REACTIVE EXTRUSION OF PHOSPHATE CROSS-LINKED RESISTANT PEA STARCHES

By GANG HUO, B.ENG., M.ENG

A Thesis Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree

Master of Applied Science

MASTER OF APPLIED SCIENCE (2016) **McMaster University**

(Chemical Engineering) **Hamilton, Ontario**

TITLE: **Reactive Extrusion of Phosphate Cross-linked Resistant Pea Starches**

AUTHOR: Gang Huo

B.Eng. (Beihang University)

M.Eng. (Beihang University)

SUPERVISOR: Dr. Michael R. Thompson

NUMBER OF PAGES: xviii, 99

LAY ABSTRACT

The Canadian food industry is increasingly interested in the potential to probe new avenues to produce enzyme-resistant food starches from pulses starches. Although extrusion cooking is commonly used for manufacturing cereals, snacks and other food products, no research has been reported on using an extruder to rapidly produce resistant pulse starches for functional food ingredients. This study aimed to develop an effective reactive extrusion process to produce phosphate cross-linked pea starches with enhanced enzyme resistance (i.e., increased slowly digestible starch (SDS) and resistant starch (RS) content) based on an examination of the effects of reaction conditions on the properties of extrusion products. Two types of commercially available pea starches, NutriPea and Meelunie, were chosen as subjects of the research. The cross-linked pea starches under optimized conditions achieved a significant but moderate increase either in RS content (for NutriPea) or in SDS content (for Meelunie) compared to their native counterparts. However, RS and SDS content could not be improved simultaneously at least based on these pea starches and the reactive extrusion processes in this study.

ABSTRACT

The primary objectives of this study were to develop an effective reactive extrusion process to produce granular phosphorylated pea starches with enhanced enzyme resistance, and examine the effects of bulk phosphorylation conditions on the morphology, physicochemical and functional properties of extruded pea starch phosphates. Two types of commercially available pea starches (NutriPea and Meelunie) were chosen as the research subjects in this study with differing native resistance. A number of methods including optical microscopy, SEM, ICP-OES, Englyst method, DSC and rapid visco analysis (RVA) were used to characterize the morphology and properties of extruded pea starches.

The effects of feed formulations and extrusion conditions on phosphorus incorporation and Englyst digestion profiles were examined systemically. The results showed that phosphorus content and digestion profiles were highly dependent on the feed moisture. Enzyme resistance did not positively correlate with phosphorus content for extruded pea starch phosphates in contrast to their counterparts prepared by conventional aqueous slurry. This was because extrusion processing can markedly increase the susceptibility of pea starch granules to enzymatic digestion. Lower feed moisture content (40%) gave lower phosphorus content, significantly lower RDS content, and higher SDS and/or RS content. Bulk phosphorylation in the extruder resulted in decreased RS2 content but increased RS4 content. Screw geometry with excessive mixing index was not desirable in terms of producing resistant starch. High screw speeds (150rpm and 200rpm) and low feed rate (1.02kg/h) brought about higher yields of SDS and RS in spite of lower phosphorus incorporation.

Reactive extrusion of pea starches under optimized conditions achieved a significant but moderate increase either in RS content (from 18.67% to 22.57% for NutriPea) or in SDS content (from 37.18% to 42.23% for Meelunie) compared to their native counterparts. However, RS and SDS content could not be improved simultaneously at least based on these pea starches and the reactive extrusion processes in this study. The optical and SEM micrographs confirmed that the granule integrity was largely retained after optimized reactive extrusion process. DSC thermograms found no significant correlation between gelatinization characteristics and Englyst digestion profiles. Evidenced by RVA pasting profiles, NutriPea pea starch phosphates exhibited enhanced thermal and shear stability in comparison to their native counterpart.

A novel foaming injection technology of cross-linking reagents solution was pioneeringly introduced to uniformly coat all starch particles at the lowest moisture level possible during the continuous production of granular NutriPea pea starch phosphates. Yet, the resulting phosphorus incorporation was much lower than expected and would require further studies.

ACKNOWLEDGEMENTS

First of all, I would like to express my most sincere gratitude to my supervisor, Dr. Michael Thompson, for his continuous guidance, patience and encouragement during my Master's studies at McMaster University.

I would also like to express my appreciation to Pulse Science Cluster Program under Agriculture and Agri-Food Canada for their generous funding to my research. I am particularly grateful to Dr. Liu Qiang, our project leader, and Ms. Elizabeth Donner for their valuable help on in vitro digestibility analysis and RVA.

I would like to thank Dr. Shiping Zhu for giving me the permission to use their high precision electronic balances; Dr. Robert Pelton for allowing me to use the Milli-Q water in the interfacial engineering lab; Ms. Elizabeth Takacs, lab manager of MMRI, for her training on DSC; Mr. Douglas Keller for his safety advice on handling strong acids, Mr. Paul Gatt for his technical support in troubleshooting of the twin-screw extruder; Dr. Xiaogang Li in Materials Science and Engineering Department for his training and help on ICP-OES; Mr. Christopher Butcher from CCEM for his training on SEM and Zeiss Axioplan2 microscope, and Ms. Kristina Trollip, Ms. Michelle Whalen, and Ms. Linda Ellis for their administrative assistance. Also, I would like to thank Yang Liu and Ali Goger for helping me run extrusion experiments.

It has been my great pleasure to work with so many lovely friends at McMaster: Samuel Grimshaw, Wing Hei Lam, Zeinab Mousavi, Daniel LeClair,

Trevor West, Wensen Xu, Shuiliang Wu, Huiying, Li, Dong Yang, Qiang Fu, Yuanhua Li, Xiaofei Dong, Darko Ljubic, Meng Li, Lei Lei, He Zhu, Qiang Ren, Farhad Zafari and others for their friendship and support during my study.

Finally, I would like to thank my beloved wife and daughter for their eternal love and support throughout all the ups and downs in my life. I wish to dedicate this thesis to them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AACC	American Association of Cereal Chemists
AFM	Atomic Force Microscopy
AM	Amylose
ANN	Annealing
AOAC	Association of Official Analytical Chemists
AP	Amylopectin
BDV	Breakdown Viscosity
CFR	Code of Federal Regulation
CI	Confidence Interval
CL	Cross-linking
DS	Degree of Substitution
DSC	Differential Scanning Calorimetry
DSMP	Distarch Monophosphate
EDXRF	Energy Dispersive X-ray Fluorescence Spectrometry
EPI	Epichlorohydrin
FTIR	Fourier Transform Infrared
FV	Final Viscosity
ΔH	Enthalpy of Gelatinization
HMT	Heat-Moisture Treatment
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
MSMP	Monostarch Monophosphate
NMR	Nuclear Magnetic Resonance

PV	Peak Viscosity
RDS	Rapidly Digestible Starch
RO^-	Starch Alcoholate Ion
RS	Resistant Starch
RTD	Residence Time Distribution
RVA	Rapid Visco Analysis
SAXS	Small Angle X-ray Scattering
SBV	Setback Viscosity
SCFA	Short Chain Fatty Acids
SDS	Slowly Digestible Starch
SEM	Scanning Electron Microscopy
SSE	Single Screw Extruder
STMP	Sodium Trimetaphosphate
STPP	Sodium Tripolyphosphate
TSE	Twin Screw Extruder
T_c	Closure Temperature of Gelatinization
T_o	Onset Temperature of Gelatinization
T_p	Peak Temperature of Gelatinization
TDF	Total Dietary Fiber
TEM	Transmission Electron Microscopy
WAXS	Wide Angle X-ray Scattering
XRD	X-ray Diffraction

CHAPTER 1 INTRODUCTION

1.1 Background

Canada is the second largest producer of pulses in the world, being the largest producer of peas and the second greatest producer of lentil. The Canadian pulse industry has grown rapidly in recent years and is now valued at over one billion dollars per year. However, most pulses starches are mainly available as by-products of protein extraction and used in industrial applications instead of food applications. Over the past decades there has been considerably growing research interest in resistant starch (RS) due to its unique physiological and health benefits, distinguished by the production of short chain fatty acids (SCFA) after fermentation in the large intestine (Dupuis et al, 2014; Englyst et al., 1992; Erlingen et al., 1993; Nugent, 2005; Thompson, 2000). Hence, the Canadian food industry is increasingly interested in the potential to explore possible avenues for enhancing the functional and nutritional properties, especially the enzyme-resistance, of pulses starches to incorporate them into food products (Hoover et al., 2010). This project is part of a large national research program in Canada, Pulse Science Cluster 2 , which is funded by Agriculture and Agri-Food Canada.

Extensive research on cereal, potato and cassava starches has made them readily available for food and non-food applications. However, a literature survey (Hoover et al., 2010) has shown that with the exception of a few starches, 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 intensive research on the composition, structure, physicochemical properties and modifications of pulse starches, mostly from Canadian grown pulses, such as pea, chickpea, lentil and beans. Both physical (hydrothermal) and solution chemical methods were used to modify the pulse starches by these two authors. Recently, some Chinese and African researchers' studies on the in-vitro digestibility of phosphate cross-linked pulse starches revealed that cross-linking modification in aqueous slurry significantly increased RS content in pulse starches (Sankon et al., 2013; Shi et al., 2013).

