TWIN - SCREW EXTRUSION FOR A FUNCTIONAL FOOD INGREDIENT

TWIN - SCREW EXTRUSION FOR THE PRODUCTION OF LIPID COMPLEXED PEA STARCH AS A FUNCTIONAL FOOD INGREDIENT

BY

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TITLE: Twin-Screw Extrusion for the Production of Lipid Complexed Pea Starch as a Functional Food Ingredient

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LAY ABSTRACT

Incorporation of pulses into food products has been a major area of Canadian research for its potential to create new avenues of enzyme resistant food starches. Extrusion cooking is commonly used in industry for producing various food products such as snacks and cereals but little research has been reported on using an extruder to rapidly produce resistant pulse starches as a functional ingredient on a large scale; resistant starch is a functional food beneficial to humans in the same manner as insoluble fiber but exhibits improved textural properties. This study aimed to develop an effective reactive extrusion process to produce lipid complexed pea starches with enhanced enzyme resistance (i.e. increased slowly digestible starch (SDS) and resistant starch (RS) content) by an examination of the effects of reaction conditions on the properties of extrusion products. The lipid complexed pea starches under optimized conditions achieved a significant but moderate increase in either RS content or SDS content depending on the sample formulation compared to their native counterparts. However, RS and SDS content could not simultaneously be improved in this study.

ABSTRACT

Canada is a major global producer of pulse products including pulse starch, which notably contributes to a healthy diet. Strategically, Canada is taking steps to research methods of adding greater value to these crop products, and functional foods like resistant starch are particularly interesting. The primary objectives of this study were to develop an effective reactive extrusion process to produce gelatinized lipid complexed pea starches with enhanced enzyme resistance and examine the effects of bulk lipid complexing conditions on physicochemical and functional properties of extruded pea starches. One type of commercially available pea starch, Nutri-Pea, was chosen as the research subject in this study. A number of methods including; Englyst digestion method, differential scanning calorimetry (DSC), infrared spectroscopy (FTIR), contact angle, titrations, residence time distribution (RTD) and rapid visco analysis (RVA) were used to characterize the properties of extruded pea starches.

The effects of feed formulation and extrusion conditions on lipid complexing and Englyst digestion profiles were systematically examined on two mixing devices. An extensive kinetics study was conducted on a lab scale twin-screw compounder, DSM-Xplore. The process was then scaled up to produce bulk lipid complexed pea starch on a Leistritz twin-screw extruder. The results showed that lipid complexing and digestion profiles were highly dependent on feed moisture and induced screw shear. Reactive extrusion of pea starches under optimized conditions achieved a significant but moderate increase in either resistant starch (RS) content (from 13.3% to 20.2%) or slowly digestible starch (SDS) content (from 7.85% to 23.3%) compared to their native

counterparts. However, RS and SDS content could not be improved simultaneously based on the pea starch and extrusion process in this study. Increased degree of substitution (DS) was found for myristic acid complexed pea starches (nominal DS= ~0.8) when compared to palmitic acid complexed pea starch (nominal DS= ~0.5). Contact angle measurements, FTIR and DSC thermograms confirmed the presence of lipids. Lipid complexed starch films showed increasing hydrophobicity with increasing lipid content.

As an alternative product compared to functional foods, the modified starch was considered as a biodegradable film for industrial applications. The material was produced at the highest moisture content for extruded native starch and two concentrations of lipid complexed starch using an intensive screw design. Preliminary results show that increasing lipid content and adding 1% glycerol to samples decreases the force per film thickness required to puncture films. However further investigation is required to determine effect of heat and moisture deformation.

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ABBREVIATIONS

AACC	American Association of Cereal Chemists
AGU	Anhydroglucose Unit
ALC	Amylose-Lipid Complex
AM	Amylose
ANN	Annealing
AP	Amylopectin
BDV	Breakdown Viscosity
CI	Confidence Interval
DP	Degree of Polymerization
DS	Degree of Substitution
DSB	Dry Starch Basis
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared
FV	Final Viscosity
MWD	Molecular Weight Distribution
NMR	Nuclear Magnetic Resonance
PV	Peak Viscosity
RDS	Rapidly Digestible Starch
RS	Resistant Starch
RTD	Residence Time Distribution
RVA	Rapid Visco Analysis

SBV	Setback Viscosity
SDS	Slowly Digestible Starch
SCFA	Short Chain Fatty Acid
SSE	Single Screw Extruder
TSE	Twin Screw Extruder

CHAPTER 1 INTRODUCTION

1.1 Background

Incorporation of pulses into food products has been a major area of Canadian research in the last few years for its potential to create new avenues of enzyme resistant food starches. Canada is the second largest producer of pulses in the world and is the greatest producer of peas, valuing the Canadian pulse industry at over one billion dollars per year. In recent years, there has been considerable growth in research in the area of resistant starch (RS) for its unique physiological and health benefits. These health benefits are a result of the production of short chain fatty acids (SCFA) after fermentation in the large intestine. Due to the abundance of pulse resources available, the Canadian food industry is increasingly pursuing possible avenues for enhancing the functional and nutritional properties of pulse starches for incorporation into food products (Hoover et al., 2010). This project is part of a national research program in Canada, funded by Agriculture and Agri-Food Canada, called the Pulse Science Cluster 2.

Increased consumer demand for high quality, nutritional food products has led to an expansion in the use of novel technologies and ingredients. Extrusion cooking is commonly used in industry for producing various food products such as snacks and cereals but little research has been reported on using an extruder to rapidly produce resistant pulse starches as a functional ingredient on a large scale.

While there is extensive research on cereal, potato and cassava starches for food and non-food applications there is still insufficient information on the structure and physicochemical properties of many pulse starches.

Hoover et al. (2010) and Maaran et al. (2014) have conducted extensive research on the composition, structure, modification methods and physicochemical properties of Canadian grown pulses such as pea, lentil, chickpea and bean. These researchers investigated both physical and chemical methods of pulse starch modification. A small number of studies have been reported in the extruder with food applications in mind however not for the preparation of resistant pea starch (Landerito & Wang, 2005; Hasjim & Jane, 2009; Huth et al., 2000; Sarawong et al., 2014). To date, no research has been reported on using twin-screw extrusion to increase enzyme resistance of pulse starches by bulk lipid complexing on a large scale for functional food applications.

For the production of lipid complexed resistant food starch the desired end-product from the extruder should be gelatinized starch. Therefore, the pulse starch granules are expected to be disrupted as a result of gelatinization and an intensive shear extrusion process. Feed formulation (e.g. feed moisture, lipid content and type) and extrusion conditions (e.g. extrusion instrument, extruder temperature and screw design) will be optimized. This thesis will focus on the extrusion of lipid complexed resistant pulse starches for functional food as well as industrial applications. Methods such as Fat Acidity titrations, *in-vitro* digestibility (Englyst

method), residence time distribution (RTD) and rapid visco analysis (RVA) were used to characterize the extrudates.

1.2 Research Objective

The main objective of this thesis was to develop an effective extrusion process to produce lipid complexed pea starch with improved SDS and/or RS content for use as a functional food ingredient. Furthermore, the effects of varied complexing and processing conditions on physicochemical and functional properties of extruded starches was extensively examined. One type of commercially available pea starch was studied, Accu-Gel^{TM} which was generously donated by Nutri-Pea Limited. The research included the following major components:

- To conduct an extensive kinetics study on a lab scale bench top extruder to produce cooked lipid complexed pea starch for use as a functional food ingredient.
- To scale up and develop an effective twin-screw extrusion process to rapidly produce cooked lipid complexed pea starch with enhanced enzyme resistance.
- Investigate the effects of feed formulations and extrusion variables on the functional and physical properties (bound lipid content, pasting properties and Englyst digestion profiles) of extruded pea starch complexes.

- To explore alternative applications for the material other than for food.

1.3 Thesis Outline

This thesis consists of five chapters. Chapter 1 is a brief introduction about the research and project objectives. Chapter 2 is a comprehensive review of relevant literature and presents related advances in studies on structure, properties, characterization, preparation and applications of lipid complexed starches. Chapter 2 also reviews relevant extrusion processes. Chapter 3 provides a description of experimental parameters such as materials, sample preparation/processing and characterization methods of lipid complexed pea starch extrudates. Chapter 4 presents and discusses in detail the experimental results of this project. This includes a detailed investigation on the effects of changing material and processing parameters with respect to sample digestion and viscosity. Additionally, Chapter 4 briefly explores the materials' potential to be used for alternative applications. The final chapter of this thesis summarizes the conclusions of this research and provides recommendations for future work in the area of extruded lipid complexed pea starches.

CHAPTER 2 LITERATURE REVIEW

2.1 Morphology and Structure of Pulse Starches

2.1.1 Morphology and Chemical Structure of Pulse Starches

Pulses are the dicotyledonous seed of plants which belong to the Legumionsae family and account for approximately 16,000-19,000 species including commodity staples such as lentil, pea, chickpea and a variety of beans (Ratnayake, Hoover, Shahidi, Perera & Jane, 2001). Canada is the second largest producer of pulses in the world and in particular is the largest producer of peas, resulting in a rapidly growing pulse industry. The Canadian pulse industry is currently valued at over one billion dollars per year leading to extensive opportunities in broadening value-added activities to high-end processing (Hoover et al., 2010). The main storage carbohydrate in pulse seeds is starch as it accounts for 22-45% of the seed itself (Chung et al., 2010; Hoover & Sosulski, 1991; Hoover et al., 2010). In many parts of the world, in particular developing countries, pulses are consumed whole because of their high protein content (20-50%) in comparison to other crops (Singh, Sandhu & Kaur, 2004). This makes them a significant food source which is both a rich and inexpensive source of protein and excellent source of B-complex vitamins and minerals (Jood, Chauhan, & Kapoor, 1988).

In the last ten years, resistant starch (RS) has drawn significant attention due to its associated physiological and health benefits, especially in foods containing high dietary fibre levels. Intake of RS is said to provide such benefits as a result of

the production of short chain fatty acids (SCFA) following fermentation in the colon (Dupuis et al., 2014; Englyst et al., 1992; Eerlingen, et al., 1993; Nugent, 2005; Thompson, 2000). This has led to increasing concern by the Canadian food industry to investigate effective methods of boosting the nutritional and functional properties, such as enzyme resistance, of pulse starch for incorporation into food products (Hoover et al., 2010).

While there is extensive research on cereal, potato and cassava starches for food and non-food applications there is still insufficient information on the structure and physicochemical properties of many pulse starches making it difficult to interpret their properties (Hoover et al., 2010). Most pulse starches consist of oval granules, which range in size between 5 to 85 microns; however, spherical, elliptical, round and irregular granules have also been observed (Hoover et al., 2010; Maaran et al., 2014; Pérez & Bertoft, 2010). Furthermore, the granule surfaces of pulse starches are usually smooth without the presence of fissures or pin holes (Chung et al., 2008; Hoover & Manuel, 1996; Hoover & Ratnayake, 2002; Singh et al., 2004). Wrinkled pea starch granules have shown extensive damage resulting in splitting and the exposure of the internal layers (Bertoft et al., 1993; Zhou et al., 2004).

Starch is comprised of two major macromolecular components, amylose (AM) and amylopectin (AP), see Figure 2.1.1 for their chemical structures (Xie et al., 2014). Amylose is a linear polysaccharide while amylopectin is a branch polysaccharide. The ratio of these two components is dependent on the starch

source. Most normal starches are comprised of 20-30% amylose and 70-80% amylopectin, while waxy starches have 0-5% amylose and high amylose starches contain 35-70% (Hoover et al., 2010; Maaran et al., 2014; Pérez & Bertoft, 2010; Shannon & Garwood, 1984). In general, amylopectin has a weight average molecular weight in the order of 10^7-10^9 daltons (Aberle, Burchard, Worwerg & Radosta, 1994). The AM content of pulse starches is 35-65%, this is higher than that of potato (21-25%) or cereal starches (20-35%) (Belitz et al., 2009; BeMiller & Whistler, 2009). Branching structure is directly related to degradation and enzymatic resistance as a result of chain splitting, especially with respect to highly branched amylopectin chains. Since amylose is less branched than amylopectin, high-amylose starches tend to be more resistant to digestion than low-amylose starch.

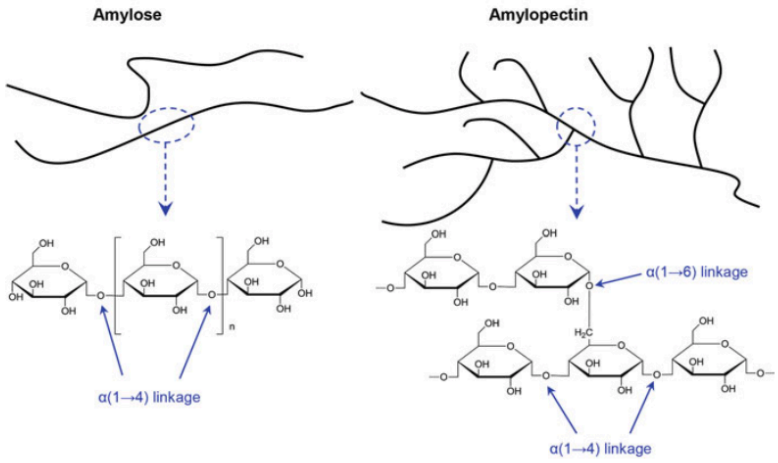

Figure 2.1.1 Chemical structure of amylose and amylopectin molecules of starch, taken from Xie et al. (2014)

Amylose and amylopectin have non-overlapping size and molecular weight distributions (MWD). Amylose molecules are predominately linked

through α-(1-4)-glycosidic linkages and tend to form more linear hydrogen bonded structures while amylopectin molecules exhibit α-(1-6)-glycosidic linkages forming branched structures of approximately 4000 glucose units (BeMiller & Whistler, 2009; Martin & Smith, 1995). Amylopectin structure has currently been regarded as having short amylopectin chains forming double helices and associating into clusters (Robin, Mercier, Charbonniere & Guilbot, 1974). The clusters pack together forming a structure of alternating crystalline and amorphous lamellae which can further be classified into three types; A, B and C as can be seen in Figure 2.1.2 (Hizukuri, 1986). As seen in Figure 2.1.2, alternating concentric hard semi-crystalline shells and soft amorphous shells form the lowest scale of granule architecture, while the thickness of the shells gradually decreases towards the granule surface. The granule structure involves radial amorphous channels, classified as blocklet structure, which is greater in semi-crystalline shells than in amorphous shells. One blocklet structure in semi-crystalline shells consists of a number of amorphous zones and crystalline lamellae. A-chains do not carry other chains through C-6 linkage and are found to be the shortest (DP 6-12; DP=degree of polymerization). B- chains carry other A and/or B-chains and can be classified into B_1, B_2, B_3 and B_4 depending on their length and the number of clusters they span (Hizukuri, 1986). There is evidence that the most exterior chains, A and B_1, form double helices within native granules, which are packed into lamellae crystallites or a single C-chain carries the only reducing end-group of each macromolecule (Bertoft, 2004).

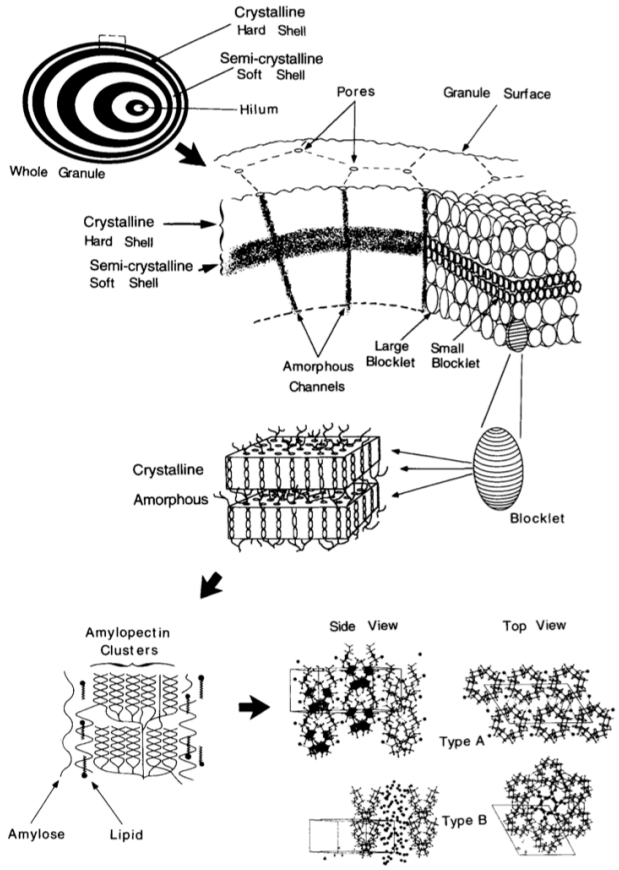

Figure 2.1.2. Starch granule structure at different levels of magnification, taken from Gallant et al. (1997)

2.1.2 Crystallinity of Pulse Starches

As in many synthetic polymers, starch granules are far from being considered perfect crystals. To best determine the crystalline structure of starch, X-ray diffraction (XRD) and related techniques are used to observe the granules. The amylopectin molecules inside starch granules crystallize into three types, A, B and C. A-type starches are found in cereals, while B-type starches are found in tubers and amylose-rich starches and C-type starches are found in legumes. With the exception of wrinkled pea starch, most pulses exhibit a C-type X-ray diffraction. Furthermore, V-type crystal structure can occur in starches which

form complexes between individual amylose helices and lipids, often these starches are gelatinized (Gallant et al., 1992; Buléon et al., 1998). Studies have shown that the B polymorphs are arranged centrally with the A-polymorph located peripherally within the granules for pea starches (Bogracheva, Morris, Ring and Hedley, 1998). The crystallinity of pulse starches is in the range of 17.0-34.0%, in particular 17.4-24.2% for smooth pea and 34% for wrinkled pea (Colonna & Mercier, 1984; Ratnayake et al, 2001). Crystallinity differences in pulse starches can be attributed to (1) crystallite size, (2) number of crystallites that are arranged in a crystalline array, (3) moisture content and (4) polymorphic content (Hoover et al., 2010). When investigating the fine structure of amylose and amylopectin in pulse starches there is inadequate information reported therefore making it difficult to directly compare pulses to cereal or tuber starches.

2.1.3 Gelatinization of Pulse Starches

When starch is heated in the presence of excess water, it undergoes an order-disorder phase transition called gelatinization. This transition occurs over a temperature range which is starch-source specific. Gelatinization is associated with the diffusion of water into the granules; water uptake in the amorphous background region; hydration and radial swelling of the starch granule; loss of birefringence; uptake of heat; loss of crystalline order; uncoiling and dissociation of double helices; and amylose leaching (Donovan, 1979; Hoover & Hadziyec, 1981; Jenkins & Donald, 1998; Waigh, Gidley, Komanshek & Donald, 2000).

Gelatinization transition temperatures (T_o (onset), T_p (mid-point) and T_c (conclusion)) and the enthalpy of gelatinization (ΔH) have been found to be influenced by the molecular architecture of the crystalline region. This corresponds to the distribution of amylopectin short chains (DP 6-11) and not by the proportion of crystalline region which corresponds to the amylose-to-amylopectin ratio (Noda, Takahata, Sato, Ikoma & Mochida, 1996). This study found that at low T_o, T_p, T_c and ΔH the presence of abundant short amylopectin chains is reflected, while Cooke and Gidley (1992) postulated that ΔH reflects the loss of double helical order rather than a loss in crystallinity. However, Tester and Morrison (1990a & 1990b) postulated that ΔH reflects the overall crystallinity (i.e. quality and amount of starch crystallites) of amylopectin. The gelatinization parameters of pulse starch can be determined using differential scanning calorimetry (DSC); however, it is difficult to determine whether these DSC parameters truly represent the species in general. This is due to limited information on amylose chain length and amylopectin branch chain length distribution, and therefore it is not possible to discuss the influence of molecular structure on DSC parameters of pulse starches (Hoover et al., 2010).

2.1.4 Retrogradation of Pulse Starches

Upon cooling after an order-disorder phase transition via gelatinization, the starch chains (amylose and amylopectin) in the resulting paste interact, leading to the formation of an ordered structure. These molecular interactions are

called retrogradation and are accompanied by an increase in the degree of crystallinity and gel firmness, exudation of water (syneresis) and the appearance of a B-type X-ray pattern (Hoover, 1995; Miles, Morris, Orford & Ring, 1985). This short-term development of a gel structure and crystallinity in starch gels during retrogradation is dominated by irreversible gelation when T< 100°C and crystallization (inside the gelatinized granules) involving amylopectin. Methods such as rheology, sensory textural evaluation, DSC, turbidimetry, syneresis, X-ray diffraction, nuclear magnetic resonance (NMR) and light spectroscopy have been used to examine retrogradation (Karim, Norziah & Seow, 2000). Retrogradation of pulse starches has predominately been determined by measuring the amount of water exuded when a frozen gelatinized starch gel is thawed at room temperature, while methods such as DSC, turbidity, X-ray and NMR have been used to a limited extent. Therefore, it is difficult to state the rate and extent of pulse retrogradation since each of these methods provide a different measure and description of the phenomenon and many studies have only focused on a single cultivar. Based on syneresis data many researchers have concluded that pulse starches retrograde to a greater extent in comparison to cereal or tuber starches, which could be as a result of their higher amylose content as well as differences in their molecular structure (Hoover et al., 2010).

