X-ray micro-computed tomography (μCT) evaluation of bubble structure to determine quality of dough and bread made from roasted wheat flour

by

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ABSTRACT

Roasting of cereals has been shown to improve its sensory properties, increase its shelf life and inactivate proteolytic enzymes promoting increased loaf volume. The breadmaking process is a series of aeration stages which ultimately affects the final crumb structure of the bread. X-ray micro-computed tomography (µCT) is a non-invasive technique capable of producing high quality three-dimensional images enabling microstructural evaluation of food products.

The aims of this study were to characterise white flour produced from roasted wheat differing in hardness and protein content using rheological and physicochemical analyses; to determine the optimal roasting conditions that would minimise the effect on the protein properties of the produced white flour; to evaluate the freeze drying of dough as a suitable sample preparation procedure to maintain the structure of the samples during X-ray µCT scanning for efficient analysis of the bubble structure of dough and foam properties of 10 g bread loaves produced from roasted wheat flour; and to evaluate the quality and shelf life of bread loaves prepared from roasted wheat flour by means of C-Cell and texture analysis.

Hard, medium and soft textured wheat kernels were roasted for 140 s at 180°C using a forced convection continuous tumble (FCCT) roaster. This resulted in the largest reductions in hectolitre mass (7.36 hg/hl), flour yield (2.33%) and moisture content (2.87%) for the hard wheat. The largest increase in damaged starch (4.54%) and flour ash (0.06%) was observed for the hard and soft wheats, respectively. Roasting resulted in gluten protein changes as a gluten network could not be formed during dough mixing with the roasted flours. The use of composite flours (80% untreated flour and 20% flour from roasted wheat) displayed the largest increase in water absorption capacity (5.2%) for the medium textured wheat. Improved alveograph P/L ratios and higher levels of free starch was observed for the hard and medium textured wheat.

The central composite design (CCD) showed no significant differences (p>0.05) for protein content, mixograph peak time and peak height for either the high or low protein roasted wheat. The roasting conditions chosen (based on trends observed) for X-ray µCT evaluation was 90°C and 86 Hz (*ca.* 130 s) as this combination maximised protein content and peak height and minimised peak time.

To evaluate the bubble structure of dough and the foam structure of bread, 20- and 40 min proofed dough as well as 10 g bread samples, produced from roasted wheat flour based on the CCD, were subjected to X-ray µCT. The use of 10 g dough and bread samples enabled scanning at a much higher resolution. A finer crumb structure and softer texture was observed for the bread produced from roasted wheat flour due to decreased strut thicknesses. Lower mixograph peak heights and increased porosity suggested a weaker gluten strength for the roasted wheat samples. The roasting conditions used did not negatively impact the foam properties of the breads.

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C-Cell analysis showed a coarser crumb structure and a darker crumb colour for breads produced from roasted wheat flour although this did not negatively impact the breads texture. More importantly, texture analysis showed the use of flour produced from roasted wheat resulted in a softer bread (i.e., lower firmness) with an increased shelf life.

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OPSOMMING

Die rooster van grane verbeter die sensoriese eienskappe en verleng die rakleeftyd daarvan. Dit inaktiveer ook proteolitiese ensieme wat verhoogde brood volume bevorder. Die broodmaakproses is 'n reeks deurlugting fases wat uiteindelik die finale krummelstruktuur van die brood beïnvloed. X-straal mikro-berekende tomografie (µBT) is 'n nie-vernietigende tegniek wat in staat is om driedimensionele beelde van hoë gehalte te produseer wat dit moontlik maak om die mikrostruktuur van voedselprodukte te evalueer.

Die doelwitte van hierdie studie was om wit broodmeel, geproduseer van geroosterde koring wat verskil in hardheid en proteïeninhoud, te karakteriseer met behulp van reologiese- en fisies-chemiese ontledings; om die optimale rooster kondisies te bepaal wat die effek op die proteïeneienskappe van die geproduseerde wit meel sal verminder; om die vriesdroog van deeg te evalueer as 'n geskikte monstervoorbereidings prosedure om die struktuur van die deegmonsters tydens X-straal µBT skandering te handhaaf vir 'n doeltreffende analise van die borrelstruktuur van deeg en skuim eienskappe van 10 g brode geproduseer van geroosterde koringmeel; en om die kwaliteit en rakleeftyd van brood berei vanaf geroosterde koringmeel te evalueer deur middel van C-Cell en tekstuur ontleding.

Harde-, medium- en sagte-tekstuur koringpitte is vir 140 s by 180°C gerooster met behulp van 'n geforseerde konveksie aaneenlopende tuimel (GKAT) rooster. Dit het gelei tot die grootste afname in hektolitermassa (7.36 hg / hL), meel opbrengs (2.33%) en voginhoud (2.87%) vir harde koring. Die grootste toename in beskadigde stysel (4.54%) en asinhoud (0.06%) is onderskeidelik waargeneem vir die harde en sagte korings. Rooster het gelei tot veranderinge in glutenproteïene, siende dat 'n glutennetwerk nie gevorm kon word, tydens die deegmengproses, met die geroosterde meel nie. Die gebruik van saamgestelde meel (80% onbehandelde meel en 20% meel van geroosterde koring) het die grootste toename in waterabsorpsie kapasiteit (5.2%) vir die medium-tekstuur koring getoon. Verbeterde alveografie P/L verhoudings en hoër vlakke van vrye stysel is waargeneem vir die harde- en medium-tekstuur korings.

Die sentrale saamgestelde ontwerp (SSO) het geen beduidende verskille ($p > 0.05$) getoon vir proteïeninhoud, miksograaf piektyd en -piekhoogte vir die hoë of lae proteïen geroosterde koring nie. Die gekose rooster kondisies (gebaseer op neigings waargeneem) vir X-straal µBT evaluering was 90°C en 86 Hz (ongeveer 130 s), siende dat hierdie kombinasie die proteïeninhoud en piekhoogte gemaksimeer het en piektyd geminimaliseer het.

Om die borrelstruktuur van die deeg- en skuimstruktuur van die brood te evalueer, is deeg wat vir 20- en 40 min gerys is sowel as 10 g brood monsters, vervaardig met geroosterde koringmeel gebaseer op die SSO, blootgestel aan X-straal µBT skandering. Die gebruik van 10 g deeg- en broodmonsters het skandering teen 'n hoër resolusie moontlik gemaak. ‘n Fyner krummel struktuur en 'n sagter tekstuur is waargeneem vir die brood wat van geroosterde koringmeel

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geproduseer is as gevolg van ‘n dunner borrel selwand. Laer miksograaf piekhoogte en verhoogde porositeit dui op 'n swakker gluten sterkte vir die geroosterde koringmonsters. Die rooster kondisies wat gebruik is het nie die skuimeienskappe van die brood negatief beïnvloed nie.

C-Cell analise het 'n growwer krummelstruktuur en 'n donkerder krummelkleur getoon vir brode wat met geroosterde koringmeel geproduseer is, alhoewel dit nie die brood tekstuur negatief beïnvloed het nie. Belangriker nog, die tekstuur analise het getoon dat die gebruik van meel geproduseer vanaf geroosterde koring gelei het tot 'n sagter brood (dit wil sê 'n laer fermheid) met 'n verlengde rakleeftyd.

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Thank you that you were always there

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RESEARCH CONTRIBUTIONS

Conference Contributions

Germishuys, Z., Deleu, L.J., Delcour, J.A. & Manley, M. (2019). Rheological characterisation of flour produced from roasted wheat. 23rd SAAFoST Biennial International Congress and Exhibition. Johannesburg, South Africa.

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Research Outputs

Germishuys, Z., Delcour, J.A., Deleu, L.J. & Manley, M. (2020). Characterization of white flour produced from roasted wheats differing in hardness and protein content. Cereal Chemistry, **97**, 339-348. https://doi.org/10.1002/cche.10250

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Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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ABBREVIATIONS

%	Percentage
°C	Degrees Celsius
µA	Microampere
µCT	Micro-computed tomography
µm	Micrometre
2D	Two-dimensional
3D	Three-dimensional
AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
BSD	Bubble size distribution
CCD	Central composite design
cm	Centimetre
cm^3	Cubic centimetre
CO_2	Carbon dioxide
CT	Computed tomography
DDT	Dough development time
FCCT	Forced convection continuous tumble
Fig.	Figure
FOV	Field-of-view
g	Gram
g/cm^3	Gram per cubic centimetre
g/min	Gram per minute
h	Hour
HLM	Hectolitre mass
HTST	High-temperature short-time
Hz	Hertz

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J	Joule
kg	Kilogram
$kg.hL^{-1}$	Kilogram per hectolitre
kV	Kilovolt
L	Extensibility
LSD	Least significant difference
max	Maximum
mb	Moisture base
min	Minute
mL	Millilitre
mm	Millimetre
mm^2	Square millimetre
mm^3	Cubic millimetre
MRI	Magnetic resonance imaging
ms	Millisecond
NaCl	Sodium chloride
P	Stability
P/L	Curve configuration ratio
PSD	Particle size distribution
ROI, ROIs	Region-of-interest, Regions-of-interest
RSM	Response surface methodology
s	Second
SEM	Scanning electron microscopy
VOI, VOIs	Volume-of-interest, Volumes-of-interest
W	Deformation energy
WAC	Water absorption capacity
WBC	Water binding capacity

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CHAPTER 1
INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most important cereal crops in the world and currently the second most produced crop following maize. It forms part of the staple diet for the majority of the global population. This is attributed to its nutritional value, agronomical adaptability and it being an excellent source of energy. The flour obtained from milled wheat is used to produce an array of different products including but not limited to pasta, cakes and biscuits (Raigar *et al.*, 2017). Wheat flour is further unique in the sense that a viscoelastic dough can be prepared from it to produce bread (Mondal and Datta, 2008).

Bread is one of the most widely consumed food products in the world. This is due to its affordability as well as the fact that it is fortified with vitamins and minerals (in South Africa) required for a healthy diet (FoodStuff-SA, 2017). During the term spanning from October 2018 to September 2019, approximately 2.37 billion loaves of bread were manufactured in South Africa. This was a 4% increase from the previous year. Of these 2.37 billion loaves baked, approximately 49.2% were from white flour (SAGL, 2020).

Roasting within the food industry is generally performed to improve and or alter the quality of a product as well as to increase its processing efficiency (Chung *et al.*, 2011). The roasting of cereal grains is traditionally practised in India to improve the grains' organoleptic properties as well as increase its shelf life (Murthy *et al.*, 2008). Roasting is considered to be a high-temperature short-time (HTST) process where both heat and mass transfer take place (Fadai *et al.*, 2017). During the roasting process, wheat kernels puff up as a result of the high internal pressure changing both their moisture content and volume (Schoeman *et al.*, 2016a; Schoeman and Manley, 2019).

Several different roasting techniques exist and have been applied to a range of different cereal grains to achieve a desired outcome. These techniques include fluidised bed (Murthy *et al.*, 2008), sand and microwave (Sharma and Gujral, 2011; Qu *et al.*, 2017), screw conveyer (Jogihalli *et al.*, 2017) and oven (Mariotti *et al.*, 2006; Schoeman and Manley, 2019) roasting. These techniques have various drawbacks as they may be tedious to operate, unhygienic, have low productivity and lead to non-uniform roasting of the product (Murthy *et al.*, 2008).

Forced convection continuous tumble (FCCT) roasting is an emerging roasting technique that eliminates most of these drawbacks. FCCT roasting uses superheated steam, produced from moisture in the product, to roast the sample. The process is energy efficient as the superheated steam is continuously recirculated through the thermally insulated rotating cylinder. As the product is continuously mixed, the roasting process is much more uniform (Flinn, 2012; Schoeman *et al.*, 2016a).

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Roasting leads to several physical and chemical changes within the wheat which can be either beneficial or detrimental to the final product. These changes greatly depend on the roasting method and conditions (roasting time and temperature) used.

Roasting has been shown to improve the flavour, colour and texture of cereal grains (Murthy *et al.*, 2008). Additionally, it also increases the grains' shelf life by lowering the water activity and thus retarding the growth of spoilage microorganisms (Ranganathan *et al.*, 2014). Roasting has further been shown to inactivate proteolytic enzymes promoting an increase in loaf volume (Vázquez *et al.*, 2001). Furthermore, an increase in softness and springiness and a decrease in chewiness has also been observed for steamed bread produced from wheat that had been subjected to microwave heating (Qu *et al.*, 2017).

Roasting may also result in detrimental changes in the wheat. Wheat is rich in carbohydrates and as such it is susceptible to acrylamide formation during roasting (Muttucumaru *et al.*, 2006; Claus *et al.*, 2008). Acrylamide, a potentially carcinogenic compound, is formed as a result of interactions between reducing sugars and amino acids during the Maillard reaction (Nematollahi *et al.*, 2019). Roasting can also influence the milling or flour yield of the wheat. Schoeman and Manley (2019) observed a reduction in flour yield for wheat roasted at 180°C using an oven and FCCT roaster.

Starch and protein, the two main components in flour, are also affected by roasting. Depending on the severity of these changes it could directly influence the quality of the bread. Schoeman and Manley (2019) observed a decrease in protein content for wheat roasted at 180°C using the oven and FCCT roasting methods. As a result of the decreased protein content the mixing time of the dough increased. Vázquez *et al.* (2001) also observed an increase in mixing time for wheat roasted between 40 and 100°C.

With reference to starch, Lorenz *et al.* (1993) reported an increase in the water absorption capacity (WAC) for starch from roasted wheat. This resulted in a longer mixing time and a decreased bread loaf volume. The increased WAC leads to starch absorbing more water and proteins having to compete for the same water. If the gluten proteins cannot properly hydrate a strong gluten network cannot be formed (Barrera *et al.*, 2007).

The texture and thus quality of a loaf of bread is greatly dependent on its pore/crumb structure and how these pores/bubbles are distributed throughout the loaf (Scanlon and Zghal, 2001). The final crumb structure of bread starts with the entrainment of air cells during mixing (Bellido *et al.*, 2006). As mixing continues, the incorporated air cells break up which results in an increase in the number of cells and a decrease in the size of the cells. These cells act as nucleation sites for CO_2 gas production by yeast during the proofing stage (Mills *et al.*, 2003). It is further imperative that enough cells are incorporated during mixing as no further occlusion of gas cells take place in succeeding stages (Scanlon and Zghal, 2001).

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During proofing, gluten plays an important role in bubble formation. The gluten network, formed during mixing, stretches to form thin films encasing the air bubbles. Additionally, gluten also plays a role in bubble stabilisation (Mills *et al.*, 2003). During proofing, the size and separation of bubbles influences the rate of disproportionation of these bubbles (Bellido *et al.*, 2006; Chakrabarti-Bell *et al.*, 2014). Disproportionation refers to the mass transfer of gas from smaller to larger bubbles resulting in a coarser foam structure. This process is driven by the Laplace pressure within the bubbles (Mills *et al.*, 2003; Sroan *et al.*, 2009; Koksel *et al.*, 2016). The pressure is generated by the tension on the bubble surface which contracts, keeping it spherical. When two bubbles come into close proximity to one another a net transfer of CO_2 gas takes place from the smaller to the larger bubbles (Mills *et al.*, 2003).

At the end of proofing and the start of baking, the bubbles within the dough undergo a rapid expansion as a result of increased CO_2 gas production and steam formation. This results in coalescence of the bubbles due to failures in the gluten-starch matrix which form the bubble walls. Coalescence refers to the process where two or more adjacent cells merge resulting in a decrease in the number of cells and an increase in the mean cell size (Mills *et al.*, 2003). The failure in the gluten-starch film results in instabilities in the bread's foam structure. The addition of fat/shortening to the dough formulation can help stabilise these bubbles against coalescence. Lipid crystals move to the gas-liquid interface and come into direct contact with the gas bubbles. These lipid crystals melt during the baking stage allowing bubble growth with minimal rupturing (Brooker, 1996; Autio and Laurikainen, 1997; Mills *et al.*, 2003).

During baking, the dough heats up and reaches a critical temperature where starch gelatinization occurs. This freezes the breads foam structure preventing any further bubble expansion from occurring (Mondal and Datta, 2008). The bubbles rupture leading to a rapid loss of gas subsequently forming a network of interconnecting cells (Mills *et al.*, 2003).

Upon cooling of the baked bread, the process of staling starts. Bread staling refers to the time-dependent loss in quality of texture and flavour and correlates with the shelf life of the bread. Staling results in crumb firming and softening of the bread crust. At present, staling is thought to be the result of water transfer within the bread as well as retrogradation of the starch molecules (León *et al.*, 2006; Besbes *et al.*, 2013). The texture of a loaf of bread is an important quality attribute (Scanlon and Zghal, 2001), therefore as staling affects the texture of the bread it results in a decrease in consumer acceptance (Gray and Bemiller, 2003).

Evaluation of the texture and shelf life of bread is important to determine its quality. Several studies have evaluated the foam structure of bread slices by means of a C-Cell instrument (Sroan *et al.*, 2009; Ktenioudaki *et al.*, 2010; van Riemsdijk *et al.*, 2011; McCann and Day, 2013). C-Cell is a digital imaging system used to determine the quality of the bread crumb. Additionally, texture analysis has also been used to determine the effect of staling on the bread crumb as well as to study the shelf life of bread in terms of its firmness.

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Studying the bubble structure of dough traditionally involved freezing the dough and sectioning it, but this affects the integrity of the sample (Bellido *et al.*, 2006). Additionally, magnetic resonance micro-imaging (Van Duynhoven *et al.*, 2003) and electron microscopy (Bache and Donald, 1998) have also been used, but these too have shortcomings due to their limitation of two dimensions and invasive nature.

In recent years X-ray micro-computed tomography (µCT) has been used with great success to study the microstructure of a range of different food products (Whitworth and Alava, 1999; Falcone *et al.*, 2004; Babin *et al.*, 2006; Bellido *et al.*, 2006; Van Dyck *et al.*, 2014; Koksel *et al.*, 2016; Schoeman *et al.*, 2016a). It is a non-invasive technique capable of producing high quality three dimensional (3D) images. X-ray µCT works on the principle of rotating a sample between a fixed X-ray source and a detector while back projection data is captured at each rotation step. The data is used to obtain cross-sectional images which can in turn be used to generate a 3D representation of the sample (Schoeman *et al.*, 2016b).

The greater aim of this study was to evaluate the bubble structure of dough and the foam structure of bread produced from roasted wheat flour by means of X-ray micro-computed tomography to determine its quality. The specific objectives that was set out to achieve this aim were to:

1. characterise white flour produced from roasted wheat differing in hardness and protein content by means of rheological and physicochemical analyses;
2. determine the optimal roasting conditions that would minimise the effect on the protein properties of the produced white flour;
3. evaluate (1) the freeze drying of dough as a suitable sample preparation procedure that would maintain the structure of the samples during X-ray µCT scanning for efficient analysis of its bubble structure and (2) the foam properties of 10 g bread loaves produced from roasted wheat flour; and
4. evaluate the quality and shelf life of bread loaves prepared from roasted wheat flour by means of C-Cell and texture analysis.

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CHAPTER 2

Literature Review

Wheat roasting, dough bubble structure formation and X-ray micro-computed tomography evaluation – a review

2.1 INTRODUCTION

Wheat (*Triticum aestivum L.*) is a unique cereal grain as in its milled form (wheat flour) it has the ability to form a viscoelastic dough with the addition of water and energy input in the form of mixing. This viscoelastic dough can be used to produce a global staple food, namely bread (Mondal and Datta, 2008). Bread is one of the most widely consumed food products in the world due to its affordability as well as the fact that it is fortified with vitamins and mineral (in South Africa) (FoodStuff-SA, 2017). It is estimated that approximately 2.37 billion loaves of bread were produced in South Africa in the 2018/2019 term of which almost half were from white flour (SAGL, 2020).

This review will address the dough formulation and the function of each of the ingredients. Secondly, the breadmaking process will be discussed with specific reference to the effect each of the different stages has on the bubble structure of the dough and the foam structure of the bread. Additionally, the quality and shelf life of bread in terms of bread staling as well as the evaluation thereof by means of C-Cell and texture analysis will be discussed. This review will furthermore explore the roasting of cereal grains and how these grains are affected by the roasting process. Finally, X-ray micro-computed tomography (µCT) will be explored as a technique for evaluating the bubble structure of dough and foam structure of bread.

2.2 DOUGH FORMULATION

Dough is considered to be a complex viscoelastic system consisting of a range of different ingredients and comprising of three different phases, namely liquid, solid and gas (Jekle and Becker, 2011). The dough formulation comprises of both essential and non-essential ingredients. Essential ingredients required in the breadmaking process include flour, water, salt and yeast (Goesaert *et al.*, 2005; Mondal and Datta, 2008). Ingredients considered to be non-essential, and added to improve/enhance shelf life and palatability include sugar, fat, enzymes and improvers (Avramenko *et al.*, 2018). Of all the essential ingredients, flour and water are the most important as these have the greatest effect on the texture and the crumb of the bread (Mondal and Datta, 2008). In the following section the essential ingredients, as well as the non-essential ingredients will be discussed.

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2.2.1 Flour

Flour is the main ingredient in dough and is unique in the sense that a viscoelastic dough can be formed when water is added and mixed (Gan *et al.*, 1995). Flour mainly consists of starch, water and protein and these give structure to the dough (Avramenko *et al.*, 2018). There are also some minor components present such as non-starch polysaccharides and lipids which contribute to the quality aspect of the dough (Goesaert *et al.*, 2005; Mondal and Datta, 2008).

The viscoelastic properties obtained when mixing flour and water allow for gas bubbles to be entrained in the dough. These unique properties are made possible by the wheat storage protein gluten. Gluten is primarily comprised of two protein classes, namely glutenins and gliadins (Wieser, 2007). Glutenin contributes to the strength and elasticity of the dough, aiding in its cell retention properties. Gliadin contributes to the viscosity and extensibility of the dough making the expansion of gas cells possible (He *et al.*, 1992; Joye *et al.*, 2009; Avramenko *et al.*, 2018).

The protein content of flour greatly influences the quality of the bread produced from it. Dough produced from flour containing high protein contents have been shown to have improved viscoelastic properties and to entrain a larger amount of gas bubbles. This results in bread loaves with a larger volume and better quality (Campbell *et al.*, 2001; Chin and Campbell, 2005; Koksel and Scanlon, 2012).

The most abundant component present in wheat flour is starch, contributing around 70 - 75% of the flour content. Starch is comprised of two polysaccharide molecules namely amylose, a small molecule with minimal branching and amylopectin, a much larger and highly branched molecule (Goesaert *et al.*, 2005). During wheat milling, damage to the starch occurs resulting in a loss of birefringence, an increase in the water absorption capacity of the starch as well as a greater susceptibility to hydrolysis by enzymes (Delcour and Hoseney, 2010a).

During the development of the dough, the gluten proteins form a continuous viscoelastic network by means of disulphide bonding and noncovalent interactions, such as hydrogen and ionic bonding, hydrophobic interactions and van der Waals forces (Salvador *et al.*, 2006). During mixing, proteins become hydrated, partially unravel and re-orient in the direction of shear, developing strong protein-protein interactions (Salvador *et al.*, 2006; Avramenko *et al.*, 2018). Embedded in this gluten matrix is a second phase comprising of damaged as well as intact starch granules, water and water-soluble components. Starch is capable of absorbing large amounts of water and when heated the starch granules swell and gelatinise which reinforces the gluten network (Goesaert *et al.*, 2005; Avramenko *et al.*, 2018).

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2.2.2 Water

One of the most fundamental events in the breadmaking process is the addition of water to the flour during mixing as this is responsible for forming a cohesive and viscoelastic dough. Water functions to hydrate the gluten proteins and starch as well as to dissolve soluble flour components and provide a medium within the dough for chemical and biochemical reactions to take place, which ultimately affect the shelf life of the baked bread (Gan *et al.*, 1995; Autio and Laurikainen, 1997; Avramenko *et al.*, 2018). The amount of water added greatly influences the rheological properties of the dough (Gan *et al.*, 1995). MacRitchie (1976) showed that below a water content of 35% almost no gas is retained by the dough. When the water content is increased up to around 44% the dough's gas retention properties greatly improve.

2.2.3 Salt

Salt (NaCl) plays an important role in the breadmaking process. It strengthens the gluten network and increases dough stability (Belz *et al.*, 2012). Salt also increases mixing time resulting in increased protein-protein interactions. Salt further retards the rate of fermentation by yeast leading to a decrease in the rate of bubble expansion (Calderón-Domínguez *et al.*, 2005; Farahnaky and Hill, 2007; Mondal and Datta, 2008). This effect has been observed with increases in salt content and has been attributed to salt-induced inhibition of the yeast (Lynch *et al.*, 2009). When the salt content is increased it hinders the yeasts metabolic activity as a result of increased osmotic pressure causing loss of water from the yeast cells (Hutton, 2002; Miller and Hoseney, 2008). Salt further has the added benefit of enhancing the flavour of the bread as well as prolonging its shelf life by decreasing the water activity thus acting as a preservative (Miller and Hoseney, 2008; Belz *et al.*, 2012).

2.2.4 Yeast

Yeast (*Saccharomyces cerevisiae)*, a facultative anaerobic microorganism which can metabolise both in the presence and absence of oxygen (favouring aerobic metabolism), is the leavening agent used in breadmaking (Avramenko *et al.*, 2018). Yeast is active at temperatures ranging from 0–55°C but is typically most active between 35–40°C. Yeast converts simple carbohydrates into CO_2 gas and ethanol. The CO_2 gas produced by the yeast provides the leavening action to the dough and subsequently the bread. During the proofing/fermentation stage the CO_2 gas expands the bubbles within the dough (Mills *et al.*, 2003; Jha *et al.*, 2017; Avramenko *et al.*, 2018). During fermentation yeast further produces by-products which impart flavour to the bread (Avramenko *et al.*, 2018).

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2.2.5 Fat/Shortening

The addition of fat or shortening to dough results in bread loaves with improved loaf volume and a finer and more uniform crumb structure with thinner cell walls (Brooker, 1996; Watanabe *et al.*, 2003). When lipid crystals move from the lipid phase to the gas-liquid interface they come into direct contact with the gas in the bubbles. As gas cells expand during fermentation more crystals are absorbed to the bubble surface. These crystals melt during baking which allow the bubbles to grow without rupturing. This allows for a large volume and fine crumb structure to be achieved in the baked bread loaf (Brooker, 1996; Autio and Laurikainen, 1997). In addition, lipids further stabilise gas cells within the dough resulting in greater air incorporation during mixing (Brooker, 1996). It has been shown that the use of fat comprised of a large number of small crystals displayed improved gas cell stabilisation when compared to fewer crystals of a larger size (Brooker, 1996; Autio and Laurikainen, 1997).

