Rheology and water mobility of low sodium bread doughs prepared with crosslinking enzymes and organic acids

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ABSTRACT

New regulations from the Government of Canada regarding sodium limits in foods have generated technical challenges for products such as bread, which relies on sodium chloride (NaCl) as one of four essential ingredients. NaCl also has particular importance in proper gluten network development. The focus of this work was three-pronged. First, was to assess the effectiveness of crosslinking enzymes for improving gluten network strength/development and reducing stickiness in low-sodium model dough systems. Secondly, this work looked to understand the relationship between organic acids which can be produced by yeast and stickiness and dough handling characteristics. Finally, this project examined the role that water plays in these characteristics and attempted to determine if handling characteristics could be linked with water mobility characteristics of doughs. This foundational work was completed to deepen mechanistic understanding of this complex system for improvement of low-sodium bread doughs in line with new regulations.

The first body of work (Chapter 3) examined the effectiveness of two crosslinking enzymes, glucose oxidase (GO) and transglutaminase (TG) at improving dough handling characteristics and reducing stickiness in low sodium doughs prepared with two different Canada Western Red Spring (CWRS) cultivars Pembina and Harvest, which were developed by Agriculture and Agri-Food Canada (AAFC). The cultivars were chosen due to their opposing characteristics; Pembina had previously shown strong dough handling and low stickiness in reduced-salt systems, whereas Harvest had high stickiness and poor dough handling under those conditions. Two concentrations of each enzyme were examined (0.001% and 0.01% GO, and 0.01% and 0.5%TG by flour wt.) and two levels of salt were assessed (1.0% and 2.0% NaCl by flour wt.). Both TG and GO were able to improve dough rheology and reduce dough stickiness, however, TG only produced improvements at the 0.5% level, whereas GO was effective at both the 0.001% and 0.01% levels. Investigation into the crosslinking of the enzymes was completed; free thiol content was reduced significantly by GO inclusion but not by TG inclusion. This was expected due to the respective mechanisms of the enzymes, as GO crosslinks proteins indirectly by forming disulfide bonds with free thiol groups using H_2O_2 produced from glucose oxidation, whereas TG crosslinks proteins directly by forming a covalent bond between lysine and glutamine residues. Glutenin macropolymer (GMP) content found that there was significantly more %GMP

in samples with GO compared to controls, and in some samples with TG. Overall, it was found that both GO and TG were effective at improving investigated parameters (dough rheology, stickiness), however, GO was more effective than TG at lower concentrations. Cultivar was significant in the case of every investigated characteristic, and enzymes produced more significant changes in the characteristics of samples produced with the weaker flour (Harvest) and at low salt levels (1.0% NaCl).

The second study (Chapter 4) examined slightly more complex model doughs; they contained a variety of organic acids which can be produced by yeast; acetic, citric, fumaric, lactic, succinic, or the bread improver ascorbic acid at levels of 1.2mmol/100g flour. Only low salt level (1.0% NaCl by flour wt.) and the low concentration of GO (0.001% by flour wt.) were assessed due to the previous work of Chapter 3. Both cultivars (Harvest and Pembina) were assessed in this work. Several parameters were assessed including dough stickiness, rheology, %GMP, and freezable water content (FWC). The inclusion of these acids (excluding ascorbic acid) had negative effects on dough rheology and increased dough stickiness but did not have large effects on %GMP and minimally increased FWC. Ascorbic acid trends were different than other acid trends, which was expected due to its use as an oxidizing agent for increasing dough strength, however, it did not produce improvements when used in tandem with GO. The inclusion of GO improved dough rheology and reduced dough stickiness as expected, and when it was included with these acids (excluding ascorbic acid) samples showed behavior in between the observed results of GO without acid, and control samples without either acid or GO. Cultivar remained an important factor in all samples, with Pembina having superior rheology, lower stickiness, higher %GMP and lower FWC in comparison to Harvest dough samples. GO had improving effects on the dough properties despite the additional inclusion of the organic acids, although it was not as pronounced as in Chapter 3.

The final study (Chapter 5) examined the molecular mobility and diffusion properties of water in model dough systems by low-field ^{1}H nuclear magnetic resonance (NMR) spectroscopy. This work assessed model doughs based primarily on the model of Chapter 4 but only included acetic, fumaric, and succinic acids, did not include a no-enzyme control, and included a yeast control (3.0% by flour wt.). It was determined that acid inclusion did not affect the overall structure of the doughs significantly, and that it was very slightly affected by flour type. Molecular motion on the MHz timescale, which relates to water molecule tumbling, was significantly lower in

doughs containing acid or for those prepared with Pembina flour. Use of acids and Pembina flour also resulted in a reduction of motion at the polymer surfaces in comparison to other samples on the MHz timescale. Motion on the kHz timescale (relating to protein side chain motion) was significantly altered by Pembina doughs and acid inclusion, however, it was not determined if this motion became faster or slower. The effect of acid type was statistically non-significant for all parameters. Diffusion characteristics were not altered by any formulation changes except yeast inclusion. Yeast dough trends were similar to those of acid inclusion, however, generally more significantly different than non-yeast samples including acids. Overall, the inclusion of acids reduces motion significantly on the MHz timescale and alters it significantly on the kHz timescale but does not appear to affect the overall structure significantly, which suggests that acids are mostly active at the surfaces of the polymers such as protein side chains.

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LIST OF SYMBOLS AND ABBREVIATIONS

AACCI	American Association of Cereal Chemistry International
AAFC	Agriculture and Agri-food Canada
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BU	Brabender Units
CPMG	Carr-Purcell-Meiboom-Gill
CNHR	Canada Northern Hard Red
CWRS	Canada Western Red Spring
Da	Dalton
dB	Decibel
d.b.	Dry basis
DSC	Differential scanning calorimetry
FAB	Farinograph water absorption
FID	Free induction decay
FTIR	Fourier transform infrared spectroscopy
FWC	Freezable water content
G′	Elastic modulus (storage modulus)
G″	Viscous modulus (loss modulus)
|G*|	Complex modulus
GMP	Glutenin macropolymer
GO	Glucose oxidase
GODU	Glucose oxidase units
GSH	Glutathione
HI	Hardness index
HMW	High molecular weight
HMW-GS	High molecular weight-glutenin subunit
Hz	Hertz
IR	Inversion Recovery
J	Creep compliance

J_{el}	Relative elasticity
J_{max}	Maximum deformation (maximum creep compliance)
J_r	Recovery compliance
kDa	Kilodalton
kHz	Kilohertz
kPa	Kilopascal
LMW	Low molecular weight
LMW-GS	Low molecular weight-glutenin subunit
LVR	Linear viscoelastic region (linear viscoelastic regime)
m.b.	Moisture basis
MHz	Megahertz
mPa	Millipascal
MRI	Magnetic Resonance Imaging
NMR	Nuclear magnetic resonance
NSP	Non-starch polysaccharide
Pa	Pascal
PO	Pyranose oxidase
R^2	Coefficient of determination of linear regression
r.f.	Radio frequency
RVU	Rapid viscoanalyzer units
SCKS	Single kernel characterization system
SD	Standard deviation
SI	Système international d'unités (International system of units)
SWA	Sales weighted average
T_1	Transverse relaxation (spin-lattice relaxation)
$T_{1\rho}$	Rotating frame relaxation time
T_2	Longitudinal relaxation (spin-spin relaxation)
T_2^*	Decay signal resulting from FID
T_{2A}	T_2 component representing bound water
T_{2B}	T_2 component representing more freee water
Tan δ	Loss tangent

TG	Transglutaminase
TGA	Thermogravimetric analysis
tq	Torque
U	Units of enzyme activity; the amount that catalyzes 1 μmol/min
w.b.	Wet basis
WE	Water extractable
WU	Water unextractable
XYL	Xylanase
α	Alpha
β	Beta
δ	Delta (phase angle)
γ	Gamma (strain)
φ	Phi (phase angle)
σ	Sigma (stress)
τ	Tau (shear stress)
μmol	Micromole

1. INTRODUCTION

1.1 Summary

Due to concerns over the high sodium intakes in Canada, the federal government has reduced the amount of sodium allowed in food products, including baked goods (Health Canada, 2018). For bread, this is a processing issue, as salt (sodium chloride) is one of four essential ingredients, the others being water, flour, and yeast. Salt performs many functions for bread; flavour enhancement, yeast control, preservation, and possibly most importantly, a critical role in gluten network development (Belz et al., 2012). Salt reduction can result in very sticky doughs which can adhere to processing and mixing equipment, which can result in quality defects, inefficiencies in production, and increased costs (Beck et al., 2012a; Belz et al., 2012). The mechanisms behind dough stickiness are also somewhat poorly understood, which contributes to the difficulty in finding solutions. Due to these issues and the incoming regulations, manufacturers require action on reducing stickiness and improving quality of the final reduced sodium products.

The overall goal of this project was to assess the feasibility of strengthening enzymes to reduce dough stickiness and improve rheological behaviour of low sodium dough, which should improve final product quality. Additionally, this project looked to deepen the current understanding of the relationship of water mobility and water association in doughs and handling and stickiness within dough. The basis of this project used a very simple dough model, beginning with only flour, water, salt, and crosslinking enzymes; transglutaminase (TG) or glucose oxidase (GO). In subsequent work, some organic acids were added to the model to make it slightly more complex but without using yeast, both to better understand specific components, and also simplify the testing and dough model in comparison to doughs including yeast. The overall hypotheses are that enzymes are able to improve gluten network strength and decrease stickiness, and that water mobility and association are a factor in the stickiness observed, particularly at low salt levels. There were three main branches of study in this work: (1) examining the effect of enzyme type (TG or GO) on dough handling in low sodium conditions, (2) examining how yeast produced organic acids interact with enzymes in low sodium doughs, and (3) examining the water mobility and diffusion characteristics

of these model doughs to see if a link could be drawn between water motion in dough and previously observed dough handling characteristics.

1.2 Hypotheses

The following hypotheses were tested in this project:

a) The inclusion of both TG and GO would increase the amount of crosslinking between proteins in the system and strengthen the gluten network as a result. This would improve dough handling and reduce stickiness, particularly at the low sodium level. Increase in enzyme concentration will have a greater impact on dough handling properties, and TG will also have a greater effect than GO.

b) The inclusion of the selected organic acids will increase dough stickiness and decrease dough strength and have poorer dough handling characteristics. The inclusion of ascorbic acid will be the exception, as it is an oxidising agent used for improving dough strength. Glucose oxidase inclusion will partially combat these effects, and synergistic effects on improving dough could be observed when glucose oxidase and ascorbic acid are included together.

c) There will be a correlation between stickiness/poor dough handling and freezable water content in dough samples.

d) Water mobility will be higher in samples which show poorer rheological behaviour and higher stickiness, and the amount of bound water will have the opposite effect. Higher diffusion will be seen in samples with less desirable characteristics.

1.3 Objectives

This project aimed to examine how some crosslinking enzymes (TG and GO) will remedy stickiness issues present in low sodium bread doughs. The project examined two enzymes specifically: TG and GO, and two Canada Western Red Spring (CWRS) cultivars which have previously shown good dough handling and low stickiness (Pembina) and poor dough handling and high stickiness (Harvest) at reduced salt levels (Yovchev et al., 2017). As of August 1, 2018,

both Harvest and Pembina have been reclassified into Canada Northern Hard Red (CNHR) market class (Canadian Grains Commission, 2018). For the duration of this project, they were classified as CWRS, and the seed year used (2013) occurred during their classification as CWRS. Therefore, throughout this thesis they will be described as CWRS instead of their new classification, CNHR. The project also looked to increase the complexity of the simple dough model to reach something closer to bread dough without using yeast, to better identify potential mechanisms, and avoid complications of yeast within handling testing. Water mobility, and association, and its role in stickiness is also of particular interest. Specific project objectives were:

a) To assess and compare the effectiveness of TG and GO on improving dough handling characteristics and reducing dough stickiness of samples with both Pembina and Harvest at low sodium levels.

b) To investigate a slightly more complex dough model which also contains yeast-produced organic acids to assess how they interact with the chosen crosslinking enzymes, and also how they affect stickiness and dough handling.

c) To investigate the water mobility and diffusion characteristics of these slightly more complex doughs and attempt to link water characteristics with dough stickiness and handling characteristics previously observed, and also compare yeast samples to those from the organic acid dough model.

2. LITERATURE SURVEY[1]

2.1 Dough formulation

Bread dough is a complex formulation of many different ingredients, all of which provide unique functional roles to the dough, and the final product. The ingredients of dough can be separated into two main categories: essential and non-essential. Essential ingredients are those which are required for dough formation (flour, yeast, water, and salt), and non-essential ingredients are those which may be added to provide some other functional role, likely related to either sensory characteristics (e.g. colour, flavour, etc.) or processing improvements (Belderok et al., 2000; Collado-Fernandéz, 2003a; Sluimer, 2005). Non-essential ingredients include a large variety of compounds such as lipids, emulsifiers, enzymes, carbohydrates, redox reagents, dairy products, antioxidants, colours, gums and hydrocolloids, and flours/proteins from other crops (Sluimer, 2005; Edwards, 2007; Delcour & Hoseney, 2010; Cauvain, 2012).

2.1.1 Flour

Flour is the major component of bread and dough, and it has many important critical functions in the final product (Mondal & Datta 2008; Delcour & Hoseney, 2010). In bread products, wheat is the most important grain, and the most common crop from which bread and other baked goods are produced (Edwards, 2007). There are several critical components of wheat flour which contribute to its functionality in bread and dough, including starch (~70-75% of wheat flour), water (~14% of wheat flour), proteins (10-12% of wheat flour), non-starch polysaccharides (NSPs; ~2-3% of wheat flour) and lipids (~2% of wheat flour) (Goesaert et al., 2005). In its simplest form, dough is formed by the combination of wheat flour, water, salt, and yeast (*Saccharomyces cerivisiae*) to produce a viscoelastic dough with gas holding capabilities which is then baked to produce bread (Belderok et al., 2000; Collado-Fernàndez, 2003a). The hydration of

[1] A portion of this literature review has been published in Functional Food Reviews.
Avramenko, N., Smith, M. A., Hopkins, E. J., Duizer, L., Nickerson, M. T., Rousseau, D., & Yada, R. Y. (2015). Challenges and opportunities in food science and technology in developing delivering sodium reduced products: bread, a case study. *Functional Food Reviews*, *7*(1), 19-30.

the wheat flour with water is primarily responsible for the formation of the viscoelastic dough (Delcour & Hoseney, 2010).

The predominant group of proteins within wheat flour are gluten proteins (80-85% of the total wheat protein) with the remaining protein in wheat being a highly diverse group (Veraverbeke & Delcour, 2002). Gluten proteins are divided into two main groups; gliadins (molecular mass of 30 – 80 kDa), and glutenins (molecular mass of 80 – 20000 kDa) (Veraverbeke & Delcour, 2002; Sluimer, 2005). As a group, gluten protein has poor solubilisation in water and is found to be high in non-polar amino acids; according to the Osborne classification scheme they are classified as prolamins (soluble in alcohols) (Veraverbeke & Delcour, 2002; Goesaert et al., 2005; Sluimer, 2005). Gliadin proteins only have a single subunit (monomeric), and are considered to confer plasticity/viscosity to dough (Veraverbeke & Delcour, 2002). Alternatively, glutenins have multiple subunits (polymeric) the most prominent of which are high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS), and provide elasticity and dough strength (Shewry et al., 2002; Veraverbeke & Delcour, 2002; Wieser, 2007). HMW-GS are thought to have significant effects on the final quality of bread products, as they are largely responsible for gluten network development, and integral in development of the large, insoluble glutenin macropolymer in conjunction with LMW-GS (Wieser, 2007; Kontogiorgos, 2011; Dai et al., 2013). Hydration of gluten proteins produces viscoelastic dough which provides many of the unique properties of dough (Wieser, 2007; Delcour et al., 2012). Generally, bread quality is correlated with protein content in the flour, and higher protein, harder wheats are preferred for breadmaking compared to softer, low protein ones (Delcour & Hoseney, 2010).

Starch is the most significant component in wheat flour by weight, and it plays an important role in bread production and final structure (Goesaert et al., 2005). Starch is comprised of two main polymers both of which have the subunit glucose: amylose (mostly linear chains ranging in size from ~80 thousand to ~1 million Da or (~500-6000 glucose units, ~25% of wheat starch) and amylopectin (highly branched chains of up to ~1 billion Da or ~600 thousand glucose units, ~75% of wheat starch), and is insoluble in water (Sluimer 2005; Delcour & Hoseney, 2010). These both contain α-(1,4) linkages between adjacent glucose subunits, with α-(1,6) linkages at branch points on amylopectin polymers (some branch points on amylose polymers also, but significantly fewer), which can be cleaved by enzymes such as amylases (Goesaert et al., 2005; Cauvain, 2012). The critical role of starch in the breadmaking process occurs during baking; at increased temperatures

and in the presence of water, starch granules will begin to take up water, swell, and eventually lose their crystalline structure and become amorphous in a process known as starch gelatinisation (Goesaert et al., 2005; Sluimer, 2005). Gelatinisation of wheat starch occurs at ~55 - 60°C, and no higher than 90°C during the baking process (Collado-Fernàndez, 2003c). This gelatinisation process is critical to the bread crumb formation, and breads with lots of damaged/broken starch (which can be fermented by yeast) will have a poor, sticky crumb (Sluimer, 2005). In addition to the formation of crumb, starch is also important in bread staling, as the retrogradation of starch (the association of amylopectin molecules via hydrogen bonding) results in the exudation of water from the product, and firmer crust (Goesaert et al., 2005; Sluimer, 2005). While amylose can also retrograde, the process, due to its lack of steric hindrance, occurs much faster with amylose than amylopectin, and therefore, most of the amylose is already retrograded by the time of cooling, and it has impacts on the initial bread crumb, and not on bread staling (Goesaert et al., 2005; Delcour & Hoseney, 2010).

Non-starch polysaccharides (NSPs) and lipids within the flour are also believed to have impacts on the final product. One particular NSP which is believed to have an important impact is arabinoxylan, which is a viscosity-increasing NSP that appears to aid gas retention by slowing the movement of CO_2, and thus retaining more CO_2 in the dough (Delcour & Hoseney, 2010). Additionally, arabinoxylan can alter the viscoelastic properties of the dough, depending upon the type of arabinoxylan (water-unextractable, WU, or water-extractable, WE), which can increase dough stiffness and consistency, and reducing mixing time, as well, WE arabinoxylan has also been known to decrease extensibility of dough (Goesaert et al., 2005). Lipids within the flour, particularly polar lipids, can have detrimental effects on bread volume, and in recent literature, some utilisation of lipases to break these compounds down has been utilised (Goesaert et al., 2005; Delcour & Hoseney, 2010). However, the full scope of how utilising lipases in the breadmaking process affect the end product has not been investigated fully (Goesaert et al., 2005; Delcour & Hoseney, 2010). Wheat flour lipids also have significant impacts on the final characteristics of the bread, and they have been found to be particularly significant in affecting final loaf volume (Pareyt et al., 2011).

2.1.2 Water

Water is the second most abundant ingredient in wheat flour bread, and it has large effects on the overall texture and structure of the final product (Mondal & Datta, 2008). Water can both be helpful (crucial in starch gelatinisation), however, also problematic, as too much water causes dough handling issues; in yeast breads with higher water contents, the crumb tends to be coarser and the end product has a greater number of larger CO_2 bubbles (Sluimer, 2005; Mondal & Datta, 2008). Without water, proteins (gluten in particular), starch and other important components are not hydrated (Collado-Fernàndez, 2003a; Delcour & Hoseney, 2010; Cauvain, 2012; Delcour et al., 2012). This viscoelastic dough which is produced with the addition of water and wheat flour is what produces the gluten network which has gas holding capabilities (Delcour & Hoseney, 2010). The mineral composition of the water can also impact the final properties of the bread; water high in carbonates and sulfates tend to increase the strength (resistance and firmness) of the gluten network, and improve the gas retention, produce a finer grain, and increase the volume of the end product (Collado-Fernàndez, 2003a).

2.1.3 Yeast

Baker's yeast (*Saccharomyces cerevisiae*) is an essential ingredient which leavens the product by fermenting carbohydrate compounds into CO_2 and alcohol compounds, and significantly increases the volume (Sluimer, 2005; Delcour & Hoseney, 2010; Cauvain, 2012). Fermentable carbohydrate compounds include sugars such as sucrose, as well as products of damaged starch (e.g. maltose, dextrose), but not intact starch (Edwards, 2007). Temperature has a critical impact on the activity of yeast; at 4°C yeast has no fermentation activity, from 20-40°C, yeast fermentation is plentiful, and at 55°C, the yeast will die, thus, it is important to work with these temperatures during bread production (Collado-Fernàndez, 2003b). This reaction is exothermic, and from one glucose molecule, two molecules of CO_2 and two molecules of ethanol are produced (Sluimer, 2005). Fermentation can also be inhibited by certain compounds which may be present in the dough, such as preservatives like calcium propionate, acetic acid, or salt (Collado-Fernàndez, 2003b; Belz et al., 2012). In addition to this critical role, yeast has large impacts on the rheological properties of the dough (Collado-Fernàndez, 2003a); yeast acts similarly to an oxidising agent in that it will increase the elasticity of the dough (Belderok et al., 2000; Delcour & Hoseney, 2010).

2.1.4 Sodium chloride

Salt (NaCl), while not as significant in amount as water or flour (only 1-2%), plays a critical functional role in bread making (Mondal & Datta, 2008). The primary function of salt is to improve the strength of the gluten network, as well as to control the fermentation process and improve the flavour and texture (Edwards, 2007; Mondal & Datta, 2008; Belz et al., 2012). Salt also plays an important role in preservation, as it acts to reduce the water activity of the bread and prevents microbial and mould growth (Samapundo et al., 2010; Belz et al., 2012). Salt has also been shown to initially inhibit the gelatinization of starch by allowing the granules to swell to a greater extent prior to bursting (Salvador et al., 2005). Salt slows the hydration of gluten proteins by screening the individual charges on amino acid subunits. This charge screening reduces the attraction of the amino acids to water, which results in a slower hydration rate of the gluten proteins (Collado-Fernàndez, 2003a; Sluimer, 2005; Beck et al., 2012a; Belz et al., 2012). The slower hydration rate results in a dough with superior gas holding capacity (Collado-Fernàndez, 2003a; Sluimer, 2005; Beck et al., 2012a; Belz et al., 2012). In addition to that, the charge shielding which salt ions produce results in less electrostatic repulsion between gluten proteins, which then leads to greater gluten-gluten interactions which strengthen the gluten network (Belz et al., 2012). Decreasing the salt concentration also generates a large processing issue, as the dough which is produced is quite sticky and difficult to process, due in part to the reduced charge screening and increased rate of hydration of gluten proteins that results from lower levels of salt (Beck et al., 2012a; Belz et al., 2012). Reduction in salt can have several negative impacts on dough and bread beyond stickiness. It can result in bread with poor texture and crumb, partially because salt acts to control the fermentation process, and with reduced levels, larger CO_2 bubbles exist which leads to a more uneven crumb (Hutton, 2002; Lynch et al., 2009; Belz et al., 2012). Additionally, reducing the salt has been shown to decrease the machinability of the dough (resistance to extensibility and reduction in elasticity) (Belz et al., 2012).

2.1.5 Non-essential ingredients

The roles of various non-essential ingredients are summarized in Table 2.1.

Table 2.1 Role of non-essential bread dough ingredients (from Avramenko et al., 2015).

Ingredient	Function/Role in Bread	Examples
Lipids	Softening agent/plasticizer: - Generates a product with a finer, softer, and more elastic grain, and increased final product volume.[1,2,3,4] - Increases the plasticity of the bread dough, which allows for a reduction in the amount water necessary.[1,4] - Improves slicing characteristics of the product and reduces staling.[2,4]	Margarine, ghee, shortening, fractionated oils.[2,3]
Emulsifiers	Softening agent: - Effects vary depending upon which emulsifier is chosen.[1,5] - Produces a finer crumb, with increased uniformity in crumb size.[1,4,5] - Produces superior dough handling and strength.[1,2,5] - Produces superior hydration rate, gas retention, loaf volume, and water absorption.[1,2,5] - Improves product shelf-life and delays staling.[2]	Sodium or calcium stearoyl-2-lactylate (SSL, E481 or E482), monoglycerides (E471), lecithin, (E322), esters from monoglycerides or diacetyltartaric acid (DATA esters; E472e), etc.[1,3]
Sugars	Fermentation/Flavour: - Increases fermentation by providing a source of energy for the yeast.[1,2] - Improves tenderness, elasticity, and stability of the dough.[1,4] - Increases sweetness, and browning of the final product.[1,3,4]	Sucrose, dextrose, high fructose corn syrup,[3] invert sugar, lactose.[4]
Dairy Products	Flavour/Softening: - Increases browning (by introducing lactose), improves crust softness, and improve loaf volume.[1,4] - Can increase shelf-life.[1]	Milk, skim-milk powder, whey powder, etc.[1]
Oxidants	Gas Retention/Structure: - Oxidation of –SH groups to disulfide bonds, which generates superior rheological properties and improves gas retention, as well as strengthens the gluten structure.[1,3] - Improves oven spring, volume, and grain quality/final product softness.[1,3,4]	Ascorbic acid (E300)[1,4], azodicarbonamide.[4]
Enzymes	Crumb Structure: - Increases volume, improves crumb (less crumbly), decreases staling.[1,6] - In some formulations, can be utilised in place of oxidising agents.[3]	α-amylase,[1,6] hemicellulases, proteases, lipases,[3] glucose oxidase,[7] lipoxygenase.[4]
Preservatives	Shelf-life: - Reduces microbial and mold growth, particularly important in high moisture content breads.[1,8]	Calcium propionate (E282),[8] vinegar, sorbic acid (E200).[1]

(1) Collado-Fernàndez, 2003a; (2) Mondal & Datta, 2008; (3) Cauvain, 2012; (4) Sluimer, 2005; (5) Stampfli & Nersten, 1995; (6) Goesaert et al., 2005; (7) Bonet et al., 2006; (8) Delcour & Hoseney, 2010.

2.2 The process of breadmaking

Breadmaking is a complicated process which can be completed several different ways, but generally comprises some basic similarities. The process can be broadly divided into three main sections (with differing steps dependent upon the method of bread making utilised): dough formation, fermentation, and baking (Collado-Fernàndez, 2003c; Delcour & Hoseney, 2010). Several different types of methods also exist, such as sponge and dough, straight dough, or Chorleywood (mechanical dough development) (Mondal & Datta, 2008). While each method may contain slightly different steps, and times for various procedures, these three main categories are the basis for breadmaking. Differences in breadmaking systems are often due to the intensity of the mixer, which alters the fermentation needs of the product (Millar & Tucker, 2012). Modern bread production typically uses higher intensity mixers as it removes the need for a bulk fermentation step and allows for faster production times, but it can still produce a quality product (Millar & Tucker, 2012).

2.2.1 Dough formation

Dough formation is also known as kneading or mixing. This stage aims to generate a homogeneous, extensible mass which hydrates some important components (gluten, starches) and dissolves others within water (Belderok et al., 2000; Collado-Fernàndez, 2003c; Sluimer, 2005). This process is also critical for development of the gluten network, addition and incorporation of air into the dough matrix, and generating the varied rheological properties (viscosity, elasticity, etc.) and gas retention properties of the dough (Collado-Fernàndez, 2003c; Delcour & Hoseney, 2010). The mixing process allows for gluten protein subunits to hold water and swell, and the mechanical action provides interaction between individual particles which results in both chemical bonding (hydrogen bonding between groups such as –OH or –SH, and covalent bonding in the form of disulfide bonds) as well as physical interaction which can be referred to as entangling of protein molecules (Sluimer, 2005; Wieser, 2012). The increase in bonding and interaction between gluten proteins generates the “gluten complex” which aids and improves the elasticity and viscoelastic properties of the dough throughout the mixing process (Sluimer, 2005). A resting period of ~30 min after mixing will result in amylase enzymes degrading some of the starch granules, which generates softer dough (Edwards, 2007).

There are several methodologies to assess how to mix doughs to proper development; mixing to a specific amount of energy input or time have been the two most commonly used, however, mixing to a specific dough consistency or temperature have also been used (Millar & Tucker, 2012). Developing doughs to a fixed energy input is the most commonly used method in industry, and with high-intensity mixing instruments requires only a very short time to complete, often less than 5 min (Millar & Tucker, 2012). The amount of mixing is critical to the development of the dough, as there are concerns with both under and overmixing. If dough is undermixed, proteins and starches are not completely hydrated, and therefore, provide no useful function within the dough (Delcour & Hoseney, 2010). If dough is overmixed, the dough produced will be sticky and wet (with a sheen appearance). The latter is because the gluten network being developed is being consistently broken down (after hydration) (Delcour & Hoseney, 2010). The breakdown of disulfide linkages is a major concern of overmixing, as it can produce reactive thiyl radicals which can form undesirable linkages in the dough (Delcour & Hoseney, 2010). Oxidation is also believed to play a role in breakdown of the gluten network, and as a result, mixing in a nitrogen atmosphere has been incorporated into some systems (Delcour & Hoseney, 2010), however, some systems require oxygen for the activity of bread improvers such as ascorbic acid, which must be oxidized to produce a strengthening effect (Koehler, 2003). The intensity of the mixing (speed and mechanical shear) also appear to play a critical role in the development of dough (Sluimer, 2005).

2.2.2 Fermentation

Fermentation processes in bread are critical for several important aspects of dough development; most important the activity of the yeast (*S. cerevisiae*), and the development of proper gas retention properties (Belderok et al., 2000; Mondal & Datta, 2008). There is typically more than one fermentation phase during the baking of bread, separated by punching/kneading/remixing phases. The two main ones are bulk fermentation (or the initial fermentation) and the final fermentation (also known as proofing) (Belderok et al., 2000; Collado-Fernàndez, 2003b). Yeast plays an important role in the rheological properties of dough; dough after yeast activity tends to be become more elastic and flexible (Belderok et al., 2000; Delcour & Hoseney, 2010). The main purpose of the fermentation step is to activate the yeast to breakdown various carbohydrates into CO_2 and alcohols (Collado-Fernàndez, 2003b; Delcour & Hoseney, 2010; Cauvain, 2012). Fermentation is an anaerobic process, so when the dough contains oxygen

little fermentation occurs; the oxygen must first be utilised for respiration by the yeast prior to the switchover to anaerobic metabolic processes (Sluimer, 2005; Delcour & Hoseney, 2010). A second lag time exists between the initial production of CO_2 and the rising of the dough, because initially the CO_2 is dissolved into the aqueous phase, therefore, this phase must be saturated before CO_2 will become present in a gaseous phase, and volume increases are observed within the dough (Sluimer, 2005; Delcour & Hoseney, 2010). These newly formed CO_2 molecules cannot generate new bubbles within the dough matrix so CO_2 is forced to migrate from the yeast towards air bubbles which were generated during the mixing stage (Collado-Fernàndez, 2003b). This migration process causes the pressure in the bubbles to increase, which in turn causes an increase in volume of the dough (Collado-Fernàndez, 2003b; Sluimer, 2005; Delcour & Hoseney, 2010). During this fermentation process, dough is typically kneaded/remixed/punched, for two reasons; firstly, because this physical manipulation of the dough divides the gas cells which are present, allowing for smaller cells to be produced which in turn give a finer grain to the final product, and secondly, this allows for yeast and carbohydrates to come into contact through mixing (Collado-Fernàndez, 2003b; Sluimer, 2005, Delcour & Hoseney, 2010). The second critical function is that it causes the gluten network to become more rigid, which gives better textural characteristics in the final product (Sluimer, 2005). This process also causes large amounts of CO_2 to be expelled from the product (Delcour & Hoseney, 2010). The generation of new gas cells is more important, however, and further fermentation allows for further production of CO_2 (Delcour & Hoseney, 2010). The number and length of fermentations and punching/remixing phases differ depending upon the method of bread production utilised, however, traditionally, the last step before baking is the final proofing (Edwards, 2007).

In addition to its critical role in the fermentation step of breadmaking, yeast also produces several metabolites during this process, and these metabolites can have significant impacts on a variety of dough and bread characteristics, such as texture, flavour, and aroma (Heitmann et al., 2018). Organic acids are some of the metabolites produced by yeast during fermentation, and they can be produced during the glyoxylate cycle (citric acid cycle) (Hietmann et al., 2018) with succinic acid being the most prevalent acid produced at levels up to 1.6mmol/100g flour, followed by acetic acid (0.2mmol/100g flour) (Jayaram et al., 2013). It has been suggested that succinic acid in particular was responsible for the pH decrease observed during dough fermentation (Jayaram et al., 2013). Lactic acid has also been shown to be present in concentrations of 0.16mmol/100g flour,

however, this appeared to be previously occurring in the flour as levels did not change after fermentation (Jayaram et al., 2013). The authors also reported a significant effect of succinic acid on yeast-less dough rheology; finding that mixing times and gluten agglomeration, strength, and extensibility were all decreased with its inclusion (Jayaram et al., 2014). Yeast metabolites other than CO_2 appear to have significant effects on dough characteristics and final quality, and should be considered when modelling doughs, especially those not containing yeast.

2.2.3 Baking

Baking is the final step in bread production, and it consists of heating the dough to induce chemical and physical changes which result in the final desired product. Many changes occur in dough during the baking process; the gelatinisation of starch, formation of volatile aromatic compounds, as well as the denaturation of proteins, as the temperatures used for baking are high (200 - 275°C) (Belderok et al., 2000; Collado-Fernàndez, 2003c). The gelatinisation of starch is a critical process, as it is primarily responsible for crumb development (Goesaert et al., 2005). Due to the size and shape of a loaf of bread, dough on the surface will contact more heat and be under higher temperatures than the dough in the centre of the loaf. This means that on the surface of the bread, there is a significantly higher temperature, which generates colour and aroma of the crust, primarily via the Maillard reaction (reaction between reducing carbohydrates, such as glucose, with primary amines, such as amino acids, in particular lysine due to its ε-amine group), as well as caramelisation (reaction between two carbohydrates) which can produce highly reactive aldehyde compounds (Collado-Fernàndez, 2003c; Sluimer, 2005). Most of the volatile compounds are removed during the baking process (due to high temperatures), however, factors such as the gas-retention capabilities, strength of the gluten network, and permeability of the dough will affect the aroma and volatile retention (Collado-Fernàndez, 2003c).

The quality of the final product is heavily affected by the quality and composition of the dough, specifically, the amount and quality of the gluten proteins in the flour, the amounts of sugars, yeast, salt, in addition to any nonessential ingredients which may be included in the product, such as lipids or emulsifiers, as discussed in Table 2.1. One of the main results of baking is the increase in loaf volume, which occurs due to several chemical processes occurring during bread production; the retention of CO_2 within the matrix, evaporation of water molecules, and the alteration of the protein matrix to increase its elasticity (and therefore allow for expansion in the

form of loaf rising) (Collado-Fernàndez, 2003c). During the baking process, the properties of the initial ingredients are critical, as weak protein structure will result in poor gas retention, and too much damaged starch can result in both over-fermentation (a factor in poor final structure) as well as a subpar crumb structure, and other problems with the product (Veraverbeke & Delcour, 2002; Liu & Scanlon, 2003; Goesaert et al., 2005).

2.3 Health Canada's sodium reduction strategy

The average sodium intake for Canadians is 3400 mg/day, which is more than double the recommended intake of 1500 mg/day for those aged 14 – 50 years (Heath Canada, 2017). This is a concern, as the high sodium intake of the majority of citizens can be linked to several health concerns, such as hypertension (high blood pressure), cardiac and vascular damage (cardiovascular disease), bone damage (harmful effects on bone metabolism and calcium), increased risk of certain cancers, such as stomach, and the increased intake of sodium can also cause more severe asthma (Health Canada, 2017). To develop a plan to reduce sodium, and therefore reduce care costs and improve the health of Canadians, the government formed the Sodium Working Group (SWG) in 2007, which produced the Sodium Reduction Strategy Report, which was instrumental in developing *Health Canada's Guiding Benchmark Sodium Reduction Levels for Processed Foods* (Health Canada, 2010; 2012b). These guidelines were established after consultation with industry and health experts, and they provide very specific reduction targets for foods in a variety of categories taking into consideration the quality of the final product as determined by several factors including microbiological safety, acceptability and sensory quality. To determine the impact that sodium had from various products, they took the sodium from the nutrition facts label and the relative market share of each category by means of a sales weighted average (SWA). From this and the Canadian Community Health Survey (2004), they determined that breads and other bread-like products had the largest contribution to sodium intake (Health Canada, 2012b). While the amount of sodium present in a loaf of bread may not be that substantial, the contribution results from the significant volume of consumption of these products. The reduction for 2016, as suggested by these guidelines, was to alter the sodium content of bread from 469 mg Na/100 g bread (traditional) to 330 mg Na/100 g bread, which would be a reduction of approximately 30% (Health Canada, 2018). In the progress evaluation from Health Canada assessing product reformulation for these new standards from 2012-2016, it was noted that pan breads met the phase

I target of sodium reduction; 430 mg Na/100 g, however, this is still significantly shy of the final target as it is only an 8% reduction and the largest technical challenges remain (Health Canada, 2018). While this reduction of sodium could have cost and health benefits for Canadians, the processing problems that reduction poses are a necessary challenge to overcome to meet these guidelines.