2.2 Resistant Starches

Starch has been known to resist digestion by α-amylase. Three classes of dietary starches have been proposed by Englyst et al. (1992): (1) rapidly digestible starch (RDS), (2) slowly digested starch (SDS) and (3) resistant starch (RS). RDS is likely to be digested in the human intestine, SDS is likely to be slowly and completely digested in the small intestine and RS is likely to resist digestion in the small intestine by a healthy human. Resistant starches can be broken down further into five forms depending on the source of resistance such as: RS1 – physically inaccessible starch that is entrapped in a non-digestible matrix such as whole or partially milled grains and seeds, RS2 – native starch granules that are highly resistant to digestion by α-amylase until gelatinized such as raw starch and high amylose starches, RS3 – retrograded starch (or amylose crystals), RS4 – starches that are chemically modified to resist enzymatic digestion and RS5 – amylose-lipid complexes which form a helical structure containing a fatty acid tail within the central cavity (Englyst et al., 1992; Eerlingen et al., 1993; Hasjim, Ai & Jane, 2013; Nugent, 2005; Thompson, 2000).

Various modification methods used to prepare native starches can lead to the formation of resistant starches for use as a functional food ingredient. This can include physical, chemical, enzymatic or a combination of these methods.

There are a number of *in-vitro* methods to determine RS in an attempt to mimic human digestion of starch (AACC, 2000; Akerberg et al., 1998; AOAC, 2000, 2002; Champ et al., 1999, 2003; Englyst et al., 1992; Goni et al., 1996;

McCleary & Monaghan, 2002; Muir & O'Dea, 1992, 1993; Prosky et al., 1985). In all methods, the first step is to remove all digestible starch from the product using pancreatic α-amylase/ amyloglucosidase or thermally stable α-amylase (McCleary & Rossiter, 2004). The proposed methods predominantly differed in terms of enzymes used, digestion time/temperature and pH of incubation or a combination of these parameters. Due to these differing techniques, reported RS contents in literature vary because *in-vitro* RS for foods are method dependent. While each method has its advantages and disadvantages, the Englyst method has been predominately used for starchy foods due to its accuracy to corresponding in vivo data (Englyst et al., 1992; Dupuis et al., 2014). The Englyst method simulates the gastrointestinal tract by employing a guar gum solution in an acetate buffer, pH 5.2, to provide viscosity. Peristalsis of the digestive tract is also simulated by vortex shaking the solution with glass beads. RDS and SDS are determined by measuring glucose respectively at 20 min and 120 min during incubation at 37°C with porcine pancreatic α-amylase, amyloglucosidase and invertase. To determine RS, the values of RDS and SDS are deducted from the total starch content. This represents the fraction of starch not hydrolyzed after 120 min of incubation.

In the United States, Japan and Australia, resistant starch is incorporated into definitions for dietary fiber by the American Association of Cereal Chemists (AACC). Method 99.1.43 by the Association of Official Analytical Chemists (AOAC) or Total Dietary Fiber (TDF) Determination in Foods are also commonly

used to measure RS as TDF (AOAC, 2000; de Vries, 2004; Prosky et al., 1985). The AOAC method digests starch using heat stable α-amylase during boiling and determines RS as a weight difference between the hydrolyzed sample and the original sample.

The RDS, SDS and RS fractions in pulse starches can be determined using the Englyst et al. (1992) and AACC (2000) methods, see Table 2.2.1. The two measurement techniques differ with respect to hydrolysis time and enzyme source. As a result of differing testing methods, it is difficult to make meaningful conclusions and comparisons of the RS content in pulse starches. In this research, one type of commercialized native pea starch, Accu-Gel™ by Nutri-Pea, was investigated with an initial RS content of 18.67±0.60%. RS content was subsequently measured for extruded lipid complexed pea starches to determine the performance of the modification process.

Table 2.2.1. Method comparison of rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS) content of pulse starches (Hoover et al., 2010)

Starch Source	Method	Digestible Starch (%)		RS (%)
		RDS	SDS	
Black gram	Englyst	9.5	29.6	60.9
Chickpea	Englyst	10.9-23.5	34.8-45.7	33.5-54.3
Chickpea	AACC	21.5-29.9	45.7-57.7	8.14-18.4
Kidney bean	AACC	11.7	65.7	17.2
Lentil	Englyst	5.2-14.8	29.7-41.5	43.7-65.2
Lentil	AACC	16.0-16.9	58.3-62.2	13.0-13.2
Mung bean	Englyst	9.7	40	50.3
Navy bean	Englyst	8.2	32.3	59.4
Navy Bean	AACC	12.4	65.8	21.9
Pea (smooth)	AACC	18.2-23.8	53.7-59.0	8.1-12.6
Pea (smooth)	Englyst	8.1-19.2	33.9-40.2	40.5-58.0
Pigeon Pea	Englyst	4.2	16.9	78.9

2.3 Lipid Complexing of Starch

2.3.1 Chemistry of Amylose-Lipid Complexes

Amylose- lipid complexes (ALCs) have been classified as resistant starch types III (Eerlingen & Delcour, 1995) or V (Jiang et al., 2010). When starch interacts with lipids, amylose and long branch chains of amylopectin form single-helical complexes with fatty acids (Jane & Robyt, 1984; Ai et al., 2013). When the linear starch chain is in a helical-complex structure with a complexed fatty acid in the helical cavity, starch binding and cleavage by amylase are prevented (Birt et al., 2018). Additionally, ALCs entangle amylopectin molecules restricting starch granule swelling and enzyme hydrolysis (Hasjim et al., 2010; Seneviratne & Biliaderis, 1991). These complexes are naturally occurring in small quantities in starch but can be enhanced by the addition of fatty acids that promote formation of complexes during heat processing (Morrison et al., 1993; Obiro et al., 2012). ALC formation has been determined to be an instant reaction and type V resistant starches are considered thermally stable.

ACLs are defined as the inclusion of lipid molecules in amylose (Panyoo & Emmambux, 2016). Single-helix amyloses that are co-crystallized with compounds such as fatty acids or alcohols are referred to as V-amylose (Godet et al., 1993). Amylose-lipid complexes contain six glycosyl residues per turn and a guest molecule inside the helix, see Figure 2.3.1 for schematic. V-amyloses can be dry or hydrated (Rappenecker & Zugenmaier, 1981).

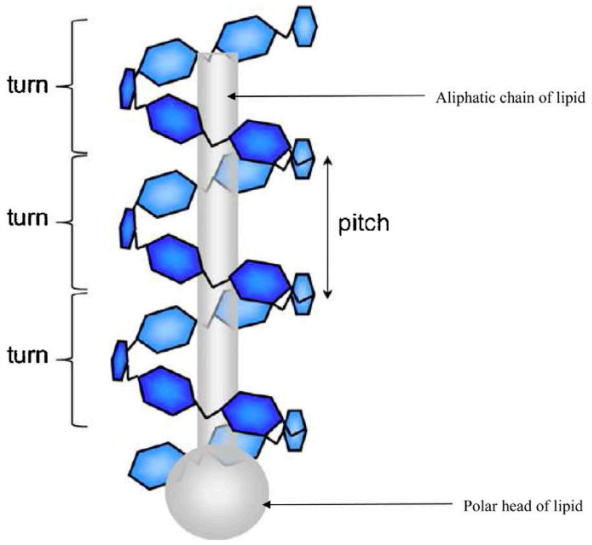

Figure 2.3.1. Amylose-lipid complex structure, taken from Panyoo & Emmambux (2016)

There are several types of lipids which have the ability to complex with amylose, such as monoglycerides, fatty acids and their esters. This is especially true when the hydrocarbon portion is inserted into the helical cavity of amylose (López, de Vries & Marrink, 2012). Lipid methylene groups and glycosidic linkages line the inner surface of amylose-lipid complex with hydrophilic hydroxyl groups pointing away from the helix (Immel & Lichtenthaler, 2000).

V-amylose is classified as either type I or type II depending on the melting temperature of crystalline components (Biliaderis & Galloway, 1989). In general melting temperatures range between 94-104°C and 115-121°C for type I and type II respectively. Type I complexes are made up of partially ordered structure lacking any distinct crystalline regions while type II complexes have distinctly crystalline/semi-crystalline structures (Biliaderis & Seneviratne, 1990). Type II

can further be broken down into Types IIa and IIb which differ in the degree of crystallinity or the perfection of the ordered domains. Type IIa complexes melt at approximately 115°C while Type IIb melt around 121°C (Karkalas et al., 1995). In 1989, Biliaderis and Galloway suggested that Type II complexes are superstructures of several Type I complexes which have crystallized together. Rappenecker and Zugenmaier suggested that Type IIb is the most stable form of amylose-lipid complexes.

2.3.2 Production of Amylose- Lipid Complexes

Amylose-lipid complexes can be produced by traditional, enzymatic and thermo-mechanical processing methods. Seo et al used the traditional method of solubilizing amylose in solvents such as DMSO or KOH followed by a reduction of pH using HCl. The starch slurry was then heated to 121°C and cooled to 60 and 90°C. Lipids were dissolved in absolute ethanol and were added to the cooled starch solution and centrifuged. Seo then washed the precipitates with hot water to remove excess starch and fatty acids. This yielded Type I and II ALCs at processing conditions of 60 and 90°C respectively. Lalush et al. conducted a similar experiment in 2005 and obtained distinct nanoscale spherical shapes with 150nm diameters and elongated structures with widths of 43-160nm.

Enzymatic methods have also been reported by Putsey et al. in 2009 in which glucose-1-phosphate was used as a primer and a fatty acid with a polymerization enzyme, phosphorylase, to promote glycosidic bonds. This

method yielded customizable short-chain ALCs. Furthermore, it identified that the primer is first polymerized to produce an amylose chain of sufficient length to account for the presence of lipid and then chain extension occurs to produce insoluble ALCs. The ALCs produced in this method were predominately Type I (Panyoo & Emmambux, 2016).

There are many alternative methods which can also be used to produce ALCs in the area of thermal processing technologies. These technologies include: steam-jet cooking, homogenization, pasting, and extrusion. All of these methods have been shown to be "greener" methods of chemistry when compared to traditional or enzymatic methods. However, it shall be noted that the traditional and enzymatic approaches are still suitable for lab-scale production of pure ALCs (Panyoo & Emmambux, 2016).

During steam-jet cooking, water dispersions of granular starch are pumped continuously through a water heater and are immediately heated with steam under temperature and shear conditions (Fanta, Kenar & Felker, 2015). Fanta et al. showed that spherulites are formed during steam-jet cooking of high-amylose corn maize starch with palmitic acid (Fanta et al., 2006, 2008). It was determined that micrometre sized spherulite formations were a result of crystallization of the helical inclusion complexes formed from amylose and native lipid material in maize starch. Fanta showed that jet-steam cooking conditions that influence spherulite formation are starch concentration, lipid presence and cooling conditions. It was also determined that sub-micron particles could be obtained

with rapid cooling using ice to 25°C and that disc-shaped particles along with spherical particles as a mixture could be obtained with slow cooling to 46°C and stirring. Spherulite growth was relatively rapid in slow-cooled dispersions due to the amylose/fatty acid complex being at maximal concentration. As the dispersion continued to rapidly cool, less complex material was available to form spherulites and therefore spherulites formed slowly thereafter. Studies conducted by Fanta et al. obtained nanoparticles of amylose-oleic acid with diameters ranging from 63-375nm using this method with cooling rates varied between 10s to 110min. Lastly, the nanoparticle size was influenced by the concentration of starch and the rate at which the hot, jet-cooked dispersions were cooled (Panyoo & Emmambux, 2016).

Meng et al. (2014) investigated the formation of ALCs in corn starch with fatty acids using high-pressure homogenization during heating. The study showed that the complexation index, an indirect method of measuring ALC based on a measurement of uncomplexed amylose using iodine, was 63% higher for maize starch homogenized at 100MPa with lauric acid when compared to un-homogenized defatted maize starch. Le Thanh-Blicharz et al. identified complexation enhancement in two ways: (1) homogenization disintegrates starch granules to release amylose and (2) homogenization improves fatty acid dispersion in the starch, increasing its contact with amylose in the starch granule. Meng et al. determined that ALC formation increased with homogenization and that the enthalpy of un-homogenized starch-fatty acid complex was lower than

that of homogenized complexes. This exhibited that high-pressure homogenization contributes to complex formation. From DSC thermographs, the melting temperature (100°C) of the starch-lauric acid complex showed that Type I ALCs were formed in homogenized and non-homogenized mixtures of starch and lipid.

Another method of amylose-lipid complexing is wet-heat processing. In this method, continuous heating of a starch-lipid-water mixture is conducted in various types of equipment, such as a rapid visco anyalzer (RVA) or rheometers, under controlled temperature and stirring conditions (Panyoo & Emmambux, 2016). The conditions which influence ALC formation are holding time and starch type (Nelles et al., 2000; D'Silva et al., 2011; Wokadala et al., 2012). Nelles et al. described the formation of biphasic pasting phenomena during pasting of commercial maize flour by observing the characteristic paste peak viscosity after pasting for a short heating time (<15 min) and at a second, higher peak paste viscosity for a longer heating time (>30 min). D'Silva et al. also observed biphasic pasting when teff starch was pasted with exogenous stearic acid for a prolonged time. Both of these studies suggest that the starch biphasic pasting curve resulted from the presence of endogenous and/or additional fatty acids. This was concluded because the biphasic curve did not occur when defatted maize or teff starch was pasted alone. Wokadala et al. identified the biphasic phenomena as a result of ALC presence when pasting maize and teff starches for 11.5-130 min with and without the presence of exogenous stearic acid, followed by hydrolysis

of thermo-stable α-amylase in an RVA. Wokadala observed Type I ALCs for short pasting times while Type II ALCs were observed for long pasting times using DSC.

The last method to commonly produce ALCs is high-temperature, short time extrusion cooking processing. In extrusion cooking moistened starch and/or proteinaceous food materials are plasticized and cooked in a heated barrel. Extrusion cooking uses a combination of moisture, temperature, pressure and mechanical shear to conduct molecular and/or chemical modifications (Singh, Gamlath & Wakeling, 2007). Experimental conditions such as barrel temperature, screw speed, amylose type and feed moisture have been found to influence the formation of ALCs (De Pilli et al., 2008 & 2011; Genkina et al., 2015). De Pilli et al. determined that optimal formation of starch-lipid complexes in a twin-screw extruder occurred at a barrel temperature of 128°C, water feed content of 21% and feed rate of 2.79kg/h for a starch and oleic acid. This system produced Type I ALCs, which was also observed when three maize varieties containing differing amylose contents (20-53%) and a high internal lipid level (~7% DSB) were extruded in a single screw extruder by Genkina et al. (2015). De Pilli suggested that the formation of ALCs was a result of high temperature and high moisture, which aids in promoting gelatinization and pasting thus increasing the amylose availability for complexation. Furthermore, Thachil et al. (2014) showed that high amylose (45%) corn extrudates have higher ALCs when compared to native corn starch extrudates after twin-screw extrusion. This supports that the degree of lipid

binding is amylose content dependent, which has also been reported by Bhatnagar & Hanna (1994) who saw increased amylose-lipid complexation in response to increased amylose content in corn extrudates with exogenous fatty acid.

2.4 Food Applications of Lipid Complexed Resistant Starches

The distinct health benefits of resistant starch have been well documented to include prevention of colon cancer, reduced probability of gall stones, hypoglycemic effects, hypocholesterolemic effects, decreased risk factor for cardiovascular disease, decreased colonic pH and increased mineral absorption (Hoover & Zhou, 2003; Nugent, 2005; Sajilata et al., 2006; Fuentes-Zaragoza et al., 2010). It is estimated that the human body requires 10-20% RS in its daily carbohydrate intake to take advantage of these physiological benefits (Dupuis et al., 2014). As touched upon earlier, RS appears to be highly resistant to mammalian enzymes and is therefore included within the definition dietary fiber by AACC and is actually measure as a fiber in the AOAC method (AOAC, 2000; Jones, 2000). However, the Prosky fiber method uses commercially manufactured sources of resistant starch as vehicles to enrich TDF content of food products (Prosky et al., 1985).

In general, resistant starches have a white appearance, bland flavour and small particle sizes. Due to their desirable physicochemical properties, they are suitable in a variety of foods. These properties include the ability to form gels, swell, increase viscosity and water binding capacity (Fausto et al., 1997).

Resistant starches are categorized as functional food ingredients which lower the calorific value of foods and are useful in products for coeliac disease, as bulk laxatives or in oral rehydration therapies. Resistant starches improve texture, appearance, flavour and mouth feel when compared to traditional high-fiber products. Due to these properties, resistant starches have been successfully used as dietary fiber fortification ingredients in bread-making, a texture modifier for baked goods, a crisping agent and a functional ingredient in extruded cereal snacks, pizza crusts, pastas and some beverages. By fortifying foods with resistant starch this provides opportunity for nutritionists and consumer product developers to promote healthy eating as well as the ability to increase fiber intake with a variety of appetizing, high quality and accessible foods (Fuentes-Zaragoza et al., 2010; Sajilata et al., 2006; Yue & Waring, 1998).

Amylose-lipid complexes are classified as a form of resistant starch. The specific health benefits of ALCs have been reported to include: (1) hypoglycemia effects (Hasjim et al., 2010; Lau et al., 2016), (2) potential for colon cancer reduction (Zhao et al., 2011& 2014) and (3) encapsulation of bioactive compounds (Ma et al., 2011). Wang et al. (2016) used porcine pancreatic α-amylase and amyloglucosidase to measure the digestibility of wet-heat treated wheat starch and waxy wheat starch with and without addition of fatty acids. The results showed decreased digestibility after 120min for fatty acid modified starch samples compared to pure starch samples. These results were confirmed by Ai et al. (2014) and Kawai et al. (2012) and is said to be a result of the formation of

complexes between starch and fatty acids, which are more resistant to amylase digestion. The resistance mechanism of ALC digestion has not yet been established; however, it is suggested that hydrolytic enzymes cannot form enzyme-substrate complexes since ALCs have compact structures whose α-(1-4)-glycosidic bonds are not exposed for enzyme substrate complex formation (Panyoo & Emmambux, 2016). Hasjim et al. conducted a human study which investigated the consumption of white bread containing ALCs. Results showed lower plasma glucose and insulin levels when compared to regular white bread. Furthermore, results showed that bread with ALCs decreased post-prandial blood glucose and reduced the risk of developing insulin resistance. In another study by Lau et al. (2016), which looked at bread with ALCs of different lipids, it was determined that there was no effect of lipid type on glucose response. Results showed lower glucose response when compared to bread without lipid which could be due to two factors: (1) delay in gastric emptying rate due to higher osmolarity of lauric and myristic acids (Clegg et al., 2012) and (2) increase in resistant starch due to formation of ACLs.

Amylose-lipid complexes have also been shown to suppress colon cancer cells (Zhao et al., 2011). Zhao showed that cooked lipid modified starch effectively reduced azoxymethane-induced preneoplastic lesions, precursors of colon cancer, in rat colon. This suggests that ALCs could potentially suppress colon carcinogenesis. The mechanism for the inhibition of colon cancer involves changing the gene expression of colon cells induced by consuming ALCs;

however, it is still not clear how this occurs. Birt et al. (2013) however identified that butyrate, a short-chain fatty acid produced by bacterial fermentation of ALCs and other large carbohydrates in the large intestine, mediates the process. Butyrate was shown to have potential gut anti-cancer properties, reduces proliferation, increases apoptosis, enhances immune surveillance and provides anti-inflammatory effects in the colorectal cancer cell.

Bioactive compounds can be encapsulated in starch by forming ALCs when they are in the form of fatty acid esters. Ma et al. (2011) encapsulated ascorbyl palmitate, retinyl palmitate and phytosterol esters with amylose by forming ALCs. Ascorbyl palmitate resulted in the highest complexation. While there are studies on encapsulating bioactive compounds by amylose to form ALCs, many do not investigate how these bioactive compounds are protected from the acidic stomach environment or how they release inside the small or large intestine. Studies have suggested that they have limited release within the small intestine but will mostly release in the large intestine.

