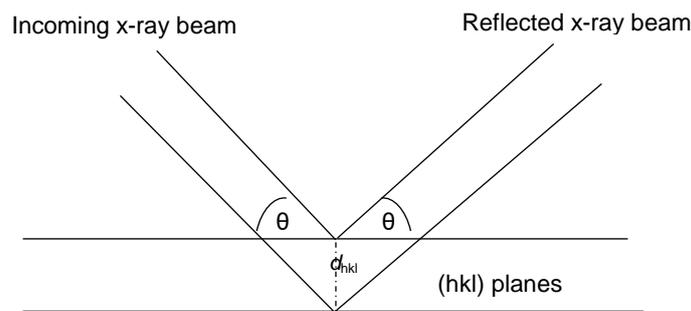


Figure 2.3.10 Visualization of Bragg's law.

The sample is orientated through all the possible planes to measure the scattered intensities. In other words, the sample is rotated so that the incident beam scatters off multiple points of the sample. The intensities of these reflected X-ray beams are then measured by a detector and translated into diffractograms. A more in depth review on the mathematics behind X-Ray Diffraction has been written by Messerschmidt (2007).

The integration of the areas of the peaks is then used to determine the percentage crystallinity of the sample. The amorphous area (area I) is determined from the base line of the diffractogram to the tail base line of each peak (Hayakawa *et al.*, 1997) (Figure 2.3.11). The crystalline area (area II) is then the sum of all the area peaks from the tail to tail base line (Hayakawa *et al.*, 1997). The percentage crystallinity is calculated using equation 2.3.2 (Yoo & Jane, 2002):

$$\% \text{ Crystallinity} = \frac{\text{area II}}{(\text{area II} + \text{area I})} \times 100$$

Equation 2.3.2

This information can be used to compare the structure of various samples and with relevance to wheat flour, it gives an indication of the amylose to amylopectin ratios (Zobel, 1988; Hayakawa *et al.*, 1997; Yoo & Jane, 2002).

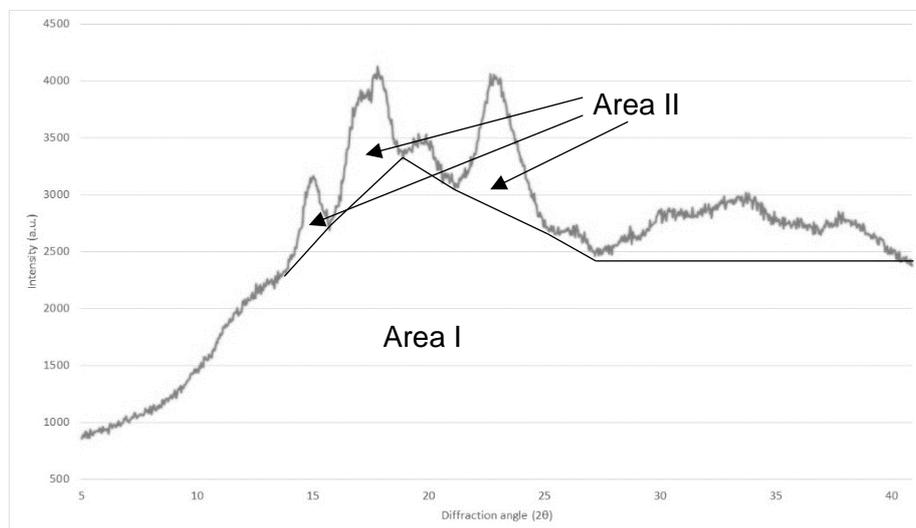


Figure 2.3.11 Illustration of amorphous (area I) and crystalline (area II) areas on a diffractogram.

2.3.3.2. X-Ray Diffraction and waxy wheats

XRD is commonly used to compare the structural characteristics of different non-waxy and waxy wheat samples. The patterns seen in diffractograms of wheat indicate whether the starch is A, B, C or V type. The percentage crystallinity or relative degree of crystallinity of the starch present in the wheat, is a result of the amylose content and the branching and length of the outer chains of the amylopectin (Xurun *et al.*, 2015).

Both non-waxy wheat and waxy wheat show a typical A-type pattern diffractogram (Kim *et al.*, 2003; Zhang *et al.*, 2013; Wang *et al.*, 2015; Xurun *et al.*, 2015). An A-type pattern shows distinct peaks at $2\theta = 15, 17-18$ and 23° (Figure 2.3.12) (Shi & Seib, 1992; Zhang *et al.*, 2013; Xurun *et al.*, 2015). The main difference between non-waxy wheats and waxy wheats was normally seen at around $2\theta = 20^\circ$ (Figure 2.3.12). The waxy wheats often lacked a peak at this diffraction angle or the intensity was much weaker (Hayakawa *et al.*, 1997; Yoo & Jane, 2002; Kim *et al.*, 2003; Zhang *et al.*, 2013). This peak reflects the amylose-lipid complexes found in wheat; and as waxy wheats completely lack amylose, these complexes would not form (Kim *et al.*, 2003).

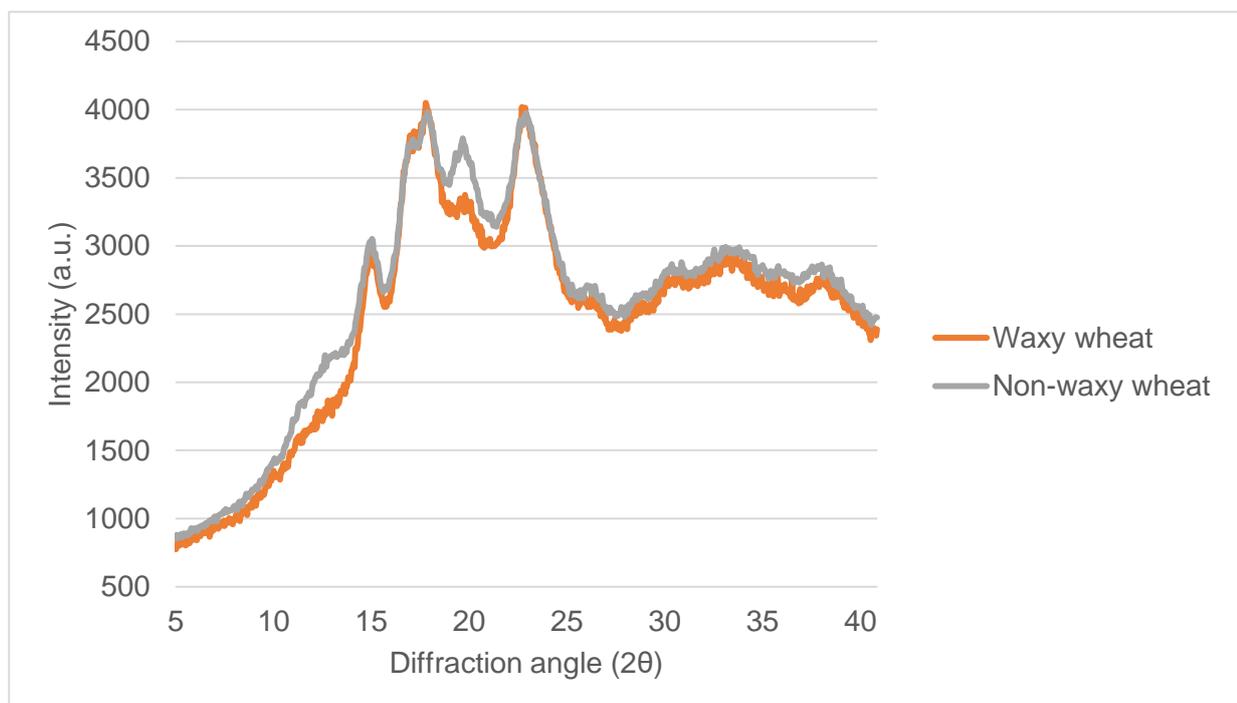


Figure 2.3.12 Example of diffractograms of both non-waxy (NWS) and waxy wheats (WWS).

The range of percentage crystallinity of waxy wheat was between 33.71% and 40.0% and for non-waxy wheat, between 21.6% and 28.9% (Kim *et al.*, 2003; Zhang *et al.*, 2013; Wang *et al.*, 2015; Xurun *et al.*, 2015). This higher degree of crystallinity for the waxy wheats is expected due to their higher amounts of amylopectin and thus their higher degree of branching and double helices. Yoo & Jane (2002) report crystallinities which are much lower than in other researcher's reports and waxy wheats were reported to have a percentage crystallinity of 18% and 13% reported for non-waxy

wheat. It is unclear exactly why these values differ so much from the others but it is probably due to the fact that the areas used to define the crystalline and amorphous areas (Figure 2.3.11) were quite different from the other studies and thus resulted in much lower crystallinities. Another possibility is that the type of detector used by the authors measured a much lower intensity from the scattered beams and as a result, the peaks would have been smaller, resulting in a smaller crystalline area. XRD can be used to compare the structure of different wheat samples and provides information about the structure of the amylopectin and the content of amylose.

2.4. Starch pasting properties

2.4.1. Principles of Rapid Visco Analyser (RVA)

The Rapid Visco Analyser (RVA) was first developed by Ross *et al.* (1987) in order to measure the degree of sprout damage in wheat kernels. The method was developed to improve the method of the Brabender Viscoamylograph (BVA) which was already in use (Thiewes & Steeneken, 1997). The RVA has many advantages over the BVA, including its faster processing time and the requirement of a smaller sample of flour (Deffenbaugh & Walker, 1989; Thiewes & Steeneken, 1997). The RVA equipment is also more durable and easier to use than the BVA (Deffenbaugh & Walker, 1989) and thus it has become the more popular choice for determining not only sprout damage but also starch quality and more importantly, the pasting properties of starch (Deffenbaugh & Walker, 1989; Batey & Curtin, 2000; Juhász & Salgó, 2008).

RVA is a rheological method where flour suspended in water is subjected to a fixed heating and cooling programme. The resulting viscosity of the starch, measured in centipoise (cP), is then recorded as a function of temperature and time. The viscosity is measured by the resistance of the starch suspension to a plastic paddle which rotates in an aluminium can (Suh, 2003). The plastic paddle also applies a mechanical shear force to the starch granules which affects the viscosity.

The viscograms give the following information: peak viscosity, peak time, pasting temperature, trough, breakdown, setback and final viscosity (Figure 2.4.1). The pasting temperature is the temperature at which the starch granules begin the uptake of water and thus the granules swell and gelatinise (Juhász & Salgó, 2008). This temperature indicates the minimum temperature at which the starch should be cooked to obtain a quality product (Newport Scientific, 2010). This information also allows for the calculation of energy costs.

During gelatinisation, hydrogen bonds in the starch double helices are broken down and reformed with water (Tester & Karkalas, 1996). The starch granules then begin to swell and the soluble polysaccharides, namely amylose, begin to leach out of the granule (Tester & Morrison, 1990). Due to the leakage of amylose, the remaining polysaccharides - which are amylopectin - begin to absorb even more water and this is what leads to an increase in viscosity (Hoseney & Delcour, 2010). The peak viscosity is a result of the pasting of starch granules which follows starch gelatinisation (Zhang *et al.*, 2013). The peak viscosity occurs after the initial heating of the starch suspension and indicates that the granules are at their optimal balance between swelling and rigidity (Thiewes & Steeneken,

1997; Juhász & Salgó, 2008). This means that the starch granule is still intact and absorbing water and has not yet ruptured (Hoseney & Delcour, 2010), thus the peak viscosity gives an indication of the water holding capacity of the starch (Newport Scientific, 2010). This information allows bakers to predict the quality of bread baked from the particular starch. It also allows them to determine what the viscosity of the dough will be and if it is suitable for the kneading equipment.

Due to the stirring action of the paddle, the soluble starch begins to align with the direction of the rotation (Newport Scientific, 2010). The shear force and the exposure to a constant high temperature (holding temperature) causes the granules to break down further, resulting in a reduction in viscosity (Hoseney & Delcour, 2010; Newport Scientific, 2010). This property of starch is called shear thinning (Lai *et al.*, 2000). The extent to which a starch paste breaks down is dependent on the holding temperature, the shear rate from the plastic paddle, the chemical composition of the starch and the enzymes which are present (Newport Scientific, 2010). It is thus important that the heating programme and the stirring rate are maintained to the standard proposed by the American Association of Cereal Chemists (AACC, 1999a) so that all RVA tests can be compared accurately (Doublier *et al.*, 1987).

The minimum viscosity reached after the holding period is known as the trough, the holding strength, or the hot paste viscosity. The difference between the peak viscosity and the trough is known as the breakdown (Juhász & Salgó, 2008). The breakdown gives an indication of the starches' resistance to mixing (shear force) and can aid bakers in determining the suitability of the flour for baking (Newport Scientific, 2010).

The holding period is followed by a cooling period. During the cooling period, the viscosity increases once again and results in a final viscosity (Newport Scientific, 2010). The increase in viscosity is due to a process called starch retrogradation. This is where the amylose polymers re-associate with one another and the amylopectin polymers re-crystallise to form a gel (Ottenhof & Farhat, 2004; Newport Scientific, 2010). This region between the trough and the final viscosity is called the setback region and this, together with the final viscosity, gives an indication of the texture of the product which is being produced with the particular starch (Thiewes & Steeneken, 1997; Newport Scientific, 2010).

2.4.2. Waxy wheat and the Rapid Visco Analyser

The RVA has been used in many waxy wheat studies to evaluate the pasting properties of the starch within the wheat, as well as to determine general starch quality. The literature reviewed was found to have little differences in its conclusions and these are summarised in Table 2.4.1. Viscograms illustrating the differences in curve shape between waxy wheat and non-waxy wheat can be seen in (Figure 2.4.1). Zhang *et al.* (2013) found that a waxy wheat sample reached its peak viscosity at 73.6°C as opposed to a non-waxy wheat sample whose peak was at 94.7 °C. Due to the quick increase in viscosity observed, it could be noted that the waxy wheat starch begins to absorb water and gelatinise much sooner and at a lower temperature than non-waxy wheat. The breakdown was

also much larger in the waxy wheat and had a lower final viscosity illustrating that waxy wheat starch is less stable after gelatinisation, but retrogrades slower.

In a similar study, Garimella Purna *et al.* (2015) obtained results that mirrored that of the study conducted by Zhang *et al.* (2013). The pasting temperatures of the waxy wheats were found to be in the same range ($\sim 70^{\circ}\text{C}$) and again the breakdown was more prominent and the setback viscosity lower. The larger breakdown is the result of the waxy wheat starch granules not being as rigid as those of the non-waxy wheat starch. The absence of amylose was the reason for the lower final viscosity as its absence did not enable the starch to form a gel matrix quickly.

It was found in more than one study, that there was a strong negative correlation between the amount of amylose in the wheat starch and the peak viscosity (Sasaki *et al.*, 2000; Yoo & Jane, 2002). This is due to the fact that the amylopectin polymer is the main cause of the absorption of water due to its double helical conformation (Tester & Morrison, 1990). This accounts for the result found in various studies that waxy wheat starch granules swell quickly to develop a higher viscosity (Table 2.4.1). Some studies, however, showed a lower peak viscosity than non-waxy wheat. Upon inspection it was determined that what caused this difference was the use of pure starch (with a higher peak viscosity) as opposed to flour (with a lower peak viscosity). As it is the starch's absorption of water which creates viscosity, it can be determined that when flour is used, the proteins present are competing with the starch granules for the water (Caramanico *et al.*, 2017). This means that the starch absorbs less water and thus results in a lower viscosity.

While most studies used the AACC 76-21.01 (AACC, 1999a) RVA method for testing starch pasting, a few had slight variations: particularly in their holding times. If the holding phase is continued for too long, it may exaggerate the breakdown of the starch due to the shear thinning properties of starch (Batey & Curtin, 2000). This may hinder the comparison of the results between studies. An example of this deviation from the AACC method is Sasaki *et al.* (2000) whose holding time was 5 min in comparison to the approved 3 min 30 s.

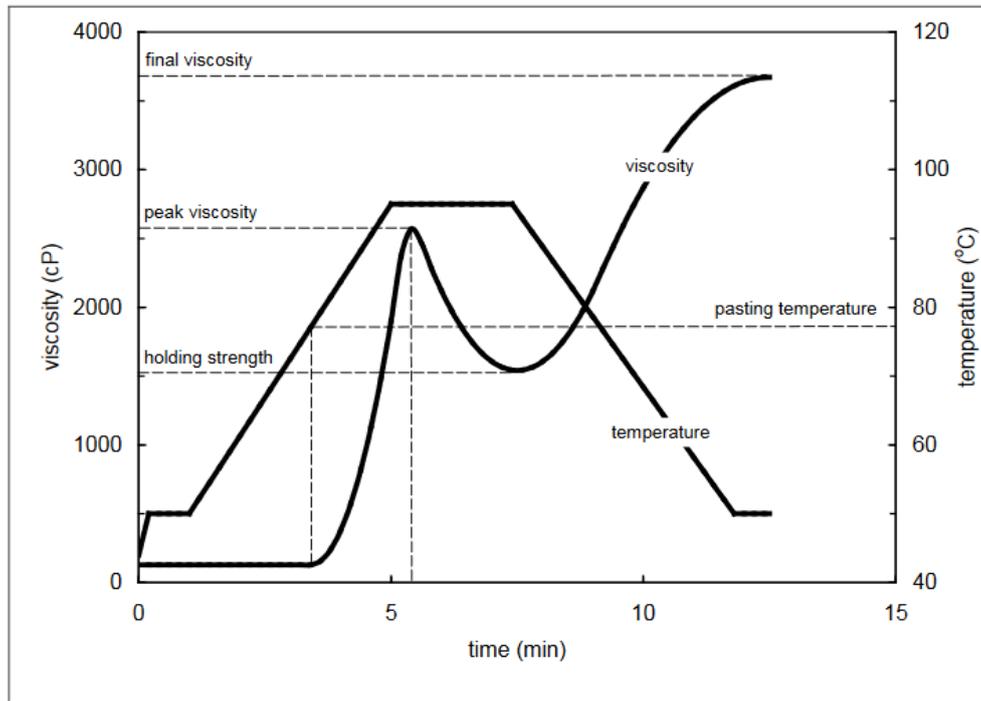


Figure 2.4.1 A typical viscomogram formed using RVA and the temperature profile applied to the flour suspension (Newport Scientific, 2010).

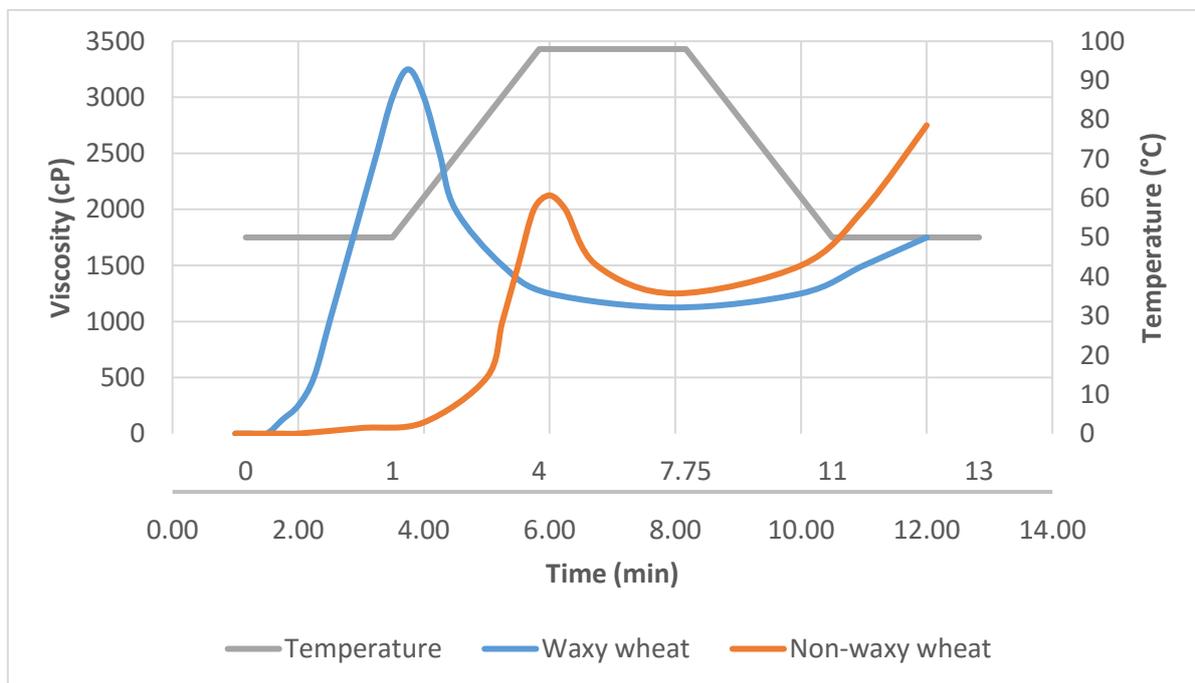


Figure 2.4.2 Viscomograms demonstrating the difference between waxy wheat and non-waxy wheat.

Table 2.4.1 Summary of RVA results from waxy wheat studies.

Viscogram property	Result of waxy wheat	Result of non-waxy wheat	Reference	General remarks
Pasting temperature (°C)	68.6	68.6	(Guo <i>et al.</i> , 2003)	The pasting temperature for non-waxy wheats is higher than for waxy wheats.
	66.18	85.28	(Li <i>et al.</i> , 2016)	
	66.7 – 67.0	67.6	(Caramanico <i>et al.</i> , 2017)	
	67.0 – 68.7	-	(Wang <i>et al.</i> , 2015)	
	65.2	66.5	(Kim <i>et al.</i> , 2003)	
	67.8	87.5	(Zhang <i>et al.</i> , 2014)	
	62.5	85.0 – 90.6	(Yoo & Jane, 2002)	
Peak time (min)	3.4	5.9 – 6	(Graybosch <i>et al.</i> , 2000)	The peak time for waxy wheats was shorter than for the non-waxy wheats.
	3.20	6.60	(Li <i>et al.</i> , 2016)	
	2.8	3.7	(Kim <i>et al.</i> , 2003)	
	4.2 -4.7	9.4	(Chakraborty <i>et al.</i> , 2004)	
Peak Temperature (°C)	82.2	95	(Guo <i>et al.</i> , 2003)	Cooler temperatures are needed for waxy wheats to reach peak viscosity, compared to non-waxy wheats.
	76.4	94.9	(Takata <i>et al.</i> , 2005)	
	78.3 – 82.0	95.00	(Caramanico <i>et al.</i> , 2017)	
	71.8	82.8	(Kim <i>et al.</i> , 2003)	
	70.3	93.2	(Zhang <i>et al.</i> , 2014)	

Peak Viscosity (RVU)	228.8	212.2 – 235.0	(Graybosch <i>et al.</i> , 2000)	Most studies found waxy wheats to have a higher viscosity than non-waxy wheat.
	211	186-219	(Guo <i>et al.</i> , 2003)	
	151.4	156.9	(Sasaki <i>et al.</i> , 2000)	
	302	201	(Kim <i>et al.</i> , 2003)	
	251	156	(Takata <i>et al.</i> , 2005)	
	266-333	214	(Chakraborty <i>et al.</i> , 2004)	
	270 - 274	152	(Bhattacharya <i>et al.</i> , 2002)	
	230	96 - 122	(Yoo & Jane, 2002)	
Peak Viscosity (cP)	4143	2228	(Li <i>et al.</i> , 2016)	
	2377 – 2398	3119	(Caramanico <i>et al.</i> , 2017)	
	459	364	(Zhang <i>et al.</i> , 2014)	
	4022 – 4243	-	(Wang <i>et al.</i> , 2015)	
	472 - 2011	1971	(Garimella Purna <i>et al.</i> , 2015)	
Trough Viscosity (RVU)	83.1	47.6	(Kim <i>et al.</i> , 2003)	Most studies found that waxy wheats had a lower trough viscosity than non-waxy wheats.
	94 - 97	110	(Bhattacharya <i>et al.</i> , 2002)	
Trough Viscosity (cP)	1362	1826	(Li <i>et al.</i> , 2016)	
	1372 - 1413	-	(Wang <i>et al.</i> , 2015)	

	312	234	(Zhang <i>et al.</i> , 2014)	
	38 - 734	1125	(Garimella Purna <i>et al.</i> , 2015)	
Breakdown (RVU)	139.4	81.3 -90.2	(Graybosch <i>et al.</i> , 2000)	Waxy wheats were found to have a larger breakdown value than non-waxy wheats.
	137	68-75	(Guo <i>et al.</i> , 2003)	
	154	28	(Takata <i>et al.</i> , 2005)	
	219	153	(Kim <i>et al.</i> , 2003)	
	187 - 245	92	(Chakraborty <i>et al.</i> , 2004)	
	176- 177	42	(Bhattacharya <i>et al.</i> , 2002)	
Breakdown (cP)	2781	402	(Li <i>et al.</i> , 2016)	
	1384-1419	1293	(Caramanico <i>et al.</i> , 2017)	
	2644-2830	-	(Wang <i>et al.</i> , 2015)	
	147	130	(Zhang <i>et al.</i> , 2014)	
Final Viscosity (RVU)	118.1	245.6 – 256.1	(Graybosch <i>et al.</i> , 2000)	A lower final viscosity was found for waxy wheats, compared to non-waxy wheats.
	101	221 – 283	(Guo <i>et al.</i> , 2003)	
	110	113	(Kim <i>et al.</i> , 2003)	
	124 - 126	197	(Bhattacharya <i>et al.</i> , 2002)	
Final Viscosity (cP)	1890	2514	(Li <i>et al.</i> , 2016)	

	1341-1383	3197	(Caramanico <i>et al.</i> , 2017)	
	1659 - 1702	-	(Wang <i>et al.</i> , 2015)	
	73 - 1038	2118	(Garimella Purna <i>et al.</i> , 2015)	
	472	548	(Zhang <i>et al.</i> , 2014)	
Setback Viscosity (RVU)	28.7	111.3 – 114.7	(Graybosch <i>et al.</i> , 2000)	A smaller setback value was found for waxy wheats, compared to non-waxy wheats.
	26	110 – 132	(Guo <i>et al.</i> , 2003)	
	16	75	(Takata <i>et al.</i> , 2005)	
	48 - 54	147	(Chakraborty <i>et al.</i> , 2004)	
	29 - 30	87	(Bhattacharya <i>et al.</i> , 2002)	
	26.7	65.5	(Kim <i>et al.</i> , 2003)	
Setback (cP)	527	688	(Li <i>et al.</i> , 2016)	
	369 -383	1371	(Caramanico <i>et al.</i> , 2017)	
	246 - 329	-	(Wang <i>et al.</i> , 2015)	
	35 - 305	994	(Garimella Purna <i>et al.</i> , 2015)	
	160	314	(Zhang <i>et al.</i> , 2014)	

2.5. Dough rheology

2.5.1. Rheological properties of dough

Wheat is the only grain that can create a unique viscoelastic dough that is suitable for leavened baked products (Hoseney, 1994). This is due to the fact that wheat contains the protein, gluten. Gluten is made up of single chain prolamins called gliadin and multi chain glutelins called glutenin (Hoseney, 1994; Dobraszczyk, 2003). The gliadins are responsible for the cohesive nature (visco) of wheat dough and the glutenins are responsible for the resistance to extension (elasticity) (Hoseney, 1994).