2.4 Challenges associated with low salt bread

Salt is one of the four essential ingredients in bread production as described in section 2.1.4, and thus, reducing the amount of salt in bread can be problematic for a variety of reasons, from the impact of salt on processing (dough stickiness), to sensory qualities and food safety. Due to the incoming legislation aimed at reducing the levels of sodium in bread and other products, it is important that these challenges can be overcome to provide a safe, acceptable product to consumers, for which the processing is not compromised by technical challenges. Sensory characteristics of the final product, processing issues relating in particular to dough stickiness, and safety concerns with the reduction of sodium are some of the main challenges which will have to be addressed for these products in the future.

2.4.1 Dough stickiness

One of the major issues with reducing the salt levels in bread is a processing concern; reduction of sodium generates a sticky dough. This problem is the result of inadequate cohesive forces (interactions within the dough) and too many adhesive forces (interactions between the external surface (e.g. mixing bowl) and the dough), which results in the dough mixture adhering to the surface of equipment in commercial facilities and causing several problems, many of which are costly and adversely affect processing (Dobraszczyk, 1997; Adhikari et al., 2001; van Velzen, 2003). It is not only the excess of adhesive forces that causes stickiness, as high adhesive forces alone will not cause stickiness, but the conjunction of high adhesive forces and low cohesive forces that cause stickiness (Hoseney & Smewing, 1999). Rheology is able to investigate cohesive forces, and texture analysis such as Chen and Hoseney's stickiness cell (1995) can help identify adhesive forces (Hoseney & Smewing, 1999). Salt is a crucial ingredient in strengthening the gluten network; a process that can be affected by several factors which include:

i) mixing conditions (shear, time, temperature, etc.);
ii) bread formulation (flour, salt, sugar, fat, etc.);
iii) amount of protein hydration;
iv) quality of the proteins utilised, as well as composition of said proteins;
v) flour milling conditions (how damaged the starch is);
vi) amount of water-soluble pentosans;
vii) enzyme activities, specifically α-amylase and proteolytic enzymes, and;
viii) the utilisation of disease resistant wheat varieties containing the chromosome translocation of 1B/1R (Dhaliwal et al., 1990; Chen & Hoseney, 1995; Hoseney & Smewing, 1999; Adhikari et al., 2001).

All of these factors contribute in some fashion to dough stickiness, and the amount that they contribute can vary depending upon the type of wheat and processing conditions. Some compounds have previously been identified as causing stickiness within dough (such as ferulic acid esterified to a hexose), however these compounds do not account for all the stickiness issues within dough, and the mechanisms remains undetermined (van Velzen et al., 2003). Water has been linked to stickiness in a variety of foods (Adhikari et al., 2001), and it has been suggested that it may play a role in the stickiness mechanism of doughs, particularly the level of hydration of the gluten network (van Velzen et al., 2003). Water content is critical to the development of a strong gluten network (Skendi et al., 2010). Different types of water also play separate roles in dough; adsorbed water is water that is involved in the development of the gluten network and other structures directly by hydrating and absorption, whereas free water relates more to the viscous and flow properties of the dough (Roman-Gutierrez et al., 2002; Lu & Seetharaman, 2013). Therefore, the balance of these two types of water is critical to dough development and viscoelasticity which also relates to the stickiness (Lu & Seetharaman, 2013).

In general, to overcome the challenge of sticky dough phenomenon, salt has been added at higher levels (1.8 – 2.1%) in bread, however, this is not acceptable under new government standards to lower sodium in bread products (Farahnaky & Hill, 2007; Health Canada, 2018). Salt is critical because it works to shield the charges generated by amino acids on the gluten proteins, and therefore, allows them to interact to a greater degree (increases protein-protein interactions), which results in a stronger, more viscoelastic gluten network because it increases the amount of

crosslinking, as well as generates greater aggregation (thicker polymers), as visualised in Figure 2.1A (Belz et al., 2012). Additionally, the charge screening effect of salt reduces the amount of water which is held by the gluten proteins, as well as by other constituents of the dough, such as starch, pentosans, etc. (Lynch et al., 2009; Beck et al., 2011). Therefore, the reduction of salt causes an increase in both hydration of the gluten proteins as well as more water mobility throughout the dough, which results in stickiness of the dough, as shown in Figure 2.1B. Research conducted by Beck et al. (2012a) utilised confocal scanning laser microscopy to examine the gluten network of dough, and found that the reduction in salt content caused the gluten proteins to become less connected (initially they were elongated fibrils), demonstrating that at reduced salt content, gluten fibres have lower crosslinking and are thinner, as illustrated in Figure 2.1B.

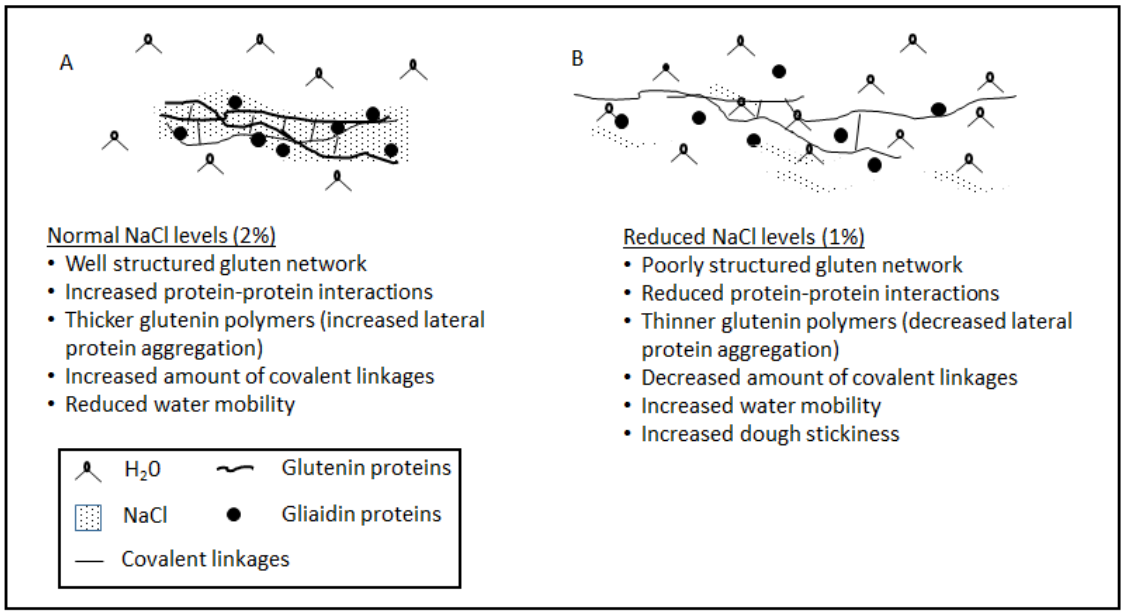

Figure 2.1 Structure of the gluten network under conditions of normal NaCl levels (2% NaCl) and reduced NaCl levels (2%) (from Avramenko et al., 2015).

2.4.2 Sensory concerns

In addition to having significant impacts on the processing and structural aspects of bread, salt reduction has been shown to have negative effects on the sensory aspects of the final bread products. In a study conducted by Lynch et al. (2009), the reduction of salt from 1.2% to 0% was shown to result in a product with “sour/acidic”, “yeasty” and “sough dough” flavours. The authors

did report that breads with 0.3% and 0.6% salt exhibited very similar sensory properties (Lynch et al., 2009). A previous study also examined differing salt contents of bread (0.25%, 0.63%, and 1.06%), and found that the most preferred sample was the bread containing 1.06% NaCl, and that consumers did not find the 0.25% sample acceptable (Hellemann, 1990). Other authors have also reported that without salt, the final product lacks flavour, as well, the crust formation of the final product can be poor (Belderok et al., 2000; Collado-Fernàndez, 2003c; Sluimer, 2005; Kilcast & Angus, 2007; Belz et al., 2012). In addition to flavour, reduction/removal of salt also had effects on other aspects of the final bread product; it generates a poor crumb structure (negatively altering texture) and also increases the effects of staling, resulting in a stiffer bread product (Lynch et al., 2009). As well, Czuchajowska et al. (1989) found that the reduction of salt decreased the overall loaf volume, however, this effect was not observed by Lynch et al. (2009), and it is possible these differences could be accounted for by differing wheat varieties and flour qualities.

2.4.3 Shelf-life and food safety concerns

In addition to its many other functional roles, salt in bread plays a critical role in preservation and reduction of microbial activity. Bread is a product with high water activity (typical a_w values are 0.96 – 0.98), and as such, it is susceptible to microbial spoilage (Smith et al., 2004; Belz et al., 2012). Salts such as NaCl act to reduce the water activity in foods by increasing the osmotic pressure of the environment, which results in fluid losses from the cells and can cause loss of cellular function, so NaCl can be used to control yeast (Belz et al., 2012). Therefore, reduction of salt in bread products results in a product with higher water activity and greater susceptibility to microbial spoilage and other food safety and preservation concerns. The majority of research relating to low-salt bread has investigated the impacts on other functional roles of salt in bread, and not the impacts on food safety and preservation (Lynch et al, 2009; Belz et al., 2012).

Lynch et al. (2009) examined the staling characteristics of bread and found that as the salt level in bread was reduced; the staling process was expedited, indicating a reduction in preservation of the product. A second study investigated the effects of reducing the salt level on the growth of fungi in bread (*Penicillium roqueforti* and *Aspergillus niger*) (Samapundo et al., 2010). The authors observed that by reducing the NaCl by 30%, the colonies grew faster and had a reduced lag time (Samapundo et al., 2010). However, this did not translate to significantly faster growing colonies of *P. roqueforti* in a baking trial, which led the authors to suggest that the

reduction in NaCl was not substantial enough to have an impact (Samapundo et al., 2010). A third study examined breads at various salt levels (0%, 0.1%, 0.2%, and 1.2% or standard), and found that the shelf-life of reduced and low salt breads were significantly decreased in comparison to the control bread, being reduced from 5-6 days to only 2 days (Markus et al., 2012).

While the studies are limited in number, there is a trend indicating that reducing the salt level in bread can have a negative impact on shelf-life and microbial safety which presents challenges to bakers. Therefore, alternative methods to extend preservation of these products need to be considered. Markus et al. (2012) examined the utilization of sourdough fermentation and calcium propionate as methods of increasing shelf-life while reducing salt content, and found both, but particularly the sourdough fermentation technique to be effective. Others have investigated the use of other salt replacement compounds as a possibility for maintaining shelf-life (Bidlas & Lambert, 2008; Samapundo et al., 2010). Organic acids have also been considered as a possible method of helping to control mould in baked goods (Corsetti et al., 2000; Marín et al., 2002). In addition to additives, improved packaging, such as modified atmosphere packing, gas packaging, or packages developed with new, improved polymeric materials have potential for use (Smith et al., 2004). Several of these studies have been completed at standard salt content for bread or other baked goods (Corsetti et al., 2000; Marín et al., 2002; Guynot et al., 2005), and as such, future studies should consider more the reduced salt level. The reduction of salt in bread presents certain challenges for preservation of bread, which will have to be considered and addressed in future product formulations. Additional challenges are raised due to the interest in “clean label” products which do not contain additives not from natural sources. This means that the most desirable future solutions will not include artificial shelf-extenders, which reduces potential choices.

2.5 Strategies to improve low salt bread

As a result of the processing and final product concerns related to low salt bread, many strategies have been employed to attempt to resolve these issues and produce a consumer acceptable product which is easily processed and low in salt. Some of these strategies relate to utilising alternative salt compounds to replace sodium chloride, others relate to changing the flavour, or the utilisation of enzymes to help reduce dough stickiness and maintain the flavour profile that consumers expect from a bread product.

2.5.1 Reduction of sodium by replacing sodium chloride with other salts

Alternative salts, such as potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) chloride have been widely considered and studied in research as replacement compounds for the sodium chloride presently used in bread to maintain quality but reduce sodium content. How well these salts manage to result in acceptable dough handling relates to how well the ions induce protein-protein aggregation which is necessary to produce a stronger gluten network, without sodium ions present which typically have this function in bread with regular salt levels. Similar to Na^+, the charges on these ions act to decrease the degree of hydration of the gluten proteins, and increase the order of the structure, however, their effectiveness is altered by their position in the lyotropic series, which rates from high degree of stabilising effect to low degree of stabilising effect in the following order: $K^+ = Na^+ > Ca^{2+} > Mg^{2+}$ (Preston, 1989; Miller & Hoseney, 2008). Therefore, KCl seems to be the most similar to NaCl for producing comparable bread quality and dough handling characteristics; Kaur et al. (2011) found that complete replacement of NaCl with KCl did not decrease stickiness of dough and actually increased hydration of gluten proteins, however, partial replacement (25-50%) led to acceptable dough handling characteristics. However, low levels of KCl (as low as 10–20% NaCl replacement) can produce metallic/bitter flavours which make the products unacceptable (Salovaara, 1982; Miller & Hoseney, 2008; Beck et al., 2012b; Belz et al., 2012). Salovaara (1982) found that utilising $CaCl_2$ and $MgCl_2$ had significantly shorter peak mixing times than NaCl, and produced a weak gluten network. $CaCl_2$ and $MgCl_2$ have also been known to produce poor flavours would could result in an unacceptable product (Beck et al., 2012b). While KCl has some potential in replacing salt, its off flavours limit its usefulness in this regard.

2.5.2 Use of ascorbic acid to combat dough stickiness

Ascorbic acid has been used as an additive in bread since 1935 to improve dough strength and loaf volume of breads produced with weaker flours (Grosch & Wieser, 1999). In its native form, ascorbic acid does not function as a bread improver. However, it can be rapidly oxidised to form dehydroascorbic acid, which can then increase the number of disulfide linkages in gluten development by oxidising glutathione (GSH), a small three peptide thiol (Glu-Cys-Gly) which interferes with the formation of additional disulfide bonds, to its disulfide form (GSSG) (Grosch & Wieser, 1999; Koehler, 2003; Franco et al., 2007). GSHs be found in the aleurone layer and

germ of wheat that can act to prevent further polymerization of glutenin proteins, thereby interfering with gluten network development (Wieser, 2012). Unlike other bread improvers, such as azodicarbonamide or potassium bromate, ascorbic acid requires oxygen to be useful, as it must be oxidized to dehydroascorbic acid prior to functioning, which also may be an issue during fermentation as yeast turns dough into an anaerobic environment (Wieser, 2012). However, it is difficult to overdose ascorbic acid (where the excess ascorbic acid begins to act as a reducing agent after the oxygen has all been utilized to form dehydroascorbic acid) at typical inclusion levels in bread of 40-100 mg/kg flour (Millar & Tucker, 2012), as overdosing does not begin to occur until ~200 mg/kg flour (Xiuzhen & Sieb, 1998). The permitted limit of ascorbic acid to include in bread products in Canada is 200 ppm (Health Canada, 2012a). Some work with ascorbic acid at reduced salt levels has been completed; Aamodt et al. (2003) added ascorbic acid to doughs prepared without salt and found strength improvements with its inclusion, and at 1.5% salt levels (by flour wt.) Dagdelen and Gocmen (2007) also found improvements in loaf volume with its addition. However, this work focused primarily on final loaf quality and rheology, but not dough stickiness. Additionally, while ascorbic acid has been effectively used in bread, the trend of "clean label" for many food products may also push for alternative improvers, such as enzymes, which do not have to be listed on a food ingredient label.

2.5.3 Utilisation of enzymes to combat dough stickiness resulting from low salt bread

Incorporation of enzymes into a bread formulation have also been shown to reduce the stickiness of dough when utilised with regular sodium levels, so there is potential for improvement in low sodium products. A variety of enzymes and enzyme cocktails have been examined as bread improvers by different approaches; some affect proteins, starches, pentosans, lipids etc. A few of the main enzymes which have been investigated as bread improvers include glucose oxidase, xylanase, transglutaminase, proteases and α-amylase (Caballero et al., 2007; Steffolani et al., 2010).

Glucose oxidase (GO) is one of the main enzymes which has been examined as a bread improver and it could have an impact in the reduction of dough stickiness. GO acts as an oxidising agent; this enzyme catalyses the oxidation reaction of α-D-glucose to H_2O_2 and δ-gluconolactone, after which, the H_2O_2 produced can react with thiol groups within the dough to form disulfide bonds and strengthen the gluten network (Steffolani et al., 2010). In addition to crosslinking

proteins, this reaction can also crosslink other substituents in the dough, such as arabinoxylans (pentosans) and promote the formation of dityrosine crosslinks (Decamps et al., 2012). Several authors have examined the effects that GO has on dough and final bread characteristics, although most of the current research has related to a standard salt level in bread (~2% NaCl). Bonet et al. (2006) incorporated GO into flour prior to mixing with other ingredients at levels of 0.001%, 0.005%, and 0.010%, and found that while the incorporation of GO did not significantly alter the water absorption, the GO did act to increase the stability of the dough to overmixing, however, too much GO caused the gluten network to become too reinforced and resulted in the loss of gas holding capacity, as well as other quality defects. Caballero et al. (2007) also tested the impacts of GO by adding 3 mg GO/100 g of flour (0.030% GO) and found that the rheological behaviour of the dough was not significantly affected by this addition, however, it did create a final product with a more elastic and cohesive crumb. This study also examined the impacts of utilising several types of enzymes in the attempt to create a synergy, and the authors found that this was the case when GO was combined with a protease as there were improvements in the loaf volume and the height/width ratio of the bread (Caballero et al., 2007). Decamps et al. (2012) utilised both GO and pyranose oxidase (PO) as bread improvers and found similar results to others; the addition of oxidising enzymes GO and PO increased the resistance of the dough to extension, and dough which was proofed showed higher volume with GO and PO inclusion, however, at the highest concentration of enzymes, the volume was significantly lower. Steffolani et al. (2010) examined the utilisation of several enzymes in bread production, one of them being GO, included at levels of 0.001% and 0.01%. The authors found that in the presence of GO, water soluble pentosans were either degraded or lost solubility, there was increased crosslinking between proteins (which formed larger protein aggregates), as well as GO increasing the dough development time and subsequent stability of the dough (Steffolani et al., 2010). GO also had an impact on the final bread quality; while it did not alter the final volume at 0.001%, it did generate a softer and less chewy crumb than the control, however, these problems were not noted with 0.010% GO (Steffolani et al., 2010). Dagdelen and Gocmen (2007) assessed GO and ascorbic acid inclusion at slightly reduced salt levels (1.5% by flour wt.) and found dough improvements, however research on low salt levels with enzymes remains less studied.

Xylanase (XYL) refers to a class of enzymes, some of which (e.g. endoxylanases) will hydrolyse water insoluble pentosans such as arabinoxylans. This causes the polymers to become

water soluble, which then has a positive impact on dough and bread quality at full salt content (Steffolani et al., 2012; Yang et al., 2014). Caballero et al. (2007) examined the effects that XYL has on bread and dough quality by adding it to standard salt bread at 6 mg XYL/100 g flour, or 0.060% XYL. The authors found that XYL had a significant effect on dough rheology after 180 min of incubation, and that it decreased both the elastic and viscous moduli (Caballero et al., 2007). In terms of final product quality, XYL resulted in a softer crumb, and decreased the effects of staling (Caballero et al., 2007). The resulting softer crumb could be a problem for low sodium bread, as it may worsen the stickiness and processing concerns in low sodium bread by binding more water to compounds in the dough. Other studies which included XYL used it in combination with other enzymes, and will be discussed later.

Transglutaminase (TG) is an enzyme known for crosslinking various food proteins via catalysis of an acyl-transfer reaction, and in the case of dough, has been known to crosslink gluten proteins to form very large and insoluble polymers, by crosslinking glutamine to lysine (Caballero et al., 2007; Steffolani et al., 2008). Steffolani et al. (2008) added TG at various levels to dough and examined its effects on dough rheology and glutenin macropolymer (GMP), and found that at high doses, amounts of GMP increased significantly. When the rheological properties of the dough were examined, the authors found that addition of TG at higher concentrations improved dough strength (due to increased crosslinking of gluten proteins) (Steffolani et al., 2008). Caballero et al. (2007) reported similar results from their investigation with TG (added at 500mg/100g flour or 0.5%), as the dough was strengthened with the inclusion, as well, increases in both the elastic and viscous moduli were observed. On final bread characteristics, TG was noted to increase the final loaf volume and provided greater crumb uniformity; however, it also significantly increased the hardness and chewiness of the bread crumb (Caballero et al., 2007).

Individual enzymes have been shown to have significant impacts on both the dough and the final loaf quality of bread, however, certain enzymes have shown some negative impacts such as TG forming a network which is too strong and gives poor final texture (Caballero et al., 2007), and as such, several authors have examined the possibility of utilising several enzymes simultaneously to hopefully provide a synergistic effect and improve the product. One such study by Steffolani et al. (2012), incorporated GO, XYL, and α-amylase (to hydrolyse starch) at levels of 3.7mg GO, 8.9mg XYL, and 10.5mg α-amylase per 100 g flour in bread dough, and they examined the effects of these enzymes on stickiness of the dough by texture profile analyser. The

authors found that XYL increased the stickiness of the dough, while GO decreased dough stickiness, and α-amylase had no significant effect on dough stickiness, by mechanisms discussed previously (Steffolani et al., 2012). Yang et al. (2014) incorporated GO, papain (a protease) and XYL into bread dough (0.01% XYL, 0.005% papain, 0.008% GO) to examine the effects on rheological properties. The authors found that at this proportion of enzymes, the GO was able to negate some of the negative effects on dough properties of the XYL and papain; however, this system was at full salt level, so it would likely have different results in a low salt system (Yang et al., 2014). Caballero et al. (2007) examined the combination of several different enzymes (TG/α-amylase, TG/XYL, TG/protease, GO/protease, α-amylase/protease, and XYL/protease) added at levels of 3mg GO, 6mg XYL, 1mg α-amylase, 5μL protease, and 500mg TG per 100 g of flour at 2% salt level. Of these combinations, TG/XYL showed a significant effects relating to the viscoelastic properties of the dough; the effects of these two enzymes counteracted each other (XYL softened the dough by breaking down arabinoxylans while TG strengthened/hardened the dough by increased crosslinking), which led to dough that did not have the excessive strength resulting from the use of TG alone (Caballero et al., 2007). The current literature into the utilisation of enzymes, both individually and in combination with others has primarily been conducted on bread with full salt levels, not reduced salt levels, so selection of enzymes which may aid, or the need for enzymes such as XYL to counterbalance effects of TG may or may not be necessary.

2.6 Rheology as a method for understanding dough handling

2.6.1 Rheology basics

Rheology is a broad field of study which examines how matter flows and deforms (Delcour & Hoseney, 2010). Viscoelastic materials such as bread dough are more complex to study than materials with simple rheology such as water or steel, which exhibit viscous flow and ideal elasticity, respectively (Delcour & Hoseney, 2010). Viscoelastic materials have viscous flow when shear is applied and elastic recovery upon the removal of that stress, however, the elastic recovery is not instantaneous (Delcour & Hoseney, 2010). Classical rheological testing of doughs has been utilized for several years using a variety of methods. Farinograph and mixograph testing is still used to characterise flours particularly in regards to water absorption values, and the extensigraph and alveograph are still used for uniaxial and biaxial extension testing which can relate to rheological properties (Delcour & Hoseney, 2010). The largest drawback of these empirical

methods is that they are only valid for the machines from which the results are produced as in the case of mixograph and farinograph testing, the stress cannot be calculated at any specific point due to the limited amount of dough being in contact with the mixing pins at any given time during mixing (Delcour & Hoseney, 2010). Farinograph absorption values (FAB) are still utilised to determine optimal water concentrations for dough handling; FAB values indicate how much water is needed to result in a centering of the peak farinograph curve of 500 Brabender Units (BU), although the BU value depends upon the country (Cauvain & Young, 2003). Optimal water addition is useful in determining the dough handling properties (the strength, extensibility, stickiness and other characteristics of doughs) which may affect their final baking quality. Prior to the increase in computer use, some rheological methods were difficult to gain valuable information from, but improvements in technology have made it feasible for use is experiments (Weipert, 1990).

2.6.2 Dynamic rheology

Dynamic rheology can be applied to dough systems to gain a greater understanding of the viscoelastic nature of doughs (Delcour & Hoseney, 2010). The basis of rheology rests on understanding the relationship between stress and strain and how those forces affect matter. Stress (σ) is equal to the force (F) applied over the area (A) it is applied ($\sigma=F/A$), with the SI units of N/m^2, or Pa, and strain (γ) is the change in length (extension) over original length, ($\gamma=\Delta L/L$), or the amount to which deformation occurs in the material (Janmey & Schliwa, 2008). The ratio of stress to strain is also a critical one for rheology (Janmey & Schliwa, 2008). From these basic definitions, there are several other important factors in rheological work which can determine moduli relevant to dough studies; shear stress (τ) is the stress force which is parallel to the surface of the material (Janmey & Schliwa, 2008). Rheological testing can be completed within the linear viscoelastic region (LVR) as it simplifies calculations and provides different information than large deformation rheology. In the LVR there is a linear stress response with increasing strain amplitude, and G' should remain constant (Hackley & Ferraris, 2001; Vlachopoulos & Polychronopoulos, 2012). For dough, remaining within the LVR means that the dough structure is not destroyed, which improves understanding of the structure (Jekle & Becker, 2011).

Oscillatory rheology is one type of rheological testing which employs rotational stress to samples and can provide information about materials for both the linear and nonlinear viscoelastic

regimes (Fang & Choi, 2012). This can be completed as a frequency sweep, ranging from lower to higher frequencies, at constant strain values (Salvador et al., 2005). When materials are tested, sinusoidal strain curves and shear stress curves can be plotted and the resulting phase angle (δ) is how out of sync those curves are, with fully elastic materials having a δ of 0°, and ideally viscous liquids having a δ of 90° (Xiao et al., 2011). Viscoelastic materials have properties of both fluid-like or viscous materials and elastic or solid-like materials as described by the dynamic storage (G′) and loss (G″) moduli, respectively (Delcour & Hoseney, 2010). G′ represents the energy which is stored during an oscillatory cycle, and loss modulus represents the energy lost during that same cycle (Delcour & Hoseney, 2010). From this, the ratio of G′ to G″ is a variable called the loss tangent or loss factor (tan δ; tan δ = G″/G′), which is an indicator of the relative elasticity or viscosity of the material; a higher G′ value indicates a more elastic material and vice versa (Mezger, 2006). The complex modulus (G*) is a combination of G′ and *i*G″, where *i* represents an imaginary number (Madsen et al., 2008), and it can be used as an indication of dough stiffness (Jekle & Becker, 2011). These parameters are all useful in evaluating the nature of a viscoelastic material, which in turn can help understand its other properties and possibly be linked to its final product quality.

Creep recovery or creep compliance is a rheological testing method which applies a constant shear stress for a set amount of time (t), and then it is often followed by a recovery compliance where at another time (t_0) the stress is removed, and the material is allowed recovery for a time (t_r) (Dealy & Wang, 2013). Creep compliance (J) is defined by the following equation:

$$J(t) = \gamma(t)\tau_0^{-1} \quad \text{(Eq. 2.1)}$$

where γ is strain, and τ_0 is the applied stress (constant) during the procedure (Jekle & Becker, 2011). The maximum deformation, or J_{max}, is the creep compliance at the end of the application of stress (Jekle & Becker, 2011). The recovery compliance (J_r) value is taken at the end of the recovery period, and using it can produce a value for relative elasticity (J_{el}), which is defined by the following equation:

$$J_{el} = J_r(J_{max})^{-1}. \quad \text{(Eq. 2.2)}$$

Unlike oscillatory rheology, which often maintains a constant strain and has changing stress, creep compliance and recovery compliance have changing strain values and constant stress values, as discussed above, so examining both can provide a deeper understanding of dough rheology, as stress examines forces, and strain examines deformation (Dealy & Wang, 2013).

2.6.3 Applications of rheology in dough studies

Rheology has had widespread application in dough systems for assessment of dough parameters (Salvador et al., 2005; Song & Zheng, 2006; Jekle & Becker, 2011). These tests can provide some insight into the structural strength, alterations doughs may have during temperature changes, and relative viscoelasticity of the doughs, however, the baking properties of dough are not necessarily reflected well in the rheological results particularly with respect to G′ of doughs (Weipert, 1990; Autio et al., 2001). Some authors have shown a good correlation with some rheological experiments and baking, such as creep testing of dough, which Van Bockstaele et al. (2008) found to have an r^2 of 0.74 between creep-recovery and bread volume. One of the largest drawbacks of this type of rheology is that it is very difficult to do properly with doughs containing active yeast, as the heterogeneity of yeast results in continuous changes in the dough structure and system during testing which can be difficult to measure accurately, therefore experimental designs are often without yeast, have inactive yeast, or have had the systems stabilized prior to testing (Salvador et al., 2005). While baking results may not always correlate, it can be very useful for understanding simple flour-water systems which may improve formulation and understanding of specific ingredients roles on rheological development to aid in production of some other bread products such as low-sodium bread (Salvador et al., 2005).

2.7 The use of ^{1}H nuclear magnetic resonance (NMR) for water studies in bread dough systems

2.7.1 Basics of ^{1}H NMR

Nuclear magnetic resonance (NMR) spectroscopy techniques have been in development since the 1970s and are able to provide information on a wide array of topics including medicine and materials work (Callaghan, 1991). NMR requires atoms which have an odd atomic number or mass, such as ^{1}H, ^{13}C, ^{15}N, or ^{19}F, however, ^{1}H and ^{13}C are the most common types used, in part due to their abundance in nature (Callaghan, 1991; Balci, 2005). Electrons have a spin of either -½ or +½, which is usually represented as a spin orientation of either an up or down arrow (Balci, 2005). In the case of elements which contain an even number of electrons and neutrons, such as ^{12}C, the atom has zero nuclear spin and cannot produce NMR spectra, whereas ^{1}H and ^{13}C both have a nuclear spin of ½ (Balci, 2005). The ½ nuclear spin has a magnetic field, and when an

external magnetic field is applied, the nuclei will align either in parallel or in antiparallel to the field, and this can be utilized to determine information about compounds and molecular motion (Callaghan, 1991; Balci, 2005). This information is determined by the production of NMR signals in these externally applied magnetic field gradients, and the signals are produced due to the excitation of the nuclei into a higher energy level, and the decay of that excitation, also known as relaxation from excited to ground state can be measured and assessed to provide useful information (Callaghan, 1991; McMurry, 2011). Relaxation efficiency refers to how fast the relaxation occurs, and is related to the physical properties of the matter being studied which is why investigation into relaxation parameters can provide information about materials (Keeler, 2002).

^{1}H NMR assesses parameters relating to protons and can be used to investigate the molecular motion of water due to the proton signal that water can produce (Separovic et al., 1998). Additionally, depending upon the parameters of the experiment, NMR can be sensitive to different timescales of motion, such as MHz, or the motions of smaller molecules such as water, or kHz, which is the motion of larger molecules such as protein side chains (Kishore et al., 2012; Chen, 2015). T_1 and T_2 are two important relaxation times which represent transverse relaxation and longitudinal relaxation, respectively (Bosmans et al., 2012). Transverse and longitudinal refer to vectors by which their magnetization occurs about the external magnetic field; transverse is perpendicular to the external field, and longitudinal is parallel to that field (Schild, 1990). T_1 assesses the relaxation times of spin-lattice interactions, which is the interaction of ^{1}H and the "lattice" which is everything else in the system which does not produce a ^{1}H signal in the NMR, which for the case of bread would be protein, starch, lipid, etc., and this parameter can be utilized to help assess how tightly associated the water within the system is with some of these components (Bosmans et al., 2012). Alternatively, T_2 represents spin-spin relaxation which is the relaxation time relating to water-water interactions or water tumbling, and it can be useful in the understanding of water mobility within dough, particularly when it is broken down to its sub components such as T_{2A} and T_{2B} which represent more bound and free water, respectively (Bosmans et al., 2012; Lu & Seetharaman, 2013). Both values relate to motion on the MHz timescale, which is indicative of molecular motion such as water tumbling within a system, and therefore has value in examining the water mobility parameters within food systems such as dough (Kishore et al., 2012). These parameters and their relationship is also indicative of the state of the material; T_1 and T_2 are relatively equivalent in the case of liquids (non-viscous) and as the material

becomes increasingly solid, T_1 becomes much larger than T_2 (Chinachoti et al., 2008). These values are often discussed in terms of correlation times, which are defined as the time it takes for a molecule to rotate 180°, which can occur and be assessed due to spin echo experiments such as those used for identifying T_1 and T_2 relaxation times (Chinachoti et al., 2008). Mobile water has a longer relaxation time when compared to bound water (Linlaud et al., 2011). Motion on other timescales, such as the kHz timescale can also be examined by assessing parameters such as $T_{1\rho}$ or rotating frame relaxation time which investigates motion on this timescale (Callaghan, 1991; Chen, 2015). These investigations are completed using pulse sequences which will be discussed in the next section.

2.7.2 ^{1}H NMR pulse sequences used to assess water mobility and other morphology in dough

Much of the NMR work done has assessed structure of organic compounds, however, to obtain information on the molecular motion as described above, pulse sequences can be utilized (Callaghan, 1991). For the study of water in bread, more focus is placed on pulse sequences which can provide information about water motion and association of water with various components of the dough and final bread product (Assifaoui et al., 2006a; Doona & Baik, 2007; Bosmans et al., 2012). Free induction decay (FID) is one of the simplest pulse sequences which can be used to determine information regarding morphology, as it only utilises a single $90°_x$ radio frequency (r.f.) pulse to excite the sample and then measure the subsequent relaxation, however, due to this it is susceptible to magnetic field inhomogeneity (Callaghan, 1991). FID can provide T_2^*, which is a decay signal resulting from spin-spin and spin-lattice interactions and magnet homogeneity, which provides insight into the fineness of pores in the structure and overall homogeneity (Callaghan, 1991; Chen et al., 2005; De Guio et al., 2009). To assess T_1 and T_2, specifically, several pulse sequences have been developed. Inversion recovery (IR) assesses T_1; it is a pulse sequence which uses two pulses; the first pulse ($180°_x$) inverts the magnetization vector, and it is followed by a second pulse ($90°_x$) to assess the longitudinal magnetization (Callaghan, 1991). T_2 relaxation can be assessed by a Carr-Purcell-Meiboom-Gill (GPMG) echo train, which begins with a $90°_x$ pulse to produce transverse magnetization, has the signal turned off, then four $180°_y$ pulses are used to produce echoes which decrease in intensity with each additional $180°_y$ pulse, and the length of the sequence, and the echoes are able to deal with the magnet inhomogeneity which FID cannot, and therefore, this pulse sequence is able to determine T_2 (and sub categories of T_2 such as T_{2A} and

T_{2B}) (Callaghan, 1991; Assifaoui et al., 2006a). Other motion parameters can be assessed, such as motion on the kHz timescale which is often indicative of protein side chain or macromolecular motion (Chen 2015). This work can be completed with a spin-lock pulse sequence which assesses the rotating frame relaxation time ($T_{1\rho}$). This sequence uses an r.f. field to produce transverse magnetization, and it assesses the resulting decay (Callaghan, 1991). A variety of pulse sequences can be utilized depending upon the interest of the subject matter, and the physical properties (i.e. is it a liquid or a solid) (Callaghan, 1991), and those described above are ones of interest to water and motion within bread.

2.7.3 Diffusion studies by ^{1}H NMR

Some previous work in diffusion of water has been completed in bread and dough systems, however, the work has been quite limited, unlike the work assessing water associations (as represented by protons) (Umbach et al., 1992; Wang et al., 2004). A pulse sequence called the "three-pulse sequence" developed by Kimmich and Fischer (1994) can help to understand self-diffusion of protons with systems such as dough; two pulses produce an echo signal, followed by a third pulse which produces a second echo and the ratio of the amplitudes of those two echoes can be used to determine proton self-diffusion. The previous work has assessed how gluten affects self-diffusion of protons in bread and dough; Umbach et al. (1992) found that gluten slowed diffusion more than starch in doughs, and Wang et al. (2004) determined that gluten did not affect diffusion significantly in final baked goods. However, work on the self-diffusion of protons in different formulations of doughs has not been reported, and there are gaps in the literature in ^{1}H NMR diffusion studies which may aid in the understand of the water-dough-quality relationships.

2.7.4 Previous applications of ^{1}H NMR in dough and bread studies

Some applications of ^{1}H NMR have been utilized in bread and dough studies for the investigation of water mobility, as protons can be used to represent water mobility. Several approaches have been examined, but of interest in the assignment of proton populations to different components of the dough, such as T_{2A} and T_{2B}; which represent more tightly bound and less tightly bound water (Assifaoui et al., 2006a; Assifaoui et al., 2006b; Bosmans et al., 2012; Simmons & Vodovotz, 2012; Lu & Seetharaman, 2013; Rondeau-Mouro et al., 2015). Bosmans et al. (2012) investigated the ^{1}H NMR properties of doughs and breads and linked different proton populations

throughout the process, characterizing these populations as bound, somewhat mobile, and free water. Much of this work examined different additive or component effects on T_2 values of doughs (Simmons & Vodovotz, 2012; Lu & Seetharaman, 2013; Hemdane et al., 2017), however, others examined more fundamental aspects such as temperature changes (Rondeau-Mouro et al., 2015), or hydrocolloid interaction (Linlaud et al., 2011). This work assessing proton populations is valuable for understanding more about the relationship between water and doughs, however, only a few authors have attempted to link select water properties with other observed dough characteristics such as rheology (Blanchard et al., 2012; Hemdane et al., 2017). In general, ^{1}H NMR has been shown to be a useful tool for the further understanding of the relationship between water and dough/bread, however, there are still gaps which need to be filled.