A 99:1(w/w) mixture of sodium trimetaphosphate (STMP)/sodium tripolyphosphate (STPP) with sodium sulphate (Na_2SO_4) and base (sodium hydroxide, NaOH) have been the most popular phosphorylating reagents for preparing food-grade cross-linked starch. This is because the federally regulated maximum permissible level of residual phosphorus (0.4%) in starch food ingredients is 10 times greater when using the combination of mostly STMP and minimal STPP compared with using STMP alone (CFR, 2001). Processing as an aqueous slurry has been the most common approach to preparing food-grade phosphate cross-linked RS4 resistant starches (Woo and Seib, 2002). Greatly enhanced RS4 (AOAC dietary fiber) content is often reported in un-gelatinized starches. Comparatively, few studies have studied the granular state reaction in an extruder and mainly for biodegradable polymers, pharmaceutical or industrial applications (Murua-Pagola et al., 2009; Nabeshima &

Grossmann, 2001; O'Brien et al., 2009; Seker & Hanna, 2003a, 2003b, 2004, 2005, 2006). A small number of studies in the extruder have been reported with food applications in mind though not for the preparation of resistant pulse starches (Hasjim and Jane, 2009; Huth et al., 2000; Kim et al., 1999; Landerito & Wang, 2005; Sarawong et al., 2014). Until now, no research has been reported on using twin-screw extrusion to increase the enzyme resistance of pulse starches by rapid granular state phosphorylation for functional food applications.

For the production of phosphate cross-linked resistant food starch, the desired end-product from the extruder should be granular starch. Therefore, the pulse starch granules are expected to largely remain intact after the rapid cross-linking reaction in the extruder by optimizing the feed formulations (e.g., feed moisture, STMP/STPP and Na_2SO_4 levels) and extrusion conditions (e.g., screw design, screw speed, feed rate, etc.). This thesis will focus on the reactive extrusion of phosphate cross-linked resistant pulse starches for functional food applications. A number of methods including ICP-OES, in vitro digestibility (Englyst method), light microscopy, SEM, DSC, residence time distribution (RTD), and rapid visco analysis (RVA) were used to characterize the reactive extrusion process as well as the morphology and properties of extruded pea starch phosphates.

1.2 Research objectives

The main objective of this thesis was to develop an effective reactive extrusion process to produce granular phosphorylated pulse starches with enhanced SDS and/or

RS content as functional food ingredients. Subsequently, the effects of varied bulk phosphorylation conditions on morphology, physicochemical (e.g., phosphorus content) and functional properties (e.g., SDS and RS content) of extruded pulse starch phosphates were intensively examined. Two types of commercially available pea starches (NutriPea and Meelunie) were chosen as the research subjects in this study. The research included the following major tasks:

- To develop an effective reactive twin-screw extrusion process to rapidly produce granular phosphorylated cross-linked pea starches with enhanced enzyme resistance.
- Investigating and illuminating the effects of feed formulations and extrusion variables on the morphology and properties (phosphorus contents, Englyst digestion profiles, gelatinization characteristics, and pasting properties) of extruded pea starch phosphates.

1.3 Thesis outline

This thesis consisted of five chapters. Chapter 1 is a brief introduction about the research background and objectives. Chapter 2 is a pertinent literature review which presents the recent advances in studies on morphology & structure, properties, characterization, preparation and food applications of cross-linked resistant pulse starches as well as relevant reactive extrusion processes. Chapter 3 describes the materials, the preparation and characterization methods of various pea starch phosphates. Chapter 4 reports the morphology and properties of pea starch phosphates prepared by various methods, and systematically elucidates the effects of feed

compositions and extrusion parameters on the reactive process. The experimental results are discussed in details in this chapter. The final chapter summarizes the conclusions on the reactive extrusion of phosphorylated resistant pea starches research and presents some recommendations for future research work.

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 edible seeds of plants belonging to the Leguminosae family which has 16,000–19,000 species including the commonly known species like pea, chickpea, lentil, and various beans. Starch is the main storage carbohydrate (22–45%) in pulses (Chung, Liu, & Hoover, 2010; Hoover & Sosulski, 1991; Hoover et al., 2010). Canada is the second largest producer of pulses in the world, being the greatest producer of peas and the second largest producer of lentil. The Canadian pulse industry has grown rapidly in recent years and is now valued at over one billion dollars per year. There are tremendous opportunities to broaden value-added activities in Canadian pulses to high-end processing (Hoover et al., 2010). In the past decades resistant starch (RS) has attracted considerable attention due to the associated physiological and health benefits of appropriate RS intake, resulting from the production of short chain fatty acids (SCFA) after fermentation in the colon (Dupuis et al, 2014; Englyst et al., 1992; Erlingen et al., 1993; Nugent, 2005; Thompson, 2000). Currently, the Canadian food industry is increasingly concerned about the potential to explore more effective approaches to augment the functional and nutritional properties (e.g., enzyme resistance) of pulses starches to incorporate them into food products (Hoover et al., 2010).

Extensive research on cereal, potato and cassava starches has made them

readily available for food and non-food applications. However, a literature survey (Hoover et al., 2010) has shown that with the exception of a few starches, there is still insufficient information on the structure and physicochemical properties of many pulse starches. As shown in Figure 2.1, most pulse starches have oval granules with sizes ranging from 5 to 85 microns, although spherical, round, elliptical and irregularly shaped granules are also found (Hoover et al., 2010; Maaran et al., 2014; Perez & Bertoft, 2010). As indicated by (a-e) in Figure 2.1, intact native bean starch granules display birefringence as maltese cross under microscope with polarized illumination. Granule surfaces of pulse starches are generally smooth with no evidence of fissures or pin holes (Chung et al., 2008; Hoover & Manuel, 1995; Hoover & Ratnayake, 2002; Singh et al., 2004). Granules of wrinkled pea starch have been naturally shown to be extensively damaged resulting in splitting and exposure of the internal layering (Bertoft et al. 1993; Zhou et al., 2004).

The two major macromolecular components of starch are amylose (AM) (a linear polysaccharide) and amylopectin (AP) (a branched polysaccharide). The ratio between amylose and amylopectin varies depending on the starch source. Normal starches contain about 20-30% amylose and 70-80% amylopectin. Waxy starches have approximately 0–5% amylose, whereas high amylose starches contain 35–70% amylose (Hoover et al., 2010; Maaran et al., 2014; Perez & Bertoft, 2010). The amylose content in pulse starches is 35-65%, which is higher than that in normal potato (21-25%) and cereal starches (20-35%) (Belitz et al., 2009; BeMiller & Whistler, 2009).

