Matrix Effect on Fat Crystallization in Laminated Bakery Products

by

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The impact of a croissant matrix on fat crystallization was determined by analyzing the polymorphism using powder x-ray diffraction (XRD), solid fat content (SFC) by pulsed nuclear magnetic resonance (p-NMR) and melting behaviour by differential scanning calorimetry (DSC). Roll-in shortenings of varying composition were used to prepare croissants. XRD revealed polymorphic conversion (from β’ to the β form) occurs when fat is baked within the matrix, and the extent of conversion depends on the fat’s composition. In addition, the fat contained within a croissant will have a significantly lower SFC and a greater temperature is required for complete melting. The same fats were then baked in the presence of isolated croissant components (wheat starch, gelatinized wheat starch, gluten and a formed gluten network) and the cooled samples were analyzed using the same methods. Overall, the results suggested that changes in crystallization behaviour are caused by an interaction between fat and gelatinized wheat starch.
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<tr>
<td>XRD</td>
<td>Powder X-ray diffraction</td>
</tr>
<tr>
<td>SFC</td>
<td>Solid fat content</td>
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<tr>
<td>p-NMR</td>
<td>Pulsed nuclear magnetic resonance</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>TAG(s)</td>
<td>Triacylglycerols</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>TFA</td>
<td>Trans fatty acids</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acids</td>
</tr>
<tr>
<td>UFA</td>
<td>Unsaturated fatty acids</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>$T_c$</td>
<td>Crystallization temperature</td>
</tr>
<tr>
<td>NH</td>
<td>Non-hydrogenated shortening</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogenated shortening</td>
</tr>
<tr>
<td>+St</td>
<td>Samples containing crystalline wheat starch</td>
</tr>
<tr>
<td>+GSt</td>
<td>Samples containing gelatinized wheat starch</td>
</tr>
<tr>
<td>+G</td>
<td>Samples containing gluten</td>
</tr>
<tr>
<td>+GN</td>
<td>Samples containing formed gluten network</td>
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1.0 Introduction

Laminated doughs are high fat bakery products, consisting of many thin, alternating layers of fat and dough formed by repeated rolling and folding. The extensive rolling and folding that takes place has given the fats used in lamination the name of “roll-ins.” Upon baking, the layering causes each individual dough layer to bake separately, creating the characteristic visual separation of layers and flaky texture. Types of laminated doughs include croissants, known for their distinct shape, Danishes, often filled with fruit, and puff pastry, used in a variety of applications from sweet to savoury. Croissants, the primary focus of this thesis, can contain approximately 30-40% fat by weight, therefore the properties of the fat have the ability to impact the overall quality of the products (Lai & Lin, 2006). The fat serves many purposes in these products, contributing functionality as well as taste and lubricity. Crystallization characteristics of these fats can be studied as an indicator of functionality, including the solid fat content (SFC) as an indication of the hardness and rolling ability at a given temperature, and crystal polymorphism which is often used in the food industry as an indicator of optimum plasticity. Of the three major polymorphic forms occurring in fats (α, β' and β), optimum plasticity of roll-in fats has been correlated to the presence of β' crystals (Macias-Rodriguez & Marangoni, 2016a). The polymorphic form present will also directly affect the melting temperature of a fat (Marangoni & Wesdorp, 2013a).

While butter is often thought of as the standard for laminated dough preparation, it is not favourable for large scale production due to its high cost and narrow plastic range. In the industry, roll-in shortenings manufactured by partial hydrogenation from different oils were first utilized, and produced highly accepted laminated dough products. However, these shortenings contain high proportions of trans fatty acids (TFA), which have a known association with heart disease and other chronic diseases (Keys, Anderson, & Grande, 1965; Lichtenstein, 2014; Mensink & Katan, 1992; Mensink, Zock, Kester, & Katan, 2003; Thompson, Minihane, & Williams, 2011). This has led to the removal of partially hydrogenated shortenings in favour of non-hydrogenated shortenings produced via interesterification or by blending oils with high melting fractions (Gibon & Kellens, 2014; McNeill, 2014). These shortenings, however, have not provided the same sensorial acceptability as the partially hydrogenated shortenings, leaving many consumers unsatisfied with the alternatives (Garcia-Macias, Gordon, Frazier, Smith, &
Gambelli, 2011, 2012; Simovic, Pajin, Seres, & Filipovic, 2009; F. C. Wang, Gravelle, Blake, & Marangoni, 2016). Continuing research indicates that increasing consumption of unsaturated fatty acids (UFA) in favour of saturated fatty acids (SFA) is correlated with positive health effects (Hu, Manson, & Willett, 2001; Lunn & Theobald, 2006). This has led to new efforts to produce a roll-in shortening containing primarily UFA, however studies have found that these shortenings have limitations when it comes to functionality (Acevedo & Marangoni, 2014; Blake & Marangoni, 2015; Garcia-Macias et al., 2011, 2012; Simovic et al., 2009). At this point, no roll-in shortening developed meets both the health and functionality criteria.

The focus now must be to understand how hydrogenated roll-in shortenings behave once incorporated into these products and ultimately to develop a TFA free, and low SFA roll-in shortening that behaves in the same way. It cannot be assumed that the properties of a roll-in shortening prior to incorporation remain after baking. First, the high temperatures required in baking are sufficient to completely melt any existing fat crystals, erasing their prior crystalline form and allowing for changes in crystallization behaviour upon cooling within the matrix. Second, melting and cooling the fat in the presence of the other ingredients creates opportunity for ingredient interactions. Notable interactions between lipid materials and starch, as well as lipid materials and proteins have been documented, meaning there is great potential for the croissant components (particularly wheat starch and gluten) to interact with the roll-in fats and alter the crystallization behaviour (Eliasson, 1994; Goesaert et al., 2005; Karel, 1973; Pareyt, Finnie, Putseys, & Delcour, 2011; Tang & Copeland, 2007). Storage time is also a variable given the known changes that occur in bakery products, including starch retrogradation and the migration and loss of moisture, which could impact the fat crystallization behaviour over a croissants’ week long shelf life.

Until now, the crystallization properties of a fat contained within a baked matrix have never been investigated. In this work, the impact of baking a commonly used roll-in fat into a laminated dough matrix on a fat’s crystallization behaviour was investigated, including polymorphism (by XRD), melting behaviour (by DSC) and SFC (by p-NMR). The use of these techniques applied to fat contained within bakery products, specifically croissants has not been documented, therefore sample preparation techniques were developed accordingly. Four different fats were used to prepare four different types of croissants: butter, two different hydrogenated shortenings (termed A & B) and one non-hydrogenated shortening. The
shortenings used were all specialized for lamination. The inclusion of both hydrogenated and non-hydrogenated shortenings allowed for speculative assessment of why the products produced vary significantly in perceived acceptability. Texture analysis for product firmness, using a Texture Analyzer fit with a knife blade attachment, also shed light on potential sensory consequences of any changes in crystallization behaviour occurring in the matrix. For a complete investigation, comparison of samples of each fat baked for the same time and at the same temperature used in baking the croissants ensured that any noted differences between bulk fat and fat within a croissant were not simply caused by melting and recrystallization. In order to determine which components are responsible for any observed interaction, this research evaluated potential for interactions between the roll-in shortenings and either wheat starch or gluten. Given the noted differences observed between shortenings of different composition, and whether or not they had been hydrogenated, this investigation focused on the behaviour of one hydrogenated shortening and one non-hydrogenated shortening. Mixtures of each shortening with either crystalline wheat starch (St) or gluten (G) were prepared. However, since croissant preparation requires the roll-in shortenings to be layered with pre-made dough, in which flour is hydrated, this means that the gluten contained would have formed a network, and the starch would gelatinize upon baking. As such, interactions with gelatinized wheat starch (GSt) or a formed gluten network (GN) were accounted for by first mixing starch or gluten with distilled water before baking in order to best simulate a fat interacting with prepared dough. All results were compared with those previously obtained from the respective croissants.

1.1 Objectives

- Identify sample preparation techniques for croissants by XRD, p-NMR and DSC
- Analyze the crystallization behaviour of fats contained within croissants
- Determine if there is an interaction between fat and croissant matrix components
- Investigate and identify component(s) that are interacting
1.2 Hypotheses

- The crystallization behaviour of fats will be different within a croissant matrix compared to when simply heated and cooled on their own
- An interaction between one or more croissant components causes changes in crystallization behaviour
- The interaction will cause the fat to form the most stable polymorphic form
- The interaction will be between starch or gluten, suspected based on the large proportion of wheat flour in the ingredient composition
1.3 References


2.0 Literature Review

2.1 Introduction into Fat Crystallization

Solid fats, including roll-in fats, are semisolid materials made of triacylglycerides (TAG) molecules where a solid fat crystal network entraps liquid oil within. Each fat will have a specific crystallization and melting temperature. When the system is in liquid state and is cooled below the melting temperature, the system becomes undercooled. This is equivalent to supersaturation, and at this point it is energetically favourable to change from liquid to solid state (Marangoni & Wesdorp, 2013b; Rousset, 2002). Fat crystallization begins with nucleation, when the TAG molecules aggregate to form stable nuclei. However, components in the system, such as impurities, can act as a site on which fat can crystallize, and in this case it is more energetically favourable to crystallize directly on that surface rather than form nuclei, and as such, the rate of crystallization is increased (Coupland, 2002; Rousset, 2002). Crystal size, and by extension the number of crystals formed, are highly dependent on the nucleation rate therefore any factors that alter this rate have the potential to alter the properties of the TAG system.

Once nucleation has begun, the crystal size depends on whether the dominating force is crystal growth or nucleation. Larger crystals form when fat simply attaches to existing crystals or nuclei, and this is favoured when slow cooling rates are used. However, different processing conditions can be modified to alter the crystallization process and favour formation of smaller crystals, including rapid cooling with added shear force during cooling. Crystal size will affect the rheological properties of a fat, including plasticity, which is an important characteristic to consider in many food applications (Narine & Marangoni, 1999).

After nucleation has occurred, the fat crystals that form can take on different forms, a phenomenon known as polymorphism. The three major polymorphic forms occurring in fats are: \( \alpha \) (hexagonal), \( \beta' \) (orthorhombic), and \( \beta \) (triclinic), listed in increasing order of melting point, density and stability, and classified according to their subcell structure. The polymorphic form(s) present will directly affect the melting point of a fat and have also been correlated to the rheological properties, therefore making the polymorphism of a fat relevant in terms of functionality and potential application (Marangoni & Wesdorp, 2013a).
2.2 Properties of Laminating Shortenings

Laminating shortenings are solid fats used to make high fat (on average 40% fat by weight) dough and pastry products, including yeast-leavened Danishes and croissants, as well as unleavened puff pastry. The shortenings used are commonly high in saturated fat content and are therefore of interest for saturated fat reduction (Ergun, Thomson, & Huebner-Keese, 2016; Lai & Lin, 2006). The properties of a roll-in fat which achieve optimum functionality have been well established. First, roll-in fats require optimum plasticity at the specific temperature at which the croissants will be prepared due to the rolling that will take place. The fats must be soft and spreadable to facilitate layering without tearing the dough layers, but not so soft that they leak out under the pressure. A minimum SFC is also required to entrap and stabilize small bubbles of air. The layering of fat within the wheat dough results in a structure with air bubbles of non-uniform size dispersed horizontally within the matrix (Deligny & Lucas, 2015). This aids in the separation of the dough layers creating the visible rise and yielding an airy, flaky appearance.

The SFC of a fat is one factor that gives a good indication of the hardness and rolling ability of a fat at a given temperature. The fatty acid composition of a fat governs the SFC at particular temperatures, with respect to the proportions of saturated (SFA), unsaturated (UFA) and trans fatty acids (TFA). It is commonly understood that fats high in SFA or TFA will be solid at ambient temperatures and have a higher SFC, while fats high in UFA will have a low SFC at ambient temperatures, and exist in oil form. The fatty acid content is highly variable based on the fat source and can be used to predict the plasticity of a fat, as well as the SFC at particular temperatures. Roll-in shortenings are produced with a SFC ranging from 10-40% over a range of 33.3-10.0°C, making them plastic and workable in a wider range than other bakery fats (Baldwin, Baldry, & Johansen, 1972; Lai & Lin, 2006).