2.2.6 Sugar

Sucrose is a disaccharide comprised of the two monosaccharides α-D-glucose and β-D-fructose. Although sugar is not an essential ingredient in the breadmaking process, the addition of it can be beneficial. Sugar promotes the production of CO_2 gas by yeast during the fermentation stage of the breadmaking process (Mondal and Datta, 2008; Trinh *et al.*, 2015). It further contributes to browning of the crust during baking as a result of the Maillard reaction. In addition, sugar contributes to the sweetness of the bread and preserves moisture through moisture absorption (Trinh *et al.*, 2015; Avramenko *et al.*, 2018).

2.3 BREADMAKING PROCESS

The breadmaking process and thus the formation of the final foam structure (or bubble structure) within a loaf of bread can be thought of as a series of processes which directly impacts the texture and appearance of the bread. There are three main processes involved in breadmaking and these include the formation of the dough, fermentation or proofing and baking (Autio and Laurikainen, 1997; Scanlon and Zghal, 2001).

Gas bubbles play an imperative role in breadmaking as they make up approximately 9–20% of the total volume of the dough at the end of mixing (Whitworth and Alava, 1999; Romano *et al.*, 2013). Subsequent stages following mixing, such as punching sheeting and moulding are responsible for further subdivision of these gas bubbles (Gan *et al.*, 1995). At the end of proofing the total volume of gas bubbles accounts for approximately 70–75% of the dough's volume, as a result of bubble inflation due to CO_2 gas production by the yeast. This volume further increases to approximately 75–85% of the total loaf volume at the end of baking (Campbell and Shah, 1999).

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During the mixing process there are three stages that determine the number, size and distribution of bubbles within the dough. These include gas entrainment or gas occlusion, gas disentrainment as well as bubble break-up (Elgeti *et al.*, 2017; Sadot *et al.*, 2017). The first two stages ultimately determine the volume of gas within the dough whereas the last stage affects the bubble size distribution within dough (Campbell and Shah, 1999; Chin *et al.*, 2004; Trinh *et al.*, 2013).

2.3.1 Gas cell formation

Mixing

Dough formation starts with the mixing process, which serves several functions. Mixing firstly combines the ingredients into a uniform dough, it encourages hydration of the ingredients, it incorporates air bubbles into the dough creating the gas nuclei for CO_2 expansion, it evenly distributes the yeast within the dough and it is responsible for developing the gluten network (Autio and Laurikainen, 1997; Scanlon and Zghal, 2001).

When water is added to the dry ingredients during the initial stage of mixing, most of the water is absorbed via hydrophilic groups on the protein molecules. The extent of water uptake by starch will depend on the amount of shearing damage caused during the milling process (Scanlon and Zghal, 2001).

The formation of a continuous viscoelastic network during dough development is as a result of the gluten proteins. During mixing, proteins are hydrated, partially unravelled and re-oriented in the direction of shear thus developing strong protein-protein interactions. Disulphide and hydrogen bonds as well as hydrophobic interactions stabilise these protein-protein interactions forming the viscoelastic network (Avramenko *et al.*, 2018).

The final crumb structure of bread is greatly dependent on the initial formation of or entrainment of gas cells into the dough during the mixing process. All processing steps following mixing, which involve the manipulation and retention of these gas cells, further contribute to the final crumb structure. During mixing, air is incorporated into the liquid phase of the dough creating gas cells. As the mixing process continues, the incorporated air cells/bubbles start to break up resulting in an increase in the number of cells and a decrease in the size of the cells present in the dough (Mills *et al.*, 2003).

The size of the bubbles within the dough created by the mixing process is crucial as it directly influences bubbles growth during proofing/fermentation (Mills *et al.*, 2003). The bubbles formed during mixing further act as nucleation sites for CO_2 gas production during proofing (Mills *et al.*, 2003).

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CO_2 gas produced by the yeast, within the liquid phase of the dough during proofing, diffuses into the nuclei as a result of a concentration gradient that is formed. This results in the expansion of the nuclei into gas cells giving rise to the dough and reducing its density (Scanlon and Zghal, 2001; Mills *et al.*, 2003).

As no further occlusion of gas cells take place during the succeeding stages of the breadmaking process, it is imperative that enough gas bubbles are incorporated into the dough during mixing (Scanlon and Zghal, 2001). It has been estimated that approximately 10^{12}–10^{14} m^3 bubbles are formed during mixing with sizes ranging from 10–100 µm. Only approximately 1 % of the gas bubbles formed during mixing end up in the final bread loaf, suggesting that the remainder of the bubbles disappear in the processing steps following mixing (Liu and Scanlan, 2003).

The disappearance of gas cells post-mixing is mainly as a result of two different processes, namely disproportionation and coalescence (Liu and Scanlan, 2003; Chakrabarti-Bell *et al.*, 2014).

Disproportionation

Disproportionation refers to the mass transfer of gas from smaller to larger bubbles, leading to coarsening of the foam structure (Mills *et al.*, 2003). This process is as a result of the Laplace pressure within the bubbles. This pressure is generated by the tension on the bubble surface which acts around the bubble contracting it and keeping it spherical. This contraction of the bubbles induces pressure increases within the bubbles and is known as the Laplace pressure. Laplace pressure is defined by the Young-Laplace equation:

$$\Delta P = \frac{2\gamma}{r}$$

where:

ΔP = the difference in Laplace pressure between the inside and the outside of the bubble

γ = the bubble surface tension

r = the radius of the bubble

From this equation it is clear that ΔP will be greater for smaller bubbles (i.e., bubbles with a smaller radius). Consequently, when two bubbles come into close proximity, a net transfer of CO_2 gas will take place from the smaller to the larger bubbles. This will ultimately result in the disappearance of the smaller bubbles, coarsening the foam structure over time.

This equation further explains why yeast are not capable of creating new cells. The pressure within a cell is greater than that outside the cell and even more so the smaller the cell is. If yeast

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was to create a new cell, the radius of that new cell would have to start at zero. The yeast would thus have to generate an infinite amount of pressure in order to create the new cell.

2.3.2 Fermentation

Fermentation is an essential stage in the breadmaking process and a very important link between mixing and baking. Yeast (Saccharomyces cerevisiae) is the leavening agent most frequently used in breadmaking and converts simple fermentable sugars into CO_2 gas and alcohol (Autio and Laurikainen, 1997; Cafarelli *et al.*, 2014). Carbon dioxide gas production by yeast occurs in stages. The net chemical reaction of glucose fermentation by yeast is depicted below:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

where: $C_6H_{12}O_6$ is glucose,

C_2H_5OH is ethanol, and

CO_2 is carbon dioxide.

Although carbon dioxide gas and ethanol are the main products produced by yeast during fermentation, yeast also produces hydrogen peroxide as well as aroma and flavour compounds.

During fermentation the dough development process continues as the gas cell membranes are continuously stretched in a biaxial way. This greatly affects the protein structure as the gluten network is hardened (Belz *et al.*, 2012). Additionally, fermentation also results in a more elastic gluten matrix. This allows the gas cells to withstand expansion.

The fermentation process consists of several phases or steps that each further change or manipulate the gas bubbles. Following mixing, dough is divided into portions of similar weight and rounded into smooth balls. Between dividing and rounding of the dough an intermediate proofing step is performed commonly referred to as the first proof or resting period (Jha *et al.*, 2017; Avramenko *et al.*, 2018).

The resting period serves several functions. Resting firstly serves to develop the gas retaining properties of the dough. Secondly it activates the yeast to produce CO_2 gas and lastly it relaxes the gluten polymers making dough shaping easier (Marsh and Cauvain, 2007; Jha *et al.*, 2017; Avramenko *et al.*, 2018). Resting further leads to increases in bubble size as a results of CO_2 gas diffusion into the bubbles. The increase in bubble size results in an increase in the porosity of the dough (Shehzad *et al.*, 2010; Jha *et al.*, 2017). Porosity is defined as the ratio of the void volume (i.e. the gas inside the bubbles) to the total volume (i.e. gas and material volume) (Wang *et al.*, 2011).

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Jha *et al.* (2017) observed that after mixing, the porosity of the dough was 13.48%. The porosity of the dough significantly increased to 20.01% following a resting period of 20 min. The authors postulated the increase in porosity to be as a result of an influx of CO_2 gas into the cells created during mixing. It was further reported that during the resting period the number of bubbles in the 50–250 µm range decreased whereas the number of bubbles in the 250–2500 µm increased.

Following resting and rounding, the dough balls undergo sheeting and moulding. During the sheeting process the dough balls are flattened whereafter the flattened dough is curled up into a cylinder and seam sealed by the sheeting rollers. Sheeting functions to expel CO_2 gas from the dough, to redistribute the yeast throughout the dough and lastly to reorganise the protein network (Autio and Laurikainen, 1997; Avramenko *et al.*, 2018).

Once the dough has been moulded, it is placed into bread pans and the final proofing stage commences. During this stage even more CO_2 gas and ethanol are produced by the yeast. This causes further expansion of the bubbles resulting in the dough rising. The final proof is usually performed at a temperature between 35–40°C as yeast is most active in this range. The quality of the gluten network greatly affects the amount of CO_2 gas retained within the proofed dough (Avramenko *et al.*, 2018).

Coalescence

At the end of the proofing stage and the beginning of baking, the gas bubbles experience a rapid expansion phase. This rapid expansion phase is the result of an increase in CO_2 gas production as well as steam formation. The increase in size and volume of the gas bubbles result in failures in the gluten-starch matrix which form the walls of the bubbles (Mills *et al.*, 2003). This further results in instabilities of the breads foam structure at the end of the proofing stage (Gan *et al.*, 1995; Mills *et al.*, 2003) and in turn leads to coalescence of the bubbles (Mills *et al.*, 2003; Miś *et al.*, 2018).

Coalescence is defined as the process whereby two or more adjacent cells merge, resulting in a decrease in their numbers while the mean cell size increases. Coalescence greatly affects the texture of the crumb as it increases the heterogeneity of the gas cells in terms of their volume (Miś *et al.*, 2018).

2.3.3 Baking

Baking is one of the most important steps in the breadmaking process. A range of different biological, physical and chemical changes take place during the baking process and temperature plays an important role in these changes (Mondal and Datta, 2008). The culmination of these changes ultimately provides the oven spring of the bread loaf and determines the crumb or foam structure of the bread.

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At the start of baking the maximum fermentation rate of the yeast is reached at around 35–70°C. The increased CO_2 gas production results in the saturation of the liquid phase of the dough. Subsequently, the CO_2 gas is transferred to the gas cells within the dough. This results in the expansion of the cells, increasing their volume and leading to rising of the dough (Mondal and Datta, 2008; Avramenko *et al.*, 2018).

When the temperature of the dough reaches the 60–70°C point, the yeast start dying off. From this point on no more CO_2 gas is produced. This is also considered a critical temperature as gelatinisation of the starch occurs at around 60°C (Mills *et al.*, 2003). During gelatinisation, the starch granules swell and lose their crystallinity. This can be observed by the disappearance of the Maltese cross (birefringence) (Avramenko *et al.*, 2018). As heating continues the starch reaches a point where it can no longer absorb more water and subsequently bursts (Gan *et al.*, 1995; Mills *et al.*, 2003).

In addition to starch gelatinisation, at around 70°C the gluten proteins start to denature. This results in the gluten proteins becoming stiff and forming a gel. Although denaturation of the gluten may contribute to the final crumb structure of the bread it is ultimately the gelatinised starch which is responsible for halting any further bubble growth essentially freezing the foam structure (Mills *et al.*, 2003).

This results in the rupture of the starch-gluten matrix which surround the bubbles forming an interconnecting network of gas cells leading to an open sponge structure. Rupturing of the bubbles further allows steam and gas to escape (Gan *et al.*, 1995; Avramenko *et al.*, 2018). In the event that the starch-protein matrix does not rupture it will result in the collapse of the foam structure (Mills *et al.*, 2003). This will negatively affect the final quality and texture of the bread.

2.4 QUALITY AND SHELF LIFE

Bread is one of the most widely consumer food products in the world and forms part of the staple diet of the majority of the global population. Its quality is thus imperative from a consumer's perspective. The texture of a loaf of bread greatly influences this perspective in terms of quality and acceptability (Scanlon and Zghal, 2001). The texture of a bread is comprised of different properties which are related to the crumb structure. These properties include the size of the pores (or bubbles), how these pores are distributed throughout the bread as well as the overall porosity of bread (i.e. the ratio of air volume to the total volume) (Scanlon and Zghal, 2001).

2.4.1 Bread staling

The main process which is responsible for the loss of quality of a bread is staling. Bread staling is referred to as the undesirable changes in terms of loss of quality and flavour that take place between the time the bread is taken out of the oven to when it is consumed. The main undesirable

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changes that take place during staling include crumb firming, softening of the crust as well as loss of flavour (Aguirre *et al.*, 2011; Torrieri *et al.*, 2014). These changes all negatively impact the consumers acceptability of the bread in terms of its organoleptic properties (Gray and Bemiller, 2003).

A staggering amount of research has been conducted over the last several decades in an attempt to determine the mechanism(s) by which bread staling occurs (Le-Bail *et al.*, 2009; Aguirre *et al.*, 2011; Besbes *et al.*, 2013a; Curti *et al.*, 2014; Amigo *et al.*, 2016; Rathnayake *et al.*, 2018), but still no definitive mechanism has been agreed upon (Gray and Bemiller, 2003; Fadda *et al.*, 2014). Bread staling is thought to be the result of a combination of different processes. Currently there are two main processes thought to contribute to bread staling (León *et al.*, 2006; Besbes *et al.*, 2013a).

The first is the migration of moisture within the bread. This process affects both the crumb as well as the crust of the bread. When moisture migrates from the crumb to the crust it results in the crumb becoming dry and losing its softness (Curti *et al.*, 2011, 2014). Additionally, the migration of moisture to the crust results in the crust losing its fresh and crisp texture becoming soft and leathery.

The second process is retrogradation of the starch molecules (Hug-Iten *et al.*, 2003). Starch mainly consists of two fractions, namely amylose (a linear polymer) and amylopectin (a highly branched polymer) (Delcour and Hoseney, 2010b). These two polysaccharides are responsible for bread staling (Hug-Iten *et al.*, 2003). During baking the starch granules swell and amylose starts to diffuse out. Upon cooling of the bread, the amylose chains link forming a crystalline structure. This provides the initial shape, firmness and strength to the bread loaf (Wang *et al.*, 2015).

During storage of the bread, amylopectin undergoes retrogradation. Retrogradation refers to the process whereby amylopectin recrystalises upon cooling. This results in the loss of moisture ultimately drying out and firming the bread. As this process is slow and occurs over a period of a few days, amylopectin appears to be the main contributor in the bread staling process (León *et al.*, 2006; Wang *et al.*, 2015).

2.4.2 Evaluation of bread quality and texture

Determining the texture and shelf life of a bread is of utmost importance to the baking industry to ensure a quality product is delivered to the end consumer. Within the baking industry the degree of aeration or the foam structure of bread slices can be evaluated by means of separating the pore structure from the cell wall structure. This allows for quantification of the visual texture of the bread. This quantification process is made possible by the use of a C-Cell instrument (van Riemsdijk *et al.*, 2011). This instrument is a digital imaging system, combined with image analysis, and used

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worldwide by the baking industry to analyse the crumb structure of the bread to determine its quality.

Several studies have been performed using C-Cell to evaluate and understand the breadmaking process and how changes in the process affect the final loaf of bread. Sroan *et al.* (2009) determined, by means of C-Cell, that different levels of flour lipids resulted in insignificant variations in the average cell elongation and the number of gas cells present in the bread. The authors indicated that lipids may not be causing variations in the gluten-starch matrix. Another study investigated eight wheat varieties from different geographical regions to examine their rheological properties and use this data to discriminate between the different varieties. Furthermore, the breadmaking potential of these different wheat varieties was predicted using C-Cell (Ktenioudaki *et al.*, 2010). McCann and Day (2013) investigated the effect of different sodium chloride (NaCl) concentrations on the gluten network formation during mixing and how these concentrations affected the rheological properties of the dough. Additionally, test baking was performed, and C-Cell was used to gain insight into the effect of the different NaCl concentrations on baking performance.

In addition to C-Cell, texture analysis is used to determine the rate and or effect of staling on the bread crumb. This involves measuring the hardness and resilience or springiness of the crumb by applying a force and measuring the deformation of the bread slices. Texture analysis can further be used to study the shelf life of bread in terms of its firmness. This is due to the fact that firmness is a widely used indicator that a loaf of bread is losing its freshness and thus becoming stale (i.e. reaching the end of its shelf-life) (Gray and Bemiller, 2003).

A number of studies have been performed where texture analysis was utilised to evaluate bread staling. Purhagen et al, (2011) conducted a study using texture analysis, in combination with other tests, to evaluate the staling rate of bread as effected by the addition of different flours/starches to the baking formulation. Curti et al. (2014) investigated the effect of different levels of gluten on bread staling using texture analysis among others. Another study assessed differences in the staling process of white wheat bread as affected by maltogenic amylase and storage time using texture analysis as one of the analytical techniques (Amigo *et al.*, 2016).

2.5 ROASTING

Within the food industry, roasting is commonly performed with the aim of improving or altering the quality of a product and/or to increase its processing efficiency (Chung *et al.*, 2011). Roasting is defined as a high-temperature short-time (HTST) process where both heat and mass transfer takes place (Fadai *et al.*, 2017). During the roasting process wheat kernels puff up as a result of the high internal pressure. This changes both the volume and the moisture of the kernel (Schoeman *et al.*, 2016a; Schoeman and Manley, 2019).

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2.5.1 Roasting techniques

Several different roasting techniques exist and have been applied to a range of different products to achieve a desired outcome. There is an abundance of literature relating to roasting of different cereals using different roasting methods. In the current review the different roasting techniques will not be discussed as such but rather the different products and how these techniques were used. Below follows the most important findings of some of these studies.

The effect of fluidised bed roasting on sorghums functional, sensory and microstructural properties was evaluated by Ranganathan *et al.* (2014). The roasted sorghum displayed a higher stability to retrogradation and an increased water absorption capacity making it suitable for instant mixes, porridges and soups.

An investigation into sand roasting vs. microwave cooking of barley and its effects on antioxidant properties was performed by Sharma and Gujral (2011). The authors reported that both roasting methods significantly affected the antioxidant properties of the barley although sand roasted barley displayed a higher antioxidant activity than that of microwaved barley.

Qu *et al.* (2017) evaluated the effects of microwave cooking of wheat on its gluten content, enzyme activity, storage stability and rheological properties. It was reported that microwave heating of wheat resulted in increased dough development time, increased starch damage as well as improved gluten quality which was dependent on the heating time.

Lastly, Mariotti *et al.* (2006) evaluated the effect of gun puffing on five different cereal grains of which wheat was included. The authors reported significant changes in starch structure and water holding capacity. It was suggested that flour from the roasted grain be incorporated into snack foods. It was further suggested that the addition of small amounts of flour from puffed cereals to bread dough could slow down the staling process resulting in bread staying softer for longer.

These techniques have various drawbacks as they may be tedious to operate, have low productivity and lead to non-uniform roasting of the cereal grains (Murthy *et al.*, 2008). Forced convection continuous tumble (FCCT) roasting can eliminate most of these drawbacks.

2.5.2 Forced convection continuous tumble roasting

Forced convection continuous tumble roasting works on the principle that hot air is forced through the product producing superheated stream from the moisture within the product. As the superheated steam is continuously recirculated through the thermally insulated roasting cylinder, the process is very energy efficient. Due to the fact that the product is continuously mixed as a result of the rotating cylinder, the roasting process is much more uniform. The superheated steam further enhances even roasting of the product.

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Additional advantages of using a FCCT roaster include that a range of different products can be roasted, the operation is continuous and can run 24 hours a day with minimal supervision, it has accurate temperature control and in the event of a power failure the roaster will automatically shut down and will continue roasting as soon as the power is restored (Flinn, 2012).

2.5.3 Effect of roasting on wheat

Roasting leads to several chemical as well as physical changes within the wheat. These changes can be either beneficial or detrimental to the final product. The degree to which these changes take place is greatly dependent on the roasting method and conditions (i.e., roasting time and temperature) used.

Beneficial changes

Roasting has been shown to improve the organoleptic properties, flavour, colour and texture of cereal grains (Murthy *et al.*, 2008). Roasting further increases the storage life of the wheat by lowering the water activity, effectively retarding the growth of spoilage microorganisms (Ranganathan *et al.*, 2014). Additionally, roasting also enhances nutrient availability and antioxidant activity (Chung *et al.*, 2011). Roasting, between 55–60°C has further demonstrated its capability to inactivate proteolytic enzymes. The inactivation of proteolytic enzymes promotes an increase in loaf volume. Vázquez *et al.* (2001) found that roasting wheat at 60°C using a fluidised bed system resulted in a 20 cm^3 increase in the loaf volume of the bread when compared to the control. Qu *et al.* (2017) observed an increase in softness and springiness and a decrease in chewiness for steamed bread that had been produced from wheat subjected to microwave heating for 20 s. The authors further noted that wheat microwaved for longer than 20 s resulted in a loss of functionality due to changes in the gluten protein.

Detrimental changes

Although roasting of wheat results in numerous beneficial changes, there are also some detrimental changes that occur. As wheat is rich in carbohydrates, it is susceptible to acrylamide formation during roasting as a result of the high temperatures used (Muttucumaru *et al.*, 2006; Claus *et al.*, 2008). Acrylamide is a potentially carcinogenic compound formed from interactions between reducing sugars and amino acids during the Maillard reaction (Nematollahi *et al.*, 2019). As the roasting time and temperatures increase, so does the concentration of acrylamide formed (Claus *et al.*, 2008). The wheats moisture content after roasting is a critical indicator of acrylamide formation. Acrylamide has only been detected when the final moisture content was below 6%. This was based on roasting conditions where the roasting temperature was above 180°C and the roasting time between 5 and 60 min (Taeymans *et al.*, 2004; Elmore *et al.*, 2005; Gökmen and Şenyuva, 2006).

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Roasting could potentially induce changes with regard to the milling and flour yield obtained from roasted wheat (Raigar *et al.*, 2017). From a financial standpoint this could impact the milling industry in terms of profit loss (Kong and Baik, 2016). A study conducted by Schoeman and Manley (2019) displayed reductions in flour yield for wheat that had been roasted at 180°C using an oven (8.52% reduction) and a forced convection continuous tumble (FCCT) roaster (5.59% reduction). Roasting results in weakening of the internal structure of the wheat due to internal moisture diffusion (Dutta *et al.*, 2015). This results in porosity increases and reduced mechanical strength (Schoeman *et al.*, 2016a). Subsequently more endosperm remains attached to the bran after milling as the breaking line is affected thus increasing flour yield loss (Kahyaoglu *et al.*, 2010).

The two main components of flour namely, starch and protein are also affected by roasting. This could directly influence the quality of the bread produced from said flour should these changes be severe. Schoeman and Manley (2019) noted a decrease in protein content for wheat roasted at 180°C using both oven and FCCT roasting methods. The consequences of the decreased protein content were observed in the mixing time or dough development time (DDT) of the dough. Both roasting methods resulted in wheat flour with increased mixing times, i.e., the time required for optimum gluten development to occur was longer than that of the unroasted controls. Similar results were reported by Vázquez *et al.* (2001) where the authors observed that as the roasting temperature increased from 40°C to 100°C so did the mixing time.

In the event that proteins are altered due to a thermal process, such as roasting, their conformation changes which inevitably affects the dough rheology. Schofield *et al.* (1983) found that heat treatment of gluten decreased the solubility of the protein. This indicated that the gluten polymers were aggregating by means of sulfhydryl (SH) – disulphide (SS) reactions. Additionally, decreases in surface hydrophobicity have also been noted as a result of hydrophobic interactions which stabilise the aggregates (Stathopoulos *et al.*, 2008; Mann *et al.*, 2014).

The effect of damaged starch on flour and subsequently bread quality cannot be evaluated independently without taking the influence of heat damaged proteins into consideration. Lorenz *et al.* (1993) reported a decrease in bread loaf volume for wheat subjected to heat treatment at a range of temperatures. The authors further noted that the damaged starch displayed a higher water absorption capacity (WAC) and longer mixing time compared to the flour produced from unroasted wheat. Comparable results were reported by Schoeman and Manley (2019) where increased WAC was noted for flour produced from wheat roasted using an oven and FCCT roaster. In addition, the DDT of these flours were also longer than that observed for the control wheat flour.

Damaged starch of roasted wheat is capable of absorbing more water, compared to unroasted wheat, as a result of the roasted wheats' starch being partially gelatinised (Barrera *et al.*, 2007; Schoeman and Manley, 2019). This increase in WAC can be problematic during mixing as the starches absorb more water leaving the proteins to compete for some of the water. If the

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proteins are not able to properly hydrate during mixing, a strong gluten network cannot be formed, ultimately leading to a reduced final loaf volume (Barrera *et al.*, 2007). The decreased loaf volume can to a degree be rectified by increasing the mixing time. This provides the proteins more time to develop a proper gluten network (Lorenz *et al.*, 1993; Barrera *et al.*, 2007).

2.6 Bubble structure evaluation

The bubble structure within dough and subsequently the crumb structure of bread greatly influences it quality and texture (Scanlon and Zghal, 2001). Furthermore, the breadmaking process is a series of aeration stages which all influence the number and size of the bubbles in the dough (Besbes *et al.*, 2013b). It is therefore imperative to understand how each of the different stages in the breadmaking process affect these bubbles. This is of even greater importance when it is considered that gas cells entrained during mixing are the only nucleation sites available of gas bubble expansion during proofing (Autio and Laurikainen, 1997).

Dough exhibits extremely complex rheological properties and the size and number of bubbles in the dough will greatly influence its rheology. An example of this would include the rate at which disproportionation occurs which is greatly influenced by the size of the bubbles as well as the distance of separation between the different bubbles (Bellido *et al.*, 2006).

Acquiring data on the size and distribution of bubbles within dough can be rather challenging due to the fragile nature of the microstructure within the dough as well as the fact that the microstructure of the dough continuously changes due to CO_2 gas production by yeast (Bellido *et al.*, 2006).

2.6.1 Traditional methods

Traditionally, studying the bubble structure of dough and foam structure of bread involved using invasive techniques which could compromise the internal structure as well as the overall integrity of the samples. One such method involved freezing dough samples with liquid nitrogen for a few minutes whereafter the samples were smashed to produce 1 cm fragments. These fragments were stored in liquid nitrogen until required for analysis. Prior to analyses the fragments were cut into thin sections by means of a cryostat microtome and mounted onto microscope slides. These samples were then imaged using a scanning electron microscope (SEM) and an optical microscope. In the case where samples needed to be transported to the analysis facility, they were frozen with liquid nitrogen and stored in a freezer at -20°C prior to transporting. Freezing of the dough pieces resulted in the formation of cracks due to thermal stresses (Whitworth and Alava, 1999).