3. EFFECT OF ENZYMATIC CROSSLINKING ON THE HANDLING PROPERTIES OF DOUGH AS A FUNCTION OF NaCl LEVELS FOR CWRS VARIETIES, PEMBINA AND HARVEST

3.1 Abstract

The effects of transglutaminase (TG) or glucose oxidase (GO) on the handling properties of model bread doughs were examined at both standard (2.0% wt. by flour) and reduced (1.0% wt.) NaCl levels using two CWRS cultivars; Pembina and Harvest. The reduction in NaCl level had negative effects on dough rheology and stickiness, however, the inclusion of GO (0.001% and 0.01% by flour wt.) or TG (only at the 0.5% by flour wt.) was able to improve dough strength and reduce stickiness. GO appeared to be more effective than TG (at 0.01%) at equivalent concentrations for improving dough handling properties. Cultivar had significant effects; Harvest flour (weaker dough strength, higher stickiness) was more impacted by salt reduction and enzyme inclusion compared to Pembina flour (higher dough strength, lower stickiness). Crosslinking assays showed significant differences in glutenin macropolymer (GMP) content in doughs prepared with GO, and doughs prepared with different flours. Additionally, significantly fewer free thiol groups were found in dough produced with GO compared to dough without any enzymes and those with TG. GO appears to have potential for use in bread dough to reduce stickiness and increase the strength of bread doughs produced at lower salt concentrations, especially for doughs prepared with weaker dough property cultivars.

3.2 Introduction

High dietary sodium has become a significant concern around the globe due to its association with a variety of health issues such as hypertension and cardiovascular diseases (CVD) (O'Donnell et al., 2015). In Canada, the average sodium intake is more than double (3400 mg/day) the recommended daily intake (1500 mg/day) provided by Health Canada (2017). In order to help consumers reduce their intake, Health Canada has impending restrictions on the amount of sodium allowed in food products, such as baked goods, dairy and seafood products, and canned goods (Health Canada, 2018). The removal of sodium is problematic for processing and final product quality in the case of bread and other baked goods. For bread, NaCl is one of the main four ingredients (flour, water, yeast, salt), and it is responsible for many important quality

characteristics of bread products including improved dough strength (Belz et al., 2012). During dough mixing, sodium ions screen charges to reduce electrostatic repulsion between gluten proteins (Belz et al., 2012). This leads to greater protein-protein interactions within the dough as glutenin polymers become aligned and crosslinked via intermolecular disulfide bonds, hydrogen bonding and hydrophobic interactions (Beck et al., 2012a; Belz et al., 2012).

According to Health Canada, nutritional targets aim to reduce sodium levels from 469 mg/100 g bread to 330 mg/100 g bread (Health Canada, 2018); however a 30% reduction in sodium can have a significant effect on dough and bread quality. Studies involving low sodium dough formulations have shown poor gluten network development and fermentation control, and poor loaf quality with respect to its flavour, texture and shelf life (Belz et al., 2012; Mondal & Datta, 2008). Furthermore, formulations where sodium is reduced produce significantly stickier dough, which results in processing and handling issues in automated bakeries, as the dough adheres to processing equipment causing costly disruptions in the line and the need for additional cleaning (Dobraszczyk, 1997; van Velzen, 2003).

Several strategies have been explored to reduce sodium levels in bread; however, not all studies have assessed reduction at the levels being recommended by Health Canada. Complete or partial replacement of NaCl with alternative salts, such as potassium (K^+), calcium (Ca^{2+}), or magnesium (Mg^{2+}) chloride have been investigated and have shown that dough has acceptable handling characteristics but significant defects in sensory and flavour characteristics (Beck et al., 2012b; Kaur et al., 2011). Enzymes have also been a significant area of study to improve some flour defects, focusing on modification of different flour components such as proteins, lipids, starches, or arabinoxylans (Caballero et al., 2007; Steffolani et al., 2010). The issues caused by reducing salt are highly linked to dough strength, therefore crosslinking enzymes such as glucose oxidase (GO) and transglutaminase (TG), which can confer additional strength to the protein network, have been studied. Studies have investigated effects on weaker flours and some have been conducted at lower salt levels (1.5% by flour wt.) (Decamps et al., 2012), but generally samples are examined at regular salt levels (2% by flour wt.). Inclusion of these enzymes have shown increases in dough strength (Bonet et al., 2006; Caballero et al., 2007; Steffolani et al., 2010). TG and GO can improve dough strength by crosslinking the gluten network and increasing protein-protein interactions to compensate for weakened protein interactions arising from the lack of charge screening provided by the sodium chloride ions (Belz et al., 2012). TG, an acyl-transfer

enzyme, acts directly on the protein and forms covalent isopeptide linkages between glutamine residues and the ε-amino group of lysine (Keillor et al., 2014). The mechanism of GO is indirect; the enzyme oxidises α-D-glucose into δ-gluconolactone and hydrogen peroxide (H_2O_2), and the H_2O_2 is then able to oxidise free thiol moieties on proteins to form additional disulfide linkages (Rasiah et al., 2005). Therefore, it is hypothesized that these enzymes could be utilized to promote greater protein-protein interactions by additional crosslinking and strengthen the gluten network even at the reduced charge screening which is observed at lesser sodium chloride concentrations, thus improving the final product quality of low salt bread products. The aim of this research was to examine the effects that these crosslinking enzymes have on simple model dough systems with respect to dough handling and stickiness characteristics, and to determine if these enzymes improve these characteristics at reduced NaCl levels.

3.3 Materials and methods

3.3.1 Materials

The two flour cultivars selected for this study were developed by Agriculture and Agri-food Canada: Pembina [12.6% protein and 61.5% Farinograph water absorption (FAB), both based on 14% wet basis] and Harvest [13.0% protein and 64.9% FAB] (Avramenko, 2017), samples of which were kindly provided by the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK, Canada). Protein and FAB levels were determined using the American Association of Cereal Chemists International (AACCI) methods 46-30.01 and 54-21.02, respectively. All grain was grown at the Kernen Crop Research Farm (University of Saskatchewan), and milled into flour at the Grain Innovation Laboratory (University of Saskatchewan) using a Buhler Mill (AACCI method 26-21.02). Cultivars were selected based on a rheological, stickiness and baking examination of dough prepared at two different NaCl levels (1.0 and 2.0% by wt. flour) involving 37 different varieties (Yovchev et al., 2017). The authors reported dough prepared from Pembina had strong handling characteristics and low stickiness at both NaCl levels, whereas dough prepared from Harvest showed weaker handling characteristics and high stickiness at both NaCl levels (Yovchev et al., 2017). Glucose oxidase (GO) (Gluzyme® Mono 10000 BG) and transglutaminase (TG) (Activa® TI) were kindly donated by Novozymes (Novozymes, Denmark) and Ajinomoto (Ajinomoto North America Inc., IA, USA), respectively.

All other chemicals were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and were reagent grade.

3.3.2 Dough preparation

Dough samples were prepared with a 10 g mixograph (TMCO National Mfg., Lincoln, NE, USA), by mixing to peak tolerance. A constant moisture content (determined by a farinograph to get the farinograph absorption value (FAB)) was utilized. A simple dough formulation was used comprised of flour, water, NaCl (either 1.0% or 2.0% by flour wt.), and either GO (0%, 0.001%, or 0.01% by flour wt.) or TG (0%, 0.01%, or 0.5% by flour wt.). Enzyme amounts were based on those used by Steffolani et al. (2010). Our preliminary experiments involving dough rheology suggest higher levels of TG were needed than GO to obtain comparable dough strengths, therefore a concentration range that overlapped was selected. After mixing, the dough was rested in small plastic enclosed containers for 1 h at room temperature (21-23°C) to allow for enzyme activity to occur. After resting, dough was tested. For freeze-dried samples, dough was prepared the same as fresh samples and rested to allow for enzyme activity, and then frozen at -30°C prior to freeze-drying. Freeze-drying was completed by POS Biosciences (Saskatoon, SK, Canada). All dough samples were prepared and tested in triplicate.

3.3.3 Dough rheology

Following the method of Jekle and Becker (2011), the rheological properties of the dough samples were measured in two parts; first with an oscillatory frequency sweep, then followed by a creep recovery test using an AR-1000 rheometer equipped with a 40 mm parallel plate fixture, 2 mm gap, and temperature of 25°C (TA Instruments, New Castle, USA). A dough sample (~5 g) was placed under the plate and after lowering the fixture to gap width, excess dough was trimmed off and the exterior of the dough was coated with paraffin oil using a pipette after the plate at been lowered to gap height to ensure that it would remain moist for the duration of the experiment. The oscillatory frequency sweep occurred within the linear viscoelastic regime but creep recovery did not. A 10 min equilibrium period was followed by the oscillatory frequency sweep (ranging from 0.1 to 100.0 Hz, with a constant strain of 0.1%). This was followed by a second 10 min equilibrium period prior to the creep recovery test. The creep recovery step consisted of a constant shear (τ_0 = 250 Pa) for 180 s, followed by the removal of that shear (τ_0 = 0 Pa) to allow the dough to recover

for 360 s. At 1.0 Hz, the complex modulus (|G|*) and the loss tangent (tan δ) was reported from the oscillatory frequency step for comparative purposes. J defined as creep compliance, and relative elasticity of the dough (J_{el}), which is a measurement of the elasticity of the material and indicates stored mechanical energy of the dough (taken at t = 360 s), and maximum dough deformation (J_{max}), which indicates the deformation observed (taken at t = 180 s) were recorded from the creep recovery test (Jekle & Becker, 2011).

3.3.4 Dough stickiness

The assessment of dough stickiness was based on the method, cell, and adhesion fixture of Chen and Hoseney (1995) with a TA.XT2 texture analyser (Texture Technologies Corp., South Hamilton, MA, USA). After preparation on the mixograph and after resting, the dough was placed into the Chen and Hoseney cell, and the dough poking through the mesh was scraped away to increase consistency of testing. The dough was then extruded to a height of ~1 mm, and allowed to rest for 30 s while covered. After this rest period, the probe was placed just above the surface of the dough, and the force (N) which was needed to separate the probe from the dough surface was considered as the stickiness value.

3.3.5 Glutenin macropolymer (GMP)

The extraction and quantification of glutenin macropolymer (GMP) was based on the method of Skerritt et al. (1999), as described in the work of Steffolani et al. (2010). The freeze-dried dough was suspended in 1.5 mL of 1.5% SDS (w/v) and stirred for 1 h prior to centrifugation (30 min at 4430 x *g*) using a VWR clinical 200 centrifuge (VWR International, Radnor, PA, USA), all occurring at room temperature (21-23°C). After centrifugation, the supernatant was poured off, and the remaining solids were analysed by micro-Kjeldahl (Labconco, Kansas City, MO, USA, modified AOAC 960.52). The protein factor for wheat (N factor of 5.7) was utilized, and the values were presented as %GMP.

3.3.6 Free sulfhydryl content

The free sulfhydryl content of freeze-dried dough samples was assessed by a combination of methods (Bak et al., 1996; Hanft & Koeler, 2006), as described in the work of Steffolani et al. (2010). A mixture of Ellman's reagent (50 μL) and freeze-dried dough (50 mg) were mixed for

25 min (room temperature, 21-23°C) in 1.5 mL of buffer (3 mM EDTA, 8 M Urea, 1.0% SDS, 0.2 M Tris-HCl, NaOH used to adjust to pH 8). After mixing, the samples were centrifuged for 10 min (3000 x *g*) using an Eppendorf 5424 centrifuge (Eppendorf, Hamburg, Germany), and the absorbance values of the supernatant were recorded at 412 nm using a Genesys 10 ultraviolet-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The path length of the cuvettes was 1 cm, and the extinction coefficient used was 13600 $M^{-1}cm^{-1}$, which is the extinction coefficient of the 2-nitro-5-thiobenzoate anion (Bak et al., 1996).

3.3.7 Statistics

Statistics were reported averages of triplicates ± one standard deviation (SD). A three-way analysis of variance (ANOVA) was performed to test the main effects of cultivar (flour), NaCl concentration and enzyme inclusion, along with their associated interactions to determine significant statistical differences among the rheological, stickiness, and crosslinking data at the 0.01% (by flour wt.) level of enzyme. A different ANOVA analysis was performed for doughs prepared with glucose oxidase and transglutaminase because the concentration of the enzyme used was different for the rheology and stickiness data. R software was utilized to complete the statistical analysis (R Foundation for Statistical Computing, Vienna, Austria).

3.4 Results

3.4.1 Crosslinking with glucose oxidase

Rheological data for doughs prepared using Pembina or Harvest flours as a function of NaCl and glucose oxidase (GO) concentration is presented in Figure 3.1. The oscillatory shear data was generally found to be less sensitive to changes in dough formulations than creep and stress recovery data, as evident by fewer significant main effects and associated interaction terms within the analysis of variance (Table 3.1). Overall, doughs appeared stronger when prepared with Pembina (|G*|=18.6kPa) compared to Harvest (|G*|=13.8kPa), regardless of the NaCl or GO level (p<0.001) (Figure 3.1A). Doughs also became stronger with increased GO concentration (regardless of the flour type and NaCl level); |G*| increased from 10.9kPa (control) to 18.0kPa (0.001% GO) to 19.7kPa (0.01% GO) (p<0.001) (Figure 3.1A). A significant 2-way interaction term involving flour-type and enzyme level within the tan δ data (p<0.001) indicated that a different trend occurred for each flour (Figure 3.1B). For both flours, tan δ decreased from 0.30

(control) to 0.28 with the addition of GO (regardless of the flour-type and NaCl level), with very little changes in tan δ occurring between the two GO levels (Figure 3.1B). However, in the case of Pembina, tan δ was more stable with NaCl content, whereas for Harvest it was always slightly lower at the 2.0% NaCl level for the control and the 0.001% GO level (Figure 3.1B). At the 0.01% GO level, doughs prepared with Harvest had tan δ values which were independent of NaCl level (Figure 3.1B).

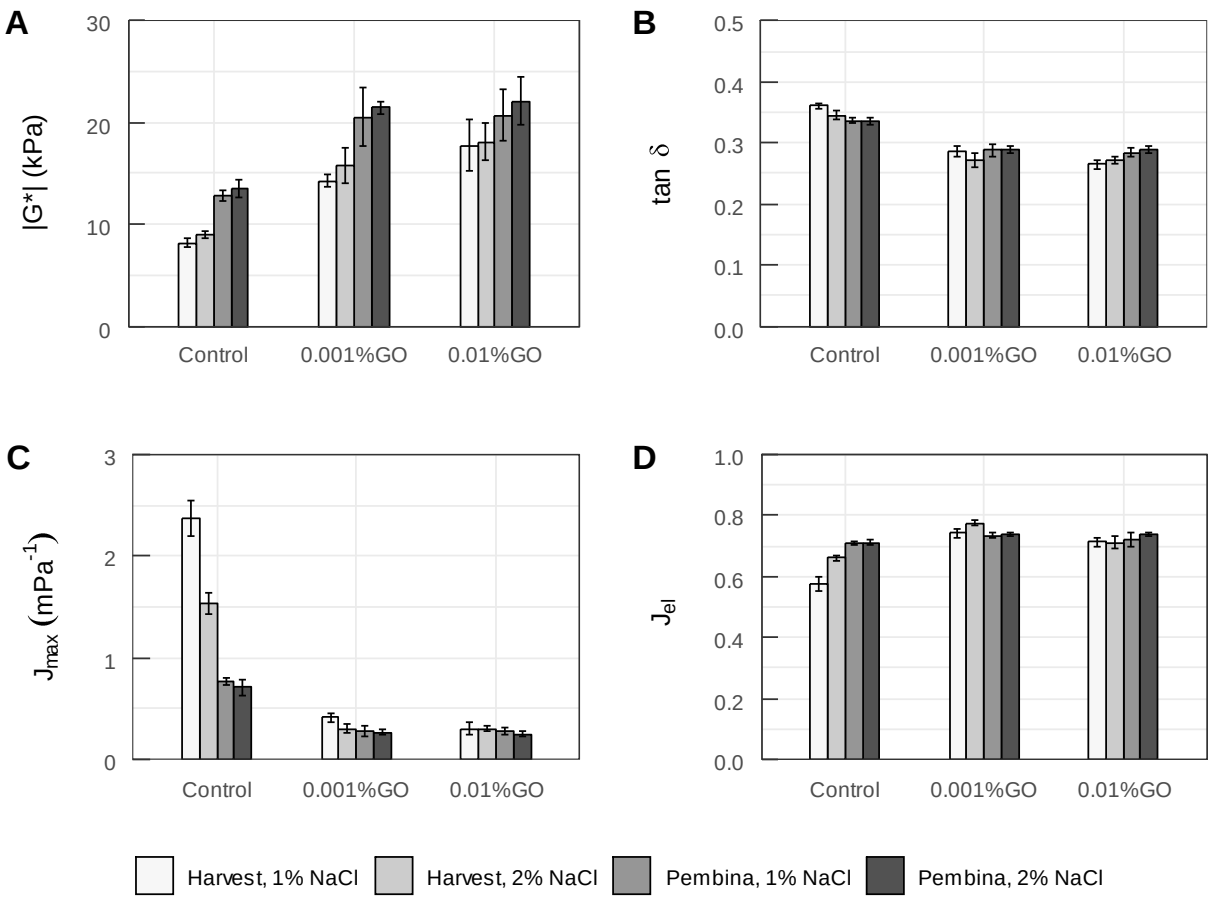

Figure 3.1 Complex modulus, |G*| (A) loss tangent, tan δ (B), maximum deformation, J_{max} (C), and relative elasticity, J_{el} (D), of doughs prepared with Harvest and Pembina flours containing either no enzyme (control) or GO at different concentrations (0.001 or 0.01% by flour wt.), and either 1.0 or 2.0% NaCl (by flour wt.). Values are the mean ± 1 standard deviation (n=3).

Table 3.1 *p*-values of dough samples prepared with no enzyme, GO, or TG, at either 1.0 or 2.0% NaCl, produced with either Harvest or Pembina flour for rheology and stickiness.

Effect/ Interaction	*p*-values									
	GO					TG				
	|G*|[1]	**tan δ[2]**	**J_{max}[3]**	**J_{el}[4]**	**Stickiness**	**|G*|**	**tan δ**	**J_{max}**	**J_{el}**	**Stickiness**
S[5]	NS[8]	NS	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.01
C[6]	<0.001	NS	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
E[7]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S:C	NS	<0.05	<0.001	<0.01	<0.05	NS	NS	<0.01	<0.05	<0.05
S:E	NS	NS	<0.001	<0.05	NS	NS	NS	<0.01	NS	NS
C:E	NS	<0.001	<0.001	<0.001	NS	NS	<0.001	<0.001	<0.001	NS
S:C:E	NS	NS	<0.001	<0.001	NS	NS	NS	<0.01	<0.01	NS

[1]Complex modulus

[2]Loss tangent

[3]Maximum deformation

[4]Relative elasticity

[5]Salt

[6]Cultivar

[7]Enzyme

[8]Not significant

An analysis of variance examining flour-type, NaCl level and enzyme concentration found all these main effects along with all associated 2- and 3-way interactions were significant for both creep and relaxation parameters (Table 3.1). In the absence of added GO, Harvest experienced the greatest change in response to NaCl and the greatest amount of dough deformation relative to doughs prepared with Pembina, suggesting it was a much weaker system. J_{max} decreased from 1.03 to 0.71mPa^{-1} and J_{el} increased from 0.68 to 0.72 as NaCl levels increased from 1.0% to 2.0%, respectively, in the case of doughs prepared with Harvest (Figure 3.1C,D). In contrast, doughs prepared with Pembina were less sensitive to the NaCl level; J_{max} decreased from 0.44 to 0.41mPa^{-1} and J_{el} increased from 0.72 to 0.73 as NaCl levels increased from 1.0% to 2.0%, respectively (Figure 3.1C,D). Overall, the addition of GO led to the strengthening of all doughs, as evident by lower J_{max} and J_{el} values relative to the control. Few differences were observed between cultivars, NaCl levels and enzyme concentration when GO was added within the creep relaxation data (Figure 3.1C,D), with the exception of the weakest dough system (Harvest flour, 1.0% NaCl, no GO) which showed reduced dough strength (Figure 3.1C). In summary, oscillatory shear data and creep relaxation data indicated that doughs prepared with Pembina flour were overall stronger than those from Harvest, and that the addition of GO acted to strengthen the dough. Differences between the two GO levels were minimal in terms of GO effects on dough strength for both cultivars. Overall, the effect of NaCl level was primarily observed in the absence of GO with doughs prepared with Harvest flours, whereas its effect on Pembina was minimal.

Dough stickiness was also evaluated on the same dough systems (Figure 3.2). An analysis of variance found that cultivar, NaCl concentration and GO level, along with the interaction between flour-type and NaCl concentration were significant (Table 3.1). Overall, dough stickiness decreased from 0.47N (control) to 0.34N (0.001% GO) and then to 0.31N (0.01% GO), regardless of the cultivar and NaCl concentration (Figure 3.2). Overall, stickiness was decreased from 0.45 to 0.4 N (regardless of the GO level) in doughs prepared with Harvest cultivar at the 1.0 and 2.0% NaCl, respectively. In contrast, doughs prepared with Pembina flour were less sensitive to NaCl level where stickiness values decreased from 0.33 to 0.31N (p<0.05) in doughs prepared with 1.0 and 2.0% NaCl concentration, respectively (regardless of the GO level) (Figure 3.2). Overall, dough that displayed greater rheological strength showed less stickiness, with a negative correlation of -0.92 between |G*| and stickiness as determined by a correlation test (Table 3.2).

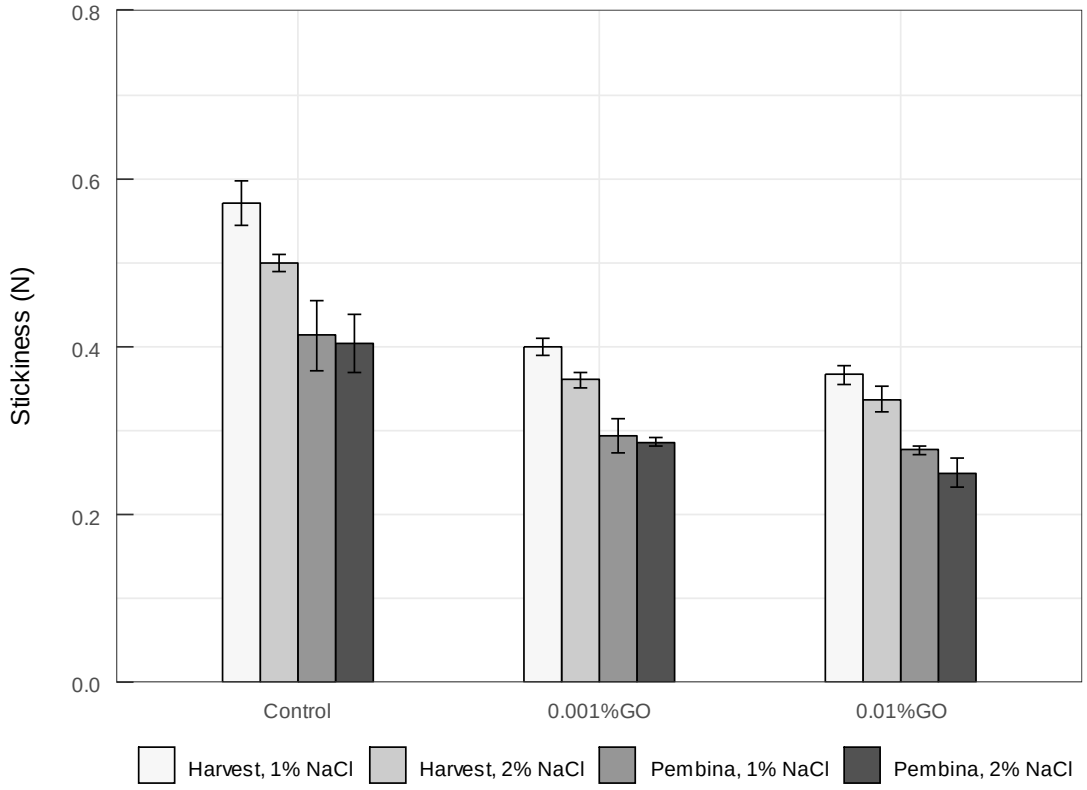

Figure 3.2 Stickiness of doughs prepared with Harvest and Pembina flours containing either no enzyme (control) or GO at different concentrations (0.001 or 0.01% by flour wt.), and either 1.0 or 2.0% NaCl (by flour wt.). Values provided are the mean ± 1 standard deviation (n=3).

Table 3.2 Pearson correlation for doughs prepared with no enzyme (control) or with GO (0.001, or 0.01% by flour wt.), either 1.0 or 2.0% NaCl, and either Harvest or Pembina flour.

	|G*|[1]	**tan δ**[2]	**J_{max}**[3]	**J_{el}**[4]	**Stickiness**
|G*|	1.00				
tan δ	-0.77***	1.00			
J_{max}	-0.82***	0.85***	1.00		
J_{el}	0.63***	-0.73***	-0.91***	1.00	
Stickiness	-0.92***	0.74***	0.88***	-0.76***	1.00

[1]Complex modulus, [2]loss tangent, [3]maximum deformation, [4]relative elasticity

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

3.4.2 Crosslinking with transglutaminase

Rheological data for doughs prepared using Pembina or Harvest flours as a function of NaCl and transglutaminase (TG) concentration is presented in Figure 3.3. An analysis of variance for all main effects and associated interaction terms for both oscillatory shear and creep relaxation data is presented in Table 3.1. Overall, doughs prepared with Pembina (|G*|=15.5kPa) were stronger than those prepared with Harvest (|G*|=10.9kPa) (regardless of the NaCl level and TG concentration); doughs prepared at the 2.0% NaCl level were stronger than those prepared at the 1.0% NaCl level; |G*|=13.7kPa; tan δ=0.31, |G*|=12.7kPa; tan δ=0.32 respectively (regardless of cultivar and TG concentration); and doughs prepared with increasing TG concentration from 0% TG (|G*|=10.9kPa; tan δ= .34) to 0.01% TG (|G*|=10.5kPa; tan δ=0.35) to 0.05% TG (|G*|=18.1kPa; tan δ=0.26) showed stronger behaviour (regardless of the flour-type and NaCl level) (Figure 3.3A,B). Greater NaCl dependence was evident in the data relative to that of GO, since the 0.01% TG level mostly likely did not have a high enough enzyme concentration to alter the behavior relative to the control.

Similar to creep relaxation data involving GO, doughs with TG showed all main effects and most of the associated interactions to be significant for J_{max} and J_{el} data (Table 3.1). For both parameters, the control and the 0.01% TG level were similar in magnitude for each dough system and showed NaCl dependence. For instance, J_{max} decreased from 2.10 to 1.48mPa^{-1} as the NaCl level increased from 1.0 to 2.0% NaCl respectively for Harvest, and from 0.87 to 0.71mPa^{-1} for Pembina (Figure 3.3C). Similarly, J_{el} increased from 0.61 to 0.67mPa^{-1} as the NaCl level increased from 1.0 to 2.0% NaCl respectively for Harvest, and from 0.70 to 0.72mPa^{-1} for Pembina (Figure 3.3D). However, as the concentration of TG was raised to 0.05% TG, no differences between flour-type and NaCl level were observed. Relative to the control/0.01% TG dough systems, J_{max} was reduced to 0.19 mPa^{-1} and J_{el} was increased to 0.81 (Figure 3.3C,D).

Dough stickiness was also evaluated on similar dough systems (Figure 3.4), with similar parameters identified as being significant as with GO (Table 3.1). Overall, dough stickiness was similar for the control and the 0.01% TG level (0.47N), then declined at the 0.05% TG level (0.35N) regardless of the cultivar and NaCl concentration (Figure 3.4). Overall, stickiness increased from 0.46 to 0.50N (regardless of the TG level) in doughs prepared with Harvest flour at the 1.0 and 2.0% NaCl, respectively. In contrast, doughs prepared with Pembina were less sensitive to the NaCl level where stickiness values increased from 0.37 to 0.38N in doughs

prepared with 1.0 and 2.0% NaCl concentration, respectively (regardless of the TG level) (Figure 3.4). Similar negative correlations were observed between |G*| and stickiness (-0.89) as with the GO data (Table 3.3).

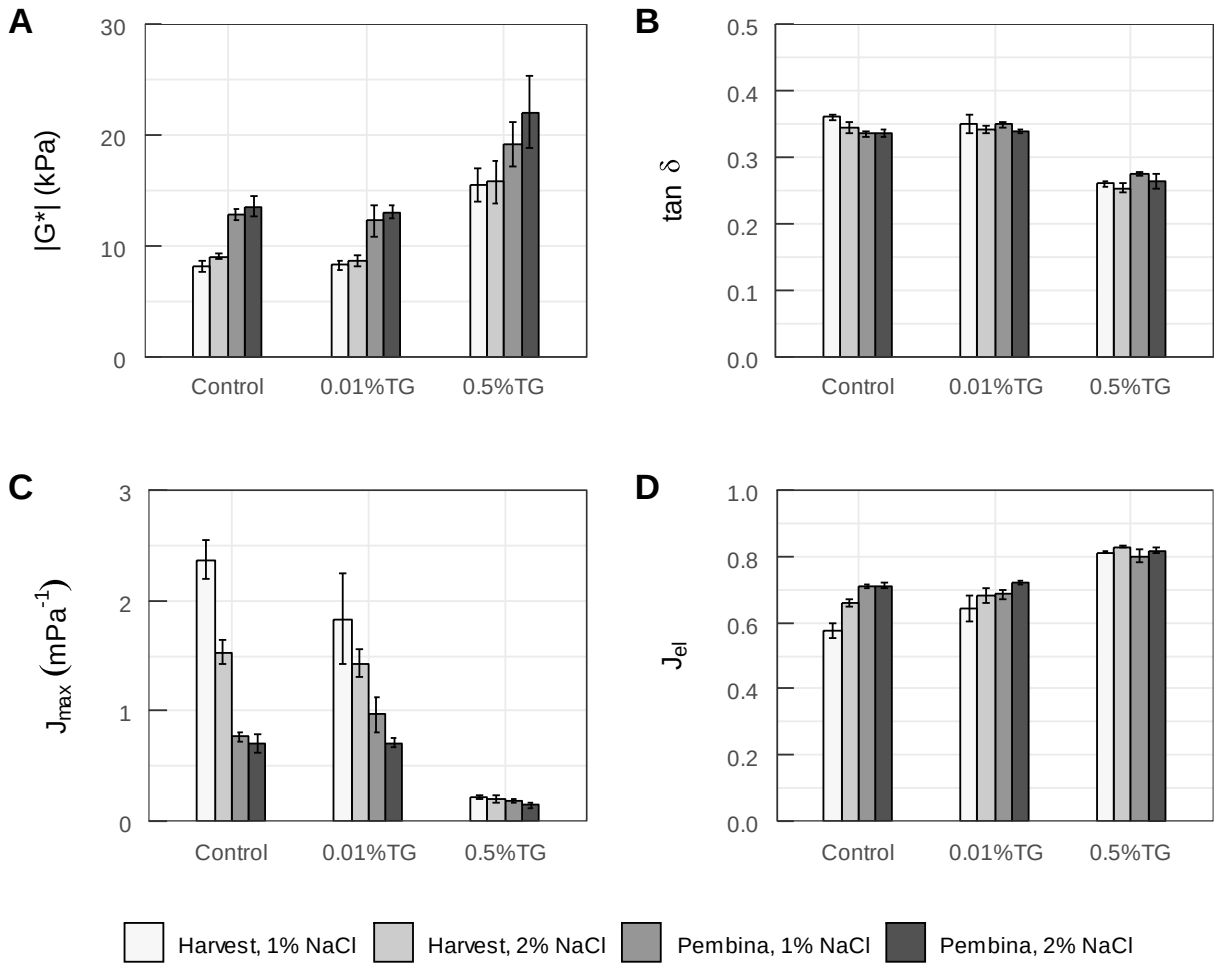

Figure 3.3 Complex modulus, |G*| (A) loss tangent, tan δ (B), maximum deformation, J_{max} (C), and relative elasticity, J_{el} (D), of doughs prepared with Harvest and Pembina flours containing either no enzyme (control) or TG at different concentrations (0.01 or 0.5% by flour wt.), and either 1.0 or 2.0% NaCl (by flour wt.). Values provided are the mean ± 1 standard deviation (n=3).

Table 3.3 Pearson correlation for doughs prepared with TG (0%, 0.01%, or 0.05% by flour wt.), either 1.0 or 2.0% NaCl, and either Harvest or Pembina Flour.

	|G*|[1]	**tan δ**[2]	**J_{max}**[3]	**J_{el}**[4]	**Stickiness**
|G*|	1.00				
tan δ	-0.81***	1.00			
J_{max}	-0.85***	0.82***	1.00		
J_{el}	0.82***	-0.93***	-0.95***	1.00	
Stickiness	-0.89***	0.72***	0.91***	-0.85***	1.00

[1]Complex modulus, [2]loss tangent, [3]maximum deformation, [4]relative elasticity

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

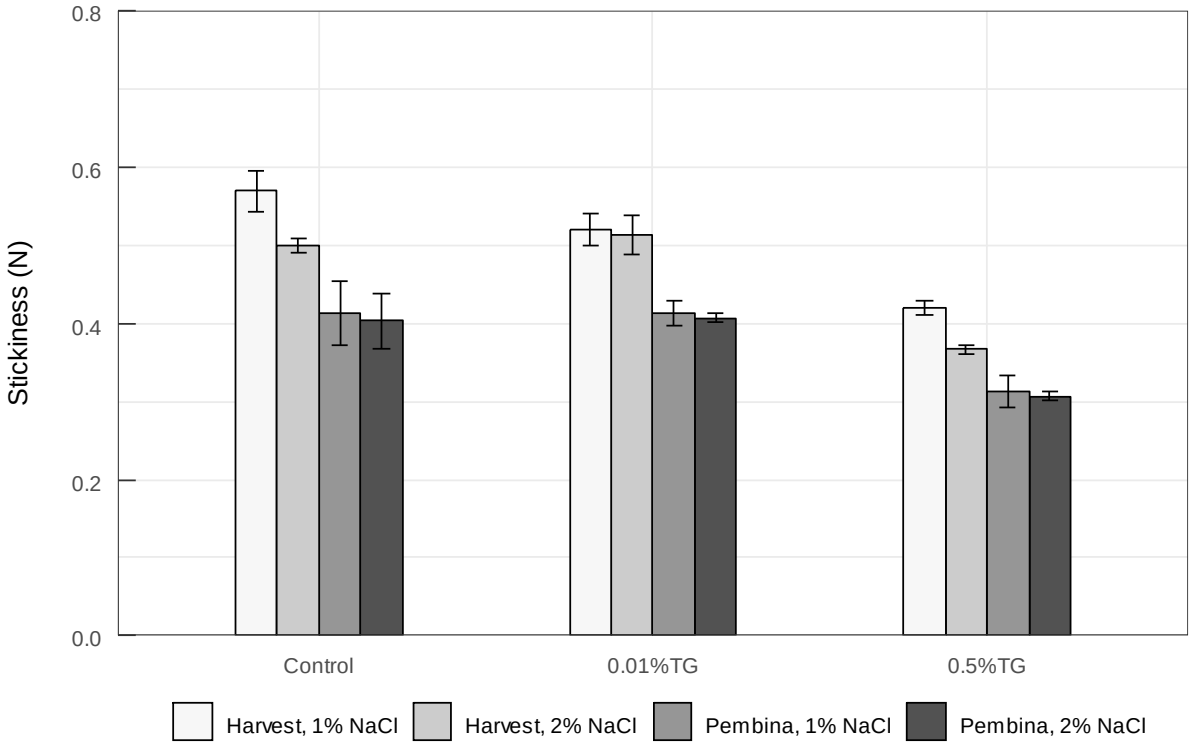

Figure 3.4 Stickiness of doughs prepared with Harvest and Pembina flours containing either no enzyme (control) or TG at different concentrations (0.01 or 0.5% by flour wt.), and either 1.0 or 2.0% NaCl (by flour wt.). Values provided are the mean ± 1 standard deviation (n=3).

3.4.3 Extent of crosslinking at the 0.01% enzyme level

Overall, GO was more effective at crosslinking the gluten proteins than TG at equivalent mass concentrations, regardless of the flour-type and NaCl level. The glutenin macropolymer (GMP) is composed of high molecular weight glutenin subunits (HMW-GS) covalently bonded via disulfide linkages to low molecular weight (LMW)-GS (Dai et al., 2013). While LMW-GS are important for the development of GMP, HMW-GS is generally thought to be very critical in the formation of the gluten network structure (Dai et al., 2013). Don et al. (2006) have shown that quantity of GMP increases with the increase in HMW-GS content. As such, it can also serve as an indirect measure of crosslinking, especially with the addition of enzymes which would promote the formation of larger polymers. In the current study, GMP was found to increase for doughs prepared with Harvest from 3.41 to 6.31% as the GO level increases from 0 to 0.01%, respectively, and with Pembina from 5.57 to 5.60%, respectively (p<0.001) indicating a greater extent of glutenin crosslinking (Figure 3.5A). The NaCl level had no effect (p<0.05) on the %GMP. In contrast, at the 0.01% TG level no difference in %GMP was evident between that of the controls for both cultivars (p>0.05).