However, SFC is not the sole factor controlling the functionality of a fat (Marangoni & Rousseau, 1998). Another factor involved in the plasticity and functionality of roll-in fats is polymorphism. Fat polymorphism is often used as an indicator for fat functionality in the food industry, and in the case of laminated doughs, optimum plasticity has been correlated to the presence of $\beta'$ crystals. These crystals tend to be smaller in size with needle-like shapes, and create strong, plastic shortenings (Macias-Rodriguez & Marangoni, 2016a). It has also been suggested that fats stable in the $\beta'$ form have a greater ability to retain separate layers during
rolling and manufacturing (Garcia-Macias et al., 2011). Conversion to β polymorphs, the most stable crystal form, can occur but these crystals are often larger and associated with hard and brittle textures making them undesirable for rolling (Baldwin et al., 1972; DeMan, DeMan, & Blackman, 1991). The presence of β’ polymorphism has become standard for laminating or roll-in shortenings.

The fatty acid composition and TAG composition of roll in fats can vary significantly. In two studies by Garcia-Macias et al., different fat blends composed primarily of either soybean and shea or palm fractions were used to prepare puff pastry (Garcia-Macias et al., 2011, 2012). These blends all had approximately the same content of SFA (30%) but had different FA and TAG compositions. The resultant puff pastry behaved differently with regards to puff pastry height and hardness, which puts emphasis on the fact that fat composition is a factor in the performance of a fat used in lamination.

Recent work by Macias-Rodriguez and Marangoni thoroughly characterizes the rheological characteristics of roll-in shortenings and compared the results to all-purpose shortenings commonly used in cakes and other non-rolling applications (Macias-Rodriguez & Marangoni, 2016a, 2016b, 2017). The authors noted that all types of shortenings displayed similarities in polymorphism, SFC, melting behaviour, as well as large deformation mechanical behaviour. However, there were distinct differences in the nonlinear rheological behaviour between the types of shortenings, where roll-in shortenings displayed greater viscous behaviour. This behaviour was correlated to the microstructure of layered crystalline aggregates identified using ultra small angle x-ray scattering (USAXS) unique to laminating shortenings, stating that this structure allows for greater viscous dissipation during the lamination process. The authors describe that this behaviour can be thought of as a “rheological fingerprint,” where shortening alternatives can be developed with this behaviour in mind.

2.3 Laminated Doughs

Many types of laminated dough products exist, including puff pastry, Danishes and croissants. They all characteristically contain numerous alternating layers of fat and dough created by repeated rolling and folding, but will differ in the softness of the dough, the layering process used and content of yeast. For each type of laminated dough, different layering methods
exist, each involving distinct folding patterns and a varying number of resultant layers. These methods can be done by manual rolling or machine sheeting, however industrial production will employ machine roller systems to ensure the desired thickness is achieved and uniform (Lai & Lin, 2006). By creating these thin layers of solid fat within the dough, the fat is then able to act as a moisture barrier during baking. When the moisture in the dough turns to vapour and expands, the many hydrophobic layers of fat prevent this vapour from simply escaping, and as a result, the layers begin to expand (Baldwin et al., 1972; Renzetti, de Harder, & Jurgens, 2016; Rogers, 2004). Laminated doughs possess the capability of rising 80-600% of their initial height (Deligny & Lucas, 2015). For context, oven-rising bread dough will only rise to 20-100% of the initial height.

Deligny & Lucas investigated the effect of the number of layers on the final product (Deligny & Lucas, 2015). Their work revealed that a minimum number of fat layers result in baked products with few, large air pockets. Increasing the number of folds, and by extension the number of layers, increased the height of the final product. However, there was a maximum number of folds to which this relationship applied. Exceeding this number of folds resulted in fat layers that were too thin to act as water vapour barriers and thus rising was limited.

Renzetti et al. demonstrated the importance of the layered structure in laminated doughs, with respect to the extent of rising and flakiness of the final product (Renzetti et al., 2016). The authors determined that the highest lift and flaky texture corresponded to the doughs that contained the best formed layered structure, identified using microscopy. The rolling and sheeting process involves significant strain and deformation rates. The ability to achieve a desired layered structure depends on the materials ability to respond to this stress. Layers which were not uniformly formed or had been broken during rolling contributed to compromised lift and flakiness. This study put emphasis on the type of fat used and its consistency both initially and during working or rolling, where they were able to identify which fats would perform the best in regards to forming the best layered structure.
2.4 Wheat Flour and its Components

Wheat flour is composed of starch, water, protein, non-starch polysaccharides and lipids. Starch is the major component (70-75%), followed by water (14%), protein (10-12%), polysaccharides (2-3%) and finally the lipid portion (1.5-2%) (Frazier, Daniels, & Eggitt, 1981; Goesaert et al., 2005). For starch, the primary components are amylose and amyllopectin, two different polymers of D-glucose. Amylose is a linear polymer of glucopyranose linked together by α(1,4) glycosidic bonds, while amyllopectin is a branched polymer of glucopyranose linked together by α(1,4) glycosidic bonds with branches linked by α(1,6) bonds (Goesaert et al., 2005; S. Wang, Li, Copeland, Niu, & Wang, 2015). In breadmaking, starch contributes to water absorption but does not contribute much functionality overall, acting mainly as a filler within the network of protein that forms after hydration. The protein content of wheat flour is integral to breadmaking, specifically due to the content of gluten. Wheat flour proteins are distinguished into two main groups: gluten proteins and non-gluten proteins. However, the non-gluten only comprise approximately 15-20% of the total proteins, and their role in breadmaking remains unclear (Goesaert et al., 2005; Veraverbeke & Delcour, 2002). It is the gluten proteins that are responsible for network formation, and of these proteins, there are two distinguishable fractions, gliadins, which are monomeric, and glutenins, which are polymeric. Changes in the relative proportions of glutenin and gliadin cause variations in the protein network formation during breadmaking (Goesaert et al., 2005; Marchetti, Cardós, Campaña, & Ferrero, 2012). The gluten network that forms is viscoelastic, giving bread dough the ability to retain gas cells as they expand during leavening and baking, allowing for significant expansion or rising (Marchetti et al., 2012). The non-starch polysaccharides include arabinoxylans, β-glucan, cellulose and arabinogalactan peptides, which can effect dough consistency, development time, gas cell stabilization during baking, and loaf volume (Gan, Ellist, & Schofield, 1995; Goesaert et al., 2005). Finally, the lipid portion is divided into starch lipids and non-starch lipids. Starch lipids, primarily lysophospholipids, are strongly bound to starch, while non-starch lipids, which represent the majority of the total lipids, are primarily TAGs, and are either free or associated with proteins. Overall, wheat flour lipids contribute to gas cell stability and gluten network formation in breadmaking (Frazier et al., 1981; Goesaert et al., 2005; Lasztity, Bekes, Orsi, Smied, & Ember-Karpati, 1996).
2.4.1 Starch Retrogradation

The process of retrogradation occurs naturally in baked goods over time and is often considered detrimental due to its association with bread staling and firming. However, it is desired in other applications such as dehydrated mashed potatoes and breakfast cereals due to the sensory and structural properties contributed (Karim, Norziah, & Seow, 2000; S. Wang et al., 2015). Native starch exists in a semi-crystalline form, and will produce characteristic peaks in an XRD spectrum. When starch is hydrated and heated, gelatinization occurs, where granules swell and lose their crystallinity. This gelatinized state is associated with physical changes including increased viscosity and gel formation (Ratnayake & Jackson, 2007; S. Wang et al., 2015). After gelatinization, storage time allows for amylose and amylopectin chains to precipitate from their gelled state and re-associate into ordered structures, a process known as retrogradation. Retrogradation has also been described as the return of starch components to their granular or crystalline state, evident by XRD as the loss of peaks in the spectra after gelatinization, and the return of a weak pattern over time. (Bayer, Cagiao, & Baltá Calleja, 2006; Miles, Morris, Orford, & Ring, 1985; Morris, 1990; Roulet, MacInnes, Würsch, Sanchez, & Raemy, 1988). For this reason, XRD is one method used in the analysis of starch retrogradation, in addition to thermal analysis and rheological methods (Karim et al., 2000).

2.4.2 Lipid-Starch Interactions

Beyond laminated dough products, there are many food products in which starch and lipids are major components and have the opportunity to interact. This led to the documentation of many types of interactions between lipids and starch, including the addition of fat to baked goods to reduce moisture loss and increase the shelf life, as well as the addition of fat to alter the rheological properties of starch (Eliasson & Wahlgren, 2004; Tang & Copeland, 2007). Fatty acids and monoglycerides have been shown to increase the viscosity of starch pastes using rapid visco analyzer (RVA) techniques (Liang, King, & Shih, 2002; Ravi, Manohar, & Rao, 1999; Tang & Copeland, 2007; Zhang & Hamaker, 2003).

Many of these observed effects resulting from starch-lipid interactions are explained by the well-established formation of amylose-lipid and amylopectin-lipid complexes, which form when starch is gelatinized in the presence of lipids, particularly monoglycerides and fatty acids.
Formation of these complexes changes properties of the starch, including modification of the solubility of starch in water, and retarding starch retrogradation (Riisom, Krog, & Eriksen, 1984; Tang & Copeland, 2007). The structure of these complexes can be described as a helix formed by glucosyl residues around a hydrophobic centre. Amylose-lipid complexes have melting temperatures ranging from 95-115°C, at which point they dissociate. Upon cooling, the materials recrystallize and again form the complexes, demonstrating reversible thermal behaviour (Bulpin, Welsh, & Morris, 1982; Putseys, Lamberts, & Delcour, 2010). Amylopectin-lipid complexes do not show any endothermic peaks upon melting during DSC analysis and are therefore more difficult to identify. Many factors dictate the extent of complex formation, including the content of unsaturated versus saturated monoglycerides and fatty acids, the type of starch and the rate of gelatinisation (Eliasson & Krog, 1985; Krog & Nybo Jensen, 1970; Riisom et al., 1984). These factors also influence the melting behaviour of the resultant complexes, where longer differences in chain length and the time-temperature treatment used in formation will directly affect the melting behaviour of the complexes (Eliasson & Wahlgren, 2004; Karkalas, Ma, Morrison, & Pethrick, 1995; Siswoyo & Morita, 2002).

2.4.3 Lipid-Protein Interactions

Protein molecules are very long and are composed of both hydrophobic and hydrophilic regions, therefore, they could interact with either type of material. These different and alternating sections are responsible for creating the secondary, tertiary and quaternary structure of proteins. Proteins also have non-polar side chains that can interact with lipids, and for this reason proteins are known to have oil and fat binding properties. In foods, proteins often interact with lipid components to form emulsions. For example, emulsion stabilization in products such as dairy occurs due to protein-lipid interactions (Karel, 1973; Pomeranz & Chung, 1978). Dairy is an oil-in-water emulsion where lipoprotein complexes surround fat globules and stabilize them in the system.

The well-known interaction between lipids (when in large quantities) and gluten is the inhibition of gluten network formation, desirable in certain bakery applications including cookies and pie crusts. However, wheat flour contains a small portion of lipids itself, and this content has been shown to interact with gluten by hydrophobic or hydrophilic bonds with gliadin and
glutenin respectively, contributing to dough formation and loaf volume (Frazier et al., 1981; Lasztity et al., 1996; MacRitchie, 1977; McCann, Small, Batey, Wrigley, & Day, 2009; Pomeranz & Chung, 1978). It was determined that non-polar lipids exist on the surface of starch granules or in small lipid droplets throughout bread dough, while polar lipids were present within the protein matrix itself, identified using confocal scanning laser microscopy. Together, gluten and polar lipids form a three dimensional matrix that surrounds starch granules and has excellent gas cell stabilization abilities (Li, Dobraszczyk, & Wilde, 2004).

2.5 Challenges in Improving the Fatty Acid Profile of Roll-in Shortenings

Solid fat plays a distinct and essential role in a large variety of food products. This includes functional roles, as well as providing characteristic mouthfeel and texture. In general, the high melting TAGs are made up of a combination of SFAs and/or TFAs. In contrast, low melting TAGs are primarily composed of UFAs (Marangoni & Garti, 2011). While lipids are an important component of the diet, it is recommended to limit consumption of SFAs and especially TFAs due to their well-documented association with adverse effects on cardiovascular health (Mensink et al., 2003; Mozaffarian, Katan, Ascherio, Stampfer, & Willett, 2006). For some time, liquid edible oils have been hydrogenated to create solid fats, including many of the shortenings used for lamination, however this method of production leads to the production of TFAs, the consumption of which is more concerning than the SFAs they were created to replace (Mozaffarian et al., 2006). In contrast, there are recognized beneficial health effects from increasing UFA consumption (Lunn & Theobald, 2006). For this reason, the concept of replacing SFAs and TFAs with UFAs in foods is gaining popularity. However, direct replacement of solid fat with oils is usually not an option due to differences between their physical and sensory properties. In the case of laminating shortenings, solid fat is instrumental, responsible for being able to roll the fat while maintaining individual and separate layers, for the rising phenomenon, and for mouthfeel and texture upon consumption.