Dough is formed when wheat flour and water are mixed together. The water causes the hydration of the flour's components, in particular starch and proteins. The gluten structure, which creates the rheological properties of the dough, can only be formed if the gluten is hydrated sufficiently (Millar, 2003). The resulting dough will be able to be stretched and then partially return to its original shape (Hoseney & Delcour, 2010).

The mechanical action of mixing will supply energy into the dough system via deformation (Belton, 2003), as well as speed up the development of the gluten network (Hoseney & Delcour, 2010). The dough will reach a peak strength which is when it is at its optimal for bread making (Millar, 2003). If mechanical mixing is continued after this peak, the dough will begin to break down. This is caused by the mechanical force causing bonds in the gluten network to break (Belton, 2003). The dough will become more extensible and less elastic, resulting in a sticky dough that is not ideal for bread making (Millar, 2003).

The rheological study of dough is used to gain insight into the performance of the dough during processing as well as its quality (Dobraszczyk & Morgenstern, 2003). It can also be used to predict the final loaf quality.

2.5.2. Farinograph and Mixograph

2.5.2.1. Principles of the Farinograph and Mixograph

The Farinograph and the Mixograph work on very similar principles. Flour and water are added together and the torque resistance against a mixing paddle is measured as the dough develops (Migliori & Correra, 2013). A graph is produced for both methods, which gives information such as dough development time, water absorbance of the flour and the dough stability (Rasper & Walker, 2000; Migliori & Correra, 2013).

The Farinograph consists of two z-shaped blades which rotate at constant but different speeds (Rasper & Walker, 2000) and the dough is subjected to a stretch and chop action in order to be developed (Migliori & Correra, 2013). The Mixograph on the other hand, consists of four pins attached to an arm which rotate around three stationary pins which are attached to the bottom of a mixing bowl (Rasper & Walker, 2000). The dough is subjected to more of a 'pull, fold and re-pull' mixing action (Rasper & Walker, 2000).

The Farinogram curve created by the Farinograph gives much information about the development of the dough (Figure 2.5.1). The main points measured are the arrival time, peak time, departure time, water absorption, stability time and mixing tolerance index. The arrival time is the time which it takes for the curve to reach 500 Brabender units (BU) and measures the rate at which the flour absorbs the water added (Rasper & Walker, 2000; Migliori & Correra, 2013). The peak time is the time it takes for the dough to reach its maximum resistance to the paddles and indicates the time it takes for the dough to be at its optimal consistency (Oliver & Allen, 1992). This is also where the water absorption is measured and indicates the amount of water needed to mix the optimum dough consistency (Rasper & Walker, 2000; De Groot, G., 2016, Laboratory Manager, Sensako, Bethlehem, South Africa, personal communication). The test for a given sample may have to be repeated to ensure that the correct amount of water is added. Often too little or too much water is added initially. It can be determined that the correct amount of water has been added by examining the curve and observing that the 500 BU line is in the middle of the curve (AACC, 1999b). The departure time is the time it takes for the curve to drop below the 500 BU and the stability time is the difference between the arrival time and the departure time (Migliori & Correra, 2013). The stability time indicates the strength of the flour and the longer it is, the stronger the flour (Zhang *et al.*, 2014). The mixing tolerance index is measured as the difference between the peak time resistance and the resistance five minutes after the peak time (Migliori & Correra, 2013).

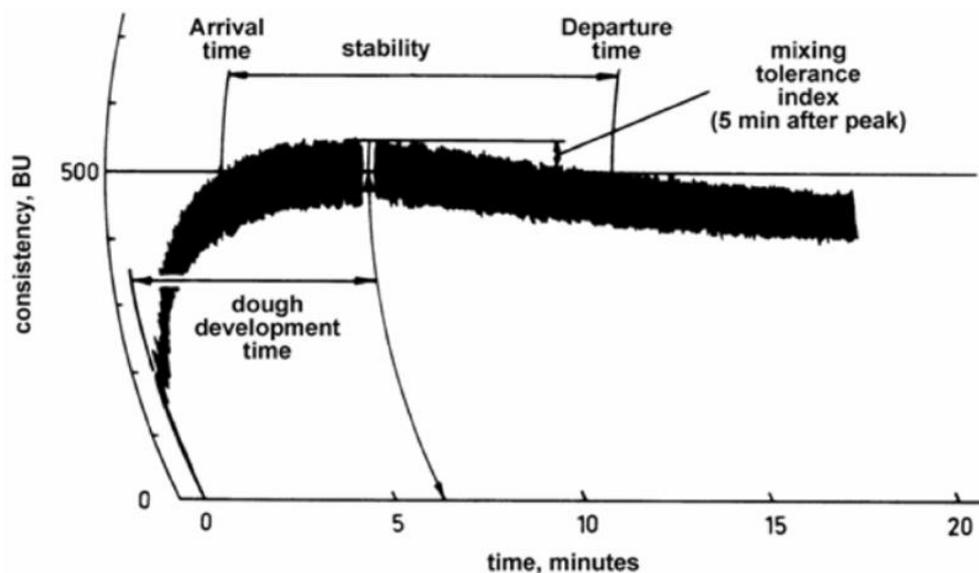


Figure 2.5.1 A Farinogram created by the Farinograph showing the measurements taken (Brabender, 2015).

The Mixograph differs in that a set amount of water is added to each run, based on the moisture content of the flour and thus only one run of each sample is completed (AACC, 1999c). A curve is also produced and software is used to analyse the Mixograms (Fig. 2.5.2) (Martinant *et al.*, 1998).

The curve consists of two envelopes and a midline and the software uses the top envelope and the midline in the analysis of the Mixograms (Martinant *et al.*, 1998).

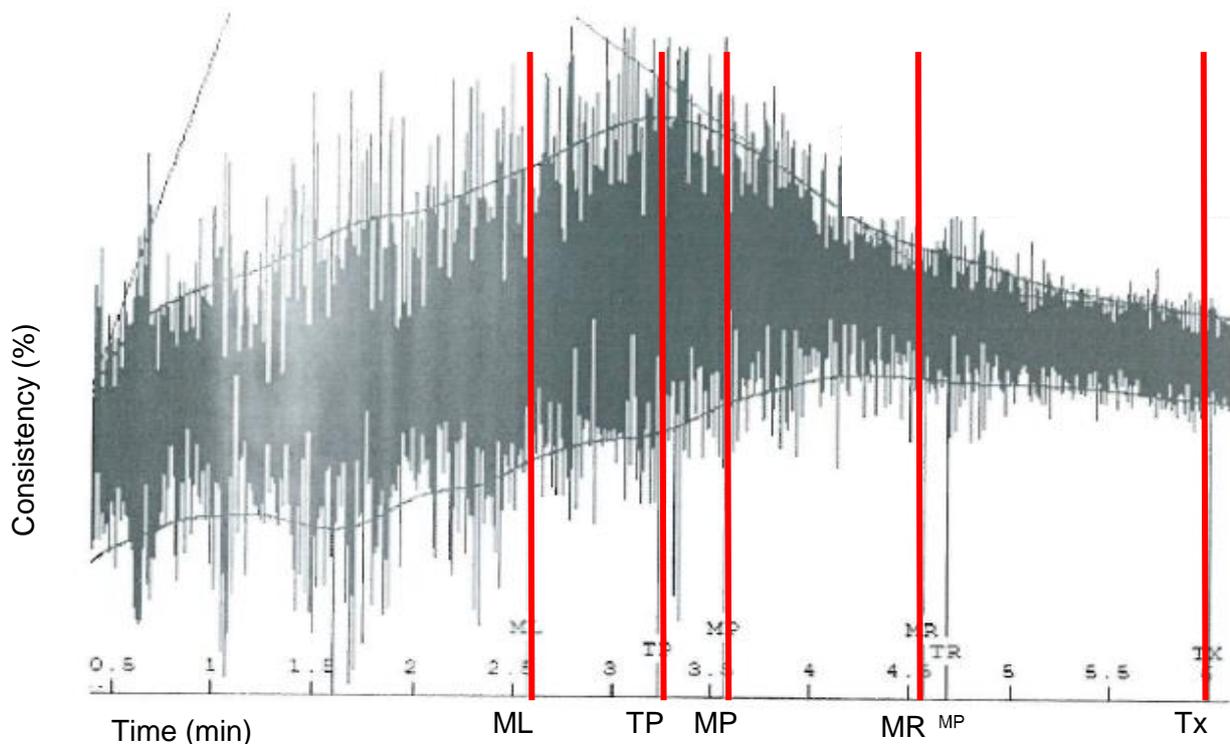


Figure 2.5.2 A Mixograph showing a few measurements off the curve where ML is the height left of the midpoint, TP is the peak time, MP is the height at the midpoint, MR is the height to the right of the midpoint and Tx is the height at 6 min.

The peak time (TP) indicates the time it takes for the dough to reach its optimal consistency and the height of the peak (MP) demonstrates the strength of the flour (Rasper & Walker, 2000). All information to the right of the midline peak value indicates the mixing tolerance of the dough.

The Mixograph is a quicker method than the Farinograph as it mixes more roughly and each sample only needs to be run once to ensure the correct amount of water is added. It is, however, harder to standardise the Mixograph compared to the Farinograph, as it has more individual components which each need to be standardised (Rasper & Walker, 2000).

2.5.2.2. Waxy wheat and the Farinograph and Mixograph

As both the Farinograph and the Mixograph provide a lot of information about the development of dough, most researchers only pick a few parameters to assist them in determining the characteristics of flour.

The three main parameters selected for analyses for the Farinograph are water absorption, the arrival time and stability time. The water absorption of the waxy wheats has been found to be higher than that of the non-waxy wheat (Guo *et al.*, 2003; Takata *et al.*, 2005; Blake *et al.*, 2015; Caramanico *et al.*, 2017) (Table 2.5.1). This is because waxy wheats have more amylopectin than the non-waxy wheat and amylopectin is considered to be the component most responsible for water absorption (Tester & Morrison, 1990; Zhang *et al.*, 2014).

It is the development time where a difference in results can be seen (Table 2.5.1). Morita *et al.* (2002) and Takata *et al.* (2005), found that waxy wheats had a longer development time than non-waxy wheats. This differs from many other researchers who found that waxy wheat doughs developed much faster (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Zhang *et al.*, 2014; Blake *et al.*, 2015). The two factors which affect the arrival time are the protein content and the starch composition i.e. amylose: amylopectin ratios. When studying waxy wheats, the protein content should not be significantly different between samples so that only the effect of the change in starch composition is observed in the results. According to in Morita *et al.*, (2002), the protein contents between the non-waxy and the waxy wheats were significantly different, with the waxy wheat protein being higher. It is possible that due to this discrepancy, the waxy wheat took longer to develop to its optimal consistency, as it was a stronger flour. In contrast, this cannot be said of Takata *et al.*, (2005) who was working with near isogenic lines, so it is possible that the full waxy genetics were not being expressed.

The stability time and the mixing tolerance index provide similar information regarding the strength of the dough. The stability time of the waxy wheat was lower than that of non-waxy wheat (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Morita *et al.*, 2002a; Takata *et al.*, 2005; Zhang *et al.*, 2014; Blake *et al.*, 2015). This indicates that waxy wheat creates a weaker dough, is very sensitive to overmixing and will quickly lose its optimal viscosity. The mixing tolerance index was found to be larger (Abdel-Aal *et al.*, 2002; Morita *et al.*, 2002; Takata *et al.*, 2005; Blake *et al.*, 2015), indicating that the dough lost resistance to the paddle more as time went on, than the non-waxy wheats. This again emphasizes that waxy wheat has a weaker dough and breaks down much faster with continued mixing.

Table 2.5.1 Summary of Farinograph results from waxy wheat studies

Farinogram property	Result of waxy wheat	Result of non-waxy wheat	Reference	General remarks
Water absorption (%)	79.5	59.5 – 59.7	(Guo <i>et al.</i> , 2003)	Waxy wheats had a higher percentage of water absorption than non-waxy wheats.
	72.1	63.0	(Takata <i>et al.</i> , 2005)	
	79.3	65.8	(Morita <i>et al.</i> , 2002a)	
	87.0	66.0	(Van Hung <i>et al.</i> , 2007)	
	68.4	66.2	(Blake <i>et al.</i> , 2015)	
	64.7	58.2 – 62.5	(Qin <i>et al.</i> , 2009)	
	69.5 – 70.6	52.5	(Caramanico <i>et al.</i> , 2017)	
	76	60 - 67	(Abdel-Aal <i>et al.</i> , 2002)	
	77.0 – 77.7	66.8	(Bhattacharya <i>et al.</i> , 2002)	
	68.7	58.7	(Zhang <i>et al.</i> , 2014)	
Water absorption (g/kg)	756	669	(Niu <i>et al.</i> , 2017)	
Development time (min)	5.0	4.0 – 7.0	(Guo <i>et al.</i> , 2003)	With one or two exceptions, waxy wheats had a faster dough development time non-waxy wheats.
	9.9	6.1	(Takata <i>et al.</i> , 2005)	
	3.70	2.50	(Morita <i>et al.</i> , 2002a)	
	5.0	17.0	(Van Hung <i>et al.</i> , 2007)	

	2.7	7.5	(Blake <i>et al.</i> , 2015)	
	4.5 – 4.8	25.8	(Bhattacharya <i>et al.</i> , 2002)	
	5.0	4.8- 11.7	(Qin <i>et al.</i> , 2009)	
	1.5	2.1	(Zhang <i>et al.</i> , 2014)	
	2.6 – 2.9	1.8	(Caramanico <i>et al.</i> , 2017)	
Stability time (min)	2.1	17.8 – 52.5	(Guo <i>et al.</i> , 2003)	Waxy wheats remained at optimal dough consistency for a much shorter time than non-waxy wheats.
	4.9	12.8 – 25.4	(Qin <i>et al.</i> , 2009)	
	5.83	7.33	(Morita <i>et al.</i> , 2002a)	
	8.1	18.0	(Takata <i>et al.</i> , 2005)	
	3.5	17.0	(Van Hung <i>et al.</i> , 2007)	
	3.7	8.3	(Niu <i>et al.</i> , 2017)	
	2.2 – 2.4	4.3	(Caramanico <i>et al.</i> , 2017)	
	1.2	2.0 – 9.4	(Abdel-Aal <i>et al.</i> , 2002)	
	3.0 – 3.3	29.8	(Bhattacharya <i>et al.</i> , 2002)	
	1.4	2.7	(Zhang <i>et al.</i> , 2014)	
Stability (cm)	2.4	>9.5	(Blake <i>et al.</i> , 2015)	

Weakness (BU)	39.0	12.0 – 18.0	(Qin <i>et al.</i> , 2009)	With one exception, the weakness of the waxy wheats. was much larger than that of the non-waxy wheats.
	150	30 - 120	(Abdel-Aal <i>et al.</i> , 2002)	
	72	33	(Takata <i>et al.</i> , 2005)	
	103.8	68.8	(Morita <i>et al.</i> , 2002a)	
	140	30	(Van Hung <i>et al.</i> , 2007)	
	85	20	(Blake <i>et al.</i> , 2015)	
	96.2	131.4	(Zhang <i>et al.</i> , 2014)	
	136-206	63	(Caramanico <i>et al.</i> , 2017)	

Table 2.5.2 Summary of Mixograph results from waxy wheat studies

Mixogram property	Result of waxy wheat	Result of non-waxy wheat	Reference	General remarks
Water absorption (%)	67.0	63.0	(Guo <i>et al.</i> , 2003)	Waxy wheats absorbed more water than non-waxy wheats.
Peak time (min)	2.0	4.3 – 5.7	(Guo <i>et al.</i> , 2003)	Waxy wheats reached optimal dough consistency faster than non-waxy wheats.
	4.2	4.1	(Takata <i>et al.</i> , 2005)	
	5.4	10.5	(Graybosch <i>et al.</i> , 2016)	
	1.8 – 2.1	4.2	(Jung <i>et al.</i> , 2015)	

	1.4	1.8 – 2.3	(Abdel-Aal <i>et al.</i> , 2002)	
	3.6 – 4.7	3.7 – 4.0	(Jonnala <i>et al.</i> , 2010)	
Peak height (%)	38.1	39.4	(Takata <i>et al.</i> , 2005)	Inconclusive effect of waxy wheat on the peak height compared to non-waxy wheat.
	59.9	50.4 – 61.7	(Abdel-Aal <i>et al.</i> , 2002)	
Peak band width (%)	22.5	27.3	(Takata <i>et al.</i> , 2005)	A lower peak band width seen for waxy wheats compared to non-waxy wheats.
	14.4	9.4 – 22.7	(Abdel-Aal <i>et al.</i> , 2002)	
Envelope area	33.9	48.8	(Takata <i>et al.</i> , 2005)	Inconclusive effect of waxy wheat on the peak height compared to non-waxy wheat.
	313	271 - 374	(Abdel-Aal <i>et al.</i> , 2002)	
Mixing tolerance (mm)	12.7	9.5	(Graybosch <i>et al.</i> , 2016)	Inconclusive effect of waxy wheat on the peak height compared to non-waxy wheat.
	12.7 – 23.0	17.0	(Jung <i>et al.</i> , 2015)	

From the large variety of different results acquired from the Mixograph, the most common information used by researchers are peak time (TP), peak height (MP) and the height of the curve at specified time (Tx). The peak time results indicate the same results as the Farinogram in that this time was also shorter, again confirming the faster dough development time of waxy wheat (Abdel-Aal *et al.*, 2002; Guo *et al.*, 2003; Takata *et al.*, 2005; Guan *et al.*, 2009; Jung *et al.*, 2015; Graybosch *et al.*, 2016). The peak height, which shows the strength of the dough, has been found to be lower than that of non-waxy wheat flour (Abdel-Aal *et al.*, 2002; Takata *et al.*, 2005).

The height at a specified time is sometimes also referred to as the Mixograph tolerance and recorded in millimetres (Graybosch *et al.*, 2016). This time can vary between 6 and 8 min and as long as this is kept constant within a study this poses no problems. It does, however, mean that this result cannot be compared to other researchers' results. This measure is normally found to be larger in waxy wheat, showing that the dough has less tolerance to overmixing (Abdel-Aal *et al.*, 2002; Jung *et al.*, 2015; Graybosch *et al.*, 2016).

2.5.3. Alveograph

2.5.3.1. Principles of the Alveograph

The Alveograph is one of the best rheology tests used to imitate the conditions which the dough will be subjected to during processing and is used primarily to test the gluten strength of the dough (Mirsaeedghazi *et al.*, 2008). The Alveograph blows air into dough, which has been moulded, to create a bubble (Hoseney & Delcour, 2010). The pressure inside the bubble is recorded as a function of the time in which the bubble remains inflated before it ruptures (Rasper & Walker, 2000). This biaxial extension of the dough mirrors the same physical conditions which a gas cell within the dough will be experiencing during fermentation and oven rise (Rasper & Walker, 2000; Dobraszczyk, 2003).

The Alveogram (Figure 2.5.3) created gives substantial information as to how dough will react during fermentation and gas production. The variables are simply annotated as P, L, P/L, and W, where P is the over pressure, L is the length of the curve, P/L is the curve configuration ratio and W is the deformation energy (Agyare *et al.*, 2005). P indicates the dough's ability to resist deformation, whereas L shows the extent of the extensibility of the dough (CHOPIN Technologies, 2014). The W value is derived from the area under the curve (S) and gives information about the strength of the dough as it is a measure of the work involved to blow the bubble (Faridi & Rasper, 1987; Hajslova & Alldrick, 2003.) P/L shows the balance between the strength and the extensibility of the dough and gives an indication of what the shape of the Alveogram curve will be (Faridi & Rasper, 1987).

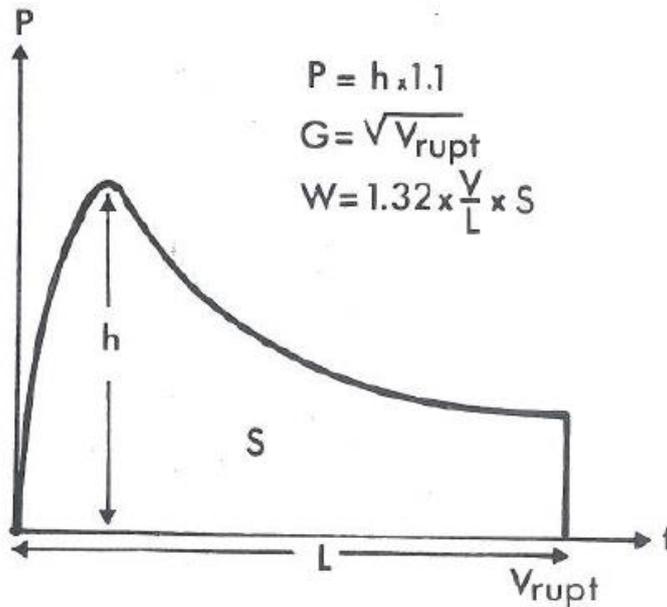


Figure 2.5.3 An Alveogram created by the Alveograph showing the measurements procured from it (Faridi & Rasper, 1987).

2.5.3.2. Waxy wheat and the Alveograph and Extensograph

The Alveograph has yet to be used to predict the baking properties of waxy wheat. The Extensograph has been used infrequently but it does not give as clear information on how the dough will react due to gas expansion. This is due to the fact that the Extensograph is only stretched in one direction (uniaxially) compared to the biaxial extension of the Alveograph (Faridi & Rasper, 1987). The biaxial extension is a more accurate prediction of how a gas bubble will expand as the dough rises and thus gives a better idea of how the dough will behave during processing. The Extensograph operates by placing a hook into a piece of dough and stretching it until it breaks (Hoseney & Delcour, 2010). A curve is created which measures the resistance (EU) of the dough to the distance (cm) to which it has been stretched.

Zhang *et al.* (2014) did a small amount of research on waxy wheat using the Extensograph. They found that the area under the curve produced by the Extensograph was larger for waxy wheat than for non-waxy wheat. However the waxy wheat and the non-waxy wheat were both deemed to have good baking properties as their areas were greater than 50 cm², which is the recommended threshold for good baking properties (Zhang *et al.*, 2014). The resistance/extensibility (R/E) ratio of the waxy wheat dough was found to be lower than that of non-waxy wheat (Zhang *et al.*, 2014). This is beneficial as a higher R/E ratio results in a dough that cannot expand sufficiently and will result in a poor loaf quality. The addition of waxy wheat could therefore improve the baking potential of non-waxy wheat (Zhang *et al.*, 2014).

The results from Zhang *et al.* (2014) can aid future researchers by giving them an idea of results which the Alveograph could produce for waxy wheat dough. Further research on waxy wheats using

the Alveograph should be conducted in order to aid in the prediction of bread making qualities in a commercial setting.

2.6. Bread loaf testing

2.6.1. Crumb structure and loaf volume

2.6.1.1. Principles of crumb structure formation

A basic bread recipe consists of four main ingredients namely flour, water, yeast and salt (Scanlon & Zghal, 2001). A dough is formed from these ingredients and is left to ferment. During fermentation, the yeast reacts with the glucose molecules found in the flour and produces carbon dioxide (CO₂) and ethanol (Hoseney & Delcour, 2010). Both these fermentation by-products are made in the liquid phase of the dough and diffuse into nuclei present in the dough to form gas cells (Scanlon & Zghal, 2001; Hoseney & Delcour, 2010). The nuclei are created during kneading and mixing, at which point small amounts of air are incorporated into the dough (Hoseney & Delcour, 2010).

After the initial fermentation, the dough is punched down and the gas cells are evenly distributed throughout the dough (Scanlon & Zghal, 2001). The dough is then left to prove for a second time and it is during this process that the final crumb structure of the dough is defined (Scanlon & Zghal, 2001). During baking, the crumb structure is set as the chemical components undergo thermal transitions. The gas cells expand to determine the aeration and crumb structure found in bread (Dobraszczyk & Morgenstern, 2003); the starch gelatinises and the proteins aggregate (Scanlon & Zghal, 2001). Both the water and the alcohol evaporate, leaving a bread which was once a moist dough but is now a solid - yet soft - foodstuff.

2.6.1.2. The C-Cell

C-Cell digital image analysis was developed by Calibre Control international (Warrington, UK) to replace the many imaging techniques used by researchers and industry to evaluate the crumb structure of bread. While some researchers were opting for the route of photographing or photocopying the sliced bread and then visually inspecting them (Lee *et al.*, 2001; Morita *et al.*, 2002b; Hayakawa *et al.*, 2004), others were developing their own in-laboratory software to analyse the pictures taken (Sapirstein *et al.*, 1994; Zghal *et al.*, 1999). This led to many inconsistencies in analysis as it was often neither objective nor consistent. The use of C-Cell provides quantitative information about the crumb structure and standardises the analysis of the crumb across all researchers and industries (Whitworth *et al.*, 2005).

Bread slice images are captured by placing them in an imaging cabinet which has a black background and is void of all natural light (Whitworth *et al.*, 2005). An optical system then illuminates the slices from two sides at a shallow angle (Whitworth *et al.*, 2005). Specifically designed software is then used to analyse the crumb structure in terms of the slice dimensions and shape, brightness and cell structure (Whitworth *et al.*, 2005). Cell area, size and elongation are measured and

analysed. Cells which are significantly larger than the average are deemed holes (Whitworth *et al.*, 2005). While it is difficult to define the exact parameters which describe a good loaf quality, a preferred sandwich loaf has a high loaf volume, a fine crumb structure and a bright slice (Cauvain, 2003). In essence, smaller cell size and area is preferred with a higher number of cells and a lower number of holes. The slice area should be higher indicating a larger loaf volume. The C-Cell also has the potential to measure the concavity of the sides of the loaf of bread which is an indication of loaf collapse, an unideal appearance in bread.