CHAPTER 3 EXPERIMENTAL

3.1 Materials

Native pea starch, Accu-Gel™ was graciously donated by Nutri-Pea Limited (Portage la Prairie, MB). Accu-Gel™ is a food grade pea starch with a typical initial moisture content ranging from 11.7% to 13.0% (on a dry starch basis, DSB). This is based on a moisture content measurement conducted using a Mettler-Toledo HG63 moisture analyzer. Lipids used for the starch modification were supplied by Alfa Aesar (Pittsburgh, PA) and Sigma Aldrich (St. Louis, MO). These lipids include Palmitic Acid, otherwise referred to as Hexadecanoic Acid (>95% purity) and Myristic Acid, otherwise referred to as Tetradecanoic Acid, (98% purity). Both lipids were supplied as odourless white powders. Sample purification was conducted after extrusion processing using Isopropyl Alcohol supplied by Caledon Laboratory Chemicals (Georgetown, ON). Toluene (C.A.S. No: 108-88-3), Isopropyl Alcohol (C.A.S. No: 67-63-0) and Potassium Hydroxide pellets (C.A.S. No: 1310-58-3) required for titration solutions were also supplied by Caledon Laboratory Chemicals. Phenolphthalein (C.A.S. No: 77-09-8) pH indicator used in the titration solution was supplied by Sigma Aldrich. All water used was Milli-Q water.

3.2 Preparation of Lipid Complexed Pea Starches Using Xplore DSM Twin Screw Compounder (Kinetics Study)

An extensive kinetics study was performed on a bench-top sized Twin Screw Compounder, DSM Xplore Micro 15cc (Netherlands). Feed moisture content of Nutri-Pea pea starch samples was regulated to 80% prior to lipid complexing and gelatinization in the Xplore Compounder. Raw starch was blended with distilled water to achieve 80% moisture and respective lipid (palmitic or myristic acid) content of 2%, 4%, 8% or 12%. Due to instrument capabilities and sample quality, moisture could not be varied for these experiments. The Xplore compounder is a closed loop system therefore all materials were required to be blended and fed into the chamber in one step. Since it was a closed loop system with a recycle loop, residence time (or cook time) was determined by the user. Residence times of 3 or 5 minutes were used for experiments. All experiments were conducted at 110 RPM and instrument temperature was set to 90, 100 or 110°C. These temperatures accounted for gelatinization to occur but prevented damage to the lipids. Table 3.2.1 summarizes the formulations and compounding conditions for all kinetics experiments.

Table 3.2.1. Formulations and extrusion conditions for Xplore kinetics study

Sample Name	Lipid	Lipid Content (%)	Temperature (°C)	Resonance Time (min)
X2P - 90 - 5	Palmitic	2	90	5
X4P - 90 - 5	Palmitic	4	90	5
X8P - 90 - 5	Palmitic	8	90	5
X12P - 90 - 5	Palmitic	12	90	5

X2P - 100 - 5	Palmitic	2	100	5
X4P - 100 - 5	Palmitic	4	100	5
X8P - 100 - 5	Palmitic	8	100	5
X2P - 110 - 5	Palmitic	2	110	5
X2P - 110 - 5	Palmitic	4	110	5
X8P - 110 - 5	Palmitic	8	110	5
X2P - 90 - 3	Palmitic	2	90	3
X4P - 90 - 3	Palmitic	4	90	3
X8P - 90 - 3	Palmitic	8	90	3
X2P - 100 - 3	Palmitic	2	100	3
X4P - 100 - 3	Palmitic	4	100	3
X8P - 100 - 3	Palmitic	8	100	3
X2P - 110 - 3	Palmitic	2	110	3
X4P - 110 - 3	Palmitic	4	110	3
X8P - 110 - 3	Palmitic	8	110	3
X2M - 90 - 5	Myristic	2	90	5
X4M - 90 - 5	Myristic	4	90	5
X8M - 90 - 5	Myristic	8	90	5
X12M - 90 - 5	Myristic	12	90	5
X2M - 100 - 5	Myristic	2	100	5
X4M - 100 - 5	Myristic	4	100	5
X8M - 100 - 5	Myristic	8	100	5
X2M - 110 - 5	Myristic	2	110	5
X2M - 110 - 5	Myristic	4	110	5
X8M - 110 - 5	Myristic	8	110	5
X2M - 90 - 3	Myristic	2	90	3
X4M - 90 - 3	Myristic	4	90	3
X8M - 90 - 3	Myristic	8	90	3
X2M - 100 - 3	Myristic	2	100	3
X4M - 100 - 3	Myristic	4	100	3
X8M - 100 - 3	Myristic	8	100	3
X2M - 110 - 3	Myristic	2	110	3
X4M - 110 - 3	Myristic	4	110	3
X8M - 110 - 3	Myristic	8	110	3

3.3 Preparation of Lipid Complexed Pea Starches Using Twin Screw Extruder (Scale Up)

Feed moisture content of Nutri-Pea pea starch samples (1000g DSB) was regulated to 30% prior to the bulk lipid complexing and gelatinization process. Raw starch was pre-blended overnight (12hrs) with water and the respective lipid (palmitic or myristic acid) content of 2%, 4% or 8%. This allowed for the starch granules to absorb additional moisture and swell. Formulations were kept frozen overnight in sealed plastic bags in a -50°C freezer. The required additional water to reach 40%, 60% or 80% total moisture during processing was achieved using a high-pressure syringe pump (Teledyne Isco; Lincoln, NE, USA), which pumped Milli-Q water. Pump flowrate was determined for each formulation respectively, with respect to starch feed-rate and weight. Starch feed-rate was kept constant at 3kg/h for all experiments and starch was gravimetrically fed by a DDSR20 (Brabender Technologie Inc.; Mississauga, ON) solids feeder. The extruder screw speed was kept constant at 250 RPM. Bulk lipid complexing and gelatinization of pre-mixed starch samples was done using a Leistritz ZSE-HP 27mm 40 L/D co-rotating intermeshing twin-screw extruder (American Leistritz Extrusion Corp.; Sommerville, NJ). Table 3.3.1 summarizes the formulations and extrusion conditions for all twin-screw extruder experiments.

Table 3.3.1. Formulations and extrusion conditions for Leistritz study

Sample Name	Screw Design	Lipid	Lipid Content (%)	Total Moisture Content/Pump Flowrate	Extrusion Conditions
TSE NS40A	A	N/A	N/A	40%, 10.15 ml/min	3.00 kg/h, 250 RPM
TSE NS60A	A	N/A	N/A	60%, 20.15 ml/min	3.00 kg/h, 250 RPM
TSE NS80A	A	N/A	N/A	80%, 30.15 ml/min	3.00 kg/h, 250 RPM
TSE 2M40A	A	Myristic	2	40%, 10.353 ml/min	3.00 kg/h, 250 RPM
TSE 2M60A	A	Myristic	2	60%, 20.553 ml/min	3.00 kg/h, 250 RPM
TSE 2M80A	A	Myristic	2	80%, 30.753 ml/min	3.00 kg/h, 250 RPM
TSE 4M40A	A	Myristic	4	40%, 10.556 ml/min	3.00 kg/h, 250 RPM
TSE 4M60A	A	Myristic	4	60%, 20.956 ml/min	3.00 kg/h, 250 RPM
TSE 4M80A	A	Myristic	4	80%, 31.356 ml/min	3.00 kg/h, 250 RPM
TSE 8M40A	A	Myristic	8	40%, 10.962 ml/min	3.00 kg/h, 250 RPM
TSE 8M60A	A	Myristic	8	60%, 21.762 ml/min	3.00 kg/h, 250 RPM
TSE 8M80A	A	Myristic	8	80%, 32.562 ml/min	3.00 kg/h, 250 RPM
TSE 2P40A	A	Palmitic	2	40%, 10.353 ml/min	3.00 kg/h, 250 RPM
TSE 2P60A	A	Palmitic	2	60%, 20.553 ml/min	3.00 kg/h, 250 RPM
TSE 2P80A	A	Palmitic	2	80%, 30.753 ml/min	3.00 kg/h, 250 RPM
TSE 4P40A	A	Palmitic	4	40%, 10.556 ml/min	3.00 kg/h, 250 RPM
TSE 4P60A	A	Palmitic	4	60%, 20.956 ml/min	3.00 kg/h, 250 RPM
TSE 4P80A	A	Palmitic	4	80%, 31.356 ml/min	3.00 kg/h, 250 RPM
TSE 8P40A	A	Palmitic	8	40%, 10.962 ml/min	3.00 kg/h, 250 RPM
TSE 8P60A	A	Palmitic	8	60%, 21.762 ml/min	3.00 kg/h, 250 RPM
TSE 8P80A	A	Palmitic	8	80%, 32.562 ml/min	3.00 kg/h, 250 RPM
TSE NS40B	B	N/A	N/A	40%, 10.15 ml/min	3.00 kg/h, 250 RPM
TSE 2P40B	B	Palmitic	2	40%, 10.353 ml/min	3.00 kg/h, 250 RPM
TSE 4P40B	B	Palmitic	4	40%, 10.556 ml/min	3.00 kg/h, 250 RPM
TSE 8P40B	B	Palmitic	8	40%, 10.962 ml/min	3.00 kg/h, 250 RPM
TSE NS80B	B	N/A	N/A	80%, 30.15 ml/min	3.00 kg/h, 250 RPM
TSE 2P80B	B	Palmitic	2	80%, 30.753 ml/min	3.00 kg/h, 250 RPM
TSE 4P80B	B	Palmitic	4	80%, 31.356 ml/min	3.00 kg/h, 250 RPM
TSE 8P80B	B	Palmitic	8	80%, 32.562 ml/min	3.00 kg/h, 250 RPM

A flat temperature profile of 90°C was applied to the nine heating zones of the extruder barrel for all samples in this study. Two high-shear screw designs (Figures 3.3.1 and 3.3.2) were examined. Extruder load, extrudate melt temperature and product moisture content were measured and recorded for each sample taken. Figure 3.3.3 shows experimental equipment setup.

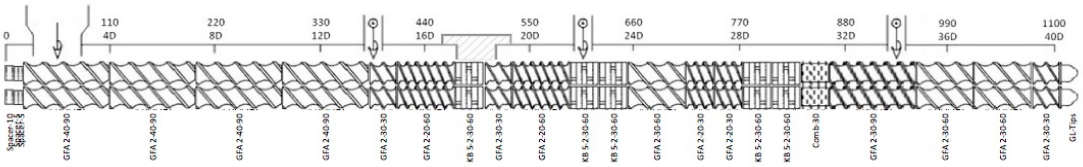

Figure 3.3.1. Schematic of screw design A

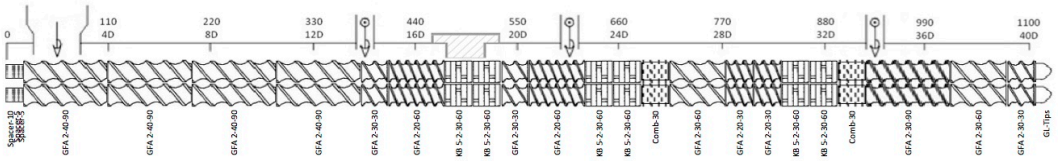

Figure 3.3.2. Schematic of screw design B

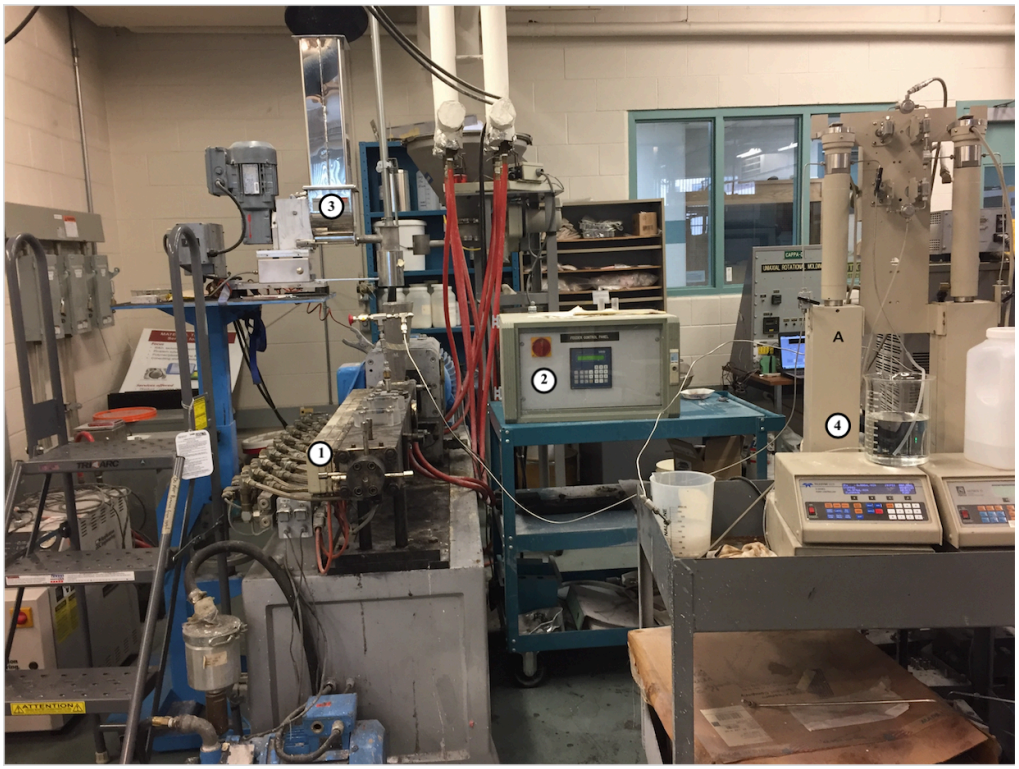

Figure 3.3.3. Image of laboratory experimental Leistritz setup: (1) extruder, (2) feeder controller, (3) gravimetric feeder for starch/lipid blends and (4) syringe water pump

3.4 Extrudate Purification

Following sample processing, samples were purified in isopropyl alcohol for 48hrs to remove any excess, unbound lipid. Samples were then rinsed thoroughly with distilled water. Depending on the required characterization, samples were then dried in an oven at 40°C to a moisture content of >12%, ground and sieved through a 500 μm mesh.

3.5 Characterization

3.5.1 Thermal Properties

Differential scanning calorimetry (DSC) measurements were conducted using a Q200 differential scanning calorimeter equipped with a nitrogen

refrigerated cooling system (TA Instruments, USA). All samples were washed in isopropyl alcohol for 48hrs, rinsed, dried to less than 14% moisture and sieved through a 500μm mesh. DSC tests were conducted for extruded native and lipid complexed pea starch samples (5-9mg) which were weighed into a Tzero hermetic pan and scanned from 30°C to 120°C, cooled to 30°C and finally heated to 120°C at 10°C/min. Each sample was analyzed in duplicate. A nitrogen flow of 50ml/min was used during all DSC tests. The TA Universal Analysis 2000 software was used to analyze DSC thermograms and to identify changes in thermal transitions of extruded pea starches.

3.5.2 Fourier Transform Infrared Spectroscopy

Raw and extruded native and lipid complexed pea starch samples were ground into powders less than 500μm prior to infrared analysis. Samples were prepared using dry (120°C, 48hrs) FT-IR grade potassium bromide (KBr) with ≥99% trace metals supplied by Sigma Aldrich (St. Louis, MO) which was ground into 1-2% sample using a mortar and pestle. Samples were then pressed into pellets using a stainless-steel die and a hydraulic press which was pumped to 15,000 pounds. The prepared pellet was immediately tested to minimize hygroscopic effects of KBr in a Thermo Fisher Scientific Nicolet 6700 FTIR (Waltham, MA) in transmission mode. Spectra were collected from 64 scans between 400-4000 cm^{-1} at a resolution of 4 cm^{-1}.

3.5.3 Titration

Total fatty acid content in the starch samples was determined by a general titrimetric procedure based on a modified AACC 02-01A Fat Acidity General Method (1999) with minor modifications. Reagents used include 0.0178M KOH solution titrated against 50:50 toluene/isopropyl alcohol solution with 0.02% phenolphthalein pH indicator supplied by Sigma Aldrich (St. Louis, MO). Samples (1g) were placed in glass jars overnight with 50ml of the toluene/isopropyl solution. It shall be noted that all samples were run in triplicate to ensure accuracy. Lipid conversion was determined for each sample as well as the degree of substitution (DS) which is the average number of substituent groups attached per anhydroglucose unit (AGU), since starch is a condensation polymer. To calculate these values reference titrations were conducted for all lipid levels without the presence of starch. The proposed reaction mechanism for the starch fatty acid complex formation, Figure 3.5.1, and equations used for conversion (1) and degree of substitution (2) can be seen below.

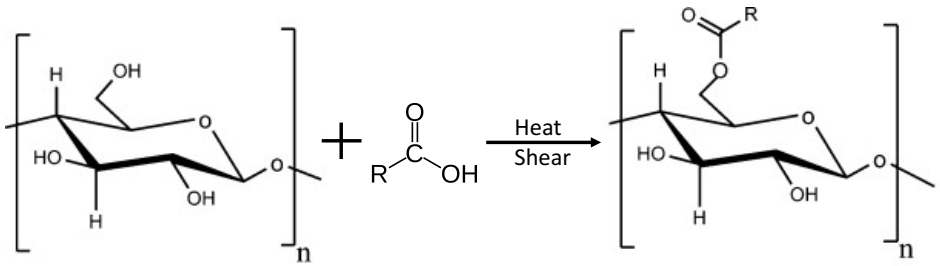

Figure 3.5.1. Proposed reaction mechanism for lipid complexed starch formation

$$\text{Conversion}=\frac{\text{mol original lipid-mol left over}}{\text{mol original lipid}} \quad (1)$$

$$\text{Degree of Substitution (DS)}=\frac{\text{mol original-mol left}}{\text{mol AGU}} \quad (2)$$

3.5.4 Contact Angle

Contact angle, a measurement used to indicate wettability, surface tension and the resulting effect of type/amount of lipid was studied for native and lipid complexed films. Post extrusion samples were pelletized, washed in isopropyl alcohol for 48hrs, rinsed and dried for film preparation. Films were prepared using a hot press set at 110°C, pressed at this temperature for 3 minutes and then cooled under pressure for 45 minutes. These films were measured for their contact angle using the most popular method, sessile drop, where the angle is determined from the tangent of the drop curvature to the base. Each drop was 3μL and the computer software program (FTÅ software, First Ten Angstroms, Inc.) recorded 26 images over a period of 50s using a CCD camera coupled to a microscope. The image analysis software was then used to calculate the contact angle. Each film was tested in triplicate and the contact angle was determined by taking an average of the angles between t_0 and t_{25} (half-way time) but no longer to minimize water absorption influencing the value.

3.5.5 In-vitro Digestibility of Pea Starch Samples

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of native and modified pea starches was determined using the Englyst et al. method from 1992 with minor modifications. A sample of starch (100mg) which had been dried to less than 14% moisture and sieved through a 500μm mesh, was taken and vortexed with a mixture of 2ml guar gum, 15 glass beads and 4ml of sodium acetate buffer solution (pH 5.2). Following continuous water bath shaking (37°C, 200 strokes/min) for 5 min, 1ml of enzyme solution was added to each test-tube which consisted of porcine pancreatic α-amylase (Sigma P7545), amyloglucosidase (Megaenzyme E-AMGDF) and invertase (Sigma I4504). This was followed by incubation in a water bath at 37°C with shaking at 200 strokes/min. Aliquots of hydrolysed solution (100μl) were taken at specified time intervals of 20 min and 120 min. These aliquots were mixed with 1ml of 50% ethanol to terminate the enzymatic reaction. The glucose released in each sample was determined using a glucose oxidase peroxidase diagnostic kit obtained by Megaenzyme and measured the absorbance of samples against the reagent at 510nm using a UV/VIS spectrophotometer. RDS was calculated for samples taken at a digestion time of 20 min by multiplying glucose (mg) released by 0.9 and dividing by 100mg. The glucose content at 120 min was determined using the same method which allowed for determination of SDS content by the difference in starch fraction at 20 and 120 min. Undigested starch, anything left after 120 min, was considered as the RS fraction, which is calculated

as a difference between the total starch content and the sum of RDS and SDS. All samples were analysed in duplicate due to time and cost constraints. It shall be noted that all *in-vitro* digestibility assays were conducted by Elizabeth Donner at Guelph Food Research Centre but results interpretation is by the author.