While shortenings containing substantial amounts of TFAs created highly acceptable products in terms of consumer acceptance, the push for the removal of TFAs from the diet has created a need for alternatives. However, substitution with other solid fats, including the use of naturally occurring highly saturated fats, such as palm, proved not to be as simple as it seemed. Research by Simovic et al. found that using low trans, yet still highly saturated, laminating
margarines in the production of puff pastry was not economical, nor nutritionally beneficial (Simovic et al., 2009). The authors found that laminating margarines containing less than 1% TFAs could only produce high quality products when the fat content was increased to 55% by weight. This is significant when compared to the common content of 30-40% fat by weight in laminated dough products (Lai & Lin, 2006). Even with significantly reduced TFA content, the overall increased fat content results in no actual health benefit.

Garcia-Macias et al. compared puff pastry made with fat blends composed of palm fractions with high oleic sunflower oil to that made with butter (Garcia-Macias et al., 2011). Each blend had a decreased SFA content, each with approximately 30%, compared to butter with approximately 65% SFA. Overall, the blends had different TAG compositions, but were each high in palmitic acid and oleic acid. Three out of four of these blends created puff pastry that was harder or tougher than that made with butter. One blend created puff pastry that was similar to that with butter in regards to texture, but all blends were still limited by the rise and specific volume of the final product. In another study, Garcia-Macias et al. analyzed four low SFA fat blends made with shea stearin, interesterified shea stearin, fully hydrogenated soybean oil and high oleic sunflower oil. Each blend again had approximately 30% SFA, and varying TAG compositions, but each was high in stearic acid and oleic acid. Similar to their first study, puff pastry prepared with each of the blends was harder and/or denser than that made with butter.

Renzetti et al. looked at the use of fats with varying levels of SFA by creating blends of palm stearin, palm oil and rapeseed oil in different ratios in the production of puff pastry (Renzetti et al., 2016). Palm stearin is the solid fat portion of palm oil and contains entirely SFA, while palm oil contains a mixture of SFA and UFA, and rapeseed oil is highly unsaturated. Six blends were created and ranged from approximately 40-55% SFA. The authors noted differences in the consistency as a result of the varying SFA content. It was suggested that fats with intermediate SFA content had the optimum consistency and were able to perform the best in regards to spreading and rolling during production. This optimum consistency translated into puff pastry with optimum structure and texture. While this work did not identify a low SFA blend for immediate use, the importance of fat consistency in regards to its performance during baking leads to possible future experiments where consistency is modified using factors other than SFA content.
2.5.1 Partial Solid Fat Replacement using Oleogels

Recently, oleogels have emerged as a novel means of employing liquid, edible oils in solid fat applications in food systems. This concept has the potential for creating foods with desirable physical and sensory properties, which also meet evolving regulations and health concerns. Canola oil is one of the many oils that can be gelled and contains only 6% saturated fatty acids, with mono- and polyunsaturated fatty acids comprising the remainder. This is one of the lowest proportions of saturated fatty acids naturally occurring in edible oils.

Ethylcellulose (EC) is a polymer capable of structuring oils into solid gel networks, which are potential alternatives to solid fat sources with improved fatty acid profiles. EC is a semi-crystalline cellulose polymer derivative, consisting of a cellulose backbone with ethoxyl substitutions at hydroxyl groups such that the substitution ratio is 2.5/2.6 (Davidovich-Pinhas, Barbut, & Marangoni, 2015; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012). It is this degree of substitution that causes the necessary lipophilicity at high temperatures. Commonly used EC range from 10, 20 or 45 cP, which is based on the average molecular weight (MW) of polymers present, where a higher MW corresponds to a greater viscosity (Dow Cellulosics, 2005). EC is of particular interest for food applications due to being tasteless, non-caloric and physiologically inert (Hughes, Marangoni, Wright, Rogers, & Rush, 2009; Zetzl, Marangoni, & Barbut, 2012). Incorporation of oleogels into food systems can both improve the fatty acid profile of the food, as well as decrease the overall fat content.

Using gelled canola oil in replacement of common solid fat sources, a significant reduction in trans and saturated fatty acid consumption is possible, without sacrificing many of the characteristic properties of foods. However, the need for solid fat in order to achieve the characteristic qualities associated with laminated dough products makes it particularly challenging to completely eliminate the saturated fatty acid content in laminating shortenings, therefore only partial fat replacement with oleogels can be considered. Dow Global Technologies LLC has submitted an application to patent laminating shortenings containing a proportion of EC oleogels, which was filed in 2014 and published in 2016 with the number US20160021898 A1 (Ergun et al., 2016). In addition, a portion of the fat was replaced with an alternative lower fat component, meaning the shortening had a lower overall fat content, in addition to the reduced saturated fatty acid content. Different formulations were described for the applications of
Danishes, puff pastry and croissants. Depending on the needs of the specific laminated application, each contained different varieties of EC, ranging from 20 to 45 cP, but each had a degree of substitution of 2.46-2.57. Each shortening blend contained at least one traditional solid fat component (butter, margarine or shortening) and an EC oleogel prepared with either canola oil or sunflower oil. Additionally, flour was added, and in some cases carboxymethyl cellulose, to increase the viscosity in order to eliminate shortening squeezing out during rolling. In some cases, water was added to facilitate adequate expansion upon baking. The exact composition depended on the laminated application. Danish pastries were produced containing a roll-in fat component with 23% oleogel (12% of 45 cP EC in canola oil) and 50% butter by weight, with the remaining portion comprised of flour. This was found to create a Danish pastry with an acceptable flaky texture. Oleogels prepared for puff pastry contained 12% of 45 cP EC in canola oil by weight. This oleogel was added at about 5% into the dough itself, which replaced butter entirely. The puff pastry roll-in shortening was composed of 35% butter and 40.5% oleogel. The remainder consisted of a solution of carboxymethyl cellulose in water, and flour. The resultant texture was also deemed acceptable, and further testing determined that CMC could be omitted while still obtaining a successful product. Finally, croissants were prepared using 7.1% of 45 cP EC in sunflower oil by weight. This decrease in EC concentration reflects the softer texture of croissant dough as compared to Danish and puff pastry doughs, and therefore the need for softer shortenings that would not tear the dough when rolling. The roll-in contained 40.3% laminating margarine, 52.7% oleogel, 1.7% water and 5.3% flour. This mixture allowed for a 60% reduction in saturated fat. The resultant croissants were reported to be indistinguishable from croissants made entirely with laminating margarine. While the published patent describes success in creating acceptable products in each laminated application, data that proves this, such as proof of comparable rise, firmness and texture, is not included. In addition, the lack of sensory analysis is a limitation and would be required before widespread acceptance and large scale availability.

Other oleogelators exist, including plant waxes, which have also been investigated for their use in laminating shortenings. These waxes are capable of gelling liquid oil at low concentrations (as low as <1% w/w) by trapping large volumes within a three dimensional network. Waxes are composed of a complex mixture of molecules, including esters of long-chain carboxylic acid and long chain alcohol derived from fatty acids and fatty alcohols hydrocarbons, free fatty acids, free fatty alcohols and other components in lesser quantities (Doan et al., 2017;
Vali, Ju, Kaimal, & Chern, 2005; Zulim Botega, Marangoni, Smith, & Goff, 2013). It is the
content wax esters that is generally considered responsible for the gelation behaviour of natural
waxes (Blake, Co, & Marangoni, 2014; Hwang, Kim, Singh, Winkler-Moser, & Liu, 2012; Patel,
Babaahmadi, Lesaffer, & Dewettinck, 2015). A wax dispersed in an oil will crystallize with
decreasing temperature, forming three-dimensional crystals of characteristic shape. One wax in
particular, rice bran wax, forms long fibrous crystals which likely contribute to its high gelation
efficiency, where it is capable of forming oleogels at concentrations as low as 1% (w/w) in
canola oil (Blake et al., 2014; Dassanayake, Kodali, Ueno, & Sato, 2009). Even still,
concentrations as low as 0.50 (w/w) were observed to cause gelation in soybean oil when
specific cooling rates were used (Hwang et al., 2012).

Blake and Marangoni reported using plant wax oleogelators to formulate laminating
shortenings, named by the authors as Coasun Laminate, and used the resulting substitute to
prepare croissants and Danishes (Blake & Marangoni, 2015). The laminating shortening
contained 7.5% rice bran wax, 6% monoglycerides (aphadim), 35% water, 0.3% sodium stearoyl
lactylate, 0.1% potassium sorbate, and the remainder was canola oil. Sodium stearoyl lactylate
was added as a co-surfactant for stabilization, and potassium sorbate was added as a preservative.
The formulated substitute was only used to laminate the products, and was not substituted for the
fat portion included in the dough. The authors claimed that the resultant products were visually
flaky and golden-brown in colour, resembling that of characteristic croissants and Danishes.
Based on unofficial sensory testing, the authors also reported that there was no waxy mouthfeel,
however the Coasun Laminate products were found to be denser than traditional croissants and
Danishes. Despite some textural differences, the authors were optimistic regarding the potential
of Coasun Laminate to be used as a laminating shortening replacement, however proper sensory
testing would shed more light on the consumer acceptance of these products, especially when
directly compared to traditional laminated products.

2.6 Conclusions

The crystallization properties of laminating shortenings are easy to determine using well
established techniques. The process of lamination then requires the shortenings to be layered
among a prepared wheat dough and baked at high temperatures that cause complete melting. It is
easy to assume that the properties of the fat after baking into dough are the same as they were in
bulk, however the melting that occurs and the presence of dough ingredients create the potential for changes in crystallization properties once cooled. The properties after baking must therefore be considered important, given the large quantity of fat in laminated bakery products and consequently the impact of fat on the quality of the final product overall. In addition, there are many types of laminating shortenings which do not all create the same quality of baked good, and therefore may have different properties after baking. Identifying key differences in their behaviour and correlating that to the quality of the final product could unveil what the desirable behaviour of laminating shortenings should be.

Despite the potential for ingredient interactions, the presence of other ingredients creates an issue when it comes to analysis, as the established methods for fat crystallization analysis do not tend to account for the presence of non-fat material. For example, components aside from fat have been known to produce peaks in a similar region as fat in an XRD spectrum, and components aside from fat will cause a signal when measuring for SFC by p-NMR. The importance of understanding any interactions between laminating shortenings and non-fat ingredients means it is necessary to have those ingredients present during analysis. Therefore, it must be determined if the conventional fat analysis techniques (e.g. XRD, DSC and p-NMR) can be performed on fat contained within a baked laminated product, and what, if any, additional steps are required to obtain usable and informative results.
2.7 References


3.0 Part I:
Matrix effects on the crystallization behaviour of butter and roll-in shortening in laminated bakery products

3.1 Materials and Methods

3.1.1 Materials

Butter and all non-fat ingredients were purchased at a local supermarket. Three different shortenings were obtained from Bunge® Canada. The composition of each shortening used, as stated by the supplier, is listed in Table 1. To maintain the fat crystal memory, the shortenings and butter were stored at 5°C prior to measurement. Wheat starch was obtained at a local supermarket, and pre-gelatinized wheat starch was obtained from ADM Food Ingredients. Prepared croissants were stored at room temperature for one week. One week was the determined shelf life as croissants became mouldy after this length of time.