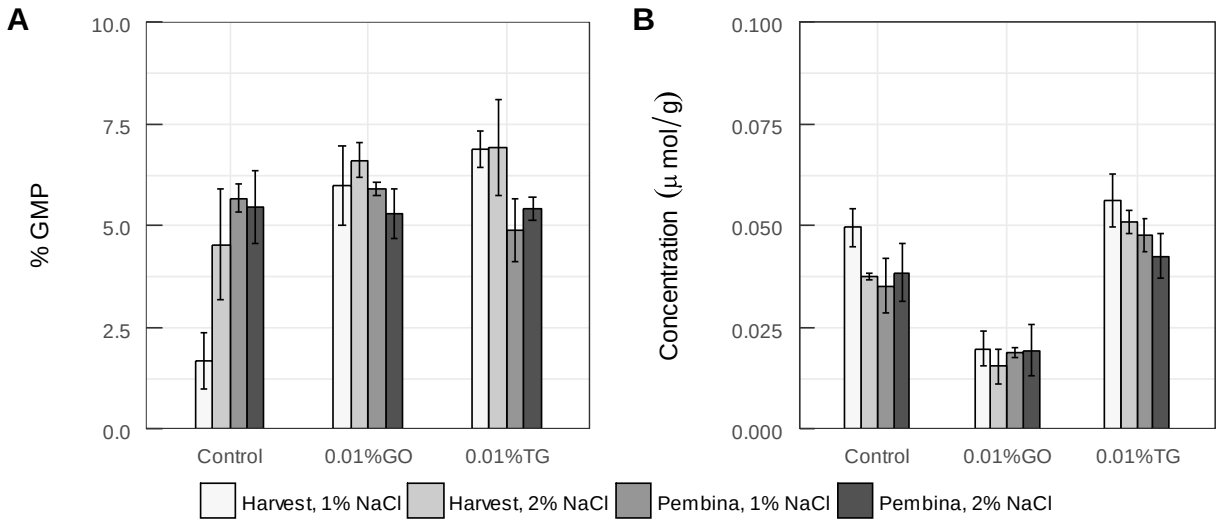

Figure 3.5 %GMP (A) and concentration of free sulfhydryl groups (B) of doughs prepared with Harvest and Pembina flours containing either no enzyme (control), 0.01% GO, or 0.01% TG, and either 1.0 or 2.0% NaCl (by flour wt.). Values provided are the mean ± 1 standard deviation (n=3).

GO crosslinks gluten proteins indirectly through the production of H_2O_2 from the oxidation of glucose, which then oxidizes free thiols to form disulfide linkages between the proteins (Rasiah et al., 2005). Therefore measuring changes in free sulfhydryl concentration within the dough can provide direct evidence of crosslinking for GO. In the case of Harvest, the free sulfhydryl concentration was found to be ~0.050 µmol/g at the 1.0% NaCl level, and ~0.038µmol/g at the 2.0% NaCl level in the absence of added enzyme. With the addition of 0.01% GO, levels of free sulfhydryl groups decreased to ~0.018µmol/g (regardless of the NaCl level) (Figure 3.5B). Whereas for Pembina, amount of free sulfhydryl groups were similar regardless of the NaCl content, and were found to decrease from ~0.037 to ~0.019µmol/g with the addition of 0.01% GO. Although the free sulfhydryl assay does not provide direct evidence of TG crosslinking, it could be hypothesized that crosslinking via an acyl-transfer reaction may induce rearrangement of the gluten proteins causing sites to be more or less available for the reaction. However, no statistical difference was observed between the controls for both Harvest and Pembina and those with added TG.

3.5 Discussion

Overall, both TG and GO were effective at dough strengthening and at reducing stickiness, especially in samples prepared with the Harvest flour, which had been shown previously to display much weaker gluten/dough strengths than Pembina. However, TG required ~5x the concentration of GO to achieve the same dough handling characteristics, which is most likely reflective of their differing modes of action. As previously mentioned, GO acts by facilitating the oxidation of free thiol groups on the proteins to form disulfide linkages (Rasiah et al., 2005), whereas TG crosslinks gluten proteins via an acyl-transfer reaction, forming an isopeptide bond (a bond between amino acid moieties that are not within the main primary protein chain) between glutamine residues and the amino group from the side chain of lysine (Zhang et al., 2009). In general, wheat proteins tend to be high and low in glutamine and lysine contents, respectively (Woychik et al., 1961), which may be one of the reasons why TG showed poorer performance in the dough systems. TG can also catalyse the reaction of glutamine with other nucleophiles which may not actually produce a crosslinking reaction (converting glutamine into glutamate if it reacts with water, for example) (Zhang et al., 2009), and this could possibly help to explain why GO was more effective at developing a stronger dough.

Other evidence of more extensive crosslinking was the greater reduction in free sulfhydryl groups and significant increases in %GMP with GO, whereas TG was similar to the control at the 0.01% enzyme level. The higher %GMP levels in doughs prepared from Pembina flour than those from Harvest flour suggests a higher amount of HMW-GS subunits, which could be one of the reasons why Pembina is less NaCl and enzyme sensitive than Harvest. These results agree with Steffolani et al. (2010) who found that both GO and TG increased the GMP content of dough samples, however, at higher concentrations (0.5% TG by flour wt.) the GMP contents were significantly reduced, due to what the authors speculated were alterations of protein solubility. In contrast, Primo-Martín et al. (2003) found a slightly reduced GMP quantity with the inclusion of GO (0.002g/100g), which the authors suggested may be the result of protein-pentosan crosslinking by GO, which could interfere with the aggregation of gluten proteins. Contradictory GMP findings in the literature as it relates to enzyme type and levels and dough strength may arise because of differences in flour-types used, which can have a significant impact on dough handling; and, due to specific enzymatic interactions within protein and non-protein constituents, such as arabinoxylans within the systems.

The strengthening effects observed from the enzyme inclusion are generally reported in the literature. However, there is little research conducted with enzymes at reduced NaCl levels. Caballero et al. (2007) did not find significant increases in the |G*| of doughs prepared with GO (0.05% GO, 2% NaCl by flour wt.). However, several other groups (Bonet et al., 2006; Decamps et al., 2012; Steffolani et al., 2010) showed that the inclusion of GO (up to 0.015%, 2% NaCl by flour wt.) led to an increase in the resistance to extension and dough mixing stability. Bonet et al. (2006) and Steffolani et al. (2010) reported that improvements to dough strength were only observed at levels >0.01% GO. In contrast to our findings and others, Caballero et al. (2007) determined TG to have a greater strengthening effect than GO; however, they utilized different enzyme concentrations than in the present study (0.003% GO/flour wt. and 0.5% TG/flour wt.).

A reduction in stickiness was observed in the case of both enzymes in the present study. Other authors have also observed reduced stickiness with the addition of enzymes such as GO and TG. Several authors observed reduced dough stickiness with the inclusion of GO (Collar et al., 1998; Steffolani et al., 2010), as well as with TG (Tseng & Lai, 2002). Tseng and Lai (2002) observed this with TG concentrations of 0.02% or 0.04% by flour wt. Collar et al. (1998) observed stickiness reductions at 0.002% GO inclusion within a sourdough system. Stickiness is the result

of greater adhesive forces at the surface of the dough relative to cohesive forces arising from protein-protein interactions within the dough. Dobraszczyk (1997) suggested that the cause of stickiness was highly related to dough rheology above other parameters. However, while several factors have been associated with dough stickiness, such as protein composition, water-unextractable arabinoxylan content, salt levels, enzyme activity and others (Beck et al., 2012a; Chen & Hoseney, 1995; van Velzen et al., 2003), a full understanding of the driving mechanism is still unknown. The reduction of stickiness within our dough systems with enzyme and higher NaCl levels is believed to be associated with a greater amount of gluten protein-protein interactions as the result of crosslinking in the case of enzymes, and as the result of charge screening by the NaCl ions of groups along the glutenin proteins to promote greater protein-protein aggregation via increased hydrophobic interaction in the case of higher NaCl levels (Belz et al., 2012).

The overall goal of this study was to examine the impact of enzyme type and concentration on the handling properties and stickiness of dough under normal and low NaCl conditions using rheological techniques with a simple dough model (i.e., no yeast), and as such the study did not include a baking trial. However, Hanft and Koehler (2006) examined the use of GO in breadmaking and found that addition at levels of up to 0.001% GO (100 U kg^{-1} enzyme) increased loaf volume in 10 g mini-loaves prepared at 2% NaCl, however, additional levels of enzyme dramatically decreased the loaf volume, suggesting that over-strengthening may be an issue if too much enzyme is added. These results were supported by Dagdelen and Gocmen (2007), where enzymes at levels of between 0.0002 and 0.0006% GO (2mg kg^{-1}–6mg kg^{-1}, 10000GODU/g) showed significant improvements in loaf volume at slightly reduced salt concentrations (1.5% NaCl by flour wt.). Caballero et al. (2007) reported that if too much enzyme (e.g., TG, added at 0.5% by weight) was added there was something of an "over-strengthening" effect which impaired the sensory properties of the final bread, and increased the overall chewiness and hardness, but that GO (added at 0.003% by weight) did not significantly alter dough characteristics, but improved bread volume and crumb quality.

3.6 Conclusions

This study examined the characteristics of simple model doughs prepared at normal and low NaCl levels with the aid of GO and TG to mitigate negative effects from the reduced salt content as it relates to dough strength and stickiness. Developing high quality, reduced sodium

bread is important for several regions of the world, such as Canada, where impending sodium regulations are restricting the amount allowed for use in bread and other food products. This work suggests the potential of GO and TG, particularly GO, at reducing the stickiness and improving dough strength under low sodium conditions, or for weaker flour cultivars which could improve their usefulness in commercial bread production. GO appears as a more promising option, particularly at lower concentrations, as it showed significant improvements in rheological behaviour and reduction of stickiness. Further investigation into understanding the causes and mechanisms of stickiness, as well as how these enzymes function in more fully formulated doughs and interact with other specific non-protein components of the dough is essential to determining the practicality of this formulation moving forward.

3.7 Linkage between enzyme studies on dough handling at a low salt level and a more complex model dough containing yeast produced organic acids

The dough model utilized for this study was very simplistic; it only contained three out of the four essential bread ingredients (flour, water and salt) and did not contain yeast due to the complexity which yeast brings, particularly with regards to dough rheology. However, the removal of yeast from the dough model means that the current simplistic model is less useful with regards to bridging the research from a simple system to a full bread dough system. Therefore, it was desired to increase the complexity of the model to be more similar to that of a full system still without the inclusion of yeast. As such, yeast-produced organic acids were selected due to their potential interference with the strengthening enzymes, and previously reported effects on doughs; alterations in pH can affect enzyme activity and efficiency, and some organic acids have reported negative effects on dough network development and strength. This increase in complexity takes steps towards a full dough system, while maintaining simplicity which can hopefully help in determining specific factors which affect the handling and stickiness properties of the doughs. The results of the enzymatic work clearly show that GO had superior effects on dough handling characteristics when compared to TG, and that there was little difference between the GO concentrations chosen, so only GO at the lower concentration was selected moving forward in the studies. Similarly, the relationship between enzymes, handling, and salt concentrations between the flour cultivars were well established in this study so only low salt concentrations were assessed moving forward.

4. EFFECTS OF GLUCOSE OXIDASE AND ORGANIC ACIDS ON THE PROPERTIES OF MODEL LOW-SODIUM DOUGH PREPARED FROM HARVEST AND PEMBINA CWRS WHEAT

4.1 Abstract

This research investigates the impact of glucose oxidase (GO) addition in the presence of organic acids (acetic, ascorbic, citric, fumaric, lactic, or succinic) in relation to a reduced salt dough system (1.0% NaCl). Parameters measured included dough rheology, stickiness, freezable water content (FWC), and percentage of glutenin macropolymers (%GMP). Two cultivars were selected: Harvest and Pembina which are known for their weak and strong dough characteristics, respectively. The inclusion of most of the acids at 1.2mmol/100g flour increased stickiness and reduced dough strength but had no effects on %GMP and little increase in FWC. The trends for ascorbic acid were dissimilar to other acids for rheology and stickiness, however, no synergistic effects were observed between it and GO. The inclusion of GO (0.001%/flour wt.) was able to mitigate some of the effects on rheology and stickiness, but GO had no effect on the freezable water content and %GMP. The mechanism of the interactions of these acids within the dough remains to be elucidated and GO appears to have potential as a low sodium bread improver, but it requires testing in complete dough systems and final bread products.

4.2 Introduction

The reduction of sodium content in foods has been one of the driving trends in the food industry over the last decade, as consumers become more aware of their health and deal with rising healthcare costs, as well as legislative reasons in some countries, such as Canada. High dietary sodium intake from processed foods has been linked to cases of hypertension, which is linked to cardiovascular disease and stroke (O'Donnell et al., 2015). Because of this, some governmental agencies are in the process of introducing sodium level restrictions in a wide range of food products, including bread (Health Canada, 2018). The reduction of sodium chloride (NaCl) in

bread however poses several processing and quality challenges, as low-sodium dough systems tend to have poorer dough development, flavour, preservation, texture, as well as poor fermentation control (Mondal & Datta, 2008; Samapundo et al., 2010; Belz et al., 2012). Various salt reduction strategies have been examined, such as the use of salt replacers or the partial replacement of NaCl with potassium chloride, however these result in defects in the final product (Kaur et al., 2011), and none are useful at the 330 mg sodium per 100 g concentration in bread proposed by Health Canada (2018). The present study examines the use of GO as a means of strengthening dough systems "weakened" by a low NaCl environment.

GO has been utilized as a bread improver previously to strengthen flours which had been deemed weak (Bonet et al., 2006; Caballero et al., 2007; Steffolani et al., 2008; Steffolani et al., 2010). GO is an oxidising compound which acts indirectly on strengthening the gluten network through the oxidisation of glucose (to δ-gluconolactone) to produce hydrogen peroxide (H_2O_2), that then crosslinks thiol groups within the gluten network (Rasiah et al., 2005). The inclusion of GO to improve bread strength and texture at a regular salt level (2%) has been well documented in the literature, where it has shown improved gluten network strength, reduced stickiness, increased stability to overmixing (Bonet et al., 2006), and improved rheological properties and final crumb structure (Caballero et al., 2007). However, some evidence of over-strengthening effects have been observed with the addition of GO (Hanft & Koehler, 2006). Ascorbic acid (in the form of dehydroascorbic acid) is also used as a bread improver, and acts as an oxidising agent to generate crosslinks between thiol and glutathione groups (Every et al., 1999; Grosch & Wieser, 1999). The use of GO and ascorbic acid in bread is widely approved for food use around the world for improving bread quality while using weaker wheat flour. However, their use within a low NaCl environment as a means to mitigate the effects of salt reduction on dough handling, alone or in tandem has largely been unexplored.

The overall goal of this research was to examine the effects of GO addition in the presence and absence of acetic, ascorbic, citric, fumaric, lactic, or succinic acids on dough handling within a low NaCl environment. During bread making, several types of organic acids are produced by yeast during the fermentation step. Of the acids being investigated, Succinic acid is the most produced acid during dough fermentation at levels up to 1.6 mmol/100 g flour (Jayaram et al., 2014), although other acids are produced, albeit in smaller amounts (Jayaram et al., 2013). Several of these acids have been shown to have effects on the structure of dough, and some have been

linked to rapid breakdown of dough structure, such as fumaric acid (Sidhu et al., 1980). The inclusion of organic acids in the present system was used to mimic some aspects of the real dough system, without the complexities of yeast.

4.3 Materials and methods

4.3.1 Materials

Grain samples of two wheat cultivars were obtained for this work from the Crop Development Centre at the University of Saskatchewan; Pembina, and Harvest both of which were developed by Agriculture and Agri-food Canada. The Pembina flour contained 12.6% protein and had a farinograph water absorption (FAB) value of 61.5% while Harvest was determined to contain 13.0% protein and have a FAB value of 64.9% (all based on 14% wb) (Avramenko, 2017). These values were determined by the American Association of Cereal Chemists International (AACCI) methods 46-30.01 (protein), and 54-21.02 (FAB). The wheat was milled at the University of Saskatchewan Grain Innovation Laboratory with a Buhler mill using AACCI method 26-21.02. Cultivar selection was based on a previous study (Yovchev et al., 2017) which examined rheological and stickiness behaviour of 37 cultivars at 1.0 and 2.0% NaCl (by flour wt.). Pembina flour was determined to have low stickiness and good dough handling at reduced salt levels, while Harvest showed poor dough strength and high stickiness at 1.0% NaCl (Yovchev et al., 2017), thus these two cultivars were selected for this study due to their opposing characteristics. The glucose oxidase (Gluzyme® Mono 10000 BG) was generously donated by Novozymes (Novozymes, Denmark). The remainder of the chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) and were reagent grade.

4.3.2 Dough preparation

Dough was prepared using a 10 g mixograph (TMCO National Mfg., Lincoln, NE, USA). The dough was mixed to peak tolerance, with constant moisture content as determined by a farinograph (FAB value). The basic formulation of the model dough system included: flour, NaCl (1.0% by flour wt.), water (by FAB value), and GO (0.001% by flour wt., or 0.0% in a control). In samples which contained organic acids in place of water, the acids (acetic, ascorbic, citric, fumaric, succinic or lactic acid) were each added at 1.2mmol/100g flour. Acid inclusion levels were selected based on some previous acid investigation by this group (Stone et al., 2017) and by levels of that

some acids can be produced by yeast as determined by Jayaram et al. (2014). Acid selection was based on some of the acids which have been found to most commonly produced by yeast during bread production (Jayaram et al., 2013) as well as those which are of interest to industry. After preparation, the dough was placed in enclosed containers at room temperature (21-23°C) for 1 h to allow for the GO reaction to proceed prior to testing. Dough was then tested. For freeze-dried samples, dough was allowed to rest for 1 h, and then frozen at -30°C prior to freeze-drying. All dough samples were produced in triplicate.

4.3.3 Dough pH

Dough pH was assessed by AOAC method (981.12) for the pH of acidified foods, for semi-solid products. pH readings were taken in duplicate, on triplicate dough samples. The pH of the control dough (no added acid) was the highest (6.1 ± 0.1), followed by dough with ascorbic acid (5.9 ± 0.1), lactic acid (5.7 ± 0.1), acetic acid (5.6 ± 0.0), succinic acid (5.6 ± 0.1), fumaric acid (5.1 ± 0.1), and citric acid (4.8 ± 0.1).

4.3.4 Dough rheology

Oscillatory shear rheometry and creep compliance testing was applied for all dough samples using an AR-1000 rheometer (TA Instruments, New Castle, DE, USA) following the method of Jekle and Becker (2011). The rheometer was equipped with a 40 mm parallel plate fixture with a 2mm gap, maintained at a constant temperature of 25°C. A ~5g sample of dough was placed on the fixture, where after setting the gap, excess was removed carefully with a plastic spatula, and paraffin oil was added to ensure the dough did not dry out during the procedure. Prior to testing, the dough was allowed to equilibrate on the instrument for 10 min. For oscillatory shear testing, an upwards frequency sweep ranging between 0.1 – 100Hz at a constant strain amplitude of 0.1% (within the linear viscoelastic regime) was applied. This strain amplitude was derived from a stress-strain sweep to determine where the dough deviated from linearity. For creep compliance testing, a constant shear stress (τ_0 = 250Pa) for 180 s was applied to the dough samples, prior to removing the shear (τ_0 = 0Pa) to observe the recovery for an additional 360 s. The complex modulus (|G*|) and loss modulus (tan δ) were recorded from the oscillatory frequency sweep (1Hz). The dough deformation (J_{max}) and relative elasticity of dough (J_{el}) were determined from the creep recovery data. Oscillatory rheology was completed in the linear viscoelastic regime,

creep recovery was not. Measurements were made on triplicate dough samples, with data being reported as the mean ± one standard deviations (n=3).

4.3.5 Dough stickiness

The stickiness of the dough was assessed using the adhesion fixture, cell and method developed by Chen and Hoseney (1995). A TA.XT2 texture analyser (Texture Technologies Corp., South Hamilton, MA, USA) was utilized for this analysis. After preparation, the dough was placed in the cell, extruded through a mesh screen to a height of 1mm, and allowed to rest (covered) for 30 s. Prior to testing, the first extruded dough was removed by a blade, and subsequent extruded dough was tested to ensure consistency of the dough. After resting, the probe was placed on the surface of the dough, and the force (N) required to separate the probe from the dough surface was recorded. Measurements were made on triplicate dough samples, with data being reported as the mean ± one standard deviations (n=3).

4.3.6 Glutenin macropolymer (GMP)

The extraction and quantification of GMP was performed using the method described in Steffolani et al. (2010) altered from Skerritt et al. (1999). A suspension of freeze-dried dough in 1.5mL of 1.5% SDS (w/v) was prepared at room temperature and mixed on a shaker plate for 1 h prior to centrifugation for 30 min at 4430*g* using a VWR clinical 200 centrifuge (VWR International, Radnor, PA, USA). The supernatant was then removed, and the pellet was analysed by micro-Kjeldahl (Labconco, Kansas City, MO, USA; AOAC 960.52). Total protein content (N factor of 5.7) was assessed, and values presented as %GMP/g dough. Measurements were made on triplicate dough samples, with data being reported as the mean ± one standard deviation (n=3).

4.3.7 Freezable water content via differential scanning calorimetry (DSC)

To determine the freezable water content (FWC) within the dough, a DSC Q2000 equipped with a refrigerated cooling system (TA Instruments, New Castle, DE, USA) was used, based on a method by Lu and Seetharaman (2013). In brief, ~15mg of dough was loaded into aluminum DSC pans which were sealed using a pan crimper press prior to loading onto the instrument. An empty reference pan was also prepared and loaded with each instrument run. Temperatures were cooled and heated at a rate of 10°C/min, with the following temperature conditions: equilibrium at 30°C

(5 min), cooling to -40°C, a second equilibrium at -40°C (5 min), and finished with a final ramp to 40°C. To determine the enthalpy of the melting peak (ΔH), TA Universal Analysis 2000 version 4.5 (TA Instruments, New Castle, DE, USA) was used. FWC was calculated by dividing the sample enthalpy by the enthalpy of pure water. The FWC values were presented on a dry weight (d.w.) basis (FWC/moisture content), using oven moisture samples of the dough. One measurement was made on triplicate dough samples, with data being reported as the mean ± one standard deviations (n=3). Dough moisture was also assessed in triplicate.

4.3.8 Statistics

Statistics are all reported as the mean ± SD using 3 separately prepared doughs. A three-way ANOVA was utilized to determine statistical differences of the main factors. Pearson correlation coefficients to determine linear relationships between variables were also completed. Statistical analysis and figures were prepared using R software (R Foundation for Statistical Computing, Vienna, Austria).

4.4 Results and discussion

4.4.1 Dough rheology

The effects of GO inclusion and organic acid type on the complex modulus (|G*|) and loss tangent (tan δ) are presented in Figures 4.1A and 4.1B, respectively. The results of a 3-way ANOVA determined that acid-type, cultivar, inclusion of GO and the interaction between acid-type and GO were all highly significant for |G*| (p<0.001) (Table 4.1). In all cases, Pembina dough was shown to have higher |G*| values (13.3 ± 2.6kPa) in comparison to Harvest (9.5 ± 2.1kPa), and, except for ascorbic acid and citric acid, samples which contained GO showed significantly higher |G*| (12.7 ± 3.2kPa) than the respective samples without (10.1 ± 2.3kPa). This indicates that the inclusion of GO increases the stiffness or strength of the dough samples. Additionally, all samples which included an organic acid had significantly lower (p<0.001) |G*| values (11.0 ± 2.5kPa), showing a reduction in dough stiffness, with the exception of ascorbic acid (11.3 ± 1.9kPa), which had higher |G*| values in comparison to controls without enzyme (10.5 ± 2.6kPa), but lower |G*| values when compared against control samples containing GO (17.4 ± 3.9kPa).

The loss tangent (tan δ) of samples showed similar results from the 3-way ANOVA: flour-type, acid-type, and GO inclusion were all significant, as well as the interaction of acid and GO,

acid and flour, and the interaction of flour and GO (p<0.001) (Table 4.1), but the 3-way interaction was not significant. Loss tangent describes whether a viscoelastic sample has liquid-like or elastic solid-like behaviour, with values >1 indicating liquid-like and values <1 indicating solid-like. Samples prepared with Pembina flour (0.33 ± 0.02), or those which contained GO (0.32 ± 0.02) had lower tan δ values in comparison to those with Harvest flour (0.34 ± 0.03) and without enzyme (0.36 ± 0.03), although differences were not observed for all acids, notably citric, succinic, and ascorbic. The inclusion of organic acids increased tan δ, or liquid-like behaviour, with the exception of ascorbic acid which did not significantly differ from the control samples without GO, however, control samples containing GO had a reduced tan δ in comparison to ascorbic acid samples also containing GO.

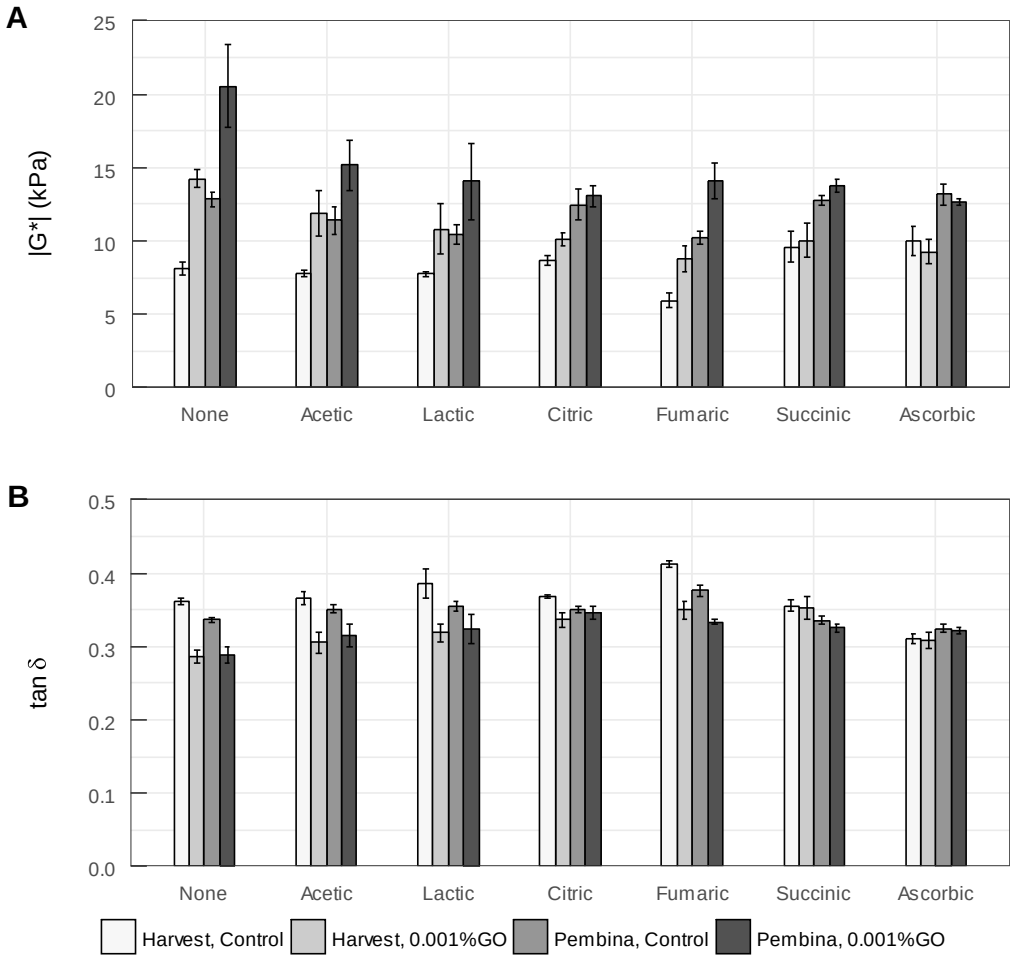

Figure 4.1 Complex modulus, |G*| (A) and loss tangent, tan δ (B) for samples prepared with Harvest and Pembina flours containing 1.0% NaCl. Values provided are the mean ± 1 standard deviation (n=3).

Table 4.1 *p*-values of dough samples prepared with and without GO, and with and without several organic acids (acetic, ascorbic, citric, fumaric, lactic, or succinic) with either Harvest or Pembina flour for rheology, FWC, %GMP and stickiness.

	***p*- values**						
Effect/Interaction	**|G*|[1]**	**tan δ[2]**	**J_{max}[3]**	**J_{el}[4]**	**Stickiness**	**FWC[5] (g ice/g d.b.)**	**%GMP[6]**
Acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Enzyme	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS
Acid:Cultivar	NS[7]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Acid:Enzyme	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cultivar:Enzyme	NS	<0.001	<0.001	<0.001	<0.001	NS	<0.05
Acid:Cultivar:Enzyme	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.05

[1]Complex modulus

[2]Loss tangent

[3]Maximum deformation

[4]Relative elasticity

[5]Freezable water content

[6]Glutenin macropolymer

[7]Not significant

The creep recovery experiment allowed the effects of GO and organic acids on the maximum deformation (J_{max}) and relative elasticity (J_{el}) of dough samples to be examined, which are shown in Figures 4.2A and 4.2B, respectively. The ANOVA results for J_{max} found that acid-type, cultivar, GO inclusion, and all interactions (acid type and cultivar, acid type and GO inclusion, cultivar and GO inclusion and the 3-way interaction) were all highly significant (p<0.001). Trends were similar to those of oscillatory rheology; the inclusion of GO (0.77 ± 0.35mPa^{-1}) reduced the deformation significantly in comparison to the samples without GO (1.84 ± 1.49mPa^{-1}), and Pembina (0.78 ± 0.34mPa^{-1}) showed lower deformation in comparison to Harvest (1.82 ± 1.50mPa^{-1}) samples. Samples containing ascorbic acid did not differ significantly from one another regardless of enzyme inclusion (1.01 ± 0.36mPa^{-1} without enzyme, 0.98 ± 0.30mPa^{-1} with GO), unlike the other acids. All variables and interactions were also highly significant (p<0.001) for relative elasticity (J_{el}) (Table 4.1). The trends of this data were the reverse of the J_{max} data because a higher J_{el} value indicates a higher relative dough elasticity, or a stronger gluten network. As per the correlation table (Table 4.2), J_{el} and J_{max} show strong negative correlation (r=-0.94, p<0.001), as do |G*| and tan δ (r=-0.73, p<0.001). The trends of ascorbic acid were not similar to those of other acids in these results, an expected outcome due to the ability of ascorbic acid to increase crosslinking in dough (Koehler, 2003).

The overall rheology trends indicated that the inclusion of GO provided stronger dough which was more resistant to deformation and had greater relative elasticity. A study completed by Caballero et al. (2007) found that the inclusion of enzyme at levels up to 0.05% GO (2% NaCl) did not significantly affect rheological properties (|G*|) in comparison to a control. In contrast, work by other authors at lower concentrations of GO (up to 0.015% by flour wt.) found that the inclusion of GO increases dough stability to overmixing and resistance of dough to extension (Bonet et al., 2006; Steffolani et al., 2010; Decamps et al., 2012). However, some of these authors only observed differences at higher concentrations of GO (minimum 0.01% by flour wt.) (Bonet et al., 2006; Steffolani et al., 2010). The findings that GO improves the stability and strength of dough is thought to be due to the ability of GO to produce additional disulfide linkages by oxidising α-D-glucose to δ-gluconolactone and hydrogen peroxide (H_2O_2). The H_2O_2 can then react with thiol groups to form additional disulfide linkages between gluten polymers. Other authors have also suggested that the inclusion of GO helps to improve dough strength by oxidising water-soluble pentosans to cause some gelation (Crowe & Rasper, 1988; Vemulapalli et al., 1998).

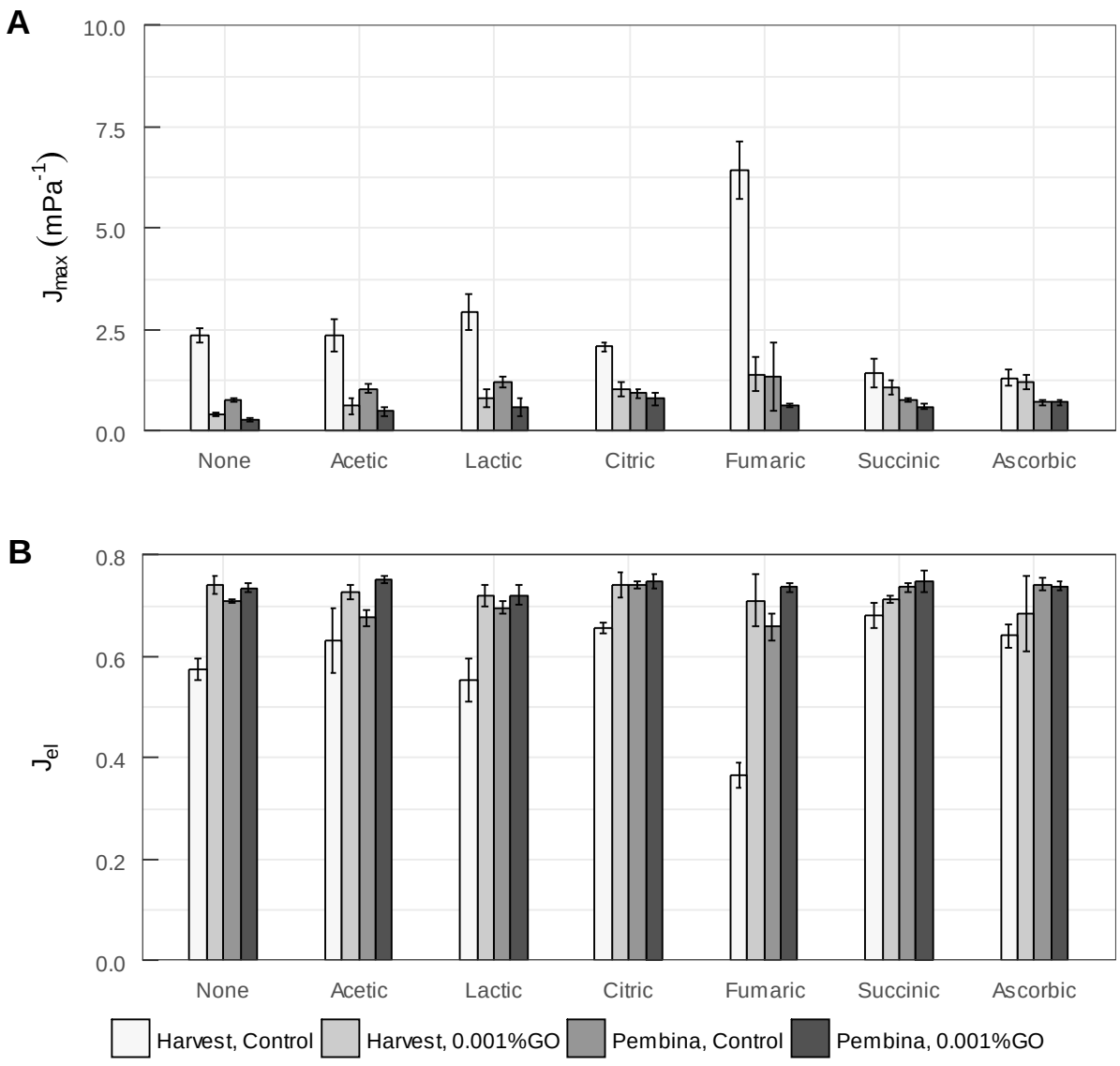

Figure 4.2 Maximum deformation, J_{max} (A) and relative elasticity, J_{el} (B) for samples prepared with Harvest and Pembina flours containing 1.0% NaCl. Values provided are the mean ± 1 standard deviation (n=3)

Table 4.2 Pearson correlation values for rheology, stickiness, %GMP, and FWC of dough samples prepared with and without GO, and with and without several organic acids (acetic, ascorbic, citric, fumaric, lactic, or succinic) and either Harvest or Pembina flour.

	FWC[1]	**|G*|[2]**	**tan δ[3]**	**J_{max}[4]**	**J_{el}[5]**	**Stickiness**	**%GMP[6]**
FWC	1.00						
|G*|	-0.56***	1.00					
tan δ	0.17	-0.73***	1.00				
J_{max}	0.39***	-0.69***	0.75***	1.00			
J_{el}	-0.35**	0.63***	-0.68***	-0.94***	1.00		
Stickiness	0.57***	-0.81***	0.55***	0.72***	-0.70***	1.00	
% GMP	-0.65***	0.62***	-0.25*	-0.37***	0.35**	-0.55***	1.00

[1]Freezable water content, [2]complex modulus, [3]loss tangent, [4]maximum deformation, [5]relative elasticity, [6]glutenin macropolymer

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

The inclusion of acid, in addition to enzyme, has been less well studied. The inclusion of acid reduced dough strength and solid-like behaviour across all samples, with the exception of ascorbic acid. Preliminary experiments indicated that the inclusion of these acids resulted in the expected decreased pH values in comparison to the control dough. This could have resulted in changes to the protonation of the proteins and altered their interactions with each other and other components of the dough, as some of the acids, such as citric acid, had a greater than 10-fold decrease in the pH of the dough (from 6.1 to 4.8). These pH changes could result in some new or reduced charge-charge interactions with the proteins and/or other components which may have resulted in reduced strength and increased deformation of the doughs if the changes were significant enough. However, pH changes are not likely to indicate the full picture, as the pH change was still within the normal range for dough (Sluimer, 2005) and citric acid was not the acid which was the most significantly different from the controls, suggesting that a pH change was likely not the most significant factor which resulted in these differences. Fumaric acid (pH 5.1) had a more significant impact on rheological parameters, particularly when no GO was included, despite reducing the pH to a lesser extent compared to citric acid. Previous work has suggested that fumaric acid can form covalent linkages with gluten proteins and disrupt the network (Sidhu

et al., 1980), and others have shown that it significantly reduced mixing times and increased breakdown of dough systems by reacting with free radicals in the dough (Han & Koh, 2011). These are potential reasons why a decrease in rheological strength is observed after acid inclusion in this study. Additionally, the action of GO appears to mitigate the effects of the fumaric acid to a similar degree as with other acids, suggesting that the mechanism of disruption that fumaric acid causes may be avoided or lessened with enzyme inclusion. It is possible that the effects of these acids are a combination of pH changes and other interactions that the acids have with the dough components, but the full mechanism or mechanisms remain to be elucidated.