As C-Cell is a relatively new technique, there are only a few studies which have utilised it to analyse the crumb structure of waxy wheat loaves. One such study by Garimella Purna *et al.* (2011) observed the number of cells, cell volume, cell wall thickness and slice brightness of waxy wheat bread. It was seen that as the amount of waxy wheat flour increases, the number of the cells decreased and the volume of the cells increased. This indicates a more open crumb grain. The study also examined the rate and total carbon dioxide (CO₂) produced during fermentation with the use of a Risograph. These results indicated that the waxy wheat starch produced 100% more CO₂ than non-waxy wheat (Garimella Purna *et al.*, 2011). The greater amount of gas produced can be attributed to the more open crumb structure, as the original gas cells of waxy wheat dough will be larger. No significant difference was found between the crust thicknesses (Garimella Purna *et al.*, 2011). Similarly, Jonnala *et al.* (2010) found that waxy wheat breads were more porous than non-waxy wheat breads and had large gas cells, supporting the findings of Garimella Purna *et al.* (2011). Other studies which did not use the C-Cell but visual inspection, also found that as the amount of waxy wheat increased, the size of the gas cells did as well (Morita *et al.*, 2002a,b; Hayakawa *et al.*, 2004).

2.6.2. Bread staling

2.6.2.1. Principles of bread staling

The aroma of a fresh loaf of bread together with a crisp crust and moist, soft crumb is what makes bread appealing to consumers (Chinachoti, 2003). The process of staling leads to a loaf which lacks a signature aroma, has a tougher or firmer crumb and a soft crust (Schiraldi & Fessas, 2001; Chinachoti, 2003). Staling cannot be attributed to any single cause and is a combination of multiple physical and chemical changes (Fadda *et al.*, 2014). Much research is still being done to understand staling in its entirety and this encompasses factors from the ingredients used (flour, fats and shortening, enzymes) to the storage and processing conditions (Fadda *et al.*, 2014). An extensive review on staling has been completed by Fadda *et al.* (2014).

It has been determined that the firming of the crumb during staling can be correlated with the retrogradation of starch (Chinachoti, 2003). Retrogradation is defined as “changes that occur in gelatinised starch from an initially amorphous state to a more ordered or crystalline state” (Gudmundsson, 1994). It involves the re-association and crystallisation of amylose followed by the re-crystallisation of amylopectin (Ottenhof & Farhat, 2004). Immediately after gelatinisation, the

amylose molecules are found in random coil formations (Ottenhof & Farhat, 2004). They soon begin to re-associate with one another to form double helices and due to the linear nature of amylose, this happens rapidly (Ottenhof & Farhat, 2004). The amylose helices aggregate to become crystalline and this creates the firmness of the crumb structure (Ottenhof & Farhat, 2004; Hosenev & Delcour, 2010). Amylopectin, on the other hand, re-crystallises much more slowly due to its highly branched nature and is responsible for later stage firming on crumb structure (Ottenhof & Farhat, 2004; Hosenev & Delcour, 2010). This leads to products made from waxy wheat to retrograde, and thus stale, more slowly and results in an extension of shelf life.

2.6.2.2. Texture analysis

A texture analyser is used to measure the freshness and quality of bread (AACC, 1999d). It does this by measuring the force required to compress a slice of bread for a predetermined distance (AACC, 1999d). Dedicated software is then used to determine the firmness and the resilience of the bread. A better quality loaf has a lower firmness and a higher resilience which will indicate that it is still soft and moist and that not too much retrogradation has taken place (Botha, L., 2016, Technical Application Manager, Anchor Yeast, Johannesburg, South Africa, personal communication).

The texture analyser is used regularly to determine if waxy wheat increases the shelf life of bread by delaying retrogradation. The Stable Micro Systems Texture Analyser (TA.XT2) was used to determine the firmness of bread slices (Lee *et al.*, 2001; Bhattacharya *et al.*, 2002; Garimella Purna *et al.*, 2011). All found that the loaf with waxy wheat was initially significantly softer than that of non-waxy. Garimella Purna *et al.* (2011) reported no difference in firmness between the waxy and non-waxy loaves on day seven. This contradicts the other research which both found that the waxy wheat resulted in a softer crumb on day seven (Lee *et al.*, 2001; Bhattacharya *et al.*, 2002). This could be due to the use of different waxy wheat cultivars as well as non-waxy wheats (Garimella Purna *et al.*, 2011).

Other instruments such as the rheometer and the uniaxial stress strain testing machine (e.g. Instron) have also been used to analyse texture. Studies using the latter instruments give similar results to those which use the texture analyser. All results show that waxy wheat created a bread with a lower initial crumb softness (day one: $36.8 - 52.9 \text{ } 10^2 \text{ N/m}^2$) compared to the non-waxy wheat (day one: $86.6 - 277.0 \text{ } 10^2 \text{ N/m}^2$) (Morita *et al.*, 2002b; Van Hung *et al.*, 2007). Waxy wheats also had a softer crumb (day three: $150.2 - 356.4 \text{ } 10^2 \text{ N/m}^2$) compared to non-waxy wheats (day three: $153.2 - 798.5 \text{ } 10^2 \text{ N/m}^2$).

2.7. Waxy wheat bread blends

2.7.1. The keyhole effect

It is seldom that researchers will use 100% waxy wheat when testing for bread quality, as it is a well-documented fact that it does not create an ideal loaf. It has been observed that after 24 hours

the loaves shrink and can lose up to 25% of their volume (Ghiasi *et al.*, 1984; Graybosch, 2001; Garimella Purna *et al.*, 2011). This phenomenon has been termed the keyhole effect, as seen in Figure 2.7.1. Garimella Purna *et al.*(2011) have hypothesised as to why this occurs. During baking the protein and starch which make up the gas cell walls in the dough undergo thermal changes; the proteins begin to crosslink and the starch gelatinises (Garimella Purna *et al.*, 2011). This results in the cell walls rupturing and allowing the gas to escape from the crumb to the crust and creates a continuous - or open crumb - structure. It was observed by Garimella Purna *et al.*(2011) that in the Scanning Electron Microscope (SEM) images of the 100% waxy wheat, the starch granules were fused. This prevents the cell walls from rupturing and prevents a continuous crumb structure from forming, as the gas never leaves the gas cell. Subsequently, as the bread cools the cell walls begin to shrink due to a negative pressure which has been created. This results in the keyhole effect and is why most bread quality research on waxy wheat is done with blends of waxy wheat and non-waxy wheat.

2.7.2. Bread quality of waxy wheat flour blends

There is abundant research which has already been conducted to determine what level of additional waxy wheat flour creates a loaf of bread which still looks appealing to the consumer but which also has a longer shelf life due to the retardation of starch retrogradation.

Bhattacharya *et al.* (2002) used ratios of 10, 20 and 30% waxy wheat flour. They found that all loaves had a lower volume than the control. The crumb texture and grain of the 10 and 20% blends were not significantly different from the control but the crumb grain was found to be large and open for the 30% blend. The whiteness of the slices decreased as the amount of waxy wheat increased.

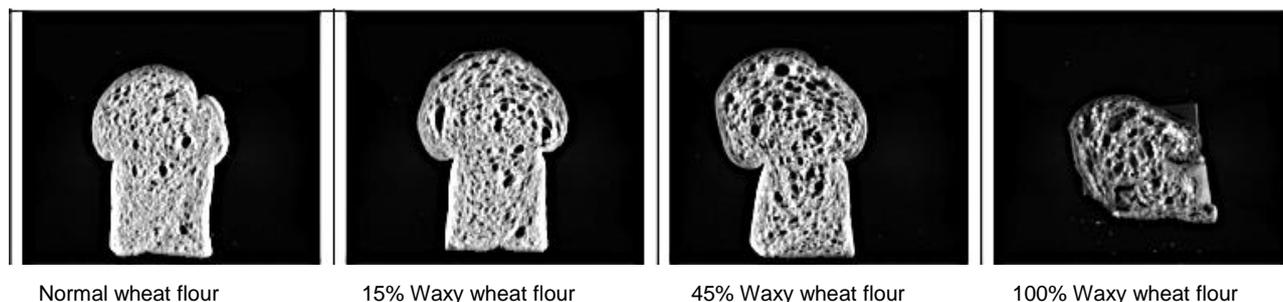


Figure 2.7.1 Illustration of the extent of the keyhole effect, with varying amounts of waxy wheat flour (Garimella Purna *et al.*, 2011)

The shelf life of the bread was determined by measuring the firmness of the bread on days zero, three and five. No differences were seen on day zero but on day three it was seen that 20 and 30% were significantly softer than the control and 10%. The same relationship between these blends were seen on day five but with an increase in firmness. The study concluded that the 20-30% blends

retarded staling but that 10% was too low to achieve this. A 30% blend has the most undesirable loaf appearance: it was recommended that a 20% blend would be the most practical for bread baking.

A study using blends of 20 and 40% was conducted by Morita *et al.* (2002b). The specific volume of the loaves for both blends was greater than that of the control and these results differ from those of Bhattacharya *et al.* (2002) who found blends to have a lower volume. While both studies used rapeseed displacement to determine volume, Bhattacharya *et al.* (2002) waited one hour before determining the volume. It is possible that these loaves had already begun to shrink slightly (keyhole) and thus were recorded as being smaller than the control. In terms of colour and crumb structure, the 20 and 40% blends were much more yellow and had a larger crumb structure than the control (Morita *et al.*, 2002b). The 40%, however, was more yellow than the 20% and the gas cells were very large. Both blends were found to be softer than the control on day seven of storage.

Further studies were conducted by Garimella Purna *et al.* (2011). Most findings were similar to those in previous studies (Bhattacharya *et al.*, 2002; Morita *et al.*, 2002b) in that as the percentage of waxy wheat increased, the crumb structure became more open and with larger cells and that on day one of storage, the blends were softer and had a higher volume than the control. This study, however, determined that on day seven there were no significant differences in firmness between the blends and the control and concluded that the addition of waxy wheat did not retard bread staling. This contradicts the above-mentioned studies (Bhattacharya *et al.*, 2002; Morita *et al.*, 2002b).

This vastly different conclusion could be as a result of myriad factors, one of which could be the large variation in chemical composition between all waxy wheat and non-waxy wheat cultivars: thus the use of different wheats in each study may have resulted in these differences. Another possible reason not considered by the authors, is that while the addition of waxy wheat retards staling, it does not prevent it. The study only measured firmness on days zero and seven and nothing in between, thus they may have not seen results of staling retardation on those days. It would be better to conclude that by day seven, effects of delayed staling are no longer seen. Other researchers who have studied the blending of waxy wheats, who have found similar results, include Hayakawa *et al.* (2004), Takata *et al.* (2005), Van Hung *et al.* (2007), Qin *et al.*, (2009), Jonnala *et al.* (2010) and Blake *et al.* (2015).

2.8. Purpose and benefits of waxy wheat

Waxy wheat is not widely used but it does have some commercial uses. Its high content of amylopectin alters its processing capabilities and this can be beneficial, particularly for Asian-style noodles, where the texture is the main contributor to the desired eating quality, which has been described as soft and elastic (Baik & Lee, 2003; Chibbar & Chakraborty, 2005). The starch component of the noodle plays the most important role in the texture and it is the swelling power of the starch which is responsible (Moss, 1980; McCormick *et al.*, 1991; Konik *et al.*, 1992; Wang & Seib, 1996; Sasaki & Matsuki, 1998). This swelling power is related to the amount of amylose present in the starch. The less amylose present, the higher the swelling power of the starch granules

(Sasaki & Matsuki, 1998). This is due to the fact that starch granule swelling is a property associated with amylopectin and that amylose acts merely as a dilutant (Tester & Morrison, 1990). Asian noodles such as the Udon noodles, also referred to as Japanese white salted noodles, benefit from the higher swelling power of waxy wheat. The result of the addition of waxy wheat flour to Asian noodles, is a noodle with a smooth, clean and shiny surface (Wang & Seib, 1996) and a soft and elastic texture (Baik & Lee, 2003) which is desirable to the target consumer.

The most frequent application for waxy wheats is in the baking industry. Waxy wheat starch is known to retrograde more slowly than non-waxy wheat and this leads to its use in increasing the shelf life of baked products (Graybosch, 1998; Maningat *et al.*, 2009). This benefits the bakers in that there would be less wastage due to stale bread which cannot to be sold (Chibbar & Chakraborty, 2005). Another way in which the extending of shelf life could be utilised is for a niche market where consumers are becoming increasingly concerned about what additives are being placed in the products they consume. The use of waxy wheat may allow bakers to reduce or eliminate the use of improvers and additives in the bread and thus would satisfy those consumers who are looking for a 'clean label' on the products they purchase. The slow retrogradation rate also increases the shelf life and organoleptic properties of baked goods which are stored in the fridge or freezer. As the demand for quick and ready prepared meals increases, the application of waxy wheats in this regard will also increase (Graybosch, 1998). Waxy wheat has also been used as a thickener for soups, sauces and gravies (Maningat *et al.*, 2009) and as a fat replacer (Graybosch, 1998). The use of waxy wheat as a fat replacer ensures that the product has a lower caloric content but maintains the same mouth feel (Guan *et al.*, 2009).

There is still much potential for waxy wheats' application on a commercial level and as people's food and meal needs change, so too will the frequency of waxy wheat usage, increase.

2.9. Conclusion

Waxy wheats show potential to be used to extend the shelf life of bread and improve the sensory properties of Asian-type noodles. The starch microstructure can effectively be seen and characterised using SEM for the granule morphology and XRD for the percentage crystallinity.

The use of RVA can successfully be used to determine the pasting properties of waxy wheat starch and shows definite differences between non-waxy and waxy wheats. The Farinograph and Mixograph both indicate that doughs made from waxy wheat reach an optimal consistency more rapidly and absorb more water, but are not as stable to overmixing as non-waxy wheat. The use of the Alveograph can provide new research opportunities for waxy wheat studies and provide important information on how the waxy wheat loaves' crumb structure will form. Finally, whilst the visual appearance of the final waxy wheat loaf is undesirable, it does increase the shelf life: using a blend of waxy and non-waxy wheats results in the best of both worlds.

Chapter 3

Materials and methods

3. Materials and Methods

3.1. Wheat samples

Four waxy wheats, namely lines 375, 376, 377 and 378, were kindly supplied by Sensako (Bethlehem, South Africa). Table 3.1 indicates the parents of each line used by Sensako to breed them. Each wheat sample was milled using a Bühler mill (Bühler Co., Uzwil, Switzerland). The waxy wheat lines were grown in completely randomised split plot designs during the 2014 and 2015 seasons. This resulted in four repeats of each line. Commercial Golden Cloud bread flour was used as a non-waxy wheat control and was measured in duplicate.

Table 3.1 Sensako SST wheat and waxy wheat parents of waxy wheat lines 375, 376, 377 and 378

Waxy wheat line	Sensako SST parent	Waxy parent
375	SST 399	BAIHUO_KANTO107_AC-MAJESTIC (N402-17)
376	SST 347	KY87C-42-8-5_Collin_ACMajestic_Kanto_107_Baihuo (N402-10)
377	SST 399	BAIHUO_KANTO107_AC-MAJESTIC (N402-17)
378	SST 356	Baihuo_Kanto_107_ACMajestic (N402-18)

3.1.1. Samples for starch characterisation

The starch from each wheat line and the control was isolated applying the method used by Zhang *et al.* (2013), with slight modifications. A smooth and homogenous dough was created by adding 120 mL of distilled water to 200 g of flour and leaving the mix to stand for 30 min. A further 500 mL of distilled water was added and each dough was scrubbed by hand to separate the starch and the gluten. The dough balls were rinsed with another 50 mL of distilled water before the slurries were passed, first through 180 µm and then 45 µm brass sieves. The filtrate was then centrifuged for 15 minutes at 3500 X g and the supernatant was discarded. After being washed twice with distilled water, the starch pellet was then suspended in a 2% sodium dodecyl sulphate (SDS) solution at a ratio of one part residue to two parts SDS for 2 h. The solution was stirred with a magnetic stirrer for the duration of the process. The solutions were once again centrifuged for 15 min at 3500 x g and the supernatant discarded. The pellet was washed twice with distilled water and then dried at 42°C for 48 hours. Once dried, the starch samples were milled with a hammer-type cyclone Laboratory Mill 3100 (Perten, Hägersten, Sweden) fitted with a 0.5 mm sieve. The samples were stored in an airtight container at room temperature until needed.

3.1.2. Samples for rheology and baking tests

The flours of each repeat of each wheat line were blended with the control in ratios of 10, 15, 20 and 25% waxy wheat to non-waxy wheat. Proximate analysis was completed on each blend using an Infratec 1421 (FOSS, Hilleroed, Denmark). The protein, moisture and ash contents were obtained.

3.2. Scanning Electron Microscope

The microstructure of both the whole kernels and the isolated starch of the samples were imaged using a Zeiss MERLIN SEM (Zeiss, Germany). The equipment used an accelerating voltage of 5 kV with a 250 pA current. The samples were placed on aluminium specimen stubs via double-sided carbon tape. These were then coated with a thin layer of palladium gold with a 5150A sputter-coater (HHV, Crawley, United Kingdom). The secondary electron images were then used to observe the starch granule morphology and the whole grain endosperm microstructure. At least three images of each sample were taken ranging from 350 X magnification to 1.50k X magnification. ImageJ 1.5j8 software (National Institute of Health, United States of America) was used to acquire a range of granule diameters of the A and B-type granules of each line of the isolated starch images. The diameters of the granules of the largest, smallest and intermediate size were measured for each image and a range determined.

3.3. X-Ray Diffraction

X-Ray Diffraction (XRD) was performed to calculate the percentage crystallinity of each of the starch samples and the control. A D8 Advance Bruker X-ray powder diffractometer (BRUKER AXS, Germany) was used, as described by Schoeman (2017). The instrument's X-ray tube had a rotating copper anode that produced Cu-K α radiation ($\lambda = 1.5406$) and functioned with a 10 mA current and a 30 kV accelerating voltage. The diffraction angle (2θ) was scanned over a region of 5 to 40° with an exposure time of 1285 s, a step size of 0.016° and measuring time of 0.5 s per point. Three replicates of each sample were scanned and the average of the three diffractograms was reported and used to determine the percentage crystallinity. EVA software (BRUKER AXS, Germany) was used to determine the crystalline and amorphous areas under the peaks. The amorphous area (area I) was considered from the base line of the Diffractogram to the tail-to-tail baseline of each peak; whereas the crystalline area (area II) was considered as the sum of all the peak areas from the tail-to-tail base line (Figure 3.1) (Hayakawa *et al.*, 1997). The crystallinity percentage was then calculated using the equation 3.3.1 (Yoo & Jane, 2002):

$$\% \text{ Crystallinity} = \frac{\text{area II}}{(\text{area II} + \text{area I})} \times 100 \quad \text{Equation 3.3.1}$$

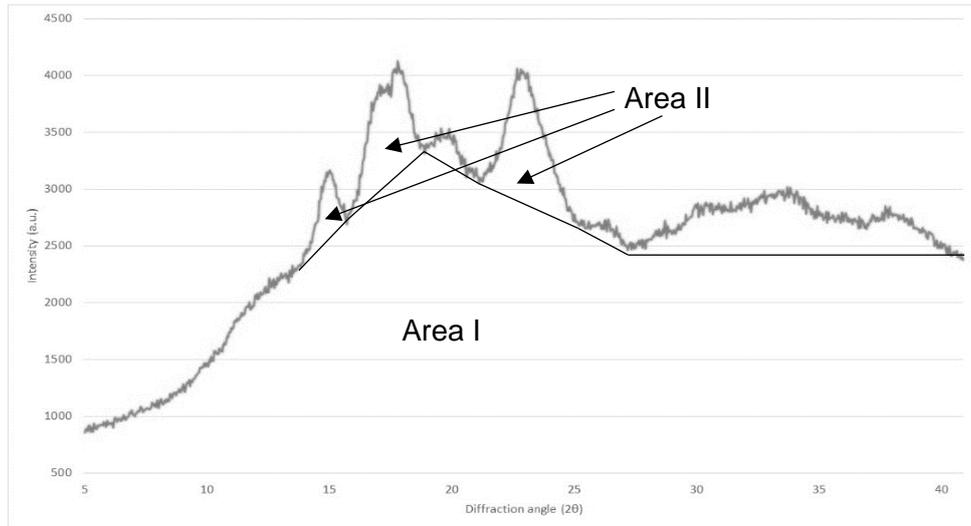


Figure 3.1 Illustration of amorphous (area I) and crystalline (area II) areas on a Diffractogram

3.4. Pasting properties

The pasting properties of each flour blend were determined using a Rapid Visco Analyser (RVA) (RVA-4, Newport Scientific, and Warriewood, Australia). A slightly modified version of the AACC 76-21.01 method (AACC, 1999a) was used and the first standard of heating and cooling cycles was selected, where the holding temperature was set to 91°C. Each flour line and blend was run in quadruplicate. Each sample's moisture content was used to determine the amount of flour added to a metal canister (ca. 3.5 g), and then 25 mL dH₂O was added to create a starch/water slurry. A plastic paddle was used to thoroughly mix the water and flour to prevent lumps, before the canister was placed into the RVA. The pre-set heating and cooling cycle then began while the paddle turned inside the canister, measuring the viscosity. The process stopped automatically after 13 min and the peak, breakdown, trough, set back and final viscosity, as well as the pasting temperature and peak time, were recorded by the ThermoLine for Windows™ (Version 3) software.

3.5. Dough Rheology

The dough rheology of the flour blends was analysed using the Farinograph, Mixograph and Alveograph. All flour blends were analysed in quadruplicate.

A Farinograph-E (Brabender GmbH & Co, Duisburg, Germany) was used to determine the arrival time (min), the water absorption and the dough stability. The AACC method 54-21.02 for constant flour weight (AACC, 1999b) was followed. Additionally, the weight of the flour was adjusted to 14% flour moisture basis (mb). Fifty grams (14% mb) of each blend was placed in a small mixing bowl and the volume of water of the expected water absorption of the sample was added via a burette.

The resistance to the dough formation was measured with two z-shaped blades in Brabender units (BU) and a Farinogram was formed. After approximately 9 min, a quick analysis of the curve was done to ensure that the 500 BU line was centred in the curve. If not, the sample was run again with a different amount of water, with adjustment dependent on whether the curve lay higher or lower than the 500 BU line. The new amount of water added was calculated under the assumption that each horizontal line (20 BU) was equivalent to 1.8 – 2.4 mL (0.6 – 0.8% absorption). The water absorption was calculated using the equation 3.5.1, where x is the mL of water added to create a centred curve and y is the mass of flour (g) equivalent to the corrected 50 g at 14% mb.

$$\text{Absorption \%} = 2(x + y - 50)$$

Equation 3.5.1

The mixing time was taken from the peak of the curve and the stability was recorded 5 minutes after this peak.

A 35 g Mixograph (National MFG Co, Lincoln, USA) was used to determine the dough's optimum mixing time (min) and mixing tolerance. The AACC 54-40.02 method (AACC, 1999c) was followed. Thirty five grams of each sample (14% mb) was placed into a mixing bowl and an amount of water determined by the following equation was added:

$$Y = 1.5X + 43.6$$

Equation 3.5.2

Where X is the protein content (14% mb) of the flour and Y is the percentage of water absorption. The torque resistance of the developing dough to the three rotating pins and four stationary pins attached to the bowl, was then measured. After 7 min, the dough was fully formed and the accompanying software was used to analyse the Mixograms created.

Finally, a Consistograph no 50.54 (Chopin, Villeneuve-la-Garenne, France) was used to analyse the bi-axial extension of the waxy wheat flour blend doughs. The AACC Alveograph 54-30.02 method was adhered to (AACC, 1999e). A 0.025 g/mL NaCl solution was added to 250 g of flour sample. The amount of NaCl solution added was determined by the moisture content of the sample. A dough was formed after 8 min of mixing and extruded from the mixing chamber onto a greased receiving plate. Three more pieces of dough were extruded and rolled 12 times, to a uniform height. All pieces were then cut into a circular shape using a specified cutter. The pieces were then placed into the Alveograph's resting compartment at 25°C. At 28 min after mixing commenced, the pieces were

placed in between two plates and air blown into the dough to create a bubble. Once the bubble popped, the dough was removed and the subsequent Alveogram was analysed. The curve gives information about the maximum over pressure (P), the average abscissa at rupture (L), the ratio of P/L and the deformation energy of the dough (W), which was calculated using equation 3.5.3, where V is the volume of air inside the bubble in mL, L is the average abscissa at rupture in mm and S is the area under the curve in cm²:

$$W = 1.32 \left(\frac{V}{L} \right) \times S$$

Equation 3.5.3

3.6. Bread baking

Bread loaves of each flour blend were baked at Anchor Yeast (Johannesburg, South Africa). Three loaves of each blend were baked and each blend was baked in quadruplicate. A control with and without a shelf life improver was also baked, each in duplicate.

A more complex formulation for the bread baking was used than for the rheology tests so as to mirror a commercial loaf more accurately (Table 3.2). The white bread control flour was the same commercial Golden Cloud flour used in the rheology testing.

A small scale version of the Chorleywood bread making method was used, as the short mixing time at a high speed best simulates the method used in South African commercial bakeries. Both dry and wet (62% water) ingredients were all placed in a spiral mixer and mixed for 2 min at a slow speed and then for a further 6 min at a faster speed. A window test was then completed to determine if the gluten had developed sufficiently. If not, the mixer was set again for 2 more minutes, or until a satisfactory window test was completed. A temperature of 28-30°C for the final dough was aimed for. The dough was then scaled into three 770 g pieces, rounded and left to rest for 10 min. The pieces of dough were then placed into the bread moulder and once moulded, placed in a bread pan. The pans were then placed into a prover at 40°C at 80% relative humidity for ca. 1 h or until the dough reached the top of the pan. The lid was slid onto the pans and they were baked at 230°C for 30 min. The loaves were then left to cool and placed into polyethylene bags and stored at room temperature.

3.6.1. Bread loaf quality

The quality of the loaves was analysed using a mono C-Cell digital image analyser (Calibre Control International, Warrington, UK). On day one, the bread was sliced using a Graef 182 Masters slicer (Graef GmbH & Co. KG, Arnsberg, Germany) to 12.5 mm. A slice was then placed in the dark drawer of the instrument and the image was taken. This was repeated until 10 slices of each loaf had been imaged. The accompanying C-Cell software then analysed the percentage concavity, slice

brightness, the number of cells and holes, the area of cells and holes (%) as well as the diameter of the cells (mm).