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It was further reported that the validity of data obtained regarding the bubble structure of dough may be questionable as freezing and sectioning or squashing the dough ultimately affects the integrity of the sample (Bellido *et al.*, 2006).

Additionally, magnetic resonance micro-imaging (MRI) have also been used to study gas cell development during proofing (Van Duynhoven *et al.*, 2003) but this technique too had some shortcomings. The dough samples were frozen at -32°C for 35 min instead of flash frozen with liquid nitrogen. Changes in the internal microstructure or bubble structure of the dough could have occurred in this period.

Therefore, in order to study the evolution of bubbles within the dough and bread as well as the distribution and size of the bubbles in the dough a non-destructive technique is required. One such technique that has been used with great success is X-ray micro-computed tomography (µCT).

2.6.2 X-ray micro-computed tomography

X-ray micro-computed tomography is a non-invasive technique that has the ability to produce high quality three-dimensional (3D) images. X-ray µCT works on the principle of image contrast produced by X-ray attenuation variations which include scattering and absorption (Lim and Barigou, 2004). When the X-ray beam passes through the sample it is attenuated and the differences in attenuation measured by the detector are attributed to density and compositional differences within the sample (Schoeman *et al.*, 2016b). During scanning, the sample rotates between a fixed X-ray source and a detector while back projection data is captured at each rotational step. This data is then used to obtain cross-sectional images which can be used to generate a 3D representation of the sample (Bellido *et al.*, 2006; Schoeman *et al.*, 2016b). A more comprehensive account of the principles and experimental setup with regard to X-ray µCT and its use to characterise food microstructure was published by Schoeman *et al.* (2016b).

Another more advanced technique, limited to only a few geographical locations, is the synchrotron X-ray instrument. A synchrotron instrument accelerates electrons to extremely high energy, periodically making them change direction and subsequently emitting energy in the X-ray wavelength. These X-rays are emitted as several thin beams of which each is directed towards a different beamline next to the accelerator. Synchrotron has the advantage of producing X-rays of much higher intensities which result in improved image quality. The image acquisition time is also greatly reduced to merely a few seconds enabling almost real-time analysis of the sample.

2.6.3 X-ray micro-computed tomography investigations

A number of investigations over the past 2 decades have made use of X-ray computed tomography (CT) and more recently X-ray micro-computed tomography (µCT) and synchrotron-based X-ray micro-computed tomography to study the bubble structure of dough and foam

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properties of bread. A few of these studies will be elaborated on below in chronological order from date of publication.

One of the first investigations to use a form of X-ray to study bubble structure of dough was performed by Whitworth and Alava (1999). The authors investigated the bubbles structure of dough at the various processing stages and further aimed at gaining quantitative information regarding the number and the size of bubbles present within the dough. X-ray computed tomography (CT) was the method employed during this study. Results from the study showed X-ray CT to be an effective method to study the bubble structure within the dough and was able to image bubbles larger than 1 mm in intact dough pieces.

From more recent literature it is known that dough contains a considerable number of bubbles that are smaller than 1 mm which was not detected in the aforementioned study. Nonetheless, this study served as the starting point or the inspiration for several other studies in which more advanced techniques with higher resolutions were developed or used.

Falcone et al. (2004) successfully illustrated the use of synchrotron-based X-ray μCT to non-destructively investigate the porous structure of a portion of a loaf of bread (25 x 25 x 12 mm in size) as well as that of a French loaf. The authors noted that due to the high resolution of the reconstructed images, sample volumes with a high density of numerical data could be rendered. This enabled quantitative analysis of the microstructure of the bread.

The first investigation into the bubble size distribution of two doughs (stiff and slack) prepared from a strong breadmaking flour using a conventional X-ray μCT instrument was performed by Bellido et al. (2006). In this study dough samples (0.5 g) were squashed between two cellophane layers at a fixed height of 2.17 mm. The findings reported, showed that dough consistency affects both the number and size distribution of bubbles entrained during the mixing process. The stiffer dough entrained less air during mixing subsequently resulting in a lower void fraction or porosity.

Babin et al. (2006) evaluated the bubble growth and foam setting during the breadmaking process by means of synchrotron-based X-ray μCT. It was reported that the development of the gas cell structure during the fermentation stage of the breadmaking process is dependent on a critical time before which the bubbles grow freely. After this critical time coalescence occurs resulting in a structure characterised by a continuous void phase possibly stabilised by liquid film walls.

An evaluation of the microstructural variations between white bread and bread with added bran by means of a conventional X-ray μCT instrument was performed by Van Dyck et al. (2014). It was reported that both the crumb and the crust of these two breads differed considerably. Furthermore, it was found that the addition of bran to the dough formulation resulted in an increased cell wall thickness as well as increases in the number of closed pores.

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Koksel et al. (2016) evaluated the bubble size distribution in non-yeasted dough. The time-dependent changes of the bubble size distribution within the dough were also quantified. Dough samples (1.0–1.5 cm in diameter) placed within in cylindrical tube were scanned by means of synchroton-based X-ray µCT. The authors reported that shortly after mixing a large number of bubbles with a median radius of 22.1 µm was observed. Approximately two and a half hours later the median bubble radius increased to 27.3 µm. These results confirmed that the transport of gas within the non-yeasted dough matrix was due to disproportionation.

2.7 CONCLUSION AND OUTLINE OF RESEARCH CHAPTERS

From this review it is clear that the bubble structure of dough, formed during the mixing phase, greatly influences the final foam structure of the bread. It was further shown that roasting of wheat could positively benefit the foam structure of the bread depending on the conditions used. Additionally, the use of FCCT roasting to study wheat microstructure has been evaluated but no evaluation of the baking performance of flour from FCCT roasted wheat have been performed. This review has also demonstrated X-ray µCT to be a suitable technique to non-destructively study the bubble structure of dough and foam structure of bread. Additionally, the use of C-Cell in combination with texture analysis has been proven to be effective in assessing the quality and shelf life of bread. Against this background, the following section will briefly outline each of the three research chapters in this study.

In the first research chapter white flour produced from roasted wheat differing in hardness and protein content was characterised by means of rheological and proximate analysis. The wheat was roasted at 180°C for 140 s using a FCCT roaster. The subsequent flour was analysed to determine its rheological properties.

The first part of the second research chapter involved determining the optimal roasting conditions to minimise the effect on the gluten protein properties. A central composite design (CCD) was used to determine the optimal roasting conditions in terms of time and temperature. The optimal conditions were used for further studies.

In the second part of the second research chapter the bubble structure of dough and foam properties of bread produced from roasted wheat flour (roasting conditions based on CCD results) was evaluated by means of X-ray µCT. Furthermore, the use of liquid nitrogen followed by freeze drying to maintain the internal structure of the dough was also evaluated.

The third research chapter evaluated the quality and shelf life of bread produced from roasted wheat flour (roasting conditions based on CCD results) by means of C-Cell and texture analysis.

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DECLARATION BY THE CANDIDATE

With regard to Chapter 3 (pp. 35–51), the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, practical analysis and writing of chapter	80

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DECLARATION BY CO-AUTHORS

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3 (pp. 35–51),

2. no other authors contributed to Chapter 3 (pp. 35–51) besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3 (pp. 33–51) of this dissertation.

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CHAPTER 3

Characterisation of white flour produced from roasted wheats differing in hardness and protein content

3.1 ABSTRACT

Roasting of cereals can improve sensory properties and increase shelf life of products made thereof. Here, the physicochemical properties of white flour from roasted wheats differing in hardness and protein content were characterised. Hard, soft and intermediate texture wheat kernels were roasted for 140 s at 180°C. Roasting of hard wheat resulted in the largest reduction in hectolitre mass (7.36 kg/hl), flour yield (2.33%) and moisture content (2.87%). The largest increase in flour ash (0.06%) and damaged starch (4.54%) was observed for the soft and hard wheats, respectively. A gluten network did not form during dough mixing with any of the resultant flours. When composite flours were prepared (80% untreated flour and 20% flour from roasted wheat), the water absorption capacity of the medium textured wheat composite flour displayed the largest decrease (5.2%) compared to the control. Hard and medium textured roasted wheats had improved Chopin P/L ratios and higher levels of free starch than the control. Prior roasting impacts wheat flour milling characteristics. Roasting has a greater effect on hard and medium textured wheats than on soft wheat. Roasting of wheat prior to milling results in flour which alone or in blends with regular flour has unique functionalities.

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3.2 INTRODUCTION

Wheat is one of the most important cereal crops in the world. It is part of the staple diet for a major part of the global population and an excellent source of energy. Wheat flour is used for producing a range of different items such as cakes, biscuits, pasta and most important of all, bread, an affordable staple food.

In the food industry, roasting is generally performed to improve or alter the quality of a product or to increase the processing efficiency (Chung *et al.*, 2011). It is a high-temperature-short-time (HTST) process in which both heat and mass transfer take place (Fadai *et al.*, 2017). During the process, puffing of wheat kernels occurs due to high internal pressures (Schoeman and Manley, 2019). Puffing changes both their volume and moisture content (Schoeman *et al.*, 2016a).

Roasting causes several physical or chemical changes within wheat grains which can either be beneficial or detrimental to the subsequent final products. It can improve the organoleptic properties as well as increase the storage life of the grains by lowering water activity and thereby retarding the growth of spoilage microorganisms (Murthy *et al.*, 2008). Improved flavour, colour and texture have been observed for roasted wheat (Murthy *et al.*, 2008) and maize (Oboh *et al.*, 2010). Roasting can also inactivate proteolytic enzymes. An increase in loaf volume was observed for breads produced from wheat that had been dried at 60°C using a fluidised bed system (Vázquez *et al.*, 2001). Steamed bread produced from wheat that had been subjected to microwave heating for 20 s displayed increased softness and springiness and decreased chewiness ((Qu *et al.*, 2017). Wheat microwaved for longer time resulted in changes in gluten which resulted in loss of functionality.

Detrimental changes caused by maize roasting include losses in both vitamin content and nutritional value (Oboh *et al.*, 2010). Acrylamide concentration increases with roasting time and temperature. This is important as the compound is potentially carcinogenic (Nematollahi *et al.*, 2019). The moisture content of roasted wheat is a critical indicator of prior acrylamide formation as it is only detected when the final moisture content is below 6% (Taeymans *et al.*, 2004).

Gluten proteins are responsible for the characteristic viscoelastic properties of wheat dough that make bread making possible (Collar and Armero, 2018). Especially relevant in the context of bread making are the changes to the wheat gluten proteins (Raigar *et al.*, 2017) which occur during roasting to a degree which depends on the severity of the process and which reduce the wheat bread making quality (Cetiner *et al.*, 2017).

Roasting techniques include sand bath, microwave (Qu *et al.*, 2017), fluidised bed (Murthy *et al.*, 2008), screw conveyer (Jogihalli *et al.*, 2017), oven as well as forced convection continuous tumble (FCCT) roasting (Schoeman and Manley, 2019). In the latter, superheated steam is produced from the water in the product. A test sample can be roasted, prior to the actual sample, to replace the hot air within the roaster with superheated steam, although this is not a pre-requisite.

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Energy is efficiently used as the superheated steam within the roaster is continuously recirculated and the roaster is thermally insulated which reduces heat loss. Hot air is forced through the product while it is continuously mixed in the rotating cylinder. Doing so ensures that roasting is uniform. The running cost of an FCCT roaster is approximately 20% less compared to that of the conventional gas fired drum type roasters when operating at the same capacity (Flinn, 2012; Schoeman *et al.*, 2016a).

Against this background, the aim of this study was to characterise white flour produced from roasted wheat differing in hardness and protein content by means of rheological and physicochemical analyses.

3.3 MATERIALS AND METHODS

3.3.1 Wheat samples

Two commercial wheat cultivars, i.e., a hard (SST 843) and a medium-texture (SST 8154) one as well as one soft wheat breeding line (LAB 316-10) (12 kg each) were obtained from Sensako (Bethlehem, South Africa). Broken kernels and impurities were removed using a Carter Day (Minneapolis, MN, USA) Dockage Tester. The individual wheat samples were subsequently mixed by pouring the 12 kg batches three times through a Boerner Divider (Seedburo Equipment, Chicago, IL, USA). After mixing, each batch was divided into four batches of 3 kg each. Two batches served as control and two were roasted. The hard, medium texture, and soft wheats had particle size indices AACC method 55-31.01; (AACC International, 2010) of respectively 55, 60 and 67%. Their protein contents were respectively 15.30, 13.87 and 12.11%.

3.3.2 Wheat roasting

Wheat was roasted by means of a R100E Forced Convection Continuous Tumble (FCCT) roaster (Roastech, Bloemfontein, South Africa). This roaster has a throughput capacity of 25 – 100 kg/h and operates at atmospheric pressure. To ensure homogenous roasting, the dry hot air in the chamber of the FCCT roaster was replaced with superheated steam. This was achieved by first roasting a 400 g wheat sample tempered with distilled water to a moisture content of 18 to 20%. Wheat samples to be roasted and used for experimental purposes were not tempered prior to roasting.

Once sufficient steam had been generated in the FCCT chamber, wheat samples were roasted at 180°C. This temperature is typical for roasting cereal grains (Schoeman and Manley, 2019). The roasting time was *ca*. 140 s. Each of the 3 kg treatment batches of the individual wheat samples were roasted separately. Roasted wheat was allowed to cool and stored in sealed containers until milled.

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3.3.3 Hectolitre mass

Hectolitre mass (HLM) (kg hl^{-1}) of control and roasted wheat samples was determined as in Guelpa et al. (2015) using the German Kern 220/222 Grain Sampler (Kern & Sohn, Balingen-Frommern, Germany).

3.3.4 Wheat milling

Milling of control and roasted wheat samples into flour was performed at PepsiCo Inc, Sub Saharan Africa, Research and Development (Essential Foods, Paarl, South Africa) using a Brabender Quadrumat Jr. (Quadruplex) mill (C.W. Brabender Instruments, South Hackensack, NJ, USA) in accordance with AACC method 26-50.01 (AACC International, 2010). Prior to milling, the 3.0 kg wheat batches were tempered to a moisture content of 15.5% for control and 14.5% for roasted wheats. Wheat tempering was performed in accordance with AACC method 26-95.01 (AACC International, 2010). The control wheat was tempered for 24 h with addition of all the water at once. The roasted wheat was tempered for 48 h with addition of half the water initially and the other half after 24 h. Before milling, the mill was run empty for 30 min. Next, a test sample (*ca*. 100 g) was run in order for the mill to reach its operating temperature. The feed rate was 75 g/min. Flour and bran were separated by the continuous reel sifter. Flour yield is the weight percentage of the white flour extracted from the tempered wheat. The flour was stored in airtight containers until further use.

3.3.5 Preparation of the composite flours

Additive free commercial white bread flour (10.0% protein content) from PepsiCo Inc, Sub Saharan Africa, Research and Development was used to prepare the composite flours from the roasted wheats in a ratio of 80% commercial white bread flour and 20% flour from roasted wheat.

3.3.6 Moisture, protein, ash and damaged starch contents

Moisture contents of control and roasted kernels were determined using the Perten Instruments (Hägersten, Sweden) Inframatic 9500 NIR Grain Analyser, and those of the resultant wheat flour samples by means of the air-oven drying AACC method 44-19.01 (AACC International, 2010).

Protein content (N x 5.7) was determined for the different flour samples in duplicate by Dumas combustion according to AACC method 46-30.01 (AACC International, 2010) with a Leco (Saint Joseph, MI, USA) TruMac N nitrogen analyser. It was calculated on a 12% moisture basis.

Ash content was determined in duplicate on flour samples using the ash-rapid (magnesium acetate) method as in AACC method 08-02.01 (AACC International, 2010). Wheat flour (3.00 ±

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0.01 g) and 5.0 mL magnesium acetate solution were transferred to pre-ignited ashing dishes. These were placed in a muffle furnace at 700°C and the flour was allowed to flame until carbonised before closing the muffle furnace. The flour samples were left until completely incinerated (45–60 min) before cooling in a desiccator and weighing.

Flour damaged starch content was determined amperometrically using the SDmatic (Chopin Technologies, Villeneuve la Garenne, France) in accordance with AACC method 76-33.01 (AACC International, 2010).

3.3.7 Particle size distribution analysis

Flour particle size distributions were determined in duplicate by means of a Beckman Coulter (Miami, FL, USA) LS 13 320 laser diffraction analyser using the dry module. A focal lens was used to measure the size distributions between 0.49 and 339.93 µm. The distributions were expressed as volume percentage fractions of the different particle size classes.

3.3.8 Rheological analyses

First, water absorption capacity (WAC, %) was determined in duplicate on control and composite flour samples using an Alveolab (CHOPIN Technologies) as in AACC method 54-50.01 (AACC International, 2010).

The mixing characteristics of control and composite flours were evaluated in duplicate using a Mixograph (National Manufacturing, Lincoln, NE, USA) as in AACC method 54-40.02 (AACC International, 2010) with a 35 g flour sample. Flour and water were mixed for 7 min. The obtained dough development time (DDT, time to peak) and peak height was determined.

Alveograph testing was in duplicate on control and composite flours. The dough's resistance to extension was measured using an Alveolab as in AACC method 54-30.02 (AACC International, 2010). Indices reported include the resistance to expansion, measured as the height of the curve (P, mm), dough extensibility measured as the length of the curve (L, mm), curve configuration ratio (P/L) and deformation energy (W). W is a measure of the work input required to inflate the dough until it ruptures and thus of the gluten strength.

3.3.9 Statistical analysis

Mixed model analysis of variance (ANOVA) was performed to compare the averages of the quantitative measurements. Control flour and flour from the roasted wheats were compared per wheat sample. The composite flours were compared with the appropriate controls. The data was reported as mean ± standard deviations. The Fisher least significant difference (LSD) test was done to perform the different post hoc analyses. Statistical analysis was performed using

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STATISTICA version 13 (Statsoft, Tulsa, OK, USA). A 95% confidence interval was used to identify significant results i.e. a 5% significance level ($p \leq 0.05$).

3.4 RESULTS AND DISCUSSION

3.4.1 Hectolitre mass

Roasting significantly ($p \leq 0.05$) impacted HLM for all roasted wheat samples (Table 3.1). HLM decreases for the hard, medium texture and soft wheats were 7.36, 5.16 and 3.98 kg/hl, respectively. Roasting increases kernel volumes due to puffing, decreases their weight due to moisture loss (Schoeman and Manley, 2019) and thus results in roasted wheat kernels having a lower HLM than their unroasted counterparts.

3.4.2 Flour yields

The flour yields for control and roasted wheat samples are depicted in Table 3.1. No significant differences in flour yield for the roasted wheat samples were observed ($p > 0.05$). Decreases in flour yield (0.79 to 2.33%) for roasted wheat samples have been attributed to process induced increases in porosity (Schoeman *et al.*, 2016b).

3.4.3 Flour moisture, protein, ash and damaged starch contents

The moisture contents of control and roasted wheat kernels and flour samples are depicted in Table 3.2. Exposure of the wheat to 180°C for *ca*. 140 s resulted in a 2.9% decrease for the hard and medium texture wheats and a 2.2% decrease for the soft wheat. While the control samples could be tempered to 15.5% moisture in 24 h, due to the low initial moisture contents of the roasted wheats the targeted 14.5% moisture content could not be achieved even after 48 h of tempering. A grain's initial moisture content plays an important role in its hydration kinetics during tempering. Lower initial moisture contents lead to slower uptake of water due to increases in the seed coat permeability to water as the water activity or moisture content increases (Delcour & Hoseney, 2010). Furthermore, at a certain moisture content and temperature, the components of the seed coat change from a glassy to a rubbery state which leads to changes in water permeability (Miano and Augusto, 2015).

Roasting resulted in significant differences ($p \leq 0.05$) in the protein content of flours obtained from hard (0.57% lower) and medium textured (0.24% lower) wheats. No significant difference in protein content (Table 3.1) for the soft wheat was noted between the control and roasted wheat flour. Schoeman & Manley (2019) reported a 0.17% lower protein content as measured with the Dumas combustion method, for flour from roasted, medium textured wheat using roasting conditions similar to those in the present study.

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Roasting resulted in significantly higher ash contents only in the flour of the medium textured wheat (0.05%) ($p \leq 0.05$) (Table 3.1). Prior to milling, wheat kernels were tempered to plasticise the bran, making it less susceptible to fragmentation during milling (Pauly *et al.*, 2013). Due to insufficient moisture absorption by the roasted wheat samples, the bran was not adequately hydrated to be fully plasticised leading to partial fragmentation during milling and increased ash contents. Lower moisture content of the roasted wheat prior to milling results in flour of higher ash content (Warechowska *et al.*, 2016).

Roasting resulted in significant increases ($p \leq 0.05$) in starch damage when milling the hard (4.54%), medium textured (4.22%) and soft (3.45%) roasted wheats (Table 3.1). Cattaneo et al. (2015) reported significantly higher starch damage contents when wheat was puffed at high temperature prior to milling. The present increases in damaged starch contents were attributed to the lower moisture content of tempered roasted wheat samples prior to milling.

The flours from the hard and medium textured roasted wheats had higher damaged starch contents than those of the soft roasted wheat. Harder wheat kernels have more densely packed endosperm with stronger starch-protein interactions than soft wheat kernels (Pauly *et al.*, 2013) which then cause more starch damage during milling.

Table 3.1 Hectoliter mass (HLM) of wheats (C), roasted wheats (R) and composition of the flour prepared from them

Sample	HLM (kg/hl)	Flour Yield (%)*	Protein (%)	Ash (%)	Damaged Starch (%)
Hard – C	82.85 ± 0.06^{a}	61.38 ± 1.69^{a}	15.30 ± 0.05^{a}	0.65 ± 0.02^{a}	11.18 ± 0.13^{a}
Hard – R	75.49 ± 1.71^{b}	59.05 ± 0.94^{a}	14.73 ± 0.03^{b}	0.68 ± 0.04^{a}	15.72 ± 0.23^{b}
Medium – C	82.45 ± 0.13^{a}	60.99 ± 0.80^{a}	13.87 ± 0.03^{a}	0.56 ± 0.01^{a}	11.04 ± 0.12^{a}
Medium – R	77.29 ± 1.17^{b}	59.76 ± 0.33^{a}	13.63 ± 0.07^{b}	0.61 ± 0.01^{b}	15.26 ± 0.34^{b}
Soft – C	80.08 ± 0.21^{a}	64.18 ± 1.21^{a}	12.11 ± 0.02^{a}	0.40 ± 0.03^{a}	7.30 ± 0.22^{a}
Soft – R	76.10 ± 0.82^{b}	63.39 ± 1.83^{a}	12.05 ± 0.08^{a}	0.46 ± 0.01^{a}	10.75 ± 0.82^{b}

Results are presented as mean ± standard deviation of duplicate determinations from two replicates. Different superscripts represent significant differences ($p \leq 0.05$) between each wheat's control and roasted flour. Protein expressed as $N \times 5.7$ on a 12% mb. C – Control, R – Roasted.

*Mean value of single measurement from two replicates

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Table 3.2 Moisture contents of wheats (C), roasted wheats (R) and flour prepared from them

Sample	Initial wheat (%)	Tempered wheat – 24 h (%)	Tempered wheat – 48 h (%)	Flour (%)
Hard wheat – C	10.40 ± 0.01	15.53 ± 0.05	-	15.29 ± 0.08
Hard wheat – R	7.53 ± 0.21	10.70 ± 0.18	13.80 ± 0.22	13.82 ± 0.09
Medium wheat – C	10.50 ± 0.01	15.50 ± 0.08	-	15.38 ± 0.09
Medium wheat – R	7.60 ± 0.18	10.73 ± 0.10	13.90 ± 0.08	13.87 ± 0.03
Soft wheat– C	10.58 ± 0.05	15.55 ± 0.06	-	13.43 ± 0.37
Soft wheat– R	8.35 ± 0.30	11.25 ± 0.13	14.10 ± 0.00	12.03 ± 0.01

Results are presented as mean ± standard deviation of duplicate determinations from two replicates. C – Control, R – Roasted.

3.4.4 Particle size distributions

The particle size distribution graphs of the flour from the hard, medium textured and soft wheats and their roasted counterparts showed bimodal distributions for all samples. The flour samples from the roasted counterparts of the hard and medium textured wheats (Figs. 3.1a & 3.1b) displayed slightly higher volume fractions of particles in a 30 µm diameter range and a slightly lower volume fraction of particles in the diameter range of 50 to 130 µm. This suggests that the former contained a higher fraction of free starch granules (Pauly *et al.*, 2013).

There were no distinct differences in particle size distribution for the control soft wheat flour and that prepared from its roasted counterpart (Figure 3.1c). A much higher volume fraction of particles in the range of 30 µm was obtained, which is typical of a soft wheat having more free starch granules (of B-type) (Pauly *et al.*, 2013). The endosperm of soft wheat is less densely packed than that of harder wheat. Therefore, during milling fractures occur through the protein-starch matrix leading to less fracturing of the starch granules (Pauly *et al.*, 2013). These results further correlate to those found for starch damage, where the soft wheat displayed least amount of starch damage after the milling process.