Table 1: Roll-in Fat Compositions (as stated by manufacturer)

<table>
<thead>
<tr>
<th>Roll-In Fat Type</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsalted Butter</td>
<td>Butter</td>
</tr>
<tr>
<td>Hydrogenated Shortening A</td>
<td>Hydrogenated vegetable and hydrogenated modified palm oil</td>
</tr>
<tr>
<td>Hydrogenated Shortening B</td>
<td>Hydrogenated soybean and cotton seed oils</td>
</tr>
<tr>
<td>Non-Hydrogenated Shortening</td>
<td>Canola oil, modified palm oil and palm kernel oil</td>
</tr>
</tbody>
</table>

3.1.2 Fatty Acid (FA) Composition

Milk fat was obtained from butter by heating gently on hot plate until bubbling ceased. Foam was skimmed off the surface and the solids were discarded, retaining only the fat portion
for analysis. Each of the roll-in fats were converted into fatty acid methyl esters (FAMEs) using the protocol described by Christie (Christie, 1982). An Agilent 6890 series GC (Agilent Technologies Inc., Wilmington DE, USE) equipped with a CP-Sil 88 capillary column (100 m x 0.25 mm x 0.20 µm), flame ionization detector (FID), split/splitless injection port and a 7683-series auto-sampler was used for the analysis of prepared FAMEs. 1-2 µg/µL of total FAMEs were dissolved into hexane and 1µL was then injected. Hydrogen was used as the carrier gas at a flow rate of 1 mL/minute. The oven began at a temperature of 110°C, increasing to 230°C at a rate of 4°C/minute and holding here for 10 minutes. The injector temperature was 240°C, and the detector temperature was 280°C. Triplicate analysis was performed and the average reported. Fatty acid (FA) composition was determined with the use of an internal standard. Quantification of FAs was done by integration of the relative peak area.

3.1.3 Triacylglycerol (TAG) Compositions

Determination of TAG composition of milk fat (obtained from butter using the same method described above) and each shortening was carried out by high performance liquid chromatography (Agilent HPLC model 110, Agilent Tech, Palo Alto, CA), equipped with a quaternary pump, autosampler and Hewlett-Packard Chem Station software (Version A.10). TAGs were detected using a refractive index detector. Samples were dissolved in a 37.5:62.5 (v/v) solution of chloroform and acetone-acetonitrile (60:40 v/v). 10 µL of each sample was injected into an Econosil column (C18, 250 x 4.6 mm) in isocratic mode at a flow rate of 1.0 mL/minute. The mobile phase was acetone-acetonitrile (60:40 v/v). Triplicate analysis was performed and the average reported. TAGs were identified by comparison with internal standards (Sigma Aldrich, Oakville, ON, Canada) and quantified by integration of the relative peak area.

3.1.4 Croissant Preparation

Butter and each shortening were used to prepare different croissants using the following steps. The formulation used is presented in Table 2. Each batch prepared 12 croissants. An image of one of the final products is presented in Figure 1.
1. Add flour, water, 1% skim milk, sugar, instant yeast, salt, and a small amount of the respective fat type to a Kitchen Aid Standmixer affixed with a dough hook attachment, and mix together at a low speed for 4 minutes to form and knead the dough.
2. Allow the dough to rise for 90 minutes at room temperature.
3. Move the dough into a refrigerator to chill for at least 1 hour.
4. Place the dough on a floured surface and roll into a square of dimensions 25 cm x 25 cm.
5. Form the roll-in portion of the respective fat into a flat square of dimensions 16 cm x 16 cm.
6. Using the envelope method of folding, position the fat square in the center of the dough square, placed such that the corners of the fat met the midpoint of the dough edges. Fold the corners of the dough around the fat square to enclose.
7. Roll the fat and dough envelope into a rectangle approximately 0.5 cm thick, and fold in thirds.
8. Repeat step 8 two more times.
9. Rest in refrigerator for at least 4 hours.
10. Roll the layered dough on a floured surface into a rectangle of dimensions 55 cm x 22 cm.
11. Cut 12 triangular shapes from the dough such that the base of each triangle is 9 cm.
12. Cut a slit at the base of each triangle and stretch the corners as the croissant is rolled into the characteristic shape. Each croissant should weigh approximately 45-50 g before baking.
13. Let the croissants rise at room temperature for 2 hours.
14. Bake at 175°C (approx. 350°F) for 20 minutes.
15. Allow to cool at room temperature in order to simulate the uncontrolled cooling conditions of the bakery industry.
Table 2: Croissant Formula

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% By Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Unbleached Flour</td>
<td>37.54%</td>
</tr>
<tr>
<td>Water</td>
<td>18.39%</td>
</tr>
<tr>
<td>1% Skim Milk</td>
<td>9.58%</td>
</tr>
<tr>
<td>Sugar</td>
<td>4.49%</td>
</tr>
<tr>
<td>Instant Yeast</td>
<td>0.76%</td>
</tr>
<tr>
<td>Salt</td>
<td>0.54%</td>
</tr>
<tr>
<td>Fat/Shortening</td>
<td>2.14%</td>
</tr>
<tr>
<td>Roll-in Fat/Shortening</td>
<td>26.81%</td>
</tr>
<tr>
<td><strong>Total fat</strong></td>
<td><strong>28.95%</strong></td>
</tr>
</tbody>
</table>

Figure 1: Cross section of croissant prepared in this study (dough according to formula from Table 2; roll-component was hydrogenated shortening A)
3.1.5 Baked Bulk Fats

Approximately 20 g of each fat was added to a 50 mL beaker. A Thermo Scientific Heratherm oven set to 175°C (approx. 350°F) was used to bake the fat for 20 minutes, the same baking conditions used for the croissants. Each sample was allowed to cool completely at room temperature in order to simulate the uncontrolled conditions of the bakery industry. Butter was cooled in an incubator set to 16°C, due to need for lower temperatures to induce crystallization.

3.1.6 Polymorphism

Polymorphism classification was achieved by powder X-ray diffraction (XRD) (Rigaku, Japan). Approximately 1-2 g of roll-in fat, baked roll-in fat or crumbled croissant sample was put onto a glass slide into the machine at room temperature (20°C). Wheat starch and pre-gelatinized wheat starch were also prepared in the same manner on glass slides. The copper lamp was operating at 40 kV and 44 mA, used with the following slits: divergence (0.57 mm), scatter (0.57 mm), and receiving (0.3 mm). Polymorphic forms were identified from the resulting spectra in the wide angle region (WAXS) from 15-25° at a scan rate of 0.20°/minute. This rate was chosen in order to balance the need for good resolution while avoiding sample cracking over time caused by drying out within the machine. MDI’s Jade 6.5 software (Rigaku, Japan) was used to examine the WAXS spectra, where the presence of characteristic peaks was used to identify the presence different polymorphic forms. The relative proportion of each polymorph present was determined through qualitative comparison of peak heights. Triplicate analysis was performed for all samples, and new batches of croissants were prepared for each analysis.

Polymorphism changes with increasing storage time were evaluated using the same conditions listed above at determined time points: 0h (freshly baked and cooled), 24h (after one day of storage) and 1w (after one week of storage). Results presented are normalized values to account for a lesser signal achieved from a croissant sample containing approximately 30% fat.

3.1.7 Thermal Behaviour

A Mettler Toledo differential scanning calorimeter (DSC) (Mettler Toledo, Mississauga, ON, Canada) was used to determine the thermal behaviour of roll-in fats and corresponding croissant samples. Baked croissant and fat samples were analyzed after 48 hours of storage to
allow for crystal stabilization. 8-10 mg of each sample was weighed into aluminum crucibles and subjected to the conditions listed below. The peak temperatures of melting (T\text{m}) were determined using STAR\text{e} software (Mettler Toledo). Triplicate analysis was performed for all samples, and new batches of croissants were prepared for each analysis. Results presented are normalized to account for a reduced signal achieved from croissant samples containing approximately 30% fat. First, the samples were held at 5°C for 10 minutes. The samples were then heated from 5 to 80°C at a rate of 5°C/minute. This was followed by isothermal holding at 80°C for 15 minutes. Finally, the samples were cooled from 80 to 0°C at a rate of 5°C/minute.

3.1.8 Solid Fat Content

The SFC of each roll-in fat and their corresponding croissant sample measured at 10.0, 21.1, 26.7, 33.3 and 37.8°C according to AOCS Official Method Cd 16b-93. Glass NMR tubes were filled with the samples. The samples were equilibrated at each temperature for 30 minutes prior to measurement. Measurements for all samples were carried out using a Bruker PC/20 Series Minispec p-NMR Analyzer (Bruker Optics Ltd., Milton, ON). Fat free, baked croissant dough was also analyzed using the same conditions to account for signals caused by other ingredients in the croissant matrix. The average SFC and standard error are reported. Triplicate analysis was performed for all samples.

3.1.9 Texture Analysis

A Model TA.XT2 texture analyzer (Stable Micro Systems, Texture Technologies Corp., Scarsdale, NY) affixed with a TA-42 knife blade attachment and a 30 kg load cell was used to measure the firmness of croissants prepared with different roll-in fats. Measurements for each croissant type were taken at the same storage time points listed previously: 0h, 24h and 1w stored at room temperature. The average height of the croissants was 4 cm. The width of the knife blade always exceeded that of the croissants. The knife blade operated at a test speed of 2.0 mm/s over a distance of 70 mm. Three different batches of each type of croissant were analyzed using three sample croissants from each batch for a total of 9 replicates per point. Results will be reported as the average maximum force required to cut each croissant as an indicator of firmness when eating.
3.1.10 Statistical Analysis

GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis and normalization of data. One-way ANOVA was performed on texture analysis data for each of the fats at every time point analyzed. The level of significance was chosen as p < 0.05.

3.2 Results and Discussion

3.2.1 Fatty Acid Composition

The fatty acid composition of each roll-in fat is summarized in Table 3. Each fat used contains a different blend of SFA and UFA, allowing for the desired functionality and spreadability (Macias-Rodriguez & Marangoni, 2016). Both hydrogenated shortenings contain a similar distribution of FAs, with hydrogenated A (vegetable and palm oil based) containing slightly more palmitic acid, and hydrogenated B (soybean and cotton seed oil based) containing slightly more trans oleic fatty acids. The non-hydrogenated shortening contains a notable proportion of 12:0, 14:0 and 16:0 SFAs, reflecting its composition high in palm and palm kernel oils. The notable difference between the non-hydrogenated shortening and the other fats is the absence of TFA. The TFA content in both hydrogenated shortenings is significant (Table 3), and still, the method of analysis used was only able to distinguish the trans 18:1 fatty acids. Given that these shortenings are blended combinations of hydrogenated and non-hydrogenated components, it is reasonable to assume that a proportion of the linoleic acid, as well as the “other” unidentified fatty acids, could also be trans fatty acids. Additionally, the nutrition facts provided by the manufacturer indicate a TFA content of 25-30% for these shortenings, which is well above recommendations to limit of TFA intake as much as possible (<1%) described by the USDA, the American Heart Association and Health Canada (American Heart Association, 2015; Health Canada, 2010; USDA, 2010). Butter contains the greatest diversity of FAs, including characteristic short chain SFA from 4:0-10:0.
Table 3: FA Composition as determined by GC

<table>
<thead>
<tr>
<th>FA (%)</th>
<th>Hydrogenated A</th>
<th>Hydrogenated B</th>
<th>Non-Hydrogenated</th>
<th>Butter</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td></td>
<td>1.4±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:0</td>
<td></td>
<td>1.6±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:0</td>
<td></td>
<td>1.2±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td></td>
<td>2.9±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>4.6±0.3</td>
<td>3.7±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>2.3±0.2</td>
<td>12.3±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:1</td>
<td></td>
<td>1.1±0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td></td>
<td>1.2±0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>19.7±1.1</td>
<td>13.3±0.03</td>
<td>29.9±0.1</td>
<td>34.5±0.2</td>
</tr>
<tr>
<td>16:1</td>
<td></td>
<td>2.0±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:0</td>
<td></td>
<td>0.5±0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>12.0±1.4</td>
<td>16.0±0.6</td>
<td>3.3±0.07</td>
<td>10.3±0.1</td>
</tr>
<tr>
<td>18:1 (c9)</td>
<td>31.0±4.1</td>
<td>26.4±1.1</td>
<td>39.4±0.9</td>
<td>20.6±0.001</td>
</tr>
<tr>
<td>18:1 (t9)</td>
<td>13.0±1.0</td>
<td>16.6±0.07</td>
<td>0.4±0.1</td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>11.7±1.2</td>
<td>11.1±0.01</td>
<td>12.9±0.4</td>
<td>1.8±0.01</td>
</tr>
<tr>
<td>18:3 (3)</td>
<td>0.6±0.2</td>
<td>0.8±0.3</td>
<td>5.2±0.8</td>
<td>0.5±0.004</td>
</tr>
<tr>
<td>Other</td>
<td>3.5±0.4</td>
<td>12.4±0.03</td>
<td>3.2±0.01</td>
<td>4.0±0.02</td>
</tr>
</tbody>
</table>

SFA  31.7  29.3  40.1  69.6  
TFA  13.0  16.6  0.0  0.4  

3.2.2 Triacylglycerol Composition

The TAG composition of each fat used is summarized in Table 4. The TAG composition of a fat is related to their lipid source and processing. While determining the presence of different isomeric FAs in TAGs (i.e. cis vs. trans) for the hydrogenated shortenings is difficult, it is conceivable that any oleic acid component in the TAG composition could also be elaidic acid in a random distribution. Both hydrogenated shortenings have generally similar TAG
composition, primarily composed of OOLE, POLe, OOO, SOO and PSS. The non-hydrogenated shortening contained many TAGs characteristic of palm oil, including POO, POP and PPP, in addition to considerable proportions of OOLn, OLeLe, OOLE and OOO. Milk fat contained the largest variety of TAG species, including several short-chain species, making it difficult to compare to the manufactured shortenings. Based on the distinct differences in TAG, as well as FA composition between butter and the shortenings, it is expected that butter croissants will possess properties dissimilar from shortening containing croissants.