Work with organic acids on dough has been limited, but some has been conducted. Wehrle et al., (1997) assessed the effect of acetic and lactic acid (1.2mmol/100g flour) on dough. The authors determined that especially in doughs with no salt, |G*| was decreased and tan δ was increased (Wehrle et al., 1997). The authors provided no mechanistic suggestion for this action except for pH changes (Wehrle et al., 1997). Seguchi et al., (1997) included gaseous acetic acid as a means to improve dough expansion and gas production, with some success, but it also decreased mixing stability. This suggests that regardless of some positive effects observed, there also are some negative effects on the gluten network and overall dough structure; the mechanism (pH related, or pH and some other mechanism) remains unidentified.

Ascorbic acid acted differently compared to the other acids included in this work, as was expected. Ascorbic acid is an oxidising agent (when in its oxidised form, dehydroascorbic acid) and has been utilised as a bread improver previously, because dehydroascorbic acid is able to generate disulfide linkages by acting as an oxidising agent (Koehler, 2003; Dagdelen & Gocman, 2007; Kornbrust et al., 2012). The inclusion of ascorbic acid appears to have little impact on the rheological properties of doughs in this study, and showed no synergistic strengthening effect when included with GO. Unlike other organic acids, the inclusion of ascorbic acid did not significantly affect the pH of the dough (pH 5.9) and it was the closest to the control when compared to other acids. Ascorbic acid without GO improved the rheological characteristics over a control; however, this effect was not increased with the addition of GO. It is possible that the high dosage of ascorbic acid used (1.2mmol/100g flour) did not allow for additional disulfide linkages to form, thus adding the GO had basically no effect. The high concentration could also have resulted in very rapid oxidation which may not have ensured the best structure possible, a suggestion made by Tang and others (2014). The high concentration may also have caused all of

the oxygen to be utilized in the conversion of ascorbic to dehydroascorbic acid, and therefore, the remaining ascorbic acid can act as a reducing agent and reduce components in the system (Millar & Tucker, 2012). These effects can be found at additions of 200mg/kg (Xiuzhen & Sieb, 1998), and the addition in our system was at ~2110mg/kg, therefore, this effect should be observed and result in a reduction of strength in the system. This can also explain some of the interactions between ascorbic acid and GO inclusion together, as GO also requires oxygen to function. As both GO and ascorbic acid have the same primary function in dough, and do not appear to have synergistic effects at the levels included, it is unlikely they would be included together. With ascorbic acid being the exception, organic acids reduced the dough stiffness, elastic behaviour, and relative elasticity, and increased the maximum deformation, while the inclusion of GO was able to partially mitigate that. In a full formula bread system, the inclusion of GO may serve to deal with some potential issues with yeast-produced acids, as Jayaram et al. (2014) found that succinic acid is considered to be the primary pH altering factor in bread, and has significant impacts on final quality, which might be exacerbated at reduced salt levels, as salt produces more tolerant dough especially at longer mixing times (Wehrle et al., 1997).

Harvest and Pembina showed significantly different rheological behaviour, with Pembina producing doughs that were stronger and had greater resistance to deformation compared to those prepared with Harvest. Harvest doughs were more sensitive to the inclusion of acids and GO than Pembina dough. GO improved dough rheology to a greater extent and the acids had a more detrimental effect on Harvest doughs when compared to Pembina, as observed especially with the J_{max} and J_{el} values of Harvest doughs containing fumaric acid. This suggests that the inclusion of GO in flours with weaker rheological attributes may be beneficial for dough production, and have the intended strengthening effects even at low concentrations. In the case of Pembina doughs, the strength of the flour appears to be more important than the inclusion of other components; while the doughs were affected by the inclusion of GO, the rheological behaviour did not change as significantly as that of the doughs produced with Harvest when GO was included.

4.4.2 Dough stickiness

Dough stickiness results are presented in Figure 4.3. The 3-way ANOVA determined that all variables (flour type, acid type, and GO inclusion) were highly significant ($p<0.001$), as were all 2-way interactions ($p<0.001$); however, the 3-way interaction was not found to be significant

(Table 4.1). Doughs prepared with Harvest flour (0.50 ± 0.09N) were stickier than those produced with Pembina flour (0.42 ± 0.07N). Except for acetic acid, all doughs prepared with acids (0.48 ± 0.09N) were significantly stickier than their control counterparts (0.42 ± 0.11N), and the inclusion of GO (0.43 ± 0.08N) decreased stickiness in comparison to those samples without (0.52 ± 0.09N), except for ascorbic acid, which remained the same after the inclusion of GO (0.57 ± 0.05N without GO, 0.54 ± 0.04N with GO).

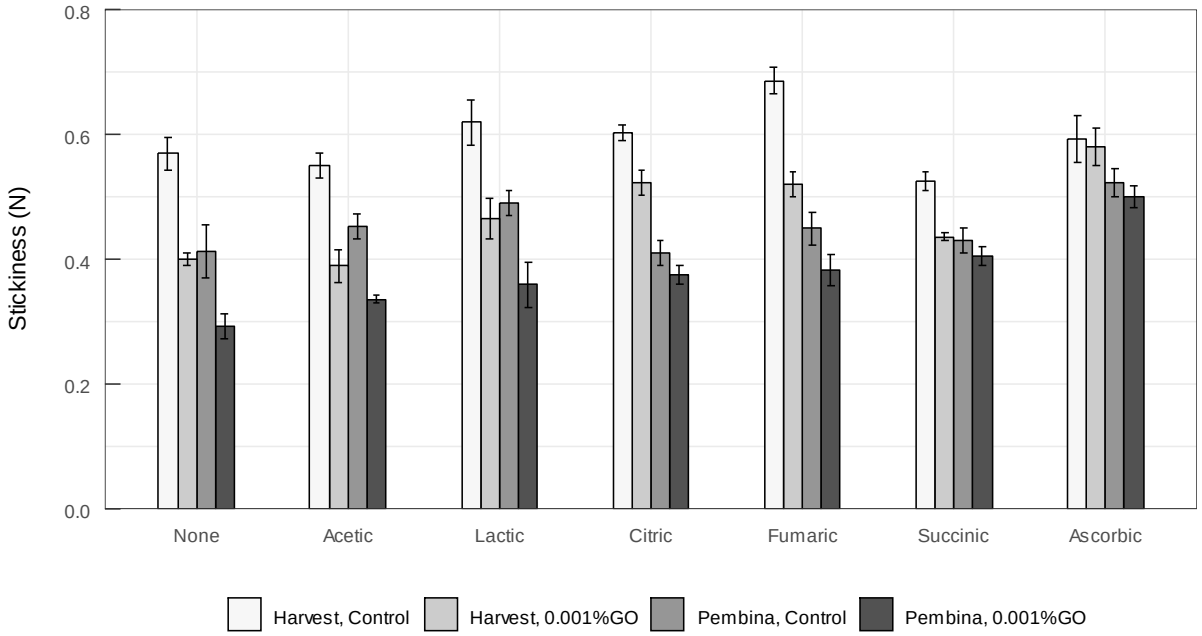

Figure 4.3 Stickiness (N) of samples prepared with Harvest and Pembina flours containing 1.0% NaCl. Values provided are the mean ± 1 standard deviation (n=3).

The trends of these results followed those of the rheology, and validate the hypothesis that the inclusion of organic acids increases dough stickiness, and that the additional inclusion of GO would mitigate this stickiness to some degree. The observed increase in stickiness supports the suggestion that the acids are interacting with the dough in some way, either by the reduction in pH resulting in protonation as well as through the potential interaction of the acids with other dough components. However, as previously discussed no mechanism has been determined. Any weakening of the structure would likely result in increased stickiness; the dough would have decreased protein-protein interaction and increased interactions between protein and water or other

dough components, so that the gluten network would suffer from these increased adhesive forces. Similar to its effect on rheology, stickiness appeared worst in fumaric acid samples (particularly Harvest without GO), and the inclusion of acetic acid had no significant effect on stickiness compared to the control. As per the correlation table (Table 4.2), stickiness showed a strong negative correlation with |G*| (r=-0.81, $p<0.001$), and J_{el} (r=-0.70, $p<0.001$), and a positive correlation with J_{max} (r=0.72, $p<0.001$) suggesting stickiness generally reflected what was found with the rheology measurements. With the exception of ascorbic acid, the inclusion of GO reduced stickiness significantly compared to doughs without the enzyme, but it did not appear to remove all stickiness associated with acid inclusion as these values were still higher than those of the control samples with GO and no acid. The increase in stickiness was minimal for many of the acids; stickiness behaviour appeared to be more affected by GO inclusion than acid inclusion or type. This aligns with the work of Jekle and Becker (2012) who assessed the stickiness parameters of doughs as they acidified them using lactic acid, and found that stickiness increased below pH 6.8 and then began to decrease at pH 5.2 which they attributed primarily to protonation changes causing changes in repulsion and attraction of dough components. Other work has also shown increased stickiness with the inclusion of acids, particularly at low salt levels (1.0% by flour wt.) albeit without any enzymes (Stone et al., 2018).

The ascorbic acid acted similarly to its effect on the rheology, which suggests that the concentration at which ascorbic acid was added into the samples was likely high enough that the GO was not able to form additional free thiol linkages, or that the ascorbic acid acted as a reducing agent and disrupted linkages with other dough components. However, the stickiness of doughs containing ascorbic acid were significantly higher than the controls, both when ascorbic acid was added alone, and with GO. This is not really expected, as ascorbic acid is added to bread products as a strengthening agent but the high concentration of ascorbic acid used could explain why higher stickiness is observed. Similar to results observed from the rheology measurements, no positive synergistic effect was found when ascorbic acid and GO were included together. Overall, this research suggests that GO can be utilized as a way to reduce stickiness in doughs, in spite of the negative effects that may be found with yeast-produced acids.

4.4.3 Freezable water content

The results of the freezable water content (FWC) measurements can be seen in Figure 4.4. The ANOVA indicated that acid-type and cultivar effects were both highly significant ($p<0.001$), however, enzyme inclusion was not ($p>0.05$) (Table 4.1). All interactions were highly significant except the interaction of cultivar with enzyme, which was not. The results show that Pembina doughs (0.427 ± 0.02g ice/g d.w.) contained less freezable water in comparison to those made with Harvest (0.479 ± 0.02g ice/g d.w.), and that the inclusion of GO did not affect this value (0.452 ± 0.04g ice/g d.w. without enzyme, 0.454 ± 0.03g ice/g d.w. with GO). Acid inclusion generally trended towards having higher FWC (0.456 ± 0.029 g ice/g d.w.) in comparison to control samples (0.433 ± 0.039g ice/g d.w.), however, in the case of acetic acid no statistical differences were observed between its inclusion and the zero acid control samples (0.443 ± 0.039g ice/g d.w. without GO, 0.448 ± 0.035g ice/g d.w. with GO). Poor rheological behaviour and dough stickiness has been linked to higher water contents (Skendi et al., 2010; Jekle & Becker, 2011). van Velzen et al. (2003) attributed some of the stickiness observed in overmixed doughs as being related to protein hydration and theorized that additional hydration caused mobility of the proteins towards the upper layers resulting in additional stickiness. The assessment of freezable water content via DSC was to assess if differences in the free water content of samples could be linked to differing rheological and stickiness behaviour found in samples. However, these results did not follow all of the trends for the rheology and stickiness data; the inclusion of GO did not significantly impact the results, unlike the stickiness and rheology results where it was found to be highly significant. The strengthening and stickiness reduction found with GO addition did not lead to a reduction in freezable water content when compared to controls. A possible explanation is that this occurs because the additional crosslinking does not actually bind up further water, but could entrap it, which may result in reduced stickiness or increased rheological strength because the entrapped water may not interfere with gluten network development or increase the adhesion. Entrapped water can be freezable (Golob et al., 2008) therefore, it should not alter the DSC results even if it is unable to interact with components of the dough which would cause weakness and/or increased stickiness unless in a manner by which it is no longer freezable.

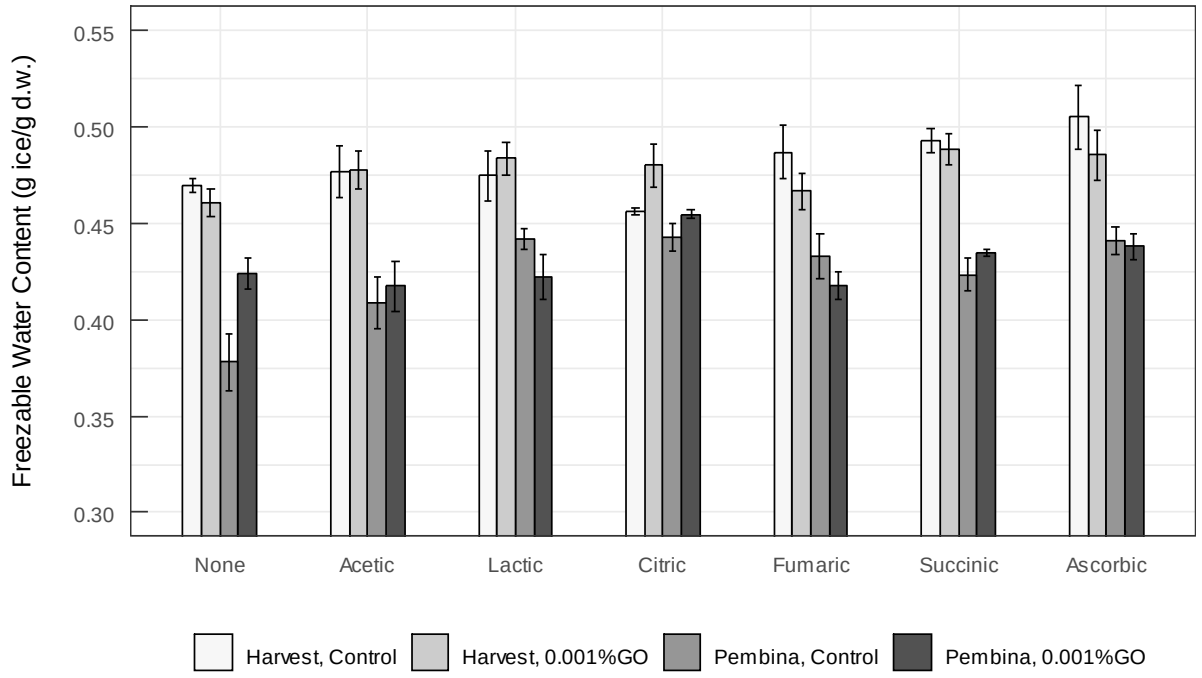

Figure 4.4 Freezable water content (FWC) (g ice/g d.w.) of samples prepared with Harvest and Pembina flours containing 1.0% NaCl. Values provided are the mean ± 1 standard deviation (n=3).

4.4.4 Glutenin macropolymer (GMP)

GMP is generally considered to be a fairly good indicator of a flour's breadmaking ability (Don et al., 2003), and it is essential for strong gluten network development (Steffolani et al., 2008). Steffolani et al. (2010) have shown that the inclusion of crosslinking enzymes can result in an increase in GMP with the inclusion of GO, however, other authors have also observed a slightly decrease in GMP with GO inclusion at low levels (0.002g/100g) (Primo-Martín et al., 2003).

The results of the GMP experiment can be seen in Figure 4.5. Results of the ANOVA show that acid-type (p<0.01) and cultivar (p<0.001) were both significant, however the inclusion of GO was not (p>0.05) (Table 4.1). All interactions were significant; however, cultivar and enzyme inclusion, and the 3-way interaction were only slightly significant (Table 4.1). While the ANOVA detected acid-type had significant effects, no significant differences were observed between acid types, or the control. Pembina had significantly higher %GMP in comparison to Harvest (3.61 ± 0.85% vs 1.70 ± 0.64% respectively). This finding is on trend with other findings of this study and

previous work by the group which suggests that Pembina produces stronger, less sticky dough in comparison to Harvest. However, no differences in %GMP were observed between dough samples containing GO and those which did not (2.60 ± 1.45% and 2.70 ± 1.14% respectively), which is contrary to other findings in this research. It is possible that at the low concentrations of GO inclusion (0.001% GO by flour wt.) there was no discernible difference in the methodology utilized, even though other experiments can detect differences in the parameters investigated. This could be the result of either GO not directly affecting the GMP, or requiring a more precise assay to observe differences. GO affects the gluten network, but can also affect other components of doughs, such as water-extractable and unextractable pentosans which may also have effects on the parameters. Therefore, the means by which these organic acids affect the dough matrix are not related to %GMP or are related to it on a level that this technique cannot discern.

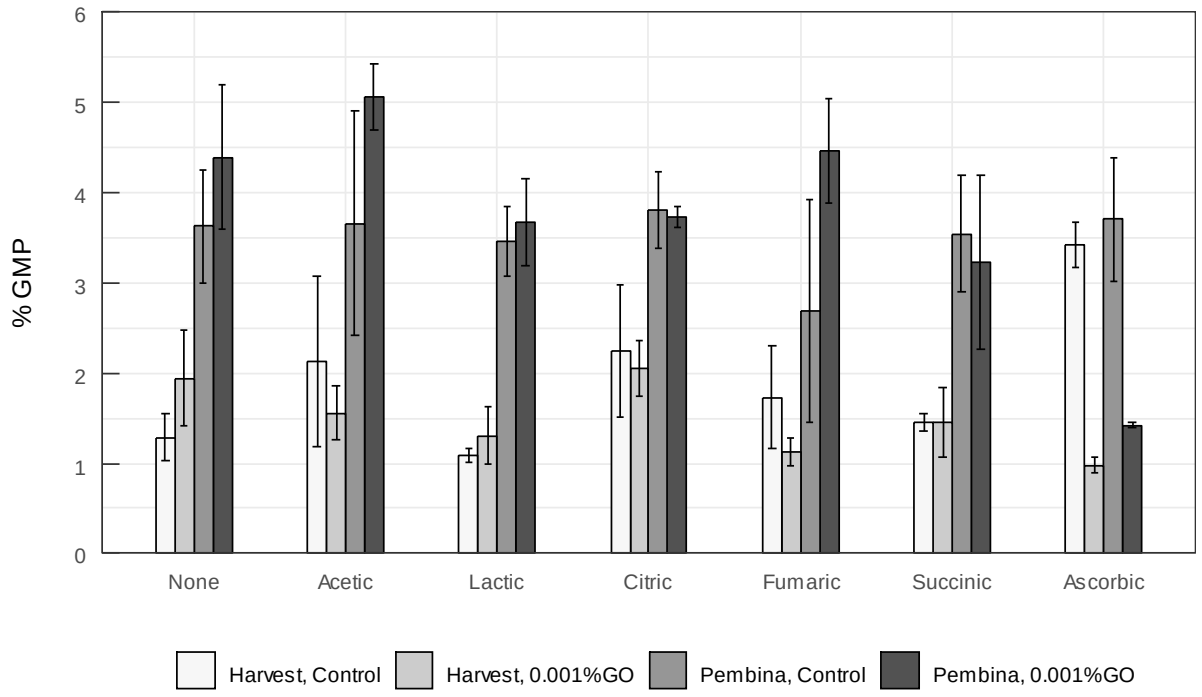

Figure 4.5 %GMP of dough samples prepared with Harvest and Pembina dough samples containing 1.0% NaCl. Values provided are the mean ± 1 standard deviation (n=3).

4.5 Conclusions

A decrease in salt concentration is a functional issue which causes production issues in bread products which need to be addressed in some manner. There is potential for the use of crosslinking enzymes such as GO to improve dough strength, but also to move towards clean labels which other oxidising agents cannot achieve as GO does not have to be labelled unlike some other oxidising agents such as azodicarbonamide. Yeast can produce several types of organic acids which can have impacts on the overall quality of the bread, and during production. This work showed that the inclusion of some of these organic acids were negative with regards to rheology and stickiness, but did affect not the amount of GMP and only slightly affected the free water content. Similarly, the inclusion of GO had significant effects on dough stickiness, and rheology, but not on GMP or freezable water content. GO also showed marked improvements in some samples with poor rheology and high stickiness, such as those dough samples which included fumaric acid. Rheology and stickiness had clear trends with acid and GO inclusion, but the linkage of water properties and these dough properties requires more characterization. Determining the complete mechanisms by which organic acids increase stickiness and weaken gluten networks requires more study. Further work with enzymes such as GO in low sodium bread systems with more complicated dough formulations and some final products to see if these enzymes can be utilized in a product as a functional replacer for salt and improve label cleanliness in bread products is also suggested.

4.6 Linkage between dough handling and stickiness characteristics of model doughs with the water mobility and association characteristics as assessed by ^{1}H nuclear magnetic resonance (NMR)

The mechanism behind dough stickiness has been investigated but not fully elucidated. There are certain factors which have been linked to stickiness, such as low-sodium, excess water, or in the case of this work, yeast-produced acid inclusion. However, the specific role(s) of water in stickiness is not completely understood. This work on the effect of organic acids on dough handling and stickiness showed that there is some relationship between the acids and these properties, however, outside of pH change there is little understanding of potential mechanisms. The DSC results provide some insight into some differences in freezable water content, but it does not consider bound water and is an incomplete picture of the relationship of water, organic acids,

and stickiness. Therefore, the use of ^{1}H NMR may help provide some insight into how water is interacting with different components of the dough, as well as mobility through the dough on a small scale. This information about water mobility aims to link dough handling characteristics/stickiness and water characteristics; to develop a better understanding of the underlying water-stickiness mechanism(s) to produce superior low-sodium bread products.

5. WATER MOBILITY AND ASSOCATION BY ^{1}H NMR AND DIFFUSION EXPERIMENTS IN SIMPLE MODEL BREAD DOUGH SYSTEMS CONTAINING ORGANIC ACIDS

5.1 Abstract

Reducing the sodium content of bread to meet desirable population health outcomes can lead to challenges in dough processing. Our objective was to better understand the relationship of water and dough components, and to see if this relationship could be linked to observed handling characteristics of low sodium doughs. The water mobility, association, and diffusion characteristics of simple model doughs containing reduced NaCl (1.0% by flour wt.), organic acids (acetic, fumaric, or succinic at 1.2mmol/100g flour or a no acid control), and a dough improver (0.001% by flour wt. glucose oxidase) using two cultivars (Canada Western Red Spring (CWRS) Pembina and Harvest) were assessed by ^{1}H NMR. It was determined that the inclusion of the acids did not significantly affect the overall structure of the dough; the polymer backbones (protein and starch) were not significantly affected, however, the inclusion of acids or use of a stronger dough cultivar (Pembina) reduced molecular motion on the MHz timescale as assessed by T_1 and T_2. Motion on the kHz timescale was also altered. Samples which contained acid or were made from Pembina flour had less mobile water than those without acids, or doughs prepared with Harvest flour. The diffusion characteristics of water in the doughs were not altered by the addition of acids or by use of different cultivars; however, diffusion was determined to be confined/restricted by the polymer matrix. These dough samples were compared to ones containing yeast and it was found that the acid inclusion trends generally followed those with yeast, which indicates that this model could be useful for investigating stickiness and dough handling mechanisms without the additional complications arising from using yeast. Overall, the inclusion of acids altered molecular motion and interactions with the side chains of the polymer backbone, and further stickiness and handling investigations should focus on these areas to expand upon the relationship of water and stickiness/dough handling, which is a particular concern in low sodium doughs.

5.2 Introduction

Sodium reduction has been a popular trend in recent years across a variety of foods, including bread products, mainly due to health concerns about high sodium intakes due to their link to hypertension, cardiovascular disease, and other issues (O'Donnell et al., 2015). Sodium reduction can pose technical challenges in several food products, including preservation, taste, and texture.

In the case of baked goods, bread in particular, reduction of sodium poses a significant challenge because salt (sodium chloride, NaCl) is one of the four essential ingredients (flour, water, salt, yeast), and its inclusion is integral for to the development of a strong gluten network and good final product quality (Mondal & Datta, 2008; Belz et al., 2012). In addition, NaCl is also important for industrial bread processing; NaCl reduction has been linked to increased dough stickiness which can result in dough handling issues (Dobraszczyk, 1997; Adhikari et al., 2001; van Velzen, 2003).

The mechanisms relating reduced NaCl to dough stickiness have not been fully elucidated. Some theories have been posited relating to various components of the dough such as water-soluble pentosans, compositional differences in protein, and enzyme activity (Chen & Hoseney, 1995; Hoseney & Smewing, 1999). Work by van Velzen et al. (2003) has shown that the degree of hydration of proteins can affect stickiness, particularly in overworked doughs. Poor rheology has also been linked to higher water content (Skendi et al., 2010; Jekle and Becker, 2011). Some authors have shown that the interference of certain organic acids, which are produced by yeast during the breadmaking process, can have negative effects on stickiness and dough rheology (Wehrle et al., 1997; Jekle and Becker, 2012; Stone et al., 2018). However, the overall mechanism that explains these results is not been fully understood; some theories have suggested that it may be related to pH changes or interactions between dough components (Wehrle et al., 1997; Han & Koh, 2011). Water characteristics of dough are also of interest; the location and association of water with different components is thought to have significant impacts on the extensibility and elasticity of doughs (Lu & Seetharaman, 2013), and the amount of free water affects mobility of water and flow characteristics significantly (Roman-Gutierrez et al., 2002).

Different analyses have been utilized to examine water properties in doughs, such as differential scanning calorimetry, DSC (Linlaud et al., 2011; Peng et al., 2017), thermogravimetric

analysis, TGA (Fessas & Schiraldi, 2001; Roozendaal et al., 2012), and ^{1}H NMR (Leung et al., 1979; Assifaoui et al., 2006a; Doona & Baik, 2007; Bosmans et al., 2012), all of which provide different information. The focus of this work is on ^{1}H NMR, which can provide insight into water's association with starches and proteins in the system based on the transverse, spin-spin relaxation times of ^{1}H (T_2) and the longitudinal, spin-lattice relaxation times (T_1) within a magnetic field. These techniques can indicate whether the water is strongly associated with polymers within the system or if the water is able move more freely within the system (Bosmans et al., 2012). Water mobility has been examined using T_2 spin-spin relaxation times in food systems, particularly in starch, by a variety of authors (Le Botlan et al., 1998; Kou et al., 2000; Chatakanonda et al., 2003; Hemdane et al. 2017), and several common proton populations have been defined; tightly bound water (T_{21}), less tightly bound water (T_{22}) and almost free water (very weakly bound) (T_{23}) (Lu & Seetharaman, 2013).

When dough handling information is combined with measurement of the diffusion characteristics of ^{1}H, it provides insight into the movement of water within doughs and can potentially produce greater understanding of the effects of organic acids on the structure of dough and any influence of the water content and mobility upon the observed increased stickiness and rheology defects. The focus of this work is on assessing the mobility of water, its association, and its diffusion characteristics in doughs prepared with different organic acids. Two cultivars were selected based on previous dough handling work. One flour had weaker dough handling characteristics (Harvest) when compared to the other (Pembina), particularly at low sodium concentrations. The intention is to better understand observed stickiness and rheological behaviour and link it to water mobility and association within the system, and then in the future, be able to use this information to design improvements for low sodium bread products.

5.3. Materials and methods

5.3.1 Materials

Two cultivars of wheat were selected for this work based on their breadmaking ability at reduced salt levels; Canada Western Red Spring (CWRS) Pembina and Harvest. Cultivar selection was based on a previous rheology, baking, and stickiness study completed by our group (Yovchev et al., 2017) which examined 37 varieties and two salt (NaCl) levels; 1.0 and 2.0% by flour wt., in which Pembina flour was determined to have strong characteristics and low stickiness at both salt

levels, and Harvest flour showed weaker handling behavior and higher stickiness at both NaCl levels. Grain samples for both cultivars was obtained from the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK, Canada). The wheat was milled with a Buhler mill at the University of Saskatchewan Grain Research Laboratory (AACCI method 26-21.02). Harvest flour contained 13.0% protein (based on 14% m.b.) and had a farinograph water absorption (FAB) value of 64.9% (based on 14% m.b.), whereas Pembina flour contained 12.7% protein (m.b.) and had a FAB value of 61.5%) (Avramenko, 2017).

Dough samples also contained a bakery enzyme (glucose oxidase) used to strengthen the dough. Novozymes (Novozymes, Denmark) graciously donated the glucose oxidase (Gluzyme® Mono 10000 BG). Other chemicals were reagent grade and obtained from Sigma-Aldrich (Oakville, ON, Canada). Yeast was procured from the local grocery store (Fleischmann's Yeast, OH, United States).

5.3.2 Dough preparation

Dough samples were prepared with a 10 g mixograph (TMCO National Mfg., Lincoln, NE). Flour, NaCl (1.0% by flour wt.), water (based on farinograph absorption value (FAB)), glucose oxidase (0.001% by flour wt.), and an organic acid (either acetic, fumaric, or succinic at 1.2mmol/100g flour, none in control), or yeast (3.0% by flour wt.) were added, and mixed until just past peak tolerance. After mixing, dough was enclosed in plastic containers for 1 hour at room temperature (21-23°C) to allow for the enzymatic reaction to proceed. After this, dough was frozen at -30°C until 1 hour before testing of each sample (frozen storage for one week), when they were removed from the freezer to be thawed to room temperature as assessed by a thermometer. All samples were prepared in triplicate.

5.3.3 ^{1}H nuclear magnetic resonance (NMR) spectroscopy

To analyse how polymers interact with water within a dough system, time domain NMR relaxometry similar to the method of Doona and Baik (2007) was employed. Approximately 1g of dough was placed in the open end of an NMR tube, and the tube was sealed with Teflon tape to ensure that the sample would not fall out of the tube during the procedure, as well as to prevent moisture loss. For the analysis, a 10 MHz Minispec MQ NMR Analyser (Bruker, Milton, ON, Canada) with a magnetic field strength of 0.24T was utilized. The sample temperature control

system was set to 25°C. A series of four experiments were completed, beginning with a 90° pulse (free induction decay, FID) sequence to determine a T_2^* value (acquisition time 10.24 ms, recycle delay 2 s, 4 scans), followed by an inversion recovery (IR) sequence to determine T_1 (using 13 recovery times in the range 0.9 ms – 366 ms, recycle delay 5 s, 4 scans each), then a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence to determine T_2 (512 echoes, separated by 0.2 ms, 8 scans), modelled as one short (T_{2A}) and one long (T_{2B}) component. Finally, a spin-lock sequence (using 12 spin-lock times in the range 0.1 to 24.9 ms, recycle delay 1 s, 4 scans each) was used to record the $T_{1\rho}$ relaxation time constant for protein side chain motion on the kHz time scale. The measurement was repeated at six different spin-lock pulse powers, with attenuation of 6 (half power), 8, 10, 12 (quarter power), 14, and 16dB compared to the 90° pulse power of the FID sequence.

5.3.4 Diffusion measurements

To determine the diffusion of water (by assessing ^{1}H signals provided by water molecules) in the dough system, and provide an idea of how water moves through the dough matrix, a magnet with a permanent magnetic field gradient (open GARField magnet; Laplacian, Abingdon, Oxfordshire, UK) coupled with a Maran DRX imaging console (Oxford Instruments, Oxfordshire, UK) was utilized. The GARField magnet was operated at a frequency of 33.1 MHz (magnetic field of 0.79T). A single-turn, homebuilt coil driven by a 1-kW CPC "MRI-plus" broadband (10-155 MHz) amplifier (CPC, New York, USA), was used to produce the radio frequency (RF) excitation within the doughs. The GARField design is based on work by Glover et al. (1999), and focuses the excitation in a thin sliver of the dough (~1.5 mm), and the measurement is sensitive to the diffusion motion of ^{1}H protons. This excitation uses three radio frequency pulses and produces two NMR echoes. The self-diffusion coefficient of ^{1}H in the dough was measured by fitting the ratio of the amplitudes of the two NMR echoes, after Kimmich and Fischer's (1994) work on the "three-pulse sequence". Times allowed for the ^{1}H diffusion between the two echoes were 7.5, 15, and 30 ms.

5.3.5 Statistics

All statistics are presented as the mean value ± one standard deviation (SD), and all dough samples were prepared in triplicate. To determine statistical differences, a two-way analysis of

variance (ANOVA) and Tukey post-hoc test were utilised. A *p*-value of <0.05 was considered to be significant. Statistical analysis and figure preparation were completed with R (R Foundation for Statistical Computing, Vienna, Austria). Data fitting of NMR signals and decays was completed using MATLAB Routines (MathWorks, MA, United States).

5.4 Results and discussion

5.4.1 ^{1}H nuclear magnetic resonance (NMR)

The purpose of using ^{1}H NMR in this work was to investigate water behaviour in the dough system. This work consistent of a four-pronged approach to examine water association within the dough system, the motion of the polymers on the MHz and kHz timescales, and to examine how the addition of various organic acids would affect the gluten network and other polymers within the dough system. These effects may become especially important at reduced salt levels, as salt reduction has negative effects on dough strength and the development of a strong gluten network (Mondal & Datta, 2008; Belz et al., 2012) in addition to increasing dough stickiness (Dobraszczyk, 1997; Adhikari et al., 2001; van Velzen et al., 2003). These experiments are unable to distinguish between the gluten network and starch within the dough system so for the context of the experiments, as a group these polymers can be referred to as the "lattice". The lattice comprises all other NMR-active nuclei not providing the ^{1}H signal, which includes carbon nuclei found in the gluten network and starch (Chinachoti et al., 2008).

5.4.1.1 Free induction decay (FID)

Other authors have used free induction decay (FID) experiments to examine doughs, but the focus has primarily been on association of proton populations with dough components (Assifaoui et al., 2006a; Doona & Baik, 2006). In our measurements, a two-component Gaussian-exponential model was used to fit the FID data. The Gaussian aspect of the fit includes background signal from the probe and is similar for all samples, and was the shorter, observed signal. The longer signal observed was the exponential fit of the data, which represents the dough sample. The data presented in Figure 5.1 is the exponential fit of the FID data. The measured T_2^* relaxation time constant or (decay constant) describes the rate of loss of signal, which can result from two types of spin interactions. In spin-spin interactions (which are described by the T_2 time constant), the magnetic dipole moments of ^{1}H spins in neighbouring water molecules interact (Callaghan,

1991). T_2^* is also affected by the inhomogeneity in the magnetic field, which prevents the FID measurement from specifically examining the longer duration spin-spin interactions (longer components of the T_2 distribution) (Callaghan, 1991). The T_2^* values can provide insight into the porous structure of the dough matrix. The fit of this data was Gaussian-Exponential, which is similar to previous work which examined dry and hydrated gluten systems (Calucci et al., 2003). Nonexponential fits are largely associated with magnetic fields which are very inhomogeneous, or structures which have larger pores and an inhomogeneous pore structure (De Guio et al., 2009). Therefore, the lack of a monoexponential fit for the dough system, in addition to microscopy work which showed that dough microstructure is not particularly homogeneous (Jekle & Becker, 2011), indicates that the dough structure is heterogeneous and lacks a fine porous structure. The lack of significant differences observed between dough samples suggested that the polymer backbone (i.e. gluten network and starch) was not significantly affected or altered by changing flour type or by the inclusion of any of the acids tested, and that the base structure remained the same. Previous work by our group has indicated that organic acids increase dough stickiness and reduce dough strength (Stone et al., 2018). When that finding is considered with those from this study, that is, all dough samples have similar T_2^* values, the inclusion of these specific acids at these concentrations does not appear to break covalent linkages and alter the overall dough structure greatly. If this is the case, then the differences in dough handling and stickiness observed may be the result of the acids' interactions with the surfaces polymers within the dough, such as protein side chains, or starch instead of breaking the bonds of dough components (i.e. starch and gluten).

Results of the two-way analysis of variance (ANOVA) suggested that cultivar was a significant determinant of this exponential component of T_2^*; however, acid inclusion, and the interaction term were not. Cultivar had a significant effect on this longer component (the exponential component) of T_2^*, which suggests that the interactions between dough ingredients differ depending upon flour type. This is reasonable as not only do the flours show differing functionalities when processed into doughs, but they also have somewhat different proximate parameters and compositions. Alterations in the interaction between the gluten network, starch and other dough ingredients could be the reason that differences in stickiness and strength are observed in handling tests.