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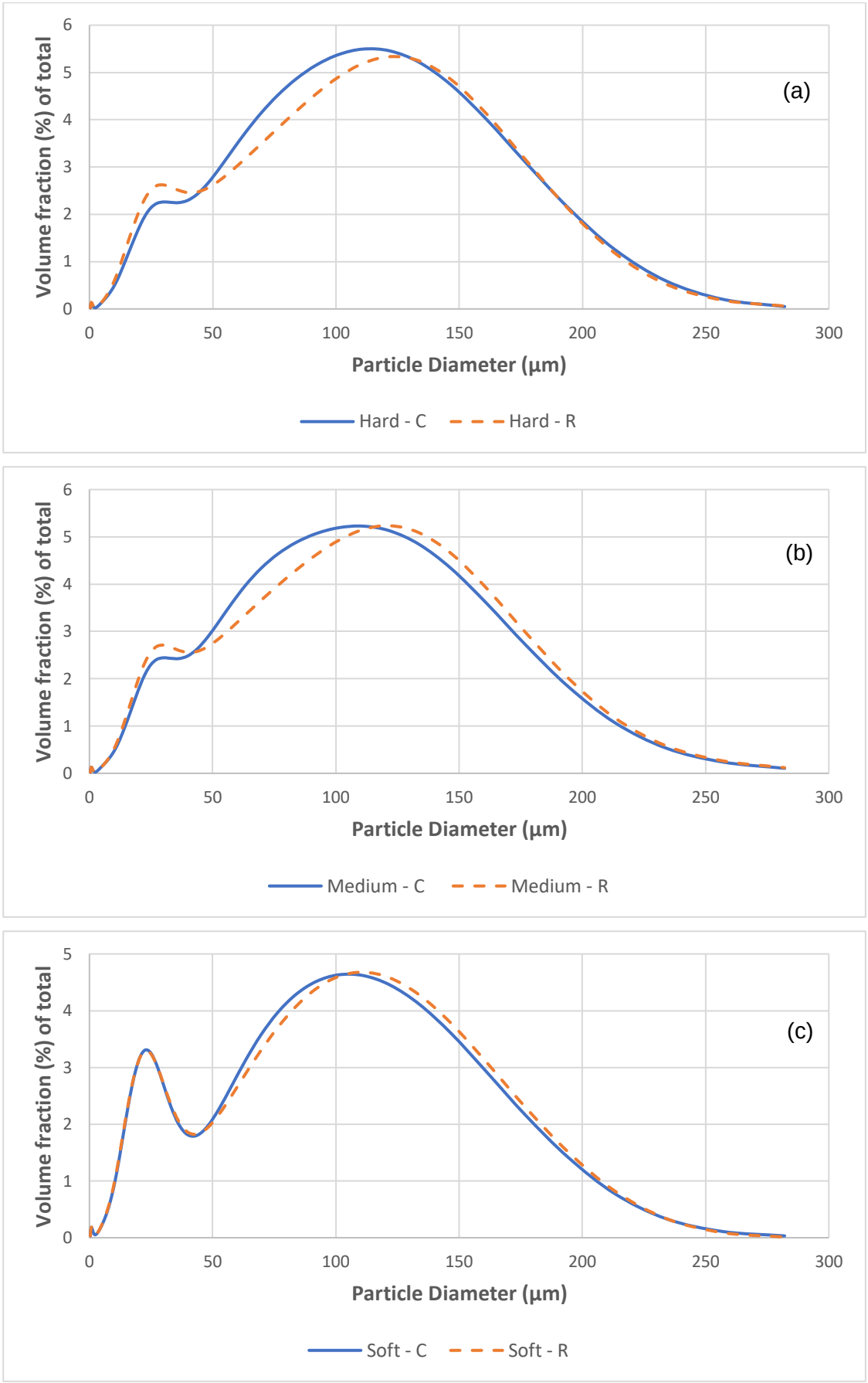

Figure 3.1 Particle size distribution of flour from control (C) and roasted (R) wheats: (a) hard, (b) medium textured and (c) soft wheat.

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3.4.5 Rheological analyses

The WAC readings of the composite flours containing flour of the hard, medium textured and soft roasted wheats were 5.2%, 4.1% and 1.0% lower than those of the corresponding controls, respectively (Table 3.3). The decreased WAC suggests that the proteins either required less water to fully hydrate or that they were damaged during roasting which made them less capable of being readily hydrated.

When mixograms were made with flour from roasted wheat it became evident that roasting had abolished the protein's gluten formation capacity. In contrast, it was possible to produce mixograms with composite flour mixtures consisting of 80% untreated commercial bread flour and 20% flour from roasted wheat.

Mixograms of the unroasted control and those of the flour composites containing flour from the roasted wheats are depicted in Figure 3.2. Mixograms a1, b1 and c1 are those of the control flours and display clear optimum gluten development indicative of good bread making quality and strong gluten network. Mixograms a2, b2 and c2 are those of the flour composites containing flour from the roasted wheats and show no distinct optimum gluten development peak, indicating a weak gluten network in the dough formed.

During mixing of flour (composites), dough resistance increased until it reaches a maximum where after it decreases. The peak time is the time for optimal gluten development and maximum water absorption by gluten and starch (Delcour and Hoseney, 2010). Lower peak times were observed for the flour composites containing flour from the hard (66 s) and medium textured (12 s) roasted wheats than for those of the corresponding controls. The flour composites containing flour from the roasted soft wheat had a peak time which exceeded that of the corresponding control by 42 s (Table 3.3). These results correlate with those by Schoeman & Manley (2019) for flour produced from medium textured roasted wheat.

Lower peak heights were observed for the flour composites containing flour from the roasted hard, medium textured and soft wheats. The differences in peak heights were 5, 4 and 1 mm, respectively. Changes in the protein during the roasting process (Bucsella *et al.*, 2016) cause less protein-protein interactions during mixing resulting in a decreased gluten strength.

Table 3.3 depicts the alveograph parameters for the unroasted control flour and the flour composites containing flour from the roasted wheats. Acceptable ranges for alveograph parameters for white bread flours are P-values (a measure of resistance to extension or elasticity) of 65 to 120 mm, L-values (a measure of extensibility) of 80 to 120 mm and P/L ratios of 0.70 to 1.50 (SAGL, 2018).

Higher P-values were noted for all flour composites containing flour from hard, medium textured and soft roasted wheats, the differences being 3, 32 and 44 mm respectively. This

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suggests an increased resistance to bubble extension. Lower L-values were observed for all flour composites containing flour from hard, medium textured and soft roasted wheats than for their control counterparts, with differences amounting to 52, 112 and 108 mm, respectively. The lower L-values suggest that doughs from the flour composites were not very extensible. An increased P-value and decreased L-value as a result of starch damage increase has been reported by Preston, Kilborn, & Dexter (1987). As damaged starch absorbs more water than undamaged starch, during alveograph testing, the increased damaged starch content may have resulted in less water being available to hydrate the gluten proteins. This subsequently results in a tighter (i.e. increased P-value) and less extensible (i.e. decreased L-value) dough (Preston *et al.*, 1987). Flour with lower protein content also is characterised by lower L and higher P values (Dexter *et al.*, 1994).

When compared to those of their respective controls, an increase in P/L ratios of 0.40, 0.66 and 2.51 was observed for the flour composites containing flour from the hard, medium textured and soft roasted wheats. This was expected considering the increase in P-values and decrease in L-values. The alveograms (Figure 3.3) depict the effect of roasting on wheats differing in hardness. As wheat hardness decreased, a decrease in P-value and increase in L-value was observed for the unroasted control flours. The opposite was observed for the flour composites containing flour from roasted wheat. The difference in P- and L-values between the flour composite containing flour from the soft roasted wheat and those of its respective control were larger than those of the hard and medium textured roasted wheats.

Considerably lower W-values were observed for all flour composites containing flour from roasted wheat, with differences amounting to 381, 185 and 74 10^{-4} J for the flour composites containing flour from the hard, medium textured and soft roasted wheats, respectively. This suggests a decreased dough strength (such as also evident from the mixograms) and thus breadmaking quality (SAGL, 2018). Baiano et al. (2008) reported that roasting wheat results in decreases in W values as well as in gluten strength. The low W-values (Table 3.3) clearly indicate the weak gluten strength of the flour composites.

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Table 3.3 Consistograph, mixograph and alveograph parameters of the unroasted control and the flour composites prepared from the roasted wheat flours

Sample	Consistograph	Mixograph		Alveograph			
	WAC (%)	Peak Time (min)	Peak Height (mm)	P (mm)	L (mm)	P/L	W (10^{-4}J)
Hard – C	60.2 ± 0.3	4.2 ± 0.1	60 ± 1	106 ± 2	151 ± 8	0.71 ± 0.04	539 ± 12
Hard – CF	55.0 ± 0.2	3.1 ± 0.4	55 ± 1	109 ± 3	99 ± 5	1.11 ± 0.05	158 ± 9
Medium – C	58.9 ± 0.2	2.9 ± 0.1	59 ± 1	72 ± 1	224 ± 6	0.32 ± 0.01	348 ± 1
Medium – CF	54.8 ± 0.2	2.7 ± 0.3	55 ± 1	104 ± 3	112 ± 30	0.98 ± 0.25	163 ± 34
Soft – C	55.9 ± 0.1	3.2 ± 0.1	56 ± 1	53 ± 1	141 ± 45	0.32 ± 0.01	207 ± 10
Soft – CF	54.9 ± 0.2	3.9 ± 0.3	55 ± 1	97 ± 2	33 ± 3	2.83 ± 0.27	133 ± 7

Results are presented as mean ± standard deviation of duplicate determinations from two replicates. C – Control, CF – Composite flour, P – resistance to extension, L – extensibility, P/L – curve configuration ratio, W – deformation energy, WAC – water absorption capacity.

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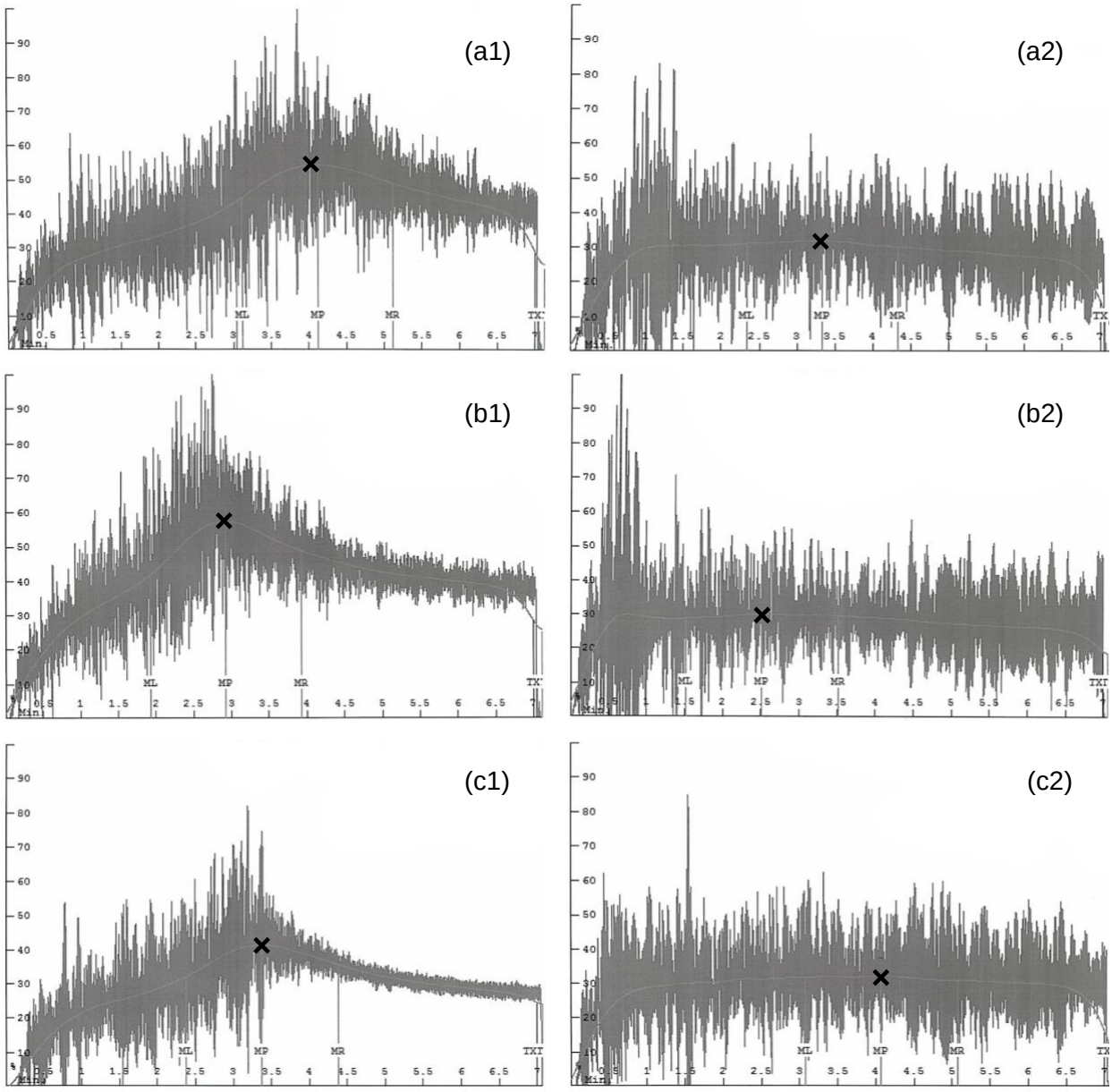

Figure 3.2 Mixograms of the flours from (a) hard, (b) medium textured and (c) soft wheats (1) and the flour composites containing 20% of flour from their roasted counterparts and 80% commercial wheat flour (2). X depicts peak time (x-axis) and peak height (y-axis)

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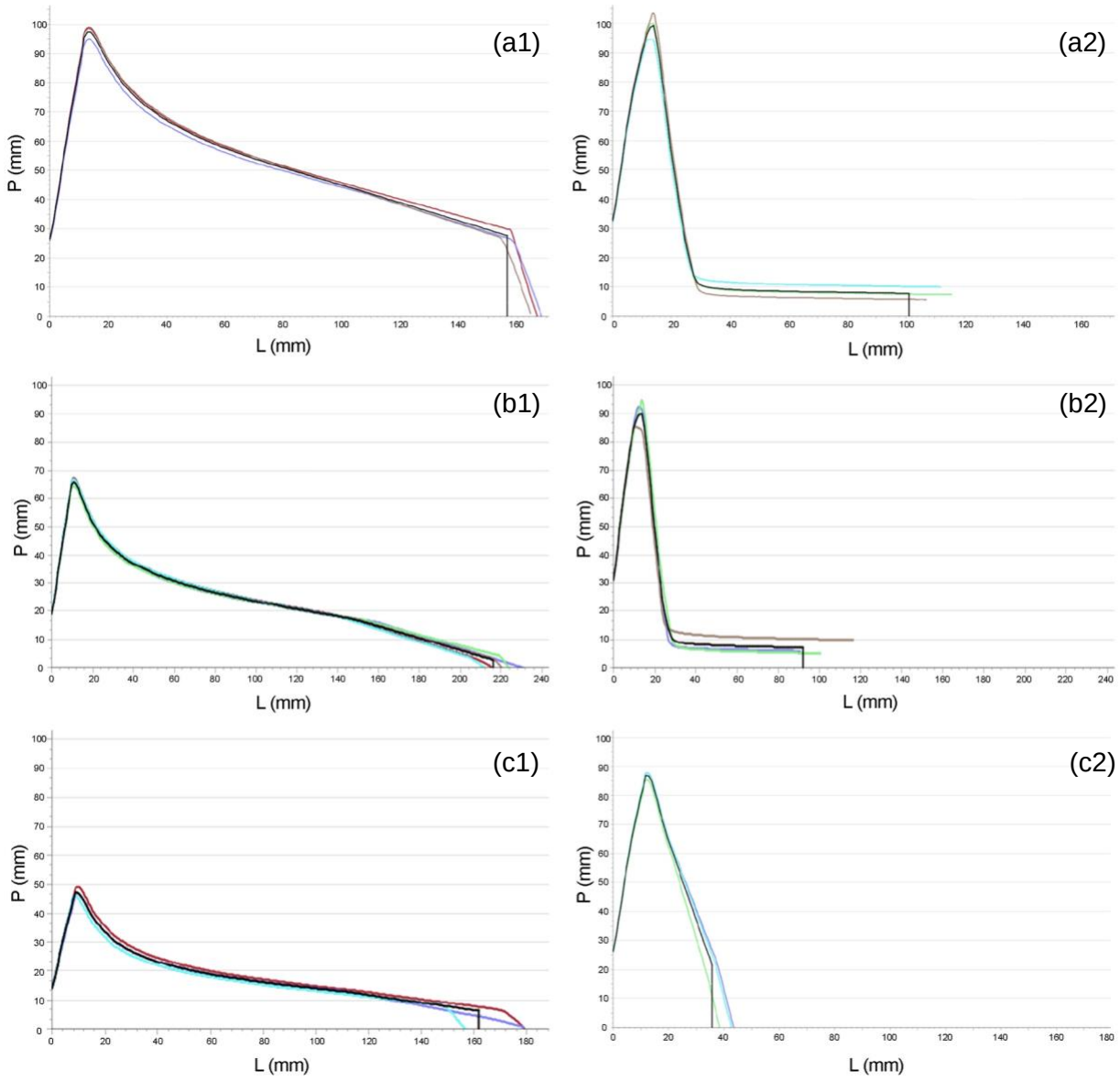

Figure 3.3 Alveograms of the flours from (a) hard, (b) medium textured and (c) soft wheats (1) and the flour composites containing 20% of flour from their roasted counterparts and 80% commercial wheat flour (2). L – extensibility, P – resistance to extension.

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3.5 CONCLUSIONS

Roasting wheat resulted in considerable moisture losses. This negatively impacted the tempering process resulting in the targeted moisture content of the roasted wheat grains not being achieved. Subsequently, significant increases in starch damage during milling was observed. Roasting also brought about changes in the protein as dough could not be formed during rheological testing. The use of flour composites consisting of 80% untreated commercial bread flour and 20% flour from roasted wheat enabled rheological evaluation of the roasted wheats. Changes in the protein resulted in decreases in WAC. Mixogram peak height and alveograph W-values both showed weakening of gluten strength. Increases in resistance to extension and decreases in extensibility, as a result of increased starch damage, positively affected the P/L ratios of the flour composites containing flour from the hard and medium textured roasted wheats. Roasting resulted in flour from the hard and medium textured wheats containing higher levels of free starch granules than that prepared from the starting materials. Although no distinct differences in particle size distribution for the soft wheat flour and its roasted counterpart was observed, both had much higher levels of free starch granules compared to the hard and medium textured wheat. In order to further study the effect of roasting on the bubble structure of dough and foam properties of bread, the use of a central composite design is required to determine the optimal roasting conditions that would minimise the effect on protein properties.

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CHAPTER 4

X-ray micro-computed tomography evaluation of bubble structure of freeze-dried dough and foam properties of bread produced from roasted wheat flour

4.1 ABSTRACT

This study evaluated the bubble structure of freeze-dried dough and foam properties of bread produced from the roasted wheat flour by means of X-ray micro-computed tomography (μCT). Wheat was roasted at 90°C for 130 s, based on a central composite design, and the subsequent flour used to produce dough. Dough was proofed for 20- and 40 min, respectively and breads were baked from the 40 min proofed dough. The use of 10 g dough and bread samples enabled scanning at a much higher resolution. Roasting resulted in a decreased strut thickness of the bread, suggesting a finer crumb structure and softer texture, compared to the control. Porosity increases were observed for the roasted wheat samples. This suggested a slightly weaker gluten strength as was observed with the lower mixograph peak height. Roasting wheat, at the conditions determined by the central composite design, did not negatively affect the foam properties of the breads produced from the roasted wheat flour.

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4.2 INTRODUCTION

Globally, wheat (*Triticum aestivum L.*) is considered one of the most important cereal crops and is currently the second most produced crop after maize. The roasting of cereals is traditionally practised in India as it increases the grains' shelf-life and improves its organoleptic properties (Murthy *et al.*, 2008). It is considered a high-temperature short-time (HTST) process where heat and mass transfer take place. Roasting can, however, also lead to detrimental changes in the grain depending on the roasting time, temperature and technique used. Roasting of wheat has been shown to result in increased porosity due to internal moisture diffusion weakening the internal structure of the kernels (Dutta *et al.*, 2015; Schoeman and Manley, 2019).

Earlier work showed that forced convection continuous tumble (FCCT) roasting of whole wheat kernels at 180°C and 80 Hz was too severe resulting in considerable moisture content losses (Germishuys *et al.*, 2020). This negatively impacted the subsequent tempering process. Furthermore, significant increases in starch damage were also observed. The greatest change brought about by these roasting conditions were the protein properties which were affected to the extent that a dough could not be formed during mixograph analysis.

Central composite design is the most commonly used experimental design utilised in response surface methodology (RSM). Response surface methodology refers to the use of mathematical modelling to obtain response surfaces which is based on experimental data. Conclusions regarding the experimental process can then be drawn from these response surfaces (Bezerra *et al.*, 2008; Granato and De Araújo Calado, 2014). These responses refer to the measured quantities being optimised and is the result of interactions between the independent experimental factors or variables as visualised by means of response surface plots (three-dimensional plot) and contour plots (two-dimensional plot) (Steinberg and Bursztyn, 2010). The main advantage of RSM is that it requires only a small number of experiments to generate a large dataset. It further considers the interaction effects between the different factors (Bezerra *et al.*, 2008).

Bread, produced from wheat flour, is one of the most widely consumed food products in the world and its characteristic foam properties has been widely studied (Scanlon and Zghal, 2001; Mondal and Datta, 2008; MacRitchie, 2016). The formation of the final foam structure within a loaf of bread is the culmination of a series of processes and it directly impacts the texture and appearance of the bread. Essentially, the formation of the crumb structure of bread, on a cellular level, starts during the mixing process when air is incorporated into the liquid phase of the dough. This creates the nuclei required for the development of the gas cells during the proofing phase. Additionally, mixing also serves to hydrate the gluten proteins and develop the gluten network (Scanlon and Zghal, 2001; Sroan *et al.*, 2009). Proofing is essential in the breadmaking process and a very important link between mixing and baking. Yeast, the leavening agent usually used, converts simple fermentable sugars into CO_2 gas and alcohol. The CO_2 gas generated within the

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liquid phase of the dough diffuses to the nuclei due to a concentration gradient resulting in the expansion of the nuclei into gas cells (Avramenko *et al.*, 2018). During baking, the dough heats up to a critical temperature where starch gelatinisation occurs, freezing the bread's foam structure and inhibiting further bubble expansion (Mondal and Datta, 2008). The bubbles subsequently rupture leading to the rapid loss of gas, ultimately forming a network of interconnecting cells (Mills *et al.*, 2003).

Traditionally, studying the bubble structure of dough involved freezing the dough and sectioning it, but these methods affect the integrity of the sample. Additional methods also employed were magnetic resonance micro-imaging (Van Duynhoven *et al.*, 2003) and electron microscopy (Bache and Donald, 1998), but these too have shortcomings due to their limitation of two dimensions as well as their invasive nature.

In recent years X-ray micro-computed tomography (µCT) has been adopted with great success to study the microstructure of foods. X-ray µCT is a non-invasive technique that has the ability to produce high quality three-dimensional (3D) images. X-ray µCT involves rotating the sample between a fixed X-ray source and detector while back projection data is captured at each rotation step. This data is used to obtain cross-sectional images that can be used to generate a three-dimensional (3D) representation of the sample (Schoeman *et al.*, 2016b). Additionally, a synchrotron X-ray µCT instrument has also been used with great success (Babin *et al.*, 2008; Koksel *et al.*, 2016). The advantage of such an instrument is its ability to produce X-rays of much higher intensities resulting in improved image quality. The image acquisition time is also considerably reduced to only a few seconds (compared to the 40–60 min required by a conventional X-ray µCT instrument) enabling almost real time analysis of the sample.

A number of investigations used X-ray µCT to study the bubble structure of dough and foam properties of bread. Whitworth and Alava (1999) investigated the bubbles within a dough structure and how it changes during processing using X-ray computed tomography (CT). Falcone et al. (2004) successfully illustrated the use of synchrotron-based X-ray µCT to non-destructively investigate the porous structure of a portion of a loaf of bread (25 x 25 x 12 mm in size) as well as that of a French loaf. Bellido et al. (2006) investigated the bubble size distribution of two doughs (stiff and slack) prepared from a strong breadmaking flour using a conventional X-ray µCT instrument. In this study 0.5 g dough samples were used which were squashed between two cellophane layers to a fixed height of 2.17 mm. They showed that the consistency of the dough affects the number and size distribution of bubbles entrained during the mixing process. The stiffer dough entrained less air which resulted in it having a lower void fraction or porosity. Babin et al. (2006) showed by means of synchrotron-based X-ray µCT that the development of the gas cell structure during fermentation is dependent on a critical time before which the bubbles grow freely. After the critical time coalescence prevails leading to a structure characterised by a continuous void phase, stabilised by liquid film walls. Van Dyck et al. (2014) studied the microstructural

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variations between white bread and bread (200 mm (l) x 110 mm (w) x 120 mm (h)) with added bran by means of a conventional X-ray µCT instrument. The authors found considerable differences in both the crumb and crust of these two breads. The addition of the bran not only increased the cell wall thickness but also resulted in an increase in the number of closed pores. Koksel et al, (2016) determined the bubble size distribution in non-yeasted dough. They further quantified the time-dependent changes of the bubble size distribution within the dough. Dough subsamples (1.0–1.5 cm in diameter) were excised from the centre of the dough, placed within cylindrical plastic containers and scanned by means of synchrotron-based X-ray µCT. Shortly after mixing a large number of bubbles with a median radius of 22.1 µm was observed. Approximately two and a half hours later the median bubble radius increased to 27.3 µm. These results confirmed that the transport of gas within the non-yeasted dough matrix was due to disproportionation.

The majority of earlier dough and bread studies were done using synchrotron-based X-ray µCT. Those that used a conventional X-ray µCT instrument either used large bread samples or very small dough samples presented to the instrument either confined in a tube or squashed between cellophane which affected the original structure of the dough. This study firstly aimed at determining the optimal roasting conditions that would minimise the effect on the protein properties of the produced white flour. Secondly, it aimed to evaluate (1) the freeze drying of dough as a suitable sample preparation procedure that would maintain the structure of the samples during X-ray µCT scanning for efficient analyse of its bubble structure and (2) the foam properties of 10 g bread loaves produced with roasted wheat flour.

4.3 MATERIALS AND METHODS

4.3.1 Wheat samples

The wheat samples for both the central composite design and X-ray µCT studies were obtained from PepsiCo Inc, Sub Saharan Africa, Research and Development facility (Essential Foods, Paarl, South Africa). Prior to dividing the samples into subsamples, broken kernels and impurities were removed using a Carter Day Dockage Tester (Carter Day International, Minneapolis, MN, USA). The wheat was subsequently mixed by pouring it three times through a Boerner Divider (Seedburo Equipment, Chicago, IL, USA).

Central composite design study: Two commercial wheats (5 kg each) differing in protein content (henceforth referred to as high and low protein wheat) were used for this part of the study. Each 5 kg batch was divided into 11 batches of 200 g each of which one served as the control (unroasted) and the remaining 10 batches served as the treatments (roasted) for the central composite design.

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X-ray µCT study: A commercial white bread wheat (40 kg) of high protein content was used for the second part of the study. The 40 kg batch was divided into four batches of 10 kg each. Two batches served as controls and two were roasted.