Table 3.2 Formulations used for controls and blends during bread baking

	Control		w/		Control		10% waxy		15% waxy		20% waxy		25% waxy	
	shelf	life	w/o	improver	flour	flour	flour	flour	flour	flour	flour	flour	flour	flour
Ingredients	%	g	%	g	%	g	%	g	%	g	%	g	%	g
White bread flour	100	200	10	200	90	180	85	170	80	160	75	150		
		0	0	0		0		0		0		0		0
Waxy Flour	-	-	-	-	10	200	15	300	20	400	25	500		
Water	62	124	62	124	62	124	62	124	62	124	62	124	62	124
		0		0		0		0		0		0		0
Bakers compressed yeast	2	40	2	40	2	40	2	40	2	40	2	40	2	40
Salt	2	40	2	40	2	40	2	40	2	40	2	40	2	40
Basic premix	1	20	1	20	1	20	1	20	1	20	1	20	1	20
Shelf life improver	0.28	5.76	-	-	-	-	-	-	-	-	-	-	-	-
	8													
Non-shelf life improver	-	-	0.1	2	0.	2	0.	2	0.	2	0.	2	0.	2
					1		1		1		1		1	

3.6.2. Shelf life testing

The extent of staling of the bread was analysed on days one, three and six using a TA.XT. plus Texture Analyser (Stable Micro System, Godalming, Surrey, United Kingdom). Two slices were placed on the instrument and the probe descended to 60% of the original sample height, remaining there for 2 seconds. This was repeated four times. The firmness was measured by the probe as the amount of force (g) required to compress the bread.

3.7. Statistical analysis

Statistical analysis was carried out using Statistica version 13.2.92.1 (StatSoft Inc., Tulsa, United States of America). One-way analysis of variance (ANOVA) was used to test for differences between the control and the waxy wheat samples. The repeated measures of bread firmness were analysed using a repeated measures ANOVA. The least significant difference test was used to evaluate the mean differences at the 5% significance level ($P \leq 0.05$). General linear models were used to determine the effect of the line and ratios at a confidence level of 95% ($P \leq 0.05$). Multivariate data analysis was also completed in the form of a correlation principal component analysis (PCA) biplot. This was done using XLstat version 19.4 (Addinsoft, United States of America).

Chapter 4

Results and Discussion

4. Results and discussion

4.1. Starch characterization

4.1.1. Scanning Electron Microscope

The Scanning Electron Microscope is used to show the microstructure of biological samples such as wheat. The starch granule morphology of the four lines and the control are seen in Figure 4.1 and Figure 4.2 respectively.

Figure 4.1 shows the isolated starch at 350X magnification. The control appeared to have a good balance between the A-type granules, which are large and disc shaped, and the B-type granules, which are small and spherical (Jane, 2009). Lines 375, 376 and 377, however, seemed to have less of the B-type and many more A-types. Line 378 looked unlike the other lines and more like the control which showed a more prominent frequency of the smaller B-type granules.

The range in size of the A-type granules across all the lines and the control did not differ greatly, although 377 did appear to have the largest range (Table 4.1). This sentiment rang true with the B-type granules as well. However it was now 376 which showed the greatest range. The ranges for all the lines were similar to those found by other researchers (Yoo & Jane, 2002; Kim *et al.*, 2003). There were no obvious differences in granule morphology between all the samples and this was in line with other research on waxy wheat starch morphology (Abdel-Aal *et al.*, 2002; Yoo & Jane, 2002; Kim *et al.*, 2003; Jung *et al.*, 2015).

Table 4.1 Range of granule size from starch isolate images from the Scanning Electron Microscope

Sample	A-Type Granules (μm)	B-Type Granules (μm)
Control	12-25	2-6
375	15-26	2-7
376	14-29	1-9
377	15-37	4-9
378	15-32	2-8

The surface of the granules and their shapes could be seen more clearly at a 1 500X magnification (Figure 4.2). With these images it became clear that not all of the protein was removed during the starch isolation and this also indicated the level of grain hardness (Morris, 2002). This is because of a protein called friabilin, or grain softness protein (GSP), which is more prominent in soft grains than hard grains (Greenwell & Schofield, 1986; Morris, 2002). This protein is also called the 'non-stick protein' as it aptly describes its function, where it impedes the association between the starch granule surface and the gluten. Friabilin binds to the starch granule via polar lipids but also binds to the gluten (Giroux and Morris, 1998; Morris, 2002)

The remains of the protein network could most clearly be seen for lines 375, 376 and 377, suggesting that they were harder grains than the control and line 378 (Figure 4.2). These three lines also

indicated more broken granules suggesting that they were more susceptible to starch damage during the milling process. The image showed that the surfaces of the granules had fewer pores and indentations than the control and line 378. These indents are generally formed as the endosperm of the kernel is developing and are, typically, impressions left by other starch granules (Wang *et al.*, 2015). Again lines 375, 376 and 377 had more of these indents, showing that the granules were more tightly packed in the endosperm.

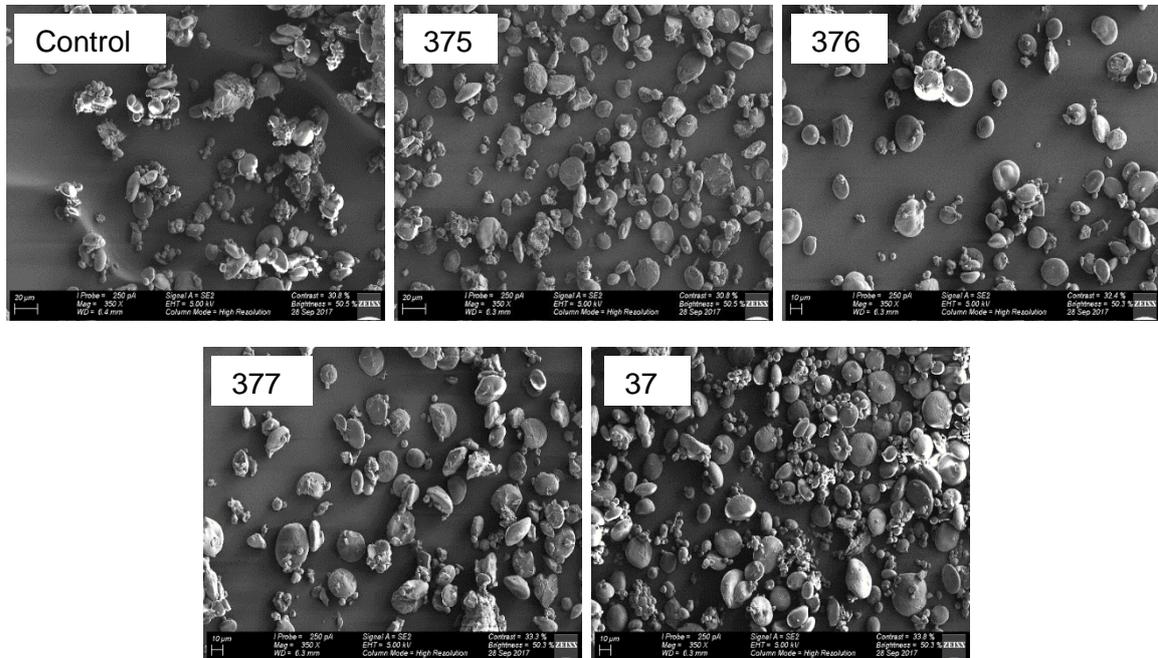


Figure 4.1 Images of starch isolates taken on the scanning electron microscope at 350 X magnification

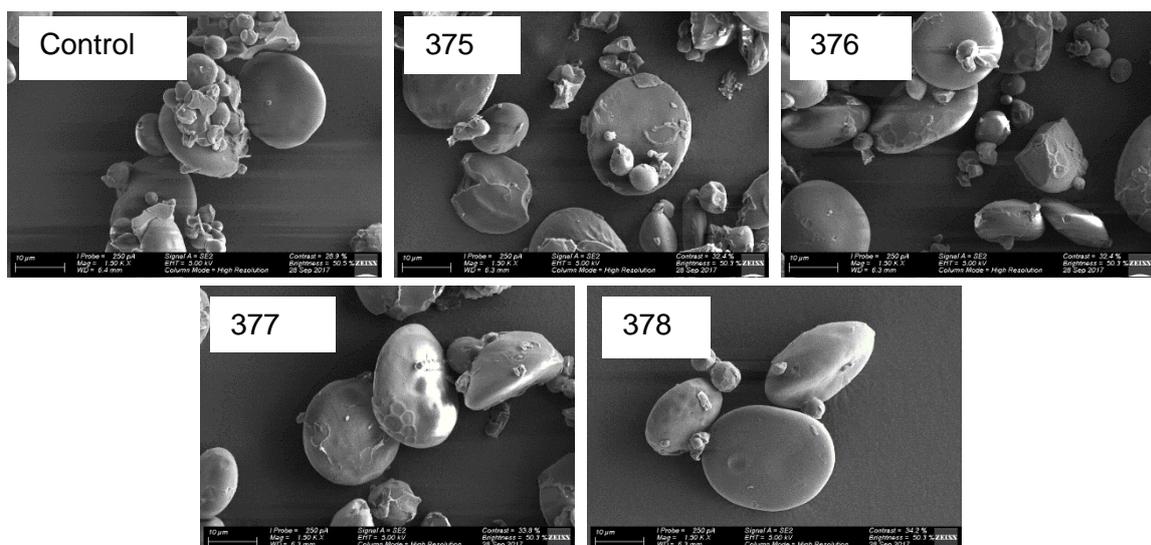


Figure 4.2 Images of starch isolates taken on the scanning electron microscope at 1500 X magnification

The tightness to which the starch is bound within the protein network in the endosperm of the kernel could be seen in images taken of the whole kernel (Figure 4.3). The image of the control showed more exposed starch granules than those of 375, 376 and 377. This indicated that during the preparation of the kernel for imaging, the protein cleanly parted from the starch granules, to expose them plainly. The protein of the other lines, however, did not part so easily from the starch granules and thus were not clearly visible. This is again the work of the non-stick protein, friabilin (Greenwell and Schofield, 1986).

Line 378 appeared to show the granules more clearly than the other waxy wheats but not as clearly as the control. More evidence of the hardness of the grains could also be seen in these images (Figure 4.3). Areas on the images, particularly 375, 376 and 377, showed parts of the endosperm comprising tightly packed starch granules in a continuous protein matrix; and no starch granules were visible. This is a clear indication of a hard grain and confirms what was seen in the images of isolated starch, which suggests that these three lines are harder than the control and 378.

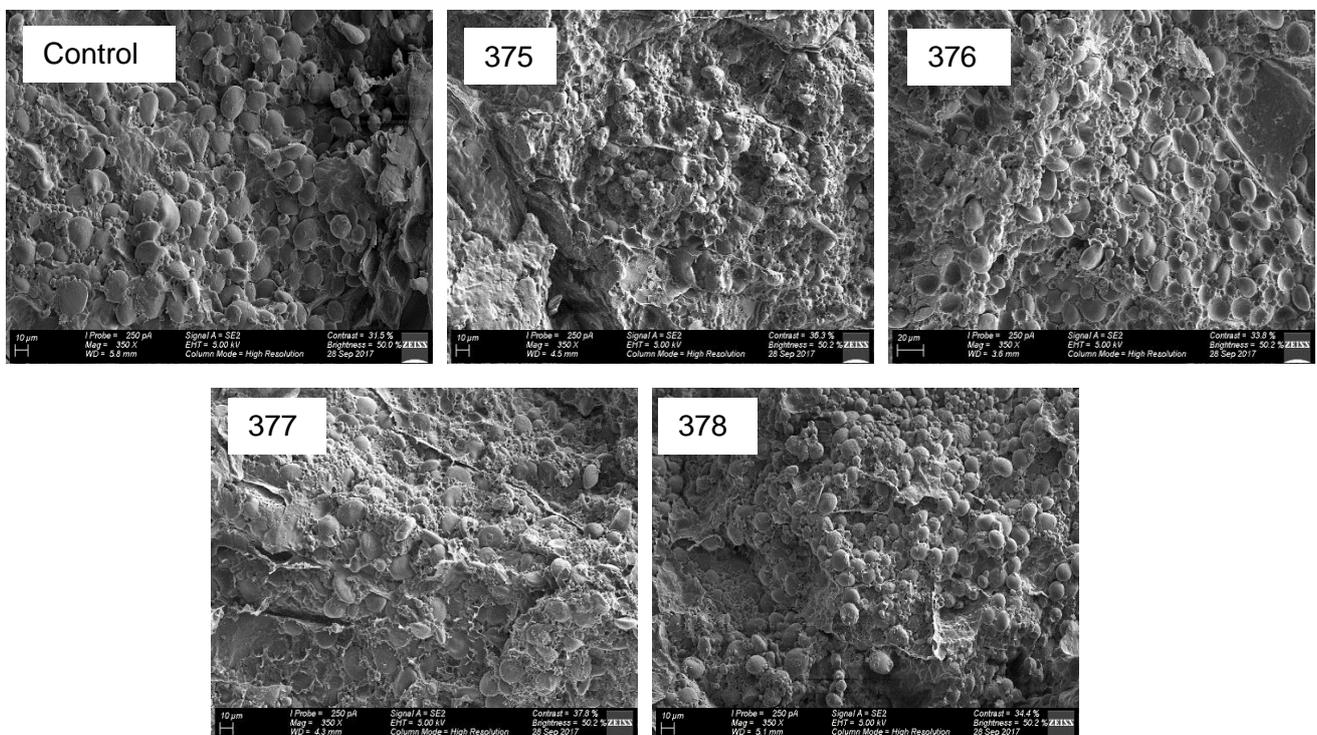


Figure 4.3 Images of whole kernels taken on the Scanning Electron Microscope at 350 X magnification.

4.1.2. X-Ray Diffraction

X-Ray Diffraction (XRD) is a useful technique in predicting the amylose content of a starch sample as well as the branching and length of the outer chains of the amylopectin (Xurun *et al.*, 2015). These starch fractions are represented as a percentage crystallinity. A starch sample with a higher

percentage crystallinity usually will have a higher amount of amylopectin due to its double helix nature (Ottenhof & Farhat, 2004).

All samples showed a typical A-type Diffractogram pattern, where single peaks were seen at $2\theta = 15^\circ$ and 23° while a doublet was seen at $2\theta = 17-18^\circ$ (Figure 4.4) (Shi & Seib, 1992; Xurun *et al.*, 2015). Another distinctive peak was seen at $2\theta = 23^\circ$, due to amylose-lipid compounds which form (Yoo & Jane, 2002). This peak is commonly the peak which differs between non-waxy wheats and waxy wheats because waxy wheats do not contain any amylose and thus cannot form these complexes. Figure 4.4 showed that lines 378, 377 and 375 had a less prominent amylose-lipid peak whereas 376 and the control had much higher peaks. It was not expected for 376 to have such a high intensity at this diffraction angle. Lines 377 and 375 showed what was most expected for a waxy wheat and the control and line 378 were the most similar.

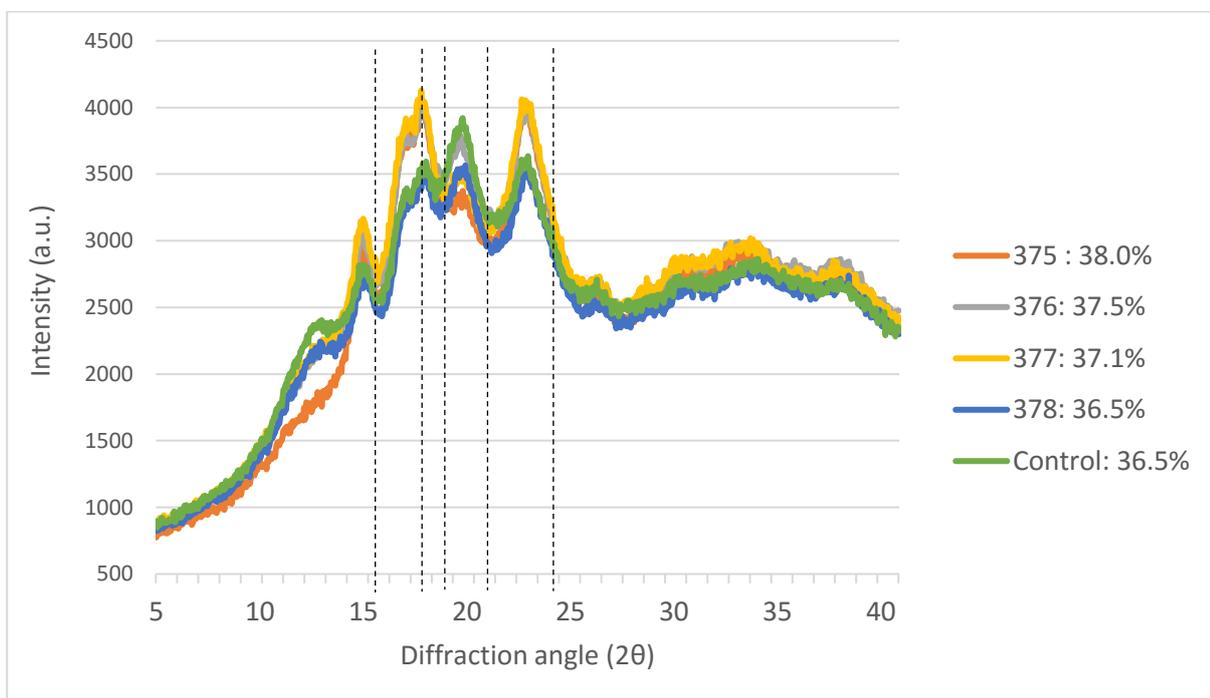


Figure 4.4 Diffractograms of each waxy wheat line and the control, as well as their percentage crystallinity

The percentage crystallinity for the control was not as was expected (Figure 4.4). It was very close in value to the waxy wheat samples which contain only amylopectin and was thus expected to have a much higher crystallinity. The control did not have similar intensities to non-waxy wheats in other studies (Figure 4.4) (Kim *et al.*, 2003; Zhang *et al.*, 2013; Wang *et al.*, 2015; Xurun *et al.*, 2015). Other studies show that the intensities for the peaks $2\theta = 17-18^\circ$ and 23° were much higher than the peak found at $2\theta = 20^\circ$. This exaggerated peak at $2\theta = 20^\circ$ may be a false impression of an increased percentage crystallinity. This peak represents the amylose-lipid complexes formed in the starch samples and while it is expected to be higher than the waxy wheats it was over pronounced in this study. It suggests that the particular sample of non-waxy wheat used for the control either has

a much higher amylose content than other wheats or more lipids, resulting in more amylose-lipid complexes.

The rest of the waxy wheat samples had a high crystallinity which were similar to one another as well as being on par with results of other waxy wheats found in other studies (Kim *et al.*, 2003; Zhang *et al.*, 2013; Wang *et al.*, 2015; Xurun *et al.*, 2015).

4.2. Starch and dough rheology

The falling numbers of blends 15, 20 and 25% of line 375 (328 – 350 s) and line 377 (327 – 352 s) were significantly lower ($P < 0.05$) than the control (378 s) (Table 4.2). Means from blend 376_10% (379 s) and blends 15, 20 and 25% from line 378 (378 – 382 s) were higher than the control. All other blends had lower means (327 – 374 s) than the control. The falling number is used to determine the amount of α -amylase activity and a lower value suggests more thereof (Graybosch *et al.*, 2000). Lines 375, 376 and 377 had lower values than the control which is expected for waxy wheats (Graybosch *et al.*, 2000; Garimella Purna, 2010) but those from line 378 were similar to the control. All blends had significantly higher ($P < 0.05$) moisture contents (12.68 – 13.48%) compared to that of the control (12.10 %) but all analyses took moisture content into account and adjusted accordingly, so it did not affect any of the results. The protein contents of all blends (11.85 – 12.48%) were higher than the control (11.60%), but not significantly so.

Table 4.2 Results from the proximate analysis of the falling number, protein and moisture contents

	Falling number (s)	Protein (%)	Moisture (%)
Control	378 ^a ±2.83	11.60±0.14	12.10 ^e ±0.28
375_10	362 ^{abc} ±17.41	11.95±0.17	12.68 ^{cd} ±0.21
375_15	350 ^{bcd} ±17.41	12.00±0.20	12.88 ^{abcd} ±0.21
375_20	343 ^{cde} ±16.63	12.10±0.20	13.03 ^{abc} ±0.25
375_25	328 ^e ±21.70	11.98±0.56	13.20 ^{abc} ±0.34
376_10	379 ^a ±13.20	12.03±0.29	13.00 ^{bcd} ±0.34
376_15	374 ^a ±17.17	12.18±0.32	13.00 ^{bcd} ±0.27
376_20	368 ^{ab} ±18.46	12.30±0.34	13.03 ^{abcd} ±0.25
376_25	366 ^{ab} ±18.39	12.45±0.30	13.05 ^{abcd} ±0.21
377_10	364 ^{ab} ±10.98	11.85±0.17	13.03 ^{abcd} ±0.55
377_15	352 ^{bcd} ±13.20	11.90±0.14	13.13 ^{abcd} ±0.68
377_20	334 ^{de} ±15.06	12.03±0.17	13.35 ^{ab} ±0.38
377_25	327 ^e ±17.63	12.08±0.13	13.48 ^a ±0.30
378_10	373 ^a ±4.12	12.08±0.32	13.20 ^{abc} ±0.28
378_15	378 ^a ±3.70	12.28±0.48	13.25 ^{abc} ±0.19
378_20	379 ^a ±6.99	12.40±0.48	13.30 ^{abc} ±0.26
378_25	382 ^a ±2.63	12.48±0.60	13.30 ^{abc} ±0.18

Sample names indicate line followed by the percentage which has been blended with the control

Values are means ± standard deviation of four replicates (n=4)c

4.2.1. Pasting properties

The heating and cooling program of the RVA which was imposed on the blends resulted in changes in viscosity. The initial heating led to the peak viscosity and peak time followed by a holding period where the temperature was maintained. This resulted in the trough viscosity. Subsequent cooling allowed starch to retrograde and viscosity increased, resulting in a final viscosity as well as the setback region, which is the difference between the trough and the final viscosity.

4.2.1.1. Peak Viscosity

All blends from lines 375 (943 – 1112 cP), 376 (1121 – 1239 cP) and 377 (955- 1055 cP) had significantly lower ($P < 0.05$) viscosities than the control (1410 cP) (Table 4.4). While the means of peak viscosity for blends 10, 15 and 25% of line 378 (1294 – 1326 cP) were not significantly lower than the control, the 20% blend (1287 cP) was ($P < 0.05$). Figure 4.5 shows how, as the amount of the waxy wheat flour in the blends increased for each line, the peak viscosity decreased. This was true for lines 375, 376 and 377. Line 378, however, did not follow this trend and instead the viscosity remained almost constant as the amount of waxy wheat flour increased. This is reiterated in Table 4.4 where line 378 had no significant differences between the four blends. The other three lines, however, did have significant differences ($P < 0.05$) between blends.

Hayakawa *et al.*, (1997) and Yasui *et al.*, (1999) likewise found that waxy wheats resulted in a lower peak viscosity but contradicting observations were found in other studies, which showed that waxy wheat gave an increased peak viscosity (Sasaki *et al.*, 2000; Abdel-Aal *et al.*, 2002; Morita *et al.*, 2002a; Yoo & Jane, 2002; Chakraborty *et al.*, 2004; Zhang *et al.*, 2013). The most prominent difference between the studies depended on whether an isolated starch or flour sample was used. Flour was used in studies where a lower peak viscosity was found (Hayakawa *et al.*, 1997; Yasui *et al.*, 1999) and isolated starch used where the viscosity was higher (Sasaki *et al.*, 2000; Abdel-Aal *et al.*, 2002; Yoo & Jane, 2002; Chakraborty *et al.*, 2004; Zhang *et al.*, 2013). These differences are due to the protein still present in the flour which competes with the starch, to absorb water (Caramanico *et al.*, 2017). As less water is absorbed by the starch, a lower peak viscosity is reached.

Another explanation for these differences in results could be due to wheat being a highly variable crop: bread wheat could have an amylose content of anywhere between 20-35% (Morita *et al.*, 2002a; Zhang *et al.*, 2013; Velisek, 2014). It is possible that the controls used in the respective studies did not have comparable amylose contents and thus gave differing pasting properties. While amylopectin is said to be the main cause of water absorption in starch (Tester & Morrison, 1990), amylose is required to make a continuous gel matrix (Hayakawa *et al.*, 1997) and so the lower peak viscosity may be a result of the inability of the waxy wheat starch to form a gel.

General linear models showed that the combined effect of the line and the blend was not significant on the peak viscosity (Table 4.3). The line and the blend did, however, have separate significant effects on the peak viscosity. When either line 375 or line 377 was added to a non-waxy

wheat, it resulted in a peak viscosity which did not differ significantly from the other. Further evidence to support this is that neither corresponding blend from the two lines differed significantly from one another i.e. 375_10 did not differ significantly from 377_10% (Figure 4.5). The addition of any amount of line 376 to a non-waxy wheat resulted in a significantly lower ($P<0.05$) peak viscosity than the addition of line 378. Table 4.3 shows that a 5% increase in the amount of waxy wheat added to a non-waxy wheat, did not have a significant effect on the peak viscosity. Only a 10% or greater increase lead to a significant decrease ($P<0.05$) in viscosity.

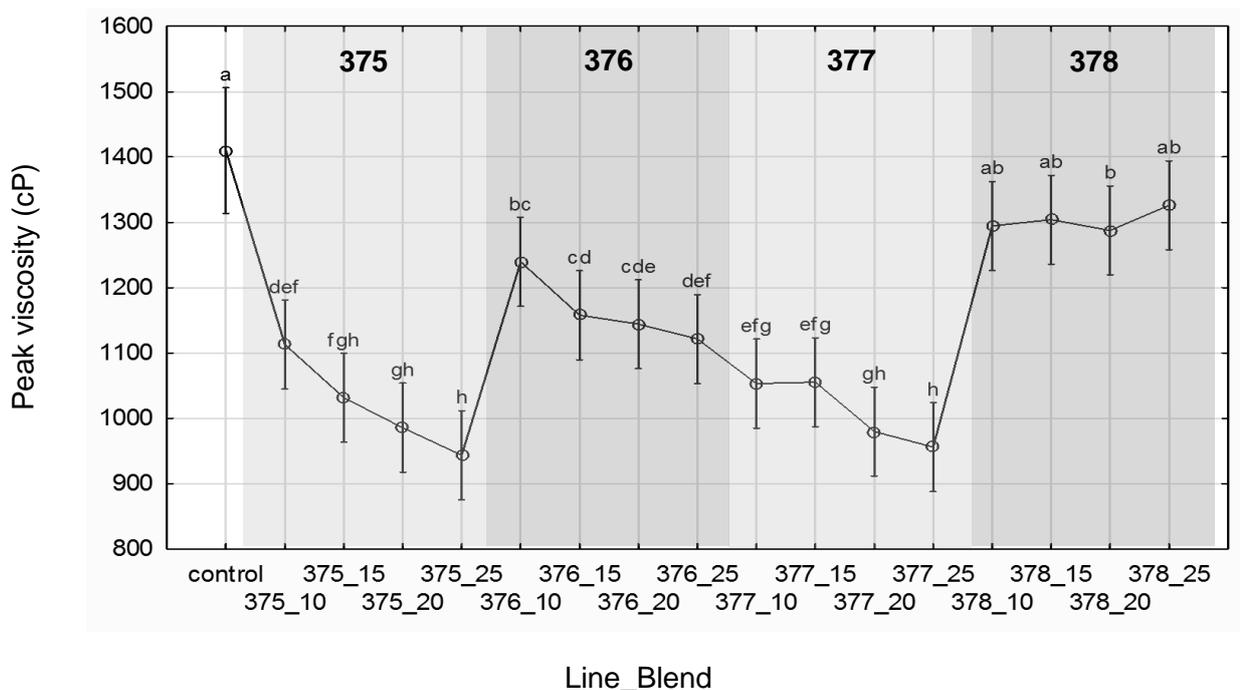


Figure 4.5 Means of peak viscosity of all lines and blends determined by the Rapid Visco Analyser. Different letters indicate significant differences ($P<0.05$) and sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control.