3.5.6 Rapid Visco Analysis (RVA)

The pasting properties of extruded and modified pea starch samples were determined using a Rapid Visco Analyzer RVA4 (Perten Instruments Australia Pty. Ltd., Warriewood, NSW, Australia) in accordance with the AACC 76-21.01 method established in 1999. All viscosity values were recorded in cP units, where 1cP is equivalent to $1mPas^{-1}$. Starch, 3g (dried to less than 14% moisture and sieved through a 500μm mesh) was added to 25ml of Milli-Q water in an aluminium sample pan. The starch slurry was equilibrated at 50°C for 1 min, heated from 50°C to 95°C at a rate of 6°C/min and held isothermal at 95°C for 5 min, followed by a cooling cycle at 6°C/min to 50°C and held isothermal for 2 min. The paddle speed was set to 960RPM for the first 10s, then decreased to 160RPM for the remainder of the experiment. Each sample was analysed in duplicate (pending available sample quantity). It shall be noted that experiments were conducted by Elizabeth Donner at Guelph Food Research Centre, but results interpretation was conducted by the author.

The standard viscogram representing the pasting profile and the RVA parameters is illustrated in Figure 3.5.2 below. The values measured from the

pasting profile were determined according to the RVA4 Manual (Perten Instruments Australia Pty. Ltd., 2010) and are as follows.

- *Breakdown viscosity (BDV)*: difference between peak and trough viscosity
- *Final viscosity (FV)*: the viscosity at the end of each run
- *Pasting temperature*: the onset temperature of rapid increase in viscosity, defined as viscosity increased by ≥24cP within 6s
- *Peak time*: the time at which peak viscosity is recorded
- *Peak viscosity (PV)*: Maximum paste viscosity achieved in the heating stage of the profile
- *Setback viscosity (SBV)*: difference between final and trough viscosity
- *Trough viscosity*: minimum paste viscosity achieved after holding at the maximum temperature

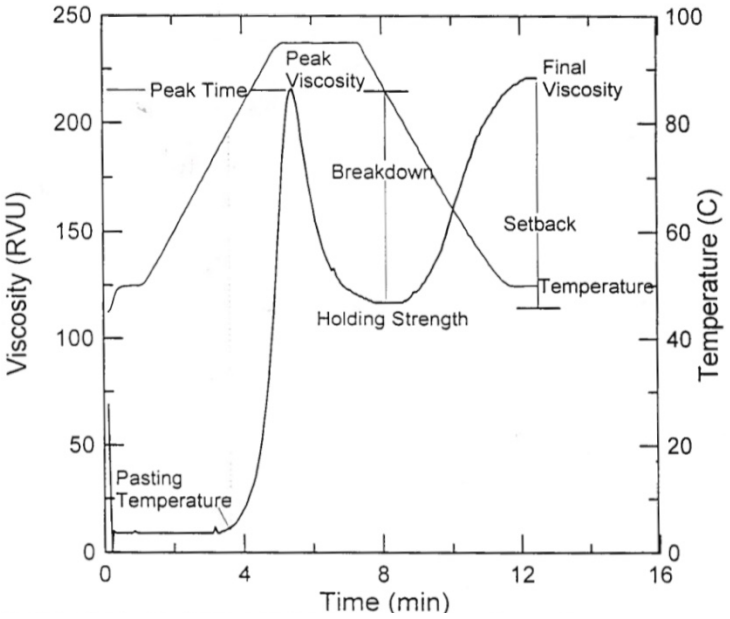

Figure 3.5.2. Typical RVA pasting curve showing the commonly measured parameters

3.5.7 Determination of Residence Time Distribution (RTD)

The extent of mixing, effect of screw design as well as reaction time within the extruder were evaluated by measuring the residence time distribution at zone 5 of the extruder and at the die. A dirac pulse stimulus response technique was conducted to obtain the RTD (Kumar et al., 2006; Li et al., 2014; Mu & Thompson, 2012). Cocoa powder was used as a tracer and 0.28g was added to the system as a pulse input through the feed port once steady state operation was achieved. At the same time that the tracer was added two cameras began filming. One camera was placed at port 5 of the extruder which had a plexi-glass viewing window over a kneading zone, while the other was positioned at the die to record the starch extrudate. Due to time and material constraints, RTDs were conducted

for two starch formulations native starch and 4% lipid complexed starch, at 80% moisture for screw designs A and B. Each formulation was run in triplicate to ensure accuracy. Next, the extracted images were analysed for colour intensity using Digital Colour Meter (Version 5.11, Apple Inc.) by recording the Adobe RGB values at time intervals of five seconds. Colour intensity was calculated by Equation 3 and was then normalized.

$$\text{Colour Intensity} = \frac{\text{R+G+B}}{3} \tag{3}$$

The RTD was described by fitting the data to an E(t) curve, which is a representation of tracer concentration (or colour intensity) at the recorded zone with time respectively. The function, Equation 4, used to fit the data was found suitable for fitting the RTD of extrusion machinery in both the conveying zones as well as kneading zones (Poulesquen et al., 2003; Zhang et al., 2008).

$$E(t) = a \times t^{-c-1} \times b^{c+1} exp\left[(b^c t^{-c} - 1) \times \left(\frac{-c-1}{c}\right)\right] \tag{4}$$

From the above equation, ‘a’ corresponds to the peak height, ‘b’ is the residence time at peak height and ‘c’ is a fitted parameter related to the peak breadth but has been determined to lack any physical interpretation. This function has been referred to as the Zusatz distribution in literature. The mean (τ) and variance (σ^2) of the normalized tracer, E(t) were determined along with the equivalent vessel number ($n=(\tau^2/\sigma^2)$) (Mu & Thompson, 2012).

3.5.8 Slow Penetration Resistance of Flexible Barrier Films

Penetration resistance is an important end-use performance measure for thin-flexible materials. A sharp-edged product can destroy the integrity of a barrier wrap which will permit entry/exit of gases, odours or unwanted contaminants and can contribute to reducing product shelf-life. Material response techniques vary rate of penetration, temperature, shape and type of probe. Using the ASTM F1306 standard, slow rate penetration resistance of a flexible barrier film was conducted on extruded pea starch samples which were prepared into films. Post extrusion samples (10g) were oven dried at 40°C and ground to a fine powder, then mixed with 10ml distilled water to form a starch paste. The paste was then hot pressed at 110°C in a 50 x 3mm square mould. The sample first encountered unpressurized heating for 5 min, followed by pressurized (6 tonnes) heating for 10 min and lastly pressurized (6 tonnes) cooling for 10 min. Sample films were made in quintuplicate and then conditioned for 48hrs in a humidity chamber set to 23°C and 50% relative humidity. Penetration tests were conducted using an Instron 3366 Mounted Mechanical Testing System (Canton, MA) with a 500kN load cell at a crosshead rate of 25mm/min as specified in the standard. The specimen clamping fixture schematic as well as a photograph of the manufactured clamping fixture can be seen in Figure 3.5.3. Samples with additional 1% glycerol content were also tested according to this same procedure.

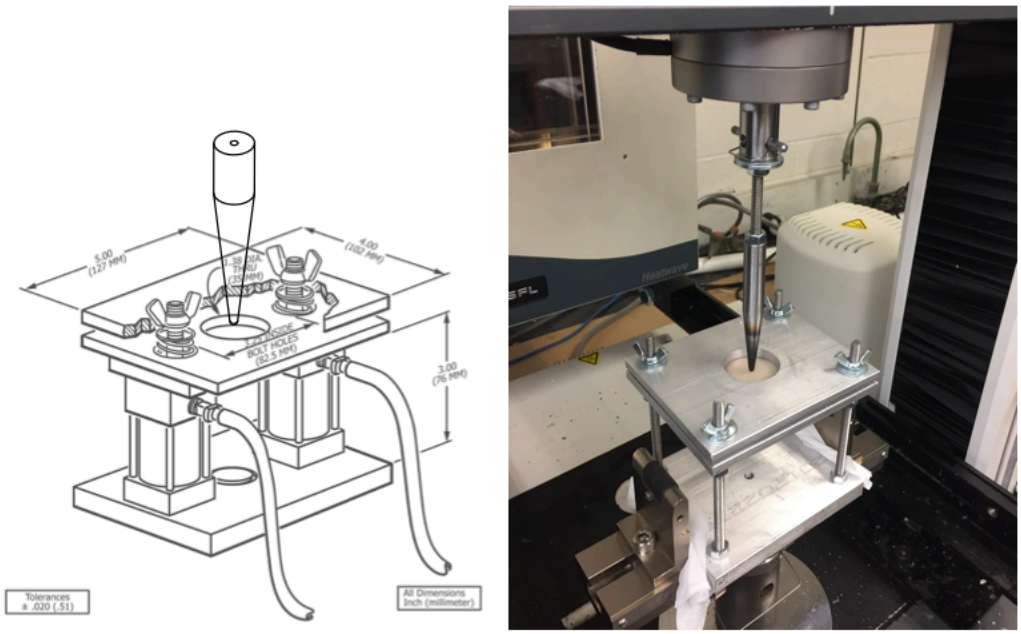

Figure 3.5.3. Schematics of the specimen clamping fixture (ASTM F1306) (left) and photo of the manufactured specimen clamping fixture mounted on Instron mechanical tester (right) are shown.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 The Bound Lipid Content

There are a variety of methods which can be used to validate the presence of added lipids in modified starches and to what extent. These methods include thermal property analysis (DSC), IR spectral analysis (FTIR), titrations to determine conversion and degree of substitution, and contact angle measurements for hydrophobicity.

4.1.1 Thermal Property Analysis

Differential scanning calorimetry (DSC) was used to determine changes in thermal properties of extruded lipid complexed pea starches in comparison to native pea starches, evaluating the influence of the reaction on crystallinity. All starches found in Figure 4.1.1, were produced on the Leistritz twin-screw extruder using screw A with 80% feed moisture.

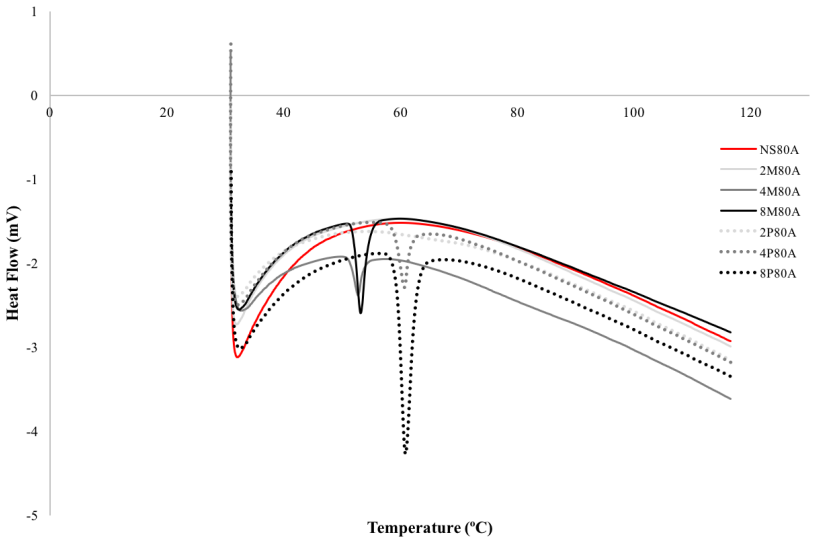

Figure 4.1.1. DSC thermogram of extruded native (NS) and lipid complexed pea starches with palmitic acid (P) and myristic acid (M) at 2, 4, 8% concentration.

From Figure 4.1.1 it is evident that the addition of lipids introduced new thermal transitions not present with the gelatinized native starch, which is consistent with the observations of Evans (1986) and Galloway et al. (1989). Increasing lipid concentration provides prominent lipid phase melting peaks at ~54°C and ~62°C for myristic and palmitic acid respectively, close to their pure melting temperatures of 54.4°C and 62.9°C. No peak is seen for the 2% lipid concentration since the bound chains at this content were insufficient to form a distinct phase in the starch matrix. As lipid content is increased from 4% to 8% palmitic acid, an increase in melting enthalpy is seen from 1.14J/g to 4.86 J/g respectively; DSC testing of a 16% palmitic acid complexed starch (data not shown) produced on screw B (TSE 16P80B) showed further increase in melting enthalpy to 19.8 J/g, which is nearly four times that of the 8% complexed sample. Melting enthalpy results were consistent for the myristic acid complexed starches, though the melting enthalpies are nearly half those of palmitic acid, being 0.47 J/g and 2.31 J/g for 4% and 8% respectively. Each sample was run through a heat-cool-heat cycle which would erase any polymer memory once the second cycle began, only the results of the second cycle are presented in Figure 4.1.1.

4.1.2 Molecular Component and Structure Identification via Infrared Absorption

IR spectra provide valuable information and validation when comparing modified starches to the native starch, as can be seen in Figure 4.1.2. Since the chemical structures of myristic and palmitic acid are similar, their characteristic

IR peaks are also very similar and correspond to wavenumbers around 3600, 3000, 1800 and 1500-1000 cm^{-1}.

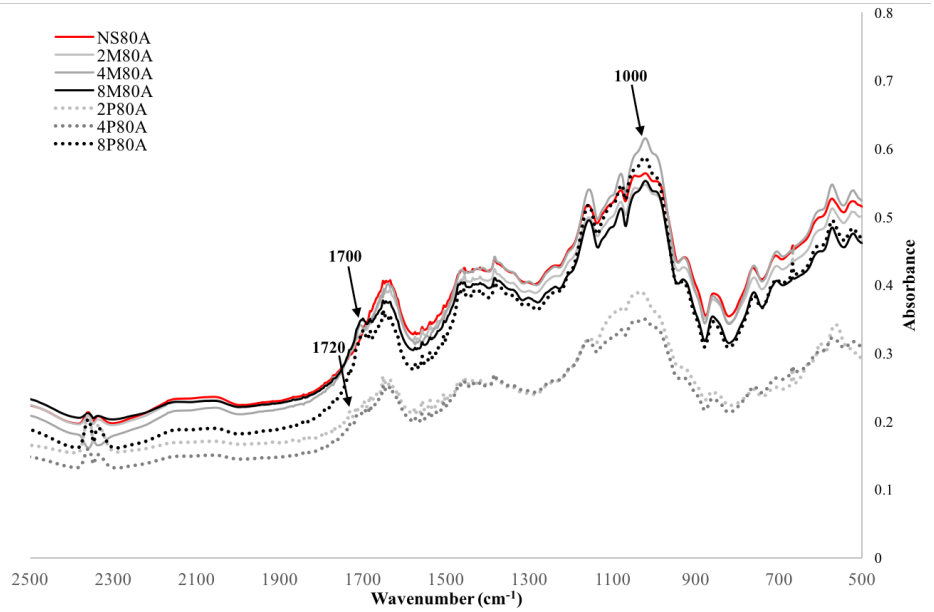

Figure 4.1.2. IR spectra for extruded native and lipid complexed pea starches

From Figure 4.1.2 it is evident that for palmitic acid modified starches the peak at 3500 cm^{-1} decreases in magnitude, assigned to the hydroxyl group stretching vibrations. This is indicative of less moisture being in those specific samples coupled with a decreasing concentration of hydrogen-bonded hydroxyl groups which were converted into ester groups. The myristic acid starches exhibit spectra closer to that of native starch, which is likely attributed to the shorter carbon chain of myristic acid. The carboxylic acid C=O stretching at 1720-1700 cm^{-1} provides the most convincing validation of lipid modification as does the broad O-H stretching between 3000-2500 cm^{-1} since all other bands appear too weak to detect. Furthermore, it is evident that as lipid concentration increases the peaks become larger in intensity.

4.1.3 Effect of Variables on the Reaction

All samples prepared on the Xplore compounder and Leistritz twin-screw extruder were titrated according to a modified AACC 02-01A Fat Acidity General Method. Based on these titrations, conversion and degree of substitution were calculated for all samples with respect to their corresponding titration standards. This provided extensive insight into the effectiveness of the reaction at two production scales. The results are summarized in Table 4.1.1. The variable 'feed moisture' was predominately studied in the Leistritz extruder; this is due to the inability to explore the variable in the Xplore compounder without jamming the device or burning the product at low levels of moisture. Although moisture levels of 30% and 60% were attempted in the Xplore, few samples could be collected till the moisture was increased to 80%. The Leistritz studies allowed experiments at three levels of moisture 40%, 60% and 80%.

Table 4.1.1. Summary of titration data, conversion (%) and degree of substitution, for Xplore compounder and Leistritz twin-screw extruder samples. All experiments conducted in triplicate.

Sample Name	Screw Design	Lipid	Lipid Content (%)	Feed Moisture (%)	Temperature (°C)	Conversion (%)	Degree of Substitution
XPLR 2P80 - 90	N/A	Palmitic	2	80	90	94.1 ± 0.04	0.532 ± 0.02
XPLR 4P80 - 90	N/A	Palmitic	4	80	90	94.6 ± 0.03	0.579 ± 0.02
XPLR 8P80 - 90	N/A	Palmitic	8	80	90	85.5 ± 0.04	0.590 ± 0.03
XPLR 2P80 - 100	N/A	Palmitic	2	80	100	88.2 ± 0.07	0.498 ± 0.04
XPLR 4P80 -100	N/A	Palmitic	4	80	100	92.3 ± 0.02	0.564 ± 0.01
XPLR 8P80 -100	N/A	Palmitic	8	80	100	83.0 ± 0.03	0.575 ± 0.02
XPLR 2P80 - 110	N/A	Palmitic	2	80	110	100 ± 0.03	0.585 ± 0.03
XPLR 4P80 -110	N/A	Palmitic	4	80	110	96.4 ± 0.03	0.580 ± 0.02
XPLR 8P80 -110	N/A	Palmitic	8	80	110	85.43 ± 0.01	0.585 ± 0.01
XPLR 2M80 - 90	N/A	Myristic	2	80	90	100 ± 0.01	0.766 ± 0.01
XPLR 4M80 - 90	N/A	Myristic	4	80	90	94.9 ± 0.01	0.754 ± 0.02
XPLR 8M80 - 90	N/A	Myristic	8	80	90	93.2 ± 0.01	0.788 ± 0.01
XPLR 2M80 - 100	N/A	Myristic	2	80	100	100 ± 0.02	0.785 ± 0.03
XPLR 4M80 -100	N/A	Myristic	4	80	100	99.6 ± 0.01	0.797 ± 0.01
XPLR 8M80 -100	N/A	Myristic	8	80	100	94.9 ± 0.01	0.807 ± 0.01
XPLR 2M80 - 110	N/A	Myristic	2	80	110	100 ± 0.06	0.765 ± 0.04
XPLR 4M80 -110	N/A	Myristic	4	80	110	93.4 ± 0.05	0.748 ± 0.05
XPLR 8M80 -110	N/A	Myristic	8	80	110	94.3 ± 0.01	0.748 ± 0.04
TSE 2P40A	A	Palmitic	2	40	90	100 ± 0.01	0.578 ± 0.01
TSE 2P60A	A	Palmitic	2	60	90	100 ± 0.01	0.593 ± 0.04
TSE 2P80A	A	Palmitic	2	80	90	96.0 ± 0.06	0.520 ± 0.03
TSE 4P40A	A	Palmitic	4	40	90	91.6 ± 0.06	0.534 ± 0.04
TSE 4P60A	A	Palmitic	4	60	90	99.2 ± 0.02	0.591 ± 0.01
TSE 4P80A	A	Palmitic	4	80	90	92.4 ± 0.07	0.559 ± 0.04
TSE 8P40A	A	Palmitic	8	40	90	88.9 ± 0.01	0.578 ± 0.01
TSE 8P60A	A	Palmitic	8	60	90	84.2 ± 0.01	0.550 ± 0.01
TSE 8P80A	A	Palmitic	8	80	90	93.6 ± 0.01	0.639 ± 0.01
TSE 2P40B	B	Palmitic	2	40	90	99.2 ± 0.05	0.492 ± 0.04
TSE 2P80B	B	Palmitic	2	80	90	99.9 ± 0.03	0.535 ± 0.02
TSE 4P40B	B	Palmitic	4	40	90	92.8 ± 0.02	0.562 ± 0.03
TSE 4P80B	B	Palmitic	4	80	90	97.0 ± 0.03	0.582 ± 0.03
TSE 8P40B	B	Palmitic	8	40	90	90.0 ± 0.02	0.591 ± 0.01
TSE 8P80B	B	Palmitic	8	80	90	95.1 ± 0.01	0.632 ± 0.04
TSE 2M40A	A	Myristic	2	40	90	100 ± 0.01	0.822 ± 0.01
TSE 2M60A	A	Myristic	2	60	90	100 ± 0.01	0.802 ± 0.02
TSE 2M80A	A	Myristic	2	80	90	100 ± 0.01	0.823 ± 0.01
TSE 4M40A	A	Myristic	4	40	90	100 ± 0.01	0.813 ± 0.01
TSE 4M60A	A	Myristic	4	60	90	96.0 ± 0.03	0.767 ± 0.02
TSE 4M80A	A	Myristic	4	80	90	99.7 ± 0.01	0.806 ± 0.01
TSE 8M40A	A	Myristic	8	40	90	68.2 ± 0.07	0.575 ± 0.06
TSE 8M60A	A	Myristic	8	60	90	67.6 ± 0.01	0.562 ± 0.01
TSE 8M80A	A	Myristic	8	80	90	79.4 ± 0.01	0.679 ± 0.01

Myristic (C_{14}) acid starches yielded higher degrees of substitution (nominal DS= ~0.8) when compared to their palmitic (C_{16}) acid counterparts (nominal DS= ~0.5). As the maximum possible DS is 3.0 for available reactive hydroxyl group per anhydroglucose unit (AGU) making up the polysaccharide chains, the data indicated that 17-27% reacted. Not included in the table, one batch of 16% palmitic acid modified pea starch was produced on screw B yielding

dramatically different values with 66.9±0.01% conversion and 0.409±0.07 degree of substitution at the die. Kapusniak & Siemion (2007) studied potato starch with linoleic (C_{18}) acid and achieved degrees of substitutions around 0.32. It appears that as the fatty acid carbon chain length increases, the degree of substitution decreases. Furthermore, when comparing degree of substitution between palmitic acid complexed samples produced on the Xplore ($DS_{XPLR\ 2M80-90} = 0.532 \pm 0.02$) and Leistritz ($DS_{TSE\ 2P80A} = 0.520 \pm 0.03$ & $DS_{TSE\ 2P80B} = 0.535 \pm 0.02$) it is evident that both devices achieve similar values when looking at corresponding formulations and operating conditions. However, myristic acid complexed samples achieved lower degrees of substitution on the Xplore ($DS_{XPLR\ 4M80-90} = 0.754 \pm 0.02$) than on the Leistritz ($DS_{TSE\ 4M80A} = 0.806 \pm 0.01$). Rajan et al. (2006) investigated esterification of starch using recovered coconut oil and determined that extrusion reactions by Brabender plasticoder was not an efficient method for esterification reactions as it yielded lower degrees of substitution when compared to methods such as solution state esterification or microwave radiation.