4.3.2 Central composite design

Optimisation of the roasting conditions were done using a central composite design (CCD) which consisted of 10 experimental runs, where the central point (110°C and 76 Hz) was repeated twice to calculate the methods' repeatability (Table 4.1). The independent variables investigated were roasting speed (Hz) and roasting temperature (°C). The treatment level ranges for the independent variables are depicted in Table 4.1. The minimum and maximum values selected for the roasting temperatures (90°C and 130°C) and roasting speeds (60 Hz and 90 Hz) were based on a preliminary study (unpublished data). The Hz, with regards to the roasting speed, refers to the alternating current delivered to the rotor motor and is directly proportional to the rotor speed (rotation of the roaster drum). The higher the Hz value, the faster the motor turns and the shorter the roasting time due to the sample moving through the roaster at a higher speed. Roasting temperatures higher than 130°C resulted in flours from which doughs could not be formed. Experimental data gathered from the central composite design was used to generate regression coefficients which were fitted to a second order polynomial equation:

$$y = \beta_0 + \beta_1.X_1 + \beta_2.X_1^2 + \beta_3.X_2 + \beta_4.X_2^2 + \beta_5.X_1.X_2$$

where β_0, β_1 and β_3, β_2 and β_4, and β_5 represent regression coefficients for the intercept, linear, quadratic and interaction terms, respectively. The response variable is represented by y, and the level of the independent variables are represented by X_1 and X_2.

Table 4.1 Levels of independent variables applied in central composite design for roasting condition optimisation

Factor	**Symbol**	**Levels**				
		-α (-1.41)	**-1**	**0**	**1**	**+α (1.41)**
Roasting Speed (Hz)	X_1	60	66	76	86	90
Roasting temperature (°C)	X_2	90	96	110	124	130

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4.3.3 Wheat roasting

To ensure a homogenous roasting process, the dry hot air within the FCCT roaster chamber (Roastech, Bloemfontein, South Africa) was replaced with sufficient superheated steam. This was achieved by roasting a 400 g wheat sample tempered with distilled water to a moisture content of 18 to 20% in accordance with AACC method 26-95.01 (AACC, 1999a). Wheat samples used for experimental purposes were not tempered prior to roasting (i.e. roasting was done on the kernels as is). The different wheat roasting conditions used for the central composite design and X-ray µCT studies are described below.

Central composite design study: Roasting of the treatment batches was performed using conditions determined by the central composite design (Table 4.1). Each of the treatment batches for the high and low protein wheat samples were roasted separately. Roasted wheat was allowed to cool and stored in sealed containers until milled.

X-ray µCT study: Wheat samples were roasted at 90°C and a roasting speed of 86 Hz (ca. 130 s). Each of the two 10 kg treatment batches were roasted separately. Roasted wheat was allowed to cool and stored in sealed containers until milled.

4.3.4 Wheat milling and flour yield

Milling of the control and roasted wheat samples, to obtain white bread flour, was performed at the PepsiCo Inc, Sub Saharan Africa, Research and Development facility. Prior to milling, the wheat batches were tempered to a targeted moisture content of 15.5% for 24 h in accordance with AACC method 26-95.01 (AACC, 1999a). Flour yield was expressed as a percentage of the weight of the white flour extracted from the total tempered wheat weight. The milling procedure for the central composite design and X-ray µCT studies, respectively were as follows.

Central composite design study: Wheat samples were milled using the Brabender Quadrumat Jr. (Quadruplex) mill (C.W. Brabender Instruments Inc., South Hackensack, NJ) in accordance with AACC method 26-50.01 (AACC, 1999b). Prior to milling, the mill was run empty for 30 min where after a 100 g test sample was run to reach its operating temperature. A continuous reel sifter separated the flour from the bran. The flour was stored in airtight containers until further use.

X-ray µCT study: Wheat samples were milled using a Bühler MLU-202 pneumatic laboratory mill (Bühler AG, Gupfenstrasse, Uzwil, Switzerland) in accordance with AACC method 26-21.02 (AACC, 1999c). The 10.0 kg control and roasted wheat batches were each split into 5.0 kg batches prior to milling to prevent overheating of the Bühler mill rollers. The flour was stored in airtight containers until further use.

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4.3.5 Moisture and protein contents

The moisture content of control and roasted whole wheat kernels were determined using the Perten Instruments (Hägersten, Sweden) Inframatic 9500 NIR Grain Analyser, whereas that of the resultant wheat flour samples were determined by means of the air-oven drying AACC method 44-19.01 (AACC, 1999d).

Protein content (N x 5.7) was determined for the different flour samples in duplicate on a 12% moisture basis by Dumas combustion according to AACC method 46-30.01 (AACC, 1999e) using a Leco (Saint Joseph, MI, USA) TruMac N nitrogen analyser.

4.3.6 Mixograph analysis

The mixing characteristics of the control and the roasted wheat flours were evaluated in duplicate using a Mixograph (National Manufacturing, Lincoln, NE, USA) in accordance with AACC method 54-40.02 (AACC, 1999f). Flour (35 g) and water were mixed for 7 min. The dough development time (DDT, time to peak) and peak height was determined.

4.3.7 Preparation of dough and bread samples for X-ray µCT analysis

Dough was mixed using a lean formulation (Table 4.2). One batch of dough was prepared for each of the two control and treatment batches. The dry ingredients (flour, salt, sugar, soy-flour, ascorbic acid and fungal α-amylase), shortening and yeast was added to the mixing bowl of a Kenwood Chef (Kenwood South Africa, Johannesburg, South Africa) where after the water was added. The ingredients were mixed for 2 min on a slow speed setting after which the speed setting was changed to the high speed to facilitate dough formation. Once optimum gluten development was reached, tested by means of windowpane formation, the dough was allowed to rest for 10 min at ambient temperature (ca. 25°C). Following resting, 10 g dough balls were prepared, flattened by hand and rolled into a cylindrical shape. The rolled pieces of dough were placed in silicone moulds and proofed at 40°C and a relative humidity of 80% using a MacAdams Convecta Oven & Prover (MacAdams International, Cape Town, South Africa). A third of the dough pieces were proofed for 20 min and the rest for 40 min. Following proofing, the dough pieces proofed for 20 min and half of the dough pieces proofed for 40 min were flash frozen with liquid nitrogen where after they were freeze dried using a VirTis Advantage Plus freeze dryer (SP Scientific, Warminster, USA) to remove the moisture. The remaining half of the dough pieces proofed for 40 min were baked instead of flash frozen. Baking was performed using a MacAdams Convecta Oven & Prover. The mini bread loaves were baked at 190°C for 6 min. After baking, mini bread loaves were allowed to cool down before packaging them in clear plastic bags until being imaged the following day.

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Table 4.2 Lean bread formulation used for preparation of dough

Ingredients	**Bread Formulation (%)**	**Scaled Weight* (g)**
Bread-flour** (14%$_{m.b.}$)	100	1000
Water	60	600
Fresh compressed yeast	2.6	26
Salt (NaCl)	2.0	20
Sugar	1.0	10
Shortening (Fat)	0.25	2.5
Soya-flour	0.2	2.0
Ascorbic acid	0.008	0.08
Fungal α-amylase	0.007	0.007

*Ingredient weights are based on their percentage of the flour weight.

**Bread flour refers to the control and roasted flour respectively

4.3.8 X-ray micro-computed tomography (µCT) image acquisition

The freeze-dried doughs and mini bread loaves were imaged using a General Electric Phoenix V|Tome|X L240 (General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorff, Germany) high-resolution X-ray computed tomography system with a tungsten target X-ray tube (Du Plessis *et al.*, 2016). The scanning parameters are listed in Table 4.3. Each sample was mounted on a piece of oasis (florist foam) at a 60° angle to stabilise it during image acquisition. A polymeric disc (10 mm thick and 25 mm in diameter) with a density of 2.15 g/cm^3 obtained from Maizey Plastic (Cape Town, South Africa) was also embedded in the oasis. The polymeric disc was used as a reference standard for relative density determinations.

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Table 4.3 Summary of X-ray µCT scanning parameters used for image acquisition

Image acquisition scanning parameters	Parameter
Voltage (kV)	100
Current (µA)	100
Magnification	6.67
Pixel size in the X- and Y axes (mm)	0.2000
Field-of-view (FOV) (mm)	60
Number of pixels in the X- and Y axes	2024
Resolution/ voxel size (µm)	30
Scan time (s)	2800
Original image greyscale intensity resolution	16-bit
Grey levels	2^{16} = 65536
Number of 2D images per sample	2800
Image acquisition time (ms)	333
Rotation sector (°)	360

4.3.9 Image processing and analysis

Following scanning of the samples, the scans were processed to reconstruct the two-dimensional (2D) images into a three-dimensional (3D) volume. This was achieved using the system supplied Datos reconstruction software (Datos|x® 2.1, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorff, Germany). The 3D volume was constructed using the 2,800 2D X-ray projection images (referred to as the image stacks) obtained during the scanning process. The 3D volume was made up of individual voxels (3D pixels) and mapped according to a 16-bit grey-value scale. The raw 3D volumes were imported into the Volume Graphics VGStudio Max 3.1 software (Volume Graphics Gmbh, Heidelberg, Germany) and used for image visualisation and analysis.

After importing the reconstructed 3D volume into VGStudio, the volume-of-interest (VOI) was extracted. This was achieved by means of a series of functions within the software. These functions included adaptive gauss filter, normalise gradient of the surface brightness of the sample and object surface determination which established the boundaries of the VOI.

With the VOI extracted, all the remaining artefacts within the scan were removed by creating regions-of-interest (ROIs) using the eroding and dilating functions, which excluded any voxels outside the sample volume. This ensured that only the sample material (i.e., the freeze-dried dough or bread) and the air entrapped within the foam-like structure would be analysed.

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Following processing of the images, analysis of the 3D volume commenced. This included a foam structure analysis, strut thickness analysis and porosity determination. The foam structure analysis was performed to analyse and visualise the size, number and distribution of the cells within the samples. Parameters recorded from the foam structure analysis included cell volume (mm^3), cell surface (mm^2) and cell diameter (µm). Strut thickness analysis measured the thickness of the material between the cells/pores in the sample. Finally, porosity determination was performed to establish the volume of the material, the volume of the air as well as the total volume of the sample. Using this data, the porosity (ratio of the air volume to the total volume) of the samples could be calculated.

4.3.10 Statistical analysis

Different statistical methods were used for the central composite design and X-ray µCT studies. The methods used were as follows.

Central composite design study: Statistical analysis was performed using STATISTICA version 13 (Statsoft, Inc., Tulsa, USA). A quadratic regression model including 2^{nd} order interaction was fitted on the data. The statistical significance of the regression model, including the factors and interactions, was determined using a 5% significance ($p \leq 0.05$) level. Standardised Pareto charts were generated to illustrate significant effects obtained for the different response values. The data was further subjected to response surface methodology where after regression equations were generated for the different responses and illustrated as two-dimensional contour plots and three-dimensional response surface plots.

X-ray µCT study: Mixed model analysis of variance (ANOVA) was performed to compare the averages of the quantitative measurements. Prior to ANOVA analysis, trimming was applied to the data of the cell volume, cell surface and cell diameter to compensate for large outliers in the mean calculation. The process encompassed sorting the data points from the smallest to the largest values where after the top 10% of the largest values were discarded. The remaining 90% of the values were used to calculate the mean for each of the three parameters. The data for the remainder of the parameters were analysed as is. For each of the parameters, comparisons were made between the controls and the treatments as well as between the different samples (20- and 40- min proofed dough and bread). For the moisture, protein and mixograph parameters, comparisons were made between the control and roasted wheat flour. The data was reported as mean ± standard deviation. The Fisher least significant difference (LSD) test was done to perform the different post hoc analyses. Statistical analysis was performed using STATISTICA version 13 (Statsoft, Tulsa, OK, USA). A 95% confidence interval was used to identify significant results i.e., a 5% significance level ($p \leq 0.05$).

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To construct the bubble size distribution graph, the individual bubble sizes for each sample were tabulated in ascending order of diameter where after they were grouped into different size classes (based on 100 µm increments) spanning the entire range of bubble sizes observed. The total number of observations within each of the size classes were expressed as a percentage of the total number of bubbles observed. It is important to note that although bubble sizes greater than 3000 µm in diameter were not plotted, they were accounted for in the statistical analyses.

4.4 RESULTS AND DISCUSSION

4.4.1 Central composite design

The effects of the two independent variables, X1: roasting speed (Hz) and X2: roasting temperature (°C) on the three response variables, protein content (%), peak time (min) and peak height (mm) were studied using response surface methodology. The results obtained from the central composite design are depicted in Tables 4.4 (flour yield, protein content, peak time and peak height) and 4.5 (initial and tempered moisture and flour moisture contents).

Flour yield

Both high and low protein roasted wheats displayed decreases in flour yield when compared to their respective controls (Table 4.4). The largest decreases in flour yield observed was 4.24% (110°C and 60 Hz) and 2.59% (96°C and 86 Hz) for the high and low protein wheats, respectively. The lowest decreases were 2.62% (90°C and 76 Hz) and 1.04% (124°C and 66 Hz) for the high and low protein wheats, respectively. These results agreed with an earlier study (Germishuys *et al.*, 2020) where a hard wheat (high protein content) displayed larger flour yield losses than a soft wheat (low protein content). The decreases in flour yield can be attributed to increases in porosity induced by the roasting process (Schoeman *et al.*, 2016a).

Moisture content

The CCD roasting conditions used proved to have had little effect on the moisture content of the whole wheat kernels after roasting (Table 4.5). Slight decreases in moisture content were observed for the high (min: 0.27; max: 0.64%) and low (min: 0.10; max 0.70%) protein roasted wheat samples when compared to their respective unroasted controls. These moisture losses were substantially lower than the losses (min: 2.2; max: 2.9%) observed in earlier work (Germishuys *et al.*, 2020). This was due to roasting temperature in this study not exceeding 130°C . Due to the small initial loss of moisture after roasting, the tempering process of the roasted wheat kernels were not impacted. All the roasted wheat samples achieved moisture contents within ±0.5% to the targeted 15.5% moisture content. Milling resulted in decreases in moisture content for the flour produced from the high (min: 0.16; max: 0.59%) and low (min: 0.11; max: 0.53%) protein roasted

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wheat samples as compared to their controls. This was also lower than the results reported by Germishuys et al. (2020).

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Table 4.4 Layout of central composite design (CCD) for optimisation of the wheat roasting conditions displaying results for flour yield, protein and mixograph peak time and peak height for the high and low protein wheats

Sample	CCD Run No.	X_1 Roasting Speed (Hz)	X_2 Roasting Temperature (°C)	Flour Yield (%)	Crude Protein* (%)	Peak Time* (min)	Peak Height* (mm)
High protein wheat flour	Control	0	0	52.31	13.76±0.09	2.7±0.1	47±0
	1 (F)	66	96	48.78	13.78±0.02	2.7±0.0	47±1
	2 (F)	66	124	49.27	13.52±0.04	3.0±0.0	44±0
	3 (F)	86	96	48.20	13.63±0.03	2.6±0.0	48±0
	4 (F)	86	124	49.45	13.55±0.01	3.0±0.0	45±0
	5 (A)	60	110	48.07	13.61±0.06	2.8±0.1	47±0
	6 (A)	90	110	48.27	13.57±0.01	2.9±0.1	48±1
	7 (A)	76	90	49.69	13.63±0.00	2.8±0.0	52±1
	8 (A)	76	130	49.36	13.24±0.04	3.3±0.2	50±0
	9 (C)	76	110	48.94	13.64±0.01	2.8±0.2	47±0
	10 (C)	76	110	49.30	13.54±0.01	2.7±0.1	48±1
Low protein wheat flour	Control	0	0	53.78	10.67±0.03	3.6±0.3	35±0
	1 (F)	66	96	52.59	10.55±0.11	4.0±0.3	34±0
	2 (F)	66	124	52.74	10.80±0.03	4.1±0.1	33±0
	3 (F)	86	96	51.19	10.56±0.07	4.0±0.2	41±0
	4 (F)	86	124	51.77	10.70±0.00	3.9±0.1	34±0
	5 (A)	60	110	52.36	10.46±0.04	4.0±0.2	35±3
	6 (A)	90	110	51.30	10.78±0.01	3.8±0.1	35±1
	7 (A)	76	90	51.51	10.62±0.01	3.8±0.3	35±0
	8 (A)	76	130	51.98	10.77±0.01	4.1±0.1	34±0
	9 (C)	76	110	52.27	10.59±0.06	3.6±0.0	34±0
	10 (C)	76	110	51.82	10.72±0.00	3.7±0.1	33±0

*Results are presented as mean ± standard deviation of duplicate determinations. Crude protein expressed as N × 5.7 on a 12% mb. (F) – factorial design point, (A) – axial point and (C) – central point.

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Table 4.5 Layout of central composite design (CCD) for optimisation of the wheat roasting conditions and results for initial and tempered wheat moisture content as well as flour moisture content for high and low protein wheats

Sample	CCD Run No.	X_1 Roasting Speed (Hz)	X_2 Roasting Temperature (°C)	Initial Wheat Moisture (%)	Tempered Wheat Moisture (%)	Flour Moisture (%)
High Protein wheat flour	Control	0	0	10.75±0.07	15.20±0.07	14.95±0.15
	1 (F)	66	96	10.39±0.03	15.30±0.07	15.01±0.16
	2 (F)	66	124	10,25±0.03	15.30±0.07	14.71±0.18
	3 (F)	86	96	10.48±0.04	15.20±0.14	15.01±0.05
	4 (F)	86	124	10.11±0.04	15.50±0.00	15.07±0.02
	5 (A)	60	110	10.33±0.03	15.30±0.07	15.12±0.03
	6 (A)	90	110	10.36±0.04	15.20±0.00	15.01±0.03
	7 (A)	76	90	10.44±0.02	15.20±0.07	14.95±0.03
	8 (A)	76	130	10.29±0.08	15.30±0.14	15.14±0.07
	9 (C)	76	110	10.11±0.02	15.30±0.07	14.80±0.14
	10 (C)	76	110	10.16±0.04	15.40±0.14	14.99±0.10
Low Protein wheat flour	Control	0	0	10.08±0.04	15.40±0.07	15.25±0.22
	1 (F)	66	96	9.85±0.03	15.40±0.07	15.02±0.06
	2 (F)	66	124	9.98±0.04	15.30±0.14	15.13±0.01
	3 (F)	86	96	9.87±0.04	15.20±0.00	15.05±0.15
	4 (F)	86	124	9.62±0.05	15.50±0.07	15.10±0.11
	5 (A)	60	110	9.40±0.05	14.90±0.07	14.49±0.09
	6 (A)	90	110	9.38±0.04	15.50±0.07	14.97±0.11
	7 (A)	76	90	9.85±0.01	15.30±0.07	15.19±0.17
	8 (A)	76	130	9.76±0.03	15.30±0.00	15.14±0.04
	9 (C)	76	110	9.84±0.07	15.20±0.07	14.88±0.09
	10 (C)	76	110	9.65±0.03	15.50±0.07	15.39±0.13

Results are presented as mean ± standard deviation of duplicate determinations. (F) – factorial design point, (A) – axial point and (C) – central point.

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Central composite design results

Standardised pareto charts provide insight into the effect of the independent variables (roasting speed and temperature) and their interactions on the dependent variables (protein content, peak time and peak height). The linear, quadratic and interaction effects of the independent variables are depicted as rectangular bars on the chart. A parameter's effect is considered significant if the bar crosses the vertical line representing the p=0.05 confidence level. Table 4.6 provides the prediction equations for the three dependent variables for the high and low protein roasted wheats.

Table 4.6 Polynomial prediction equations for the three dependent variables in the roasting of the high and low protein wheats

Sample	Dependent variable	Predictions equation	R^2-value
High protein wheat flour	Protein	$Y = 14.84080 - 0.05889X_1 + 0.00016X_1^2 + 0.02727X_2 - 0.00026X_2^2 + 0.00030X_1X_2$	0.79
	Peak time	$Y = 11.79920 - 0.07819X_1 + 0.00033X_1^2 - 0.12359X_2 + 0.00053X_2^2 + 0.00027X_1X_2$	0.92
	Peak height	$Y = 91.26341 + 0.88948X_1 - 0.00591X_1^2 - 1.35338X_2 + 0.00558X_2^2 + 0.00046X_1X_2$	0.57
Low protein wheat flour	Protein	$Y = 7.32707 + 0.06428X_1 - 0.00025X_1^2 + 0.00785X_2 + 0.00006X_2^2 - 0.00021X_1X_2$	0.62
	Peak time	$Y = 16.63421 - 0.14759X_1 + 0.00112X_1^2 - 0.13413X_2 + 0.00071X_2^2 - 0.00024X_1X_2$	0.69
	Peak height	$Y = 36.61439 - 0.24224X_1 + 0.01030X_1^2 + 0.11311X_2 + 0.00296X_2^2 - 0.01106X_1X_2$	0.71

X_1 = roasting speed (Hz) and X_2 = roasting temperature (°C)

The standardised pareto charts for protein content, peak time and peak height for both the high and low protein content wheats are depicted in Figure 4.1. These charts indicated that neither roasting temperature nor roasting speed resulted in significant differences (p>0.05) in protein content, mixograph peak time or peak height for the different roasting combinations. Although no significant differences were found, some trends were observed. It was noted that the linear followed by the quadratic effect of roasting temperature resulted in the greatest effect on both the

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protein content as well as the mixograph peak time and peak height for the high protein wheat. The low protein wheat, on the other hand, showed that the linear effect of roasting temperature followed by the linear effect of roasting speed showed the greatest effect on the protein content. The inverse of this was observed for the mixograph peak height of the low protein wheat. The quadratic effect of roasting temperature followed by the quadratic effect of roasting speed displayed the greatest effect on the mixograph peak time for the low protein wheat.

The response surface plots (Figure 4.2) further support the effects observed in the standardised pareto charts. The response surface plots for protein content for the high protein wheat showed an almost linear decrease in protein content for roasting temperatures above 110°C at the lower speed settings (i.e., longer roasting times) whereas the effects were less severe at the higher speed settings (above 80 Hz). A linear increase in protein content as the temperature increased was observed for the low protein wheat, although this was only observed for the lower speed settings (i.e., longer roasting times). An increase in protein content was also observed as the roasting speed increased (i.e., shorter roasting time).

Mixograph peak time is referred to as the time required for optimal gluten development and maximum water absorption by gluten and starch (Delcour and Hoseney, 2010). As the roasting temperature decreased, the peak time also decreased for the high protein wheat due to less impact on its protein properties and subsequent ability to form a gluten network (Raigar *et al.*, 2017). Roasting speed on the other hand displayed little effect on the peak time, with the exception of the slower speeds (55 to 70 Hz) which resulted in slightly longer peak times. The low protein wheat displayed the shortest peak time around the centre of the plot. The longest peak times were located at the extremes of the temperature (85 and 135°C) and speed (55 and 95 Hz) variables.

The effects of roasting temperature and speed on the mixograph peak height of the high and low protein wheats were similar (Figure 4.2). Both wheats displayed increases in peak height as the roasting temperature decreased and the roasting speed increased (i.e., decreasing roasting time). Peak height is used as an indication of the strength of a gluten network formed during dough mixing. Lower temperatures and shorter roasting times will have a less detrimental effect on the protein structure. Detrimental changes in the protein structure during the roasting process can result in less protein-protein interactions during the mixing process and a decreased gluten strength (Bucsella *et al.*, 2016).

Figure 4.3 depicts the contour plots for the interaction between roasting speed and roasting temperature and their effect on protein content, mixograph peak time and peak height for the high and low protein wheats. Due to no significant differences between the different treatment combinations (Figure 4.1), the optimum roasting conditions for protein content, mixograph peak time and peak height was chosen based on trends observed in the contour plots.

The optimum roasting conditions for protein content were 85–110°C and 55–85 Hz for the high protein wheat and 85–115°C and 80–95 Hz for the low protein wheat. For peak time the

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values ranged from 90–105°C and 70–90 Hz for the high protein wheat and 105–110°C and 75–80 Hz for the low protein wheat. The optimum roasting condition ranges for peak height was 85–92°C and 60–90 Hz for the high protein wheat and 85–90 °C and 90–95 Hz for the low protein wheat.

It is ideal to recommend a single roasting combination for each wheat. These recommendations would take into consideration the effect of the roasting process on the protein content, mixograph peak time and peak height. For the high protein wheat, the recommended roasting conditions would be 90°C and 86 Hz (Figs. 4.3a & 4.3d). This combination will maximise protein content and peak height and minimise peak time. Similarly, the recommended roasting conditions for the low protein wheat would be 90°C and 90 Hz. In this case preference was given to peak height as small deviations from these conditions would result in a larger decrease in peak height. The same deviation would not result in a considerable increase in peak time (Figs. 4.3e & 4.3f).