Table 4.3 General linear models of the effects of the line, blend and line*blend on the peak viscosity determined by the Rapid Visco Analyser

Effect	P-value	
Line	0	378 ^a
		376 ^b
		375 ^c
		377 ^c
Blends (%)	0.00231	10 ^a
		15 ^{ab}
		20 ^{bc}
		25 ^c
Line*Blends	0.27094	-

Effect is significant when $P<0.05$

Different letters in the same effect indicate significant differences ($P\leq 0.05$).

Table 4.4 Pasting properties of flour from all lines and blends as determined by the Rapid Visco Analyser

	Peak viscosity (cP)	Peak time (min)	Trough (cP)	Setback (cP)	Final viscosity (cP)
Control	1410 ^a ±72.12	6.93 ±0.00	1145 ^a ±2.83	340 ±104.65	1485 ^a ±107.48
375_10	1112 ^{def} ±28.09	6.82 ±0.14	865 ^{efg} ±81.20	294 ±103.04	1160 ^{defg} ±30.21
375_15	1031 ^{fgh} ±24.12	6.80 ±0.14	804 ^{fgh} ±58.51	269 ±97.56	1074 ^{efgh} ±38.66
375_20	985 ^{gh} ±37.97	6.85 ±0.08	770 ^{gh} ±56.12	260 ±98.81	1030 ^{fgh} ±57.88
375_25	943 ^h ±13.92	6.82 ±0.17	753 ^h ±42.31	245 ±62.38	998 ^h ±20.71
376_10	1239 ^{bc} ±54.84	6.90 ±0.09	979 ^{bcd} ±95.40	281 ±148.69	1260 ^{bcd} ±60.51
376_15	1157 ^{cd} ±59.32	6.87 ±0.12	913 ^{cde} ±57.43	286 ±120.93	1200 ^{cde} ±76.67
376_20	1144 ^{cde} ±34.61	6.87 ±0.09	917 ^{cde} ±57.95	283 ±103.27	1200 ^{cde} ±49.44
376_25	1121 ^{def} ±65.53	6.92 ±0.06	906 ^{def} ±32.04	261 ±50.17	1168 ^{defg} ±79.80
377_10	1053 ^{efg} ±123.58	6.92 ±0.08	803 ^{fgh} ±68.90	288 ±103.93	1091 ^{efgh} ±159.51
377_15	1055 ^{efg} ±63.63	6.92 ±0.06	802 ^{fgh} ±52.21	287 ±59.04	1089 ^{efgh} ±97.62
377_20	979 ^{gh} ±46.02	6.90 ±0.07	736 ^h ±56.77	285 ±66.75	1021 ^{fgh} ±104.24
377_25	955 ^h ±88.05	6.79 ±0.10	742 ^h ±74.31	261 ±78.47	1003 ^{gh} ±131.12
378_10	1294 ^{ab} ±85.20	6.90 ±0.12	1019 ^{abc} ±96.94	329 ±74.78	1349 ^{abc} ±171.57
378_15	1304 ^{ab} ±80.27	6.90 ±0.16	1031 ^{abc} ±87.23	322 ±79.57	1353 ^{abc} ±161.42
378_20	1287 ^b ±85.89	6.85 ±0.21	1004 ^{bcd} ±102.25	327 ±70.23	1332 ^{abc} ±169.51
378_25	1326 ^{ab} ±89.03	6.92 ±0.13	1039 ^{ab} ±130.02	321 ±27.09	1360 ^{ab} ±156.39

Sample names indicate line followed by the percentage which has been blended with the control

Values are means ± standard deviation of four replicates (n=4).

Different letters in the same column indicate significant differences (P≤0.05).

4.2.1.2. Peak Time

No significant increases or decreases could be seen for the peak times (6.79 – 6.93 min) (Table 4.4). This suggested that the addition of up 25% of any of the waxy wheat lines to non-waxy wheat, would have no significant effect on the peak time. However, Figure 4.6 shows that mean values from all blends did have a faster peak time than the control which is expected for waxy wheats. This is due to the rapid swelling caused by the amylopectin (Hayakawa *et al.*, 1997) which was also seen by Chakraborty *et al.* (2004) and Zhang *et al.* (2013). The peak time shows that waxy wheats gelatinise faster than non-waxy wheats. This information is important for bakers to predict the baking quality of flours, as well as allowing them to choose a flour with the quickest processing time. The 25% blend of line 377 resulted in the quickest peak time of 6.79 min.

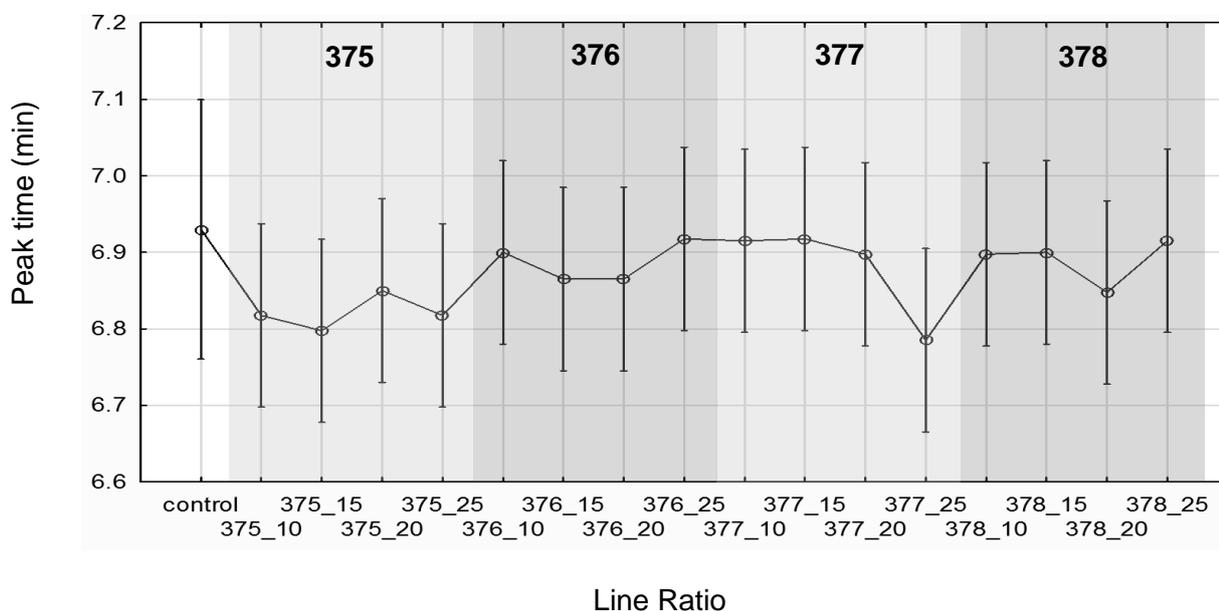


Figure 4.6 Means of peak time of all lines and blends determined by the Rapid Visco Analyser. Sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control.

4.2.1.3. Trough

The trough shows the minimum viscosity at the end of the holding period. All blends from line 375 (753 – 865 cP), 376 (906 – 979 cP) and 377 (736 – 803 cP) had significantly lower viscosities than the control (1145 cP) (Table 4.4). The mean values of the 10, 15 and 25% blends of line 378 (1019 – 1039 cP) had lower viscosities than the control but not significantly so. The 20% blend from this line, was significantly lower ($P < 0.05$) than the control and had a value of 1004.75 cP. Figure 4.7 illustrates that with lines 375, 376 and 377, as the amount of waxy wheat added to the non-waxy

wheat was increased, the trough viscosity decreased. Conversely line 378 showed little changes in viscosity as the amount of waxy wheat increased. This is furthermore demonstrated in Table 4.4 where no significant differences were seen between the blends of line 378 whereas differences were seen between blends of the other three lines ($P < 0.05$).

The low viscosity trough values can be explained due to starch's shear thinning properties (Lai *et al.*, 2000). This is due to the occurrence of starch granule breakdown, caused by mechanical stirring and is dependent on the chemical composition of the starch (Newport Scientific, 2010); thus a lower viscosity is probable for the waxy wheats compared to non-waxy wheats on account of their different starch compositions. The trough indicates how stable a starch is once it has gelatinised and how sensitive it could be to over-stirring. Thus results (Table 4.4) suggest that the blends of waxy wheat lines 375, 376 and 377 have an unstable starch gel and are susceptible to over-mixing and stirring. Line 378 would produce a starch gel comparable to the non-waxy wheat control. The 20% blend of line 377 would be the most susceptible to over-stirring with the lowest trough viscosity value of 736 cP.

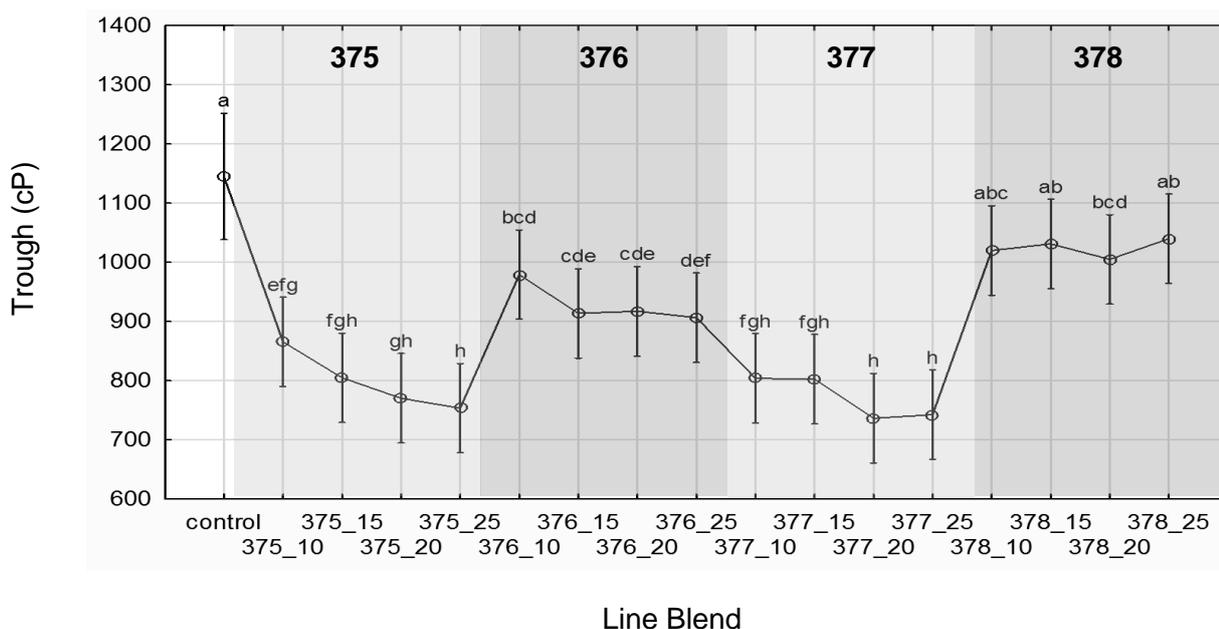


Figure 4.7 Means of trough of all lines and blends determined by the Rapid Visco Analyser. Different letters indicate significant differences ($P \leq 0.05$), and sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control

General Linear Models showed that it was only the waxy wheat lines which had a significant effect on the trough value and not the percentage thereof blended with the non-waxy wheat (Table 4.5). As with the peak viscosity, line 375 and 377 had the same effect of the viscosity of the trough. Line 378 would not decrease the viscosity as significantly as line 376.

Table 4.5 General linear models of the effects of the line, blend and line*blend on the trough viscosity determined by the Rapid Visco Analyser

Effect	P-value	
Line	0.000001	378 ^a 376 ^b 375 ^c 377 ^c
Blends (%)	0.105141	- -
Line*Blends	0.884541	- -

Effect is significant when $P < 0.05$

Different letters in the same effect indicate significant differences ($P \leq 0.05$).

4.2.1.4. Breakdown

Bakers use the breakdown value of the RVA to help determine how stable the flour is. The breakdown is the difference between the peak viscosity and the trough. Figure 4.8 shows that no significant differences were seen between the blends (190-286 cP) and the control (265 cP). It is clear, however, that as the amount of waxy wheat in the blend increased, the breakdown decreased. A lower breakdown value indicates a smaller difference between the peak viscosity and the trough. Other studies observed that waxy wheats had a larger breakdown value than a non-waxy wheat (Chakraborty *et al.*, 2004; Zhang *et al.*, 2013). Both these studies used isolated starch which resulted in waxy wheats having a higher peak viscosity; unlike this study which found that waxy wheats caused a lower peak viscosity (Figure 4.5). Consequently the difference between a higher peak viscosity and the trough value would result in a larger difference. The larger breakdown value shows a less stable starch gel which is sensitive to overmixing. As there are no significant differences in breakdown between the control and all of the blends, up to 25% of any of the waxy wheat lines could be added to a non-waxy wheat starch without having a significant effect on the breakdown.

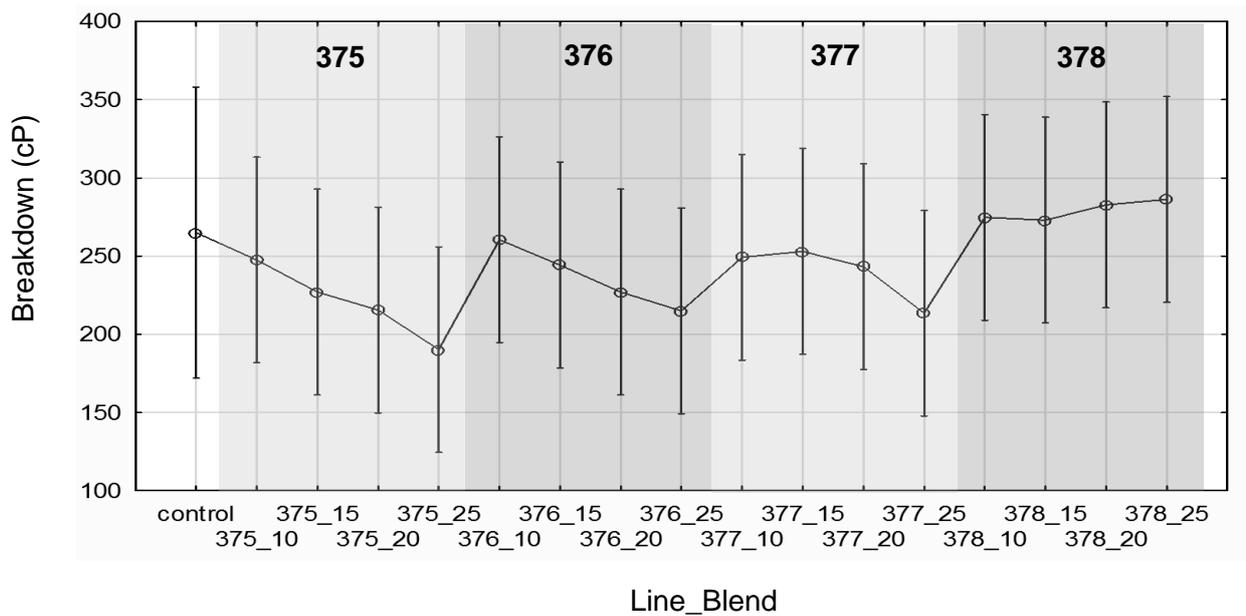


Figure 4.8 Means of breakdown of all lines and blends determined by the Rapid Visco Analyser. Sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control

4.2.1.5. Final Viscosity and setback

The final viscosity occurs after the cooling period and is normally higher than the trough. This is due to the starch retrogradation which takes place upon cooling. The amylose polymers begin to re-associate with one another and then crystallise, while the amylopectin simply re-crystallises (Ottenhof & Farhat, 2004; Newport Scientific, 2010): this causes the increase in viscosity. The setback region is the difference between the final viscosity and the trough viscosity.

No significant differences between the control (340 cP) and any of the blends (245 – 329 cP) were found for the setback region (Table 4.4) though the mean values of blends were lower than that of the control. Similar results from other studies also found the setback viscosity for waxy wheats to be lower than that of non-waxy wheat (Abdel-Aal *et al.*, 2002; Chakraborty *et al.*, 2004; Garimella Purna *et al.*, 2015; Wang *et al.*, 2015).

All blends from lines 375 (998 – 1160 cP), 376 (1168 – 1260 cP) and 377(1003 – 1091 cP) had significantly lower ($P < 0.05$) final viscosities than the control (1485 cP) (Table 4.4). Mean values of blends from line 378 were lower than the control but not significantly. Lower final viscosity values for waxy wheats compared to non-waxy wheats were also observed by Zhang *et al.* (2013). Figure 4.9 shows how, as the amount of waxy wheat added to the non-waxy wheat increases, the final viscosity decreases. This is true for all lines with the exception of line 378 where there was no significant decrease in viscosity between the blends.

The setback and the final viscosity were expected to be lower with the addition of waxy wheat, as due to the high content of amylopectin, retrogradation takes place more slowly, resulting in a lower viscosity. This gives an indication to bakers that bread baked from waxy wheats may have a softer crumb and stale slower, than bread from non-waxy wheat. The low viscosity values of the 25% blends from line 375 (998 cP) and line 377 (1003 cP) indicate that when these two blends are baked into bread, the starch will retrograde the most slowly and thus have the best potential to extend the shelf life of the loaf.

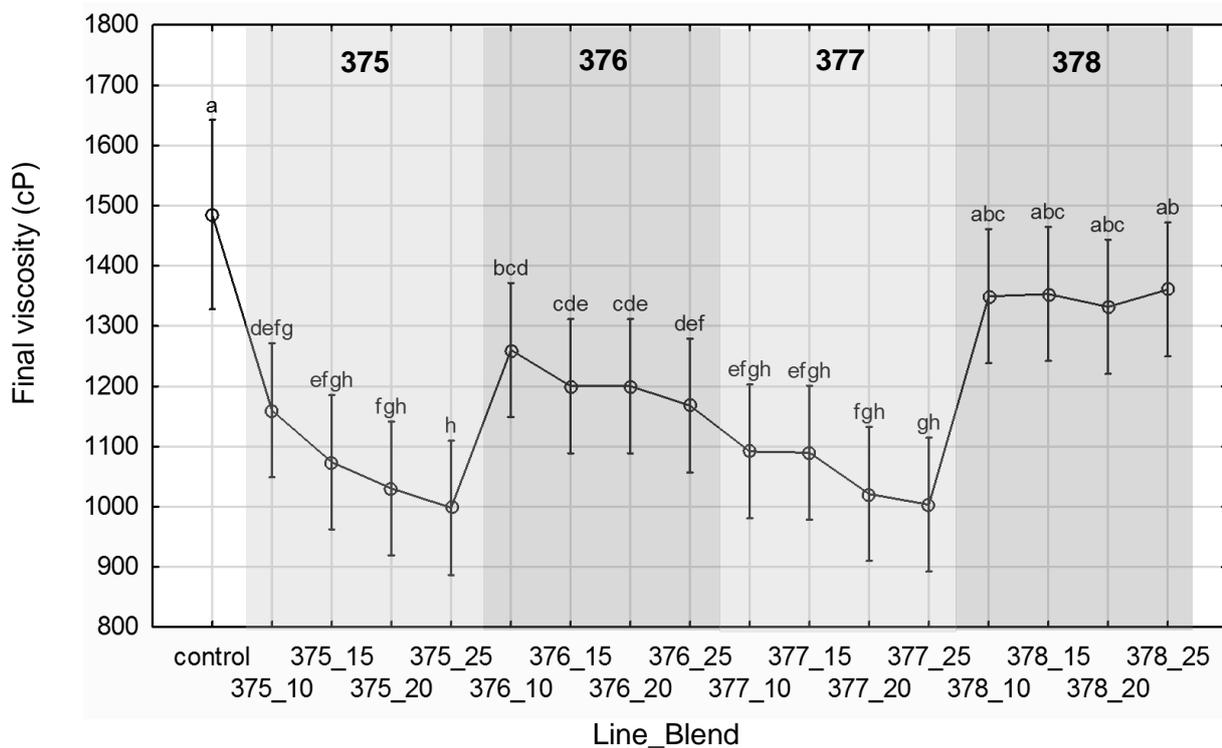


Figure 4.9 Means of final viscosity of all lines and blends determined by the Rapid Visco Analyser. Different letters indicate significant differences ($P \leq 0.05$) and sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control

The general linear models showed that only the waxy wheat lines had a significant effect on the final viscosity ($P < 0.05$) (Table 4.6). This means that any amount of each line (blends) could be added to a non-waxy wheat and it would not affect the final viscosity significantly. Likewise, no specific line and blend combination had a significant effect on decreasing the final viscosity. Lines 375 and 377 could be used interchangeably to give the same decrease in final viscosity ($P < 0.05$). Line 376 caused a more significant decrease ($P < 0.05$) in viscosity than line 378 but not as significant a decrease as lines 375 and 377.

Table 4.6 General linear models of the effects of the line, blend and line*blend on the final viscosity determined by the Rapid Visco Analyser

Effect	P-value	
Line	0.00001	378 ^a 376 ^b 375 ^c 377 ^c
Blends (%)	0.162442	- -
Line*Blends	0.954880	-

Effect is significant when $P < 0.05$

Different letters in the same effect row indicate significant differences ($P \leq 0.05$).

4.2.2. Farinograph

The Farinograph aids bakers in predicting the dough consistency of a particular flour, as well as how much water needs to be added to create a dough of optimal consistency. The dough consistency is important as it is linked to the final loaf quality.

The arrival time, water absorption and stability of the dough were analysed and it was found that for all three, no significant changes from the control were seen for all the lines and blends. This shows that the addition of up to 25% of any of the waxy wheat lines to non-waxy wheat will not significantly influence the development of a dough.

4.2.2.1. Arrival Time

The arrival time is the time it takes for the dough to develop to its optimum consistency and helps bakers decide how long dough should be kneaded for during processing. There were no significant differences between the lines blends and the control (5.5 min) (Table 4.7). Arrival time means of the 25% blend from line 375 (5.48 min) and the 15, 20 and 25% blends from line 376 (5.08 – 5.48 min) were all faster than the control. All arrival times of blends from lines 377 (5.90 – 6.28 min) and 378 (5.83 – 6.20 min) were longer than the control as well as blends 10, 15 and 20% from line 375 (5.65 – 5.78min) and 10% from line 376 (5.58 min). Morita *et al.* (2002a) and Takata *et al.* (2005) both found the arrival times for waxy wheats to be longer whereas other researchers found it to be shorter (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Zhang *et al.*, 2014; Blake *et al.*, 2015). The arrival time depends on the protein and starch contents in the flour. A longer time may be the result of a higher protein content which will make the dough stronger, resulting in more time to reach optimal consistency.

Table 4.2 shows that there were no significant differences in protein between the blends and the control and so it is the amylose: amylopectin ratios of each blend which influence the arrival time. As a faster mixing time has economic advantages, line 376 should be used, as it gave the quickest arrival time (Figure 4.10).

Table 4.7 Arrival time, water absorption and stability of doughs made from blends of lines of waxy wheats determined by the Farinograph

	Arrival time (min)	Water absorption (%)	Stability (min)
Control	5.50 ±0.00	62.98 ±1.03	8.60 ±0.42
375_10	5.78 ±0.38	62.84 ±0.81	8.93 ±0.63
375_15	5.65 ±0.17	60.43 ±5.42	8.83 ±0.74
375_20	5.68 ±0.19	63.16 ±1.03	8.68 ±0.78
375_25	5.48 ±0.25	63.33 ±1.06	8.45 ±0.90
376_10	5.58 ±0.67	62.50 ±0.80	9.13 ±1.65
376_15	5.48 ±0.68	62.63 ±0.72	8.70 ±1.31
376_20	5.38 ±0.42	62.94 ±0.51	7.95 ±1.13
376_25	5.08 ±0.51	63.15 ±0.53	8.03 ±1.90
377_10	5.93 ±0.79	62.28 ±1.23	9.48 ±1.28
377_15	5.90 ±0.45	62.48 ±1.34	9.13 ±1.43
377_20	5.98 ±0.92	62.66 ±1.25	8.88 ±2.17
377_25	6.28 ±1.50	62.45 ±1.39	9.13 ±2.90
378_10	5.95 ±0.61	61.61 ±1.03	10.10 ±1.50
378_15	5.83 ±0.49	61.68 ±0.76	10.13 ±1.73
378_20	6.15 ±0.49	61.53 ±0.72	10.48 ±0.93
378_25	6.20 ±0.47	61.43 ±0.88	10.65 ±1.25

Values are means ± standard deviation of four replicates (n=4).