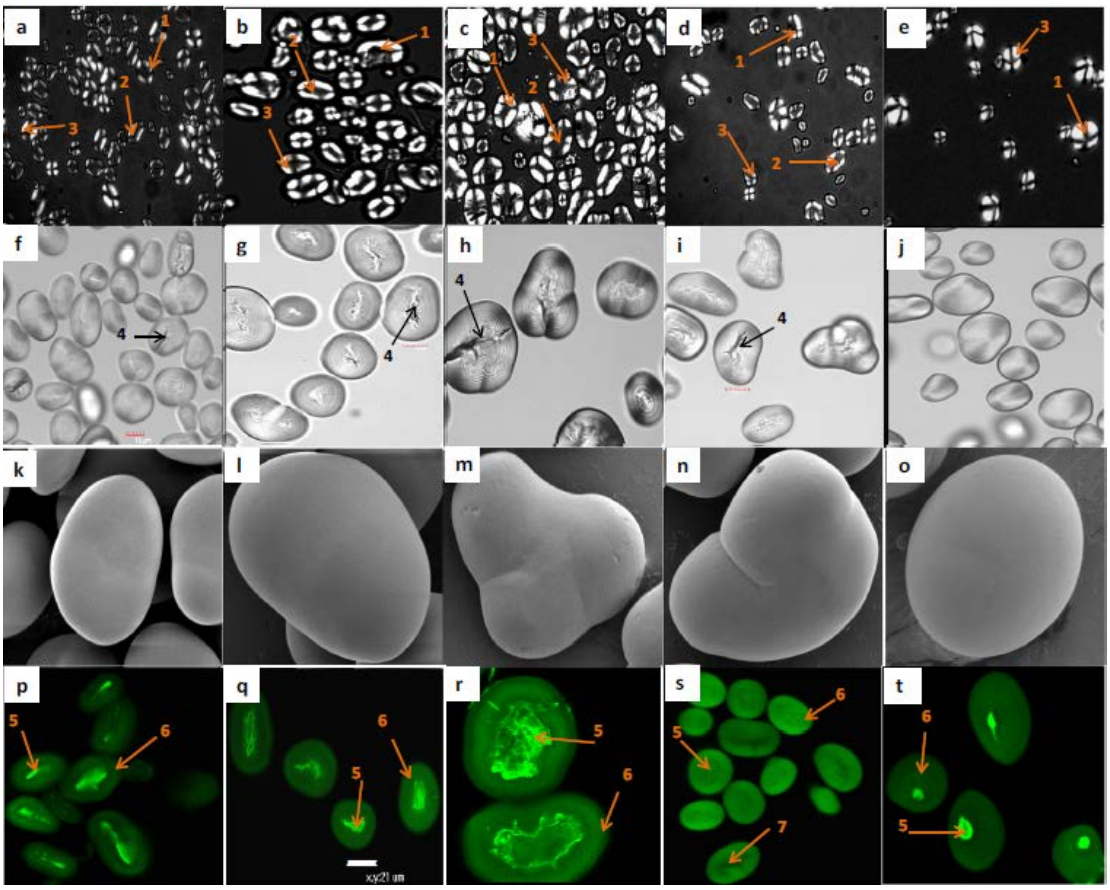

Figure 2.1 (a–e) – Polarized light microscopy (x200) of lablab bean (a), navy bean (b), rice bean (c), tepary bean (d), and velvet bean (e). Arrows 1–3 represent maltese cross (intensities and patterns), voids and quadrants, respectively. (f–j) – Bright field microscopy (x400) of lablab bean (f), navy bean (g), rice bean (h), tepary bean (i), velvet bean (j). Arrow 4 indicates different types of cracked granules. (k–o) – Scanning electron microscopy (x10,000) of lablab bean (k), navy bean (l), rice bean (m), tepary bean (n), velvet bean (o). (p–t) – Confocal laser scanning microscopy (x600) of lablab bean (p), navy bean (q), rice bean (r), tepary bean (s), velvet bean (t). Arrows 5 and 6 represent the intensity of APTS fluorescence in the hilum and peripheral regions, respectively. The dark central cracked region of tepary bean is indicated by arrow 7 (s) (Maaran et al., 2014).

The structures of linear amylose and branched amylopectin are illustrated in Figure 2.2. Amylose molecules are the predominantly α(1-4) linked α-glucan and tend to form hydrogen-bonded structure, whereas amylopectin molecules are α(1-4)-linked α-glucan joined by α(1-6)-linkage (5-6%), forming a highly branched structure (BeMiller & Whistler, 2009; Martin & Smith, 1995). Amylopectin chains are further

classified in to three types: A-chains do not carry other chains through C-6 linkage; B-chains carry other A and/or B-chains; One single C-chain carries the only reducing end-group of each macromolecule (Bertoft, 2004). There is inadequate information on the fine structure of amylose and amylopectin of pulse starches. So it is hard to make any sound comparison with the counterparts of cereal and tuber starches.

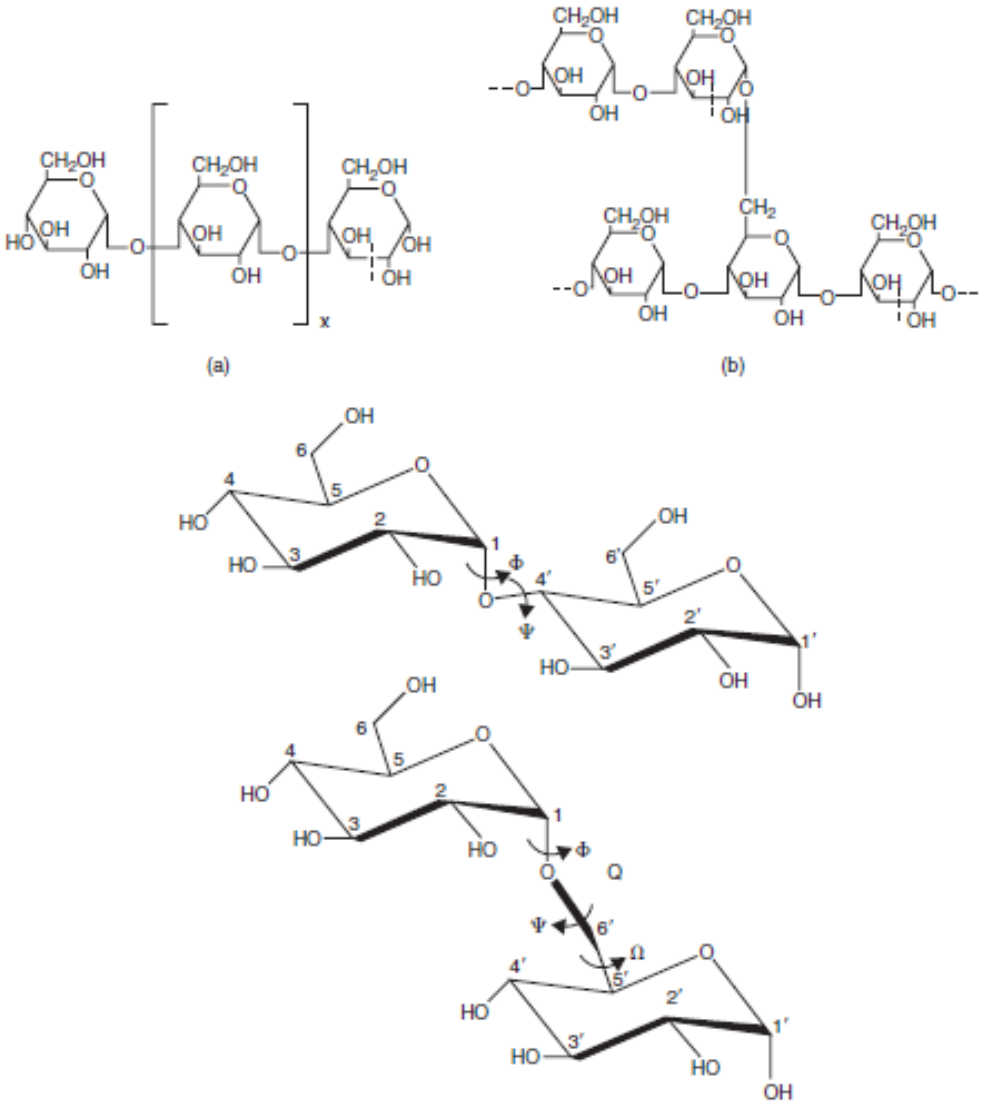

Figure 2.2 The structures of linear amylose (a) and branched amylopectin (b) (BeMiller & Whistler, 2009)

2.1.2 Crystallinity and polymorphism of pulse starches

Like many synthetic polymers, starch granules are far from being perfect

crystals. Starch is biosynthesized as semi-crystalline granules with different polymorphic forms and levels of crystallinity. For amylopectin-rich starches it is generally understood that the origin of crystallinity is credited to the intertwining of the outer chains of amylopectin in the form of double helices which join together to form crystalline lamellae (French, 1972). Based on the model postulated by French (1984) about starch structure, it is commonly believed that starch granules are composed of concentric alternating 120-400nm thick amorphous and semi-crystalline radial growth rings (Perez & Bertoft, 2010). The semi-crystalline rings are composed of a lamellar structure of alternating crystalline and amorphous regions with a repeat distance of 9–11 nm, whilst the amorphous rings consist of amylose and amylopectin in a disordered conformation. The crystalline lamellae are believed to be formed by double helices of amylopectin side chains packed laterally into a crystalline lattice, while amorphous zones are mainly composed of branching points of amylopectin molecules, and amylose and amylopectin molecules in a disordered conformation (French, 1984; Maaran et al., 2014; Robin, 1974). Amylose molecules may also appear in amylopectin clusters and pass across both crystalline and amorphous zones.

Most useful information about the crystalline structure of starch granules is obtained using X-ray diffraction (XRD) and related techniques. Wide angle X-ray scattering (WAXS) and small angle X-ray scattering (SAXS) are employed in parallel to reveal the complex superstructures of the granule and determine the crystallinity and polymorphism (Tester et al., 2004a). The amylopectin molecules inside starch granules have been shown (Hizukuri et al., 1983) to crystallize into either A-type

(cereal starches), B-type (tuber, root, high amylose cereal starches and retrograded starches) or a C-type (pulse starches). With the exception of wrinkled pea starch, most pulse starches exhibit a C-type X-ray diffraction, which is an intermediate between the A- and B-types. In addition, a V-type crystal structure can occur due to complexation of single amylose helices with lipids, often in gelatinized starches and in some native maize, rice and oat starches (Gallant et al., 1992; Buleon et al., 1998). The crystallinity of pulse starches is in the range 17.0–34.0%. Crystallinity differences among pulse starches could be influenced by: (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).

2.1.3 The blockets model

Considerable evidence has been accumulated from SEM (Gallant et al., 1992, 1997), TEM, enzyme hydrolysis studies (Helbert & Chanzy, 1996) and AFM (Baldwin et al., 1998; Gallant et al., 1997), which suggests that there exists an intermediate level of starch granular structure between the growth rings and the amylopectin lamellae. It is hypothesized that the crystalline and amorphous lamellae of the amylopectin are grouped into larger, discrete and elongated spherical structures, which have been designated as "blocklets". By gathering past and recent knowledge established over the decades by a variety of microscopic and other analytical techniques, an overall picture of starch granule internal structure and organization is illustrated in Figure 2.3 (Gallant et al., 1997).