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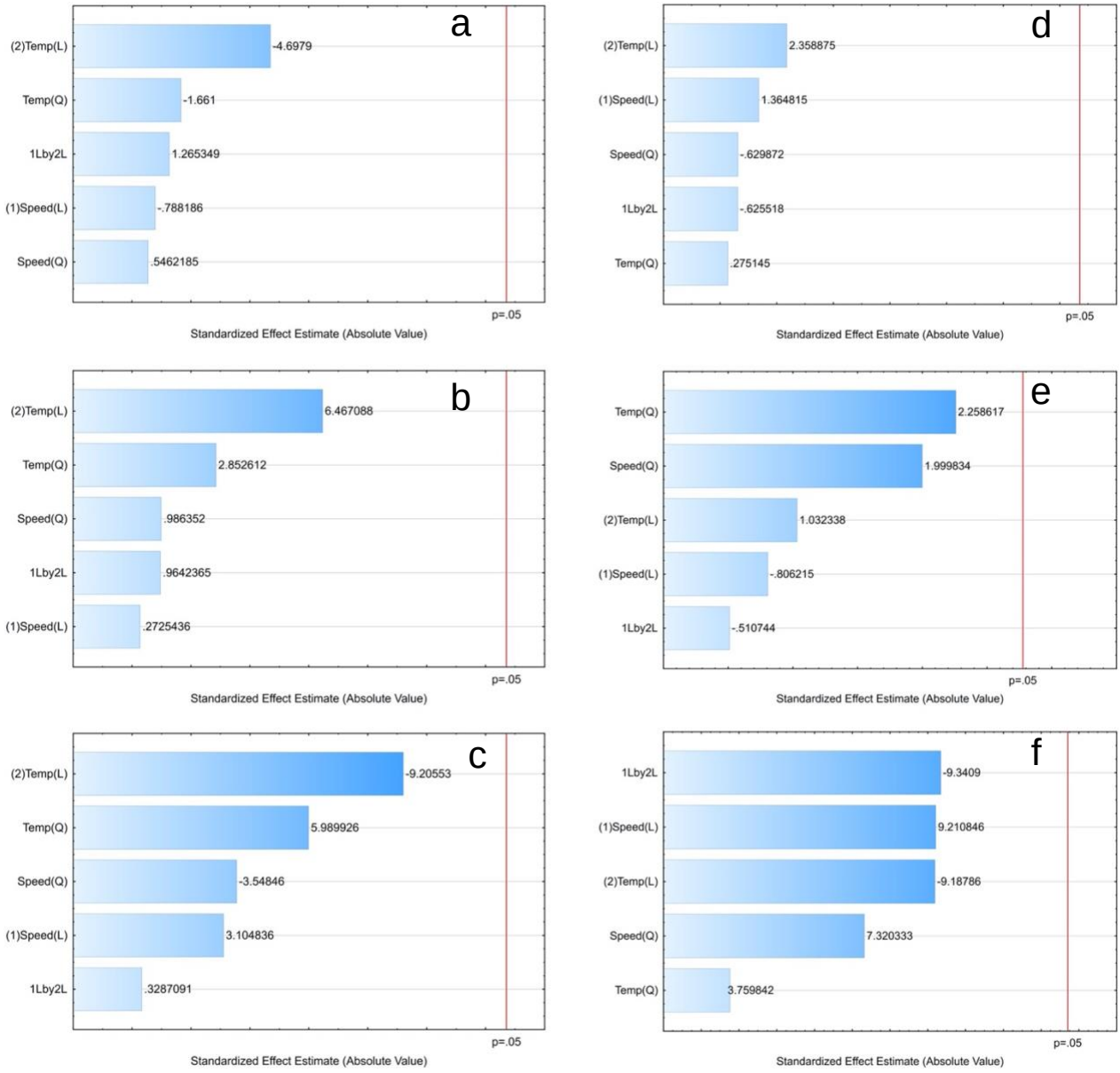

Figure 4.1 Standardised Pareto charts showing the linear, quadratic and interaction effects for (a) protein content, (b) peak time and (c) peak height of the high protein wheat flour and (d) protein content, (e) peak time and (f) peak height of the low protein content wheat flour. L = linear effect; Q = quadratic effect; LxL = interaction effect.

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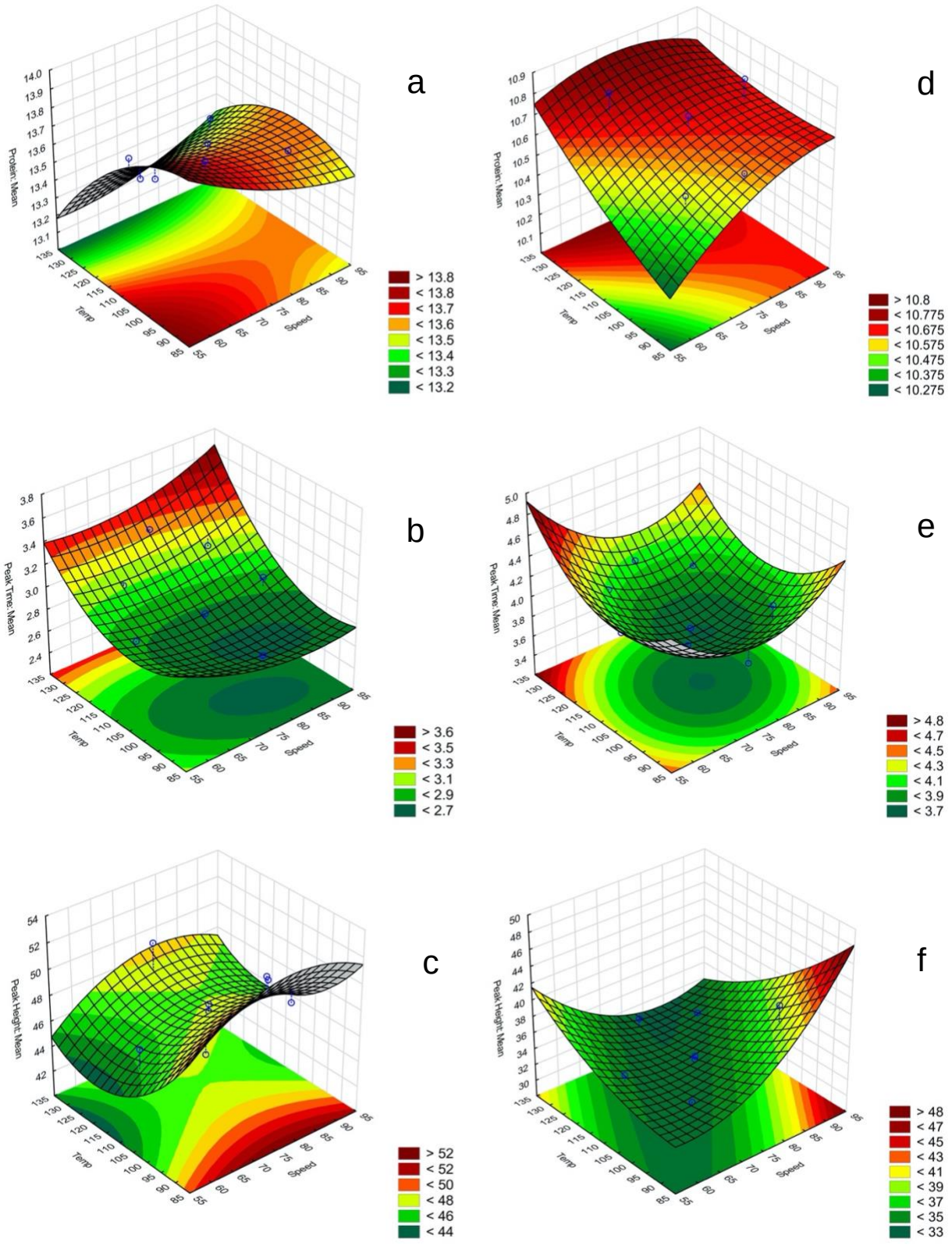

Figure 4.2 Response surface plots for (a) protein content, (b) peak time and (c) peak height of the high protein wheat flour and (d) protein content, (e) peak time and (f) peak height of the low protein content wheat flour.

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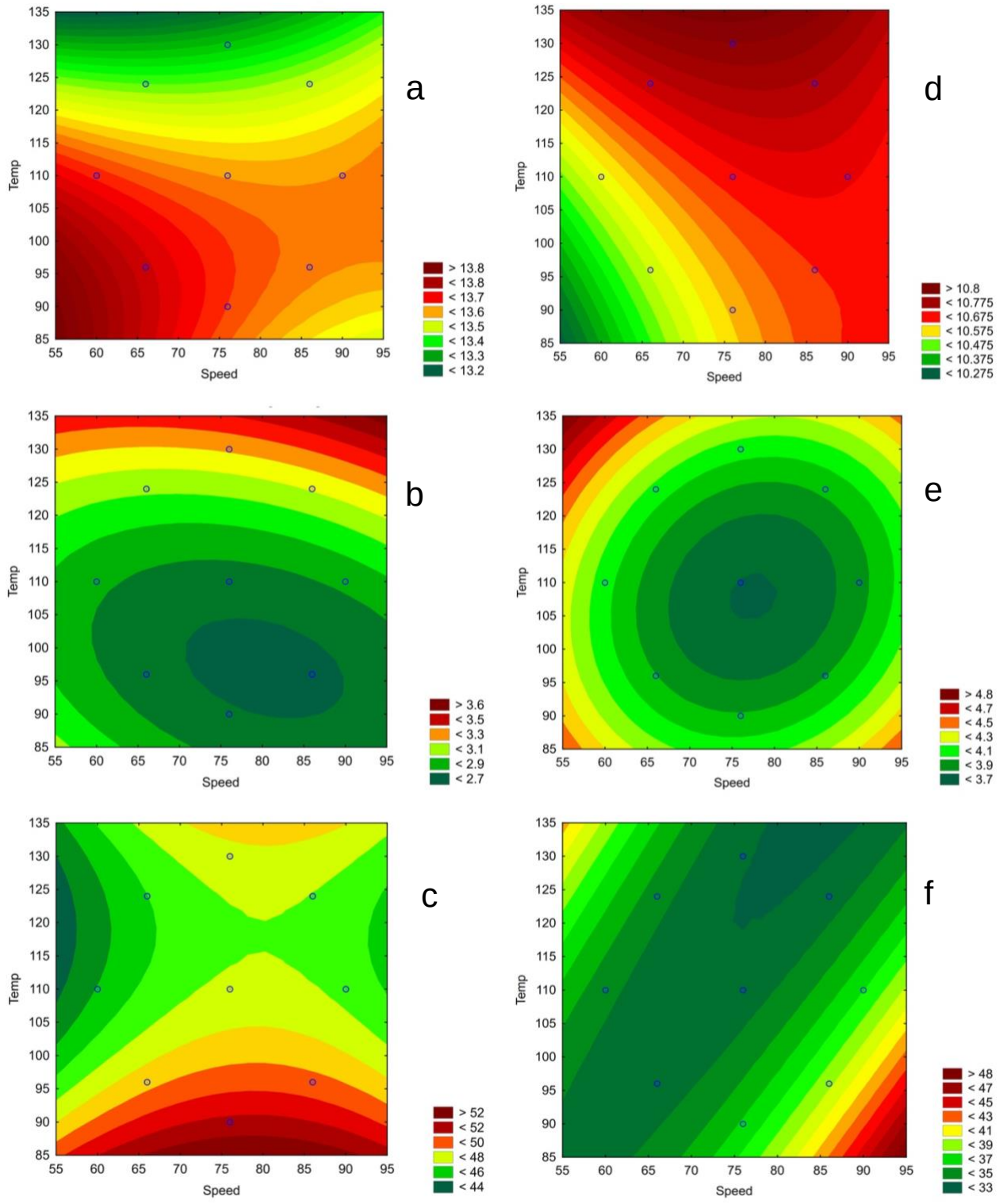

Figure 4.3 Contour plots for (a) protein content, (b) peak time and (c) peak height of the high protein wheat flour and (d) protein content, (e) peak time and (f) peak height of the low protein content wheat flour.

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4.4.2 X-ray µCT analysis

Flour yield, moisture and protein contents and mixograph analysis

A slight increase of 0.18% in flour yield was observed for the roasted wheat flour although this increase was not significant ($p>0.05$) (Table 4.7).

The roasting conditions used (90°C and 86 Hz), based on the results of the central composite design, resulted in a significant decrease ($p\leq0.05$) of 0.86% in wheat moisture content for the roasted wheat sample (Table 4.7). Although the decrease was significant, the tempering process of the roasted wheat kernels were not impacted. Both the control and roasted wheat samples reached the optimum tempered moisture content of 15.0–15.5%. Significant differences ($p\leq0.05$) in flour moisture content was observed between the control and roasted wheat flour samples. This could be expected as the tempered moisture content of the control was 0.48% lower than that of the roasted wheat samples. Roasting resulted in a 0.07% decrease in protein content of the roasted wheat samples ($p\leq0.05$).

No significant differences ($p>0.05$) were observed for mixograph peak time between the control and the roasted wheat flour, although the roasted wheat flour displayed an increase in peak time of 3 s (Table 4.7). No significant differences ($p>0.05$) in mixograph peak height were observed between the control and roasted wheat flour, although there was a decrease of 1 mm in peak height for the roasted wheat flour. Changes in the protein properties during the roasting process (Bucsella *et al.*, 2016) cause less protein-protein interactions during mixing resulting in a decreased gluten strength. The current roasting conditions had an insignificant effect on the protein-protein interactions.

X-ray µCT results

The X-ray µCT instrument used in this study required a scan time of approximately 46 min. This posed the problem of maintaining the integrity of dough samples during the scan time which was overcome by flash freezing the dough samples using liquid nitrogen where after the samples were freeze dried. This ensured that the structural integrity of dough samples would be maintained throughout the scanning process. The aforementioned processes were performed in such a way to minimise deformation of the dough samples. A full-size bread loaf could be imaged, but at the cost of resolution. Preparing 10 g bread loaves, similar to the size of the dough samples, resulted in much higher resolution images to be achieved during X-ray µCT scanning as compared to a full-size bread loaf. The higher resolution enables the detection of smaller bubbles within the dough.

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Table 4.7 Initial and tempered wheat moisture content, flour moisture content, flour yield, flour protein content and mixograph peak time and peak height for the control and roasted wheat flours

						Mixograph	
	Wheat Moisture (%)	**Tempered Moisture (%)**	**Flour Moisture (%)**	**Milling Yield (%)**	**Flour Protein (%)**	**Peak Time (min)**	**Peak Height (mm)**
Control	13.08±0.00[a]	15.05±0.10[a]	14.78±0.07[a]	65.99±0.79[a]	12.25±0.06[a]	3.51±0.10[a]	51±1[a]
Treatment	12.22±0.12[b]	15.53±0.05[a]	14.95±0.06[b]	66.17±0.51[a]	12.18±0.05[a]	3.56±0.15[a]	50±1[a]

Results are presented as mean ± standard deviation of duplicate determinations from two replicates.

An increase in mean cell volume of 3% ($p>0.05$) was observed in the 20-min proofed dough prepared with roasted wheat flour compared to the control (Table 4.8). A significant increase ($p\leq0.05$) in mean cell volume was observed for both the 40-min proof dough (15%) and the bread (25%) prepared from roasted flour, compared to the control. Figure 4.4 depicts the 2D slice images obtained from the centre of the 3D volume (i.e., the centre of the dough/bread) illustrating the cell volumes (as per the foam structure analysis) for the different samples. The 20-min proofed dough displayed mostly small bubbles with the exception of the large cells that can be observed for the treatment sample. These large cells stemmed from the way in which the dough pieces were prepared for panning. When the 10 g dough pieces were flattened and rolled up, a large air pocket got trapped between the dough layers.

The mean cell volume of the 40-min proofed dough, for both the control and roasted wheat flour, significantly ($p\leq0.05$) increased from that of the 20-min proofed dough. The decrease in small bubbles and increase in larger size bubbles (i.e., increase in cell volume) is attributed to the mass transfer of gas from the small bubbles to the larger ones. This process, known as disproportionation, is driven by the Laplace pressure within the bubbles (Mills *et al.*, 2003; Koksel *et al.*, 2016). The large air pockets observed in the 20-min proofed dough treatment sample (ca. 560–600 mm^3) seemed to have sub-divided into smaller cells (Figure 4.4). The control sample of the 40-min proofed dough still appeared to have an air pocket in the dough although it was much smaller (ca. 220–240 mm^3) than that of the 20-min proofed dough treatment sample. When observing the 2D images of the bread, for both the control and roasted wheat flour, one can see a significant ($p\leq0.05$) decrease in the mean cell volumes from that of the 40-min proofed dough. This was unexpected. During the early stages of baking the gas bubbles experience a more rapid expansion phase resulting from an increase in CO_2 production as well as the formation of steam. Both these factors increase the bubbles' tendency to undergo coalescence as a result of failure in the gluten-starch matrix which make up the cell walls of the bubbles (Mills *et al.*, 2003; Turbin-Orger *et al.*, 2012). During coalescence, adjacent gas cells merge giving rise to a decrease in the

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number of gas cells present in the dough and an increase in the mean cell size of these bubbles (Miś *et al.*, 2018). The reason for the contrast of the results observed in the current study to what would be expected could be as a result of the foam structure analysis. As the software is searching for closed cells (i.e., bubbles) it may have misinterpreted the connecting cells as separate cells. This would directly influence the number and size of the cells it reports, effectively inflating the results.

A slight decrease of 1% in mean cell surface area was observed for the roasted wheat flour for the 20-min proofed dough although this decrease was not significant ($p>0.05$) (Table 4.8). Significant ($p\leq0.05$) increases of 9% and 14% in mean cell surface area were observed between the control and roasted wheat flour for the 40-min proofed dough and the bread, respectively. These results could be expected as the cell surface area would increase as the volume of the cells increase (Cafarelli *et al.*, 2014).

No significant differences ($p>0.05$) were observed between the control and roasted wheat flour in terms of the mean cell diameter for both the 20- and 40-min proofed dough (Table 4.8). Although not significant, increases of 1% and 4% were observed for the 20- and 40 min proofed dough respectively for the roasted wheat flour. A significant increase ($p\leq0.05$) of 7% was observed for the bread produced from the roasted wheat flour compared to its control.

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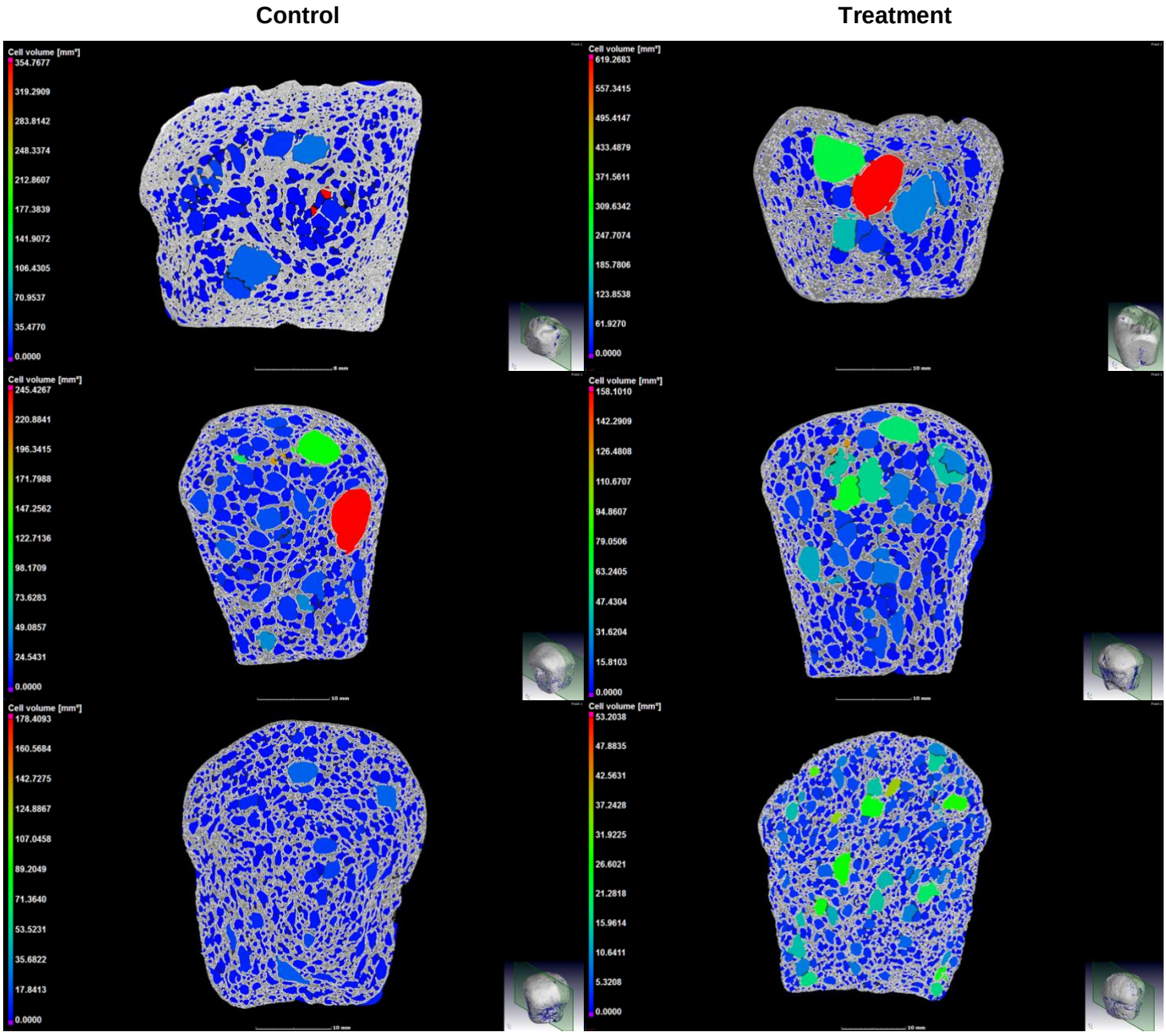

Figure 4.4 2D slice images (centre slice) taken from the 3D volume illustrating the cell volumes (as per the foam structure analysis) of the different samples. Different colour cells represent different cell volumes as depicted in the colour scale to the right of the image.

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Table 4.8 Mean and percentage increase/decrease in X-ray µCT parameters for the 20- and 40 min proofed dough as well as the bread

Sample	20-min Proofed dough	40-min Proofed dough	Bread
Cell Volume (mm^3)			
Control	0.32±0.01^{d}	0.62±0.01^{b}	0.48±0.04^{c}
Treatment	0.33±0.05^{d}	0.71±0.04^{a}	0.60±0.00^{b}
% Increase / Decrease	3	15	25
Cell Surface (mm^2)			
Control	6.72±0.01^{c}	7.84±0.21^{b}	6.54±0.09^{c}
Treatment	6.66±0.27^{c}	8.51±0.38^{a}	7.47±0.14^{b}
% Increase / Decrease	-1	9	14
Cell Diameter (µm)			
Control	776.58±4.72^{c}	947.51±0.07^{a}	887.31±22.69^{b}
Treatment	787.54±34.79^{c}	986.73±19.06^{a}	945.81±0.65^{a}
% Increase / Decrease	1	4	7
Strut Thickness (mm)			
Control	0.32±0.01^{ab}	0.29±0.01^{bc}	0.25±0.00^{cd}
Treatment	0.34±0.02^{a}	0.29±0.01^{b}	0.24±0.02^{d}
% Increase / Decrease	6	0	-4
Defect Volume (mm^3)			
Control	7014.94±793.39^{d}	13328.49±589.09^{abc}	16760.49±2090.11^{ab}
Treatment	8002.79±1145.97^{cd}	10369.60±5585.26^{bcd}	18563.91±183.33^{a}
% Increase / Decrease	14	-22	11
Porosity (%)			
Control	52.21±2.61^{b}	67.83±1.15^{a}	65.90±2.33^{a}
Treatment	54.74±3.61^{b}	69.63±1.13^{a}	74.88±9.04^{a}
% Increase / Decrease	5	3	14

Results are presented as mean ± standard deviation from two replicates. Different superscripts represent significant differences ($p \leq 0.05$) for each parameter respectively.

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Figure 4.5 depicts the bubble size distribution (BSD) of the freeze-dried dough proofed at 20- and 40 min as well as the bread for both the control and treatment samples as determined by foam structure analysis performed on the 3D volumes. The normalised frequency percentage of bubbles grouped in size classes of 100 µm increments are plotted against the diameters (µm). This provides an easier way of visualising the size distribution of the thousands of bubbles within the samples. It is clear that the 20-min proofed dough displayed the largest volume fractions of bubbles in the 700 µm diameter range (peak of the curve). This was followed by the bread and lastly the 40-min proofed dough. In all three of these instances the control samples displayed higher volume fractions compared to their roasted counterparts. With reference to the larger size bubbles (1000–2000 µm in diameter), an increase in volume fractions of these bubble sizes can be observed for both the 40-min proofed dough as well as the bread when compared to the 20-min proofed dough. This is expected as increases in proofing time results in bubble expansion facilitated by CO_2 produced by the yeast as well as the process of disproportionation (Mills *et al.*, 2003; Jha *et al.*, 2017). During baking the starch-protein matrix surrounding the gas cells/bubbles rupture resulting in the interconnection of adjacent gas cells giving rise to a fine crumb structure (Gan *et al.*, 1995; Mills *et al.*, 2003). It was also noted that the treatment samples displayed slightly higher volume fractions of bubbles in the 1000–2000 µm range compared to their respective controls.

Strut thickness refers to the thickness of the bubbles' cell walls that encase the air. The thickness of the cell walls may impact its mechanical strength as well as its deflection at break. Scanlon & Zghal (2001) illustrated that greater mechanical strength and deflection at break may result from thinner cell walls, due to its flexibility, leading to a softer crumb structure. No significant differences ($p>0.05$) for mean strut thickness were observed between the control and roasted wheat flour for either the 20- or 40 min proofed dough or the bread (Table 4.8). Although not significant, a 6% increase in strut thickness was observed for the 20-min proofed dough, produced from the roasted wheat flour, increasing from 0.32 mm for the control to 0.34 mm for the roasted wheat flour. This sample also displayed the largest strut thickness of all the samples.

The strut thickness of the 40-min proofed dough was lower than that of the 20-min proofed dough with both the control and roasted wheat flour having a mean strut thickness of 0.29 mm. This decrease in strut thickness as proofing time increases (Figure 4.6) is in agreement with an earlier study performed by Babin et al. (2006) who investigated bubble growth and foam setting during breadmaking. They found that strut thickness decreased as proofing time increased. They reported that after 20- and 40 min of proofing the strut thicknesses within the dough were 0.28 mm and 0.26 mm, respectively. The strut thicknesses reported in their study was lower than those observed in the current study, however it must be noted that their sample size (in terms of the volume of dough used) was much smaller (diameter × h = 9 mm × 4 mm).