Sample names indicate line followed by the percentage which has been blended with the control

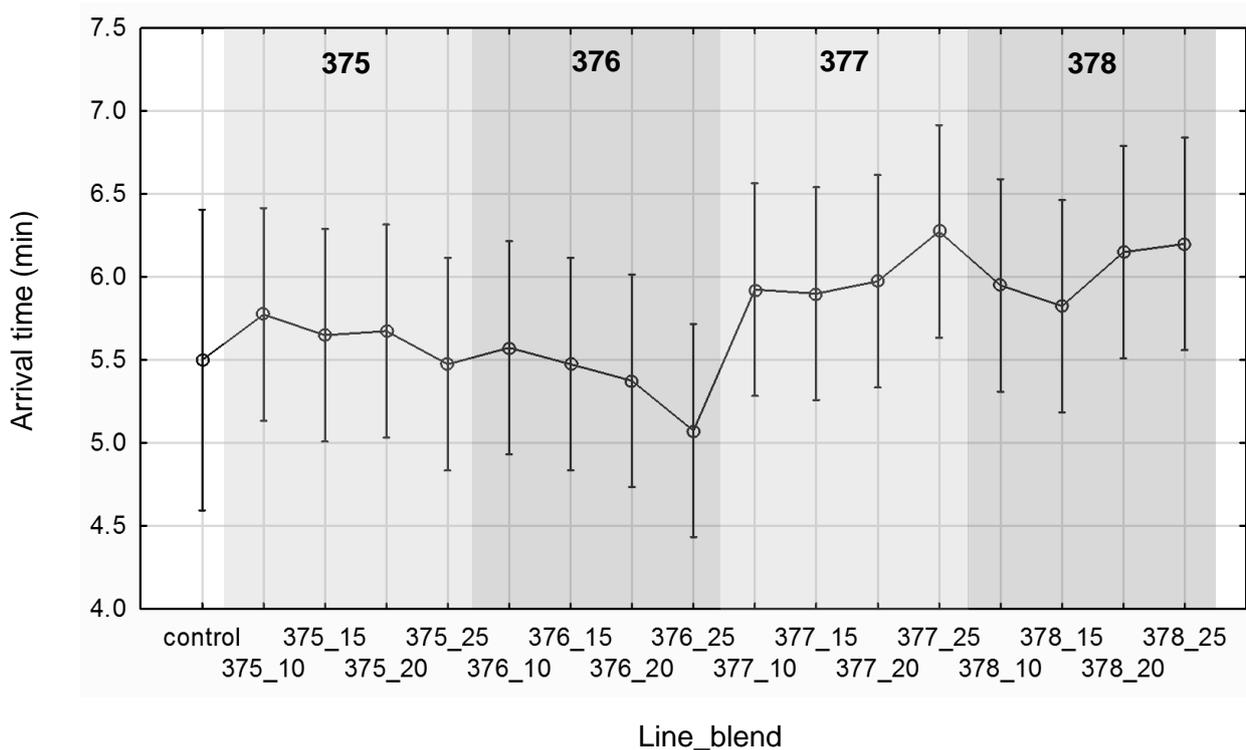


Figure 4.10 Means of arrival time of all lines and blends determined by the Farinograph. Sample names specify the waxy wheat line, followed by the percentage thereof which has been blended with the control

4.2.2.2. Water absorption

The water absorption indicates how much water needs to be added for a specific flour to create an optimal dough. Again, no significant differences were seen between the blends and control (62.98%) (Table 4.7). Lines 375 (60.43 – 63.33%), 376 (62.50 – 63.15%) and 377 (62.28 – 62.66%) showed that as the amount of waxy wheat added to non-waxy wheat increased, the water absorption increased too (Figure 4.11). This was in agreement with other studies which also found that more water needed to be added to waxy wheat flour to reach an optimal dough (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Morita *et al.*, 2002a; Takata *et al.*, 2005; Zhang *et al.*, 2014; Blake *et al.*, 2015; Xurun *et al.*, 2015). This is due to the higher amounts of amylopectin, which is said to be the main driver of water absorption in starch (Tester & Morrison, 1990). The water absorption of line 378 (61.43 – 61.68%) did not increase as the amount of waxy wheat increased (Figure 4.11). Instead there was little change between the four blends which suggests that an increased addition of this waxy wheat line to non-waxy flour did not increase the level of amylopectin present and thus did not alter the water absorption. Blend 375_15 (60.43 ± 5.42%) (Figure 4.11) appeared to be an outlier due to its low mean value and its high standard deviation, suggesting that human error may have occurred during one or more of the four replications of this specific blend.

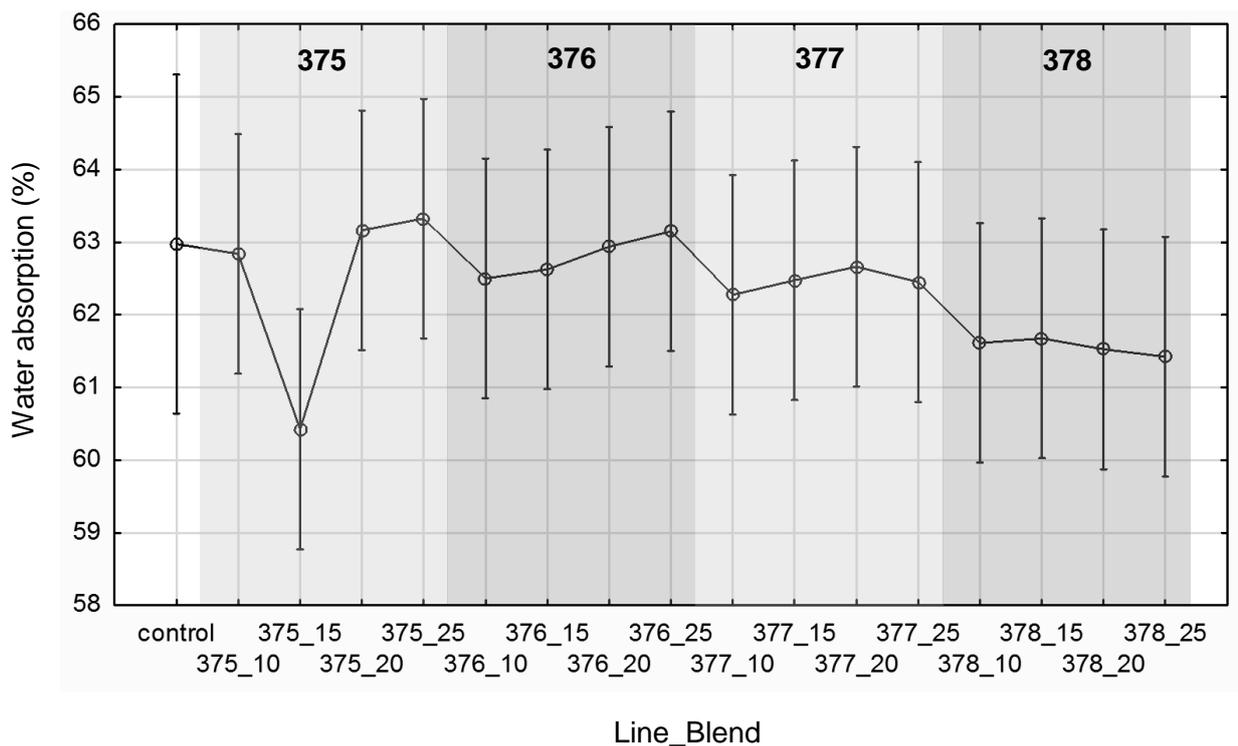


Figure 4.11 Means of water absorption of all lines and blends determined by the Farinograph. Sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control

4.2.2.3. Stability

The stability time indicates the strength of the flour and the higher the value is, the stronger the flour (Zhang *et al.*, 2014). No significant differences were seen between any of the blends and the control (Table 4.7). The means, however, from the 25% blend of line 375 (8.45 min) as well as the 20 and 25% blends from line 376 (7.95 – 8.03 min) were lower than the control (8.60 min). All the other the blends were greater than the control, with a range of 8.68 – 10.65 min. Figure 4.12 shows that for line 375, 376 and 378, the stability decreases slightly as the addition of waxy wheat increases. Line 378, increases stability to some extent as the amount of waxy wheat added increases. Other research has showed that 100% waxy wheat and waxy wheat blends have caused a less stable dough which was more susceptible to overmixing (Abdel-Aal *et al.*, 2002; Bhattacharya *et al.*, 2002; Morita *et al.*, 2002a; Takata *et al.*, 2005; Zhang *et al.*, 2014; Blake *et al.*, 2015). This was contradictory to this study, where only three blends: 375_20% (8.45 min), 376_20% (7.95 min) and 376_25% (8.03 min), had a lower stability than the control (8.60 min). Thus, up to 25% of any of the lines of waxy wheat could be added to non-waxy wheat flour without detrimentally decreasing the stability of the dough.

The general linear models showed that line 375, 376 and 377 had the same effect on the stability of dough (Table 4.8). Line 378 created a significantly more stable dough than the other three lines ($P < 0.05$).

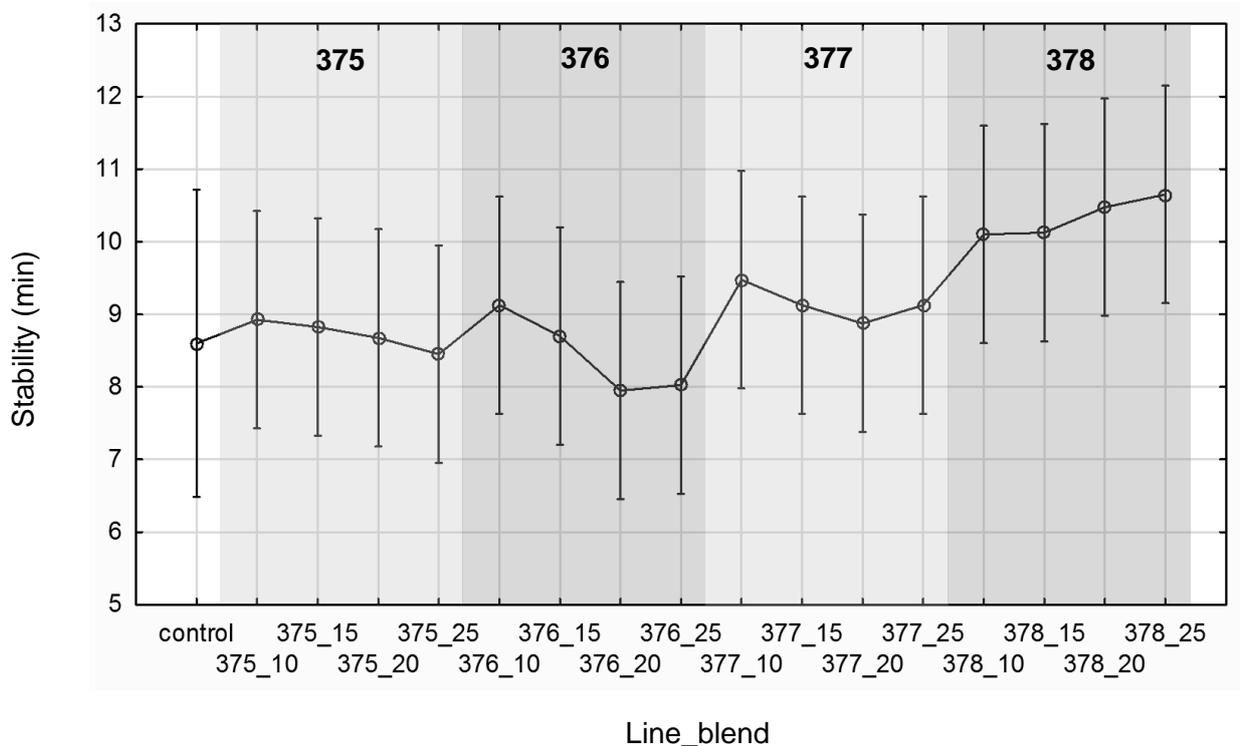


Figure 4.12 Means of stability of all lines and blends determined by the Farinograph. Sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control

Table 4.8 General linear models of the effects of the line, blend and line*blend on the stability determined by the Farinograph

Effect	P-value	
Line	0.004648	378 ^a 376 ^b 375 ^b 377 ^b
Blends (%)	0.872846	- -
Line*Blends	0.991302	- -

Effect is significant when $P < 0.05$

Different letters in the same effect indicate significant differences ($P \leq 0.05$).

4.2.3. Mixograph

4.2.3.1. Peak Time

Like the Farinograph's peak time, the Mixograph gives information about how long it will take to develop the optimal dough. No significant differences between the blends and the control were seen (Table 4.9). All blends, with the exception of 377_25% (3.59 min), had faster peak times than the control (3.49 min), with a range of times between 2.86 min and 3.47 min. Similarly, other researchers found that waxy wheats produced shorter peak times than non-waxy wheats (Abdel-Aal *et al.*, 2002; Guo *et al.*, 2003; Takata *et al.*, 2005; Guan *et al.*, 2009; Jung *et al.*, 2015; Graybosch *et al.*, 2016).

Table 4.9 Peak time, peak height and tail height of doughs made from blends of lines of waxy wheats determined by the Mixograph

	Peak time (min)	Peak height (%)	Tail height (%)
Control	3.49 ±0.28	62.12 ±2.79	50.02 ± 5.02
375_10	3.23 ±0.14	60.84 ±1.63	48.30 ±1.64
375_15	3.30 ±0.16	61.81 ±2.06	48.71 ±2.16
375_20	3.25 ±0.23	61.81 ±0.88	48.44±1.34
375_25	3.25 ±0.28	61.82 ±1.85	48.45 ±2.07
376_10	3.11 ±0.09	59.73 ±1.38	46.09 ±0.80
376_15	3.01±0.10	59.87 ±2.09	46.09 ±1.06
376_20	2.95 ±0.11	61.65 ±0.95	46.23 ±0.76
376_25	2.86 ±0.10	62.42 ±1.28	46.04 ±0.30
377_10	3.42 ±0.34	58.28 ±1.63	47.19 ±1.11
377_15	3.41 ±0.44	59.26 ±1.53	47.85 ±1.98
377_20	3.47 ±0.71	59.36 ±2.84	47.99 ±2.14
377_25	3.59 ±0.94	60.73 ±4.28	49.24 ±2.86
378_10	3.41 ±0.37	61.97 ±3.10	49.71±4.19
378_15	3.33 ±0.25	63.07 ±4.36	49.60 ±3.05
378_20	3.32 ±0.34	63.28 ±4.38	49.59 ±3.76
378_25	3.27 ±0.26	63.51 ±4.62	49.05 ±3.98

Sample names indicate line followed by the percentage which has been blended with the control

Values are means ± standard deviation of four replicates (n=4).

For lines 376 and 378, as the added amount of waxy wheat increased, the time taken to develop an optimal dough decreased (Figure 4.13). No obvious increase or decrease in peak time was seen for line 375 as the percentage of waxy wheat increased (Figure 4.13). The peak time increased slightly as the amount of waxy wheats increased for line 377, which was an unexpected result and was not in agreement with other research conducted on waxy wheats (Abdel-Aal *et al.*, 2002; Guo *et al.*,

2003; Takata *et al.*, 2005; Guan *et al.*, 2009; Jung *et al.*, 2015; Graybosch *et al.*, 2016). Even though it was an unexpected result, it was not a significant increase in time compared to the control and thus would still create an optimal dough in an acceptable time.

Line 376 had the quickest peak times (2.86 – 3.11 min) which was in agreement with the Farinograph’s mixing time (Figure 4.10) and would thus have the most economic benefits for bakers. General linear models showed that line 375, 377 and 378 had the same effect on the peak time as one another (Table 4.10). Line 376 gave a significantly lower peak time than the other three lines ($P < 0.05$).

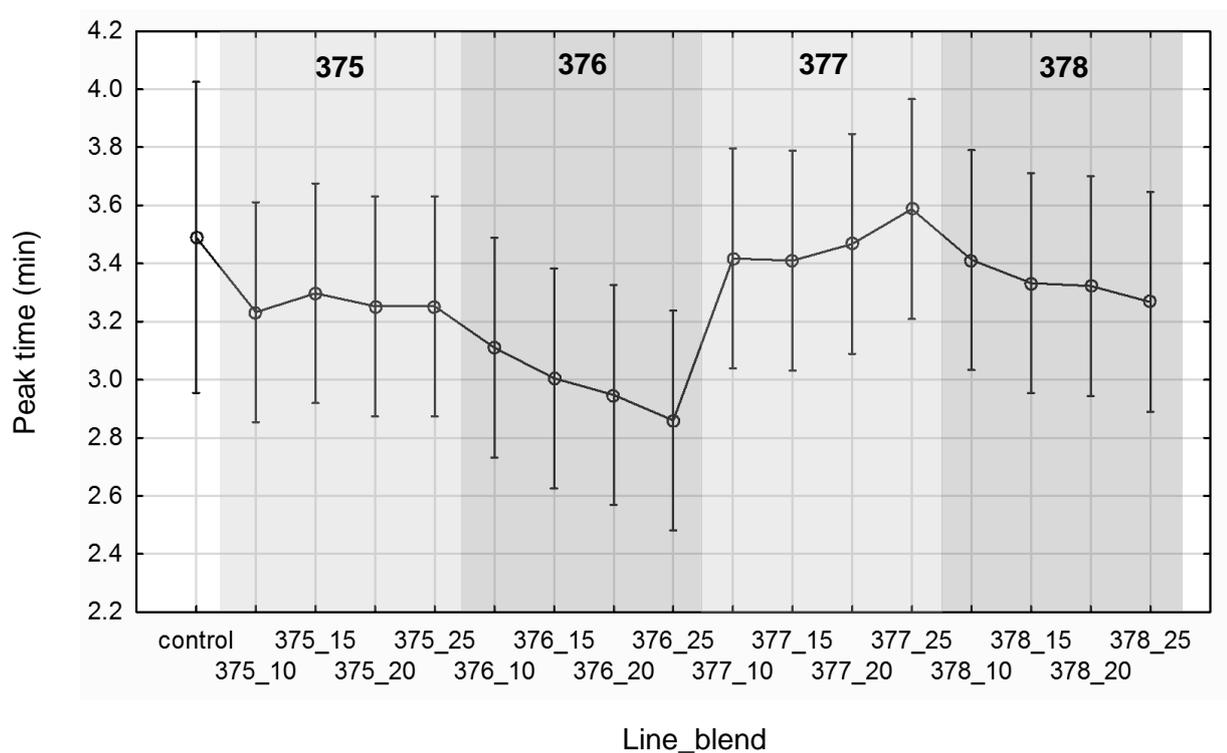


Figure 4.13 Means of peak time of all lines and blends determined by the Mixograph. Sample names specify the waxy wheat line followed by the percentage thereof which has been blended with the control

Table 4.10 General linear models of the effects of the line, blend and line*blend on the peak time determined by the Mixograph

Effect	P-value	
Line	0.005493	375 ^a 377 ^a 378 ^a 376 ^b
Blends (%)	0.981041	- -
Line*Blends	0.994714	- -

Effect is significant when $P < 0.05$

Different letters in the same effect row indicate significant differences ($P \leq 0.05$).

4.2.3.2. Peak Height

The peak height indicates the strength of the dough (Rasper & Walker, 2000). No significant differences were seen between the blends and the control (Table 4.9). The mean values of blend 376_25% (62.42%) and the 15, 20 and 25% blends from line 378 (63.07 – 63.51%) were higher than that of the control (62.12%) while all the other blends were lower (58.28 – 61.97%). Other studies observed that waxy wheats created doughs with a lower peak height than those developed with a non-waxy wheat (Abdel-Aal *et al.*, 2002; Takata *et al.*, 2005). Line 378 created the strongest dough (61.97 – 63.51%) of the waxy wheat lines followed by line 376 (59.73 – 62.42%).

The strength of the dough is attributed to the protein content (Dobraszczyk & Morgenstern, 2003). As there were no significant differences in protein between the blends and the control (Table 4.2), it was not this which caused the majority of the weaker waxy wheat blends to have weaker dough than the control. It was more likely that the quality of the gluten network was hindering the strength. This is because both the starch and the protein require hydration during dough formation: hence they begin to compete for the available water (Caramanico *et al.*, 2017). As waxy wheats contain only amylopectin - which is responsible for water uptake in starch granules – they absorb more water, leaving less water available to the gluten and consequently a weaker gluten network forms, resulting in a weaker dough. Graybosch *et al.* (2016) speculated that due to the fact that few people have bred and studied waxy wheats comprehensively, little time or effort has gone into examining the genetic background: thus waxy wheats, with an inferior dough strength, are continuing to be bred.

4.2.3.3. Tail Height

The tail height was recorded at 6 min after the peak height. While there are many different recordings after this point, they all indicate the mixing tolerance of the dough. It was found that none of the blends differed significantly from the control (Table 4.9). The mean tail height values of the blends (46.04 – 49.71%) were all lower than that of the control (50.02%). This indicated that waxy wheat caused the dough to be less tolerant to overmixing and that the strength of the dough broke down much faster than non-waxy wheat. Other researchers found similar results (Abdel-Aal *et al.*, 2002; Takata *et al.*, 2005; Jonnala *et al.*, 2010), where the consistency of the dough was much weaker for waxy wheat.

Figure 4.14 showed how for lines 375, 376 and 378 that as the amount of waxy wheat increased in the blend, there were only subtle changes to the mixing tolerance. Nevertheless, it was clear that all blends from line 376 (46.04 – 46.23%) had a weaker tolerance than lines 375 (47.30 – 48.71%) and 378 (49.05 - 49.71%). Line 377 (47.19 – 49.24%) showed an increase in tolerance to overmixing as the amount of waxy wheat added increased (Figure 4.14). This contradicts other findings that waxy wheat decreases the mixing tolerance of a dough, but as there was no significant increase between the blends, it could be attributed to instrumental variation (Abdel-Aal *et al.*, 2002; Takata *et al.*, 2005; Jonnala *et al.*, 2010).

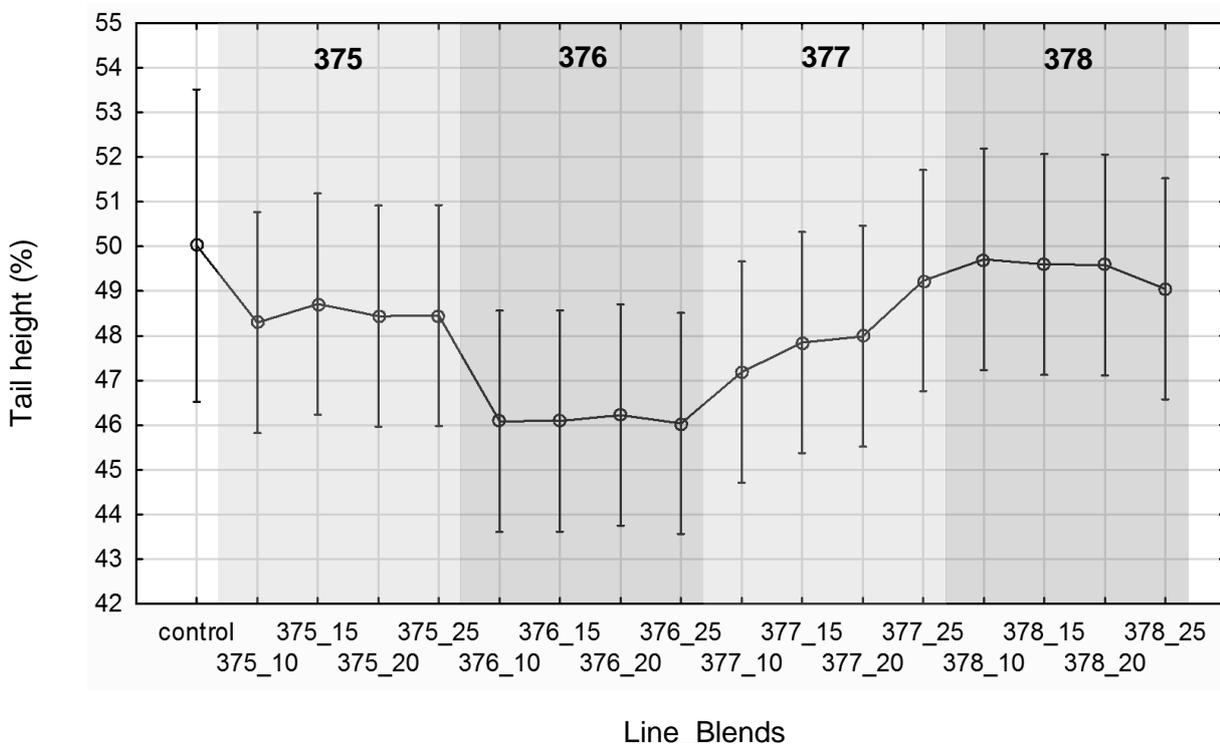


Figure 4.14 Means of tail heights of all lines and blends determined by the Mixograph. Sample names specify the waxy wheat line, followed by the percentage thereof which has been blended with the control

General linear models (Table 4.11) showed that lines 375, 377 and 378 had the same effect on the tail height. Line 376 gave the greatest reduction in tail height and thus the weakest mixing tolerance compared to the other three lines ($P < 0.05$) (Table 4.11). This weak mixing tolerance is due to a weaker gluten matrix and corresponds with the results of the peak height showing that a weaker dough is less tolerant to overmixing.

Table 4.11 General linear models of the effects of the line, blend and line*blend on the peak time determined by the Mixograph

Effect	P-value	
Line	0.002128	375 ^a 377 ^a 378 ^a 376 ^b
Blends (%)	0.976446	- -
Line*Blends	0.995427	- -

Effect is significant when $P < 0.05$

Different letters in the same effect row indicate significant differences ($P \leq 0.05$).

4.2.4. Alveograph

The Alveograph gives the best indication of how a dough will behave during proofing and baking due to its biaxial extension (Table 4.12) (Mirsaeedghazi *et al.*, 2008).

4.2.4.1. Stability

The P value gives an indication of the extent to which the dough will resist the expansion of gas (CHOPIN Technologies, 2014) or how much pressure it can withstand before the bubble blown bursts (Garimella Purna, 2010). The waxy wheat blends proved not to be significantly different from the control (Table 4.12). Blends 15, 20 and 25% from line 378 (90.75 – 92.75 mm) had lower P values than the control (93.50 mm) while all other blends were either equal to, or higher, with a range of 93.50 – 104.25 mm. This showed that the addition of waxy wheat increased the P value in comparison to a non-waxy wheat. An addition of up to 25 % increased or decreased the value significantly from a 10% addition on waxy wheat. This means that the waxy wheat causes the dough to be able to resist gas expansion better and can withstand a higher pressure inside the bubble (Garimella Purna, 2010).