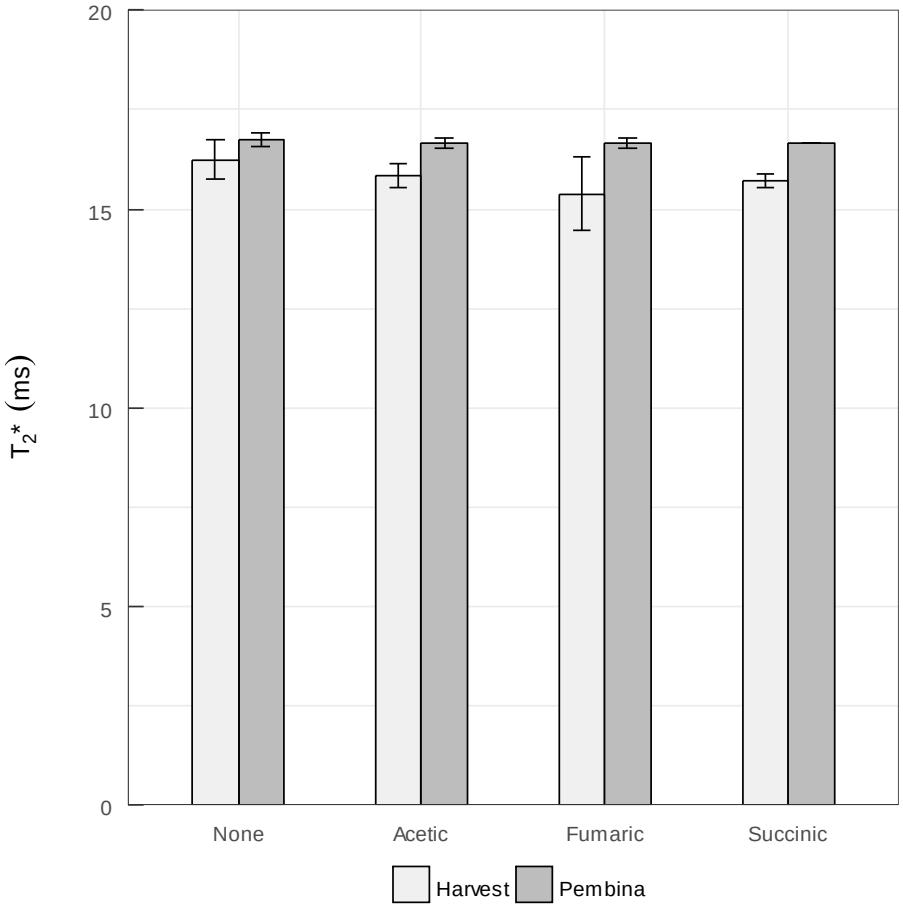

Figure 5.1 T_2* values of the exponential component of a Gaussian-exponential fit for dough samples prepared with Harvest or Pembina flour at 1.0% NaCl containing 0.001% GO (both by flour wt.) and either no acid, acetic acid, fumaric acid, or succinic acid. Values provided are the mean ± 1 standard deviation (n=3).

5.4.1.2 Carr-Purcell-Meiboom-Gill (CPMG)

A CPMG sequence was used to assess the T_2 components of the dough: T_{2B} (T_2 long component) which is indicative of mobile, but not completely free water, and the T_{2A} (T_2 short component) which is associated with tightly bound water (Lu & Seetharaman, 2013). The longer, exponential component of the FID decay (section 5.4.1.1) is a combination of the T_{2A}-, T_{2B}- and magnetic field inhomogeneity-mediated decays of signal. The CPMG measurement allows the magnetic field effects to be removed entirely and the T_{2A} and T_{2B} components to be resolved. Differences between the samples were only observed in the $T_{2B,}$ reported below. Other authors

have primarily used the CPMG sequence to assign proton populations within dough systems, and have also observed these two populations, T_{2A} and T_{2B} (Assifaoui et al., 2006a; Assifaoui et al., 2006b; Doona & Baik, 2006). Other proton populations have also been identified, such as those which are rigidly associated with starch (Assifaoui et al., 2006a; Assifaoui et al., 2006b; Bosmans et al., 2012; Serial et al., 2016) or associated with almost completely free water, T_{23} (Lu & Seetharaman, 2013). This CPMG testing provides insight into the molecular mobility of water within the dough system on the MHz timescale (Kishore et al., 2002), as well as quantifying the bound and unbound fraction of water (A1 ratio; the fraction of bound ^{1}H/total ^{1}H). A higher A1 ratio suggests a greater quantity of tightly bound water. The results of the CPMG experiments are seen in Figure 5.2, which shows (A) T_{2B} and (B) the A1 ratio. The statistical analysis suggests that both cultivar and acid inclusion were highly significant (p<0.001) for T_{2B}, however, the interaction between the terms was not statistically significant. For the A1 ratios, only cultivar was found to be highly significant (p<0.001), acid inclusion was somewhat significant (p<0.05) and the interaction term was not significant.

The results of the T_2 experiments determined that the inclusion of acid or the use of Pembina flour over Harvest produced lower T_{2B} values which suggests longer correlation times (slower molecular tumbling) in both cases. However, the differences observed are much larger for acid inclusion in comparison to cultivar differences. While acid inclusion had significant effects on T_2 values, the type of acid included did not. The decrease in T_{2B} values for samples containing acid, and those prepared with Pembina flour showed a shift towards lower mobility on the MHz timescale in comparison to those samples which had higher T_{2B} values (and, therefore, shorter correlation times). In Pembina doughs and doughs treated with acid, the mobile component of water had reduced mobility. The A1 ratio showed significant differences with respect to cultivar; Pembina flour produced slightly higher A1 ratios in comparison to Harvest flour (0.35 and 0.32 respectively), which suggests that overall Pembina flours contain slightly more bound water when compared to Harvest flours. This is in part expected based on experimental design as water was added based on FAB values, which means more water was added to Harvest samples over Pembina to hydrate the doughs optimally, but this should not account for all observed differences between cultivars. The other differences observed might be expected due to differing flour qualities; amounts of starch and gluten within the systems, and differing compositions of those (damaged starch, different amino acid composition, different amounts of non-starch polysaccharides) which

provide different functional groups and could affect the ratio of bound/unbound water in the systems. Leung et al. (1979) examined different flour effects on T_{2A} and T_{2B} components in doughs prepared to optimum consistency (different water contents) and found minimal differences between them, despite rheological differences, concluding that the NMR method was not precise. Our observations could be due to improved equipment since this study, or greater differences in starting materials in comparison to those they selected. Overall, the use of Pembina flour or the addition of acids causes the mobile water component in the dough to have reduced mobility when compared to samples without acid or prepared with Harvest flour.

Furthermore, doughs prepared with Pembina flour have been shown to have strong rheological properties and low stickiness (Yovchev et al., 2017), whereas organic acids have been shown to negatively affect the rheology and stickiness (Stone et al., 2018). Increased water content has been thought to explain the increase in stickiness observed in some dough systems (van Velzen et al., 2003; Skendi et al., 2010; Jekle & Becker, 2011). The reduction in water mobility observed in these samples does not appear to link closely to the stickiness and rheological behaviour observed in other work suggesting that more information than just CPMG experiments may be needed to identify the linkages.

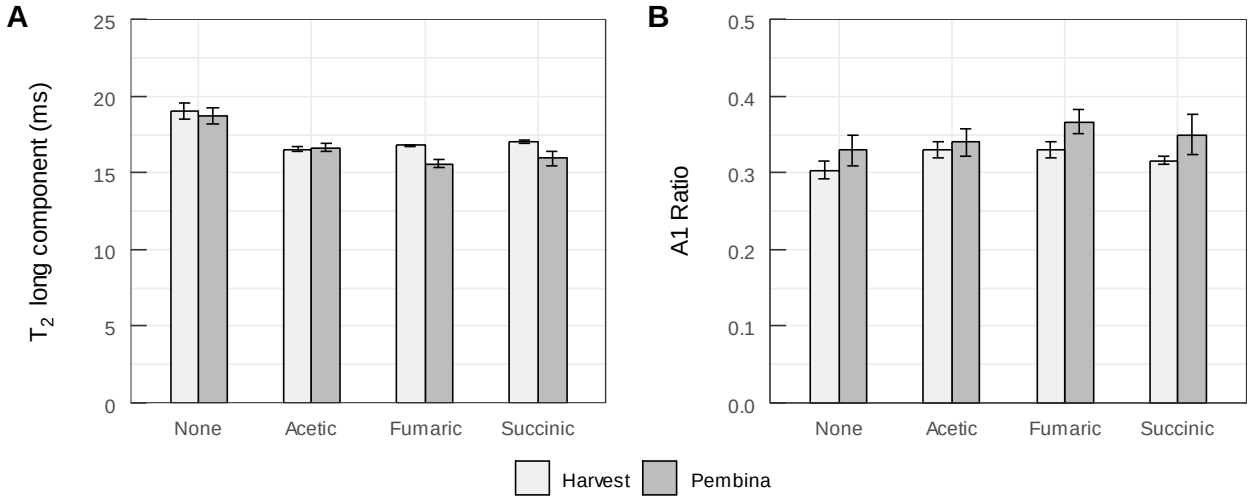

Figure 5.2 T_{2B} (or T_2 long component) values (A) and A1 ratios (B) for dough samples prepared with Harvest or Pembina flour at 1.0% NaCl containing 0.001% GO (both by flour wt.) and either no acid, acetic acid, fumaric acid, or succinic acid. Values provided are the mean ± 1 standard deviation (n=3).

5.4.1.3 Inversion recovery (IR)

Inversion recovery (IR) was used to assess the T_1 values of the dough system. The T_1 value is the spin-lattice relaxation time of the system and provides an indication of ^1H interaction with the lattice (nuclei which do not produce ^1H signals, such as carbon in the gluten network and starch) (Chinachoti et al., 2008). The results of this experiment are presented in Figure 5.3, and the two-way ANOVA indicates that both acid type and cultivar effects were highly significant ($p<0.001$), however the interaction term was not ($p>0.05$). T_1 values for Pembina were lower than those for Harvest (65.0 ms and 73.4 ms, respectively) and those containing no acid were significantly higher than those with (79.0 ms and 67.8 ms, respectively).

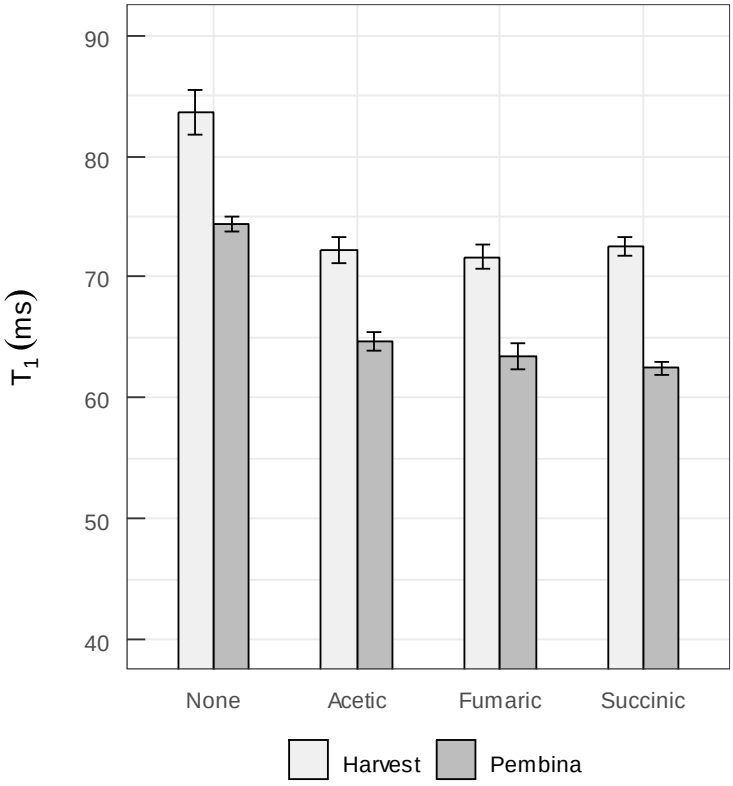

Figure 5.3 T_1 values for dough samples prepared with Harvest or Pembina flour at 1.0% NaCl containing 0.001% GO (both by flour wt.) and either no acid, acetic acid, fumaric acid, or succinic acid. Values provided are the mean ± 1 standard deviation (n=3).

Figure 5.4 shows the predicted variation of T_1 and T_2 with the correlation time, or the time it takes for a molecule to rotate 180°. At the highest relaxation efficiency, T_1 values are minimized,

which indicates a maximum interaction of protons with the lattice and this is generally associated with reduced water mobility compared to bulk water, which has T_1 and T_2 values on the order of seconds (Simmons & Vodovotz, 2012). The schematic of Figure 5.4 can be used to help explain the motion of water on the MHz timescale based on this relationship. Generally, longer correlation times are expected of larger molecules (Keshari & Wilson, 2014). T_2 values are smaller than T_1 values because magnetic dipole-dipole interactions between ^1H nuclei contribute to the spin-spin relaxation (Rummeny et al., 2011). In mobile liquids, at short correlation times, the dipole-dipole interaction are averaged out by rapid molecular tumbling, so that T_1 and T_2 are nearly the same (Figure 5.4). Correlation times are affected by temperature changes; increasing the temperature decreases the correlation times (Cavanagh et al., 1995).

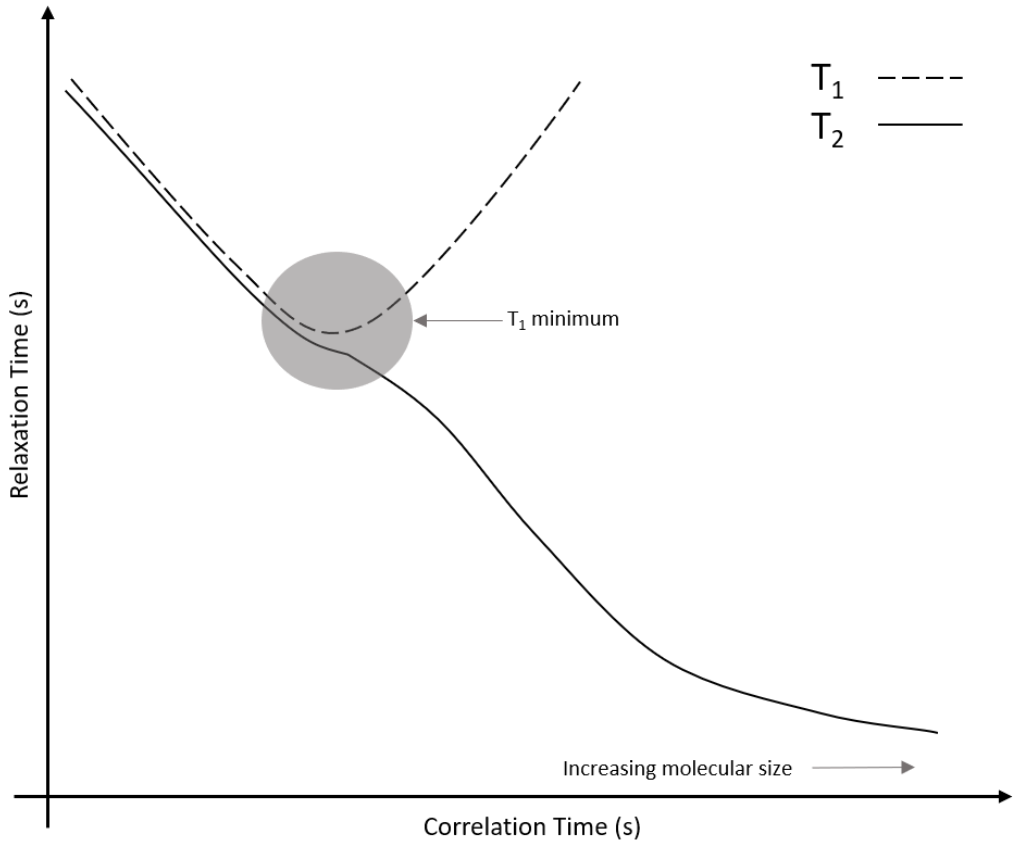

Figure 5.4 A schematic of T_1 and T_2 values plotted against correlation times. The figure was adapted from Keshari and Wilson (2014) and based on the work of Bloembergen et al. (1948).

T_1 values in Figure 5.3 were recorded at 25°C, but another set of samples was examined at 40°C (data not shown) to determine whether the T_1 values were approaching the minimum or moving away from the minimum of Figure 5.4. Increasing the temperature mostly resulted in small increases in T_1 values, indicating that the dough samples in the current measurements are to the left of the T_1 minimum. This finding, in conjunction with the T_{2B} values decreasing in samples which also had decreasing T_1 values, indicates that the inclusion of acids or the use of Pembina flour shifts the samples to higher correlation times, from the left towards the T_1 minimum in Figure 5.4, which suggests that the inclusion of acid is not breaking down the structure of the dough into smaller molecules. This approach to the T_1 minimum also suggests that these samples (prepared with Pembina flour or with acid inclusion) have reduced molecular motion on the MHz timescale, in comparison to those without acid or produced with Harvest flour. A change in flour type appears to reduce the T_1 values to a greater extent than acid, and acid type appears to have no effect. Decreased amount of spin-lattice interactions, or water-polymer backbone (gluten network, starch, etc.) interactions indicate a reduction in motion at the surface of the polymer. This could be due to greater water-binding (to reduce molecular motion), or swelling of the gluten proteins, which has been known to occur with organic acids in dough systems (Upson & Calvin, 1916; Jayaram et al., 2014). The A1 ratio results suggest there is evidence for greater binding but increases in bound water were minimal, therefore it is possible that colloidal swelling is at least partially responsible; swollen hydrogels have been shown to restrict water mobility and water diffusion rates (Alam et al., 2014). Lower water addition in Pembina based on experimental design could explain why it has reduced mobility compared to Harvest. The lower T_1 values observed for acid are possibly due to the pH changes which occur; preliminary work has shown that pH drops from ~6 to 5.1 – 5.6 depending on choice of acid (data not included). pH reduction would result in minor protonation and may have some small effects on overall protein charge (for example, histidine has a pKa of 6.0) (Nelson & Cox, 2008). The pH changes could affect water binding and may result in the changes to water mobility observed, however, it is unclear how this relates to stickiness and dough handling without further investigation.

5.4.1.4 Spinlock

The final aspect of this testing was spinlock, which was utilized to observe $T_{1\rho}$, or spin-lattice relaxation in the rotating-frame. $T_{1\rho}$ assesses motion on the kHz timescale, which is generally associated with slower motion in the lattice, often with macromolecular motion such as those of proteins or side chains (Chen, 2015). Figure 5.5 shows the variation of $T_{1\rho}$ with spin-lock pulse power (the attenuation of which is measured in dB). The higher the spin-lock pulse power (left hand side of the plot), the higher the frequency of rotation of magnetization caused by the spin-lock pulse (f_1) and the faster the kHz timescale motions being assessed. Where the $T_{1\rho}$ is long (at 8dB), f_1 is poorly matched to the frequencies of kHz timescale polymer motion. Statistics were compared across all samples at the 8dB level, since all doughs showed similar dependencies upon the level of attenuation (dB). It was found that the cultivar and acid inclusion effects were highly significant (p<0.001), and the cultivar-acid interaction was significant (p<0.05). Overall, the inclusion of any type of acid reduced the $T_{1\rho}$ values across all dB levels in comparison to the no-acid control. Pembina had lower $T_{1\rho}$ values across all dB levels in comparison to Harvest doughs. For all samples, relaxation efficiency was found to be lowest at 8dB (highest $T_{1\rho}$ values), and relaxation efficiency improved on either side of 8dB. In the limit of very low spin-lock pulse power (16dB), the measured relaxation rate approaches T_2 (Hills, 1998) and the single exponential time constant in Figure 5.5 is expected to be a weighted average of T_{2A} and T_{2B}. When fitting the data, it was noted that a simple monoexponential model did not represent *all* the data effectively; however, a more complicated biexponential model represented a smaller fraction of the data well, so the simpler model was utilized.

At 8dB, the $T_{1\rho}$ relaxation is least efficient, which indicates that the side-chain motions are at a frequency furthest from f_1. The data indicate that kHz timescale motion is altered by the presence of acid and by the use of Pembina flour. In both cases the $T_{1\rho}$ relaxation is more efficient, which is likely an indication of some change in the intermediate polymer motion, such as the mobility of side chains (bringing the frequency of kHz timescale motions closer to f_1). The data are not sufficient to distinguish between an increase or a decrease in side chain mobility. While the changes observed with cultivar are relatively small, there are significant motion changes observed with the addition of acids. However, more work is necessary to characterize the specifics of these changes.

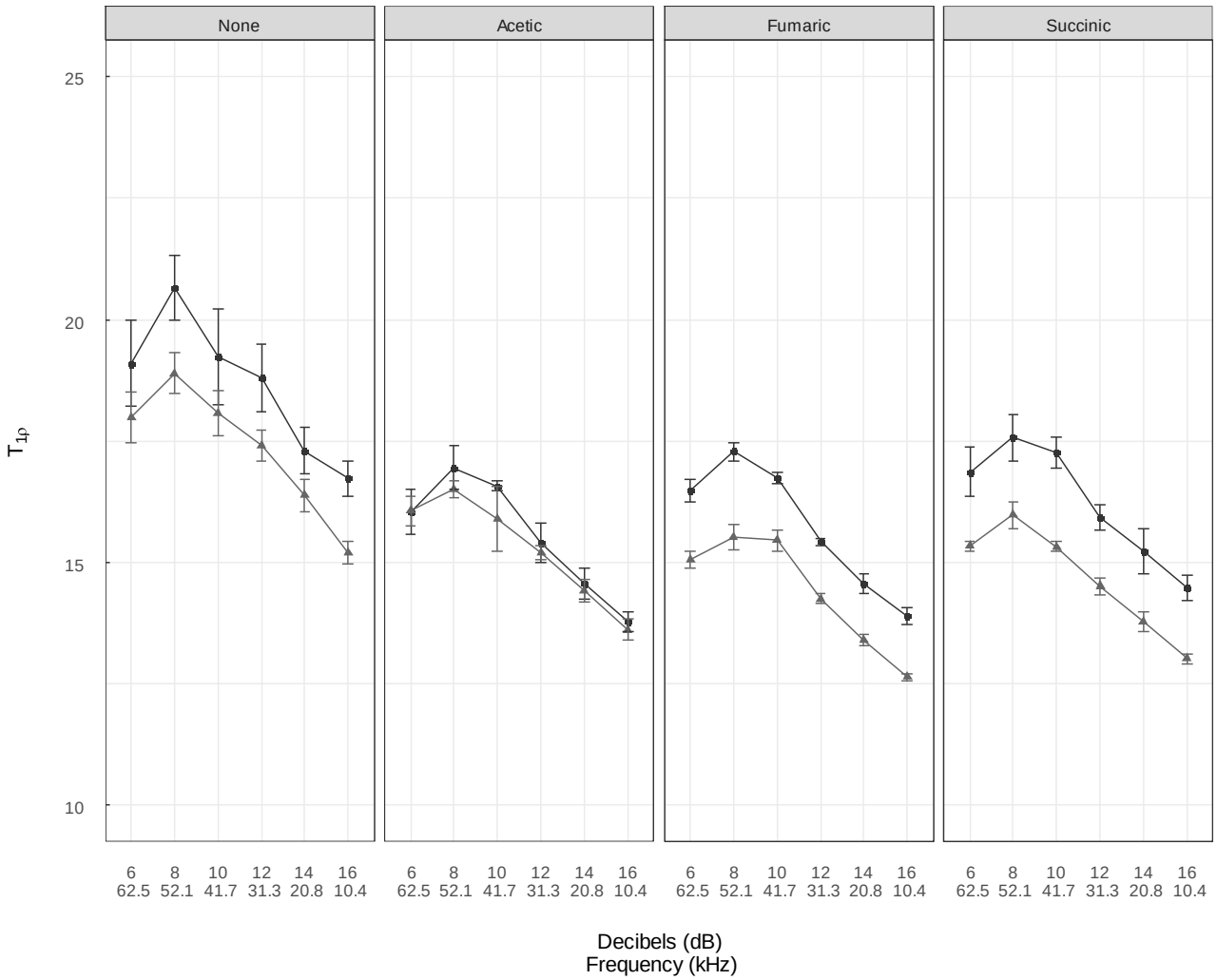

Figure 5.5 $T_{1\rho}$ values for dough samples prepared with Harvest or Pembina flour at 1.0% NaCl containing 0.001% GO (both by flour wt.) and either no acid, acetic acid, fumaric acid, or succinic acid and spinlock pulse power levels decreasing from 6-16dB (attenuation compared to the 90° pulse power). At low attenuation the pulse power is high and the frequency of rotation of magnetization caused by the spin-lock pulse (f_1) is high. Where the $T_{1\rho}$ is long (at 8dB), the frequencies of kHz timescale molecular motion are poorly matched to f_1. Values provided are the mean ± 1 standard deviation (n=3).

If the motion changes are slower, it is possible that this is due to colloidal swelling which can be caused by acid addition, as discussed above in the IR results (section 5.4.1.3 above). However, an increase in kHz timescale motion with the inclusion of acids, would be consistent with an increase in side chain motion of proteins within the gluten network, which could result in increased stickiness. This idea is visualized in Figure 5.6. This concept may explain why there is decreased motion on the MHz timescale (water molecule tumbling, and water interaction with the polymer surfaces), as there would be increased interaction of water within the protein side chains to reduce motion on a different timescale (reduce MHz motion and increase kHz motion). The increased kHz motion could contribute to the stickiness observed by others when organic acids were included (Stone et al., 2018). However, this idea fails to address the lower $T_{1\rho}$ values observed for Pembina flour versus Harvest, as doughs prepared with Pembina flour show superior handling characteristics and reduced stickiness compared to Harvest. A greater range of spin-lock pulse powers will be required to say definitively if motion was faster on the kHz timescale with the addition of organic acids, and to distinguish what is occurring with the different cultivars.

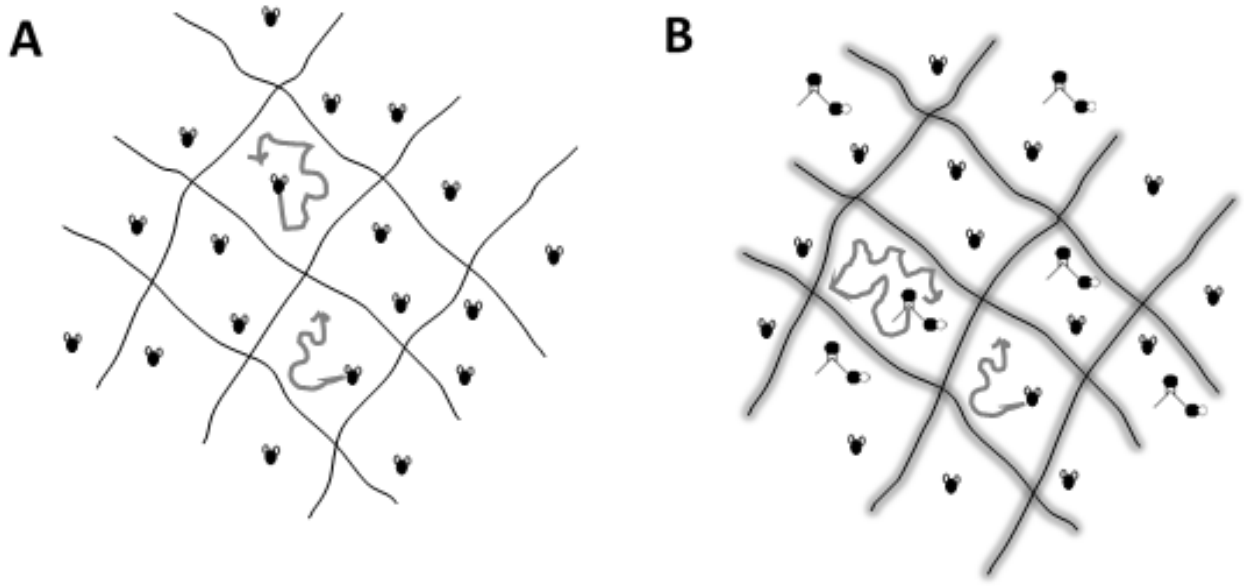

Figure 5.6 Proposed potential mechanism schematic of action of acids on gluten network which may be a cause in increased stickiness and the determined rheological behaviour. (A) Without acid, (B) with an organic acid.

5.4.2 Diffusion

The second body of work examined how protons diffuse throughout the dough system, and aimed to assess how restricted the diffusion of water would be within a dough system taking ^{1}H diffusion to be indicative of water diffusion. Previous diffusion work in dough systems has been completed by Umbach et al. (1992), who found that the gluten slowed the diffusion of water to a greater extent than starch. Work has also been completed in the final baked goods; increasing gluten content did not slow diffusion as observed by Wang et al. (2004). To our knowledge, diffusion of water by NMR with respect to dough formulation alterations has not been previously studied. The diffusion times examined were 7.5, 15, and 30 ms, and the data were fit to a simple model of two diffusing species (faster and slower diffusers). The 30 ms data were not well represented by this simple model, but the signal-to-noise ratio of these data did not justify a more complicated model: only the fits to 7.5 and 15 ms data are presented here. The diffusion of all protons within the system is restricted, as both the faster ($3.22x10^{-4}$ mm^2/s) and slower ($6.21x10^{-6}$ mm^2/s) diffusion coefficients are much lower than that of free water ($2.3x10^{-3}$ mm^2/s at 25°C); the faster diffusion coefficient is only 14% of the diffusion coefficient of free water, and the slower diffusion was 0.27% the value. Restricted diffusion is likely caused by a combination of interactions with the polymer network, including simple steric hindrance and adsorption, van der Waals forces, and ionic interactions. Slow diffusion ratios of the samples are presented in Figure 5.7; the slow diffusion ratio is the ratio of slower (confined) diffusers to faster (relatively unconfined diffusers) in a system.

Results of the statistical analysis showed that the inclusion of acid, the cultivar, diffusion time, and any of the interactions were not statistically significant. This suggests, at the precision of this experiment, that the inclusion of acid, and the differing cultivar, which show differences in other aspects of this study, do not alter the diffusion of water in the system. Therefore, any differences observed in dough handling and other water related characteristics are due to other mechanisms, or on a scale not detectable by this diffusion measurement.

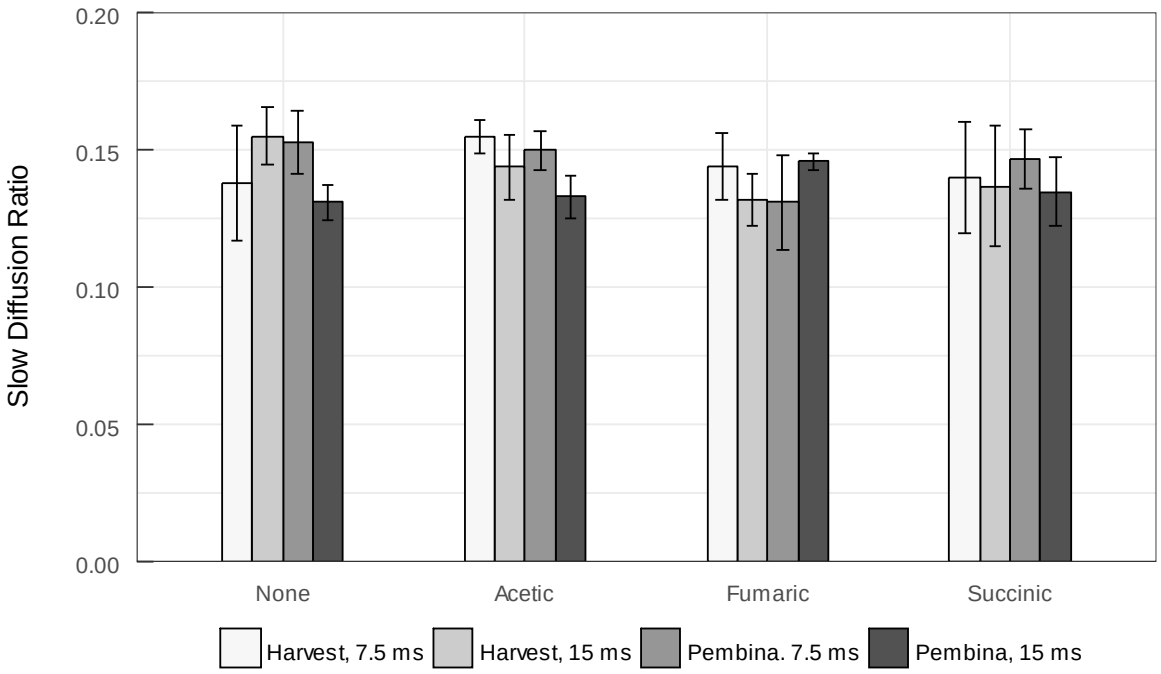

Figure 5.7 Ratio of the fraction of slow diffusers for dough samples prepared with Harvest or Pembina flour at 1.0% NaCl containing 0.001% GO (both by flour wt.) and either no acid, acetic acid, fumaric acid, or succinic acid. Values provided are the mean ± 1 standard deviation (n=3).

5.4.3 Yeast

Yeast was included as a secondary control to compare to the inclusion of organic acids which can be produced during bread production (Table 5.1). The purpose of the organic acid model was to simplify the dough as a way to improve understanding of the stickiness with a less complex system. The yeast samples were prepared to get an idea of how well this more simplistic model dough would mimic doughs which contain yeast. In general the yeast samples showed similar trends to the acid inclusion results; yeast inclusion had a larger reducing effect on the T_1, T_{2B}, and spinlock values (increased relaxation efficiency) and increased the A1 ratio and slow diffusion ratio. This suggests that the inclusion of acids is likely a suitable, simple model to provide valuable insights into the mechanisms of stickiness and dough handling in real dough systems. The simplified doughs still give an accurate indication of the trends that are occurring and may provide insights that are relevant to the more complex doughs, and by extension, the final bread products.

Table 5.1 Values of ^{1}H NMR (FID, IR, CPMG, spinlock) and diffusion parameters for dough samples prepared with 1.0% NaCl, 0.001% GO, and 3.0% yeast (by flour wt.) with either Harvest or Pembina flour. Compared to control samples containing no yeast. Presented as average ± 1 standard deviation (n=3).

	Control		**Yeast**	
	Harvest	**Pembina**	**Harvest**	**Pembina**
T_2* (ms)	16.25 ± 0.49	16.74 ± 0.17	16.03 ± 0.18	16.17 ± 0.19
T_1 (ms)	83.6 ± 1.8	74.4 ± 0.6	67.0 ± 1.0	59.9 ± 0.3
T_2, long (=T_{2B}) (ms)	19.0 ± 0.6	18.8 ± 0.5	16.6 ± 0.3	15.6 ± 0.8
A1 Ratio	0.31 ± 0.01	0.33 ± 0.02	0.36 ± 0.03	0.40 ± 0.00
Slow Diffusion Ratio:				
7.5 ms	0.138 ± 0.021	0.153 ± 0.011	0.173 ± 0.015	0.173 ± 0.017
15 ms	0.155 ± 0.011	0.131 ± 0.007	0.159 ± 0.014	0.178 ± 0.007
Spinlock:				
6 dB	19.10 ± 0.88	17.98 ± 0.53	15.68 ± 0.49	14.66 ± 0.25
8 dB	20.65 ± 0.68	18.89 ± 0.42	16.14 ± 0.85	14.86 ± 0.26
10 dB	19.23 ± 0.98	18.08 ± 0.47	15.57 ± 0.59	14.08 ± 0.31
12 dB	18.81 ± 0.71	17.40 ± 0.33	14.79 ± 0.45	13.52 ± 0.39
14 dB	17.30 ± 0.47	16.38 ± 0.34	13.81 ± 0.31	12.75 ± 0.38
16 dB	16.73 ± 0.36	15.20 ± 0.23	12.97 ± 0.34	12.01 ± 0.43

5.5 Conclusions

This work provided insight into some of the roles that yeast-produced acids play in water mobility and water interaction within doughs, and may help to elucidate the mechanisms of dough stickiness and rheology. It is clear that the inclusion of these acids alters the motion of the doughs on the MHz and kHz timescales and interactions occur on the polymer surfaces and with side chains; however, the inclusion does not have significant effects on the overall structure, polymer backbones and diffusion behaviour of water molecules within the dough. This indicates that handling changes and stickiness can be linked to interaction/alteration which occurs at the surfaces of the polymers or side chain interactions. Colloidal swelling may play a role in slowing the motion of water in doughs containing organic acids, and this swelling may cause or partially result in the increased stickiness and poor dough handling that has been observed by others, but this hypothesis

requires further investigation. The idea that acid inclusion increases side chain motion and creates stickiness is conceivable but is not definitively supported by this experimental data since it fails to address the differences seen in side chain motion for different flour samples. Further understanding of the reasons for increased stickiness and poor dough handling are important for future work in reducing stickiness in low sodium bread and developing approaches for acceptable final product quality.

6. GENERAL DISCUSSION

6.1 Overview

This thesis was split into three main sections aimed at improving the understanding of the relationship between water and stickiness within doughs using a simplistic dough model, and investigating potential uses of enzymes in combating dough stickiness. The first piece of work aimed to understand if crosslinking enzymes can improve dough strength and reduce stickiness in low sodium doughs, particularly doughs produced with weaker gluten cultivars. The second body of work increased the complexity of the simple dough model slightly by including organic acids commonly produced by yeast in dough. The latter work was designed to gain a better understanding of the interactions which may occur between glucose oxidase (GO) and the selected acids. The third piece of work examined water mobility in these doughs by ^{1}H nuclear magnetic resonance (NMR). This was investigated to better understand the relationship between water and dough attributes, and it was hoped it would link the water attributes to observed dough handling characteristics.