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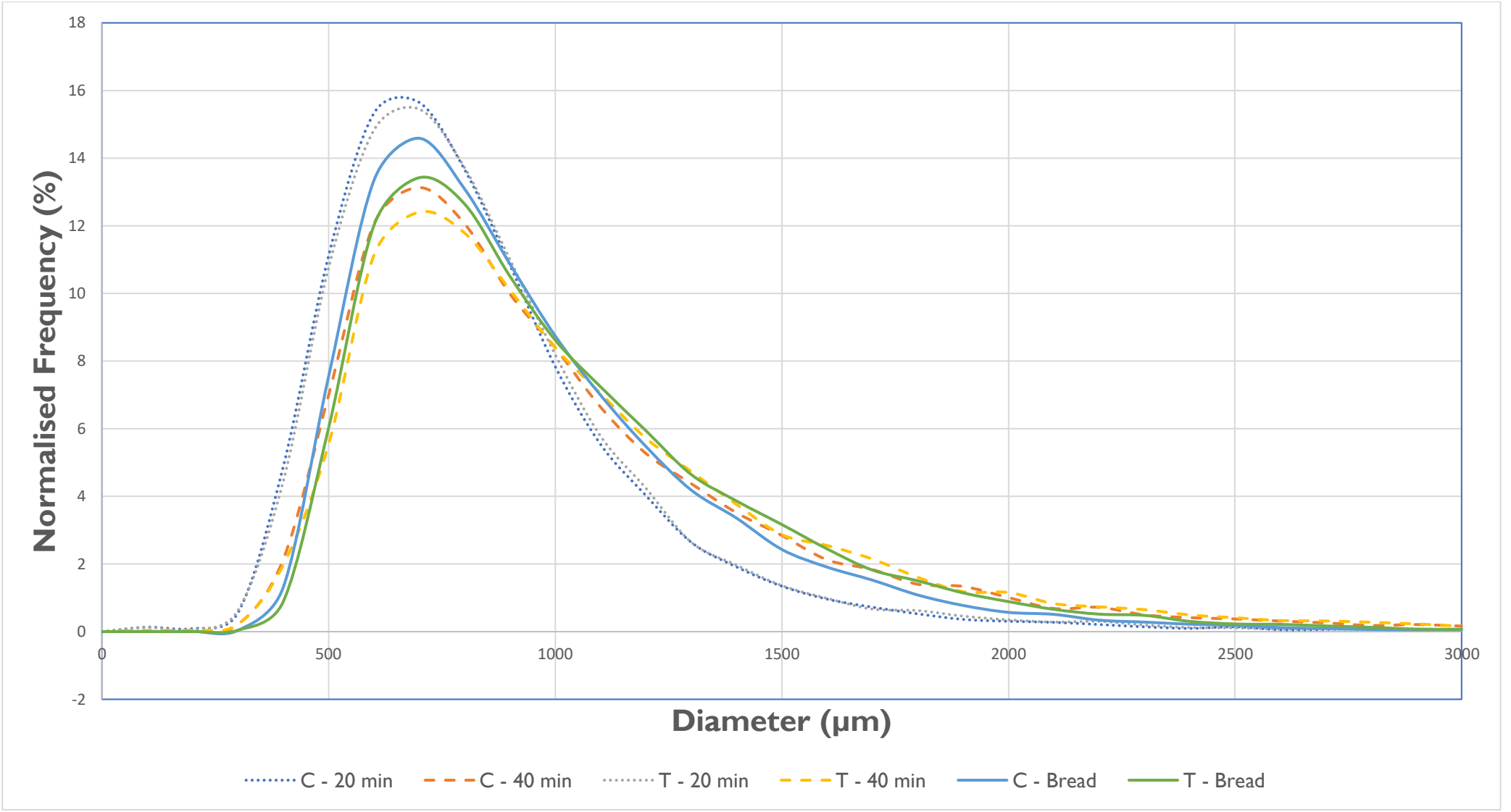

Figure 4.5 Bubble size distribution (BSD) of the freeze-dried dough proofed for 20- and 40 min as well as the bread for both the control and treatment samples as determined by X-ray µCT.

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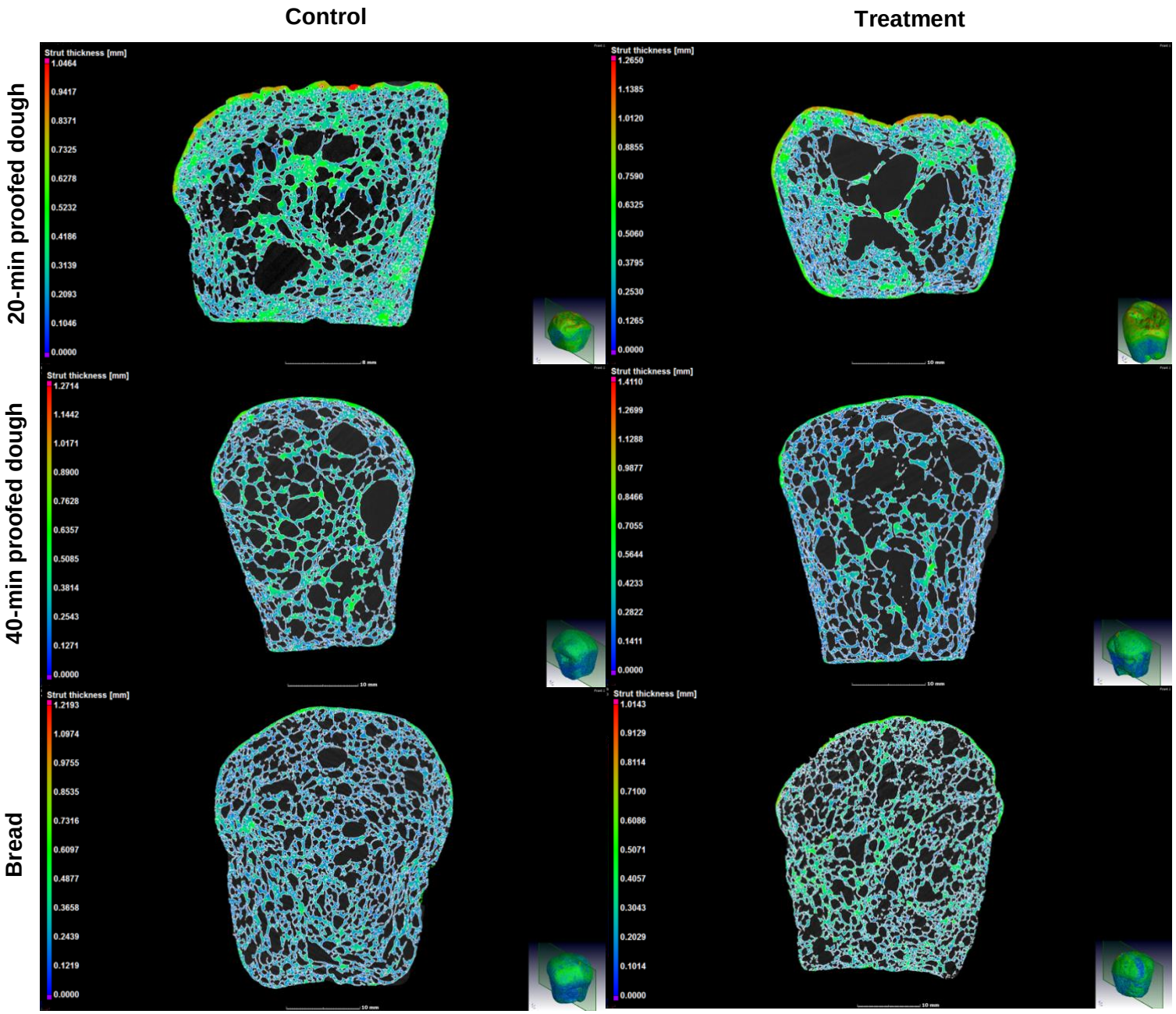

Figure 4.6 2D slice images (centre slice) taken from the 3D volume illustrating the strut thickness of the different samples. Different colours represent different strut thicknesses as depicted in the colour scale to the right of the image. Strut thicknesses shown in blue are thinner than those in green.

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A 4% decrease in mean strut thickness was observed for the bread sample, decreasing from 0.25 mm to 0.24 mm for the roasted compared to the control wheat flour sample. The bread also displayed the thinnest strut thickness compared to the 20- and 40 min proofed dough. A thin strut thickness would cause a finer crumb structure, ultimately resulting in a softer and more elastic crumb texture (Rathnayake *et al.*, 2018). As no significant differences in strut thickness were observed between the control and roasted wheat flour samples, roasting of the wheat would not affect the quality of the bread loaves prepared from the resultant flour.

Increases of 14% and 11% was observed for defect (air) volume for the 20-min proofed dough and the bread (Table 4.8). A decrease of 22%, on the other hand, was observed for the defect volume of the 40-min proofed dough. Once again, no significant differences ($p>0.05$) were observed between the control and roasted wheat flour for defect volume. As the proofing time increased up until the end of baking, the defect volume also increased. This could be expected as during proofing yeast produce CO_2 which diffuse into the cells inflating the bubbles and increasing the volume within them (Scanlon and Zghal, 2001; Avramenko *et al.*, 2018). As proofing time increases, the volume of air within the dough also increases leading to a higher defect volume.

Porosity is defined as the ratio of the defect volume (i.e., air volume) to the total volume of the dough (i.e., air- and material volume). The porosity of a bread greatly influences its loaf volume and crumb texture. A low porosity (i.e., higher volume of material to air) would result in a dense loaf of bread. As expected, the porosity of the 20-min proofed dough was significantly lower ($p\leq0.05$) than that of the 40-min proofed dough and the bread.

Porosity determination of the 3D volumes showed that the porosity of the roasted wheat sample for both the 20- and 40 min proofed dough as well as the bread was higher ($p>0.05$) than that of their control counterparts (Table 4.8). An investigation into the bubble size distribution of wheat flour, using two different dough formulations, concluded that the stiffer dough (i.e. the stronger dough) entrained less air resulting in a reduction in porosity (Bellido *et al.*, 2006). In the current study, the roasted samples produced a weaker dough and subsequently a weaker gluten network as their porosities were higher compared to their respective controls. These results are in agreement with the mixograph peak height for the control and roasted wheat flour. Mixograph analysis showed that the roasted wheat flour had a peak height of 50 mm whereas the control wheat flour has a peak height of 51 mm, although these did not significantly differ ($p>0.05$).

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4.5 CONCLUSIONS

No significant differences for protein content, mixograph peak time and peak height were observed as no severe roasting conditions were included. The roasting conditions chosen for the X-ray µCT part of the study was 90°C and 86 Hz. This combination will maximise protein content and peak height and minimise peak time.

The use of liquid nitrogen to flash freeze the 20- and 40 min proofed dough samples followed by freeze drying aided in maintaining the structural integrity of the dough samples during scanning. Furthermore, the 10 g samples made it possible to study the dough and bread samples at a much higher resolution than would be possible with a full-size piece of dough and bread loaf. This higher resolution enabled the detection of smaller bubbles. Most notable were the decreased strut thickness of the bread produced from the roasted wheat flour, suggesting a finer crumb structure and softer texture. When taking into consideration the lower mixograph peak heights of the dough, it suggests these samples had a weaker gluten network compared to their controls. Secondly, porosity increases for all the treatment samples were observed. Roasting at 90°C and a roasting speed of 86 Hz did not negatively affect the foam properties of the breads baked from the resultant flour. On the contrary, an improvement in the foam properties of bread in terms of the crumb fineness and texture was observed. X-ray µCT is expensive, time-consuming and requires technically trained personnel to operate the instrument and analyse the data. Therefore, the use of C-Cell as an alternative technique to effectively study the foam properties of bread was investigated.

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CHAPTER 5

Evaluation of quality and shelf life of breads produced from roasted wheat flour by means of C-Cell and texture analysis

5.1 ABSTRACT

The quality and shelf life of bread is important as it directly influences its texture. This study evaluated the quality and shelf life of bread produced from roasted wheat flour by means of C-Cell and texture analysis. Bread loaves were baked using flour produced from unroasted and roasted (90°C for 130 s using a forced convection continuous tumble roaster) wheat and subjected to C-Cell and texture analysis. C-Cell analysis showed the breads produced from the roasted wheat flour had a coarser crumb structure and a darker crumb colour compared to the control breads although this did not negatively impact the texture of these breads. The control bread samples displayed larger hole areas compared to the treatment samples although the volume of the holes observed for the control samples were lower than that of the treatment samples. Therefore, as volume accounts for the depth of the hole, the treatment samples thus displayed deeper holes. More importantly, it was shown that the use of flour produced from roasted wheat resulted in a softer bread (i.e., lower firmness) with an increased shelf life. Breads produced from the roasted wheat flour displayed the lowest overall firmness, and on the fourth day of shelf life testing it had not yet reached the firmness observed for the control breads on day three of testing.

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5.2 INTRODUCTION

Bread is one of the most widely consumed food products in the world and forms part of the staple diet of the majority of the global population. The texture of a loaf of bread is an important attribute with reference to its quality (Scanlon and Zghal, 2001). The main process responsible for the loss in quality of a bread is staling. Bread staling refers to the time-dependent loss in quality of texture and flavour and can thus be directly correlated to the shelf life of the bread.

Roasting of wheat and other cereals are traditionally practised to increase its shelf life as well as improve its organoleptic properties (Murthy *et al.*, 2008). In addition, roasting is also performed to improve product quality and increase processing efficiency (Chung *et al.*, 2011). Beneficial changes linked to roasting of wheat have been described by Murthy et al, (2008) and included improved flavour, texture and colour. Bread produced from roasted wheat flour displayed a decreased strut thickness which suggested a finer crumb structure and softer texture as well as an increase in porosity as measured with X-ray micro-computed tomography (µCT) (section 4.4.2, pp. 77, 80). Roasting has also been shown to inactivate proteolytic enzymes which lead to an increase in loaf volume (Vázquez *et al.*, 2001). Qu et al, (2017) further reported an increase in the softness and springiness of steamed bread that had been produced from wheat heated by means of a microwave.

Roasting has also been shown to have detrimental effects, the degree to which is dependent on the roasting method, time and temperature. Germishuys et al, (2020) characterised flour produced from wheat roasted at 180°C for 140 s using a forced convection continuous tumble (FCCT) roaster. Rheological and physicochemical results showed considerable moisture loss which negatively impacted the wheat tempering process prior to milling. Roasting at these conditions further resulted in significant starch damage and changes in protein properties as a dough could not be formed during mixograph testing.

Texture, with reference to bread, encompasses properties such as its pore structure and how these pores are distributed as well as the overall porosity of the loaf (Scanlon and Zghal, 2001). Staling, which changes the texture of the bread, leads to crumb firming, softening of the bread crust and loss of flavour (Torrieri *et al.*, 2014). Currently the process of bread staling is thought to be a combination of different processes. The two main processes thought to cause staling is the transfer of water within the bread as well as retrogradation of the starch molecules (León *et al.*, 2006; Besbes *et al.*, 2013). Bread staling is further associated with a decrease in consumer acceptability in terms of the organoleptic properties of the bread (Gray and Bemiller, 2003).

A common method to quantify the visual texture of bread slices is to separate the pore structure from the cell wall structure where after relevant features can be identified from two-dimensional scans. This quantification process is made possible through the use of a C-Cell instrument (van Riemsdijk *et al.*, 2011). The C-Cell instrument is a digital imaging system used

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worldwide by the bread industry, among others, to analyse the crumb structure of bread to determine its quality. The results obtained from C-Cell has been used to understand the rheological properties required to stabilize expanding gas cells (Sroan *et al.*, 2009), to investigate the rheological properties of wheat varieties from different geographical regions (Ktenioudaki *et al.*, 2010) and to study the effect of different sodium chloride concentrations during mixing and how this impacted baking performance (McCann and Day, 2013).

In addition to C-Cell, a commonly used technique to determine the rate and/or effect of staling on the bread crumb (i.e., the shelf life of the bread) is texture analysis. This involves measuring the hardness and resilience/springiness of the crumb. Texture analysis has been used to evaluate the staling rate of bread as effected by the addition of different flours/starches to the baking formulation (Purhagen *et al.*, 2011), to investigate the effect of different levels of gluten on bread staling (Curti *et al.*, 2014) and to assess differences in the staling process of white wheat bread as affected by maltogenic amylase and storage time (Amigo *et al.*, 2016).

The aim of this study was to evaluate the quality and shelf life of bread loaves prepared from roasted wheat flour by means of C-Cell and texture analysis.

5.3 MATERIALS AND METHODS

5.3.1 Wheat sample preparation

Wheat samples as described in (section 4.3.1, p. 55) were used. The wheat samples were roasted at 90°C for 130 s and milled as described in (section 4.3.3, p. 57). The subsequent control and roasted wheat flour, which included two control and two roasted wheat flour batches, were utilised. The moisture content of the control and roasted wheat flours were 14.78 and 14.95% respectively whereas the protein contents were 12.25 and 12.18% respectively, making these flours suitable for breadmaking (Agricultural Product Standards Act, 2019).

5.3.2 Wet and dry gluten content

Wet and dry gluten analyses of the control and roasted wheat flours were performed in duplicate using the Perten Glutomatic system (Perten, Hägersten, Sweden) in accordance with AACC method 38-12.02 (AACC International, 2010). The wet gluten was washed by the automatic washing apparatus and centrifuged on a sieve under standardised conditions. The wet gluten forced through the sieve as well as the total wet gluten were both weighed. The total wet gluten was dried under standardised conditions and weighed. These values were used to calculate the wet and dry gluten content, the gluten index and water binding capacity for the respective flours.

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5.3.3 Damaged starch contents

Damaged starch content analysis of the control and roasted wheat flours were performed amperometrically using the SDmatic (CHOPIN Technologies, Villeneuve la Garenne, France) in accordance with AACC method 76-33.01 (AACC International, 2010). The analysis was performed in duplicate.

5.3.4 Breadmaking process

Baking of the bread loaves for this study was performed at the baking facility at Anchor Yeast Head Quarters in Johannesburg. Table 5.1 depicts the bread formulation used. Six loaves of bread were produced from each of the two control and treatment flour batches (i.e., 24 loaves in total) of which two loaves from each was used for analysis on three different shelf life days (days 2, 3 and 4). The ingredients used for the breads included flour, salt, sugar, soya flour (Product name: Breadsoy Flour, Impilo Products (PTY) LTD, Pretoria, South Africa), ascorbic acid (Bragan Chemicals (PTY) LTD, Johannesburg, South Africa), fungal α-amylase (Product name: Bakezyme P500, Anchor Yeast, Johannesburg South Africa), shortening (Product name: Planto Industrial, Sime Barby Hanson & Knight, Boksburg, South Africa) and yeast (Product name: Baker's Compressed Yeast, Anchor Yeast, Johannesburg South Africa). The ingredients were added to the mixing bowl of a Morton mixer (Morton Mixers & Blenders Ltd., Scotland, United Kingdom) where after water was added. The ingredients were mixed for 2 min using the slowest speed setting after which the speed setting was changed to the higher speed to facilitate dough formation. Once optimum gluten development was reached the dough was sectioned into 770 g dough balls and allowed to rest for 10 min at ambient temperature (ca. 25°C). Following resting, the dough balls were sheeted and rolled into a cylindrical shape using a MacAdams Moulder (MacAdams International, Cape Town, South Africa) and placed into bread pans (280 mm length x 110 mm height x 110 mm width). The dough was proofed at 40°C and a relative humidity of 80% for 50 min where after baking occurred. Breads were baked at 220°C for 30 min using a Tom Chandley Compacta Deck Oven (Tom Chandley Ltd, Manchester, United Kingdom). After baking, breads were allowed to cool down. Prior to packaging them in clear plastic bags the baking height of the loaves were measured, and the oven spring (difference between baking height and pan height) was calculated.

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Table 5.1 Bread formulation and scaled weights used for the production of the control and treatment bread loaves

Ingredients	Bread Formulation (%)	Scaled Weight* (g)
Bread-flour** (14%$_{m.b.}$)	100	3000
Water	60	1800
Fresh compressed yeast	2.6	78
Salt (NaCl)	2.0	60
Sugar	1.0	30
Shortening (Fat)	0.25	7.5
Soya-flour	0.2	6.0
Ascorbic acid	0.008	0.24
Fungal α-amylase	0.007	0.021

*Ingredient weights are based on their percentage of the flour weight. **Bread flour refers to the control and roasted flour respectively

5.3.5 C-cell visual analysis

The quality of the bread loaves was evaluated by means of C-Cell analysis using a C-Cell digital image analyser (Calibre Control International, Warrington, UK). The bread loaves were analysed on shelf life days two, three and four. On each of these days, two loaves from each control and treatment batch were sliced into 12.5 mm thickness slices using a Graef 182 Master slicer (Graef GmbH & Co. KG, Arnsberg, Germany). After slicing, ten slices from each bread loaf were subjected to C-Cell analysis. Image acquisition of the bread slices were performed by placing individual slices into the drawer of the instrument, which is designed to exclude external light. The built-in camera then captured surface images of each slice which was analysed by the accompanying software (C-Cell Colour, Version 3). The parameters measured during C-Cell analysis included slice area (mm^2), slice brightness, number of cells, area of cells (mm^2), volume of cells (mm^3), cell diameter (mm), cell wall thickness, number of holes, area of holes (mm^2) as well as the volume of holes (mm^3).

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5.3.6 Texture analysis

The texture of the breads, i.e., firmness and resilience, were evaluated by means of a TA.XTplus texture analyser (Stable Micro System, Godalming, Surrey, United Kingdom) using a 35 mm diameter cylindrical probe. A modified version of AACC Method 74-09.01 (AACC International, 2010) was used. Texture analysis was conducted on shelf life days two, three and four. Two slices of bread (12.5 mm diameter each) were placed on top of each other and placed onto the sample platform of the instrument. The firmness of the bread was measured by descending the cylindrical probe to 60% of the sample height before retracting it. The firmness was measured as the force (g) required to compress the bread slices. The resilience was calculated as the percentage recovery (i.e., the final height of the two slices after compression divided by the original height).

5.3.7 Statistical analysis

Mixed model analysis of variance (ANOVA) was performed to compare the averages of the quantitative measurements. The data was reported as mean ± standard deviation. The Fisher least significant difference (LSD) test was done to perform the different post hoc analyses. Statistical analysis was performed using STATISTICA version 13 (Statsoft, Tulsa, OK, USA). A 95% confidence interval was used to identify significant results i.e., a 5% significance level ($p \leq 0.05$).

5.4 RESULTS AND DISCUSSION

5.4.1 Wet and dry gluten and damaged starch content

No significant differences ($p > 0.05$) were observed between the control and treatment bread samples for either the wet or dry gluten content, gluten index or water binding capacity (Table 5.2). No significant differences ($p > 0.05$) in starch damage were observed between the control and roasted wheat flour samples. Although not significant, the flour from the roasted wheat did display a slightly higher amount of damaged starch. Germishuys et al, (2020) observed similar results in terms of an increase in damages starch content. The authors observed increases between 3.45 and 4.54%, which was much higher than currently reported, but this was attributed to the considerably higher roasting temperature (180°C) used. A study involving puffing of wheat at high temperatures (360 - 390°C) prior to milling also reported an increase in damaged starch (Cattaneo *et al.*, 2015).

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Table 5.2 Starch damage, wet and dry gluten, gluten index and water binding capacity of the control and roasted wheat flours

	Starch Damage (%)	**Wet Gluten (%)**	**Dry Gluten (%)**	**Gluten Index**	**WBC (%)**
Control	7.23±0.61[a]	28.96±1.47[a]	10.04±0.24[a]	89.60±5.99[a]	18.92±1.36[a]
Treatment	8.13±0.31[a]	28.68±0.65[a]	10.26±0.22[a]	91.17±5.28[a]	18.43±0.46[a]

Results are presented as mean ± standard deviation from two replicates. Different superscripts represent significant differences *(*p≤0.05) for each parameter respectively. WBC – Water Binding Capacity.

5.4.2 C-cell visual analysis

The results from the C-Cell analysis of the control and treatment bread loaves for the three different shelf life days are depicted in Table 5.3.

No significant differences (p>0.05) in slice area were observed between the control and treatment breads for either of the three shelf life testing days. Furthermore, no significant differences (p>0.05) in slice area were observed between the different days of testing for any of the samples. The slice area, to a degree, provides an indication of the bread volume. As there were no significant differences observed for slice area, it can be assumed that the volume of the different bread loaves would also be relatively consistent.

Significant differences (p≤0.05) were observed between the control and treatment samples for number of cells present within the bread slices. On all three days of shelf life testing the breads produced from roasted wheat flour displayed lower numbers of cells present which suggests that these breads had a coarser crumb structure. The control breads thus had a finer crumb structure. No significant differences (p>0.05) in number of cells were observed between the different shelf life testing days for either the control or treatment breads.

Significant differences (p≤0.05) were observed for cell diameter between the control and treatment samples on all three shelf life testing days. The diameter of the cells within a loaf of bread directly affects its crumb structure, with smaller cell diameters resulting in a finer crumb structure (van Riemsdijk *et al.*, 2011) and subsequently a softer bread (Scanlon and Zghal, 2001). The breads produced from the roasted wheat flour displayed greater cell diameters than their control counterparts, suggesting they had a coarser crumb structure. These results confirm what was observed for the number of cells present within the bread slices. Similar results were observed with X-ray µCT analysis (section 4.4.2, p. 74) where the bread samples produced from the roasted wheat flour displayed larger cell diameters compared to their control counterparts. Furthermore, the treatment bread samples displayed higher volume fractions of bubbles in the larger size ranges compared to the controls as depicted by the bubble size distribution graph constructed from the X-

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ray µCT results (section 4.4.2, pp. 77–78). No significant differences ($p>0.05$) in cell diameter were observed between the different testing days for the treatment breads. With reference to the control breads, a significant difference ($p\leq0.05$) in cell diameter was observed between the third and fourth days of shelf life testing.

The area of the cells was expressed as a percentage of the total slice area. Significant differences ($p\leq0.05$) with regard to area of cells were observed between the control and treatment bread samples. Overall, the breads produced from the roasted wheat flour displayed a larger cell area compared to the control breads. Similar results were observed with X-ray µCT analysis where the treatment bread samples displayed a larger cell surface area compared to its control counterparts (section 4.4.2, p. 74). No significant differences ($p>0.05$) in area of cells were observed between the different testing days for either the control or the treatment samples.

Significant differences ($p\leq0.05$) were observed for the average cell volume between the control and treatment bread samples for all three shelf life testing days. The slices of the breads produced from the roasted wheat flour displayed greater cell volumes than those of the control samples, suggesting a coarser crumb structure as was observed for cell diameter. The results from the cell volumes, cell diameters and the area of cells are all associated with one another as it would be expected that as the cell diameters increase the cell volumes and thus area of the cells would also increase (Cafarelli *et al.*, 2014). Furthermore, the cell volume also provides a better indication of the coarseness of the crumb texture as it takes into consideration the depth of the cells, which is not the case for cell diameter. Similar results were observed with X-ray µCT analysis where both the cell diameters and cell volumes were larger for breads produces from the roasted wheat flour (section 4.4.2, pp. 73–74).

The porosity of a bread is defined as the ratio of the defect (air) volume to the total volume of the bread (i.e., air- and material volume) (Wang *et al.*, 2011). When considering the area and volume of the cells, these two parameters could be associated with the porosity of the bread. As both the area and volume of the cells was larger for the treatment breads, it can be assumed that the porosity of these breads would then also be higher than that of their control counterparts. The X-ray µCT study results confirm this assumption as it was observed that the breads produced from the roasted wheat flour displayed a larger porosity than that of the controls, as measured with X-ray µCT (section 4.4.2, p. 80).

Significant differences ($p\leq0.05$) were observed for wall thickness between the control and treatment samples on all three shelf life testing days. Breads produced from the roasted wheat flour displayed wall thicknesses of 0.01 mm thicker compared to the control breads. With reference to the strut thicknesses as measured with X-ray µCT, the treatment breads displayed a 0.01 mm thinner cell wall compared to the control samples (section 4.4.2, p. 77). This study made use of 770 g bread loaves whereas 10 g bread loaves were used for X-ray µCT, which explains the differences in cell wall thickness observed. Increase in cell wall thickness may negatively impact a

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consumer's acceptability of a loaf of bread as thicker cell walls result in a firmer (i.e., less soft) and less elastic textured bread. No significant differences ($p>0.05$) in cell wall thickness were observed for either the control or treatment samples between the different testing days.