Table 4: TAG composition as determined by HPLC

<table>
<thead>
<tr>
<th>TAG (%)</th>
<th>Hydrogenated A</th>
<th>Hydrogenated B</th>
<th>Non-Hydrogenated</th>
<th>Butter</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaOBu</td>
<td>3.2±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMBu + PLaBu</td>
<td>0.6±0.001</td>
<td>0.5±0.07</td>
<td></td>
<td>2.8±0.3</td>
</tr>
<tr>
<td>BuOM + MMCp + PLaBu</td>
<td></td>
<td></td>
<td></td>
<td>3.0±0.2</td>
</tr>
<tr>
<td>LLL</td>
<td></td>
<td></td>
<td>0.9±0.06</td>
<td></td>
</tr>
<tr>
<td>PMBu</td>
<td></td>
<td></td>
<td></td>
<td>3.5±0.01</td>
</tr>
<tr>
<td>BuOO</td>
<td></td>
<td></td>
<td></td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>MOCp</td>
<td></td>
<td></td>
<td></td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>PMCp + POBu</td>
<td></td>
<td></td>
<td></td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>PPBu + SMBu</td>
<td></td>
<td></td>
<td></td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>LnLnLn</td>
<td>0.6±0.01</td>
<td>0.7±0.2</td>
<td>2.6±0.3</td>
<td></td>
</tr>
<tr>
<td>LeLeLe</td>
<td>2.8±0.1</td>
<td>2.9±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OOLn</td>
<td></td>
<td></td>
<td>8.0±0.6</td>
<td></td>
</tr>
<tr>
<td>OLeLe</td>
<td>6.2±0.1</td>
<td>5.2±0.2</td>
<td>14.0±0.6</td>
<td></td>
</tr>
<tr>
<td>PLeLe</td>
<td>3.1±0.06</td>
<td>3.1±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLeP</td>
<td></td>
<td></td>
<td>1.8±0.4</td>
<td></td>
</tr>
<tr>
<td>OOLE</td>
<td>14.2±0.4</td>
<td>11.7±0.5</td>
<td>18.0±1.8</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>POLe</td>
<td>7.3±0.5</td>
<td>7.4±0.3</td>
<td>3.5±0.4</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>PLeP</td>
<td></td>
<td></td>
<td>1.8±0.3</td>
<td></td>
</tr>
<tr>
<td>PPLe</td>
<td></td>
<td></td>
<td>2.9±0.01</td>
<td></td>
</tr>
</tbody>
</table>
Table 4 Continued:

<table>
<thead>
<tr>
<th>TAG (%)</th>
<th>Hydrogenated A</th>
<th>Hydrogenated B</th>
<th>Non-Hydrogenated</th>
<th>Butter</th>
</tr>
</thead>
<tbody>
<tr>
<td>POM</td>
<td>1.2±0.3</td>
<td></td>
<td>2.9±0.02</td>
<td></td>
</tr>
<tr>
<td>PPM</td>
<td>3.7±0.2</td>
<td>1.6±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OOO</td>
<td>24.8±0.08</td>
<td>26.2±0.7</td>
<td>16.0±1.0</td>
<td>2.6±0.08</td>
</tr>
<tr>
<td>POO</td>
<td>14.0±0.7</td>
<td>17.1±0.06</td>
<td>5.0±0.6</td>
<td>4.0±0.09</td>
</tr>
<tr>
<td>POP</td>
<td></td>
<td>6.8±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPO</td>
<td></td>
<td>2.7±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPP</td>
<td>5.1±0.1</td>
<td>0.9±0.2</td>
<td>9.8±0.3</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>SOO</td>
<td>5.5±0.9</td>
<td>8.4±0.1</td>
<td>1.4±0.5</td>
<td>3.6±0.08</td>
</tr>
<tr>
<td>POS</td>
<td>2.0±0.3</td>
<td>3.2±0.09</td>
<td>1.2±0.4</td>
<td>4.4±0.2</td>
</tr>
<tr>
<td>PPS</td>
<td>6.8±0.06</td>
<td>2.4±0.2</td>
<td>1.7±0.08</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>SOS</td>
<td>0.8±0.5</td>
<td></td>
<td>2.2±0.02</td>
<td></td>
</tr>
<tr>
<td>PSS</td>
<td>4.3±0.2</td>
<td>4.9±0.3</td>
<td>1.2±0.01</td>
<td></td>
</tr>
<tr>
<td>SSS</td>
<td>3.9±0.06</td>
<td></td>
<td>0.4±0.0</td>
<td></td>
</tr>
</tbody>
</table>

Bu – Butyric acid, Cp – Caprylic acid, La – Lauric acid, Le – Linoleic acid, Ln – Linolenic acid, O – Oleic acid, P – Palmitic acid, M – Myristic acid, S – Stearic acid

3.2.3 Polymorphism

Three principal polymorphic forms can exist in fats: α, β' and β, where α crystals are the least stable and β are the most. Most roll-in shortenings are manufactured to contain β' polymorphs, but conversion to the most stable crystal form can occur when conditions are favourable. Characteristic Bragg’s peaks in the spectra corresponding to d-spacing values of 4.2 Å, 4.3 Å and 3.8 Å, indicate the presence of β' polymorphs for all roll-in fats (Fig 2) (Marangoni & Wesdorp, 2013). Only in the case of the non-hydrogenated shortening is there a small peak appearing at 4.6 Å, indicative of β polymorphs. Based on relative peak sizes, the non-hydrogenated shortening contains an approximately equal proportion of β' and β polymorphs.
Initial XRD measurements of croissants were taken approximately 48 hours after baking to allow some crystal stabilization to occur. In figure 2, the apparent development of a peak at 4.6 Å is evident for all croissant types, signifying the development of β crystals. This peak is small in the case of hydrogenated A, hydrogenated B and butter, but large in the case of the non-hydrogenated shortening. In addition, the size of the peaks 4.2 Å and 4.3 Å for non-hydrogenated shortening croissants is reduced, indicating a substantial conversion to β crystals.

**Figure 2**: Wide angle X-ray spectra of roll-in fats in bulk, after baking, and baked into a croissant (Cr): a) hydrogenated shortening A, b) hydrogenated shortening B, c) butter and d) non-hydrogenated shortening
When each fat was baked using the same conditions as for the croissants, the XRD spectra contained peaks of roughly the same size and position as compared to the same fats before the baking treatment (Fig. 2). Values presented in Table 5 are the calculated ratios of the area of peaks corresponding to β polymorphs to the area of peaks corresponding to β' polymorphs. Values closer to 0 denote content of primarily β' polymorphs and values closer to 1.0 denote content of primarily β polymorphs. From these values, it is clear that baked fats do not experience significant polymorphic conversion over one week of storage, with the exception of the non-hydrogenated shortening, which shifts to a greater proportion of β polymorphs over time.

Croissants are consumed from the point they are baked and cooled, until the end of their one week shelf life, making the properties of a fat throughout this time relevant. With increasing storage time, the position and size of the peaks present in the XRD spectra for each of the bulk baked fats is largely unchanging, indicating polymorphic stability over one week of storage (fig. 3). However, the corresponding XRD spectra for croissant samples at the same time points indicate that this stability is lost in the presence of a croissant matrix (fig 4). Each of the four fats initially form predominantly β' polymorphs, an occurrence that is similar to that of the same fats when baked in bulk, but polymorphic conversion becomes distinct after 24 hours of storage time has passed. Croissants prepared with either of the hydrogenated shortenings show only minor conversion from β' to β polymorphs, with the appearance of a small peak corresponding to a d-spacing of 4.6 Å becoming more distinct over one week of storage. This is not so much an increase in height or peak area, reflected in the unchanging values from Table 5, but by visual inspection of the spectra, this peak does become noticeable with increasing storage. Butter croissants experience moderate conversion from β' to β polymorphs, but it is the non-hydrogenated shortening that experiences the most notable polymorphic conversion, evident by the significant peak growing in height at 4.6 Å and near loss of the peak at 4.2 Å over storage, and the notable increase in values in Table 5. Additionally, the appearance of a peak at 3.7 Å separate from that at 3.8 Å indicates that β polymorphs are predominating (Marangoni & Wesdorp, 2013).
**Figure 3**: Wide angle X-ray spectra of baked roll-in fats over one week of storage: a) hydrogenated shortening A, b) hydrogenated shortening B, c) butter and d) non-hydrogenated shortening
Figure 4: Wide angle X-ray spectra of croissants over one week of storage prepared from a) hydrogenated shortening A, b) hydrogenated shortening B, c) butter and d) non-hydrogenated shortening
Table 5: Ratios of $\beta$ to $\beta'$ peak areas\(^1\) with increasing storage time for baked fats and croissants

<table>
<thead>
<tr>
<th>Type</th>
<th>Baked Fats</th>
<th></th>
<th>Croissants</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>24h</td>
<td>1 week</td>
<td>0h</td>
<td>24h</td>
<td>1 week</td>
</tr>
<tr>
<td>Hyd A</td>
<td>0.25 ± 0.01</td>
<td>0.24 ± 0.004</td>
<td>0.24 ± 0.001</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.01</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>Hyd B</td>
<td>0.29 ± 0.005</td>
<td>0.28 ± 0.01</td>
<td>0.28 ± 0.005</td>
<td>0.31 ± 0.003</td>
<td>0.32 ± 0.001</td>
<td>0.32 ± 0.008</td>
</tr>
<tr>
<td>Non-Hyd</td>
<td>0.35 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.39 ± 0.01</td>
<td>0.34 ± 0.005</td>
<td>0.42 ± 0.05</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>Butter</td>
<td>0.42 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td>0.37 ± 0.007</td>
<td>0.38 ± 0.04</td>
<td>0.41 ± 0.01</td>
</tr>
</tbody>
</table>

\(^1\) $\beta'$ considered peaks: 4.3, 4.2, 3.8; considered $\beta$ peaks: 4.6, 3.7

The differences seen between fats simply melted at high temperatures versus the same fats baked into a croissant matrix indicate that there is a component within the croissant matrix which influences formation of at least a small proportion of $\beta$ polymorphs after 24 hours of storage. This matrix interaction seems to have a greater effect on the polymorphism of the non-hydrogenated shortening when compared to the hydrogenated fats and butter. This could be due to the presence of different TAGs in each roll-in fat source, as certain TAGs may form $\beta$ polymorphs more readily within the croissant matrix. It is also possible that certain TAGs interact more with the croissant matrix, resulting in the formation of stable $\beta$ polymorphs. The opposite may also be true, where the lack of one, or a combination of specific TAGs could be the cause of polymorphic instability.

A peak corresponding to a d-spacing value of 5.2 Å becomes notable in the spectra for each croissant type after at least 24 hours of storage, and more prominent after one week. Initially, it was assumed that this resulted from the conversion to $\beta$ crystals over time due to established associations with this peak and this polymorphic form (Marangoni & Wesdorp, 2013). However, after one week of storage a peak at 5.8 Å also begins to appear. Together, peaks at 5.2 Å and 5.8 Å are also two of the characteristic peaks of crystalline wheat starch (fig 5a). Wheat starch is gelatinized during baking due to the moisture in the croissant dough, causing it to lose its crystallinity and no longer produce any peaks in an XRD spectrum (fig 5b). However, starch retrogradation occurs over storage, when amylose and amylopectin chains will precipitate from their gelled state and recrystallize into ordered structures. For this reason, the phenomenon
of retrogradation has also been described as the return of starch components to their granular or crystalline state, and causes a weak pattern to again appear in the XRD spectra (Bayer, Cagiao, & Baltá Calleja, 2006; Miles, Morris, Orford, & Ring, 1985; Morris, 1990; Roulet, MacInnes, Würsch, Sanchez, & Raemy, 1988). Therefore, it is assumed that the starch retrogradation increasing with time is responsible for the appearance and growth of these peaks at 5.2 Å and 5.8 Å, in addition to one at 3.8 Å which would overlap with the same peak produced by β and β’ polymorphs. Interestingly, the occurrence of starch retrogradation coincides with the formation of β polymorphs over storage time. It is therefore possible that the polymorphic conversion is caused, at least in part, by the presence of retrograded starch. Determining whether this is a direct relationship or simply a coincidence is an objective for future work.