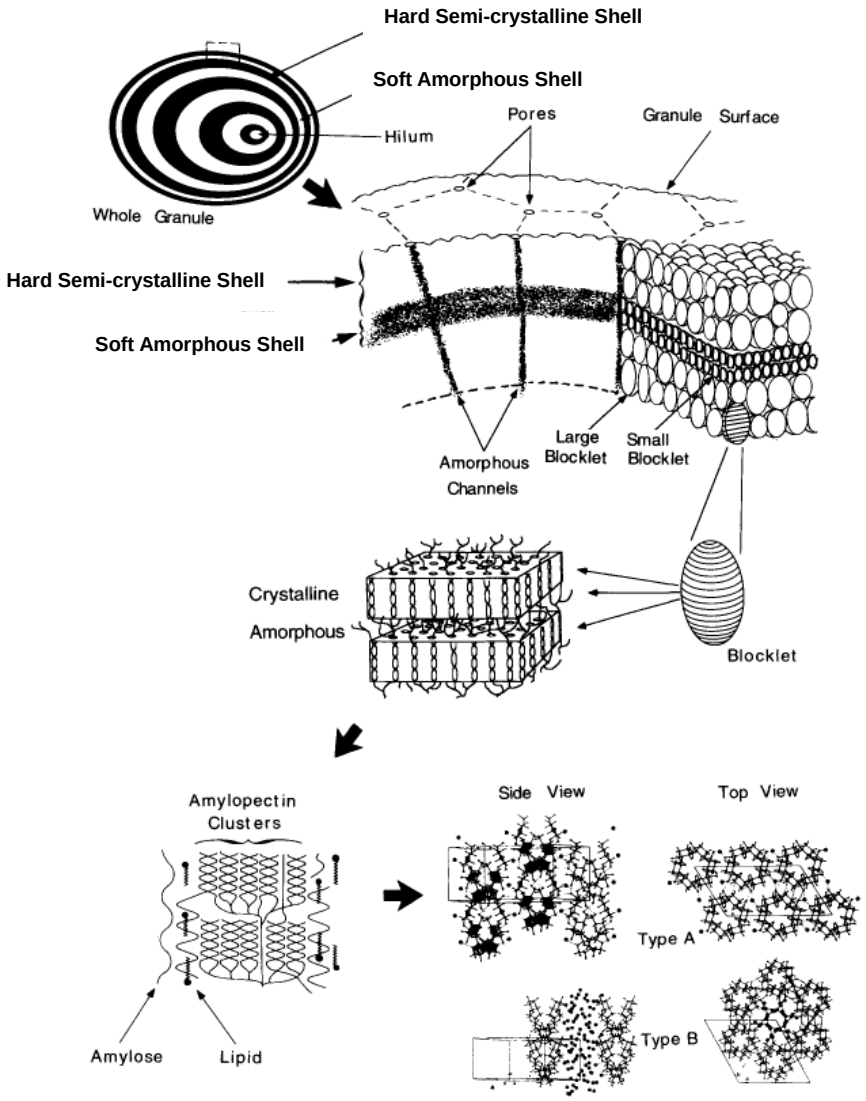

Figure 2.3 Overview of starch granule structure at different levels of magnification featured by the blockets model. Taken from Gallant et al. (1997)

As shown in Figure 2.3, alternating concentric hard semi-crystalline shells and soft amorphous shells form the lowest level of granule architecture. The thickness of the shells gradually declines towards the granule surface. At the next level of granule organization, the blocket structure is displayed, involving with the radial amorphous channels. Blocket size is bigger in semi-crystalline shells than in amorphous shells. Additionally, one blocket in semi-crystalline shells consists of a number of amorphous zones and crystalline lamellae. At the second highest level of structure,

amylopectin clusters and amylose-lipid (or protein) complexes were revealed (Blanshard, 1987). At the highest order of granule structure, the A-type and B-type crystalline structures are presented. Clearly, molecule chains of A-type and B-type starch granule are crystallized in the monoclinic lattice and hexagonal lattice, respectively. In general, the blocklets vary in diameter between 20 to 500nm depending on starch botanical origin and their location in the granule. Enzymatic digestion studies coupled with SEM observations (Gallant et al., 1992) reveal that the blocklets are larger (400-500 nm) in B-crystalline type starches (e.g., potato starch) and C-crystalline type starches (pulse starches) than in A-crystalline type starches (in which, the blocket diameter is 25-100 nm), e.g. wheat and corn starches (BeMiller & Whislter, 2009; Perez & Bertoft, 2010). Moreover, it appears that resistant starches (e.g., potato starch) tend to have larger blockets than less resistant starches (wheat starch) (Gallant et al., 1997). However, other factors such as amylose content, location as well as amylose amylopectin interplay may also have significant effects in starch resistance.

2.2 Resistant starches

2.2.1 Definition and types of resistant starches

Starch sometimes resists digestion by α-amylase. Englyst et al. (1992) proposed three classes of dietary starches: (1) rapidly digestible starch (RDS), which is likely to be digested in the human intestine; (2) slowly digested starch (SDS),

which is likely to be slowly yet completely digested in the small intestine; and (3) resistant starch (RS), which is likely to resist digestion in the small intestine.

Resistant starches refer to the portion of starch and starch degradation products not digested in the small intestine of healthy human. It is subdivided into five forms depending on the cause of resistance: RS1 - physically inaccessible starch which is entrapped in a non-digestible matrix such as whole or partly milled grains or seeds (e.g., coarse-ground grains or legumes); RS2 - some types of native starch granules which are highly resistant to digestion by α-amylase until gelatinized (e.g., in raw potatoes and bananas) and high amylose starches (e.g. high amylose corn starches); RS3 - retrograded starch (amylose crystals) (e.g., in cooled, cooked potatoes, pastas and cornflakes); RS4 - starches that are chemically modified to obtain resistance to enzymatic digestion (e.g., some starch ethers, starch esters, and cross-linked starches); RS5 - amylose-lipid complexes, a helical structure that contains a fatty acid tail within the central cavity (Englyst et al., 1992; Erlingen et al., 1993; Hasjim and Jane., 2013; Nugent, 2005; Thompson, 2000).

2.2.2 Preparation of resistant starches

Resistant starches can be prepared by the modification of native starches using physical, chemical, enzymatic or their combined methods. Different approaches have been employed to manufacture resistant starch ingredients for varied food applications. Chung et al. (2010) investigated the effect of annealing (ANN) and heat-moisture treatment (HMT) on the nutrient factions of pea, lentil and navy bean starches. Their

research demonstrated that in gelatinized pulse starches ANN and HMT decreased RDS level and increased SDS and RS levels. Ambigaipalan et al. (2014) showed that HMT at 80°C and 100°C increased the resistant starch content to nearly the same extent as enzymatic and chemical methods.

RS4 type resistant starch can be produced by chemical modifications, such as cross-linking, conversion, or substitution, which can prevent its digestion by blocking enzyme access and forming atypical linkages such as α(1-4) and α(1-6) linkages (Sajilata et al., 2006). Woo and Seib (2002) reported a solution process for making a highly cross-linked distarch phosphate RS4, achieving the RS content of 35-66% (Englyst method). Cross-linking of starch with mixtures of mostly STMP and a minor weight fraction of STPP under alkaline (sodium hydroxide) conditions increases its resistance to α-amylase digestion as phosphorus incorporation increases to 0.4-0.5%. Recently, Shi et al. (2013) and Sankon et al. (2013) studied the effect of solution cross-linking with STMP/STPP on the in vitro digestibility of pea starch and African Locust bean starch, respectively. In their research work, the RS content (Englyst method) of cross-linked pea starch and African Locust bean starch increased significantly to as high as 64% and 49%, respectively.

RS3 type resistant starch (i.e., retrograded amylose) is often found in small amounts (<5%) in many foods, such as cooked oats and cornflakes (Englyst and Cummings, 1985). Extrusion cooking is usually used for making breakfast cereals, snacks, pastas, and other food products. Most recently, Sarawong et al. (2014) studied

effects of twin-screw extrusion cooking variables and subsequent storing conditions on the RS content and functional properties of green banana flours. They observed that extrusion cooking led to a dramatic drop of RS content, decreasing from around 47% to around 1-4% (AOAC, 2002). Higher feed moisture (50% vs. 20%) gave higher RS content (3.82% vs. 1.20%) and higher amylose content (30.16% vs. 17.96%) in extruded flours. Storing of extruded flours at 4°C for 24 hours only resulted in a minimal increase in RS content (4.00% vs. 3.82%).

2.2.3 Measurement of resistant starches

A number of in vitro methods for RS determination have been proposed or used (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 an attempt to imitate starch digestion in the human. The main step of any method to measure RS content must first remove all of the digestible starch from the product using pancreatic α-amylase/amyloglucosidase or thermo-stable α-amylase (McCleary & Rossiter 2004). These methods mainly differed by the enzymes used, pH of incubation, and digestion time/temperature combination of the reactions. Therefore, the RS content reported in the literature will vary for different methods because the in vitro RS contents measured in foods are method dependent. Each method has its merits and drawbacks (Champ et al., 2003; Manigat et al., 2013). However, Englyst method has been one of the most popularly used in vitro digestibility assay procedures for starchy foods because it gives values

close to the in vivo data (Englyst et al., 1992; Dupuis et al., 2014). This method intends to mimic the action of gastrointestinal tract by employing a guar gum solution in an acetate buffer (pH5.2) to impart viscosity. By using glass beads coupled with vortex shaking, peristalsis is also simulated. RDS and SDS are determined respectively by the glucose liberated in 20 min and 120 min incubation at 37°C with porcine pancreatic α-amylase, amyloglucosidase and invertase. RS is defined as the starch fraction that is not hydrolyzed after 120 min incubation, by deducting RDS and SDS from the total starch content.