Table 4.12 Biaxial extension of all waxy wheat lines and blends as determined by the Alveograph

	P (mm)	L (mm)	P/L	W (10 ⁻⁴ J)
Control	93.50 ±14.85	76.50 ±0.71	1.23 ±0.21	35.80 ±5.66
375_10	99.50 ±7.85	83.50 ±2.65	1.19 ±0.09	40.95 ±4.19
375_15	99.75 ±7.54	83.75 ±0.50	1.19 ±0.08	41.96 ±3.81
375_20	101.25±8.26	87.00 ±4.83	1.17 ±0.15	43.39 ±3.46
375_25	104.25 ±9.00	90.25 ±3.10	1.16 ±0.12	46.33 ±4.50
376_10	95.00 ±9.38	88.25 ±0.96	1.08 ±0.10	40.23 ±4.63
376_15	94.25 ±7.50	91.50 ±3.87	1.04 ±0.12	41.20 ±2.77
376_20	95.25 ±8.38	95.00 ±2.16	1.01 ±0.11	42.45 ±3.71
376_25	93.50 ±10.21	97.00 ±4.97	0.97 ±0.15	42.45 ±4.08
377_10	99.00 ±12.19	82.75 ±7.54	1.21 ±0.24	40.48 ±3.73
377_15	102.75 ±16.80	85.50 ±11.27	1.24 ±0.38	43.28 ±3.41
377_20	104.25 ±14.41	82.00 ±10.68	1.30 ±0.30	42.65 ±4.29
377_25	102.75 ±14.55	86.25 ±15.69	1.24 ±0.36	43.70 ±3.45
378_10	94.00 ±10.07	84.75 ±6.65	1.12 ±0.20	39.18 ±2.81
378_15	92.25 ±9.64	87.00 ±9.42	1.08 ±0.20	39.04 ±4.00
378_20	92.75 ±12.15	91.00 ±10.39	1.04 ±0.25	41.26 ±4.08
378_25	90.75 ±11.35	96.00 ±11.40	0.96 ±0.21	42.13 ±3.22

P = stability ; L = extensibility ; W = strength

Sample names indicate line followed by the percentage which has been blended with the control.

Values are means ± standard deviation of four replicates (n=4).

4.2.4.2. Extensibility

The L value demonstrates the extensibility of the dough. No significant differences between the control and the blends were seen and all the blends (82 .00 – 97.00 mm) had L values which were greater than that of the control (76.50 mm) (Table 4.12). It was observed that as the amount of waxy wheat in the blends increased, the L value increased as well showing that its addition will increase the extensibility of the dough but not significantly so.

4.2.4.3. P/L Ratio

While individually the P and L values do provide valuable information about the dough, it is the P/L value that gives the best indication of how the dough will perform. It shows the balance between the strength and the extensibility of the dough (Faridi & Rasper, 1987). The P/L value is used by bakers to predict the quality of the final loaf. No significant differences were seen between the blends and the control (Table 4.12). The mean P/L values of the 15, 20 and 25% blends from line 377 (1.24 -

1.30) were larger than the control (1.23) while all the other blends had lower mean values (0.96 – 1.21).

The high value of the control could possibly be attributed to improvers added to the flour by the milling company. The control came from a commercial brand and as wheat is a highly variable crop, commercial companies often add improvers to the flour in order to give customers a consistent quality. These improvers have been known to increase the P/L value (O’Kennedy, K., 2017, Technical Specialist, Sasko, Paarl, South Africa, personal communication). These results show that the addition of up to 25% of any of the waxy wheat lines to non-waxy wheat creates a dough which is comparable to a commercial wheat and that it would have a similar final loaf quality.

4.2.4.4. Strength

The final value evaluated from the Alveograph is the area under the curve (W). It provides an overall view of the strength of the dough, as it is an indication of how much work was needed to blow the dough bubble (Faridi & Rasper, 1987; Hajselova & Alldrick, 2003). No significant differences were seen between the blends and the control. It was observed that more work (higher values) was needed to blow the bubble for all waxy wheat blends (39.04 – 46.33 10^{-4} J) compared to the control (35.80 10^{-4} J) (Table 4.12). This could indicate that smaller gas bubbles will form in the dough of the waxy wheat doughs during proofing as they required more energy to form the same size bubbles as that of non-waxy wheat. Thus a waxy wheat loaf may have a lower loaf volume due to less gas cell expansion during baking. Blend 375_25% required the highest amount of energy (46.33 10^{-4} J) and therefore may have the worst bread loaf quality whereas blend 378_15% required the least amount (39.04 10^{-4} J) resulting in a more favourable loaf appearance.

4.3. *Bread baking*

4.3.1. Bread loaf quality

The C-Cell digital images and the analysis thereof enable researchers to quantify the quality of the bread, rather than giving a subjective visual score. The data from the C-Cell also supports the starch and dough rheology tests’ predictions on the final loaf quality for a specific blend.

When dealing with waxy wheats, the total concavity of the slice is very important. This is due to what is described as the key-hole effect in which, after baking, the sides of the loaves collapse inwards, creating a keyhole shape (Ghiasi *et al.*, 1984; Graybosch, 2001; Garimella Purna *et al.*, 2011).

Blends of 10 and 15% of line 376 (3.42% and 4.20%) and all blends from line 378 (2.85 – 5.81%) had significantly lower ($P < 0.05$) percentage concavities than the control with the improver (7.36%). Blends of 20 and 25% from line 376 (6.49% and 6.53%) and blends 10 and 15% from line 377 (5.52% and 6.20%) had lower means than the control with improver but not significantly so. All blends from line 375 (7.94 – 11.32%) and blends 20 and 25% of line 377 (9.07% and 12.44%) had a larger percentage concavity than the control with the shelf life improver.

Table 4.13 Results of loaf quality from C-Cell digital image analysis

	Total Concavity (%)	Slice Brightness	Number of Cells	Number of Holes	Area of cells (%)
Control w/ improver	7.36 ^a ±1.49	154 ±6.49	7101 ±992.28	2.53 ±1.74	49.76 ± 0.34
Control w/o improver	2.74 ^c ±0.09	156 ±1.73	8921 ±1868.18	4.82 ±1.44	49.10 ± 1.27
375_10	7.94 ^{abc} ±3.17	155 ±4.51	8565 ±861.24	5.44 ±0.91	49.82 ± 1.01
375_15	10.31 ^{abc} ±3.82	154 ±4.81	8093 ±357.02	4.65 ±0.54	49.82 ± 0.54
375_20	8.99 ^{abc} ±3.20	153 ±6.34	7979 ±701.63	5.17 ±1.45	49.39 ± 1.20
375_25	11.32 ^{ab} ±1.97	153 ±6.57	7871 ±997.87	5.41 ±1.69	49.32 ± 0.66
376_10	3.42 ^c ±0.76	157 ±3.76	7969 ±364.56	4.91 ±2.54	50.37 ± 0.40
376_15	4.20 ^c ±1.11	158 ±3.81	8468 ±628.64	5.68 ±2.09	49.97 ± 0.73
376_20	6.49 ^{abc} ±2.86	159 ±5.25	8477 ±799.88	5.36 ±1.77	50.43 ± 0.75
376_25	6.53 ^{abc} ±2.08	159 ±3.96	8244 ±1226.86	4.96 ±2.19	50.49 ± 1.05
377_10	5.52 ^{abc} ±2.00	153 ±1.48	8638 ±892.72	5.49 ±0.67	49.52 ± 1.15
377_15	6.20 ^{abc} ±2.05	151 ±3.80	8066 ±573.48	4.58 ±1.30	50.07 ± 0.36
377_20	9.07 ^{abc} ±3.25	152 ±1.68	8007 ±417.02	5.45 ±1.37	49.92 ± 0.53
377_25	12.44 ^{abc} ±3.36	150 ±2.67	8253 ±598.27	5.78 ±1.56	49.19 ± 0.99
378_10	2.85 ^c ±0.44	159 ±6.49	8185 ±1250.78	4.29 ±0.60	50.13 ± 1.09
378_15	4.19 ^c ±0.69	160 ±7.20	8894 ±899.19	5.23 ±0.83	49.63 ± 0.51
378_20	2.99 ^c ±0.48	158 ±6.78	8738 ±1111.01	3.72 ±1.04	49.62 ± 0.53
378_25	5.81 ^{bc} ±1.29	158 ±6.14	8584 ±805.45	5.24 ±1.16	50.10 ± 0.33

Sample names indicate line followed by the percentage which has been blended with the control

Values are means ± standard deviation of four replicates (n=4).

Different letters in the same column indicate significant differences (P≤0.05).

The general linear models showed that both the line and the blend had a significant effect ($P < 0.05$) on the concavity of the loaf but that the combined effect was not significant (Table 4.14). The effect of lines 375 and 377 on the percentage concavity did not differ significantly from one another. This was also true for the effect of lines 376 and 378, as they had a comparable influence on the concavity. Line 375 and 377 created a more prominent keyhole effect than line 376 and 378 ($P < 0.05$). As a higher percentage concavity is associated with a negative loaf appearance, lines 376 and 378 would provide a loaf with the least negative appearance.

No significant difference in effect between 10 and 15% and between 15 and 20% was seen on the percentage concavity (Table 4.14). This suggested that an increase of only 10% of waxy wheat added to a non-waxy wheat would have a significant effect on the concavity. However 25% blends caused a significant increase compared to the 20% blends. It was observed that the effects of the addition of waxy wheat on percentage concavity were gradual up to 20% but that thereafter, the appearance of the bread became more susceptible to changes in waxy wheat amount.

Table 4.14 General linear models of percentage concavity determined by the C-Cell analyser

Effect	P-value	
Line	0.000001	375 ^a 377 ^a 376 ^b 378 ^b
Blends (%)	0.000099	25 ^a 20 ^b 15 ^{bc} 10 ^c
Line*Blends	0.310073	-

Effect is significant when $P < 0.05$

Different letters in the same effect row indicate significant differences ($P \leq 0.05$).

The blends showed no significant differences from either of the controls for slice brightness, number of cells and number of holes as well as cell area ($P < 0.05$) (Table 4.13). This is a positive result as it indicates that the addition of up to 25% waxy wheat does not change the crumb structure of the bread, nor lessen the brightness of the slice, which has been noted by other researchers to be a result of the addition of waxy wheat (Garimella Purna *et al.*, 2011).

All blends of line 375 (153 – 155) and 377 (150-153) had lower mean slice brightness values than the control without the improver (156); while all blends of line 376 (157 – 159) and 378 (158-160) had higher mean values. As a desirable loaf of bread is said to have a bright slice (Cauvain, 2003), lines 376 and 378 would give a more appealing loaf.

The mean value of the number of cells was lower than the control without improver (8921) for all blends (7871 – 8894). A fine crumb structure also determines bread loaf quality and thus a higher number of cells with smaller areas is more desirable (Cauvain, 2003). The area of the cells from all the blends (49.32 – 50.49%) were higher than the control without improver (49.10%). Thus an addition of up to 25% of waxy wheat decreased the number of cells and increased the area - but not significantly enough to create an undesirable crumb structure. A large number of holes is detrimental to final loaf quality. The mean values number of holes from blends 375_15 (4.65), 377_15% (4.58), 378_10% (4.29) and 378_20% (3.72) were lower than the control without improver (4.82) while the remainder of the blends had higher values (4.91 – 5.78). This showed that while adding waxy wheats to non-waxy wheats did increase the amount of holes in the crumb structure compared to the controls, it was not significant and so would not negatively affect the appearances of the loaves of bread.

Images taken with the C-Cell visually represent the data seen in Table 4.13 (Figure 4.15; Figure 4.16; Figure 4.17; Figure 4.18). By comparing each corresponding blend from each line to each other and the control, a clearer idea is gained of which percentage waxy wheat and which line will give the most appealing final bread loaf quality. Figure 4.15 compares the 10% waxy wheat blends. Statistically, none of them differed significantly from each other, or the control, without improver (Table 4.13). Blends from line 376 and 377 did appear to have more holes but these are unlikely to deter a consumer and so an addition of 10% waxy wheat could be used to create a satisfactory loaf.

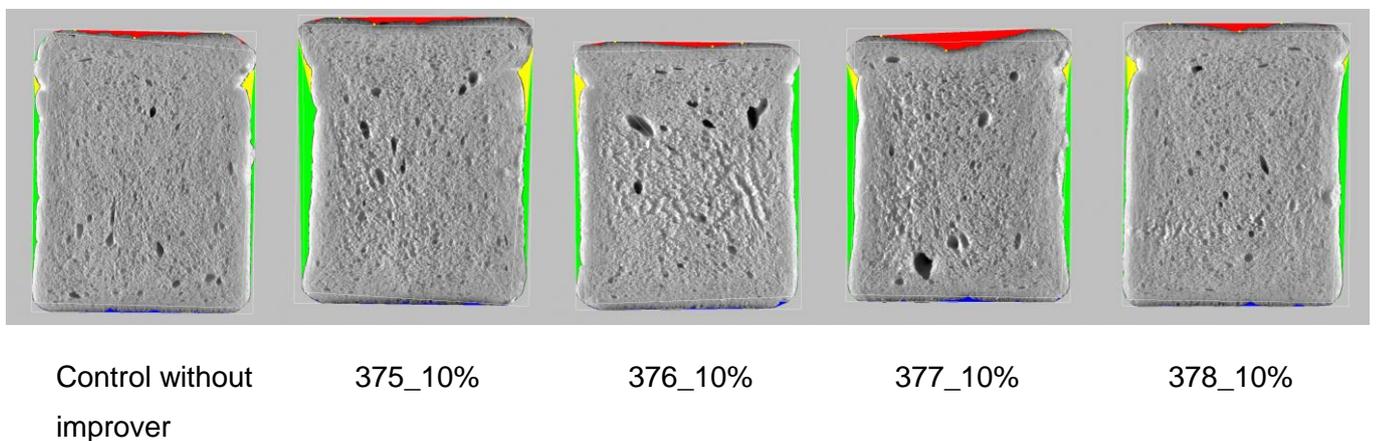


Figure 4.15 Comparison of bread loaf images of the 10% blends for all lines taken by the C-Cell

Figure 4.16 shows the 15% blends of waxy wheat and non-waxy wheat compared to the control without improver. Line 377 showed the most prominent collapse of the side of the loaf and the most holes indicating that it may create the poorest loaf appearance at this level of waxy wheat compared to the other lines. All the loaves blended with the lines of waxy wheat would nonetheless be

acceptable to consumers, as there are no significant differences in all aspects from the control without the improver (Table 4.13).

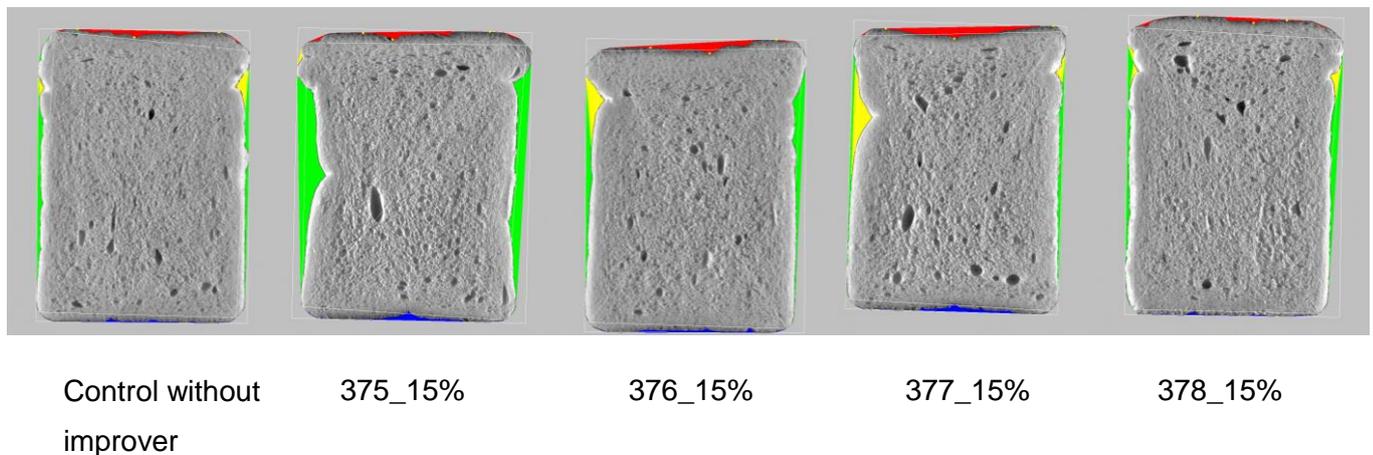


Figure 4.16 Comparison of bread loaf images of the 15% blends for all lines taken by the C-Cell

The appearance of the upper percentages of 20 and 25% of waxy wheat (Figure 4.17; Figure 4.18) were the most important as it was expected that they would have the most potential to retard starch retrogradation and increase shelf life. Other research also showed that it was this range of addition of waxy wheats that provided an increase of shelf life but a decrease in loaf appearance (Bhattacharya *et al.*, 2002; Morita *et al.*, 2002b).

Data showed that there were no significant differences seen in all the blends of 20% and the control without the improver (Table 4.13). The images reiterated this, as they were all visually appealing and had minimal shrinking of sides (Figure 4.17). Lines 375 and 378 appeared most like the control and would provide the best loaf quality for this percentage addition of waxy wheat.

The blends of 25% showed the most prominent keyhole effect and began to show how the addition of more than 25% of waxy wheat to a non-waxy wheat could be exceedingly detrimental to final loaf appearance (Figure 4.18). Line 375_25% was the only blend which had a significantly higher ($P < 0.05$) percentage concavity (11.32%) compared to the control without improver (2.74%) (Table 4.13). It is clear from the image that it had the worst visual appearance. While line 377 was not significantly different from the control with improver, it also had a prominent keyhole effect. These two lines had the worst effect on loaf appearance at a 25% addition of waxy wheat. Both lines 376 and 378 appeared very similar to the control without improver and showed that up to 25% thereof could be added to a non-waxy wheat to create a loaf that was still visually appealing to the consumer.

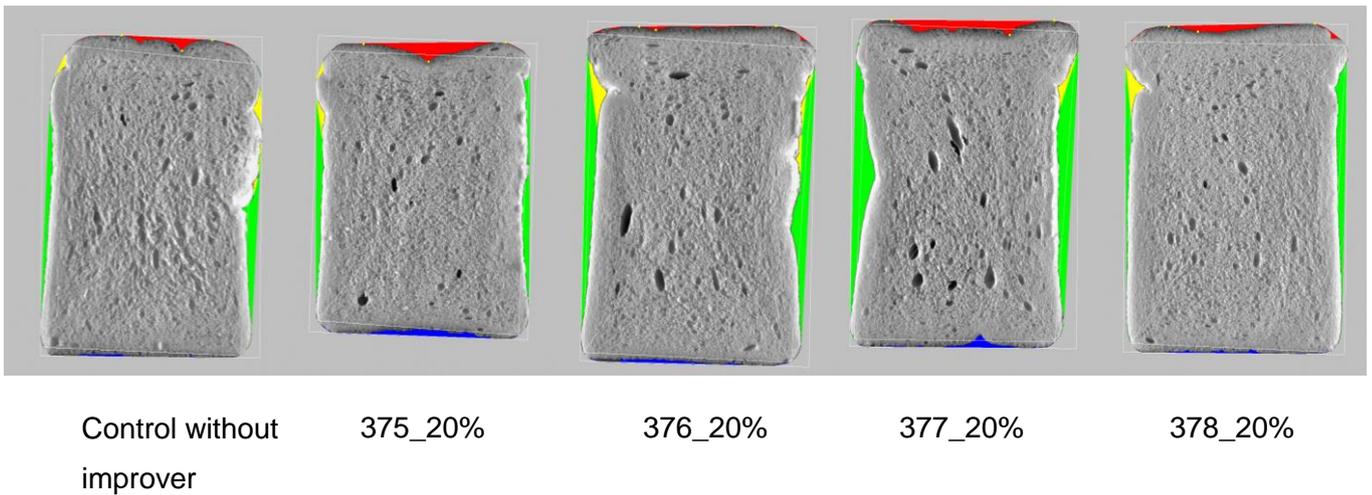


Figure 4.17 Comparison of bread loaf images of the 20% blends for all lines taken by the C-Cell

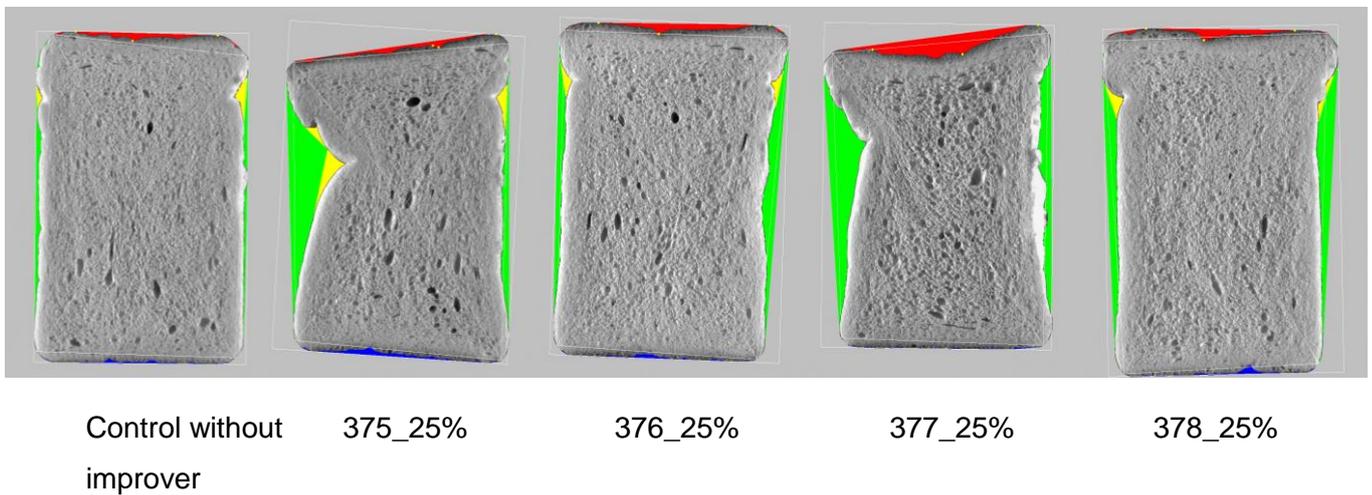


Figure 4.18 Comparison of bread loaf images of the 25% blends for all lines taken by the C-Cell

Figure 4.19 shows how when a commercial shelf life improver is used, it increases the percentage concavity and thus this aspect of loaf quality could be sacrificed in order to have fresher bread for longer. As none of the blends had a significantly higher ($P < 0.05$) percentage concavity than this control, all blends from all lines would have a comparable visual appearance to a commercially available loaf of bread.

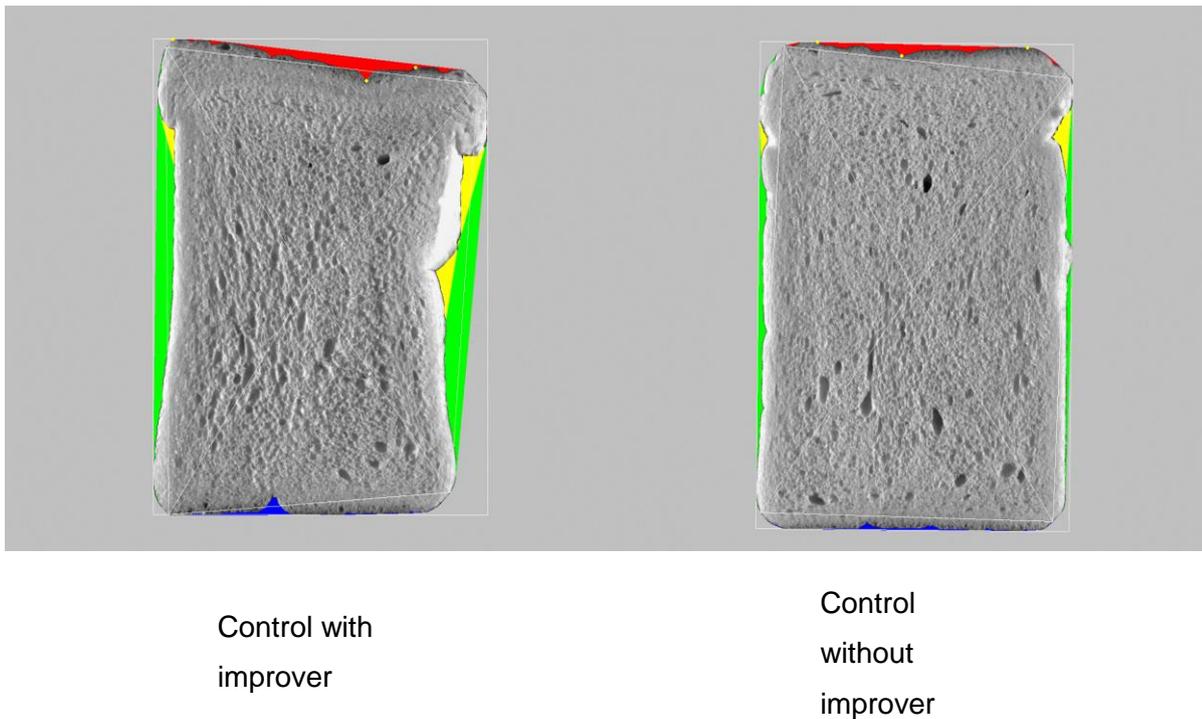


Figure 4.19 Comparison of images of control with shelf life improver and without taken by the C-Cell

4.3.2. Shelf life testing

The texture analyser helps determine the extent to which starch has retrograded and this indicates bread staleness (Chinachoti, 2003). The firmer a slice of bread is, the more starch retrogradation that has occurred. The addition of waxy wheat aims to retard starch retrogradation and thus maintain a softer loaf for longer.

No significant differences in firmness were seen between the control without the improver and the blends on day one. The mean firmness for all blends (2.00 - 2.64 g), however, was lower than the control without improver (2.70 g) (Table 4.15). One exception was blend 378_20% (2.87g) which had a higher mean value on day one. Figure 4.20 showed how, on day one, the increase in amount of waxy wheat in each blend had no significant increase or decrease on the firmness of the loaf of bread. These results showed that the addition of up to 25% of any of the waxy wheat lines created a softer initial loaf which was in agreement with other studies which found that breads baked with waxy wheat have a lower firmness on day one (Bhattacharya *et al.*, 2002; Blake *et al.*, 2015).

On day three, no significant differences were seen between the control without improver and the blends. Mean firmness values from the 10, 15 and 20% blends of line 378 (3.68 – 3.99 g) had a higher value than the control without improver (3.56 g) (Table 4.15). The remaining blends had a softer crumb on day three (2.82 – 3.33 g), than the control without improver. This showed that lines 375, 376 and 377 would still have a softer loaf than the control without improver on day three but not significantly so. Figure 4.20 shows that there was no significant increase or decrease in firmness as the amount of waxy wheat increased.