Figure 5: Wide angle X-ray spectra of a) crystalline wheat starch (obtained in isolated form at a local supermarket) and b) gelatinized wheat starch

3.2.4 Thermal Behaviour

Melting behaviour was determined for all samples. The presence of different polymorphic forms, each with their own melting point, appears as multiple peaks in the obtained DSC endotherm curve. For each fat type, it is evident that the melting temperature (T_m), or the temperature at which the maximum of each peak occurs, is not changing with the presence of the
croissant matrix (fig. 6). However, the peaks broaden, such that a higher temperature is required to completely melt the fat. In the case of butter and the non-hydrogenated shortening (fig. 6 c & d), there is an observed fractionation occurring that causes two or more smaller peaks take the place of a former larger peak. Here, the slow cooling of the fats causes higher and lower melting fractions to fractionate, resulting in two distinct melting points when heated. Since this occurs upon baking regardless of the croissant matrix, it is only the broadening of the peaks that can be considered a result of any existing matrix interactions. The broadening peaks suggest that the TAG crystals present within the croissant are more heterogeneous, existing in many different sizes. Returning to the hypothesis that certain TAGs interact with the matrix more than others, it is possible that the extent of interaction causes the TAGs to crystallize in a different manner, perhaps manifested as rapid crystallization upon cooling, encouraging the formation of β polymorphs and the promotion of heterogeneous mixture of crystal sizes and forms.
3.2.5 Solid Fat Content

The SFC of each fat decreased with increasing temperature, as expected, where butter showed the most drastic variance in SFC over the temperatures analyzed, having the largest value at 10°C and falling to almost 0% solids at 37.8°C (fig. 7a). The signal from fat free baked dough at each temperature was subtracted from that obtained for each croissant sample to account for the signal caused by the non-fat ingredients of a croissant. The results showed that the SFC contained within a croissant matrix is significantly less than a bulk fat (fig. 7b).
suspected interaction is likely causing some of the TAGs normally solid at the respective temperatures to not crystallize. Interestingly, the non-hydrogenated shortening has the greatest SFC at 21.1°C, the closest temperature to that which consumption and storage would normally occur. While the difference is not considered significant (p<0.05) as compared to the other shortenings used, the large variation in the results makes firm conclusions difficult. This variation resulted from the uneven distribution of fat throughout the croissants, where it was impossible to ensure the same amount of fat to other croissant components in each sample. Even still, the SFC values obtained for each of the bulk fats showed the opposite trend, where the non-hydrogenated shortening had the lowest SFC as compared to the other shortenings (excluding butter). Again, these results point towards this hypothesized interaction occurring to a different extent for the non-hydrogenated shortening compared with any other type of roll-in fat. The greater SFC combined with the increased content of β polymorphs in the non-hydrogenated shortening contained within the croissant matrix could account for some of the reduced acceptance of these products, potentially causing a waxy mouthfeel when compared to croissants prepared with other fats.

**Figure 7**: SFC curves as a function of temperature for: a) roll-in fats and b) croissants
3.2.6 Texture Analysis

Texture analysis allows for determination of the maximum force required to cut each sample, used as an indication of firmness and potential sensorial consequences of using different roll in fats. The maximum force during cutting occurred at the time just before the bulk of the croissant yielded to the knife attachment and two separate pieces were formed. As the knife attachment began pressing down on the croissant, the crumb structure first compressed, such that the maximum force required to cut the croissant was measured for the compact structure. However, this compression also occurs when one bites into a croissant, therefore this analysis can be used as an indicator for the results that could be attained if sensory analysis were to be performed. The croissants began with crisp edges and softened over time, however, the force required to penetrate the crust was never greater than that to completely cut the croissants in half. A one-way ANOVA (p < 0.05) was performed to determine significant differences between the all croissant types at each time point (fig. 8).

![Figure 8](image.png)

**Figure 8:** Maximum force required to cut croissants comparing croissants prepared with the different roll-in fats at different storage times. Bars with the same letter are not statistically different (p < 0.05).
Of the four fats used, only the non-hydrogenated shortening showed a significant change in firmness over one week of storage. After 24 hours, the firmness increased greatly, however it decreased once again after one week to a level which was not significantly different than when it was fresh. Only when fresh were these croissants comparable in firmness to the hydrogenated shortening or butter croissants. It is therefore conceivable to think that croissants prepared with non-hydrogenated shortening have only a 24 hour window for consumption before the matrix interactions cause quality deterioration.

Comparison also revealed differences in firmness between croissant types only appear after 24 hours, when croissants prepared with any shortening increased in firmness (to a point not significantly different from each other), while the croissants made with butter experienced a decrease in firmness. After one week, the butter croissants remained the softest. Essentially, croissants prepared shortenings with a greater SFC experienced an increase in firmness, while those prepared with butter, which has the lowest SFC, decreased in firmness. This result contradicts previous findings that detailed a reduced firming rate in bread over storage when using a shortening with a greater SFC (Rogers, Zeleznak, Lai, & Hoseney, 1988; Smith & Johansson, 2004). It is possible that because bread contains significantly less fat than croissants (% w/w), the effects noticed in these studies are simply caused by the moisture barrier created by the presence of solid fat, reducing migration as compared to a fat free dough. Since croissants contain a significantly greater amount of fat, the impact on texture caused by the properties of the fats themselves, or the results of matrix interactions occurring, overshadows the effects of firming by moisture migration.

3.3 Conclusions

In this part, two hydrogenated roll-in shortenings, one non-hydrogenated roll-in shortening and butter were used to prepared croissants. The fat crystallization properties of each fat in bulk, each fat after a baking treatment, and each fat within the matrix of croissants were compared. In each case, only the fat contained within a croissant began to convert from β' to β polymorphs over one week of storage, however conversion was only significant in croissants prepared with non-hydrogenated shortening. Peak broadening occurred in the melting endotherms for each type of fat within the croissant matrix, indicating crystal heterogeneity. A significantly decreased SFC for each type of croissant was observed as compared to that of the
same fat in bulk. Finally, variations in firmness over one week of storage indicated that croissants prepared with shortening increased in firmness over time, with greatest increase seen in croissants prepared with non-hydrogenated shortening. Given the results, it is likely that there is an interaction occurring between the roll-in fats and the croissant matrix that is causing observed differences. The results suggest that the extent of this interaction may be determined by the presence, or lack of, particular TAGs. The conclusion that shortenings will behave differently within a croissant matrix is significant considering the dependence of consumer acceptance on the type of shortening used. However, sensory analysis is required to determine conclusive relationships regarding consumer acceptability and the crystallization behaviour of the shortenings or butter.

When considering the components of the croissant matrix, the ingredient with the greatest potential for interaction is wheat flour, as it is the only ingredient present at a high enough concentration. However, the components that make up flour, protein and starch, must be considered separately. Interactions between lipids and proteins, as well as lipids and starch have been well documented, yet the noted interactions have only been observed when isolated in fat alone, not within an entire ready-to-eat matrix. Therefore more research is required to determine which components are interacting, and what type of interaction is occurring.
3.4 References


4.0 Part II:
Gelatinized Wheat Starch Influences Crystallization Behaviour and Structure of Roll-in Shortenings in Laminated Bakery Products

4.1 Materials and Methods

4.1.1 Materials

Non-fat ingredients were purchased at a local supermarket. Two different shortenings were obtained from Bunge® Canada, one hydrogenated shortening (hydrogenated vegetable and hydrogenated modified palm oil) and one non-hydrogenated shortening (canola oil, modified palm oil and palm kernel oil). To maintain the fat crystal memory, the shortenings were stored at 5°C prior to measurement. Prepared croissants were stored at room temperature for one week. One week was the determined shelf life as croissants became mouldy after this length of time.

4.1.2 Croissant Preparation

Each shortening was used to prepare different croissants. The formulation used and the preparation steps are detailed in section 3.1.4.

4.1.3 Baked Isolated Component Mixtures

Mixtures of each shortening with either gluten or wheat starch were prepared and baked in 50 mL beakers. Samples were also prepared where the gluten or wheat starch was first hydrated through direct addition of water. All samples contained components at the same ratio as what exists within a croissant (Table 6). Samples will be referred to as H (hydrogenated shortening) or NH (non-hydrogenated shortening), with added matrix components +St (crystalline wheat starch), +GSt (gelatinized wheat starch), +G (gluten), or +GN (gluten network). Each mixture was approximately 50g in total. A Thermo Scientific Heratherm oven set to 175°C (approx. 350°F) was used to bake the mixtures for 20 minutes, the same baking conditions used for croissants. Each sample was allowed to cool completely at room temperature.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Shortening</th>
<th>Starch</th>
<th>Gluten</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortening + St</td>
<td>52%</td>
<td>48%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shortening + GSt</td>
<td>39%</td>
<td>36%</td>
<td>-</td>
<td>25%</td>
</tr>
<tr>
<td>Shortening + G</td>
<td>86%</td>
<td>-</td>
<td>14%</td>
<td>-</td>
</tr>
<tr>
<td>Shortening + GN</td>
<td>56%</td>
<td>-</td>
<td>9%</td>
<td>35%</td>
</tr>
</tbody>
</table>

4.1.4 Polymorphism

Polymorphism was determined using powder X-ray diffraction (XRD) (Rigaku, Japan). Approximately 1–2 g of each sample was put onto a glass slide into the machine at room temperature. The copper lamp was operating at 40 kV and 44 mA, used with the following slits: divergence (0.57 mm), scatter (0.57 mm), and receiving (0.3 mm). Polymorphic forms were identified from the resulting spectra in the wide angle region (WAXS) from 15-25° at a scan rate of 0.20°/min. The same rate used previously for croissant samples was chosen, in order for direct comparison of intensity. MDI’s Jade 6.5 software (Rigaku, Japan) was used to examine the WAXS spectra, where the presence of characteristic peaks was used to identify the presence of different polymorphic forms. The relative proportion of each polymorph present was determined through qualitative comparison of peak heights. Polymorphism changes with increasing storage time were evaluated using the same conditions listed above at determined time points: 0h (freshly baked and cooled), 24h (after one day of storage) and 1w (after one week of storage). Triplicate analysis was performed for all samples. Results presented are normalized values to account variation in the signal achieved from samples containing different amounts of fat.

4.1.5 Solid Fat Content

The SFC of each roll-in fat and their corresponding croissant sample measured at 10.0, 21.1, 26.7, 33.3 and 37.8°C according to AOCS Official Method Cd 16b-93. Glass NMR tubes were filled with the samples. The samples were equilibrated at each temperature for 30 min prior to measurement. Measurements for all samples were carried out using a Bruker PC/20 Series Minispec p-NMR Analyzer (Bruker Optics Ltd., Milton, ON). Values achieved at 60°C
(determined to be above the melting temperature of both fats) were subtracted from all values to account for signals caused by non-fat ingredients. The average SFC and standard error are reported.

4.1.6 Rate of Crystallization

Again, the Bruker PC/20 Series Minispec p-NMR Analyzer (Bruker Optics Ltd., Milton, ON) was used. Samples in NMR tubes were first allowed to equilibrate to 60°C in a water bath. After initial measurements were taken at 60°C, samples were then moved to a water bath set to 10°C. Measurements were taken at predetermined time intervals up to 1 hour after cooling began, returning the samples to the 10°C water bath after each measurement. The obtained data was fitted using the Avrami model. Using this model is common to quantify the fat crystallization kinetics (Marangoni, 1998; Sharples, 1996; Wright, Hartel, Narine, & Marangoni, 2000). The equation is in the form:

$$\frac{SFC(t)}{SFC(\infty)} = 1 - e^{-k(t)^n}$$

where \( k \) is the Avrami constant that represents the crystallization rate constant, and \( n \) is the Avrami exponent, or index of crystallization. The constant \( k \) takes into account both nucleation and crystal growth, where a large value indicates a faster crystallization rate and vice versa. The exponent \( n \) describes the crystal growth mechanism, including the time dependence of nucleation and the dimensions in which crystal growth occurs.