In the US and some other countries such as Japan and Australia, resistant starch is included within the definitions of dietary fiber by the American Association of Cereal Chemists (AACC). The Association of Official Analytical Chemists (AOAC) method 991.43 (AOAC, 2000) or Total Dietary Fiber (TDF) Determination in Foods (Prosky et al., 1985) is commonly used to measure the resistant starch as TDF (De Vries, 2004). AOAC method involves digestion of starch with heat-stable α-amylase during boiling. In this method, RS is determined as the weight difference between the hydrolyzed and original sample. Woo and Seib (2002) adopted the AOAC method 991.43 to determine the Total Dietary Fiber (TDF) to characterize the RS4 content in solution cross-linked starches.

2.2.4 Resistant starch content of pulse starches

The RDS, SDS, and RS fractions in pulse starches have been determined by the Englyst et al. (1992) and AACC (2000) methods (Table 2.1). These two measurement methods differ in time of hydrolysis and enzyme source. Due to

different testing method, it is difficult to make a meaningful comparison of the RS content among pulse starches. In this research, two types of commercialized native pea starches (NutriPea and Meelunie) with quite different initial RS contents (18.67±0.60% and 50.83±1.69%, respectively) were investigated in terms of their bulk phosphorylation performance as well as the resulting SDS and RS content of extruded pea starch phosphates.

Table 2.1 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[a]	Englyst	9.5	29.6	60.9
Chickpea[b]	Englyst	10.9–23.5	34.8–45.7	33.5–54.3
Chickpea[c]	AACC	21.5–29.9	45.7–57.7	8.14–18.4
Kidney bean[d]	AACC	11.7	65.7	17.2
Lentil[e]	Englyst	5.2–14.8	29.7–41.5	43.7–65.2
Lentil[f]	AACC	16.0–16.9	58.3–62.2	13.0–13.2
Mung bean[g]	Englyst	9.7	40.0	50.3
Navy bean[h]	Englyst	8.2	32.3	59.4
Navy bean[i]	AACC	12.4	65.8	21.9
Pea (smooth)[j]	AACC	18.2–23.8	53.7–59.0	8.1–12.6
Pea (smooth)[k]	Englyst	8.1–19.2	33.9–40.2	40.5–58.0
Pigeon pea[l]	Englyst	4.2	16.9	78.9

2.3 Cross-linking modifications of pulse starches

Native pulse starches have poor functional properties such as low shear and acid resistance, low thermal stability and high retrogradation tendency (Hoover et al., 2010). Therefore, modification of pulse starches is necessary to tailor-make their specific functional properties, e.g. desirable digestion-resistance, to develop novel functional food ingredients and functional foods.

Starch modification is generally achieved chemically (cross-linking, substitution, acid or enzymatic hydrolysis, oxidation) and/or physically (pre-gelatinization, heat-moisture treatment, or annealing) to enhance their resistance towards mechanical shearing, high temperatures, acid and/or enzyme hydrolysis and to decrease the degree of retrogradation (Hoover et al., 2010). Chemical modification involves the introduction of functional groups into the starch molecules, resulting in markedly altered physicochemical properties (Singh et al., 2007).

2.3.1 Commonly used cross-linking reagents

Cross-linking modifications generally utilize multifunctional reagents to form either ether or ester intermolecular or intramolecular cross-links between the hydroxyl groups on adjacent starch chains. Sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), phosphoryl chloride (phosphorus oxychloride: $POCl_3$), epichlorohydrin (EPI), and adipic-acetic mixed anhydride are the common agents employed to produce cross-linked starches (Guerrero & Betancur, 1998; Hoover & Sosulski, 1986; Lim & Seib, 1993; Shi et al., 2013; Woo & Seib, 1997, 2002). Cross-linking of pulse starches has been shown to decrease α-amylose leaching, water binding capacity, amylase digestibility, granular swelling but to increase stability and the degree of set-back (Hoover et al., 2010).

Optimal reactive conditions and schemes vary according to reagent type. For reactions with STMP and STPP, starch is generally impregnated with both reagent and catalyzing base within an aqueous granule slurry (Woo & Seib, 2002), after which

slurry moisture levels can be reduced (to <15%) and granules are heated in the semi-dry state to drive the reaction (Lim & Seib, 1993). Cross-linking is favored at pH values above 8 and 10 for STPP and STMP, respectively. In contrast, $POCl_3$, EPI, and adipic–acetic mixed anhydride cross-linking reactions are conducted as aqueous granular suspensions under basic conditions at pH values of 11–12, 8, and 10.5, respectively (Kasemsuwan & Jane, 1994; Wu & Seib, 1990). Addition of a stabilizing salt (e.g., Na_2SO_4) is reported to boost reaction with $POCl_3$ and STMP (Woo & Seib, 1997; Wu & Seib, 1990). Sodium sulfate (Na_2SO_4) or sodium chloride (NaCl) is usually needed in a short time modification process to inhibit gelatinization and to accelerate the phosphorylation reaction (Woo & Seib, 2002). Besides, it is suggested that sodium sulphate not only increase absorption of alkali and cross-linking reagents into starch granules, but also generate ionic strength that enhances cross-linking reaction between negatively charged starch alkoxide and phosphoryl reactant species (Woo & Seib, 1997).

Woo and Seib (2002) reported that cross-linking of wheat starch in aqueous slurry with the maximum permissible level (CFR, 2001) of $POCl_3$ (0.1%) and EPI(0.3%) gave no AOAC dietary fiber (resistant starch) (AOAC, 2000). However, cross-linking reaction of wheat starch in aqueous slurry with 12.0% of a 99:1(w/w) mixture of STMP/STPP in 10.0% sodium sulfate gave a modified wheat starch with phosphorus (0.32%) and gave about 76% dietary fiber (resistant starch) by the AOAC method.

2.3.2 Mechanism of starch phosphorylation

The most popularly used food-grade cross-linking reagent for starch is 99:1 (w/w) STMP/STPP owing to its high phosphorylating efficiency. The mechanism (Figure 2.4) of starch phosphorylation with STMP was proposed by Sang et al. (2007) based on their study on phosphorylated resistant wheat starches by ^{31}P NMR spectroscopy. The first step of the reaction engages the ring opening of STMP by the attack of a starch alcoholate (RO^-) ion to generate the tripolyphosphate intermediate (Reaction 1, Figure 2.4). Under an alkaline condition all four ionizable hydrogens on the starch triphosphate intermediate would be negatively charged. Pyrophosphate in the completely ionized intermediate is considered a better leaving group than orthophosphate when the starch triphoshpate intermediate is attacked by hydroxide or starch alcoholate ion at pH 11.5-12.5. Attack of an RO^- or OH^- ion at the α-phosphorus of the triphosphoryl group is more effective than the attack at either the β- or γ-phosphorus atom (Reaction 2, Figure 2.4), producing only DSMP (distarch monophosphate) and MSMP (monostarch monophosphate). Sang et al. (2007) observed that the DSMP content in the solution phosphorylated starch was positively correlated with RS4 content. In contrast, at pH 10.5 the concentration of RO^- drops by 90%, leading to the slow reaction of RO^- with the starch triphosphate intermediate. The unimolecular peeling reaction appears to deprive the γ-phosphorus on the starch triphosphate intermediate (Reaction 3, Figure 2.4), resulting in the formation of MSDP (monostarch diphosphate).