None of the blends on day six differed significantly from the control without improver (4.81 g). Blends 375_25% (5.24 g) and the 15, 20 and 25% blends from line 377 (4.98 – 5.26 g) had a larger mean value than the control without improver (Table 4.15). All other blends had a lower firmness mean (3.55 – 4.80 g). It was seen for lines 375 and 377 that, as the percentage of waxy wheat added to the blend increased, so did the firmness (Figure 4.20). This contradicted what theoretically should have happened, where an increase in waxy wheat was determined to decrease the firmness (Lee *et al.*, 2001; Bhattacharya *et al.*, 2002; Garimella Purna *et al.*, 2011). It was possible that those particular lines had shorter amylopectin chains with less branching and thus completed retrogradation sooner than other waxy wheats. In other words, the amylopectin chains were so short that they behaved more like amylose. An increase of line 376 in a blend did decrease the firmness and had the most potential for creating a softer loaf on day 6. Line 378 appeared different from the other three lines of day six as there was no increase nor decrease in firmness with the increase of waxy wheat (Figure 4.20). This suggested that, as no changes occurred from one blend to the next, the starch composition of this line was the same as the control and that line 378 is possibly not a full waxy wheat.

Figure 4.20 clearly shows the advantages of the shelf life improver to reduce firmness. The addition of the improver kept the bread as soft on day six (2.31 g) as it was on day one (2.03 g) (Table 4.15). This suggests that the benefits of waxy wheat may not be applicable in a commercial setting but shows that it may be more appropriate in smaller bakeries which are not using commercial improvers. It will appeal to the artisanal bread makers who are making relatively small batches of bread and who are not wanting to utilise additives.

Table 4.15 Firmness of days one, three and six for all lines and blends as determined by the texture analyser

	Firmness (g)		
	Day 1	Day 3	Day 6
Control w/ improver	2.03 ± 0.24	2.14 ± 0.37	2.31 ± 0.27
Control w/o improver	2.70 ± 0.43	3.58 ± 1.16	4.81 ± 1.55
375_10	2.06 ± 0.19	2.99 ± 0.47	3.97 ± 0.22
375_15	2.07 ± 0.23	3.01 ± 0.40	4.63 ± 0.37
375_20	2.08 ± 0.25	3.29 ± 0.76	4.80 ± 1.10
375_25	2.00 ± 0.15	2.95 ± 0.34	5.24 ± 1.93
376_10	2.42 ± 0.44	3.60 ± 1.33	4.71 ± 1.14
376_15	2.35 ± 0.41	3.00 ± 0.65	4.03 ± 0.77
376_20	2.09 ± 0.21	2.88 ± 0.33	3.55 ± 0.61
376_25	2.18 ± 0.18	2.82 ± 0.19	3.80 ± 0.53
377_10	2.54 ± 0.63	3.19 ± 0.84	4.75 ± 1.57
377_15	2.37 ± 0.58	3.17 ± 0.91	4.98 ± 0.71
377_20	2.14 ± 0.23	3.19 ± 0.56	5.26 ± 0.98
377_25	2.21 ± 0.27	3.33 ± 0.64	5.26 ± 0.72
378_10	2.64 ± 0.19	3.99 ± 0.71	4.51 ± 0.75
378_15	2.58 ± 0.33	3.77 ± 0.71	4.58 ± 1.16
378_20	2.87 ± 0.43	3.68 ± 1.22	4.63 ± 1.72
378_25	2.38 ± 0.31	3.32 ± 0.78	4.08 ± 1.29

Sample names indicate line followed by the percentage which was blended with the control

Values are means ± standard deviation of four replicates (n=4).

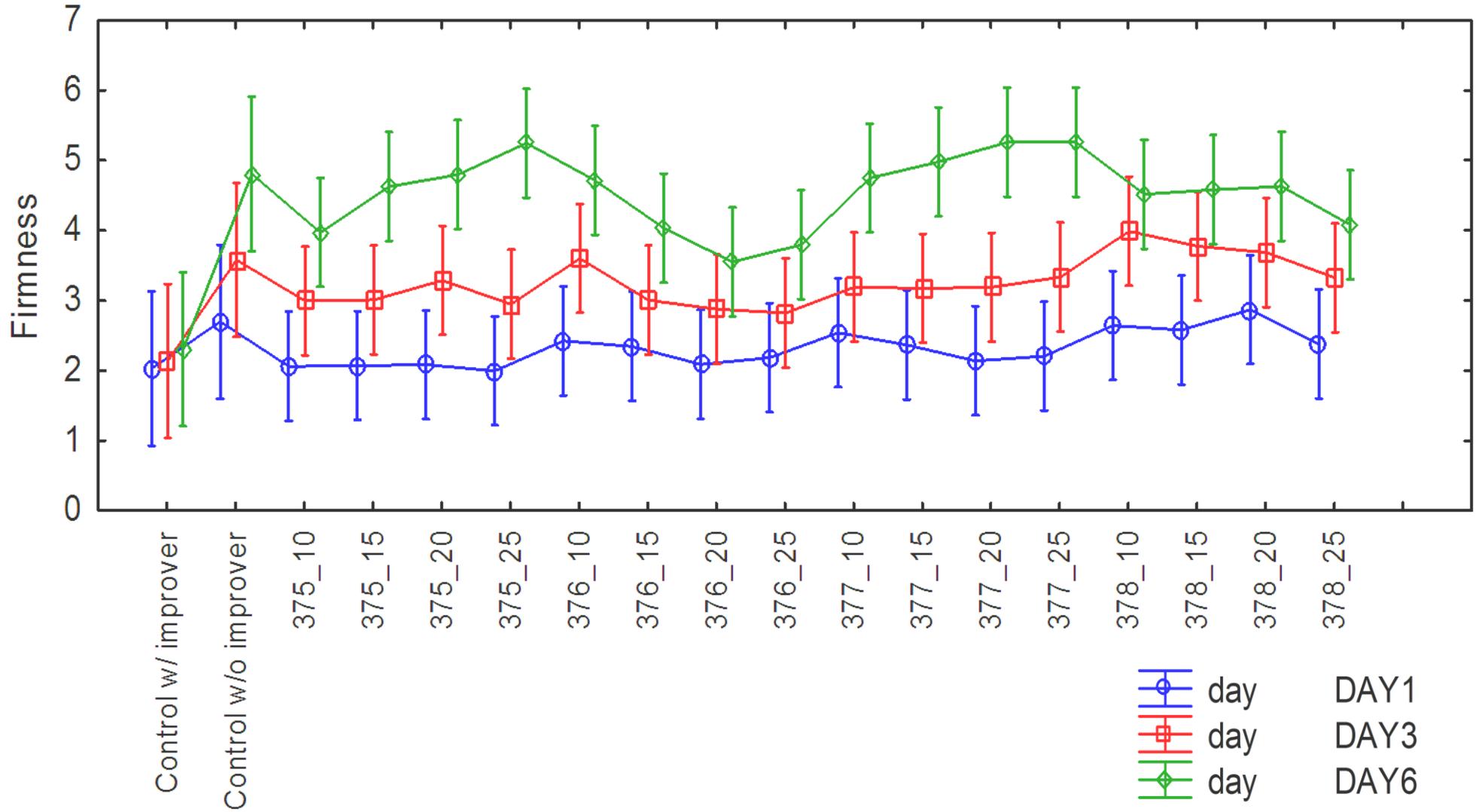


Figure 4.20 Firmness on days, one, three and six of all blends and controls, as determined by the texture analyser

4.4. *Multivariate data analysis*

A principal component analysis (PCA) was conducted using the data from the starch and dough rheology tests as well as the c-cell images (Figure 4.21). The first and third components were used as it showed the clearest clustering of the waxy wheat lines while still describing approximately 58% of the variation (Bro & Smilde, 2014). The PCA bi-plot clearly shows that blends from lines 378 corresponded with one another. This was also true of line 376. The blends from lines 375 and 377 associated less. This agreed with results from general linear models which indicated that 375 and 377 had the same effect on results whereas 376 and 378 had separate effects (Table 4.5; Table 4.6).

It was clear throughout the analysis of the dough and starch rheology and the baking and shelf life testing, that line 376 gave the most favourable outcomes. For the rheology, it mostly did not differ significantly from the control and for the shelf life testing it gave the softest loaf after day six. The PCA bi-plot further reinforces these positive results by showing that it is line 376 which is most associated with the control. C-Cell analysis showed that lines 375 and 377 had the highest percentage concavity (Table 4.13) and the PCA confirms this, as these lines are closely associated with the percentage concavity. Results from the Farinograph indicated that line 378 was the most stable of the doughs and this is demonstrated in the PCA bi-plot by the nearness of stability to the 378 line.

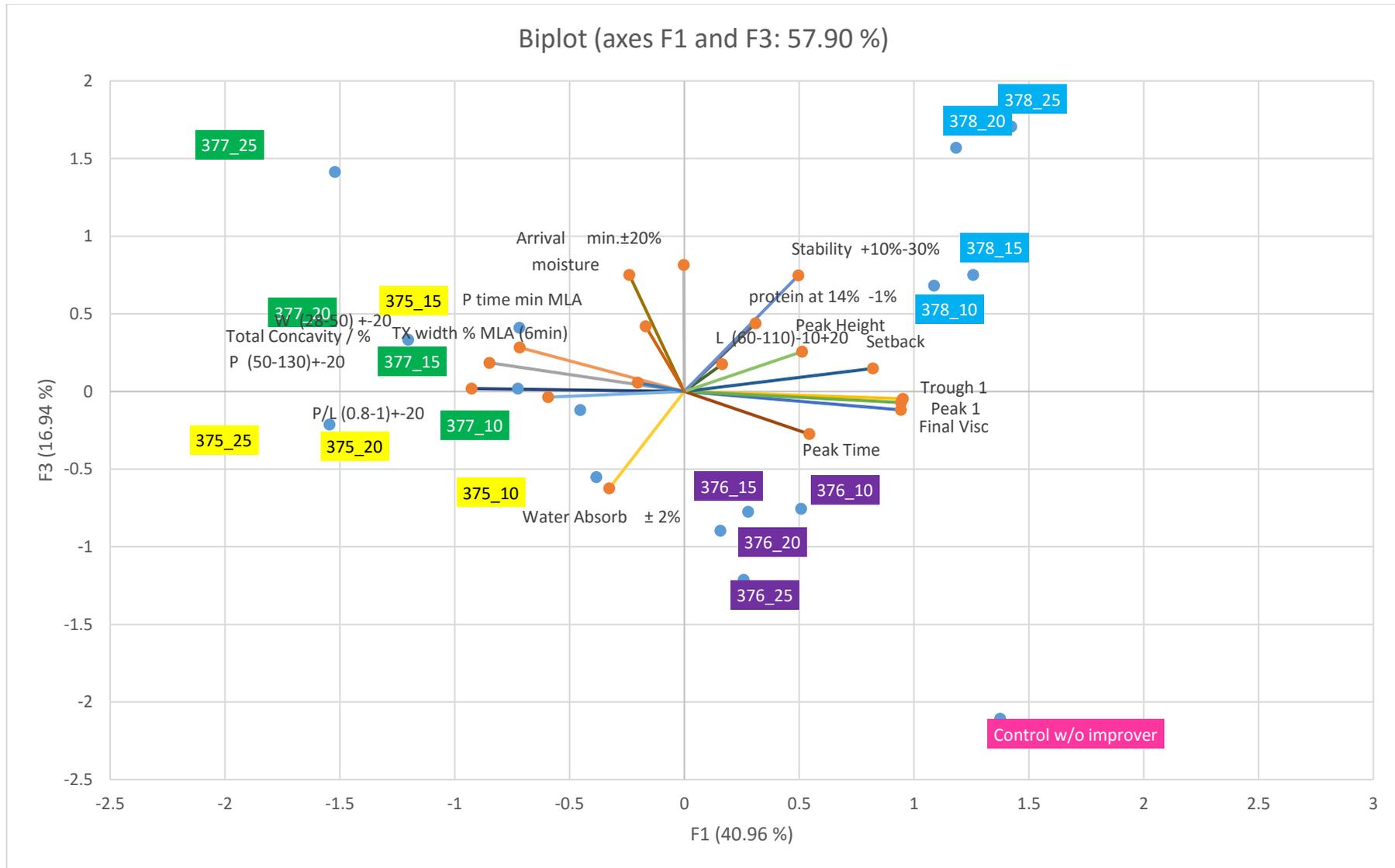


Figure 4.21 A principal component analysis bi-plot with the first and third factors (F1, F3) and describing 57.90% of the variation.

Chapter 5

Conclusions

5. Conclusions

The SEM showed that there were little to no differences in starch granule morphology between the waxy wheats and the control. Lines 375, 376 and 377 did however appear to have more B-type granules and seemed to be harder grains than the control and line 378. The Diffractograms from the XRD analysis had a typical A-type shape for all the samples. The control had unexpected peak intensities compared to other non-waxy wheat studies and this resulted in it having an unusually high percentage crystallinity. Line 376 also displayed an abnormally high amylose-lipid peak intensity and further testing should be done to uncover why. All waxy wheat lines had unexpectedly similar percentage crystallinities. Results from both SEM and XRD showed that the starch from the waxy wheat lines and the control had very similar microstructures and no definite distinctions between them could be made.

The RVA results showed that the initial processing may differ slightly from a non-waxy wheat flour but the lower final viscosities of waxy wheat indicates its potential to increase the shelf life of bread. The waxy blends from line 376 behaved most like a waxy wheat as determined by other research. It also formed the optimal dough the quickest as well as creating the strongest dough of the waxy wheats. Both the Farinograph and the Mixograph showed that it was least tolerant to overmixing but that it was not significantly different from the control and thus could be deemed acceptable for commercial processing. This line was also found at its 20 and 25% blends, to have the best P/L value and to still be visually appealing to the consumer at these levels. The texture analyser confirmed that adding up to 25% of 376 to non-waxy wheat will result in a loaf that is softer on day 6 than the control and the other waxy lines. In conclusion, the addition of between 20 – 25% of line 376 to non-waxy wheat resulted in the fastest dough development, the most appealing loaf appearance as well as the best ability to extend shelf life.

Lines 375 and 377 were found throughout to have the same effect on dough and loaf quality and often did the opposite of what they were supposed to. While they did have some positive effects, it would be recommended that they are not used to replace improvers in bread making due to having the worst visually loaf appearance.

Line 378 behaved far more like a non-waxy wheat than a waxy wheat. Further genetic analysis should be conducted on it to determine if it really is a waxy wheat and the results from this research be used to exclude it from any further waxy wheat breeding programs or research.

Waxy wheats could be used to increase the shelf life of bread whilst maintaining its visual appeal. However, the shelf life extension is no match for commercial improvers and so it is likely that their use is more applicable for small scale or artisanal bakers who want to avoid adding undesirable additives to their products.\

Chapter 6

General discussion and conclusions

6. General discussion and conclusions

Waxy wheats are a widely studied genetic mutation of wheat. Wheat is naturally highly variable as a result of its genetic background, location and environment (Shewry, 2009; Shevkani *et al.*, 2017). Consequently, it is necessary to complete studies on waxy wheat cultivars/varieties to determine their processing properties in terms of dough rheology and final baking quality. Waxy wheats are known to retard starch retrogradation which lead to a slowing of staling and an increase in shelf life (Graybosch, 1998; Maningat *et al.*, 2009). Thus it was important to explore this technological ability for each of the four waxy wheat lines in this study. When it comes to the ultimate goal of producing a quality loaf of bread with a longer shelf life, the important factors to take into consideration are the time it takes to reach optimal dough consistency, the strength of the dough and its tolerance to overmixing, the visual appeal of the loaf of bread and if it does, in fact, stale more slowly. All these things were considered in the preceding study, as well as the starch microstructure of the four waxy wheat lines.

The scanning electron microscope (SEM) and X-ray diffraction (XRD) were used to observe the microstructure of the starch. SEM images were taken of both isolated starch and the endosperm of the whole kernels and showed that there were little to no differences in the starch granule size and morphology between the waxy wheats and the control. Lines 375, 376 and 377 had fewer small and spherical B-type granules (Jane, 2009) than the control and line 378 and also had more visible protein remaining on them. This indicated that these three lines were harder grains. More broken or damaged granules were also seen on these lines, demonstrating that they are more susceptible to starch damage during milling. Further testing, such as particle size index (PSI), near infrared reflectance (NIR) and single kernel characterization system (SKCS) should be undertaken on the waxy wheat lines to confirm their grain hardness and the presence of friabilin (Morris, 2002).

Diffraction patterns from the XRD showed that both the control, a non-waxy wheat, and the waxy wheat lines were A-type polymorphs of starch. The control had an unexpectedly high percentage crystallinity considering it had more amylose than the waxy wheats and should theoretically have had a much lower percentage crystallinity than the waxy wheats (Zhang *et al.*, 2013; Wang *et al.*, 2015). It is due to the waxy wheats only having amylopectin that they had a higher percentage crystallinity, as amylopectin is a highly branched molecule which is found in its double helix, crystalline form (Xurun *et al.*, 2015; Shevkani *et al.*, 2017). Apart from the usual A-type peaks at $2\theta = 15, 17-18, 23^\circ$, all Diffraction patterns had a peak at $2\theta = 20^\circ$. This peak represents the amylose-lipid complexes which form in starch and should have a lower intensity for the waxy wheats due to their lack of amylose (Hayakawa *et al.*, 1997; Yoo & Jane, 2002). It could be hypothesised that this peak for the control was higher than expected which resulted in an increase in crystallinity. It could be theorised that the control contained

unusually high amounts of amylose and/or lipids, which caused this anomaly. Line 376 also had an unusually high peak at this diffraction angle and was more similar to the control than the other waxy wheats. It is recommended that further analysis such as amylose content and lipid content be conducted on this line and the control to explain these results. Line 378 had the lowest percentage crystallinity of the waxy wheats and line 375 had the highest suggesting that line 378 had shorter amylopectin chain lengths than line 375. This could be further confirmed by conducting size exclusion chromatography (SEC) to determine the amylopectin chain lengths of each waxy wheat line.

While SEM and XRD did illustrate the microstructure of the starch, it was rather simplistic and did not give enough information to allow the non-waxy wheat to be clearly defined from the waxy wheats. Other techniques such as X-ray micro-computed tomography (μ CT) and NIR hyperspectral imaging would enable these differences to be seen far more clearly. Data from the NIR hyperspectral imaging could also be used to create models which will non-destructively determine a waxy wheat kernel from a non-waxy wheat kernel. The Rapid Visco Analyser (RVA) investigates how the starch within the flour blends affects the processing properties when heated, cooled and stirred. The peak time found no significant differences between the blends of all the lines and the control. However, the waxy wheat blends had shorter peak times than the control. This showed that the addition of waxy wheat decreased the time it took the flour blends to reach peak viscosity. The trough and breakdown values from the RVA showed how the blends lost viscosity as a result of continued heat and stirring. The breakdown, which is the difference between the peak viscosity and the trough showed no significant differences, however the trough itself did ($P < 0.05$). Only blends from line 378's trough did not differ significantly from the control. The values for the other three lines blends were much lower than the control suggesting that the waxy wheats will reduce in viscosity more due to overmixing than a non-waxy wheat. The lack of significant difference in breakdown suggests that up to 25% waxy wheat can be added without detrimentally affecting a decrease in viscosity. These results showed that although no clear differences were seen between the starch granule morphology and percentage crystallinities, the processing properties of the waxy wheat flour blends were still affected by the changing amylose: amylopectin ratios.

The time it takes for a dough made from a blend of waxy wheat and non-waxy wheat to reach its optimal consistency can be determined from the arrival time of the Farinograph and the peak time of the Mixograph. Both methods of analysis showed that as the amount of waxy wheat increased, the time to optimal consistency shortened. As waxy wheats contain only amylopectin, which is responsible for water absorption in the starch granule (Tester and Morrison, 1990), the starch will hydrate faster due to the rapid absorption of water and this results in a faster processing time (Hayakawa *et al.*, 1997). Neither the arrival time

(Farinograph), nor the peak time (Mixograph), showed any significant differences ; therefore, up to 25% addition of waxy wheat to non-waxy wheat will not significantly speed up the dough development process. However, results show that line 376_25% will form an optimal dough the quickest. As the farinograph and the mixograph gave such similar results it would be recommended that only one need be done in future studies. This will save time for doing other analysis which will give different information.

The peak height of the Mixograph indicates the strength of the gluten network which forms in the dough. This is an important factor in dough as the gluten network is what traps the carbon dioxide produced by the yeast and so a weaker dough would have a lower loaf volume (Hoseney & Delcour, 2010). No significant differences were seen between the blends and control. Lines 375, 376 and 377, however, had lower values and were thus weaker than the control, while line 378 was stronger. No significant differences were seen in protein contents between the lines and the control and so it was not this which caused the notoriously weaker dough of a waxy wheat. The reason for the weaker dough was that both the starch and the gluten needed to be hydrated and so they competed for the available water to do so (Caramanico *et al.*, 2017). As waxy wheat has a higher content of amylopectin than non-waxy wheat, it absorbed more water leaving less for the gluten and thus producing an underdeveloped gluten network, resulting in a weak dough. Seeing that line 378 produced a stronger dough than the control, its amylose: amylopectin ratio and genetics should be tested to ensure that it is in fact a waxy wheat. Up to 25% waxy wheat can be added to non-waxy wheat without significantly reducing the strength of the dough.

The stability of the dough or the tolerance to overmixing is an important aspect when developing a dough. If mixing continues after the optimal consistency is reached, the mechanical action from the mixer will begin to break the bonds in the gluten network and will result in a weak dough that will bake to an inferior loaf (Belton, 2003). This aspect of dough making is determined by the stability (Farinograph) and the tail height (Mixograph) where no significant differences were seen. It was clear that as the amount of waxy wheat increased, the stability decreased. Line 376 was least stable for both dough rheology techniques. General linear models of the tail height show that it is only the line/cultivar that has a significant ($P < 0.05$) effect on the stability and not the amount of waxy wheat present in the blend. These results indicate that the addition of waxy wheat to non-waxy wheat had a negative effect on the doughs stability and if used commercially, close attention will have to be paid to the time which the dough is mixed for as it may quickly lose its optimal consistency.

The overall appearance of a loaf of bread is what is most important to a consumer. A loaf of sandwich bread should have a high loaf volume, a fine crumb structure and a bright slice (Cauvain, 2003). The Alveograph enables bakers to predict how the crumb structure of a loaf will appear. No significant differences between the blends and the control were seen for P, L,

P/L and W. Thus concluding that the addition of up to 25% of any of the waxy wheat lines to non-waxy wheat will not detrimentally affect the processing properties of the dough as well as the crumb structure. This absence of significant difference in crumb structure was confirmed by the results of C-Cell image analysis. No significant differences were seen in number of cells, holes and area of cells between all blends and the control without the improver. This confirms the prediction from the Alveograph that the addition of up to 25% of waxy wheat will not negatively affect the crumb structure of the final loaf. The percentage concavity from the C-Cell gives an indication of the outward appearance of the loaf of bread. A higher percentage indicates that the sides of the loaf of bread have collapsed more during cooling and this is an unwanted aesthetic by the consumer. Only blend 375_25% was significantly different ($P < 0.05$) from the control without improver and had a higher percentage. All other blends from this line and line 377 also had higher percentages than the control without improver and thus had the worst loaf appearance. The blends from lines 376 and 378 did not differ significantly from the control without improver, giving the best loaf appearance. Thus, up to 25% of lines 376 and 378 may be added to non-waxy wheat to create a loaf of bread with an acceptable outward appearance. The control with a commercial shelf life improver had a significantly higher ($P < 0.05$) percentage concavity than the control without. This indicated that the outward appearance of a loaf of bread can be sacrificed to some extent in order to increase the shelf life.

One of the most useful benefits of waxy wheats is its ability to increase the shelf life of bread by retarding starch retrogradation. The final viscosity values from the RVA indicate whether a blend and line have the possibility of extending the shelf life of bread as it shows the starch retrogradation capabilities (Thiewes and Steeneken, 1997). The viscosities of all blends from line 375, 376 and 377 were lower than that of the control and showed that it did have the potential to retard starch retrogradation. Blends from line 378 had similar final viscosities to the control indicating that it did not have as much potential as the other lines. These predictions were confirmed by results from the texture analyser taken on days one, three and six. A firmer slice indicates that more starch retrogradation has taken place (Lee *et al.*, 2001). All blends from lines 375, 376 and 377 were initially softer than the control without improver on days one and three. Blends from line 378, however, had values much closer to that of the control without improver on days one and three and some blends had a higher firmness than the control, demonstrating that this line would not improve initial loaf softness. On day six the shelf life extension possibilities of the blends became clear. For line 375 and 377, an increase in firmness was seen as the amount of waxy wheat added to the non-waxy wheat increased. This contradicted what, theoretically, should have been happening, where the firmness should have been decreasing with the increase in waxy wheat (Lee *et al.*, 2001; Bhattacharya *et al.*, 2002). It is possible that the amylopectin chain lengths are so short in

these two lines that they were behaving more like amylose and thus retrograding sooner. As the amount of line 376 increased, the firmness of the loaves decreased. This is what is expected for waxy wheats and the 376_20% blend showed the best ability to extend the shelf life. Line 378 showed little change in firmness as its amount added to non-waxy wheat increased. It was concluded that only additions of up to 25% of line 376 to non-waxy wheat had the potential to extend shelf life. None of the blends or lines on day six could compete with the shelf life extension capabilities of the improver, showing that waxy wheats have more place in artisanal bakeries than in commercial ones.

The amount of waxy wheat blended with non-waxy wheat only had a significant effect ($P < 0.05$) on the peak viscosity determined by the RVA and the percentage concavity determined by the C-Cell. This showed how adding up to 25% of waxy wheat to non-waxy wheats has a minimal effect on the starch and dough rheology - and hence on the processing qualities of the dough. However, the addition of waxy wheat does significantly ($P < 0.05$) affect the appearance of the final loaf which is an important aspect in ensuring consumer satisfaction. General linear models showed that a 10% increase in waxy wheat is required to cause a significant effect on parameters used to determine final loaf quality. Lines 375 and 377 were seen for all starch and dough rheology as well as final loaf appearance to have the same effect as one another. The addition of these two waxy wheat lines to non-waxy wheat could be used interchangeably to give the same changes to dough processing properties and final loaf quality. Ultimately lines 375 and 377 had the worst loaf appearance and the firmest loaves on day six. They would not be suitable for use when increasing the shelf life of bread. General linear models of line 378 consistently had a significantly different ($P < 0.05$) effect on all parameters from the other three lines. It also did not behave entirely as a waxy wheat line should do and further genetic testing should be done to confirm that it is in fact a full waxy wheat. It would thus not be suitable for shelf life extension of bread. Line 376 often resulted in the best dough processing capabilities such as the quickest dough development time. It also created the most visually appealing loaf particularly at its highest blends (20 and 25%) while creating the softest loaf on day six. Consequently, an addition of between 20 and 20% of line 376 to a non-waxy wheat will result in a loaf of bread which has an increased shelf life whilst still remaining visually appealing to the consumer.

Chapter 7

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7. References

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