The effect of all tested operational variables on the reaction were determined by statistical analysis using open source R software, of titration results. These results are summarized in pareto plots found in Figures 4.1.3 and 4.1.4. It should be mentioned that this was an imbalanced data set due to the moisture limitations noted above for the Xplore mixer as well as the fact that temperature remained constant for all Leistritz studies to keep the number of trials

reasonable; temperature was kept constant in the extruder since it was found to be the least sensitive variable to the reaction based on tests in the Xplore. The variables considered in the analysis included the influence of the mixing device, lipid, lipid content, moisture content and temperature on conversion and degree of substitution.

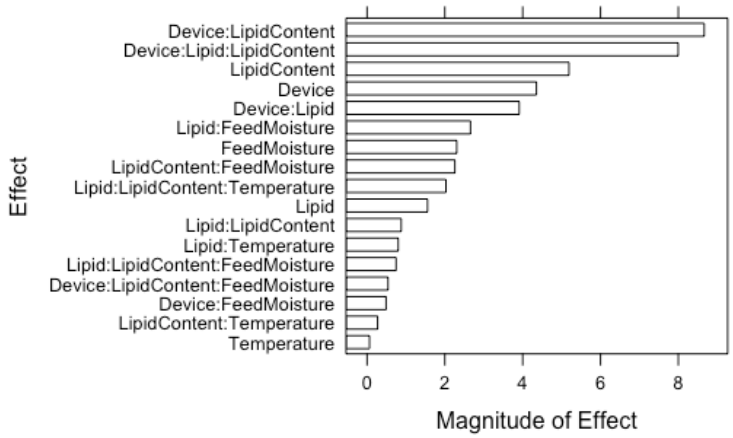

Figure 4.1.3. Statistical analysis ranking the significance of extrusion device, lipid, lipid content, moisture content and temperature to conversion (multiple R^2= 0.7395, p=0.002)

From Figure 4.1.3 it is evident that three main factor effects influence conversion the most, being the device used (Xplore, Leistritz screw A or screw B), lipid content (2,4 or 8%) and a combination of these two effects with a specific lipid (myristic or palmitic acid). The next major contributing factor is feed moisture content (40, 60 or 80%) while temperature had the least significance in terms of individual or multi-factor contributions.

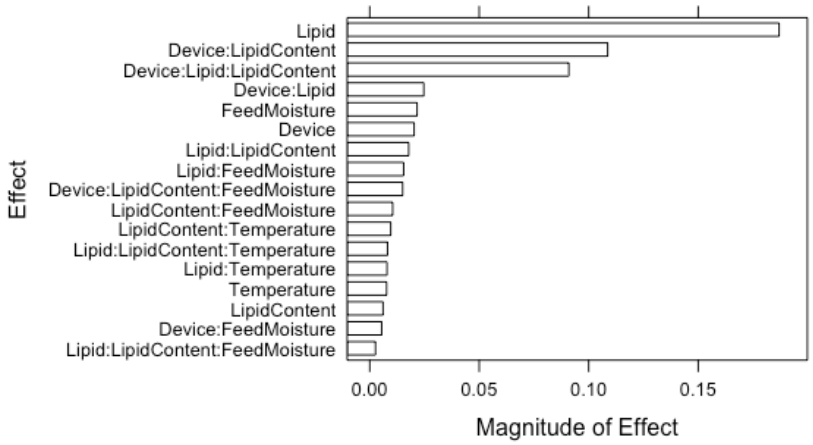

Figure 4.1.4. Statistical analysis ranking the significance of extrusion device, lipid, lipid content, moisture content and temperature to the degree of substitution (multiple R^2= 0.9324, p=1.37x10^{-9})

However, results from Figure 4.1.4 show that most effects have less significance on the degree of substitution than they do on conversion, yet individual and multi-component effects of lipid, lipid content and device have the greatest impact overall. Lipid type (myristic or palmitic acid) is the strongest individual factor influencing degree of substitution. It is postulated that the effect of lipid type differs between conversion and degree of substitution because DS is significantly affected by fatty acid carbon chain length. This is consistent with the ability of myristic acid to better react with one of the available reactive hydroxyl groups per AGU due to its shorter carbon chain which provides improved chain packing. Given more time, investigation of varied temperature on the Leistritz could be interesting to look at when coupled with its very different and intensive shear environment in comparison to the Xplore.

When looking at the effect of reaction time (3 or 5 minutes) in the Xplore (results not shown) it was determined that reaction time significantly affected

conversion and degree of substitution of myristic acid complexed starches over palmitic acid complexed starches. Thus, all titration results from the Xplore presented in Table 4.1.1 were from the 5-minute reaction time to allow the lipids to (1) completely melt and (2) sufficient time to react as well as starch gelatinization.

4.1.4 Development of Conversion and Degree of Substitution Inside TSE

To better understand the mechanism of conversion and degree of substitution for the palmitic complex reaction within the twin-screw extruder, samples were taken at three zones (4, 5 and 7) along the length of the screw and at the die. The extruder operated for five minutes at steady state then stopped, with samples removed quickly from the barrel opening at the mentioned zones. Samples were titrated according to a modified AACC 02-01A Fat Acidity General Method to determine conversion and degree of substitution of samples at each zone respectively which can be seen in Figures 4.1.5-4.1.8. All results were conducted in triplicate however error is too small to be depicted.

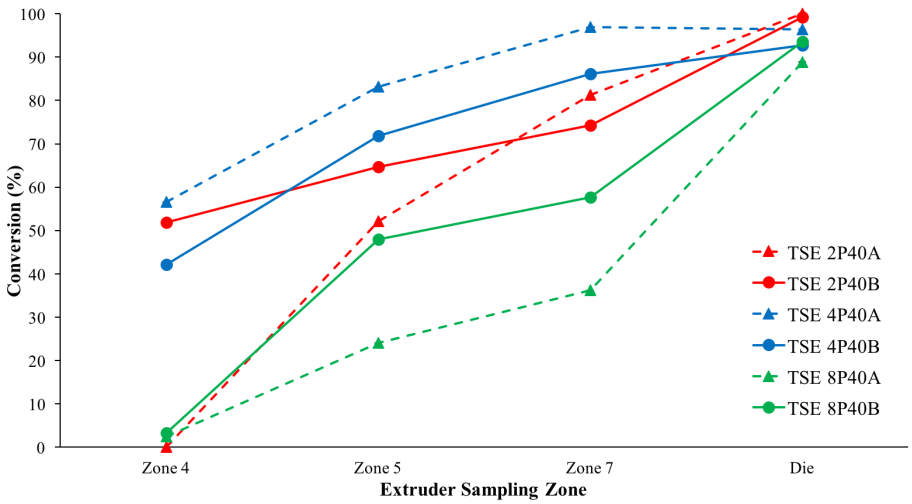

Figure 4.1.5. Lipid conversion along the length of the twin-screw extruder of lipid modified palmitic acid pea starch samples (2%, 4% and 8%) with 40% moisture produced on screws A and B. Relative standard deviation (RSD) <3%.

From Figure 4.1.5 it is evident that lipid conversion for samples made with 40% moisture progressed gradually along the length of the extruder, with conversion midway along the extruder at zone 5 varying between 20-82%. Comparatively, samples made with 80% moisture found in Figure 4.1.6 were more rapid, achieving conversions of 70% or greater by zone 5. This is attributed to the additional water content promoting gelatinization, cooking and granule disruption. Della Valle et al. (1987) identified water content as the most influential variable in extrusion since changes in the degree of fill combine the effects of screw speed and feed rate compared to other variables such as temperature and pressure. Specifically, as water content increases the viscosity of molten starch decreases and viscous dissipation decreases. Davidson et al. (1984) explained this to be a result of stress reduction due to reduced melt viscosity. For some starch extrusion studies and modification methods it is beneficial to have

low moisture contents and minimal shear to maintain granule integrity. For a lipid complexing process such as this, granule disruptions are beneficial as they disorganize the natural starch structure allowing for increased lipid binding sites, thus providing higher degrees of conversion and substitution. Moisture was not found to be the most important factor in this study, as noted in the previous section (Sec 4.1.3), since by the die the benefits of moisture are not apparent. Higher moisture appears to improve the kinetics of the reaction but not the extent of the reaction.

From Figures 4.1.5 and 4.1.6 it is evident that screw design significantly affects conversion. At a higher moisture content (80%) it is evident that increased screw shear (i.e. screw B) aids in boosting conversion early on. Samples with 40% moisture however see improved conversion with screw B only at low lipid contents. It is expected that as the degree of shear increases, the natural hull of pea starch granule is broken down, consequently creating disorder in the amylose and amylopectin chains thus increasing opportunity for lipid complexing. In general, decreasing lipid content increased conversion at the die as there were more available binding sites for the lipid to attach.

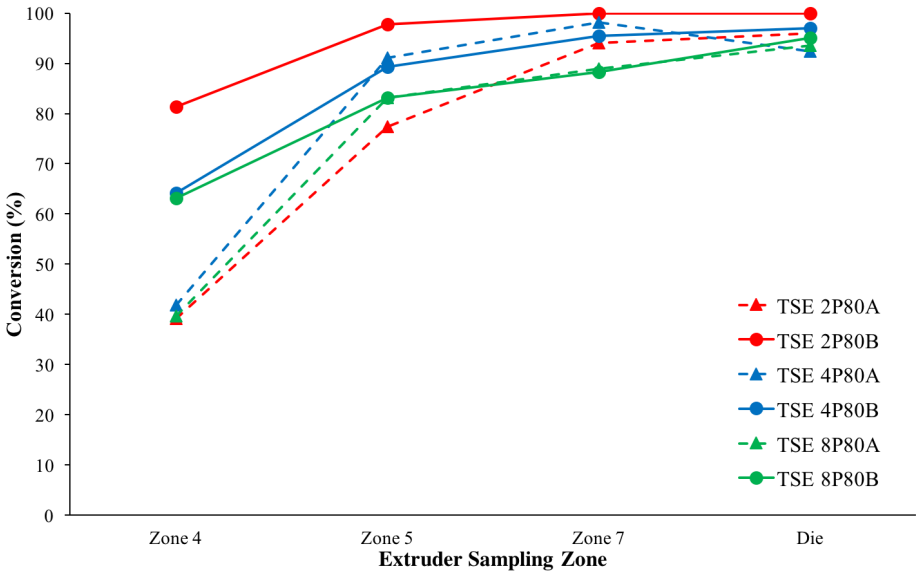

Figure 4.1.6. Lipid conversion along the length of the twin-screw extruder of lipid modified palmitic acid pea starch samples (2%, 4% and 8%) with 80% moisture produced on screws A and B. Relative standard deviation (RSD) <6%.

Degree of substitution was also calculated along the length of the extruder, which can be seen in Figures 4.1.7 and 4.1.8. Similarly, to conversion results, it is evident that lower moisture content results in more variance in the degrees of substitution while still increasing along the length of the extruder. The higher processing moisture content of 80% yields more consistent degrees of substitution values with the exception of the 2% lipid levels for both screw designs. In general, all major outliers occur at zone 4 and while the samples were all run in triplicate, further investigation is required to determine the cause of this. Due to the configuration of zone 4, with respect to its proximity to the feed zone and the screw elements within the zone, there is a low degree of fill in the area and thus there was less material to collect during screw pull outs. Furthermore, at this stage, material has not yet been fully gelatinized and is therefore still in a partially

powdered state which could provide variability in samples as they are not yet well mixed.

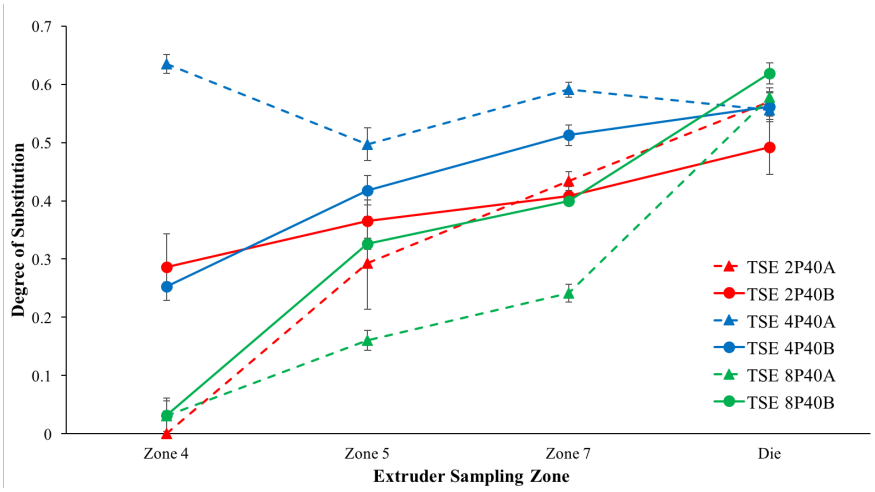

Figure 4.1.7. Degree of substitution along the length of the twin-screw extruder of lipid modified palmitic acid pea starch samples (2%, 4% and 8%) with 40% moisture produced on screws A and B. Relative standard deviation (RSD) <2%.

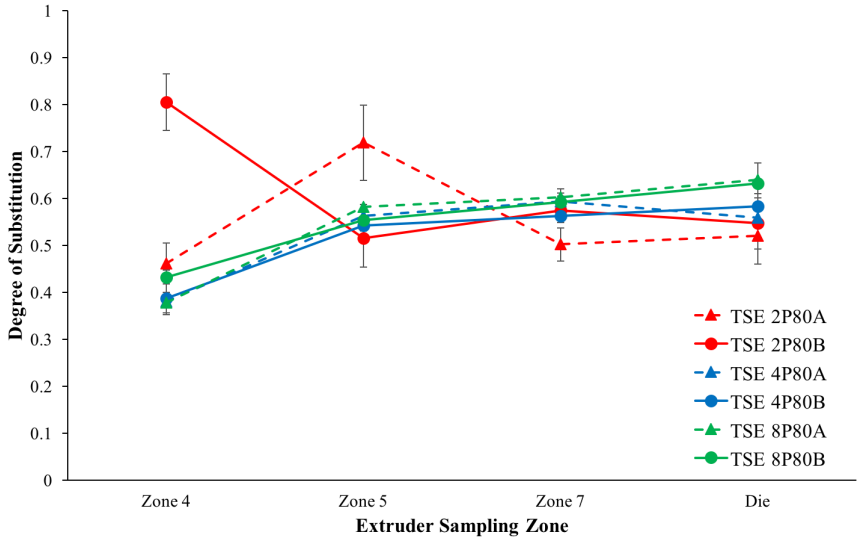

Figure 4.1.8. Degree of substitution along the length of the twin-screw extruder of lipid modified palmitic acid pea starch samples (2%, 4% and 8%) with 80% moisture produced on screws A and B. Relative standard deviation (RSD) <8%.

It is interesting to note that no matter the lipid level, processing moisture content or screw design, samples can consistently reach ≥85% conversion and degrees of substitution between 0.5-0.6 by the die. It is speculated that intense

compressive and pressure forces at the die aid in achieving these values. Additional investigation is required to determine the exact saturation point between the 8% and 16% concentrations.

From Figures 4.1.7 and 4.1.8 it is apparent that increasing screw shear (i.e. screw B) improves degree of substitution, particularly for 80% moisture content samples. For the 8% palmitic acid complexed starch with 40% moisture, a significant improvement in DS is exhibited for screw B (TSE 8P40B) by zone 5. Conversely, in Figure 4.1.8, DS is essentially mirrored for screws A and B at 4% and 8% lipid content at 80% moisture. In conclusion, it is evident that screw design, in particular increased shear, plays a more important role in achieving high conversion than degree of substitution along the length of the extruder.

4.1.4 Effect of Lipid Presence on Contact Angle

As a reference, the contact angle of native starch extruded on the twin-screw extruder with 80% moisture (TSE NS80A) is 67.4±1.28°. From Figure 4.1.9, it is evident that the greatest effects impacting contact angle occurs as a result of twin-screw extrusion processing and increasing lipid content. By increasing lipid content, film hydrophobicity increases thus increasing its surface tension. The impact of the Leistritz extruder is evident when comparing the samples prepared at 90°C (which was the only temperature shared for both devices). Decreased performance was found with the Xplore samples for both myristic and palmitic acid modified films compared to the extruder. This can be attributed to steric hindrance preventing close association of chains thus

restricting hydrogen bonding and by changing the hydrophilicity of the starch molecules which alters the ability to bond with water molecules as a result of lipid substitution. When comparing the performance of palmitic acid versus myristic acid, myristic acid films from the Xplore yield higher contact angles. For example, a 4% palmitic acid film has a contact angle of 60.76±0.22° while its myristic acid counterpart (prepared in the exact same conditions) yields an angle of 70.26±7.49°. These results are unanticipated as it is expected that the palmitic acid films would have a higher angle due to the lipid having a longer carbon chain (C_{16} versus myristic acid C_{14}). Of course, the anticipated outcome is based on the assumption that the lipid chain lies flat along the starch surface to cover more wettable area and the results do not support that assumption. This difference between lipid sources is also seen with Leistritz samples, in Figure 4.1.9, though on average the values are 10° higher in contact angles for the respective lipid concentrations.

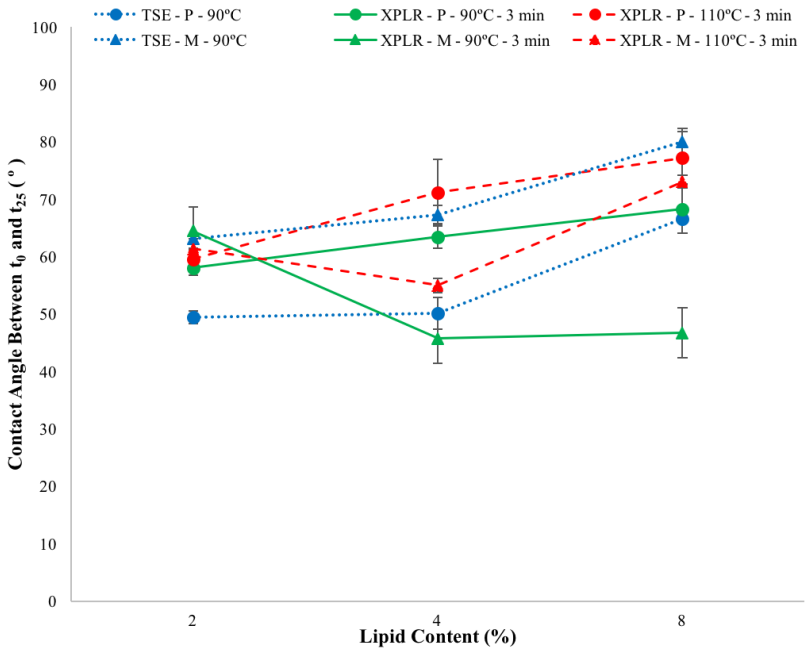

Figure 4.1.9. Effect of lipid content and extrusion technology on contact angle between time t_0 and t_{25}. All experiments conducted in triplicate.