6.2 Dough model

The dough model used in this work was a very simplistic model consisting primarily of flour, water, and salt, with some other additives depending upon the study. The model did not contain yeast (the fourth essential ingredient in bread) due to the heterogeneity it can produce with regards to rheology; active yeast results in continual changes in the dough system with regards to rheology, structure, bubble shape, formation, size, etc. (Salvador et al., 2005). Therefore, yeast-less systems were investigated (except for a set of samples in Chapter 5) to simplify the model and investigate the effects of the additives specifically on the gluten network development. This was also intended to develop a greater understanding of the interaction and mechanism of some of these components at a basic level which may have been more difficult to separate or understand with more complex models which included yeast. The ^{1}H NMR (Chapter 5) work included a yeast

sample as a control to investigate how well the trends of the no-yeast acid model matched against a yeast model, but it was not a primary focus of the study.

With respect to cultivars, two cultivars were selected based on a baking trial previously completed by members of our research group which included 37 cultivars using 1.0% and 2.0% NaCl levels (by flour wt.) (Yovchev et al., 2017). The two cultivars were selected because of their contrasting character; Pembina showed good strength and low stickiness at reduced salt levels and Harvest demonstrated the opposite (Yovchev et al., 2017). Therefore, these two were selected for this research to highlight differences in the systems and to determine how effective enzymatic inclusion would be at improving weaker gluten cultivars in comparison to stronger ones particularly at low salt levels. The two cultivars contain different flour constituents, therefore they had different water absorption capacities and optimal water contents to develop dough. Many factors can affect water absorption capacities including several which increase water absorption capacities such as higher protein content, more damaged starch, higher number of water-soluble pentosans, lower moisture content, increased flour colour, bran and/or ash level, or enzymatic activity, dependent upon the enzyme (Cauvain & Young, 2003). As a result, to produce doughs which have optimal characteristics, FAB values were used to determine optimal water addition and then applied to each dough system, which means that while they are both in optimal form, they do not contain exactly the same amount of water or flour addition. If too much water is added it can result in weaker, soft doughs, but too little will result in doughs which are too stiff and difficult to mold so optimal water addition is important for assessing dough parameters (Cauvain & Young, 2012). The system was produced on a 14% m.b. as it is a standard value for comparison of moisture-dependent testing in the field (AACC International, 1999).

The organic acid component of the model (Chapter 4) was included to attempt to mimic some of the metabolites which can be produced by yeast. Acids and levels of inclusion were selected based on some previous work completed by the group (Stone et al., 2018) in addition to work done by previous authors showing which acids were most abundantly produced by yeast during bread production; succinic, acetic, and lactic (Jayaram et al., 2013). This model is still a great simplification without the inclusion of yeast, however, it aims to investigate the relationship of these components with the development of the gluten network specifically with the goal of increasing the understanding of the stickiness mechanism(s) in order to develop superior low salt bread in full formulation. The acids were included at the same concentration (1.2mmol/100g flour)

to account for molarity of the acids as opposed to weights and therefore to deal with the differing molar masses of the acid compounds as they ranged from 60.05g/mol (acetic acid) to 192.12g/mol (citric acid).

6.3 Assessment of crosslinking enzymes

The investigation of the effectiveness of crosslinking enzymes was discussed in-depth in Chapter 3 of this thesis. This work was of interest due to the preexisting linkage between poor handling and increased stickiness, and low sodium doughs (Farahnaky & Hill, 2007; Belz et al., 2012) which becomes a greater issue in the light of the impending Health Canada restrictions on sodium in bread products (Health Canada, 2018). Beck et al. (2012) identified that the reduction in sodium results in poorer gluten network development; the structures were less interconnected than those with higher sodium contents. This is thought to be due, at least in part, to greater amount of protein-water interactions and fewer protein-protein interactions which occur because of the reduction in charge screening from salt reduction (Lynch et al., 2009). As a result, crosslinking enzymes were of interest as a potential way to improve the gluten network development in reduced sodium environments and therefore hopefully reduce the stickiness and improve rheological characteristics such as dough strength. Both glucose oxidase (GO) and transglutaminase (TG) have previously been investigated in bread as improvers for weak flour but work has not really focused on them for the purposes of alleviating issues associated with low salt systems (Bonet et al., 2006; Steffolani et al., 2008; Steffolani et al., 2010; Decamps et al., 2012). The work relating to this topic presented in this thesis determined that GO improved rheological characteristics more than TG at the equivalent level of enzyme inclusion (0.01% by flour wt.) and that stickiness was also reduced to a greater degree with this system. GO was also compared to a lower concentration which showed similar results to the higher concentration (0.001% versus 0.01% by flour wt.) however, for TG only the higher concentration (0.5% by flour wt.) was found to have much effect on these parameters. This was somewhat unexpected based on the original hypotheses of this work; it was believed that the direct crosslinking mechanism of TG would have a greater effect when compared to GO as it was shown to increase protein aggregation in some previous work by others (Steffolani et al. 2010). However, based on the rheology, stickiness, and crosslinking results it is possible that this is the case for a number of reasons. Firstly, the necessary components for mechanism of action; TG requires glutamine and lysine, and lysine tends to be low in wheat proteins (Woychik et al.,

1961; Zhang et al., 2009) whereas GO produces additional disulfide linkages (Rasiah et al., 2005). While both saw some improvements regarding the % glutenin macropolymer (GMP) particularly for Harvest samples (weaker doughs) GO appeared to be somewhat superior to TG with regards to Pembina for this value. Free thiol content was significantly lowered by GO but not by TG which is expected as GO coverts free thiol groups to disulfide linkages (indirectly) whereas TG does not, instead directly linking proteins via an acyl transfer reaction that links glutamine and lysine moieties (Zhang et al., 2009). However, the potentially small increase in free thiol seen in TG samples could be due to protein rearrangement which may result in fewer disulfide linkages, as these proteins could also crosslink with other non-glutenin proteins in the system (albumins, globulins, etc.) (Steffolani et al., 2008; Steffolani et al., 2010). Additionally, GO is an enzyme which can have activity with other dough components, such as arabinoxylans, which have been associated with dough stickiness in previous work, although they were not assessed in this study (Decamps et al., 2012). TG can also have interactions with other components which may not result in crosslinking, such as the conversion of glutamine to glutamate (by reacting glutamine with water) which would not be productive for gluten network development (Zhang et al., 2009). Finally, while by weight the inclusions of these enzymes were equivalent, the unit activity was not; GO contained ~10000 glucose oxidase units (GODU)/g, whereas TG contained activity on the scale of ~100U/g, therefore, that discrepancy could affect the results and explain why TG was so much less effective.

One of the largest factors which affected the results of this study was cultivar selection, which was significant in every aspect of the study with Pembina producing significantly better dough handling characteristics in comparison to Harvest. While the inclusion of enzymes appeared to affect the Harvest samples and low salt samples more, Pembina required less inputs to produce superior characteristics, which suggests that cultivar selection for baking may be one of the most important aspects when considering production. The need for higher quality cultivars could be an issue as it may limit use of sub-par cultivars or poor quality crop years. However, it is possible that enzymes could be used to improve the weaker dough cultivars and may not be necessary for the stronger ones. It is possible that cultivar blending could alleviate some strength and stickiness issues, but that would still not deal with any other formulation concerns with respect to flavour loss from reduced salt content, etc. This work reinforces the idea that cultivar selection remains important and that stronger dough cultivars are significantly less affected by salt changes than

weaker cultivars. This is thought to be linked to things such as the protein content and quality, gluten strength, ratio of high molecular weight-glutenin subunits (HMW-GS) to low molecular weight-glutenin subunits (LMW-GS) (Khatkar et al., 1996; Wieser, 2007; Kontogiorgos, 2011).

While these results are useful with regards to rheology and stickiness in simple models, there are flaws within the model which limit effectiveness, and concerns about the final product which are not addressed with this solution. While these enzymes can improve handling characteristics, this is not necessarily a good indication of baking quality, as the relationship between rheological behaviour and baking quality is not definitive, especially with some rheological parameters (Weipert, 1990; Autio et al., 2001; Van Bockstaele et al., 2008). Reduced salt has been linked to a variety of negative final quality characteristics such as poor crust, crumb, and stiffness (Lynch et al., 2009), so without a baking trial it is difficult to assess how well these enzymes would improve these characteristics, but some other trials have suggested there is promise in full salt formulations with regards to dough mixing stability (Bonet et al., 2006), improved crumb structure, improved bread volume (Caballero et al., 2007), and softer crumb (Steffolani et al., 2010). However, some negative effects, such as decreased gas retention have also been observed (Bonet et al., 2009). Additionally, while the function in gluten network development is one of the most important that salt offers, it also performs several other functions in bread which are not addressed by the solution of enzymes, such as flavour, both the issue with "yeasty" flavour and blandness (Lynch et al., 2009) and also issues with over-active yeast, poor shelf-life and increased food safety concerns (Samapundo et al., 2010; Markus et al., 2012). Further work into this area would need to be conducted and would require baking work and shelf-life trials. Reformulation investigation is also important considering lower salt conditions, such as work with flavour deficiencies from reducing NaCl and the inclusion "clean-label" ingredients. Enzymes work well for this framework as they are "clean-label". This work is a good foundation for suggesting the possible efficacy of enzymes in improving low salt characteristics of bread (particularly GO). However, further issues would have to be dealt with to prove its functionality in the market from the perspective of the final product, in addition to the increased production costs associated with enzymes. Additionally, this work confirms that cultivar selection remains important and will continue to have to be addressed moving forward in these types of studies as cultivars can perform very differently.

6.4 Investigation of acids with regards to dough handling

The focus of Chapter 4 of this thesis was on the effects of organic acids on dough handling and stickiness and it also began investigations into the effects of organic acids on water properties of doughs. This work was of interest for a number of reasons; yeast produces some organic acids as metabolites, and since the model does not include yeast it was one way to investigate the effects of some yeast metabolites without including yeast. This study limited the enzymes studied and level of salt due to previous work; only 1.0% NaCl (by flour wt.) was used and only a lower concentration of GO was investigated (0.001% GO by flour wt.) because in Chapter 3 GO was determined to be more effective than TG, and the differences between concentrations of GO used were not very significant. It was found that the inclusion of acids generally had negative impacts on rheology and increased dough stickiness, however, %GMP was not affected and freezable water content (FWC) was only slightly affected. The inclusion of GO had similar results to the first study on rheology and stickiness and did not affect %GMP or FWC. When combined, GO and the organic acids results landed in between only enzyme inclusion or only organic acid inclusion, as expected. The only acid to have differing behaviour was ascorbic acid, which is presently used as a bread improver, and found some improvements in rheological behaviour and stickiness. Ascorbic acid did not improve the dough handling further when it was included with GO, which is possibly due to overdosing; the concentration of ascorbic acid used in this work was much higher than what would typically be included in bread, and it is possible that it began to act as a reducing agent at this concentration (Millar & Tucker, 2012). Additionally, ascorbic acid would compete with sites used by GO for crosslinking, and therefore at high levels any positive interactions may be negated due to all sites being occupied. Ascorbic acid inclusion in this experiment was calculated to be the same molar level as the other acids included (1.2mmol/100g) to have the same level to compare all acid types, which resulted in inclusion levels much higher than the maximum addition of ascorbic acid allowed in bread, which is 200ppm (Health Canada, 2012a). However, this likely resulted in overdosing of the system which can occur at levels higher than 200ppm (Xiuzhen & Sieb, 1998). While this concentration was selected for this study to have equal addition to the doughs across all acids, future studies which examine acid effects should take the acceptable addition levels and overdosing effect into consideration. Fumaric acid did in some cases show significantly worse behaviour than the other acids, however, it was mostly mitigated when GO was added with it. It has been suggested that fumaric acid can form linkages with gluten proteins

via free radical reactions after disulfide linkages are broken and affect the network development (Sidhu et al., 1980), however it was not investigated in this work. Cultivar again played a very significant role in the results, with Pembina having more favourable results (stronger rheology, lower stickiness, etc.) than Harvest.

This acid work builds on Chapter 3 by expanding the model and investigating how effective the enzymes would be in the presence of acid. Acids have been shown to influence gluten and doughs' dating to the early 1900s; authors investigated the effects of acid, alkali and salt addition on gluten (Wood, 1907; Wood & Hardy, 1909). Other early work by Upson and Calvin (1916) determined that water absorption in gluten was increased by small concentrations of certain acids; lactic, acetic, and hydrochloric acid (0.02N, 0.04N, and 0.005N respectively), however that at higher concentrations water absorption is decreased particularly rapidly with HCl. Both lactic and acetic acid had concentrations which produced optimal swelling in gluten and produced soft, gelatinous gluten, and with all acids the addition of salt drastically reduced the swelling of gluten (Upson & Calvin, 1916). These original studies in the early 1900s dealt with gluten separate from the rest of the flour, which provides good insight into the effects of the acids on gluten but not the overall system. More recent work with acids has also been completed; Wehrle and others (1997) investigated the effects of lactic and acetic acid on dough rheology and found decreases in dough stiffness and increases in viscous behaviour of the dough. Jayaram et al. (2014) investigated the swelling properties of doughs including succinic acid and found that swelling was observed at concentrations of 10mmol/100g and higher, which is significantly higher than what was observed in this work, but noted significant characteristic changes in doughs containing succinic acid at levels comparable to what would be seen in doughs (1.6mmol/100g or less). This work showed similar results in terms of rheology, and the increase in stickiness could possibly be attributed to the increase of water absorption which might be attributed to the inclusion of acid as the concentrations of acid included were low (1.2mmol/100g) and may exhibit the swelling behaviour indicated by Upson and Calvin (1916), especially as salt levels in these doughs were low, and salt was one of the factors which reduced colloidal swelling. Changes in dough handling and characteristics have also been attributed to pH changes; the pH change was sometimes significant, becoming as low as 4.8 ± 0.1 (citric acid) from 6.1 ± 0.1 (no acid), however, the rheology and stickiness of samples do not completely follow the trends of the pH changes which suggests that some other mechanism(s) are also involved.

There are a few flaws in this study relating to actual dough systems; the most obvious is that this is only one metabolite produced by yeast, and it cannot encompass the full effects of yeast. Additionally, the enzyme would likely be added in the mixing process along with yeast, however since the acids are yeast metabolites, they would not exist in the concentrations that they are used in this study initially, except for some, which were shown to be present in the flour and not produced by yeast such as lactic acid (Jayaram et al., 2013). Therefore, the interactions of these acids with the enzyme may be more complicated than presented in the model and they may interfere with the crosslinking of the enzymes less than observed in this work in real bread systems as they will be in lower concentrations during the enzyme addition step. Additionally, this is not an accurate representation of the levels in which these acids would be present in the system; for the sake of comparing acids they were added at the same molarity however, succinic acid is the most produced acid in yeast by a factor of 10x according to Jayaram and others (2013), so the effects that these acids may have on the flour system could be different depending upon what acids are produced, in what amounts they are present, and the endogenous acids present in the flour. Overall this work provides some insights into the interactions of simple water and flour doughs and yeast-produced organic acids, however, more work needs to be done to understand the mechanism(s) of acid interference in dough development and increased stickiness.

6.5 Water mobility, acids, and linkages to other observed dough characteristics

To build upon the dough handling work of the previous study, this final body of work assessed the water mobility of doughs prepared with organic acids (Chapter 5) to attempt to link the previously observed handling characteristics with information about water mobility as determined by ^{1}H NMR. The model was based on the work in Chapter 4 but altered slightly; all dough models included low levels of GO (0.001% by flour wt.) and only 1.0% NaCl (by flour wt.), and acids were limited in comparison to Chapter 4 (acetic, fumaric, and succinic) based on the results of that work. A yeast control was also included (3.0% by flour wt.). The general findings were that the overall structure was not greatly affected by acid type or inclusion, and cultivar effects were minimal. Motion was assessed on the MHz and kHz timescales; it was found that acid inclusion and Pembina flour reduced motion on the MHz timescale, and resulted in reduction of motion at the protein polymer surfaces than non-acid and Harvest flour samples. Both acid and Pembina altered motion on the kHz timescale, but it was not determined whether the motion on

the kHz timescale was shifted faster or slower. Diffusion behaviour was not affected by acid inclusion or flour type. Results for dough containing yeast significantly differed from non-yeast samples, as expected, because the yeast was active and has several different effects other than just acid production, however, the trends were generally in the same direction as acid trends. Samples which included yeast showed higher diffusion behavior in comparison to other samples.

When this work is assessed against the dough handling and stickiness characteristics of Chapter 4, there are a few trends to discuss. First, the results of this work generally showed overall less significant differences between Pembina and Harvest compared to the previous work. This may be due to the inclusion of GO in all samples; rheological behaviour of samples was generally more similar for samples with enzyme than without; such as tan δ (Figure 4.1B) and J_{el} values (Figure 4.2B). One interesting difference was that the T_2^* (Figure 5.1) values did not correlate well with %GMP values (Figure 4.5); while Pembina appeared to have increased %GMP or an increase the amount of glutenin crosslinking in the system, it did not appear to affect the overall structure (as determined by T_2^*) significantly. However, T_2^* is an indicator of how fine the porous structure is and the overall homogeneity of the pores in the structure but it is not a direct measure of pore size, so it is likely that this can explain some of the observed discrepancies between results. It might be expected that Pembina samples have greater homogeneity of structure compared to Harvest samples, and there are some slight differences observed in T_2^* values which may explain this. It is also possible that the measurement is not precise enough to detect differences between the two cultivars. Harvest samples contained a lower bound to free water ratio (Figure 5.2B) and had higher motion at the surfaces of protein polymers (e.g. water interactions with protein backbone and side chains) (Figure 5.4) compared to Pembina samples. This could help to explain some of the stickiness behaviour if free water is at least partially causing stickiness, and it correlates well with the DSC findings from Chapter 4 which showed that Harvest samples contained higher FWC compared to Pembina samples (Figure 4.4). However, it fails to explain the acid results.

The ^{1}H NMR results for Pembina and acid samples trended in the same direction; reduced MHz motion and altered kHz motion, however, this is opposite to what was observed for the rheology and stickiness behaviour of samples in Chapter 4, as acid samples showed poorer rheological behaviour and increased stickiness in most cases. Dough stiffness (|G*|) and motion at the polymer surfaces (T_1) do not appear well correlated. The inclusion of acids resulted in relatively

similar dough stiffness (when GO was also included), however, it decreased motion at the protein surfaces. Pembina doughs also saw a decrease in motion at the polymer surfaces, however these samples had higher dough stiffness when compared to acid samples. Stickiness results were also contrasting for acid samples and Pembina; Pembina had the lowest stickiness of all samples, especially when enzyme was included without any acid. In contrast, acid inclusion tended to increase stickiness, which suggests that motion results do not necessarily paint a full picture of what is happening as dough handling trends do not correlate well with ^{1}H NMR trends. The acid and Pembina trends being so similar for NMR data and different for handling characteristics suggest that this data may not be the most reflective for understanding why these changes are occurring in dough handling. However, it should also be noted that the reasons we see motion and bound water changes in the two systems are possibly due to different mechanisms. It is also possible that other motion changes or interactions are the reason for the changes observed in handling, however, it is difficult to determine at this stage why the trends between these two bodies of work differentiate to such a great degree. pH changes observed in acid samples would have some protonation effects and those will affect interactions of components within the system and may explain why there is reduced motion; there could be increased interactions due to protonation and resulting ionic interactions. O'Connor et al. (1996) investigated cross-linked polymers by ^{1}H NMR and found that molecular tumbling motion was restricted in highly cross-linked systems, which could indicate why slightly lower MHz motion is observed in samples containing Pembina over Harvest flour. It is also possible that colloidal swelling of acids could play a role in the values observed; if colloidal swelling reduced the molecular motion in the system it would explain why there is significantly reduced motion in the systems containing acids compared to those without.

An alternative hypothesis relates to the motion of the kHz timescale, which can be attributed to larger molecular motions than water tumbling, e.g. protein side chains. There is significant change in motion for acid inclusion (and Pembina-based doughs), however, it is unknown whether this motion is increased or decreased on this time scale. In the case of increasing motion on the kHz timescale, it is supposed that while there is overall decreased molecular motion on the MHz timescale (water tumbling and interaction of water with polymers in the dough), the acid addition interacts with protein side chains which increases motion of the side chains on the kHz timescale. It is thought that this increased kHz motion results in increased stickiness. This hypothesis was visualized in Chapter 5 in Figure 5.6.

This hypothesis could explain why there is a reduction in mobile water on the MHz timescale as the water could have increased interactions with the side chains, however, increases in motion on kHz timescale could be contributing to the sticky character samples including acids exhibit. It could also be freezable as per the DSC results in Chapter 4. This would fail to explain why there are also lower $T_{1\rho}$ (kHz timescale motion) results observed for Pembina doughs also, however, there could be another mechanism at play. This idea would require additional information to support the hypothesis that motion is indeed increased on this timescale, but, it is one potential explanation which requires additional work to investigate.

In terms of the NMR data, acid trends showed more significant differences than cultivar effects, which was also different than what was observed with the rheology and stickiness results where cultivar effects tended to show more significant differences. The strengthening effect of GO, which was present in all samples, may explain why there are less differences between the two cultivars. As shown in Chapter 3, Harvest samples were more sensitive to the inclusion of GO than Pembina and saw larger improvements in rheology and stickiness with its inclusion. Having GO in all samples may have minimized differences between cultivars for the results of Chapter 5. Regardless, cultivar differences were still shown to have more significant changes even with enzyme inclusion in some parameters in Chapter 4 such as FWC, %GMP, and stickiness more so than rheological behaviour. While the NMR data provides some interesting information, it appears to be difficult to correlate the results well with the handling data with the present information which exists. Further investigation into both NMR and other water-related parameters would be necessary to develop a greater understanding of any possible connections, and to develop or disprove a hypothesis about water and dough stickiness, and its relationship with yeast-produced acid metabolites.

6.6 Summary

This work contributes to the development of some solutions to the stickiness problems in low sodium doughs; enzymes, which are also in line with new trends of “clean label” foods. GO appears promising in this regard however other flavour and baking deficiencies may have to be addressed. The attempt to understand and link yeast-produced organic acid metabolites, water, and observed stickiness in dough samples was less clear, and more work is needed to fully understand this. Hypotheses of molecular motion changes on the kHz timescale have been posited, but more

work would be required to develop a deeper understanding of these, and at present, only some conclusions can be drawn from this work. Additionally, scaling up of the model complexity to represent actual bread production will be necessary, however, further work should also be done on the simple doughs to better understand the complex nature of dough stickiness, dough handling, and water characteristics within these systems.

7. GENERAL CONCLUSIONS

The overall goal of this research was to address three objectives: to investigate the use of crosslinking enzymes to overcome stickiness and dough handling issues associated with low sodium doughs, to examine the effects of organic acids commonly produced by yeast on dough handling, stickiness, and finally to attempt to link handling characteristics with water characteristics to gain a better understanding of acid and stickiness mechanisms of the dough. The work examined dough rheology, stickiness, crosslinking, and water characteristics using a simple model dough containing a variety of acids, two enzymes, and using two cultivars with contrasting dough characteristics (weak and strong). There are some conclusions which can be drawn from the individual studies and the work as a whole.

Chapter 3 represents the first body of work, assessing the feasibility of using crosslinking enzymes to mitigate some of the issues to dough stickiness. Of the two enzymes examined for use, glucose oxidase (GO) and transglutaminase (TG), GO showed greater effectiveness at improving dough rheology when compared to TG at the same inclusion level; it increased dough stiffness and relative elasticity, and solid-like character, and reduced maximum deformation, in addition to reducing stickiness. The differences between GO inclusion levels were minimal, whereas a higher concentration of TG was required to have an impact on dough characteristics. With respect to crosslinking, GO showed increased crosslinking compared to TG via free thiol, as it is the mechanism of action of this enzyme. Both enzymes had some improvements in % glutenin macropolymer (GMP), however, effects were more significant for GO, and changes appeared to be more significant in Harvest samples over Pembina, particularly at low salt levels. Cultivar effect was highly significant across all parameters; Pembina flour had significantly reduced stickiness, and improved dough rheology compared to Harvest samples, and some improved crosslinking (%GMP). Overall, the findings of this work suggest a few key points. GO (both assessed concentrations) and TG (only the higher concentration) were able to improve dough rheology and reduce stickiness, however, GO was more effective than TG at the same concentration, and cultivar quality remains crucial in determining dough handling characteristics. Additionally, more

significant improvements were observed with the weaker cultivar (Harvest) and lower salt levels (1.0% NaCl by flour wt.) in comparison to the stronger cultivar and higher salt level (Pembina, 2.0% NaCl by flour wt.). This work provides evidence of application of enzymes in addressing low salt issues, as enzymes had previously mostly been studied for improvements of weaker dough cultivars. While it shows promise in addressing issues relating to gluten network development and stickiness which arise from sodium reduction, it does not show if the inclusion will have any effects on other deficiencies brought on from this reduction (e.g. flavour).

The second body of work is discussed in Chapter 4, and it expands on the simple dough model used in Chapter 3 using a series of organic acids, most of which can be produced by yeast during the breadmaking process (acetic, ascorbic, citric, fumaric, lactic, and succinic acid). Dough rheology, stickiness, %GMP, and freezable water content (FWC) were all assessed. Similar to the previous work, cultivar effect was significant with Pembina having superior dough handling, reduced stickiness, lower FWC, and higher %GMP compared to Harvest doughs. Acid inclusion had significant impacts; except for ascorbic acid which is in use as a bread improver, acids increased dough stickiness, have negative impacts on rheology, and slightly increased FWC. Acid inclusion had small to negligible effects on %GMP. In general, acid type did not have a significant effect. Fumaric acid showed poorer dough rheology without GO compared to others and ascorbic acid acted differently in all cases, but the other acids behaved similarly. The inclusion of GO improved dough characteristics, and when included with acids, the negative effects of the acids were somewhat mitigated. Ascorbic acid generally showed some improvements in characteristics, but it increased stickiness, reduced %GMP, and did not result in further improvements when included with GO, which is possibly due to overdosing which could have occurred due to the high level of inclusion in this study. The FWC results were not affected by GO inclusion, however, it is possible that the inclusion of this enzyme is entrapping more water, and entrapped water is still freezable, therefore, would not alter the results. In general, this work expanded upon the understanding of how simple dough rheology is affected by acids and showed that even with the acid inclusion enzymes can improve dough handling. This work leaves questions as to how the acids are affecting the system: pH change appears to be a significant factor but the doughs with the largest pH changes were not the ones that differed most from the control in terms of rheology or stickiness. Investigation into these mechanisms, as well as the relationship of water to the handling properties observed remain, and thus the last body of work aimed to address this.

The final body of work was discussed in Chapter 5, and it examines water mobility and diffusion characteristics in doughs via ^{1}H NMR. This work assessed mobility on the MHz (small molecular motions such as water tumbling) and kHz (larger molecular motions such as side chain motion) timescales, as well as diffusion of protons within the dough matrix to assess water diffusion. It was found that motion on the MHz timescale was slowed by doughs made with Pembina flour and the inclusion of acids in comparison to those made with Harvest or without acid. Heterogeneity of overall structure of the dough structure remained similar across all samples, with some minor cultivar effects, and diffusion behavior was not affected by formulation changes. Motion on the kHz timescale changed significantly with the use of Pembina flour and inclusion of acids, however, it could not be determined whether the motion change was to faster or slower motion. From these results, it is thought that pH changes had a significant impact on the assessed parameters, and the colloidal swelling observed in doughs containing acids may have also played a role, but other mechanistic reasons have yet to be determined. In general, the molecular motion results did not correlate particularly well with that of the dough handling and stickiness results of the previous study, and it cannot be determined if they're not causally linked, or if the methodology to assess them does not correlate the results well. A set of yeast controls were also produced to examine how well the organic acid dough samples modelled actual dough containing yeast. It was found that the trends were in a similar direction (except for diffusion). However, yeast results generally showed more significant changes, which was expected as the model doughs only contain organic acids. Overall, this work shows that formulation changes result in significant motion changes particularly with organic acids and cultivar selection, but some of the specifics of the motion changes require more work to be fully characterized. This work can help to build more foundational knowledge for a better understanding of dough components and water, which may help bakers reformulate in the face of sodium reduction regulations.

Overall, this research has shown that if other technological and monetary challenges can be overcome, enzymes, particularly GO, have potential for use as a bread improver in the low-sodium doughs mandated by government regulations. The amount, and how other technological challenges will be dealt with still require further work. Organic acids produced by yeast have significant effects on dough development and stickiness, and some mitigation for these effects may also be possible with enzymes. However, the mechanism of action outside of pH changes and possibly colloidal swelling requires further investigation to characterize completely. The

relationship with water and doughs remains complicated and not fully understood, however, this work shows that cultivar, and acids can affect water mobility significantly. A further understanding of the mechanism may be able to help producers formulate to limit processing issues which are exacerbated in low salt conditions.

8. FUTURE STUDIES

There are several directions that this work can progress in. Dough is a complicated system and understanding even the basic form which was examined in this work can be difficult It only becomes more complex when components such as yeast are included, and the rest of the processes needed to produce bread. Understanding aspects of the basic components can be helpful for developing solutions to issues which may arise, but there must also be a practical approach which indicates whether solutions are feasible.

On a fundamental level, more research can be conducted to better understand the mechanism of action of acids on the dough microstructure, and how this relates to other aspects of dough handling and final quality. pH is a contributing factor, and it is likely that the acid-water mobility interaction also plays a role, but those functions remain unclear as to the final effects that they have on dough. Further work in this field may lead to greater understanding of dough rheology and stickiness, in addition to understanding acid effects in doughs. Organic acids have also been considered for use as bread preservatives (Corsetti et al., 2000; Marín et al., 2002) so a greater understanding of their roles could provide insight into the feasibility of that option in reduced salt systems. Some more recent work into the understanding of swelling with the inclusions of acids has been completed (Schober et al., 2003; Jayaram et al., 2014), and further work by solvent retention capacity or microscopy could prove beneficial for understanding the extent of swelling, how much it is affecting the doughs, and possibly link it to the observed handling and water mobility characteristics such as ^{1}H NMR. Increasing complexity of the dough model for further rheological work could also be conducted; inactivation of yeast is one possibility for completing the work, although research using active yeast has also been done even though it has issues with some experimental protocols (Newberry et al., 2002; Salvador et al., 2005). While simple dough systems can be useful for understanding some fundamental components of the dough, the lack of full formulation means that there will be some nuances in the full formulation or ingredient interactions which will be missed. Assessing full formulation doughs, in addition to simple doughs and comparing the two may provide further insights.

With regards to water properties within doughs, there is also additional research which can be completed both with regards to mobility, and with water association. Fourier transform infrared spectroscopy (FTIR) could be utilized to assess the water association with the starch and gluten components within the system (Esselink et al., 2003), which could be very complementary to achieving a greater understanding of water mobility and interaction with the dough components especially when paired with ^{1}H NMR work. There is also additional ^{1}H NMR work which could be informative about molecular motions on the MHz timescales; T_2 values with differing pulse sequences (Hills, 1998) can help to differentiate molecular motions on that scale, and interactions or exchange with the lattice (non-proton components) of the system. A broader range of pulse powers (outside of the 6-16 dB range used in this work) may also help to determine how motion is changed on the kHz timescale which may help to develop hypotheses around why these motion changes occur both for differing cultivars, and in systems containing organic acids. While excess water is known to increase dough stickiness (Jekle & Becker, 2012) there remains more to understand about the relationship of water with stickiness in doughs, and dough handling.

The focus of this work is entirely on the gluten network, and how various components or additives interact with, or affect it. However, flour, dough, and bread are all comprised of many components of which gluten is a crucial one, but by no means the only critical component. Therefore, more work into understanding the function of other dough components, such as starch, water extractable (WE)- and water unextractable (WU)-arabinoxylans, and other minor constituents of flour can provide insights into other aspects which may contribute to stickiness or dough quality which have been addressed less significantly. WE-arabinoxylans are important in stabilizing the foam structure during bread production by increasing viscosity, whereas WU-arabinoxylans have the opposing effect by causing physical disruptions to gluten network development, and interfere with water absorption (Courtin & Delcour, 2002). There has been some investigation into arabinoxylans to increase water absorption in pasta doughs (Turner et al., 2008), and it was shown to improve water absorption and reduce cooked stickiness, however, that is for a significantly different application so greater understanding of the roles of both WE- and WU-arabinoxylans in dough stickiness could prove useful, both at a basic and broader level. Work on the role of starch, particularly damaged starch has been completed (Stone et al., 2017) and it was found that increased damaged starch reduces stickiness, which merits further investigation, however, breads with lots of damaged starch have shown some poor final loaf qualities such as

sticky crumb (Sluimer, 2005). Other authors have investigated the use of damaged starch in breads (1.0% NaCl by flour wt.) and found that final loaf quality had some defects; colour was dark and crumb was firm, and they used α-amylase and amyloglucosidase to improve these defects (Barrera et al., 2016). The conjunction of other enzymes with damaged starch may provide some findings of interest. Further work investigating the starch component, and all other non-gluten flour components may help to explain some of the stickiness observed or use various components to aid in maintaining quality after sodium reduction.

One of the critical components which was not addressed in this study was how these components affect the final, baked product. While a deeper understanding of the underlying mechanisms is important, rheological behavior is not a perfect indication of final product quality, and without the inclusion of yeast, the model remains incomplete for assessment of functionality in the final product. A baking trial would be essential for understanding how effective GO would be at improving final product quality and determining whether the enzyme would be effective in a full formulation system, and at what level it is necessary to be included for effectiveness. Several baking trials have been reported; however, they have primarily occurred in full salt formulations (Hanft & Koehler, 2006; Caballero et al., 2007; Dagdelen & Gocmen, 2007). Additionally, due to the highly significant effects of cultivar, it would be advised that future baking trials experiment be conducted with a range of cultivars to assess response to enzymatic activity. In addition to baking trials for examining the effectiveness of GO at improving loaf qualities (crumb, colour, etc.) investigation into shelf-life should also be performed, as the reduction of salt could impact this factor. Sensory testing would also be another important component of this testing, as it is quite likely that further formulation changes will be necessary to have an acceptable product for the consumer; while GO may be able to improve structural deficiencies, other changes such as flavour may prove to be unacceptable to consumers and other alternatives should be examined through this course of study. With the advent of “clean-label” foods, investigation into ingredients which fall into this category are desirable. The regulations restricting salt addition may result in defects which require more functional ingredients which may take precedence over “clean-label”, but this is to be determined by future work, and enzymes may play a key role in maintaining product quality and having “clean-labels”.

REFERENCES

AACC International (1999). Method 54-29.01: Approximate corrections for changing as-is farinograph absorption to 14.0% moisture basis, for constant dough weight method. In *Approved Methods of Analysis,* Eleventh Edition. St. Paul, MN: AACC International.

Aamodt, A., Magnus, E. M., & Færgestad, E. M. (2003). Effect of flour quality, ascorbic acid, and DATEM on dough rheological parameters and hearth loaves characteristics. *Food Chemistry and Toxicology, 68*, 2201-2210.

Adhikari, B., Howes, T., Bhandari, B. R., & Truong, V. (2001). Stickiness in foods: a review of mechanisms and test methods. *International Journal of Food Properties, 4*, 1-33.

Alam, T. M., Childress, K. K., Pastoor, K., & Rice, C. V. (2014). Characterization of free, restricted, and entrapped water environments in poly(*N*-isopropyl acrylamide) hydrogels via ^{1}H HRMAS PFG NMR spectroscopy. *Journal of Polymer Science, 52*, 1521-1527.

Assifaoui, A., Champion, D., Chiotelli, E., & Verel, A. (2006a). Characterization of water mobility in biscuit dough using a low-field ^{1}H NMR technique. *Carbohydrate Polymers, 64,* 197-204.

Assifaoui, A., Champion, D., Chiotelli, E., & Verel, A. (2006b). Rheological behaviour of biscuit dough in relation to water mobility. *International Journal of Food Science and Technology 41*, 124-128.

Autio, K., Flander, L., Kinnunen, A., & Heinonen, R. (2011). Bread quality relationship with rheological measurements of wheat flour dough. *Cereal Chemistry, 78,* 654-657.

Avramenko, N., Smith, M. A., Hopkins, E. J., Duizer, L., Nickerson, M. T., Rousseau, D., & Yada, R. Y. (2015). Challenges and opportunities in food science and technology in developing delivering sodium reduced products: bread, a case study. *Functional Food Reviews, 7*, 19-30.

Avramenko, N. A. (2017). *Investigating structure-function relationships of dough stickiness within low sodium bread dough formulations* (Doctoral dissertation). University of Saskatchewan, Saskatoon, SK: Canada. <http://hdl.handle.net/10388/8125>.

Bak, P., Nielsen, I.L., Thogersen, H.C., & Poulsen, C.H. (1996). A method for testing the strengthening effect of oxidative enzyme in dough. In Schofield, J. D. (Ed.), *Wheat Structure, Biochemistry, and Functionality* (pp. 361-367). New York, NY: Elsevier.

Balci, M. (2005). *Basic ^{1}H- and ^{13}C-NMR Spectroscopy* (pp. 3-133). New York, NY: Elsevier Science.

Barrera, G. N., Tadini, C. C., León, A. E., & Ribotta, P. D. (2016). Use of alpha-amylase and amyloglucosidase combinations to minimize the bread quality problems caused by high levels of damaged starch. *Journal of Food Science and Technology, 53,* 3675-3684.

Beck, M., Jekle, M., & Becker, T. (2011). Impact of sodium chloride on wheat flour dough for yeast-leavened products. II. Baking quality parameters and their relationships. *Journal of the Science of Food and Agriculture, 92,* 299-306.