4.1.7 Thermal Behaviour

A Mettler Toledo differential scanning calorimeter (DSC) (Mettler Toledo, Mississauga, ON, Canada) was used to determine the thermal behaviour all samples. Baked samples were analyzed after 48 hours of storage to allow for crystal stabilization. 8-10 mg of each sample was weighed into aluminum crucibles and subjected to the following conditions: held at 5°C for 15 minutes, heated from 5 to 80°C at a rate of 5°C/minute, held at 80°C for 15 minutes, cooled from 80 to 0°C at a rate of 5°C/minute. The peak temperatures of melting (Tm¬) were determined using STARE software (Mettler Toledo). Results presented are normalized to account for lesser signals achieved samples containing different amounts of fat. Subsequent tests were run on
samples containing shortening +GSt to identify if amylose-lipid complexes had formed in the system. For these tests, conditions used were the same as described previously except that the maximum temperature was 115°C, as this is above the melting temperature previously documented for these complexes.

4.1.8 Statistical Analysis

GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis and normalization of data. One-way ANOVA was performed on the Avrami coefficient data for each sample type. The level of significance was chosen as p < 0.05.

4.2 Results and Discussion

4.2.1 Polymorphism

There are three principal polymorphic forms that can exist in fats, α, β' and β, in order of increasing stability, and are identified using characteristic Bragg’s peaks in the XRD spectra. Peaks corresponding to d-spacing values at 4.3 Å, 4.2 Å and 3.8 Å, indicate the presence of β' polymorphs, while peaks corresponding to d-spacing values of 5.2 Å and 4.6 Å indicate the presence of β polymorphs (Marangoni & Wesdorp, 2013a). In our previous investigation, β' polymorphs were most prevalent in each roll-in shortening examined. However, conversion to the most stable crystal form (β) occurred to different extents when shortenings were baked into a croissant matrix and stored for one week. In order to identify the cause of this conversion, shortenings were baked with isolated matrix components. In Figure 9, it can be seen that after 24 hours of storage, the only samples which experienced polymorphic conversion were the croissants and the gelatinized wheat starch (+GSt) samples. For both, the non-hydrogenated (NH) shortening experienced much more notable conversion compared to the hydrogenated (H) shortening, evident by the drastic difference in relative peak height at 4.6 Å. When crystalline wheat starch (+St) is present, its previously identified characteristic peaks at 4.9 Å, 5.2 Å and 5.8 Å appear, proving that gelatinization does not occur when water is not added (Mattice & Marangoni, 2017). Neither the gluten (+G) nor the gluten network (+GN) samples experienced any polymorphic conversion for either shortening. Since our previous investigation showed that storage time is a factor for polymorphic conversion within croissants, this was again considered
for samples containing gelatinized wheat starch. Based on when the peak at 4.6 Å appears over storage time, Figure 10 a&b show that polymorphic conversion takes place only after 24 hours of storage for the non-hydrogenated shortening, but only after one week of storage for the hydrogenated shortening. Further, after one week of storage, NH+GSt had almost completely converted to the β form, evident by the near loss of peaks at 4.3 Å and 4.2 Å, and the appearance of a separate peak at 3.7 Å, also associated with β polymorphs. In comparison, the H+GSt samples only experienced very minor conversion (Marangoni & Wesdorp, 2013a). To complete the comparison, +St samples were also analyzed over one week of storage, shown in Figure 10 c&d. Here, it is apparent that there is no polymorphic conversion occurring in H+St samples, but growth of the peak at 4.6 Å does indicate conversion to some degree in NH+St samples, however not nearly to the same extent as NH+GSt samples. Based on the similarities between croissant and +GSt samples, gelatinized wheat starch is likely the cause of the observed polymorphic instability.

Figure 9: XRD spectra after 24h of storage of a) hydrogenated shortening and b) non-hydrogenated shortening, after baking without matrix components, after baking into a croissant and after baking with isolated croissant components.
In agreement with Part I, the interaction observed appears to occur at different extents based on the type of shortening. This could be explained by very different TAG composition of this shortening when compared with the other fat sources, reported in our previous study. It could be that certain TAGs in the non-hydrogenated shortening interact to a greater extent, or form β polymorphs more readily in the presence of gelatinized wheat starch. It is also possible that the lack of one, or a combination of specific TAGs could be the cause of polymorphic instability. Given that there are particular documented interactions specifically between
Similar to results previously reported from analysis of croissants, the appearance and growth of peaks at 4.9 Å, 5.2 Å and 5.8 Å occurs over storage for GSt containing samples. Given that these are two of the characteristic peaks associated with crystalline wheat starch, we previously attributed this to starch retrogradation with increasing storage time. When wheat starch is gelatinized, the characteristic peaks associated with crystalline wheat starch no longer appear in an XRD spectrum, however, retrogradation has also been described as the return of starch components to their granular or crystalline state, and causing a weak pattern to again appear in the XRD spectra (Bayer et al., 2006; Miles et al., 1985; Morris, 1990; Roulet et al., 1988). Interestingly, starch retrogradation continuing with time coincides with the increasing formation of β polymorphic crystals. It is therefore likely that the polymorphic conversion is caused in part by the either the presence of retrograded starch or the actual process of retrogradation. However, because some extent of conversion does exist with the presence of non-gelatinized, or crystalline, wheat starch over time, retrogradation cannot be solely responsible.

4.2.2 Solid Fat Content

The signal from wheat starch, gelatinized wheat starch, gluten and gluten network at each temperature cause great difficulty in determining the SFC of the sample mixtures, particularly in gelatinized wheat starch and gluten network samples due to the presence of moisture. Our previous investigation determined that the SFC contained within a croissant matrix is significantly less than a bulk fat, and the results of the gelatinized wheat starch samples generally follow this trend, particularly at higher temperatures. However, this trend was also observed for the other component samples, with the exception of +G, making it difficult to state true conclusions from these results (Table 7).
Table 7: SFC curves as a function of temperature for samples of hydrogenated shortening and non-hydrogenated shortening with and without croissant matrix components.

<table>
<thead>
<tr>
<th>Sample</th>
<th>SFC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.0°C</td>
</tr>
<tr>
<td>H shortening</td>
<td>29.0 ± 0.4</td>
</tr>
<tr>
<td>H+St</td>
<td>26.0 ± 2.4</td>
</tr>
<tr>
<td>H+GSt</td>
<td>30.8 ± 0.5</td>
</tr>
<tr>
<td>H+G</td>
<td>26.8 ± 2.1</td>
</tr>
<tr>
<td>H+GN</td>
<td>28.3 ± 3.7</td>
</tr>
<tr>
<td>NH shortening</td>
<td>26.4 ± 0.4</td>
</tr>
<tr>
<td>NH+St</td>
<td>20.7 ± 2.3</td>
</tr>
<tr>
<td>NH+GSt</td>
<td>23.9 ± 2.9</td>
</tr>
<tr>
<td>NH+G</td>
<td>35.8 ± 2.3</td>
</tr>
<tr>
<td>NH+GN</td>
<td>25.2 ± 2.3</td>
</tr>
</tbody>
</table>

4.2.3 Rate of Crystallization

The rate of crystallization was determined by measuring the SFC as samples were cooled from 60°C to 10°C. Figure 11 presents the SFC as a function of time, where the cooling rate over the elapsed time was determined to be statistically similar for all samples. The curves obtained were fit using the Avrami model, and the Avrami coefficients ($k$) and exponents ($n$) are reported in Table 8. From the results, it is evident that the SFC of a fat in the presence of gelatinized wheat starch increases at a faster rate than what occurs for the same fat in bulk based on increased $k$ values. It is also noteworthy that samples containing crystalline wheat starch did not demonstrate this behaviour. It is therefore conceivable that gelatinized wheat starch is acting as a nucleation site on which the fat can crystallize, thereby allowing crystallization to occur more quickly. Faster crystallization causes formation of less thermodynamically stable crystals,
which could be the cause of some polymorphic instability over time. Although the majority of polymorphic conversion does not occur immediately upon cooling, the fact that the existing fat crystals in the system were formed so quickly gives rise to greater instability, increasing the chance that another environmental factor, such as starch retrogradation, could cause the shift from $\beta'$ to $\beta$ crystals at a later time. The $n$ values reported in Table 8 do not show any significant changes with added crystalline or gelatinized wheat starch, indicating the crystal growth mechanism is not impacted by the presence of these components.

Figure 11: Changes in SFC as a function of time during the isothermal (10°C) of a) hydrogenated shortening and b) non-hydrogenated shortening, each in bulk, and after baking with wheat starch or gelatinized wheat starch. Solid lines correspond to the Avrami fit of the model to the data.
Table 8: Avrami variables from modelled rate of crystallization curves (Fig. 11) for each shortening in bulk and when baked with wheat starch or gelatinized wheat starch.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Avrami Coefficient (k)</th>
<th>Avrami Exponent (n)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenated A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk Fat</td>
<td>0.61 ± 0.01</td>
<td>0.64 ± 0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>+St</td>
<td>0.69 ± 0.02</td>
<td>0.58 ± 0.03</td>
<td>0.96</td>
</tr>
<tr>
<td>+GSt</td>
<td>0.92 ± 0.04</td>
<td>0.50 ± 0.04</td>
<td>0.90</td>
</tr>
<tr>
<td>Non-Hydrogenated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk Fat</td>
<td>0.61 ± 0.02</td>
<td>0.90 ± 0.05</td>
<td>0.98</td>
</tr>
<tr>
<td>+St</td>
<td>0.60 ± 0.01</td>
<td>0.89 ± 0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>+GSt</td>
<td>0.88 ± 0.06</td>
<td>0.83 ± 0.09</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Values with the same superscript letter within the same column are not statistically different (P < 0.05)

4.2.4 Thermal Behaviour

In Figure 12a, it is evident that the melting temperature ($T_m$) of the hydrogenated shortening, or the temperature at which the maximum of each peak occurs, does not change with the presence of matrix components (Table 9). In Figure 12b, the non-hydrogenated shortening undergoes an observed fractionation that causes two smaller peaks to take the place of a former larger peak (relative to the same shortening before baking as reported in our previous investigation), occurring for all samples after baking with the exception NH+St samples (Mattice & Marangoni, 2017). Additionally, both NH+G and NH+GN samples appear to undergo fractionation to a lesser extent, evident by the first peak occurring at temperatures between that of fractionated and unfractionated samples.

However, in the case of both shortenings, the presence of gelatinized wheat starch prompts the same result as when baked into a croissant, where the peaks broaden such that a higher temperature is required to completely melt the fat (Fig. 12 a&b). The peak width was represented by the difference between the onset ($T_{onset}$) of the first major melting peak, and the temperature at which melting had completed, where the curve levelled ($T_{end}$), in degrees Celsius (Table 10). For each shortening, the croissant and +GSt samples had significantly greater peak
width values when compared to all other samples. There was greater variance in the case of the non-hydrogenated shortening due to the existence of two major peaks and extent of fractionation that occurred with different samples, however it was still evident that the peak widths of croissant and +GST samples were significantly different than all other samples. The broadening peaks suggest that the TAGs crystallize heterogeneously in the presence of gelatinized wheat starch, existing in many different sizes. It is also possible that the heterogeneity is a result of the previously observed increase in crystallization rate. As stated in the previous section, it is likely that gelatinized wheat starch acts as a nucleation site on which the shortenings can crystallize. However, not necessarily all of the fat will crystallize on the gelatinized starch, but rather some extent of nucleation may be occurring within the fat itself. This theory can be used to explain the variation in crystal size, as the different processes may favour crystal growth over nucleation more than another.