Due to time constraints, contact angle measurements were not conducted for samples prepared on screw B in the Leistritz. As discussed in Section 4.1.4, it is expected that increasing shear would increase lipid attachment thus increasing hydrophobicity. This hypothesis could also be contradicted as a cast native starch thin film (boiled with minimal agitation) had a very high contact angle of 77.07±0.80° due to natural hull of the pea remaining intact, while increasing screw shear would disrupt the hull. This provides opportunity to determine a balance between maintaining the natural granule integrity while increasing shear to promote lipid attachment thus creating a modified hydrophobic surface.

4.2 Effect of Screw Design on Lipid Complexing

The extent and uniformity of the reactions within the extruder are affected by its residence time distribution (RTD). Studies on RTD in an extruder have

been used to understand the flow patterns and extruder performance, especially as mixing occurs simultaneously with a reaction. Starch processing differs in two main respects compared to synthetic polymers: (1) granular starch must be completely transformed into an amorphous and expanded phase to fully access all hydroxyl groups per AGU and (2) starch is always extruded with water (minimum of 5-40%, extruder dependent) which acts as a plasticizer for starch materials (Colonna et al., 1983). The RTD was assessed for extruded samples prepared with the same moisture content (80%), same screw speed (250RPM) and same feed rate (3kg/h) comparing native starch versus samples produced by reaction with 4% palmitic acid samples for screw designs A and B, determined at zones 5 and the die. Moments of the RTDs are presented in Table 4.2.1 and it shall be noted that all experiments were conducted in triplicate.

Table 4.2.1. Conversion and calculated RTD parameters of extruded Nutri-Pea pea starch samples (80% moisture, native and 4% palmitic acid complexed, 3kg/h and 250RPM) prepared on screws A and B. Data recorded at extruder zones 5 and die in triplicate.

Extrusion Conditions	Zone	Sample	Conversion (%)	Mean Residence Time (s)	Variance (s^2)	Equivalent Vessel Number
3kg/h, 250RPM	Zone 5	NS80A	-	43 ± 4.33	717 ± 130	2.56
		NS80B	-	55 ± 4.08	1154 ± 160	2.66
		4P80A	91.1 ± 0.01	54 ± 3.82	1120 ± 146	2.65
		4P80B	89.3 ± 0.01	44 ± 2.89	767 ± 93.8	2.58
	Die	NS80A	-	99 ± 1.44	3523 ± 93.8	2.81
		NS80B	-	120 ± 10.0	5129 ± 792	2.83
		4P80A	92.4 ± 0.07	81 ± 1.77	2407 ± 100	2.75
		4P80B	97.0 ± 0.03	143 ± 6.29	7219 ± 620	2.86

The main difference between screw A and B is that the kneading zone at the end of zone 4 was doubled and an additional comb element was added to zone 5 of screw B (see Figures 3.3.1 and 3.3.2). These modifications would give rise to

a significantly longer mean residence time and greater variance in residence time compared to screw A. Variance was influenced most at the die and showed significant increases when comparing native samples for screw A versus screw B. As suggested by Mu and Thompson (2012), equivalent vessel number was expected to decrease with increased axial mixing or axial dispersion increases with increased mixing zones. In this present study however, it is suggested that the equivalent vessel number was relatively unchanged between screws A and B at both zones 5 and the die. The mixing effect based on screw design is readily seen by the samples of native starch where no reaction was taking place; however, in the presence of the lipid complex reaction the rheology of the system will vary in correspondence to the morphology of the mixture. The decreased mean residence time of sample TSE 4P80B in zone 5 can be attributed to the higher degree of conversion by that point causing more lipid to plasticize the starch rather than act as an individual phase. Comparatively, the increased mean residence time of sample TSE 4P80A in zone 5 indicates the heterogeneity of the system due to lower mixing. By the die, increased mean residence time of sample TSE 4P80B can be attributed to the additional mixing elements after zone 5 which contribute to the increased degree of conversion and homogeneity of the system. Decreased mean residence time of sample TSE 4P80A is due to the shift of shearing elements near zone 5 thus allowing for extra conveying elements at the end of the screw (see Figure 3.3.1). The additional comb element in screw B slowed the screw down however improved mixing thus improving conversion.

The fitted RTD model to the data of each screw design was based on the assumption that mixing within the barrel is described as an additive contribution of the conveying action in the screws (main peak) and each mixing zone (secondary peaks) as seen in Figures 4.2.1 and 4.2.2 (Mu & Thompson, 2012). The E(t) curves represent the variation of the tracer concentration in zone 5 and at the die exit (Chuang & Yeh, 2004). From the inlet feed zone to the start of the first kneading section, starch only partially fills the screw channel and remained in semi-powder state. Around the last flight of the conveying element, leading into the first kneading zone, starch completely fills the screw and is transformed from a powder into a melt for both screw A and B. Cai & Diosady (1993) identified this as the transition zone and from this point to the die, was classified as the cooking zone. Examination of the samples obtained along the length of the screw revealed starch gelatinization began at the cooking zone through to the die. As seen in Figure 4.2.1, there are no visible secondary peaks which is a direct result of kneading block placement around zone 5. In both Figures 4.2.1 and 4.2.2, screw B exhibits a broader main peak. The flow of solids is complex within the kneading zones due to the compression from axial pressure as well as normal and tangential forces being applied in the intermeshing region (Thompson & Sun, 2010). Increased mixing provided by kneading zones improved heat transfer and it is hypothesized that increased shearing action developed heat through dissipation of mechanical energy. Partially stagnant flow noted by RTD analysis may be a result of decreased conveying capacity leading into kneading zones and back pressure of

the die at the end of the screw. Colonna et al. (1983) stated that mass transport without physical or chemical modification is the predominate process of the conveying zone, thereafter comminution and mixing are the major processes where physical changes to granule structure and partial degradation of molecules occur. Furthermore, literature addressing the effects of screw elements on cooking starch flours at high moisture contents is limited, making it difficult to directly compare this RTD study with other available literature (Chuang & Yeh, 2004).

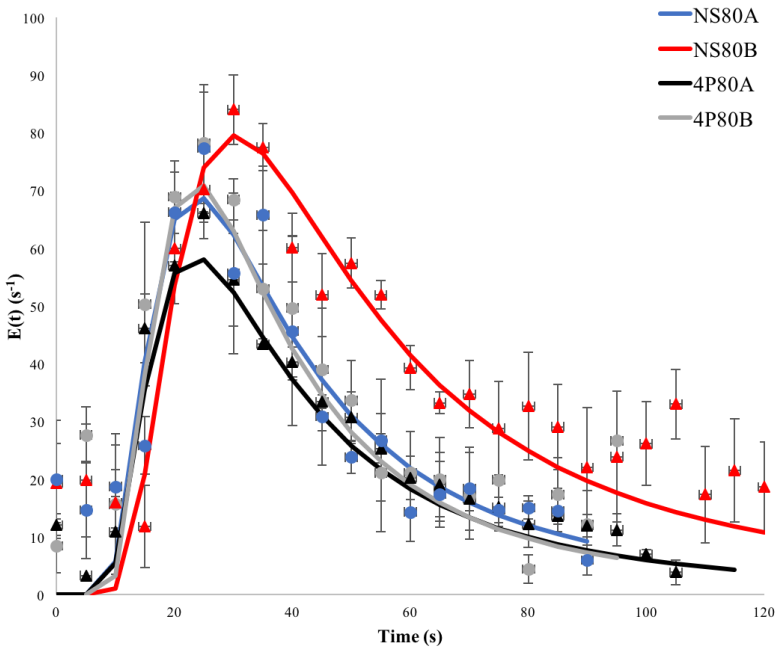

Figure 4.2.1. RTD of extruder zone 5 for screw designs A and B at fixed feed rate (3.00kg/h) and fixed screw speed (250RPM) for native (NS80) and palmitic acid complexed (4P80) pea starches.

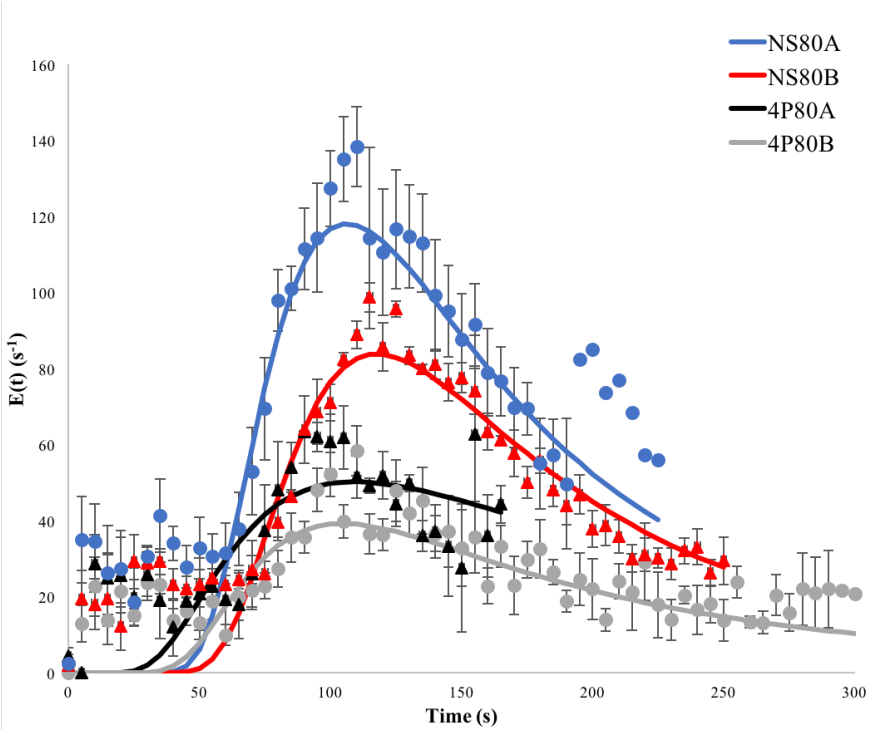

Figure 4.2.2. RTD of extruder die for screw designs A and B at fixed feed rate (3.00kg/h) and fixed screw speed (250RPM) for native (NS80) and palmitic acid complexed (4P80) pea starches.

When examining the extrudate appearance at the die in the RTD trials there was no distinguishable difference between samples from screw A and B. The native starch samples were significantly more intense in brown colour than the 4% palmitic acid modified samples. This has been attributed to the presence of lipid acting as a lubricant within the extruder, improving the incorporation of cocoa powder into the starch. The use of cocoa powder as a tracer was beneficial as it had a similar particle size to the starch powder and is hydrophobic thus would not solubilize in the presence of large amounts of water in the system.

4.3 *In Vitro* Digestibility

RDS is thought to predominately represent the amorphous starch fractions found in large quantities in freshly cooked or baked starch foods such as breads. SDS is said to be associated with physically inaccessible amorphous starch fractions and is comprised of a mixture of amorphous and semi-crystalline materials in raw starches with A-type or C-type crystalline pattern, such as in pulses, and B-type starches in granule or cooked retrograded form. RS starch fractions were believed to be related to the crystalline regions of raw starches (Lehmann & Robin, 2007; Sajilata et al., 2006). In the results presented in this study, the inherent RS fractions (amylopectin crystals) in native pea starches could be classified as RS3. When pea starches were lipid complexed in the bulk state in this study, RS5 was formed. In general, α-amylase resistance of starches is thought to be influenced by a combination of factors including, feed moisture content, lipid type and concentration and the degree of induced shear or extent of starch granule disruption. At the molecular level, the crystalline and amorphous regions, in particular the packing of amylopectin double helices affected the enzymatic susceptibility. The RS results of the trials are divided into sub-sections below to highlight the influence of different factors

4.3.1 Effect of Processing Method on Native Starch Digestion

As suggested by the table in Appendix A1 and Figure 4.3.1, extrusion cooking significantly affects the SDS and RS content of native pea starches.

Native twin-screw extruded fully gelatinized pea starches (TSE NS40A/B and TSE NS80A/B) showed significantly decreased SDS and increasing RDS values in comparison to granular pea starch samples; increasing moisture content maintained RS and minimized the decline in SDS content for the extruded samples. The decreased SDS content may be a result of disruption to the order and packing of double helices induced by a relatively high shear and high heat-moisture environment (Chung et al., 2009). Initial resistance, RS2, of raw pea starches is associated with the restrictive mobility of molecular chains or individual glucose units. However, RS2 is vulnerable to destruction by high shear thermal processing (Menezes, et al., 2011). Thus, non-reactive twin-screw extrusion showed potential to increase enzymatic susceptibility of pea starch granules. Unfortunately, native starch produced on the Xplore was not tested for digestion due to limited and poor sample quality as a result of lipid absence, thus a comparison of these devices cannot be made.

When examining cooking methods, extrusion significantly retains more of the RS content and minimizes the increase in RDS content in comparison to starch boiled in an aqueous slurry. This may be due to findings that amylopectin is affected most by shear degradation due to its branching structure (Liu et al., 2010) while Englyst and Cummings (1987) propose that less branched retrograded starch is most resistant to digestion. The aqueous slurry would not undergo rigorous shear during cooking and thus minimal granule degradation would occur.

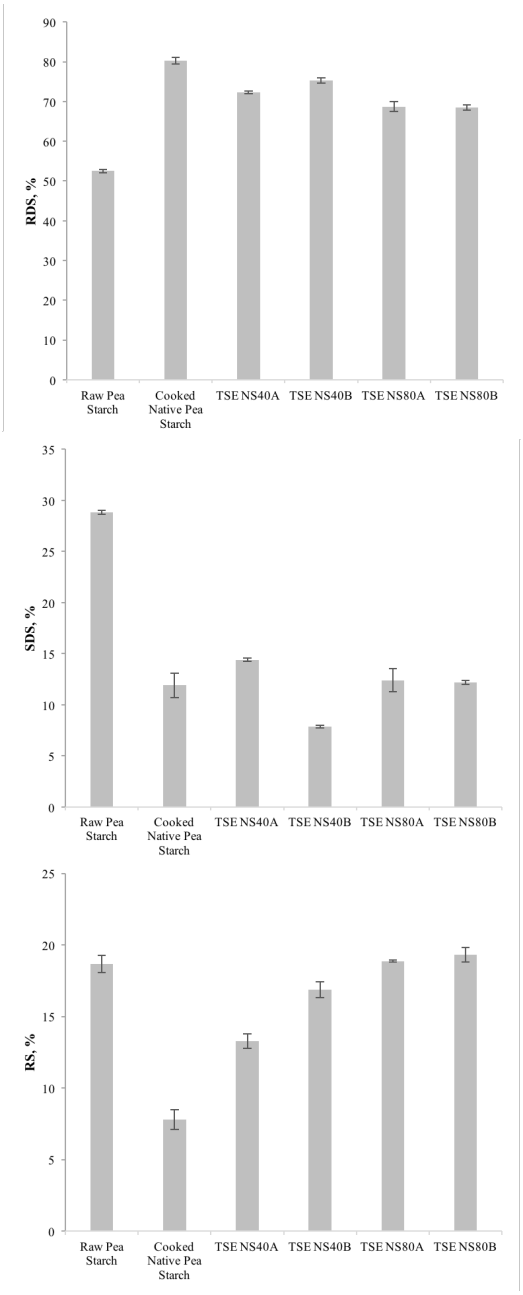

Figure 4.3.1. Nutritional fractions (RDS, SDS and RS content) of raw, boiled and extruded pea starch with moisture contents of 40% or 80%, determined by Englyst's method. The data was standardized to a total starch basis.

4.3.2 Effect of Feed Moisture Content for the Lipid Complexed Starches

The effect of feed moisture content on digestion profiles of extruded Nutri-Pea pea starch lipid complexes is presented in Figures 4.3.2 and 4.3.3. It

was observed that lower feed moisture content (40%) gave significantly lower RS content when compared with higher feed moisture content (80%). However, higher SDS contents were exhibited for lower feed moisture content (40%). In comparison, it is evident that RDS content is relatively unchanged at both levels of moisture. The increasing feed moisture aided in the extent of lipid complexing thus enhancing the formation of RS5 but also increased the extent of starch gelatinization and degradation which validates the lower SDS. Due to the high levels of feed moisture used in this study, the effects of feed moisture on starch resistance were predominant.

Sharma et al. (2015), reported that an increase in feed moisture at low levels (i.e. from 20% to 24%) did not lower the RS content of non-reactively twin-screw extruded kidney bean and field pea starches. Comparatively, Sarawong et al. (2014) observed that extrusion cooking at high feed moistures (50%) increased the amylose content and was positively correlated with RS content. Therefore, by further increasing moisture content to 80%, amylose content (long chain branching) increases, promoting RS formation.

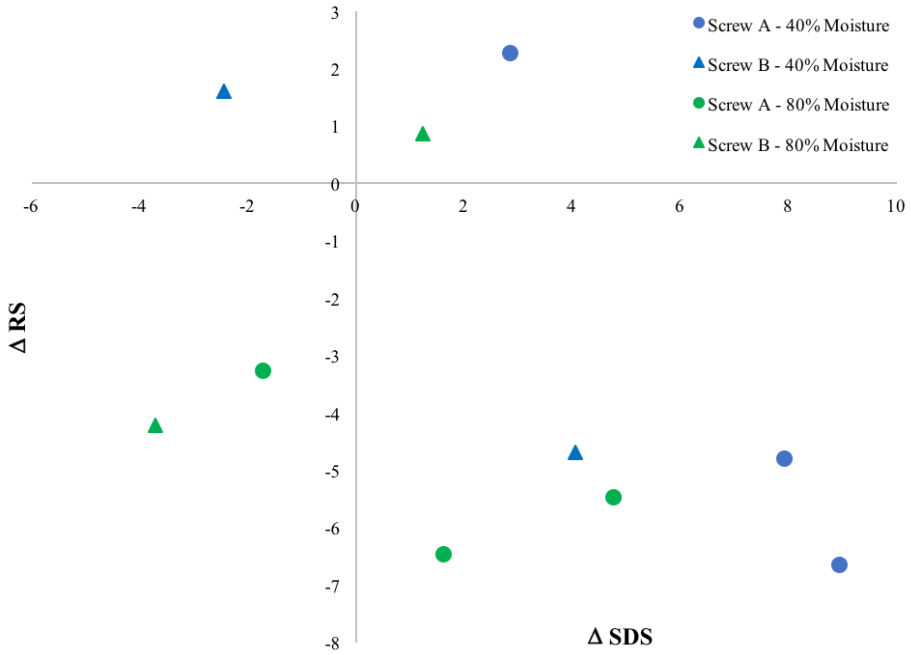

Figure 4.3.2. Effect of moisture content (40% or 80%) on SDS and RS nutritional fractions (represented as a difference from native starch nutritional fractions) of twin-screw extruded lipid complexed pea starches determined by Englyst's method for Screws A and B.

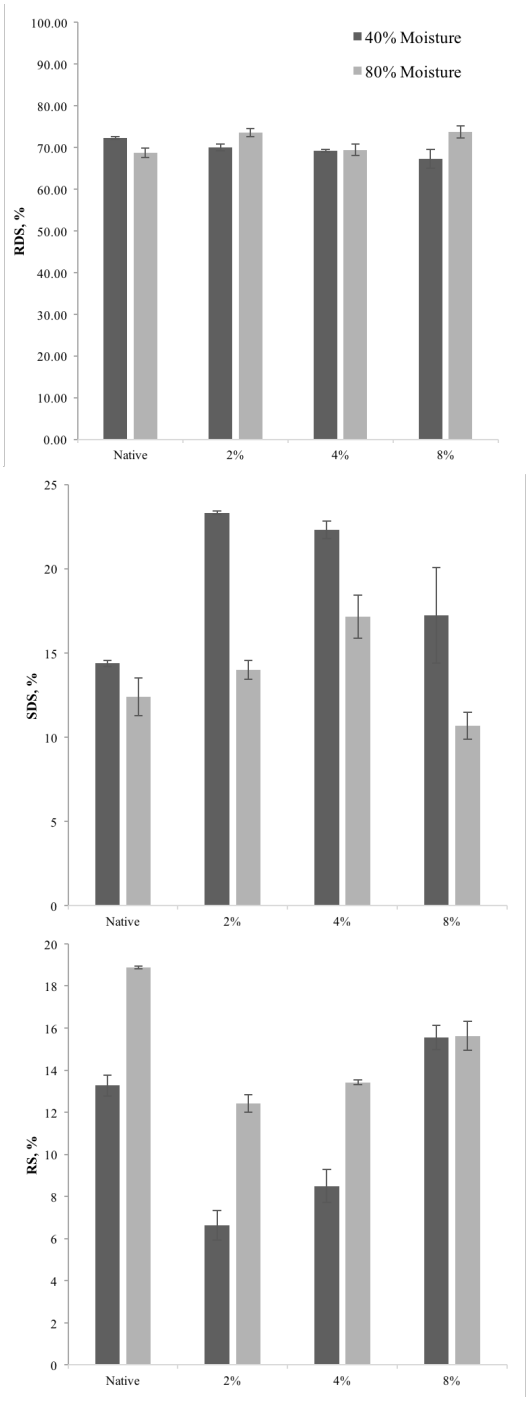

Figure 4.3.3. Nutritional fractions (RDS, SDS and RS content) with respect to variation in moisture content (40% vs. 80%) of extruded palmitic acid complexed starch samples determined by Englyst's method. The data was standardized to a total starch basis.