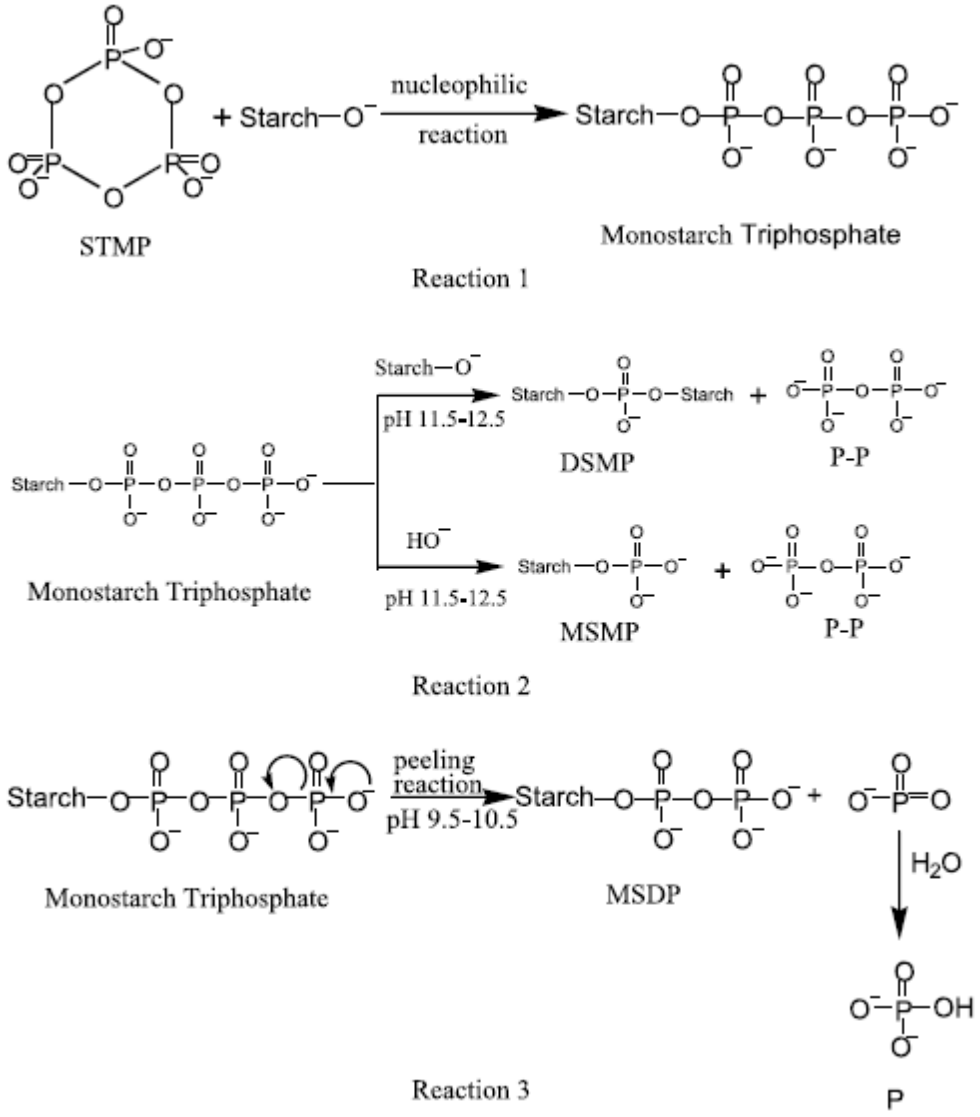

Figure 2.4 Proposed pathway of starch phosphorylation with STMP in aqueous NaOH at pH 9.5-12.5. Taken from Sang et al. (2007)

2.3.3 Cross-linking using reactive extrusion

The conventional methods of solution cross-linking modification require an excess of reagents with high concentrations of gelatinization-inhibiting salts (10%-30% Na_2SO_4 or NaCl) and may cause environmental contamination from un-reacted chemicals. Moreover, the low starch/water ratio in conventional process leads to a poor reaction selectivity (loss of reagents via side-reaction with water) and long reaction time (Tomaisk & Schilling, 2004). By contrast, reactive extrusion technology

is generally a high-temperature rapid process with the merits of high versatility and absence of effluents, that may overcome pollution by using reduced amounts of reagents (Bertolini, 2010; Landerito & Wang, 2005). In reactive extrusion, reaction rates can be multiplied because of higher starch concentrations (typically 60-80% starch), higher temperatures (often 70-140°C depending on the amylose content compared to typically 40°C-60°C in solution) and much better mixing. Reactive extrusion of starch involves a combination of physical and chemical modification, in which granular starch is heated, transported and mixed by single or twin-screw toward a die, and is derivatized in the molten state or solid state (Bertolini, 2010; Moad, 2011; Xie et al., 2006). The most widely used reactive extruders are single-screw extruders (SSE) and twin-screw extruders (TSE). TSE can be classified as co-rotating or counter-rotating and as intermeshing or non-intermeshing. Co-rotating intermeshing twin-screw extruders are mostly favored for many reactive extrusion processes owing to their greater control over mixing and residence time distribution (RTD), higher flexibility for screw designs to control reagent addition and byproduct removal, superior heat transfer and self-cleaning operation (White et al., 1987; Xie et al., 2006) .

Reactive extrusion has been applied to starch modifications including bulk phosphate cross-linking reaction using a single screw extruder or a twin screw extruder. Murua-Pagola et al. (2009), Nabeshima & Grossmann (2001), O'Brien et al. (2009) and Seker & Hanna (2003a, 2003b, 2004, 2005, 2006) studied the granular state reactive extrusion of phosphate cross-linked starches for biodegradable polymers,

pharmaceutical or industrial applications. Only a few studies have been reported to date in the field of reactive extrusion for phosphorylation with the purpose of food applications in mind. Phosphorylation of cereal starches (Kim et al., 1999; Landerito & Wang, 2005) improves swelling properties for food applications but these modified samples were not tested as α-amylase resistant starches. Some studies (Hasjim and Jane, 2009; Huth et al., 2000; Sarawong et al., 2014) have been carried out to evaluate the potential to increase resistant starch content of (maize) starches and (barley and green banana) flours by extrusion cooking for food products. However, those studies were limited to induce the formation of RS3 by lengthy sequential hydrothermal or freezing treatment after the starches or flours were fully gelatinized by extrusion cooking process. Morphological observations of these starch extrudates were particularly lacking in the mentioned studies with extrusion technologies making it unclear whether granules were actually retained in these processes. Up to now, no research has been reported on using the reactive extrusion of phosphorylated pulse starches to increase enzyme resistance for functional food applications. Hence, this thesis will pioneer the intensive research on the preparation of granular phosphorylated resistant pulse starches using twin-screw extrusion. Effective extrusion technology needs to be developed and applied to the continuous production of phosphate cross-linked granular resistant pulse starches. Also, the effects of feed compositions and extrusion conditions on the morphology and properties (e.g., phosphorus content and Englyst digestion profile) of extruded pulse starch phosphates need investigating.

2.3.4 Characterization of cross-linked starches

The phosphorus in modified starch for food use is regulated by the Code of Federal Regulation (CFR, 2001) of the U.S. Food and Drug Administration or by the Directive of the EEC (2000). If STMP/STPP is used to phosphorylate starch for food use, the modified starch cannot contain more than 0.4% phosphorus. Smith and Caruso (1964) proposed a popular method to determine the phosphorus content in phosphorylated cross-linked starch. Based on the phosphorus content, the degree of substitution (DS) for phosphate monoester and phosphate diester can be calculated accordingly (Wongsagonsup et al., 2005). The phosphorus content in cross-linked starched can also be determined by Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF) (Paltridge et al., 2012) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (AACC, 2001; Lu et al., 2011, 2012; Safford et al., 1998). Due to its high sensitivity and accuracy, ICP-OES was employed in this research to determine the phosphorus content for all of the pea starch samples. To characterize the enzyme resistance (discussed in Section 2.2.3), Englyst method (1992) was chosen in this research to determine the in vitro digestion profiles for all the pea starch samples since this profile is more informative (i.e., RDS, SDS & RS content) and conducive to examine the influences of complex extrusion conditions.

Solution cross-linked starches cannot be distinguished from their native starch counterparts based on the granule morphology because the reaction does not appear to alter granule appearance (Hoover & Sosulski, 1986; Kaur & Singh, 2006). For those

cross-linking reaction methods without shear, where the granules remain largely intact, particle attributes, such as degree of swelling, pasting properties and free-thaw stability, are often used by food chemists to indirectly characterize the extent of cross-linking. These methods are suited to bulk cross-linked starches in an extruder if the extruded granules only undergo minimal damage during extrusion processing (Kim et al., 1999; Landerito & Wang, 2005). Also, light microscopy and SEM are popular techniques to study the morphological changes of extruded starch granules compared to their native counterparts (Sharma et al., 2015). DSC is commonly used to determine the gelatinization parameters of cross-linked starches though the reported results are fairly contradictory regarding the effect of cross-linking due to differing cross-linking conditions between studies (Landerito & Wang, 2005; O'Brien et al., 2009; Shi et al., 2013; Woo & Seib, 2002; Wu & Seib, 1990). Spectroscopic techniques are generally more universal and informative for both solution and solid-state reactions. Particularly, NMR techniques (^{13}C, ^{31}P NMR) have successfully been used to establish the reaction pathways through structural changes to the starch despite the complex, lengthy enzymatic digestion procedure of sample preparation for ^{31}P NMR (Delval et al., 2004; Sang et al., 2007).

2.4 Food applications of cross-linked resistant starches

The unique health benefits of resistant starch (RS) have been well reported as prevention of colonic cancer, substrate for growth of probiotic organisms, reduction of gall stone formation, hypoglycemic effects, hypocholesterolemic effects, inhibition of

fat accumulation, and increased absorption of minerals (Hoover & Zhou, 2003; Nugent, 2005; Sajilata et al., 2006). In order for the human body to take advantage of the physiological benefits, it is estimated that daily carbohydrate intake needs to contain 10-20% RS (Dupuis et al., 2014). Resistant starch appears to be highly resistant to mammalian enzyme and is included within the definitions of dietary fiber by the American Association of Cereal Chemists (Jones, 2000), and is measured as fiber via the AOAC method (AOAC, 2000). Where the Prosky fiber method (Prosky et al., 1985) is used (US, UK, Australia and Japan) commercially-manufactured sources of resistant starch can be used as vehicles to enrich the total dietary fiber content of food products.