The crystallization behaviour of both shortenings exhibits a similar pattern, where the +St, +G and +GN samples behave like the shortenings baked in the absence of matrix components, while croissant and +GST samples demonstrate a different behaviour. While the onset temperature of these samples is unchanging, the peak maximum or crystallization temperature (T_p) occurs at a lower temperature than all other samples of the same fat. The difference in T_p is only minor, despite the onset of crystallization being the same (Fig. 12c&d), therefore we hypothesize that at that instant there is too much heat generated from the rapid crystallization to dissipate, leading to a delayed T_p due to the fact that the temperature within the sample had not yet decreased to the same point as the instrument (Table 10).
Table 9: Melting peak width represented as the average magnitude of difference between $T_{\text{end}}$ and $T_{\text{onset}}$ for each shortening after baking without matrix components, after baking into a croissant and after baking with isolated croissant components for nine replicates, with outliers removed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak width (°C)</th>
<th>Sample</th>
<th>Peak Width (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked H shortening</td>
<td>6.4$^a$ ± 2.1</td>
<td>Baked NH shortening</td>
<td>15.5$^a$ ± 2.2</td>
</tr>
<tr>
<td>H croissant</td>
<td>19.4$^b$ ± 1.4</td>
<td>NH croissant</td>
<td>23.6$^b$ ± 1.1</td>
</tr>
<tr>
<td>H+St</td>
<td>7.2$^a$ ± 1.7</td>
<td>NH+St</td>
<td>9.4$^c$ ± 2.9</td>
</tr>
<tr>
<td>H+GSt</td>
<td>14.2$^c$ ± 1.8</td>
<td>NH+GSt</td>
<td>22.0$^b$ ± 2.8</td>
</tr>
<tr>
<td>H+G</td>
<td>7.6$^a$ ± 1.8</td>
<td>NH+G</td>
<td>10.1$^c$ ± 2.8</td>
</tr>
<tr>
<td>H+GN</td>
<td>8.9$^a$ ± 2.4</td>
<td>NH+GN</td>
<td>13.0$^{bc}$ ± 3.5</td>
</tr>
</tbody>
</table>

Values with the same superscript letter within the same column are not statistically different (P < 0.05)

Table 10: Peak melting and crystallization temperatures for samples of baked hydrogenated shortening and non-hydrogenated shortening with and without croissant matrix components.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Melting (°C)</th>
<th>Crystallization (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak 1</td>
<td>Peak 2</td>
</tr>
<tr>
<td>Baked H shortening</td>
<td>50.1 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>H croissant</td>
<td>49.5 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>H+St</td>
<td>50.2 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>H+GSt</td>
<td>50.1 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>H+G</td>
<td>50.2 ± 1.0</td>
<td>-</td>
</tr>
<tr>
<td>H+GN</td>
<td>49.8 ± 0.7</td>
<td>-</td>
</tr>
<tr>
<td>Baked NH shortening</td>
<td>42.7 ± 1.8</td>
<td>50.5 ± 1.2</td>
</tr>
<tr>
<td>NH Croissant</td>
<td>42.0 ± 1.1</td>
<td>49.9 ± 0.6</td>
</tr>
<tr>
<td>NH+St</td>
<td>46.1 ± 1.2</td>
<td>-</td>
</tr>
<tr>
<td>NH+GSt</td>
<td>42.5 ± 1.3</td>
<td>50.8 ± 1.3</td>
</tr>
<tr>
<td>NH+G</td>
<td>44.5 ± 0.9</td>
<td>51.1 ± 0.2</td>
</tr>
<tr>
<td>NH+GN</td>
<td>44.4 ± 1.3</td>
<td>50.4 ± 0.9</td>
</tr>
</tbody>
</table>
Figure 12: DSC melting endotherms of a) hydrogenated shortening and b) non-hydrogenated shortening with different components, and DSC crystallization endotherms of c) hydrogenated shortening and d) non-hydrogenated shortening with different components.
Additional testing, where H+GSt and NH+GSt samples were heated further to 115°C, did not reveal any additional endothermic peaks. This indicates, that despite starch gelatinization occurring in the presence of lipid material, no amylose-lipid complexes were formed, which, as previously cited, melt at temperatures ranging from 95-115°C. This is likely a reflection of a lack of monoglycerides and free fatty acids in each of the shortenings’ composition.

4.3 Conclusions

Overall, the similarities in behaviour between +GSt and croissant samples indicate that there is an interaction that occurs between gelatinized wheat starch and roll-in shortenings. This interaction is associated with polymorphic conversion to the most stable form, an increased rate of crystallization and crystal heterogeneity as evidenced by a broader melting peak in the DSC endotherm. Consequences of this interaction are pronounced for the non-hydrogenated shortening, which appears to interact with gelatinized starch to a greater extent than the hydrogenated shortening. When considering the coincidental occurrence of polymorphic conversion with starch retrogradation, it is possible that retrogradation is at least in part responsible for the polymorphic instability observed. This therefore puts emphasis on the benefits of consuming laminated baked products as soon after baking as possible, particularly those which have been prepared using non-hydrogenated shortening. Also notable is the increased rate of crystallization that occurs in the presence of gelatinized wheat starch. Based on the results observed, it is probable that the crystallization behaviour of roll-in shortenings within a croissant matrix is most significantly impacted by this increased crystallization rate, the occurrence of starch retrogradation, or by the combination of the two.

This research allows for a better understanding of the interactive role of fats in baked laminated dough products, with the goal of aiding in the eventual development of acceptable alternatives with improved fatty acid profile. By determining that shortening composition is a factor in how it interacts with the gelatinized wheat starch within the laminated dough matrix, it is possible to tailor shortenings during development such that they behave like those which are known to create the most acceptable products. This would open the door for products that comply with evolving regulations and health concerns however still satisfy consumers.
4.4 References


5.0 Conclusions and Future Work

Laminating or roll-in shortenings serve the main purpose of creating many thin layers of shortening that separate layers of dough. Because of the extensive rolling and folding that takes place, the shortening must be very plastic and extensive research has been done to determine the properties which achieve this plasticity. However, it cannot be assumed that the properties of a roll-in shortening prior to incorporation remain after baking as the melting at high temperatures and the potential for ingredient interactions create opportunity for changes in crystallization behaviour upon cooling within the matrix.

In this work, specialized laminating or roll-in shortenings, two hydrogenated and one non-hydrogenated, as well as butter were used to prepare four different types of croissants. The fats, the same fats after baking and the respective croissants were each analyzed using the same techniques to examine the effect of the croissant matrix on fat crystallization. Each of the samples were analyzed for polymorphism using powder x-ray diffraction (XRD), SFC by pulsed nuclear magnetic resonance (p-NMR) and melting behaviour by differential scanning calorimetry (DSC).

First, given the lack of research done on the fat contained within an entire food product using these specific methods, sample preparation techniques were developed, specific to each method. For XRD, the croissants were grated into a fine crumb using a metal sieve, and the crumbs were then pressed down into the sample well in a glass XRD slide. It was determined that this fine size was needed in order to prevent having an uneven surface when applied to the slide. During measurement, the scanning rate was chosen to be slow enough to have optimal resolution, yet not so slow that crumbs dried out, avoiding surface cracking. For p-NMR, croissants were again crumbled, however the size did not need to be as small nor uniform. A metal rod just slimmer than the width of the glass p-NMR tubes was then used to press down croissant pieces to the bottom of the tube, working small portions at a time to ensure there were no air pockets. However, there were still difficulties in attaining SFC measurements due to the many non-fat ingredients in croissants that still registered a signal. Three methods were tested to best account for this additional signal. First, the signal achieved at temperatures above the melting point of fat (60°C) was subtracted from the SFC measurements. Alternatively, croissant
dough was baked in the absence of fat and the signal from this fat free dough was taken at each
temperature and subtracted from the respective SFC measurements. Indirect SFC measurements
were also taken, however the presence of moisture in the dough interfered with these results.
Overall, the results obtained when the signal of fat free dough is subtracted were determined to
be the most appropriate due to the fact that the signal from non-fat components changed with
temperature, giving this method the greatest accuracy across all temperatures. Finally, for the
DSC, it was determined that little accommodation was needed to analyze croissants, and they
were simply cut using a small blade and weighed into the aluminum crucibles.

For each type of laminating fat used, XRD revealed that the polymorphism of a roll-in
shortening or butter is different when baked within the dough matrix than when simply heated
and cooled on its own. Both hydrogenated roll-in shortenings and butter experienced only minor
changes, largely retaining their β' polymorphs, however the non-hydrogenated shortening
experienced significant conversion from β' to the β form. Interestingly, this conversion did not
take place immediately upon cooling, but after approximately 24 hours of storage time. The fat
contained within the croissants exhibited a significantly lower SFC than the same fats in bulk.
Further, DSC results demonstrated that a greater temperature was required to melt all of the fat
completely in a croissant than the same fat in bulk, observed visually as broader peaks in the
melting endotherms. Analysis of croissant firmness over storage time, measured as the maximum
force required to cut a croissant was used as an indication of potential sensory consequences.
Results suggested that only croissants prepared with non-hydrogenated shortening experienced
significant changes in firmness over one week of storage. These results indicate an interaction
occurs between the shortenings and the ingredients of the croissant matrix. Given the differences
observed between the type of roll-in fats used, the extent of interaction is potentially influenced
by the composition of the roll-in fat itself.

The same shortenings and butter were then baked in the presence of isolated croissant
components and the cooled samples were analyzed using the same methods as the croissants.
Samples did not require specialized preparation for analysis for XRD and DSC, however the
same metal rod was used to press the samples down into glass p-NMR tubes. Limitations were
noted again with the SFC measurements, due to the complexity of the mixtures and the signals
achieved from non-fat ingredients, particularly gelatinized wheat starch and gluten network
which contain moisture. While fat-free dough was analyzed to account for this in croissants,
usable measurements of fat free gelatinized wheat starch and gluten network could not be obtained, therefore more research is required to determine the most suitable method of accounting for this added signal.

Overall, the only component that prompted the same results as seen from the croissant samples was gelatinized wheat starch, causing similar changes in polymorphism and melting behaviour. Crystalline wheat starch did prompt polymorphic conversion over one week of storage, but to a much lesser extent. The samples gluten or a formed gluten network behaved as the respective fats in bulk. These results suggest that the proposed interaction occurs between roll-in shortenings or butter and the gelatinized wheat starch within the matrix. The interaction appears to cause an increased rate of crystallization identified as by a faster increase in SFC as samples were cooled from 60 to 10°C. It is possible that the observed increase in crystallization rate results in greater crystal heterogeneity. This heterogeneity was identified based on broadening peaks in the DSC endotherms, where the peak width of croissants and samples containing gelatinized wheat starch of croissant was greater than all other samples. As stated in the previous section, it is likely that gelatinized wheat starch acts as a nucleation site on which the shortenings can crystallize. However, not necessarily all of the fat will crystallize on the gelatinized starch, but rather some extent of nucleation may be occurring within the fat itself. This theory can be used to explain the variation in crystal size, as the different processes may favour crystal growth over nucleation more than another. Based on the results observed, it is probable that the crystallization behaviour of roll-in shortenings within a croissant matrix is most significantly impacted by this increased crystallization rate, the occurrence of starch retrogradation, or by the combination of the two.

The implication of this research for the food industry is to understand the interactive role of fats in these baked dough products, with the goal of aiding in the eventual development of acceptable, trans fatty acid-free alternatives. By determining that different shortenings and butter likely interact to different extents with the gelatinized wheat starch within the laminated dough matrix, it is possible that newly developed trans fatty acid free and low saturated fatty acid shortenings could be tailored such that they behave like those which create the most acceptable products. This would open the door for products that comply with evolving regulations and health concerns that are still satisfying to consumers.
Because the true nature of the interaction was not revealed, more research to uncover would be recommended in the future. Likely, this would involve microscopy, where starch retrogradation and the fat structure at a similar scale to the starch granules could be monitored over time. Additionally, the inclusion of more laminating shortenings of different compositions could be included, to further identify any differences in behaviour and structure, and relate this back to the TAG and FA composition. Another consideration is the fact that the different types of laminated dough products have slightly different compositions and differing ratios of ingredients. These products may each have unique interactions compared to that of croissants. For this reason, the laminating shortenings could be used to prepare different laminated products and examined in a similar manner.

Another major aspect is sensory analysis, which would be necessary to truly identify if the polymorphic changes over storage time cause a perceivable difference for consumers among the different shortening types. However, a different selection of shortenings would be needed for this, as the hydrogenated shortenings included in this investigation were notably high in trans fatty acids and it would not have been ethical to feed participants a large number of croissants over the week of storage time knowing that consumption of this content of trans fat has been proven on many occasions to have negative health associations.

To conclude, gelatinized wheat starch, and likely the retrogradation that it experiences over storage time, has an influence on the crystallization behaviour and structure of fat contained within croissants. Additionally, the extent of the interaction was found to depend on the composition of the shortening, specifically regarding the content of trans fatty acids. Given the difference in behaviour of shortenings previously reported to produce less desirable products, these techniques are therefore suitable to analyze laminated products produced with new and novel shortening alternatives as an indicator of potential consumer acceptance and for large scale production.