4.3.3 Effect of Lipid Type and Content for the Lipid Complexed Starch

Due to time, cost constraints and considerations of food safety, only two types of lipids were used in this study, myristic acid and palmitic acid. These saturated fatty acids are very similar in structure and have relatively close melting temperatures of 54.4°C and 62.9°C, respectively. Most importantly, these are both food grade fatty acids and can therefore be used to modify a food ingredient. The effect of lipid type on nutritional starch fractions can be seen in Figures 4.3.4-4.3.6 and have been compared to cooked native starches. Both lipid types were used in experiments on the Xplore compounder and the Leistritz twin-screw extruder, however only on screw A.

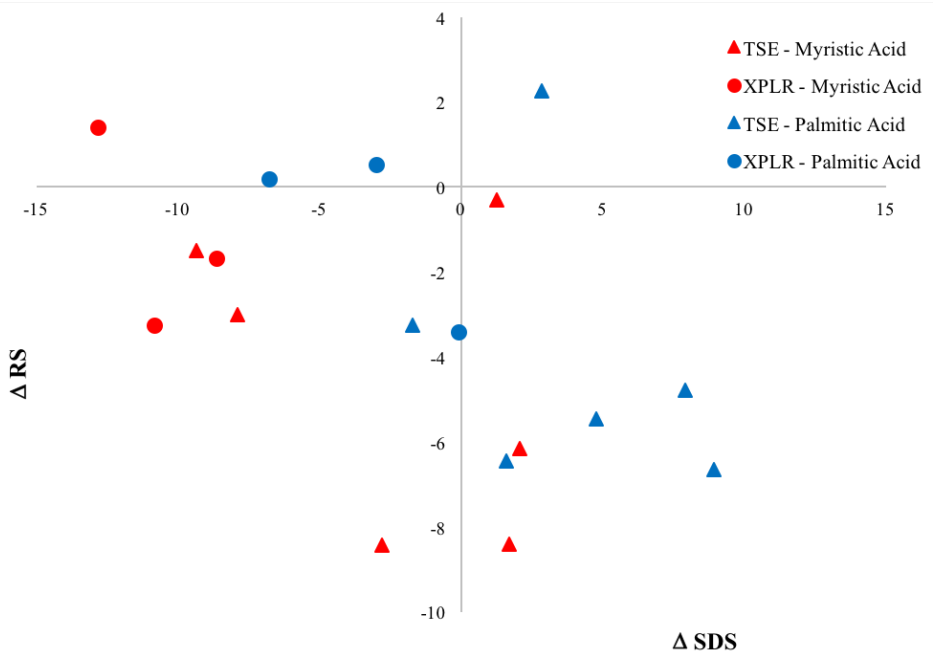

Figure 4.3.4. Effect of lipid addition and type on SDS and RS nutritional fractions (represented as a difference from native starch nutritional fractions) of extruded pea starches on Xplore compounder and Leistritz twin-screw extruder (screw A) at 80% moisture determined by Englyst's method.

When looking at the effect of lipid addition and type in Figure 4.3.4, with respect to SDS and RS content only as a difference from native starches, which were more important to this study, it is evident that in general myristic acid modified starches are prone to observe a drop in SDS content relative to an extruded native starch, while palmitic acid modified starches more often produced a rise in SDS content. This trend is prominent in Figures 4.3.5 and 4.3.6 where SDS content is <10% for all myristic acid starches and is particularly low, <5% for samples produced on the Xplore. Palmitic acid starches, in particular those produced on the twin-screw extruder, yielded high RS contents with low SDS contents which is favourable for a functional food ingredient. As discussed earlier, RDS content remains quite unchanged and therefore the increase in RS content is a result of converting the SDS fraction to a more resistant form. The highest fraction of RS was achieved for 8% palmitic acid complexed starches (TSE 8P40A & TSE 8P80A) produced on the Leistritz with screw A.

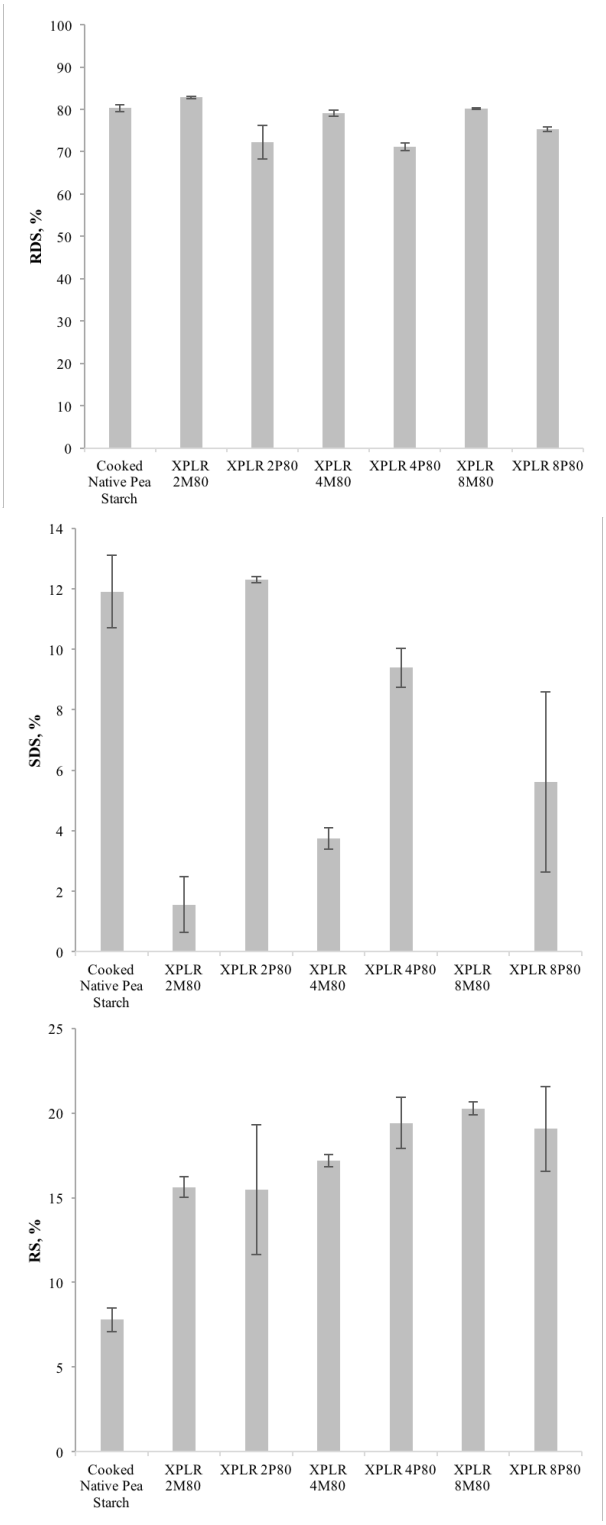

Figure 4.3.5. Nutritional fractions (RDS, SDS and RS content) with respect to lipid type of Xplore compounder extruded samples determined by Englyst's method. The data was standardized to a total starch basis.

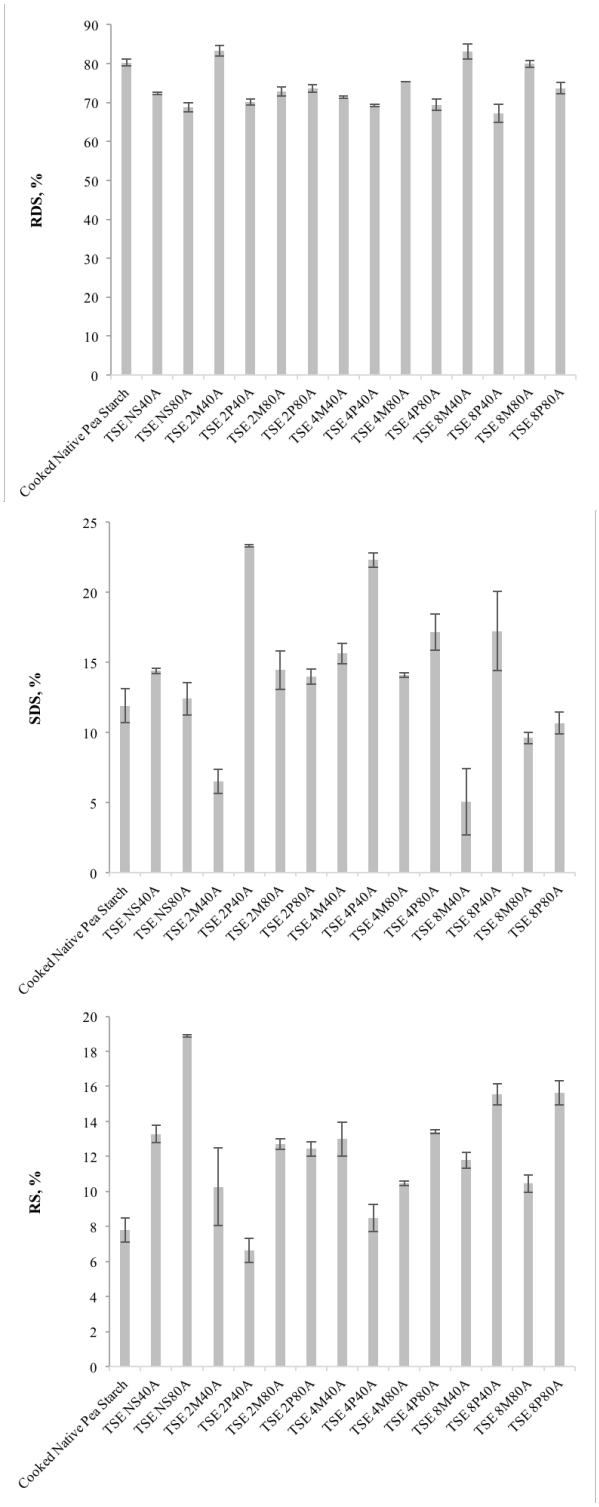

Figure 4.3.6. Nutritional fractions (RDS, SDS and RS content) with respect to lipid type of twin-screw extruded samples determined by Englyst's method. The data was standardized to a total starch basis.

4.3.4 Effect of Screw Design on In Vitro Digestibility

The effect of screw design on the digestion profiles of extruded lipid complexed samples are illustrated in Figure 4.3.7. As seen in Figure 4.3.7, SDS content decreased with Screw B while RS content increased for native, 2% and 8% lipid modified samples at both 40 and 80% moisture contents. According to the RTD parameters in Table 4.2.1, the residence time and mixing both increased with an increase in induced shear (i.e. screw B). A longer residence time led to increased moisture loss, accounting for higher SDS and RS content. Furthermore, increased lipid content provided added lubrication to the rigorous shear induced by Screw B. For screw B, it appears that the influence of mixing and shear is more important than the influence of feed moisture.

Future work including varied screw speed and/or feed rate would contribute to changes in RTD. For example, a lower feed rate would result in a lower degree of fill, longer residence time and higher moisture loss which would favour increased SDS and RS content formation. Increasing screw speed would decrease residence time and mixing. Fixed flow rates at high screw speeds lead to decreased channel fill providing freedom for particles to avalanche, this would minimize the forces that would push the particles against the barrel thus decreasing the extent of shear degradation.

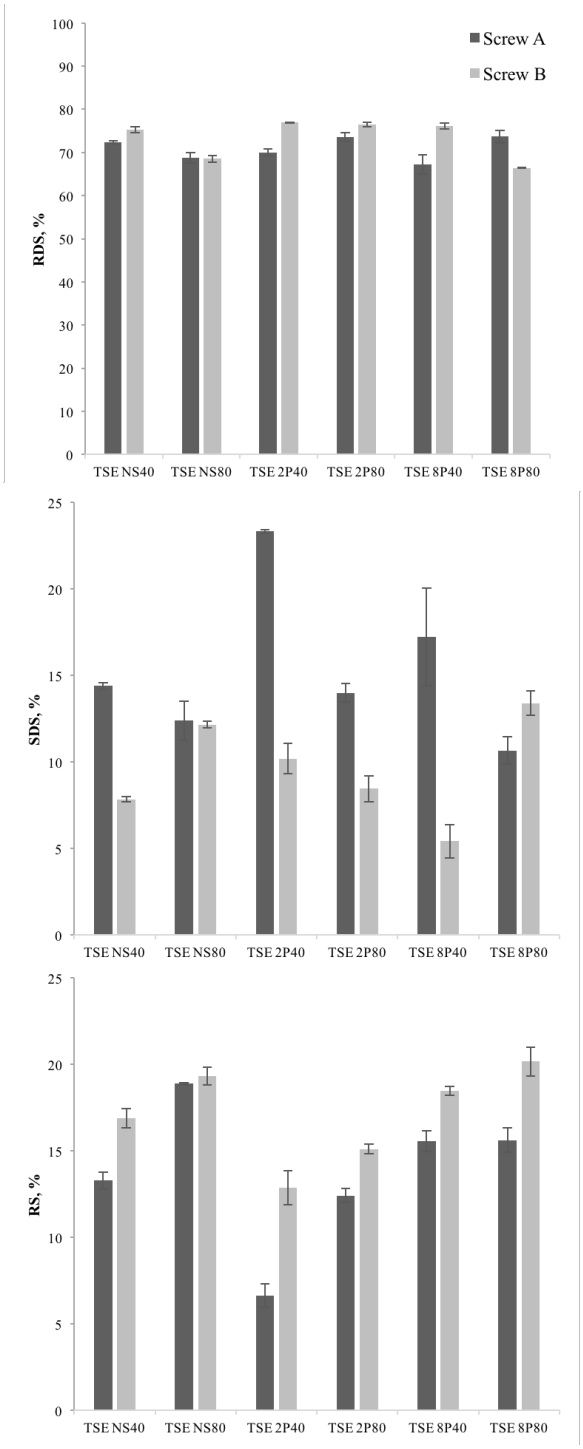

Figure 4.3.7. Nutritional fractions (RDS, SDS and RS content) with respect to screw design (A or B) of twin-screw extruded palmitic acid complexed with 40 and 80% moisture samples determined by Englyst's method. The data was standardized to a total starch basis.

4.4 RVA Pasting Profiles

The RVA pasting parameters and profiles of native and lipid complexed extruded pea starches are summarized in Tables 4.4.1-4.4.3. The phases of a typical RVA pasting curve correspond to starch granules initially absorbing water which causes swelling, followed by the disruption of granule structure under the shear force causing leaching and finally retrogradation of the molecules into a gel and/or semi-crystalline aggregate. Given that this present study does not investigate granules but instead gels, a similar explanation of pasting can be understood. Post processing, extrudates are dried and ground into fine starch powders which form pastes in the presence of water creating soft gels. The resulting gels gradually increase in viscosity providing greater mobility and loosening of the network-like structure with increasing temperature, which is later followed by re-ordering and re-association upon cooling. It can be observed that native starch extrudates (TSE NS40A, NS40B, NS80A & NS80B) showed much lower peak viscosity (PV), final viscosity (FV) and setback viscosity (SBV) as compared with a boiled (100°C, 15 min) native starch control, Cooked Native Pea Starch, in Table 4.4.1 and Figure 4.4.1. This is in agreement with the study conducted by Sharma et al. (2015). The reduced PV is attributed to partial degradation and gelatinization of starch which is due to depolymerization and molecular entanglement of starch during extrusion cooking (Hagenimana et al., 2006). Breakdown viscosity (BDV) is expected to decrease for the extruded native starches however in almost all cases, with the exception of TSE NS80A,

BDV increased. This is attributed to the increased ability to re-crystallize, from the low shear screw design (screw A) and high moisture content processing environment, in the stage called retrogradation which occurs upon cooling to gelatinized starch. Lower BDV and FV of extruded starches is attributed to lower degradation of starch fractions, molecular structure rupturing and reduction of re-crystallization tendency (Sharma et al., 2015).

Table 4.4.1. RVA parameters of native Nutri-Pea pea starch and extruded native Nutri-Pea pea starches. All samples measured in duplicate

Sample	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Pasting Temp (°C)
Native Pea Starch	982 ± 11	974 ± 11	8.5 ± 1	1741 ± 37	767 ± 26	12 ± 0	73 ± 1
TSE NS40A	986 ± 28	906 ± 27	80 ± 1	1101 ± 21	195 ± 6	10 ± 0	87 ± 0
TSE NS40B	601 ± 35	573 ± 32	28 ± 3	707 ± 31	135 ± 1	11 ± 0	-
TSE NS80A	613 ± 2	617 ± 5	-4 ± 3	819 ± 4	202 ± 1	15 ± 0	95 ± 0
TSE NS80B	855 ± 25	843 ± 32	13 ± 6	1031 ± 12	188 ± 20	12 ± 0	88 ± 6

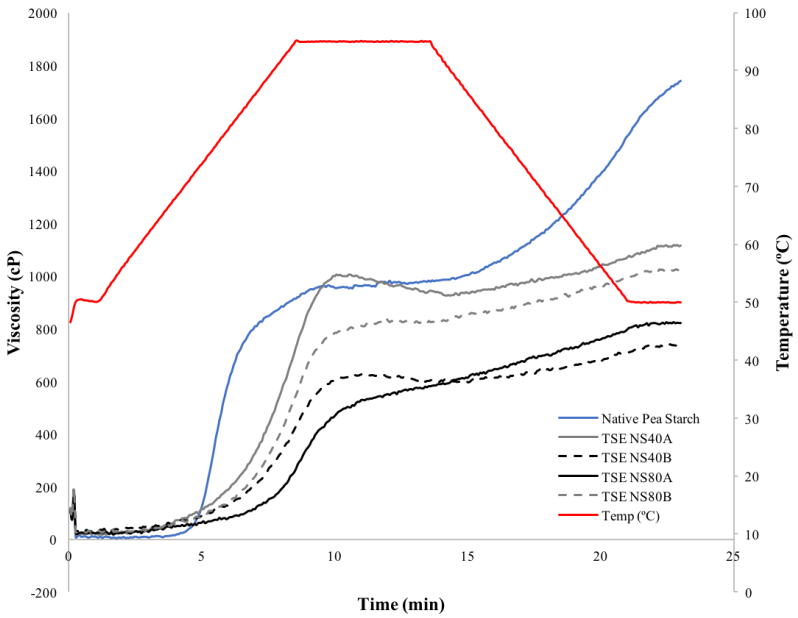

Figure 4.4.1. RVA pasting curve of native Nutri-Pea pea starch and extruded native Nutri-Pea pea starches

Changes in viscosity were investigated with respect to changes in feed moisture content. The RVA parameters and pasting profiles are summarized in Table 4.4.2 and Figure 4.4.2, respectively looking at 8% palmitic acid starches produced with 40, 60 or 80% moisture on the Leistritz twin-screw extruder using screws A and B. Samples produced with 40% moisture (TSE 8P40A and 8P40B) show increased PV and FV with increased extruder shear (i.e. Screw B). This was also demonstrated for the higher feed moisture content of 80%. Due to time constraints, 60% feed moisture content was only run on screw A. The pasting curve of TSE 8P60A is interesting as it is very similar to the curve of TSE 8P80B. Unfortunately, it cannot be confirmed whether this is an effect related to feed moisture or screw design and would require further investigation. Variations in PV can be attributed to differences in degree of complex formation as a result of feed moisture and screw design.

Table 4.4.2. Effect of feed moisture content and screw design on RVA parameters for 8% palmitic acid complexed Nutri-Pea pea starches. All samples measured in duplicate.