No significant differences ($p>0.05$) between the control and treatment samples were observed with reference to the number of holes present in the bread slices. Additionally, there were also no significant differences ($p>0.05$) in number of holes present between the different testing days for either the control or the treatment samples.

The area of the holes is defined as the total area of all the holes expressed as a percentage of the total slice area. No significant differences ($p>0.05$) in area of holes were observed between the control and treatment samples for the second or fourth day of shelf life testing, while a significant difference was observed on the third day. Overall, the control breads displayed a larger area of holes for all three shelf life testing days. A larger area of holes indicates a bread with poor textural properties. This is often as a result of a lack of elasticity in the dough resulting in an inadequate stabilization of the gas cells (van Riemsdijk *et al.*, 2011). No significant differences ($p>0.05$) in area of holes were observed between the different days of testing for either the control or treatment breads.

No significant differences ($p>0.05$) with regard to the volume of the holes were observed between the control and treatment breads for second and fourth day of shelf life testing. A significant difference ($p\leq0.05$) in volume of holes was observed between the control and treatment samples on the third day of testing. Interestingly, on the second and fourth days the treatment breads displayed higher hole volumes than the control samples, although the control samples had larger hole areas. This is attributed to the fact that volume takes into account the depth of the holes. The treatment samples on these days thus had deeper holes which would in turn increase the volume of these holes. This was also the case for the control sample on the third day which displayed a higher volume of holes compared to the treatment sample. With reference to differences in volume of holes observed between the different testing days, the only significant difference ($p\leq0.05$) was between the second and third day of testing for the treatment samples.

Significant differences ($p\leq0.05$) in brightness were observed between the control and treatment samples on the second and third shelf life testing days. No significant differences ($p>0.05$) in brightness were observed for the fourth day. The treatment samples displayed lower brightness values on the second and third testing days indicating a darker crumb structure, while the control samples displayed a darker crumb structure on fourth testing day. In terms of the different shelf life testing days, no significant differences ($p>0.05$) in brightness were observed for the control samples between any of the three testing days. A significant difference ($p\leq0.05$) in brightness was observed for the treatment samples between the third and fourth days. The lower brightness (i.e., darker crumb) of the treatment bread samples could be explained by the differences observed in the cell diameters and cell volumes of these samples. As the treatment

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bread samples displayed larger cell diameters and volumes, these could influence the brightness detected by the instrument.

When light is shined onto an object (in this case the bread slice) some of the light will be absorbed and some will be reflected. In the case of a hole, or cell in this case, it is harder for the light to enter the hole. The light beam has to travel further to illuminate the surface of the hole resulting in a less intense beam and subsequently a lower brightness detected. Taking the latter into consideration, the larger and deeper the cells within the bread slice are, the lower the brightness detected will be. Additionally, the darker crumb of the treatment bread samples could also be attributed to the roasting process. As heat is applied to the kernels during roasting, colour changes (based on the severity of the roasting conditions) take place ultimately darkening the kernels (Murthy *et al.*, 2008) and subsequently the flour.

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Table 5.3 Results from the C-Cell analysis for the control and treatment breads over the different shelf life days

Sample	Shelf life Day 2	Shelf life Day 3	Shelf life Day 4
Slice Area (mm^2)			
Control	13164.07±679.28^{a}	13157.31±757.53^{a}	13473.49±493.05^{a}
Treatment	13424.97±622.14^{a}	13076.45±768.26^{a}	13593.92±421.41^{a}
Number of Cells			
Control	9173.55±670.31^{a}	9281.03±740.10^{a}	9283.68±514.08^{a}
Treatment	8798.45±636.25^{b}	8461.50±688.39^{b}	8535.40±570.82^{b}
Cell Diameter (mm)			
Control	1.67±0.06^{de}	1.64±0.07^{e}	1.72±0.05^{cd}
Treatment	1.79±0.09^{bc}	1.84±0.10^{ab}	1.90±0.15^{a}
Area of Cells (mm^2)			
Control	53.16±0.52^{d}	53.15±0.55^{cd}	53.67±0.44^{bcd}
Treatment	53.80±0.62^{abc}	54.06±0.59^{ab}	54.39±0.99^{a}
Volume of Cells (mm^3)			
Control	5.10±0.20^{cd}	5.04±0.28^{d}	5.33±0.19^{c}
Treatment	5.61±0.31^{b}	5.87±0.38^{ab}	6.06±0.61^{a}
Wall Thickness (mm)			
Control	0.40±0.00^{b}	0.40±0.01^{b}	0.40±0.00^{b}
Treatment	0.41±0.01^{a}	0.41±0.01^{a}	0.41±0.01^{a}
Number of Holes			
Control	4.43±1.68^{a}	4.85±2.07^{a}	4.88±1.99^{a}
Treatment	3.87±1.80^{a}	4.22±2.03^{a}	4.23±1.49^{a}
Area of Holes (%)			
Control	3.82±1.13^{ab}	3.80±0.96^{a}	3.75±0.96^{ab}
Treatment	3.74±0.84^{ab}	3.22±1.16^{b}	3.63±1.51^{ab}
Volume of Holes (mm^3)			
Control	101.58±13.73^{ab}	105.79±11.66^{a}	104.21±14.96^{ab}
Treatment	104.71±13.43^{a}	91.75±17.06^{b}	104.65±26.79^{ab}
Brightness			
Control	139.64±1.01^{ac}	138.97±1.70^{ab}	139.33±2.14^{abcd}
Treatment	138.35±1.63^{bd}	137.72±2.88^{cd}	140.06±1.97^{ab}

Results are presented as mean ± standard deviation. Different superscripts represent significant differences ($p \leq 0.05$) for each parameter respectively.

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5.4.3 Texture analysis and oven spring

The results from the texture analysis of the control and treatment bread loaves for the three different shelf life days are depicted in Table 5.4.

The resilience (or springiness) of a loaf of bread is defined as its ability to recover after being subjected to compression (i.e., returning to its original shape). It relates to the height that a slice of bread recovers to, in the time between the end of the first bite and the start of the second bite. No significant differences ($p>0.05$) between the control and treatment breads were observed for either the second or third day of shelf life testing, while a significant difference ($p\leq0.05$) was observed on the fourth day. The breads produced from the roasted wheat flour displayed a lower resilience on the second and fourth day of testing. With respect to differences between the testing days, only the treatment sample displayed a 6.9% decrease in resilience between days three and four.

Increases in bread crumb firmness is the most widely used indicator that a loaf of bread is losing its freshness and thus becoming stale (i.e., reaching the end of its shelf life) (Gray and Bemiller, 2003). Significant differences ($p\leq0.05$) in firmness were observed between the control and the treatment samples. Overall, the treatment breads displayed lower firmness values over all three testing days, compared to the control samples. This indicates that the roasted wheat flour produced breads that were softer than the unroasted counterparts. With reference to changes in firmness between the different shelf life testing days, a significant increase ($p\leq0.05$) in firmness was observed for both the control and the treatment breads between the second and third day of testing. Furthermore, the control breads showed a significant ($p\leq0.05$) increase in firmness between the third to the fourth day of testing. With regard to the treatment breads, an initial spike in firmness was observed between days two and three but little change was noted between days three and four. This was not observed for the control bread samples. From the abovementioned results it is evident that even on the fourth day of testing the treatment breads had not yet reached the firmness observed for the control breads on day three. This clearly shows that flour, produced from roasted wheat using the conditions in this study, had a beneficial outcome on the resultant breads in terms of shelf life extension.

This is especially important to both the baking industry as well as the consumer as bread staling is responsible for great economic losses annually. The extension of the shelf life of bread could potentially mitigate some of these losses (Giménez *et al.*, 2007).

No significant differences ($p>0.05$) with regard to the baking height were observed between the control and treatment bread samples for either of the three testing days. Subsequently, no significant differences ($p>0.05$) with regard to oven spring were observed. The effect of time, i.e., the different shelf life days, further showed no significant ($p>0.05$) changes in the loaf height of either the control or the treatment samples. The use of flour produced from roasted wheat therefore did not influence the loaf height and as such the oven spring of the resultant breads.

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Table 5.4 Results from the texture analysis for the control and treatment breads over the different shelf life days

Sample	Shelf life Day 2	Shelf life Day 3	Shelf life Day 4
Resilience (%)			
Control	75.33±2.11^{a}	73.26±1.59^{ab}	69.67±2.62^{b}
Treatment	74.65±3.41^{a}	73.30±2.12^{ab}	66.40±2.27^{c}
Firmness (g)			
Control	274.51±15.16^{d}	366.79±23.51^{b}	447.44±28.94^{a}
Treatment	239.41±15.29^{e}	332.32±22.61^{c}	335.20±38.18^{c}
Baking Height (mm)			
Control	169.50±3.42^{a}	169.75±0.96^{a}	169.75±3.30^{a}
Treatment	170.00±5.29^{a}	168.25±4.99^{a}	169.00±2.16^{a}
Ovenspring (mm)			
Control	59.50±3.42^{a}	59.75±0.96^{a}	59.75±3.30^{a}
Treatment	60.00±5.29^{a}	58.25±4.99^{a}	59.00±2.16^{a}

Results are presented as mean ± standard deviation. Different superscripts represent significant differences ($p \leq 0.05$) for each parameter respectively.

5.5 CONCLUSIONS

The quality and shelf life of bread loaves prepared from roasted wheat flour was investigated by means of C-Cell and texture analysis. No significant differences in wet and dry gluten content, gluten index, water binding capacity or starch damage were observed. Results from C-Cell analysis showed that the breads produced from roasted wheat flour had a darker crumb, which was attributed to the larger and deeper cells present in these slices. Results pertaining to the number of cells, cell diameter and average cell volume all showed that the treatment breads displayed a coarser crumb structure compared to the control breads. The treatment breads further showed slightly thicker cell walls. Overall, the treatment breads displayed larger hole volumes whereas the control breads displayed larger hole areas. With reference to texture analysis of the samples, the treatment breads displayed a lower resilience but also a lower overall firmness. These lower firmness values indicated that the treatment breads were softer than the control breads. Although both control and treatment breads showed an initial spike in firmness, the firmness of the control bread on the third day had already surpassed that of the treatment on day

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four. The use of flour produced from roasted wheat thus resulted in a softer bread with an increased shelf life compared to its control counterparts. This is of great importance to the baking industry, as bread staling is responsible for great economic losses annually. The extension of the shelf life of bread, even only one day, has the potential to mitigate some of these losses.

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CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

Wheat (*Triticum aestivum L.*) is one of the most important cereal crops in the world and unique in the sense that a viscoelastic dough can be prepared from the milled flour to produce bread (Mondal and Datta, 2008). Bread is one of the most widely consumed food products in the world due to its affordability and the fact that it is fortified with vitamins and minerals in South Africa (FoodStuff-SA, 2017).

Roasting is a high-temperature short-time (HTST) process where both heat and mass transfer take place (Fadai et al., 2017) and is generally performed to improve the quality of a product and increase its processing efficiency (Chung et al., 2011). During roasting, wheat kernels puff up due to the high internal pressure changing both their moisture content and volume (Schoeman et al., 2016; Schoeman and Manley, 2019). Forced convection continuous tumble (FCCT) roasting is an emerging roasting technique that uses superheated steam, produced from moisture in the product, to roast the sample. This roasting method is energy efficient and much more uniform than other methods (Flinn, 2012; Schoeman et al., 2016).

Several studies have shown the benefits of roasting cereal grains which included improved flavour, colour and texture (Murthy et al., 2008), increased shelf life of the grains (Ranganathan et al., 2014) and inactivation of proteolytic enzymes promoting increased loaf volume (Vázquez et al., 2001). Additionally, detrimental effects caused by roasting have also been reported and included increased susceptibility to acrylamide formation (Muttucumaru et al., 2006; Claus et al., 2008), changes in the protein properties (Vázquez et al., 2001; Schoeman and Manley, 2019) and increased starch damage (Lorenz et al., 1993).

The breadmaking process is a series of aeration stages which ultimately affect the crumb structure of the bread. Therefore, the texture and subsequently the quality of a loaf of bread is greatly dependent on its crumb structure and how these bubbles are distributed throughout the loaf (Scanlon and Zghal, 2001). The process responsible for loss of bread quality is staling and is directly correlated to the shelf life of the bread (Besbes et al., 2013).

C-Cell and texture analysis has been used to evaluate the crumb structure and texture of bread slices (Sroan et al., 2009; Ktenioudaki et al., 2010; van Riemsdijk et al., 2011; McCann and Day, 2013). C-Cell, a digital imaging system, and texture analysis evaluates the quality and shelf life of bread, respectively.

X-ray µCT has been used with great success to study the microstructure of foods, including the bubble structure of dough (Whitworth and Alava, 1999; Bellido et al., 2006; Koksel et al., 2016) and the crumb structure of bread (Falcone et al., 2004; Babin et al., 2006; Van Dyck et al., 2014). Although these studies evaluated dough and bread structure, no literature is available on the

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bubble structure of dough and foam structure of bread produced from FCCT roasted wheat flour by means of X-ray µCT. Therefore, the main aim of this study was to evaluate the bubble structure of dough and the foam structure of bread produced from roasted wheat flour by means of X-ray µCT to determine its quality.

The first objective of this study was to characterise white flour produced from roasted wheat differing in hardness and protein content by means of rheological and physicochemical analyses. Three different wheats were used in this part of the study all differing in hardness (hard, medium-textured and soft wheat) and protein content. The wheat was roasted at 180°C for 140 s using a forced convection continuous tumble roaster. The flour obtained from the milled wheat were subjected to rheological and proximate analyses.

Roasting resulted in significant decreases ($p \leq 0.05$) in hectolitre mass (HLM) for all the roasted wheat samples. Decreases in HLM was attributed to increases in kernel volume as a result of puffing and decreases in weight due to moisture loss (Schoeman and Manley, 2019). Furthermore, decreases in flour yield were also observed for the roasted wheat samples. Roasting resulted in considerable moisture losses of 2.9% for hard and medium and 2.2% for soft wheat samples. This greatly affected the tempering process as even after 48 h of tempering none of the roasted wheat samples reached the targeted moisture content.

Additionally, significant increases ($p \leq 0.05$) in starch damage were observed for the roasted wheat flour samples. Similar results were reported for wheat that had been puffed at high temperatures (Cattaneo et al., 2015). The increased starch damage content observed in the present study was attributed to the lower moisture content of the tempered wheat samples prior to milling.

Most notably, roasting resulted in changes in the protein properties resulting in a lack of dough formation during mixograph testing. The use of flour composites which consisted of 80% unroasted commercial flour and 20% roasted wheat flour enabled rheological evaluation of the roasted wheat. All the composite flours containing roasted wheat flour displayed a decreased water absorption capacity (WAC) of which the hard wheat had the largest decrease of 5.2%. The decreased WAC suggested that either the proteins required less water to fully hydrate or that the proteins properties were changed during roasting making them less capable of being readily hydrated.

In contrast to the 100% roasted wheat flour which could not form a dough during mixograph testing, the flour composites were able to produce a dough. It was thus evident that roasting had abolished the protein's gluten forming capacity. Mixograph testing clearly showed that there were no distinct optimum gluten development peaks for the composite flours containing roasted wheat flour, compared to the controls which all showed clear optimum gluten development peaks. Furthermore, mixogram peak height and alveograph W-values both showed weakening of the gluten strength with respect to the composite flours. With reference to alveograph testing, an

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increase in resistance to extension and a decrease in extensibility was observed for the composite flours as a result of the increased starch damage. This positively affected the P/L ratios of the composite flours from both the hard and medium textured roasted wheats.

Particle size distribution showed a higher level of free starch granules present in the flour from the hard and medium textured roasted wheat as compared to their unroasted counterparts. Both the control and roasted wheat flour from the soft textured wheat showed much higher levels of free starch granules as compared to the other two wheat samples. The higher level of free starch granules is as a result of the endosperm being less densely packed in softer wheats. During milling, fractures occur through the protein-starch matrix resulting is less fracturing of the starch granules (Pauly et al., 2013).

It was clear that the roasting conditions used were too severe and abolished the protein's gluten formation capacity. To further study the effect of roasting on dough and bread characteristics, the use of a central composite design was required to determine the optimal roasting conditions. The second objective of this study was therefore to determine the optimal roasting conditions that would minimise the effect on the protein properties of the produced white flour. Two commercial wheats differing in protein content were utilised in this section of the study. The wheat samples were roasted at a range of time and temperature intervals based on a central composite design (CCD). The minimum and maximum values selected for the roasting temperatures (90°C and 130°C) and roasting speeds (60 Hz and 90 Hz) were based on a preliminary study (unpublished data). The subsequent flour was subjected to physicochemical and mixograph analyses.

Decreases of up to 4.24% (110°C and 60 Hz) and 2.59% (96°C and 86 Hz) in flour yield was observed for the high and low protein wheats, respectively. These results agreed with those observed for the hard and soft texture wheats from the first objective (Germishuys et al., 2020). These decreases were attributed to increases in porosity induced by the roasting process (Schoeman et al., 2016).

The roasting conditions as set out by the CCD proved to have little effect on the moisture content of the wheat kernels after roasting. Slight decreases in moisture content were observed for both the high (min: 0.27; max: 0.64%) and low (min: 0.10; max 0.70%) protein roasted wheat samples. These were substantially lower than previously observed (Germishuys et al., 2020) and was attributed to the lower roasting temperature used.

No significant differences ($p>0.05$) for protein content, mixograph peak time and peak height were observed for either the high of low protein roasted wheat as no severe roasting conditions were included. Although these results were not significant ($p>0.05$), some trends were observed in the contour and response surface plots.

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Roasting at the lower speed settings (i.e., longer roasting time) resulted in a decrease in protein content for the high protein wheat as the roasting temperature increased. The low protein wheat displayed increases in protein content as the roasting temperature increased. Decreases in mixograph peak time was observed for the high protein wheat as the roasting temperature decreased, with the roasting speed having little effect. The low protein wheat showed the shortest peak time around the centre of the contour plot while the longest peak times were situated at the extremes of roasting temperature and speed. Both the high and low protein wheats displayed increases in peak height as the roasting temperature decreased and the roasting speed increased. The results for peak time and peak height could be expected as the less severe the roasting conditions, the less the changes are to the protein structure. The roasting conditions chosen for the X-ray µCT part of the study was 90°C and 86 Hz (ca. 130 s). This combination will maximise protein content and peak height and minimise peak time.

The third objective of this study was to evaluate the freeze drying of dough as a suitable sample preparation procedure that would maintain the structure of the samples during X-ray µCT scanning for efficient analysis of its bubble structure. Additionally, the foam properties of 10 g bread loaves produced from roasted wheat flour were also evaluates. A commercial white bread wheat was used for this section of the study and roasted at 90°C and a speed of 86 Hz (ca. 130 s) based on the recommended conditions as per the central composite design. The wheat was milled, and the subsequent flour used to prepare a dough using a lean bread formulation whereafter the dough was sectioned into 10 g pieces. A third of the dough pieces were proofed for 20 min and the remaining for 40 min. Following proofing, the dough pieces proofed for 20 min and half of the dough pieces proofed for 40 min were flash frozen with liquid nitrogen and freeze dried to remove the moisture. The remaining half of the dough pieces proofed for 40 min were baked. All the samples were subjected to X-ray µCT scanning and image analysis.

Mixograph results showed an increase in peak time of 3 s for the roasted wheat flour samples, although the increase was not significant ($p>0.05$). Furthermore, a decrease of 1 mm in peak height was observed for the roasted wheat flour although again not significant ($p>0.05$). Conformational changes in the gluten protein during the roasting process cause less protein-protein interactions to occur during mixing resulting in a decreased gluten strength and changes in the rheological properties (Bucsella et al., 2016). The current roasting conditions had an insignificant effect on the protein-protein interactions.

The X-ray µCT instrument used in this study required a scan time of approximately 46 min per sample. This posed the problem of maintaining the integrity of dough samples during the scan. The use of liquid nitrogen to flash freeze the 20- and 40-min proofed dough samples followed by freeze drying aided in maintaining the structural integrity of the dough samples during scanning. Additionally, the use of 10 g bread loaves, similar to the size of the dough samples, resulted in a much higher resolution (30 µm) image than would have been the case when a full-size bread loaf

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would have been scanned. The higher resolution enabled the detection of smaller bubbles within the dough and bread.

Significant increases ($p \leq 0.05$) in mean cell volume, with respect to the roasted wheat flour, was observed for both the 40-min proofed dough as well as the bread. The mean cell volume also significantly increased ($p \leq 0.05$) from the 20- to the 40-min proofed dough. Significant increases ($p \leq 0.05$) in mean cell surface area were observed for the 40-min proofed dough and the bread. No significant differences ($p > 0.05$) in mean cell diameter were observed for either the 20- or 40-min proofed dough, although a significant increase ($p \leq 0.05$) was observed for the bread.

The bubble size distribution graph showed that 20-min proofed dough displayed the largest volume fraction of bubbles in the 700 µm range, whereas the 40-min proofed dough displayed the lowest. The 40-min proofed dough and the bread displayed the largest volume fraction of bubbles in the 1000 – 2000 µm range with the 20-min proofed dough displaying the lowest volume fraction. The dough produced from the roasted wheat flour displayed lower volume fractions in the 700 µm range and higher volume fractions in the range of 1000 – 2000 µm compared to their controls.

Most notable were the decreased strut thickness of the bread produced from the roasted wheat flour, which suggested a finer crumb structure and softer texture. Considering the lower mixograph peak heights of the dough, it suggests these samples had a weaker gluten network compared to their controls. Furthermore, porosity increases for all the treatment samples were observed. Roasting at 90°C and a roasting speed of 86 Hz did not negatively affect the foam properties of the breads baked from the resultant flour. On the contrary, an improvement in the foam properties of bread in terms of the crumb fineness and texture was observed.

X-ray µCT is very expensive and time-consuming, requiring trained personnel to operate the instrument and perform the data analysis. An alternative method readily used in the baking industry to determine the foam properties of bread is C-Cell. The fourth objective of this study therefore included the use of C-Cell and texture analysis to evaluate the quality and shelf life of bread loaves prepared from roasted wheat flour by means of C-Cell and texture analysis. Bread loaves were baked using flour produced from unroasted and roasted (90°C for 130 s using a forced convection continuous tumble roaster) wheat and subjected to C-Cell and texture analysis.

No significant differences ($p > 0.05$) in wet and dry gluten content, gluten index or water binding capacity were observed. Increases in starch damage were observed for the roasted wheat flour samples although this was not significant ($p > 0.05$).

C-Cell analysis showed the breads produced from roasted wheat flour to have a darker crumb, which was attributed to the larger and deeper cells present in these slices. Results pertaining to the number of cells, cell diameter and average cell volume all showed that the treatment breads displayed a coarser crumb structure compared to the control breads. Breads produced from the roasted wheat flour displayed 0.01 mm thicker cell walls as compared to the

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controls. This was the opposite of what was observed in the X-ray µCT study where the bread produced from the roasted wheat flour displayed a 0.01 mm thinner strut thickness. This study made use of 770 g bread loaves whereas 10 g bread loaves were used for X-ray µCT which explains the differences observed in cell wall thickness.

With reference to texture analysis of the samples, the treatment breads displayed a lower resilience but also a lower overall firmness. The lower firmness values indicated that the treatment breads were softer than the control breads. Even though both the control and treatment breads displayed an initial spike in firmness, as a result of amylose retrogradation, the firmness of the control bread on the third day had already surpassed that of the treatment on day four. Therefore, the use of flour produced from roasted wheat did not negatively influence the quality of the bread in terms of its crumb structure and texture. Most notably, and of great importance to the baking industry, the use of roasted wheat flour resulted in a softer bread with an increased shelf life compared to its control counterparts.

In conclusion, this study provided valuable information regarding the effect of FCCT roasting on the rheological properties and baking potential of flour produced from roasted wheat kernels. This included a decrease in strut thickness and firmness resulting in a softer and finer crumb texture and an increased shelf life, respectively. Furthermore, it was observed that the C-Cell results were comparable with the X-ray µCT results for the breads. Therefore, it can be said that to an extent C-Cell and X-ray µCT could be used interchangeably to study bread foam structure. X-ray µCT has the advantage that every bubble can be studied individually in detail, which is not the case with C-Cell as an average measurement is taken from the slice surface. In terms of practicality, C-Cell is more appropriate for the baking industry as it is more user friendly, does not require technically trained personnel, as is the case with X-ray µCT and it is also considerably less expensive. In addition, the image acquisition time for C-Cell is almost instant and analysis of the image is performed by the accompanying software.

The use of liquid nitrogen freezing followed by freeze drying maintained the internal structure of the dough, making X-ray µCT scanning possible. This study further showed that roasted wheat could still be used to produce bread of almost equal quality (based on the roasting conditions) as that of the unroasted wheat. In addition, the roasted wheat flour displayed superior properties in terms of shelf life extension. The current study provides valuable insight to the baking industry with regards to the use of roasted wheat flour for the extension of bread shelf life. The positive outcomes of an extended shelf life would have to be weighed against the economic input required to purchase and operate, in terms of energy consumption, the required roasters. As these roasters are not too expensive, the application of roasted wheat flour in bread could potentially be adopted by artisanal bakeries.

Additional recommendations for future studies include increasing the proofing time intervals to obtain a better understanding of changes in the bubble structure during the proofing process. It is

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also recommended to perform another central composite design study which include higher temperatures to determine at what time and temperature combination the proteins start losing their functionality. It would further be advantageous if the same bread samples could be used for X-ray µCT and C-Cell studies to be able to better compare the results. In the current study it was not viable due to the instruments being located in different parts of the country. It is further recommended that with the correct international collaboration, this study could be improved upon with the use of Synchrotron-based X-ray µCT which would allow for real-time scanning of the dough samples. This has the added advantage of being able to study the dough bubble structure as the process of proofing occurs and eliminate the need for freeze drying. It is recommended that a sensory evaluation be performed to determine whether a taste difference can be observed between the breads produced from the control and the roasted wheat flour and what the consumer response would be in the case of a taste difference. Additionally, the current study could also serve as the starting point for investigations related to the baking potential of heat damaged wheat instead of roasted wheat. Lastly, it would be interesting to investigate the potential of wheat roasting to eliminate any microbial contamination on the wheat to prevent the subsequent contamination of the flour. This would involve managing the roasting time and temperature to ensure elimination of microbial contaminants without impacting the protein properties. It is hoped that this study inspires further research in the field of cereal roasting and X-ray µCT as a technique to study dough and bread structure.

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