Beck, M. Jekle, M., & Becker, T. (2012a). Impact of sodium chloride on wheat flour dough for yeast-leaved products. I. Rheological attributes. *Journal of the Science of Food and Agriculture, 92,* 585-592.

Beck, M., Jekle, M., & Becker, T. (2012b). Sodium chloride – sensory, preserving and technological impact on yeast-leavened products. *International Journal of Food Science and Technology, 47,* 1798-1807.

Belderok, B., Mesdag, J., & Donner, D. A. (2000). *Bread-Making Quality of Wheat; A Century of Breeding in Europe.* Netherlands: Kluwer Academics Publishers.

Belton, P. S. (2012). The molecular basis of dough rheology. In S. P. Cauvain (Ed.) *Breadmaking,* Second Edition (pp. 337-351). Cambridge, UK: Woodhead Publishing.

Belz, M. C. E., Ryan, L. A. M., & Arendt, E. K. (2012). The impact of salt reduction in bread: a review. *Critical Reviews in Food Science and Nutrition, 52,* 514-524.

Bidlas, E., & Lambert, R. J. W. (2008). Comparing the antimicrobial effectiveness of NaCl and KCl with a view to salt/sodium replacement. *International Journal of Food Microbiology, 124,* 98-102.

Bonet, A., Rosell, C. M., Caballero, P. A., Gómez, M., Pérez-Munuera, I., & Lluch, M. A. (2006). Glucose oxidase effect on dough rheology and bread quality: a study from macroscopic to molecular level. *Food Chemistry, 99,* 406-415.

Bosmans, G. M., Lagrain, B., Deleu, L. J., Fierens, E., Hills, B. P., & Delcour, J. A. (2012). Assignments of proton populations in dough and bread using NMR relaxometry of starch, gluten, and flour model systems. *Journal of Agricultural and Food Chemistry, 60,* 5461-5470.

Blanchard, C., Labouré, H., Verel, A., & Champion, D. (2012). Study of the impact of wheat flour type, flour particle size and protein content in a cake-like dough: Proton mobility and rheological properties assessment. *Journal of Cereal Science, 56,* 691-698.

Bloembergen, N., Purcell, E. M., & Pound, R. V. (1948). Relaxation effects in nuclear magnetic resonance absorption. *Physical Review, 73,* 679-715.

Caballero, P. A., Gómez, M., & Rosell, C. M. (2007). Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. *Journal of Food Engineering, 81,* 42-53.

Callaghan, P. T. (1991). *Principles of Nuclear Magnetic Resonance Microscopy,* (p. 45). Oxford, UK: Clarendon Press.

Calucci, L., Forte, C., Galleschi, L., Geppi, M., & Ghiringhelli, S. (2003). ^{13}C and ^{1}H solid state NMR investigation of hydration effects on gluten dynamics. *International Journal of Biological Macromolecules, 32,* 179-189.

Canada Grains Commission. (2018). *Orders of the Canadian Grain Commission: Variety designation lists.* Retrieved September 2, 2018, from https://www.grainscanada.gc.ca/legislation-legislation/orders-arretes/ocgcm-maccg-en.html.

Cauvain, S. P., & Young, L. S. (2003). Water control in baking. In S. P. Cauvain (Ed.) *Bread Making,* First Edition (p. 452). Cambridge, UK: Woodhead Publishing.

Cauvain, S. P. (2012). *Breadmaking: an overview.* In S. P. Cauvain (Ed.) *Breadmaking*, Second Edition (pp. 9-31). Cambridge, UK: Woodhead Publishing.

Cavanagh, J., Fairbrother, W. J., Palmer, A. G., Skelton, N. J. (1995). *Protein NMR Spectroscopy, Principles and Practice,* (p. 21). New York, NY: Elsevier.

Chatakanonda, P., Dickinson, L. C., & Chinachoti, P. (2003). Mobility and distribution of water in cassava and potato starches by ^{1}H and ^{2}H NMR. *Journal of Agricultural and Food Chemistry, 51,* 7445-7449.

Chen, W. (2015). Errors in quantitative $T_{1\rho}$ imaging and the correction methods. *Quantitative Imaging in Medicine and Surgery, 5,* 583-591.

Chen, W. Z., & Hoseney, R. C. (1995). Development of an objective method for dough stickiness. *Lebensmittel-Wissenschaft & Technologie, 28,* 467-473.

Chinachoti, P., Vittadini, E., Chatakanonda, P., Vodovotz, Y. (2008). Characterization of molecular mobility in carbohydrate food systems by NMR. In Webb, G. A. (Ed.), *Modern Magnetic Resonance,* (pp. 1703-1712). Berlin, Germany: Springer.

Collado-Fernàndez, M. (2003a). Breadmaking processes. In B. Caballero, L. C. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of Food Science and Nutrition,* Second Edition (pp. 627-634). San Diego, CA: Academic Press.

Collado-Fernàndez, M. (2003b). Dough fermentation. In B. Caballero, L. C. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of Food Science and Nutrition,* Second Edition (pp. 627-634). San Diego, CA: Academic Press.

Collado-Fernàndez, M. (2003c). Chemistry of baking. In B. Caballero, L. C. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of Food Science and Nutrition,* Second Edition (pp. 627-634). San Diego, CA: Academic Press.

Collar, C., Andreu, P., & Martínez-Anaya, M.A. (1998). Interactive effects of flour, starter and enzyme on bread dough machinability. *European Food Research and Technology, 207,* 133-139.

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., & Rossi, J. (2000). Combined effect of sourdough and lactic acid bacteria and additives on bread firmness and staling. *Journal of Agricultural and Food Chemistry, 58,* 3044-3051.

Courtin, C. M., & Delcour, J. A. (2002). Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science, 35,* 225-243.

Crowe, N. L., & Rasper, V. F. (1988). The ability of chlorine and chlorine-related oxidants to induce oxidative gelation in wheat flour pentosans. *Journal of Cereal Science, 7,* 283-294.

Czuchajowska, Z., Pomeranz, Y., & Jeffers, H. C. (1989). Water activity and moisture-content of dough and bread. *Cereal Chemistry, 66,* 128-132.

Dai, Z., Yin, Y., Li, Y., Cao, L., & Wang, Z. (2013). Variation of high-molecular-weight glutenin subunits and glutenin macropolymer particle distribution in wheat grains produced under different water regimes. *The Crop Journal, 1,* 84-89.

Dagdelen, A. F., & Gocmen, D. (2007). Effects of glucose oxidase, hemicellulose and ascorbic acid on dough and bread quality. *Journal of Food Quality, 30,* 1009-1022.

Dealy, J. M., & Wang, J. (2013). *Melt Rheology and its Applications in the Plastics Industry,* (pp. 1-89). New York, NY: Springer International Publishing AG.

Decamps, K., Joye, I. J., Courtin, C. M., & Delcour, J. A. (2012). Glucose and pyranose oxidase improve bread dough stability. *Journal of Cereal Science, 55,* 380-384.

De Guio, F., Musse, M., Benoit-Cattin, H., Lucas, T., Davenel, A. (2009). Magnetic resonance imaging method based on magnetic susceptibility effects to estimate bubble size in alveolar products: application to bread dough during proving. *Magnetic Resonance Imaging, 27,* 577-585.

Delcour, J. A., & Hoseney, R. C. (2010). Chapter 5: Rheology of Doughs and Batters In R. C. Hoseney (Ed.), *Principles of Cereal Science and Technology,* Third Edition (pp. 87-96). St. Paul, MN: AACCI Inc.

Delcour, J. A., Joye, I. J., Pareyt, B., Wilderjans, E., Brijs, J., & Lagrain, B. (2012). Wheat gluten functionality as a quality determinant in cereal-based food products. *Annual Review of Food Science and Technology, 3,* 469-492.

Dhaliwal, A. S., Mares, D. J., & Marshall, D. R. (1990). Measurement of dough surface stickiness associated with the 1B/1R chromosome translocation in bread wheats. *Journal of Cereal Science, 12,* 165-175.

Dobraszczyk, B.J. (1997). The rheological basis of dough stickiness. *Journal of Texture Studies, 28,* 139-162.

Don, C., Lichtendonk, W., Plijter, J. J., & Hamer, R. J. (2003). Glutenin macropolymer: a gel formed by glutenin particles. *Journal of Cereal Science, 37,* 1-7.

Don., C., Mann, G., Bekes, F., & Hamer, R. J. (2006). HMW-GS affect the properties of glutenin particles and thus flour quality. *Journal of Cereal Science, 44,* 127-136.

Doona, C. J., & Baik, M. (2007). Molecular mobility in model dough systems studied by time-domain nuclear magnetic resonance spectroscopy. *Journal of Cereal Science, 45,* 257-262.

Edwards, W. P. (2007). *The Science of Bakery Products.* London, UK: The Royal Society of Chemistry.

Esselink, E. F. J., van Aalst, H., Maliepaard, M., & van Duynhoven, J. P. M. (2003). Long-term storage effect in frozen dough by spectroscopy and microscopy. *Cereal Chemistry, 80,* 396-403.

Every, D., Simmons, L., Sutton, K. H., & Ross, M. (1999). Studies on the mechanism of the ascorbic acid improver effect on bread using flour fractionation and reconstitution methods. *Journal of Cereal Science, 30,* 147-158.

Fang, F. F., & Choi, H. J. (2012). Chapter 10: Electrorheological fluids: Materials and rheology. In M. Kontopoulou (Ed.), *Applied Polymer Rheology: Polymeric Fluids with Industrial Applications,* (pp. 285-300). Hoboken, NJ: John Wiley & Sons Inc.

Farahnaky, A., & Hill, S. E. (2007). The effect of salt, water, and temperature on wheat dough rheology. *Journal of Texture Studies, 38,* 499-510.

Fessas, D., & Schiraldi, A. (2001). Water properties in wheat flour dough I: classical thermogravimetry approach. *Food Chemistry, 72,* 237-244.

Franco, R., Schoneveld, O. J., Pappa, A., & Panayiotidis, M. I. (2007). The central role of glutathione in the pathophysiology of human diseases. *Archives of Physiology and Biochemistry, 113,* 234-258.

Glover, P. M., Aptaker, P. S., Bowler J. R., Ciampi, E., & McDonald, P. J. (1999). A novel high-gradient permanent magnet for the profiling of planar films and coatings. *Journal of Magnetic Resonance, 139,* 90-97.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science and Technology, 16,* 12-30.

Golob, P., Farrell, G., Orchard, J. E. (2008). *Crop Post-Harvest: Science and Technology, Volume 1: Principles and Practice,* (p. 79). Hoboken, NJ: John Wiley & Sons Inc.

Grosch, W., & Wieser, H. (1999). Redox reactions in wheat dough as affected by ascorbic acid. *Journal of Cereal Science, 29* 1-16.

Grunewald, E., & Knight, R. (2012). Nonexponential decay of the surface-NMR signal and implications for water content estimation. *Geophysics, 77,* EN1-EN9.

Guynot, M. E., Ramos, A. J., Sanchis, V., & Marín, S. (2005). Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5-5.5). *International Journal of Food Microbiology, 101,* 161-168.

Hackley, V. A., & Ferraris, C. F. (2001). NIST Special Publication 946: Guide to rheological nomenclature: measurements in ceramic particulate systems. *National Institute of Standards and Technology.*

Han, H.-M., & Koh, B.-K. (2011). Antioxidant activity of hard wheat flour, dough and bread prepared using various processes with the addition of different phenolic acids. *Journal of Food Science and Agriculture, 91,* 604-608.

Hanft, F., & Koeler, P. (2006). Studies on the effect of glucose oxidase in bread making. *Journal of Science of Food and Agriculture, 86,* 1699-1703.

Health Canada. (2010). *Sodium reduction strategy for Canada.* Retrieved October 7, 2015, from https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/sodium/related-information/reduction-strategy/recommendations-sodium-working-group.html.

Health Canada. (2012a). 2. *List of permitted bleaching, maturing or dough conditioning agents (lists of permitted food additives).* Retrieved September 2, 2018, from https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/2-bleaching-maturing-dough-conditioning-agents.html.

Health Canada. (2012b). Guidance for the food industry on reducing sodium in processed foods. Retrieved October 7, 2015, from https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-food-industry-reducing-sodium-processed-foods-2012.html.

Health Canada. (2017). *Sodium in Canada*. Retrieved September 2, 2018, from https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/sodium.html.

Health Canada. (2018). *Sodium reduction in processed foods in Canada: An evaluation towards voluntary targets from 2012 to 2016.* Retrieved July 17, 2018, from https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-food-industry-reducing-sodium-processed-foods-progress-report-2017.html.

Heitmann, M., Zannini, E., & Arendt, E. (2018). Impact of Saccharomyces cerevisiae metabolites produced during fermentation on bread quality parameters: a review. *Critical Reviews in Food Science and Nutrition, 58,* 1152-1164.

Hemdane, S., Jacobs, P. J., Bosmans, G. M., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2017). Study of biopolymer mobility and water dynamics in wheat bran using time-domain ^{1}H NMR relaxometry. *Food Chemistry, 236*, 68-75.

Hellemann, U. (1990). Bread and butter with varying NaCl contents: hedonic responses and sensory characteristics. *Food Quality and Preference, 2,* 167-176.

Hills, B. H. *Magnetic Resonance Imaging in Food Science* (1998). Toronto, Canada: John Wiley & Sons.

Hoseney, R. C., & Smewing, J. (1999). Instrumental measurement of stickiness of doughs and other foods. *Journal of Texture Studies, 30,* 123-136.

Hutton, T. (2002). Sodium technological functions of salt in the manufacturing of food and drink products. *British Food Journal, 104,* 126-152.

Janmey, P. A., & Schliwa, M. (2008). Rheology, *Current Biology, 18*(15), R639-R641.

Jayaram, V. B., Cuyvers, S., Lagrain, B., Verstrepen, K. J., Delcour, J. A., & Courtin, C. M. (2013). Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. *Food Chemistry, 136,* 301-308.

Jayaram, V. B., Cuyvers, S., Verstrepen, K. J., Delcour, J. A., & Courtin, C. M. (2014). Succinic acid in levels products by yeast (*Saccharomyces cerevisiae*) during fermentation strongly impacts wheat bread dough properties. *Food Chemistry, 151,* 421-428.

Jekle, M. & Becker, T. (2011). Dough microstructure: novel analysis by quantification using confocal laser scanning microscopy. *Food Research International, 44,* 984-991.

Jekle, M., & Becker, T. (2012). Effects of acidification, sodium chloride, and moisture levels on wheat dough: I. Modeling of rheological and microstructural properties. *Food Biophysics, 7,* 190-199.

Kaur, A., Bala, R., Singh, B., & Rehal, J. (2011). Effect of replacement of sodium chloride with mineral salts on rheological characteristics of wheat flour. *American Journal of Food Technology, 6,* 674-684.

Keillor, J. W., Clouthier, C. M., Apperley, K. Y. P., Akbar, A., & Mulani, A. (2014). Acyl transfer mechanisms of tissue transglutaminase. *Bioorganic Chemistry, 57,* 186-197.

Keeler, J. (2002). Chapter 8: Relaxation In J. Keeler (Ed.), *Understanding NMR Spectroscopy,* (pp. 1-24). Cambridge, UK: University of Cambridge.

Keshari, K. R., & Wilson, D. M. Chemistry and biochemistry of ^{13}C hyperpolarized magnetic

resonance using dynamic nuclear polarization. *Chemical Society Reviews, 43,* 1627-1659.

Khatkar, B. S., Bell, A. E., & Shchofield, J. D. (1996). A comparative study of the inter-relationships between mixograph parameters and bread-making qualities of wheat flours and glutens. *Journal of the Science of Food and Agriculture, 72*, 71-85.

Kilcast, D., & Angus, F. (2007). In D. Kilcast & F. Angus (Eds.), *Reducing Salt in Foods: Practical Strategies*, First Edition. Cambridge, UK: Woodhead Publishing Limited.

Kimmich, R., & Fischer, E. (1994). One- and two-dimensional pulse sequences for diffusion experiments in the fringe field of superconducting magnets. *Journal of Magnetic Resonance, 106,* 229-235.

Kishore, A, I., Herberstein, M. E., Craig, C. L., & Separovic, F. (2002). Solid-state NMR relaxation studies of Australian spider silks. *Biopolymers, 61,* 287-297.

Koehler, P. (2003). Effect of ascorbic acid in dough: reaction of oxidized glutathione with reactive thiol groups of wheat glutelin. *Journal of Agricultural and Food Chemistry, 51,* 4964-4959.

Kontogiorgos, V. (2011). Microstructure of hydrated gluten network. *Food Research International, 44,* 2582-2586.

Kornbrust, B. A., Forman, T., & Matveeva, I. (2012). Chapter 19: Applications of enzymes in breadmaking. In S. P. Cauvain (Ed.), *Bread Making: Improving Quality*. Cambridge, UK: Woodhead Publishing Limited.

Kou, Y., Dickinson, L. C., & Chinachoti, P. (2000). Mobility characterization of waxy corn starch using wide-line ^{1}H nuclear magnetic resonance. *Journal of Agricultural and Food Chemistry, 48,* 5489-4595.

Le Botlan, D., Rugraff, Y., Martin, C., & Colonna, P. (1998). Quantitative determination of bound water in wheat starch by time domain NMR spectroscopy. *Carbohydrate Research, 208,* 29-36.

Leung, H. K., Magnuson, J. A., & Bruinsma, B. L. (1979). Pulsed nuclear magnetic resonance study of water mobility in flour doughs. *Journal of Food Science, 44,* 1408-1411.

Linlaud, N., Ferrer, E., Puppo, M. C., & Ferrero, C. (2011). Hydrocolloid interaction with water, protein, and starch in wheat dough. *Journal of Agricultural and Food Chemistry, 59,* 713-719.

Liu, Z., & Scanlon, M. G. (2003). Predicting mechanical properties of bread crumb. *Chemical Engineering Research & Design, 81,* 224-238.

Lu, Z., & Seetharaman, K. (2013). ^{1}H nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) studies of water mobility in dough systems containing barley flour. *Cereal Chemistry, 90*, 120-126.

Lynch, E. J., Dal Bello, F., Sheehan, E. M., Cashman, K. D., & Arendt, E. K. (2009). Fundamental studies on the reduction of salt on dough and bread characteristics. *Food Research International, 42,* 885-891.

Madsen, E. L., Frank, G. R., Hobson, M. A., Lin-Gibson, S., Hall, T. J., Jiang, J., & Stiles, T. A. (2008). Instrument for determining the complex shear modulus of soft-tissue-like materials from 10 to 300 Hz. *Physics in Medicine & Biology, 53,* 5313-5342.

Marín, S., Guynot, M. E., Neira, P., Bernandó, M., Sanchis, V., & Ramos, A. J. (2002). Risk assessment of the use of sub-optimal levels of weak-acid preservatives in the control of mould growth on bakery products. *International Journal of Food Microbiology, 79,* 203-211.

Markus, C., Belz., E., Mairinger, R., Zannini, E., Ryan, L. A. M., Cashman, K. D., & Arendt, E. K. (2012). The effect of sourdough and calcium propionate on the microbial shelf-life of salt reduced bread. *Applied Microbiology and Biotechnology, 96,* 493-501.

McMurry, J. E. (2011). Chapter 11: Structure determination: nuclear magnetic resonance spectroscopy In J. E. McMurry (Ed.), *Organic Chemistry with Biological Applications,* Second Edition (pp. 404-431). Belmont, CA: Books/Cole, Cengage Learning.

Mezger, T. G. (2006). *The Rheology Handbook: For Users of Rotational and Oscillatory Rheometers*, (p. 119). Germany: Vincentz Network GMbH & Co KG.

Millar, S., & Tucker, G. (2012). Chapter 16: Controlling bread dough development. In S. P. Cauvain (Ed.), *Breadmaking: Improving Quality.* Cambridge, UK: Woodhead Publishing Limited.

Miller, R. A., & Hoseney, R. C. (2008). Role of salt in baking. *Cereal Foods World, 53,* 4-6.

Mondal, A., & Datta, A. K. (2008). Bread baking – a review. *Journal of Food Engineering, 86,* 444-452.

Nelson, D. L., & Cox, M. M. (2008). Amino acids, peptides and proteins. In *Lehninger: Principles of Biochemistry,* Fifth Edition (p. 73). New York, United States: W. H. Freeman and Company.

Newberry, M. P., Phan-Thien, N., Larroque, O. R., Tanner, R. I., & Larsen, N. G. (2002). Dynamic and elongation rheology of yeasted bread doughs. *Cereal Chemistry, 79,* 874-879.

O'Connor, P. J., Cutié, S. S., Smith, P. B., Martin, S. J., Sammler, R. L., Harris, W. I., Marks, M. J., & Wilson, L. (1996). ^{1}H characterization of swelling in cross-linked polymer systems. *Macromolecules, 29,* 7872-7884.

O'Donnell, M., Mente, A., & Yusuf, S. (2015). Sodium intake and cardiovascular health. *Circulation Research, 116,* 1046-1057.

Pareyt, B., Finnie, S. M., Putseys, J. A., & Delcour, J. A. (2011). Lipids in bread making: sources, interactions, and impact on bread quality. *Journal of Cereal Science, 52,* 266-279.

Peng, B., Li, Y., Ding, S., & Yang, J. (2017). Characterization of textural, rheological, thermal, microstructural, and water mobility in wheat flour dough and bread affected by trehalose. *Food Chemistry, 233,* 369-377.

Preston, K. R. (1989). Effects of neutral salts of the lyotropic series on the physical dough properties of a Canadian red spring wheat flour. *Cereal Chemistry, 66,* 144-148.

Primo-Martín, C., Valera, R., Martínez-Anaya, A. (2003). Effect of pentosanase and oxidases on the characteristics of doughs and the glutenin macropolymer (GMP). *Journal of Agricultural and Food Chemistry, 51,* 4673-4679.

Rasiah, I. A., Sutton, K. H., Low, F. L., Lin, H. M. & Gerrard, J. A. (2005). Crosslinking of wheat dough proteins by glucose oxidase and the resulting effects on bread and croissants. *Food Chemistry, 89,* 325-332.

Roman-Gutierrez, A. D., Guilbert, S., & Cuq, B. (2002). Frozen and unfrozen water contents of wheat flours and their components. *Cereal Chemistry, 79,* 471-475.

Rondeau-Mouro, C., Cambert, M., Kovrlija, R, Musse, M., Lucas, T., & Mariette, F. (2015). Temperature-associated proton dynamics in wheat starch-based model systems and wheat flour dough evaluated by NMR. *Food and Bioprocess Technology, 8,* 777-790.

Roozendaal, H., Abu-hardan, M., & Frazier, R. A. (2012). Thermogravimetric analysis of water from wheat flour and wheat bran suspensions. *Journal of Food Engineering, 111,* 606-611.

Rummeny, E., Reimer, P., & Heindel, W. (2011). Basic Principles of MRI. In E. Rummeny, P. Reimer, & W. Heindel (Eds.), *MR Imaging of the Body* (p. 7). Stuttgart, Germany: Thieme.

Salovaara, H. (1982). Effect of partial sodium chloride replacement by other salts on wheat dough rheology and breadmaking. *Cereal Chemistry, 59,* 422-426.

Salvador, A., Sanz, T., & Fiszman, S. M. (2005). Dynamic rheological characteristics of wheat flour-water doughs. Effect of adding NaCl, sucrose and yeast. *Food Hydrocolloids, 20,* 780-786.

Samapundo, S., Deschuyffeleer, N., Van Laere, D., De Leyn, I., & Devlieghere, F. (2010). Effect of NaCl reduction and replacement on the growth of fungi important to the spoilage of bread. *Food Microbiology, 27,* 749-756.

Schild, H. H. (1990). *MRI Made Easy*, (p. 13). Berlin, Germany: Schering AG.

Schober, T. J., Dockery, P., Arendt, E. K. (2003). Model studies for wheat sourdough systems using gluten, lactate buffer and sodium chloride. *European Food Research and Technology, 217,* 235-243.

Seguchi, M., Hayashi, M., & Matsumoto, H. (1997). Effect of gaseous acetic acid on dough rheological and breadmaking properties. *Cereal Chemistry, 74,* 129-134.

Separovic, F., Lam, Y. H., Ke, X., & Chan, H.-K. (1998). A solid-state NMR study of protein hydration and stability. *Pharmaceutical Research, 15,* 1816-1821.

Serial, M. R., Blanco Canalis, M. S., Carpinella, M., Valentinuzzi, M. C., León, A. E., Ribotta, P. D., Acosta, R. H. (2016). Influence of the incorporation of fibers in biscuit dough on proton mobility characteristics by time domain NMR. *Food Chemistry, 192,* 950-957.

Shewry, P. R., Halford, N. G., Belton, P. S., & Tatham, A. S. (2002). The structure and properties of gluten: an elastic protein from wheat gluten. *Philosophical Transactions of the Royal Society of London, 357,* 133-142.

Sidhu, J.S., Nordin, P., & Hoseney, R. C. (1980). Mixograph studies. III. Reaction of fumaric acid with gluten proteins during dough mixing. *Cereal Chemistry, 57,* 159-163.

Simmons, A. L., & Vodovotz, Y. (2012). The effects of soy on freezable bread dough: a magnetic resonance study. *Food Chemistry, 135,* 659-664.

Skendi, A., Papageorgiou, M., & Biliaderis, C. G. (2010). Influence of water and barley β-glucan addition on wheat dough viscoelasticity. *Food Research International, 43,* 57-65.

Skerritt, J. H., Hac, L., & Bekes, F. (1999). Depolymerization of the glutenin macropolymer during dough mixing: I. Changes in levels, molecular weight distribution, and overall composition. *Cereal Chemistry, 76,* 395-401.

Sluimer, P. (2005). *Principles of Breadmaking: Functionality of Raw Materials and Process Steps.* St. Paul, MN: American Association of Cereal Chemists.

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J., & El-Khoury, A. (2004). Shelf life and safety concerns of bakery products – a review. *Critical Reviews in Food Science and Nutrition, 44,* 19-55.

Song, Y., & Zheng, Q. (2007). Dynamic rheological properties of wheat flour dough and proteins. *Trends in Food Science & Technology, 18,* 132-138.

Stampfli, L., & Nersten, B. (1995). Emulsifiers in bread making. *Food Chemistry, 52,* 353-360.

Steffolani, M. E., Pérez, G. T., Ribotta, P. D., Puppo, M. C., León, A. E. (2008). Effect of transglutaminase on properties of glutenin macropolymer and dough rheology. *Cereal Chemistry, 85,* 39-43.

Steffolani, M. E., Ribotta, P. D., Pérez, G. T., & León, A. E. (2010). Effect of glucose oxidase, transglutaminase, and pentosanase on wheat proteins: relationship with dough properties and breadmaking quality. *Journal of Cereal Science, 51,* 366-373.

Steffolani, M. E., Ribotta, P. D., Pérez, G. T., & León, A. E. (2012). Combinations of glucose oxidase, α-amylase and xylanase affect dough properties and bread quality. *International Journal of Food Science and Technology, 47,* 525-534.

Stone, A. K., Hucl, P. J., Scanlon, M. G., & Nickerson, M. T. (2017). Effect of damaged starch and NaCl level on the dough handling properties of a Canadian Western Red Spring Wheat. *Cereal Chemistry, 94,* 970-977.

Stone, A. K., Lam, R. S. H., Hopkins, E. J., Hucl, P., Scanlon, M. G., & Nickerson, M. T. (2018). Effect of organic acids and NaCl on the rheological properties of dough prepared using Pembina and Harvest CWRS wheat cultivars. *Cereal Chemistry, 95,* 478-485.

Tang, L., Yang, R., Hua, X., Yu, C., Zhang, W., & Zhao, W. (2014). Preparation of immobilized glucose oxidase and its application in improving breadmaking quality of commercial wheat flour. *Food Chemistry, 161,* 1-7.

Tseng, C.-S., & Lai, H.-M. (2002). Physicochemical properties of wheat flour dough modified by microbial transglutaminase. *Food Engineering and Physical Properties, 67,* 750-755.

Turner, M. A, Soh, C. H. N., Ganguli, N. K., & Sissons, M. J. (2008). A survery of water-extractable arabinopolymers in bread and durum wheat and the effect of water-extractable arabinoxylan on durum dough rheology and spaghetti cooking quality. *Journal of the Science of Food and Agriculture, 88,* 2551-2555.

Umbach, S. L., Davis, E. A., Gordon, J., & Callaghan, P. T. (1992). Water self-diffusion coefficients and dielectric properties determined for starch-gluten-water mixtures heated by microwave and by conventional methods. *Cereal Chemistry, 69*, 637-642.

Upson, F. W., & Calvin, J. W. (1916). The colloidal swelling of wheat gluten in relation to milling and baking. *Historical Research Bulletins of Nebraska Agricultural Experiment Station, 124*, 1-27.

Van Bockstaele, F., De Leyn, I., Eeckhout, M., & Dewettinck, K. (2008). Rheological properties of wheat flour dough and the relationship with bread volume. I. Creep-recovery measurements. *Cereal Chemistry, 85*, 753-761.

Van Velzen, E. J. J., van Duynhoven, J. P. M., Pudney, P., Weegels, P. L., & van der Maas, J. H. (2003). Factors associated with dough stickiness as sensed by attenuated total reflectance infrared spectroscopy. *Cereal Chemistry, 80*, 378-382.

Vemulapalli, V., Miller, K. A., & Hoseney, R. C. (1998). Glucose oxidase in breadmaking systems. *Cereal Chemistry, 75*, 439-442.

Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition, 42*, 179-208.

Vlachopoulos, J., & Polychronopoulos, N. (2012). Chapter 1: Basic concepts in polymer melt rheology and their importance in processing. In M. Kontopoulou (Ed.), *Applied Polymer Rheology: Polymeric Fluids with Industrial Applications,* (pp. 2-25). Hoboken, NJ: John Wiley & Sons Inc.

Wang, X., Choi, S-G., & Kerr, W. L. (2004). Water dynamics in white bread and starch gels as affected by water and gluten content. *Lebensmittel-Wissenschaft & Technologie, 37*, 377-384.

Wehrle, K., Grau, H., & Arendt, E. K. (1997). Effects of lactic acid, acetic acid, and table salt on fundamental rheological properties of wheat dough. *Cereal Chemistry, 74*, 739-744.

Weipert, D. (1990). The benefits of basic rheometry in studying dough rheology. *Cereal Chemistry, 67*, 311-317.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*, 115-119.

Wieser, H. (2012). Chapter 18: The use of redox agents in breadmaking. In S. P. Cauvain (Ed.), *Breadmaking: Improving Quality.* Cambridge, UK: Woodhead Publishing Limited.

Wood, T. B. (1907). The chemistry of strength of wheat flour. *The Journal of Agricultural Science, 2*, 139-160.

Wood, T. B., & Hardy, F. R. S. (1909). Electrolytes and colloids. The physical state of gluten. *Proceedings of the Royal Society of London B: Biological Sciences, 81*, 38-43.

Woychik, J. H., Boundy, J. A., & Dimler, R. J. (1961). Amino acid composition of proteins in wheat gluten. *Journal of Agricultural and Food Chemistry, 9*, 307-310.

Xiao, F., Amirkhanian, A. N., & Amirkikhanian, S. N. (2011). Influence of carbon nanoparticles on the rheological characteristics of short-term aged asphalt binders. *Journal of Materials in Civil Engineering, 23*, 423-431.

Xiuzhen, L., & Sieb, P. A. (1998). Assay of dehydroascorbic acid in bread and dough added as a crystalline dimer. *Cereal Chemistry, 75*, 200-206.

Yang, T., Bai, Y., Wu, F., Yang, N., Zhang, Y., Bashari, M., Jin, Z., & Xu, X. (2014). Combined effects of glucose oxidase, papain and xylanase on browning inhibition and characteristics of fresh whole wheat dough. *Journal of Cereal Science, 60,* 249-254.

Yovchev, A.G., Briggs, C., Stone, A. K., Hucl, P., Nickerson, M. T., & Scanlon, M. G. (2017). Effect of salt reduction on dough handling and the bread making quality of Canadian Western Red Spring wheat varieties. *Cereal Chemistry, 94,* 752-759.

Zhang, D., Zhu, Y., & Chen, J. (2009). Microbial transglutaminase production: understanding the mechanism. *Biotechnology and Genetic Engineering Reviews, 26,* 205-222.

APPENDIX A: CULTIVAR INFORMATION

Table A.1 Flour characterization of Pembina and Harvest CWRS wheat cultivars from the 2013 crop year. Data shown is the mean ± one standard deviation (n=2). Modified table from Avramenko (2017).

Characteristics[1]	**Flours**[2]	
	Pembina	**Harvest**
Proximate analysis		
a. Protein (% based on 14% w.b.)	12.6 ± 0.0[a]	13.0 ± 0.1[b]
b. Protein (% d.b.)	14.7 ± 0.0[a]	15.1 ± 0.1[b]
c. Lipid (% d.b.)	1.13 ± 0.01[b]	1.03 ± 0.04[ab]
d. Ash (% d.b)	0.52 ± 0.00[b]	0.53 ± 0.0[b]
Falling number (s)	475 ± 16[a]	486 ± 21[a]
SKCS – HI	67.43 ± 0.67[a]	73.53 ± 0.97[b]
Damaged starch (%)	5.97 ± 0.26[a]	7.06 ± 0.22[b]
Gluten Index (%)	84.3 ± 2.9[b]	48.6 ± 0.8[a]
a. Wet gluten (%)	36.0 ±0.6[a]	42.1 ± 0.3[b]
b. Dry gluten (%)	12.2 ± 0.3[a]	13.8 ± 0.1[b]
Rapid visco-analysis (RVU)		
a. Peak viscosity	123.3 ± 2.1[a]	140.1 ± 1.6[b]
b. Breakdown viscosity	35.5 ± 2.5[a]	36.4 ± 0.1[a]
c. Trough viscosity	87.0 ± 1.0[a]	103.7 ± 1.5[b]
d. Setback viscosity	102.3 ± 1.5[a]	119.6 ± 0.8[b]
e. Final viscosity	189.3 ± 2.5[a]	223.3 ± 0.8[b]

[1]*Abbreviations used:* wet basis (w.b.), dry basis (d.b.), single kernel characterization system (SCKS), hardness index (HI), and rapid viscoanalyzer units (RVU).

[2]Lowercase letters represent significantly ($p < 0.05$) different values within a row of values.

Table A.2 Empirical rheology characterization of Pembina and Harvest CWRS wheat cultivars from the 2013 crop year. Data presented is the mean ± one standard deviation (n=2). Modified table from Avramenko (2017).

Empirical Rheology[1]	**Flours**[2]	
	Pembina	**Harvest**
Farinograph		
Farinograph water absorption (FAB; % to 14% w.b.)	61.5 ± 0.3^{a}	64.9 ± 0.1^{b}
Dough development time (DDT; min)	6.3 ± 0.4^{b}	5.2 ± 0.3^{ab}
Mixing tolerance index (MTI; BU)	20.5 ± 2.1^{a}	33.0 ± 5.7^{a}
Stability time (STA; min)	8.9 ± 0.9^{a}	5.3 ± 0.9^{a}
Mixograph		
Baking absorption (BA; %)	62.5	63.4
Mixograph development time (MDT; min)	3.23 ± 0.02^{b}	2.68 ± 0.06^{a}
Peak dough resistance (PDR; %)	51.12 ± 2.02^{ab}	47.41 ± 0.20^{a}
Bandwidth at peak dough resistance (PWPR; %)	27.65 ± 1.41^{a}	19.99 ± 0.79^{a}
Resistance to breakdown 1 min after peak (RBD; %)	1.50 ± 0.82^{a}	1.97 ± 0.43^{a}
Bandwidth breakdown 1 min after peak (BWBD; %)	7.89 ± 4.07^{a}	3.26 ± 0.00^{a}
Work input to PDR (WIP; % tq min)	117.90 ± 6.68^{b}	85.37 ± 5.62^{a}

[1]*Abbreviations:* Barbender units (BU), and torque (tq).

[2]Lowercase letters represent significantly ($p < 0.05$) different values within a row of values.

APPENDIX B: CHAPTER 5 *P*-VALUE TABLES

Table B.1 *p*-values of dough samples prepared with Harvest or Pembina flour, at 1.0% NaCl, with 0.001% GO, and either no acid, acetic, fumaric, or succinic acid for ^{1}H NMR experiments.

Effect/Interaction	***p*-values**			
	T_2*	T_1	T_2, long (= T_{2B})	A1 Ratio
Acid	NS	<0.001	<0.001	<0.05
Flour	<0.001	<0.001	<0.001	<0.001
Acid:Flour	NS	NS	<0.05	NS

Table B.2 *p*-values of dough samples prepared with Harvest or Pembina flour, at 1.0% NaCl, with 0.001% GO, and either no acid, acetic, fumaric, or succinic acid for spinlock experiments taken at 8dB.

Effect/Interaction	***p*-values**
	$T_{1\rho}$
Acid	<0.001
Flour	<0.001
Acid:Flour	<0.05

Table B.3 *p*-values of dough samples prepared with Harvest or Pembina flour, at 1.0% NaCl, with 0.001% GO, and either no acid, acetic, fumaric, or succinic acid for diffusion experiments.

Effect/Interaction	***p*-values**
	slow diffusion ratio
Acid	NS
Flour	NS
Time	NS
Acid:Flour	NS
Acid:Time	NS
Flour:Time	NS
Acid:Flour:Time	NS