Sample	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Pasting Temp (°C)
TSE 8P40A	415 ± 5	236 ± 1	179 ± 6	255 ± 5	19 ± 6	10 ± 0	84 ± 1
TSE 8P60A	937 ± 12	542 ± 8	395 ± 4	656 ± 8	114 ± 0	11 ± 0	87 ± 0
TSE 8P80A	646 ± 2	323 ± 8	323 ± 6	323 ± 5	0 ± 3	11 ± 0	90 ± 1
TSE 8P40B	513 ± 1	291 ± 4	222 ± 3	304 ± 2	13 ± 2	11 ± 0	86 ± 3
TSE 8P80B	1067 ± 30	528 ± 21	540 ± 9	575 ± 21	48 ± 1	13 ± 0	92 ± 0

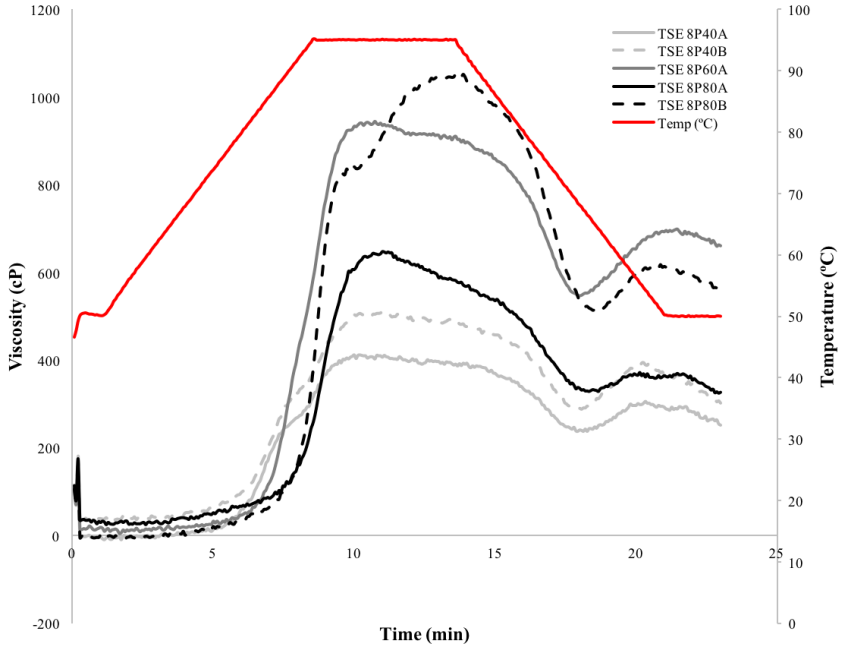

Figure 4.4.2. RVA pasting curve of 8% palmitic acid complexed Nutri-Pea pea starches with varied moisture content and screw design

Lastly, changes in viscosity were investigated with respect lipid type (myristic or palmitic acid) and content (2, 4 or 8%). The RVA parameters and pasting profiles are summarized in Table 4.4.3 and Figure 4.4.3, respectively. From Table 4.4.3 it is evident that increasing lipid content increases PV and FV. Increased PV has been attributed to the interaction between amylose and lipids as a result of amylose-lipid complex formation. It is very interesting to note that TSE NS80A has high PV and FV prior to lipid addition. During sample collection from the die, native starch samples had a very dough-like and sticky consistency in comparison to the lipid complexed samples which were more plasticized. D'Silva et al. (2011) and Wang et al. (2016) showed that the addition of lipids reduced retrogradation of starch during cooling and storage. This is attributed to the unavailability of amylose molecules in such cases to form junctions which are

responsible for short-term starch retrogradation. Oyeyinka et al. (2016) suggested that lipid addition restricts starch granule hydration and swelling. Low values of SBV indicate low tendency toward starch retrogradation, further suggesting lipid modification preventing the re-association of amylose chains during cooling and storage (Oyeyinka et al., 2016). Furthermore, similar to Liang et al. (2002) and Wang et al. (2016) who determined that some lipids increase PV over others, from Figure 4.4.3 it is evident that myristic acid complexed starches yield higher PVs over palmitic acid starches.

Table 4.4.3. Effect of lipid presence, type and content on RVA parameters for Nutri-Pea pea starches. All samples measured in duplicate.

Sample	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Pasting Temp (°C)
TSE NS80A	613 ± 2	617 ± 5	-4 ± 3	819 ± 4	202 ± 1	15 ± 0	95 ± 0
TSE 2M80A	526 ± 4	369 ± 9	158 ± 5	401 ± 0	33 ± 9	11 ± 0	95 ± 1
TSE 4M80A	912 ± 17	851 ± 23	62 ± 6	466 ± 19	-385 ± 4	11 ± 0	91 ± 1
TSE 8M80A	1525 ± 4	1393 ± 8	132 ± 12	1372 ± 27	-21 ± 35	11 ± 0	88 ± 1
TSE 2P80A	531 ± 12	312 ± 11	219 ± 23	331 ± 13	20 ± 2	12 ± 0	95 ± 0
TSE 4P80A	611 ± 29	341 ± 3	270 ± 26	369 ± 13	28 ± 11	11 ± 0	94 ± 0
TSE 8P80A	646 ± 2	323 ± 8	323 ± 6	323 ± 5	0 ± 3	11 ± 0	94 ± 1

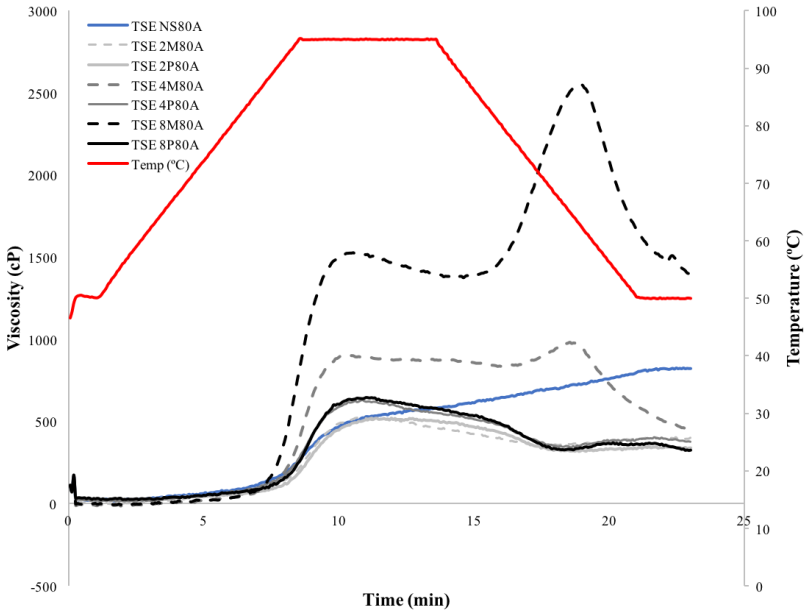

Figure 4.4.3. RVA pasting curve of native, myristic and palmitic acid lipid complexed Nutri-Pea pea starches

4.5 Alternative Applications

Due to the high lipid content of the modified starches and their ability to manufacture not only thin films but also thick films with different levels of flexibility and strength, alternative applications were considered beyond use as a functional food ingredient. As exemplified through contact angle measurements, these films can be very hydrophobic depending on the lipid level and manufacturing process. Since the material is 100% food grade and very sensitive to changes in moisture, an alternative application could be a biodegradable packaging. Preliminary experiments were conducted to test the puncturability of the films using the ASTM F1306 standard. This experiment provides valuable evidence towards using this lipid complexed pea starch material to create a biodegradable coffee cup lid or pod. In anticipation of experimenting with this

idea, one run of 16% palmitic acid modified starch was produced on the twin-screw extruder with 80% moisture on screw B. This lipid level, as well as 4% palmitic acid and native extruded starches were used to produce films for slow rate penetration. All materials were tested in quintuplicate in their pure state as well as with 1% added glycerol, which was added to increase shelf-life, act a filler and provide increased flexibility. Slow rate penetration results can be seen in Table 4.5.1 whereas force traces can be found in Figure 4.5.1 for two repetitions of each factor level.

Table 4.5.1. Force at break per film thickness and probe displacement at penetration for extruded native and lipid complexed (4% and 16% palmitic acid) starch films as well as films produced with additional 1% glycerol content.

Sample	Force at Break per Film Thickness (N/mm)	Displacement at Penetration (mm)
NS80B - Pure	67.6 ± 22.6	1.83 ± 0.75
	57.6 ± 22.6	2.37 ± 0.75
4P80B - Pure	50.7 ± 1.62	1.46 ± 0.44
	53 ± 1.62	0.83 ± 0.44
16P80B - Pure	21.8 ± 7.90	1.08 ± 0.21
	20.9 ± 7.90	1.13 ± 0.21
NS80B - Glycerol	31.4 ± 8.22	0.96 ± 0.29
	37.6 ± 8.22	1.12 ± 0.29
4P80B - Glycerol	19.1 ± 0.97	0.96 ± 0.06
	17.7 ± 0.97	1.12 ± 0.06
16P80B - Glycerol	13.7 ± 0.80	0.42 ± 0.09
	12.5 ± 0.80	0.54 ± 0.09

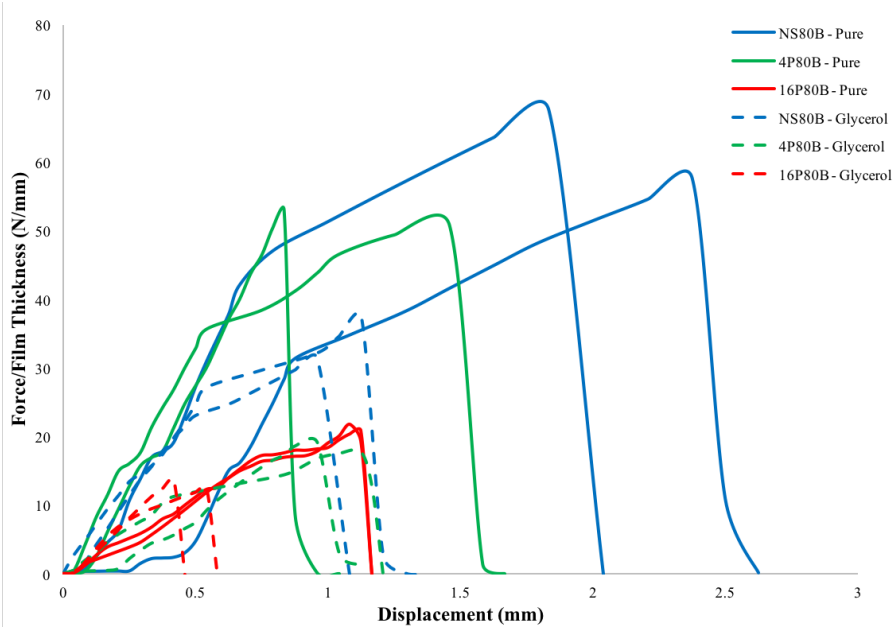

Figure 4.5.1. Graphical output of slow rate penetration for extruded native and lipid complexed (4% and 16% palmitic acid) starch films as well as films produced with additional 1% glycerol content.

From Figure 4.5.1 it is evident that increasing lipid content decreases the force required to puncture films. Furthermore, the addition of glycerol decreases the force required to puncture the film when compared to a corresponding sample formulation without glycerol. From Table 4.5.1 it is observed that the probe displacement at penetration decreases for samples with glycerol. Variation in repetitions is attributed to film inconsistencies after conditioning. Films were placed in a humidity chamber with a weighted metal plate over top to ensure that films dried flat. Despite this, films still curled on the edges making it difficult to place them in the clamping fixture, hence the increased quantity of repetitions. Films with glycerol added did not experience as much curling. As lipid content increased, especially at 16%, conditioning resulted in very dry, brittle and slightly

fragile films. However, the extruded native starch films dried to a glassy and rigid state. This physical attribute is noted in the force required to puncture films presented in Table 4.5.1 with an increase in puncture force for native starch films.

Additional investigation of heat deflection temperature, swelling/water solubility and biodegradability would be relevant to this work. Current commercialized coffee pods are recyclable; however, they still require intensive end-use processing and lack biodegradation. This proposed material is completely natural and food grade thus is not only safe for holding ingestible materials but also has a shelf-life by which when exposed to moisture and other organic mass (i.e. compost) will promote breakdown. These preliminary results indicate the potential suitability of this lipid complexed pea starch to be used for applications other than as a functional food ingredient, especially at high lipid contents.

CHAPTER 5 CONCLUSIONS

5.1 Conclusions

This thesis explored the potential to produce gelatinized lipid complexed pea starches with increased RS and/or SDS starch fractions using a rapid and continuous reactive extrusion process. Bulk lipid complexing of Nutri-Pea pea starch was investigated using a benchtop DSM-Xplore twin-screw compounder and Leistritz co-rotating intermeshing twin-screw extruder. The effects of feed formulations and extrusion conditions, in particular screw design, on lipid complexing and Englyst digestion profiles were examined intensively. As compared to boiled native starch control (SDS: 11.9 ± 1.2, RS: 7.8 ± 0.7), extruded native starch prepared with 80% moisture on a high shear screw design (SDS: 12.2 ± 0.19, RS: 19.3 ± 0.52) saw a significant increase in RS content. Increasing lipid content in palmitic acid complexed starches from 2% (RS: 6.63 ± 0.69) to 8% (RS: 15.6 ± 0.59) also provided significant improvements of RS fractions when processed with a low shear screw and 40% moisture. Myristic acid complexed starches favoured RS formation while palmitic acid complexed starches favoured SDS formation at low moisture contents. Formation of starch lipid complexes was highly dependent on feed moisture content, which consequently affects starch gelatinization, induced shear and lipid content. Fatty acid titrations were conducted to determine lipid conversion and degree of substitution of samples collected at the die. To gain a better understanding of the reaction within the extruder, samples were collected along the length of the screw

to trace conversion and degree of substitution. It was determined that all formulations could achieve ≥85% conversion by the die for two screw designs. Furthermore, it was discovered that there is an optimum concentration range for fatty acids to form complexes with starch between 8% and 16%, however the exact point of saturation requires further investigation.

Lipid presence was confirmed using characterization methods such as FTIR, DSC and contact angle measurements. As lipid content increased, hydrophobicity of starch films increased during sessile drop measurements. Moreover, it was determined that sample viscosity increased with increased processing shear or lipid content, in RVA measurements.

A novel material to produce biodegradable films for industrial applications was introduced. The material was produced at 80% moisture content for extruded native and two concentrations of lipid complexed starch using an intensive screw design. Preliminary results showed that increasing lipid content and adding 1% glycerol to samples decreased the force per film thickness required to puncture films. However further investigation is required to determine effect of heat and moisture deformation.

5.2 Recommendations

The effectiveness of using rapid bulk lipid complexing by twin-screw extrusion process to increase enzyme resistance of pea starches was relatively small in comparison to studies conducted on other types of starches. This could be

associated with the complex counteractive influences of extrusion cooking and lipid complexing. Investigation into use of alternative lipids, other commercialized pea starches with different amylose content, heat treatment (post or pre-extrusion), use of a heat stable de-branching enzyme (i.e. pullulanase) within the extruder or alternative lipid addition methods would be interesting to look at with respect to effects on decreasing digestibility. Another suggestion would be to employ additional characterization methods such as XRD, SEM, swelling power, freeze-thaw stability and alternative *in-vitro* methods of digestion such as the Prosky method (AOAC 991.43) to better reveal and understand the relationship between the structure and functional properties of extruded lipid complexed pea starches. Lastly, improved RTD measurements by which tracers are injected along the length of the extruder, as opposed to only in the feed zone, would provide a greater understanding of the effect of screw design and corresponding reaction time in each zone.

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APPENDICES

A.1 Digestion profiles of native and lipid complexed starch prepared in the Xplore compounder, twin-screw extruder and aqueous slurry

Sample	Feed Moisture (wt%)	Lipid	Lipid Content (wt%)	Complexing Conditions	Digestion Profile[a] (Englyst Method)		
					RDS (%)	SDS (%)	RS (%)
Raw Pea Starch	N/A	N/A	N/A	N/A	52.49 ± 0.39	28.84 ± 0.21	**18.67 ± 0.60**
Cooked Native Pea Starch [b]	N/A	N/A	N/A	N/A	80.30 ± 0.80	11.90 ± 1.20	**7.80 ± 0.70**
TSE NS40A	40	N/A	0	3.00kg/h, 90ºC, 250 RPM, screw A	72.33 ± 0.32	14.39 ± 0.18	**13.28 ± 0.50**
TSE NS40B	40	N/A	0	3.00kg/h, 90ºC, 250 RPM, screw B	75.27 ± 0.71	7.85 ± 0.15	**16.88 ± 0.56**
TSE NS80A	80	N/A	0	3.00kg/h, 90ºC, 250 RPM, screw A	68.73 ± 1.19	12.40 ± 1.13	**18.88 ± 0.06**
TSE NS80B	80	N/A	0	3.00kg/h, 90ºC, 250 RPM, screw B	68.52 ± 0.71	12.16 ± 0.19	**19.31 ± 0.52**
XPLR 2P80 [c]	80	Palmitic	2	90ºC, 110 RPM, 5 min	72.24 ± 3.95	12.30 ± 0.11	**15.46 ± 3.84**
XPLR 4P80 [c]	80	Palmitic	4	90ºC, 110 RPM, 5 min	71.20 ± 0.86	9.39 ± 0.64	**19.41 ± 1.51**
XPLR 4P80 - 3 [cd]	80	Palmitic	4	90ºC, 110 RPM, 3 min	75.57 ± 1.61	10.07 ± 1.02	**14.37 ± 2.63**
XPLR 8P80 [c]	80	Palmitic	8	90ºC, 110 RPM, 5 min	75.32 ± 0.49	5.61 ± 2.98	**19.07 ± 2.49**
XPLR 2M80 [c]	80	Myristic	2	90ºC, 110 RPM, 5 min	82.82 ± 0.32	1.55 ± 0.92	**15.63 ± 0.60**
XPLR 4M80 [c]	80	Myristic	4	90ºC, 110 RPM, 5 min	79.07 ± 0.71	3.74 ± 0.35	**17.19 ± 0.36**
XPLR 4M80 - 3 [cd]	80	Myristic	4	90ºC, 110 RPM, 3 min	75.14 ± 1.65	2.30 ± 0.32	**22.56 ± 1.97**
XPLR 8M80 [c]	80	Myristic	8	90ºC, 110 RPM, 5 min	80.18 ± 0.14	-0.45 ± 0.23	**20.27 ± 0.37**
TSE 2P40A	40	Palmitic	2	3.00kg/h, 90ºC, 250 RPM, screw A	70.05 ± 0.79	23.32 ± 0.10	**6.63 ± 0.69**
TSE 4P40A	40	Palmitic	4	3.00kg/h, 90ºC, 250 RPM, screw A	69.20 ± 0.26	22.31 ± 0.52	**8.49 ± 0.78**
TSE 8P40A	40	Palmitic	8	3.00kg/h, 90ºC, 250 RPM, screw A	67.23 ± 2.25	17.23 ± 2.84	**15.55 ± 0.59**
TSE 2M40A	40	Myristic	2	3.00kg/h, 90ºC, 250 RPM, screw A	83.25 ± 1.34	6.48 ± 0.87	**10.26 ± 2.21**
TSE 4M40A	40	Myristic	4	3.00kg/h, 90ºC, 250 RPM, screw A	71.80 ± 0.25	15.64 ± 0.73	**12.98 ± 0.98**
TSE 8M40A	40	Myristic	8	3.00kg/h, 90ºC, 250 RPM, screw A	83.17 ± 1.93	5.04 ± 2.39	**11.78 ± 0.46**
TSE 2P40B	40	Palmitic	2	3.00kg/h, 90ºC, 250 RPM, screw B	75.90 ± 0.61	11.91 ± 0.41	**12.19 ± 0.20**
TSE 8P40B	40	Palmitic	8	3.00kg/h, 90ºC, 250 RPM, screw B	76.11 ± 0.72	5.42 ± 0.98	**18.47 ± 0.26**
TSE 2P80A	80	Palmitic	2	3.00kg/h, 90ºC, 250 RPM, screw A	73.58 ± 0.95	13.40 ± 0.55	**12.42 ± 0.41**
TSE 4P80A	80	Palmitic	4	3.00kg/h, 90ºC, 250 RPM, screw A	69.44 ± 1.39	17.15 ± 1.28	**13.41 ± 0.11**
TSE 8P80A	80	Palmitic	8	3.00kg/h, 90ºC, 250 RPM, screw A	73.71 ± 1.48	10.67 ± 0.80	**15.62 ± 0.69**
TSE 2M80A	80	Myristic	2	3.00kg/h, 90ºC, 250 RPM, screw A	72.84 ± 1.09	14.44 ± 1.39	**12.72 ± 0.30**
TSE 4M80A	80	Myristic	4	3.00kg/h, 90ºC, 250 RPM, screw A	75.44 ± 0.02	14.09 ± 0.14	**10.47 ± 0.12**
TSE 8M80A	80	Myristic	8	3.00kg/h, 90ºC, 250 RPM, screw A	79.94 ± 0.90	9.60 ± 0.41	**10.45 ± 0.49**
TSE 2P80B	80	Palmitic	2	3.00kg/h, 90ºC, 250 RPM, screw B	76.46 ± 0.47	8.45 ± 0.74	**15.10 ± 0.27**
TSE 8P80B	80	Palmitic	8	3.00kg/h, 90ºC, 250 RPM, screw B	66.43 ± 0.13	13.39 ± 0.70	**20.17 ± 0.83**

[a] Results are means of duplicates ± standard deviation; [b] Starch boiled in aqueous slurry at 100°C for 15 min; [c] Prepared using the Xplore DSM twin-screw compounder; [d] 3 min cook time in Xplore