Resistant starches have a small particle size, white appearance, and bland flavor. Meanwhile, they have desirable physicochemical properties such as swelling, viscosity increase, gel formation, and water-binding capacity, making them useful in a variety of foods (Fausto et al., 1997). Resistant starches allow the formation of low-bulk high-fiber products with improved texture, appearance, flavor, and mouth feel (such as better organoleptic qualities) compared with traditional high-fiber products; they increase coating crispness of products and the bowl life of breakfast cereals. They are functional food ingredients lowering the calorific value of foods and useful in products for coeliacs disease, as bulk laxatives and in products for oral rehydration therapy. Some of these properties of resistant starches have been successfully used in a wide array of food products as dietary fiber fortification in bread-making, texture modifier in baked cakes (e.g. muffins and brownies), crisping

agent (e.g. in French toasts and waffles) and functional ingredient in extruded cereals, snacks (e.g., crackers and cookies), pizza crusts, pastas and healthy beverages. By formulating foods with resistant starch, food product developers and nutritionists can encourage consumers to increase their fiber intake with a variety of palatable, high quality foods that are healthy as well (Fuentes-Zaragoza et al., 2010; Sajilata et al., 2006; Yue & Waring, 1998).

Phosphorylated cross-linked starches with increased levels of resistant starch are thought to provide nutritional benefits for humans (Sang & Seib, 2006). Many chemically modified starches (RS4) made for food use contain only small amount of substituent groups and have been used as safe food ingredients. The maximum permitted levels of substitution for starch acetates, starch phosphates and hydroxypropylated starches are 2.5%, 0.4% and 10% respectively (CFR, 2001). Cross-linked starches of RS4 type, based on maize, tapioca and potato, have been useful in formulations needing pulpy texture, smoothness, flowability, low pH storage, and high temperature storage (Sajilata & Singhal, 2005). Solution cross-linked RS4 wheat starches, have been commercialized, such as Fibersym®RW (85%TDF) and FiberRite®RW (75%TDF), which are excellent sources of dietary fiber fortification for healthy human food products (Thompson et al., 2011).

CHAPTER 3 EXPERIMENTAL

3.1 Materials

Native pea starches Accu-Gel and Windmill were purchased from Nutri-Pea Limited (Portage la Prairie, MB) and Meelunie America Inc. (Southfield, MI), respectively. Accu-Gel and Windmill are food-grade pea starches derived from Canadian and Belgian yellow peas, respectively. Their typical initial moisture content ranges from 11.7% to 13.0% (on raw starch basis) based on measurement using a Mettler-Toledo HG63 moisture analyzer. Food-grade lecithin was obtained from a Canadian bulk foods retailer, Bulk Barn®. Sodium trimetaphosphate (STMP/T5008, >95% purity), sodium tripolyphosphate (STPP/72061, >98% purity), sodium sulphate (Na_2SO_4) (238597, >99% purity), and glycerol (7757, >99% purity) were obtained from Sigma Aldrich (St. Louis, MO). The aqueous sodium hydroxide (0.1N and 1N standardized) solutions were purchased from LabChem Inc. (Pittsburgh, PA). Standard phosphorus solution (Specpure #13862, 1000μg/ml P in 5% HNO_3) was obtained from Alfa Aesar (Pittsburgh, PA). Concentrated nitric acid (Baker Instra-Analyzed Reagent for trace metal analysis, 69-70%) was obtained from Avantor Performance Materials Inc. (Center Valley, PA). Concentrated hydrochloric acid (TraceMetal™ Grade, 34-37%) was obtained from Fisher Scientific Inc. (Fairlawn, NJ). All water used was Milli-Q water.

3.2 Preparation of granular pea starch phosphates using Haake batch mixer

The feed moisture content of NutriPea pea starch samples (88g, on dry starch

weight basis(db)) was regulated to 60% (db) before the bulk phosphorylation process. This is because 60% feed moisture content has been found to be the upper threshold level to obtain granular pea starch rather than thermoplastic pea starch. The necessary additional water to reach 60% feed moisture content was used to dissolve different concentrations of 99:1 (w/w) STMP/STPP (1%, 3%, 5%, 7%, and 10%) and sodium sulphate (3%). Then, the pH of the cross-linking reagents solutions was adjusted to 11.5 or 12.9 with 0.1N or 1N NaOH solution according to different experimental purposes. Next, each batch of NutriPea pea starch sample was thoroughly mixed with cross-linking (CL) reagents solution and stored at room temperature in sealed plastic bags for 20 hours. After that, bulk phosphorylation reaction of pre-mixed starch samples was done using a counter-rotating Haake batch mixer (PolyLab Rheomix 3000P with twin Roller Rotors) under various conditions as indicated in Table 3.1. Screw rotation speed was kept constant at 50rpm for all the trials in this study.

Table 3.1 Formulations of NutriPea pea starch phosphates prepared in Haake batch mixer

Sample	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	pH of CL Reagents Solution	CL Conditions
A1	1	3	11.5	95°C, 20min, 50rpm
A2	3	3	11.5	
A3	5	3	11.5	
A4	7	3	11.5	
A5	10	3	11.5	
B1	5	3	12.9	95°C, 5min, 50rpm
B2	10	3	12.9	
B3	5	3	12.9	95°C, 20min, 50rpm
B4	10	3	12.9	

3.3 Preparation of granular pea starch phosphates using twin-screw extruder

The feed moisture content of NutriPea or Meelunie pea starch samples (600-900g, on dry starch weight basis(db)) was regulated to 40%, 47%, and 50% (db) (depending on experimental purpose) prior to the bulk phosphorylation process. The necessary additional water to reach 40%-50% feed moisture content was used to dissolve different concentrations of 99:1 (w/w) STMP/STPP (3%, 5%, 7%, and 10%) and sodium sulphate (0%-3%). Then, the pH of the cross-linking reagents solutions was adjusted to 12.9 with 1N NaOH solution. Next, each batch of pea starch sample was thoroughly mixed with phosphorylating (cross-linking) reagents solution at room temperature in sealed plastic bags for about 20 minutes. After that, bulk phosphorylation reaction of pre-mixed starch samples was immediately done using a Leistritz ZSE-HP 27mm 40L/D co-rotating intermeshing twin-screw extruder (American Leistritz Extrusion Corp.; Sommerville, NJ) without a die under various extrusion conditions as indicated in Tables 3.2 and Table 3.3 (percentages of various reactants are all based on dry starch weight basis). A flat temperature profile of 95°C was applied to the nine heating zones of the extruder barrel for all the samples in this study. Two low-shear screw designs (Figure 3.1 and 3.2) were examined. Due to the poor flowability of Meelunie pea starch, it could not be extruded at low screw speed or high feed rate.

Table 3.2 Formulations of extruded NutriPea pea starch phosphates

Sample	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP(wt%)	Na_2SO_4 (wt%)	Extrusion Conditions
NE0[A)]	40	0	0	1.02kg/h, 150rpm
NE1	40	5	3	1.02kg/h, 30rpm
NE2	40	5	3	1.02kg/h, 50rpm
NE3	40	5	3	1.02kg/h, 100rpm
NE4	40	5	3	1.02kg/h, 150rpm
NE5	40	5	3	1.02kg/h, 200rpm
NE6	40	5	3	3.06kg/h, 150rpm
NE7	40	5	1	1.02kg/h, 50rpm
NE8	40	5	1	1.02kg/h, 150rpm
NE9	40	5	1	3.06kg/h, 150rpm
NE10	40	5	5	1.02kg/h, 50rpm
NE11	47	7	3	1.02kg/h, 50rpm
NE12	40	3	1	1.02kg/h, 150rpm
NE13	40	7	1	1.02kg/h, 150rpm
NE14	40	7	1	2.04kg/h, 150rpm
NE15	40	7	1	3.06kg/h, 150rpm
NE16	47	3	1	1.02kg/h, 150rpm
NE17	47	5	1	1.02kg/h, 150rpm
NE18	47	7	1	1.02kg/h, 150rpm
NE19	50	7	1	1.02kg/h, 150rpm
NE20	50	10	1	1.02kg/h, 150rpm
NE21	40	3	0	1.02kg/h, 150rpm
NE22	40	5	0	1.02kg/h, 150rpm
NE23	40	7	0	1.02kg/h, 150rpm
NE24	47	7	0	1.02kg/h, 150rpm
NE25	47	10	0	1.02kg/h, 150rpm
NE26[B)]	40	7	1	1.02kg/h, 150rpm
NE27[B)]	40	7	1	2.04kg/h, 150rpm
NE28[B)]	40	7	1	3.06kg/h, 150rpm

A).NutriPea pea starch extrudate control prepared without phosphorylation.

B).Screw design #2 was used for NE26, NE27, and NE28 while screw design #1 was used for all the other samples.

Table 3.3 Formulations of extruded Meelunie pea starch phosphates

Sample	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Extrusion Conditions
ME0[A)]	40	0	0	1.02kg/h, 150rpm
ME1	40	3	1	1.02kg/h, 150rpm
ME2	40	5	0	1.02kg/h, 150rpm
ME3	40	5	1	1.02kg/h, 150rpm
ME4	40	5	3	1.02kg/h, 150rpm
ME5	40	7	1	1.02kg/h, 150rpm
ME6	47	7	1	1.02kg/h, 150rpm
ME7	40	7	1	1.02kg/h, 250rpm
ME8	40	7	1	2.04kg/h, 150rpm
ME9[B)]	40	7	1	1.02kg/h, 150rpm
ME10[B)]	40	7	1	2.04kg/h, 150rpm

A).Meelunie pea starch extrudate control prepared without phosphorylation.

B). Screw design #2 was used for ME9 and ME10 while screw design #1 was used for all the other samples.

Figure 3.1 Photo image of screw design #1

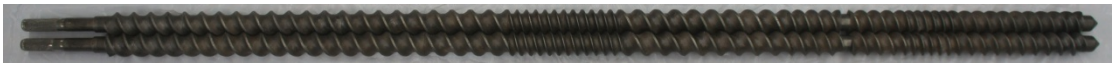

Figure 3.2 Photo image of screw design #2

Besides two-step reactive extrusion process, NutriPea pea starch phosphates were produced using one-step reactive extrusion process which was featured by the in-line foaming injection of cross-linking reagent solution. The foaming agent (i.e., lecithin) was fully dissolved in the cross-linking reagent solution in a sealed glass bottle via vibrant magnetic stirring one night prior to extrusion. The foaming extrusion conditions & formulations as well as the relevant extruder-setup are shown in Table 3.4 and Figure 3.3, respectively.

Table 3.4 Formulations of extruded NutriPea pea starch phosphates using foaming injection of cross-linking (CL) reagent solution

Sample[A)]	Feed Moisture (wt%)[a]	99:1 (w/w) STMP/STPP (wt%)[)]	Na_2SO_4 (wt%)[a]	Lecithin (wt%)	Extrusion Conditions	Flow Rate of CL Solution (mL/min)
NEF1	40	5	3	0.15	2.04kg/h, 150rpm	8.58
NEF2	40	5	3	0.15	2.04kg/h, 50rpm	8.58
NEF3	40	5	1	0.15	2.04kg/h, 150rpm	8.48
NEF4	40	5	1	0.15	2.04kg/h, 50rpm	8.48
NEF5	40	5	1	0.25	2.04kg/h, 150rpm	8.48
NEF6	40	5	1	0.25	2.04kg/h, 50rpm	8.48

A). For NEF1-NEF6, the cross-linking reagent solution was directly added during extrusion by foaming injection through the syringe pump, foaming device and side stuffer (Figure 3.3). Screw design #1 was used for NE1-NE6.

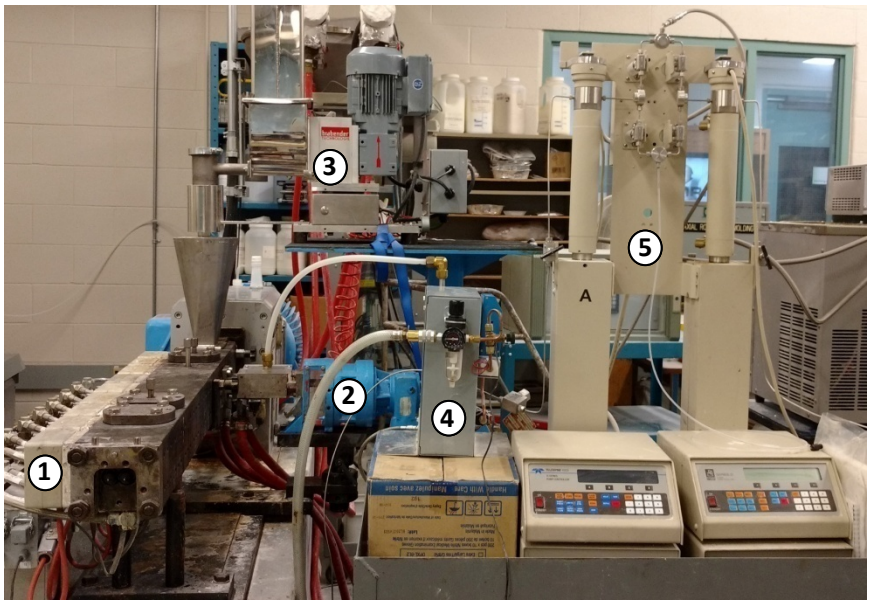

Figure 3.3 Photo image of the extruder setup using foaming injection for the addition of cross-linking reagents solution: (1) extruder, (2) side-stuffer, (3) gravimetric feeder for starch powder, (4) foaming device, (5) syringe pump.

3.4 Extrudate purification

For analysis, un-reacted phosphates were removed by dispersing about

80g-100g extrudate in 800mL-1L Milli-Q ultrapure water. After 20min of vibrant mixing, the mixture was filtered by vacuum filtration. The starch extrudate (from batch mixer or extruder) was washed 8 times following the procedure above until the conductivity of the washings reaches around 10 μs/cm. The purified pea starch phosphate was dried in an oven at 45°C for 48hr, and then was ground and sieved to reach a particle size of less than 150μm before analysis.

3. 5 Characterization

3.5.1 Light microscopy

Pea starch suspensions (water–glycerol 65:35, v/v) were observed under bright field light (magnification 300x and 600x) and crossed polarized light (magnification 600x) using a Axioplan 2 (Carl Zeiss AG, Göttingen, Germany) equipped with a real time image capture and analysis software Clemex Vision Lite 6.0.035 (Clemex Technologies Inc., Longueuil, Quebec). Pea starch granules were observed to facilitate granule size distribution measurement. The optical micrographs were analyzed using Image J version 1.46. For particle size distribution analysis, 356 NutriPea and 193 Meelunie raw starch granules in bright filed microscopy images were traced and measured, respectively. The granular size was expressed using Feret's diameter, which is well accepted in biochemistry image analysis.

3.5.2 Scanning electron microscopy (SEM)

Granule morphology of selected pea starch samples was observed using JEOL JSM-7000F and JSM-6610LV (JEOL Ltd., Japan) scanning electron microscopes at

an accelerating potential of 2-3 kV. Pea starch particles were directly dispersed on circular polished aluminum stubs and then sputter coated with a thin film (5 nm) of platinum in an argon atmosphere prior to observation in order to enhance image quality.

3.5.3 Determination of residence time distribution (RTD)

The extent of mixing and available reaction time in the extruder were evaluated by measuring the exit-age RTD. A pulse stimulus response technique was used to obtain the RTD (Kumar et al., 2006; Li et al., 2014; Mu and Thompson, 2012). A tracer was prepared by mixing 0.15g Allura red dye (Sigma 458848) with 20g native pea starch and the amount of water needed to bring the moisture content of the tracer to that of the feed material. During the reactive extrusion of pea starch, 0.5g tracer was added as a pulse input through the feed port of the extruder. At the same instant, a 16Mpixel digital camera started video recording the starch extrudate from the exit of the extruder. Next, the extracted images were analyzed for the color intensity (displayed in Lab color space) of starch extrudate using Photoshop CS5 (Ver 12.01, Adobe System Inc.; San Jose, CA, USA). The values of a and b were subtracted from the values of the control (starch extruded prior to adding the tracer). The red color intensity was calculated as the 'Index of Saturation' (Francis and Clydesdale, 1975) following:

$$c = \sqrt{(\Delta a)^2 + (\Delta b)^2} \quad (1)$$

The RTD was described by E(t) curve, which represents the variation of the concentration of tracer (i.e., color intensity) at exit of the extruder with time.

Mathematically (Lee & McCarthy, 1996),

$$E(t) = \frac{c}{\int_0^\infty c\, dt} \approx \frac{c_i}{\sum c_i \Delta t_i} \quad (2)$$

The moments of the E(t) curve, namely mean residence time (MRT) and variance (σ^2) (i.e., the mixing index), were determined according to following equations (Kumar, et al., 2006).

$$MRT = \int_0^\infty tE(t)dt = \frac{\sum t_i c_i \Delta t_i}{\sum c_i \Delta t_i} \quad (3)$$

$$\sigma^2 = \frac{\sum (t_i - \bar{t})^2 \Delta t_i}{\sum c_i \Delta t_i} \quad (4)$$

3.5.4 Determination of phosphorus content

Phosphorus content of pea starch phosphates was determined using ICP-OES in accordance with AACC method 40-75.01 (2001). A dry pea starch sample (between 1.000g and 2.000g) is weighted into a 50mL tared porcelain crucible, then placed into a muffle furnace, heated up at 300°C/h and ashed 16 hours at 520°C. After cooling, wet samples with a few drops of water to check for completeness of ashing. If the sample darkens or appears black, add 4mL concentrated HNO_3 and dry on a hot plate at moderate heat to prevent splattering. Re-ash in the furnace at 520°C for 2 hours. Then, the sample is dissolved in 10mL 3N HCl and heated up on a hot plate to digest until residue goes into solution. Next, the sample solution is transferred into a 100mL volumetric flask and diluted with Milli-Q water to the mark. Phosphorus content is measured with an Inductively Coupled Plasma-Optical Emission Spectrometer (Varian Vista Pro ICP-OES) at the wavelength of 213.618nm, 214.914nm, 253.561nm and 177.434nm against the working standard solutions which are prepared by diluting Alfa Aesar Specture solution with 0.3N HCl. Phosphorus content is determined by

averaging the phosphorus measurements at above four wavelengths.

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 pea starches and pea starch phosphates were determined according to the method of Englyst et al. (1992) with minor modifications. Pea starch (100mg) was taken, and 2 mL guar gum, 15 glass beads, and 4mL sodium acetate buffer (pH5.2) were mixed by vortexing. After continuously shaking in a water bath (37°C, 200 strokes/min) for about 5 min, 1mL of enzyme solution was added which consisted of porcine pancreatic α-amylase (Sigma P7545), amyloglucosidase (Megazyme E-AMGDF) and invertase (Sigma I4504), followed by incubation in a water bath at 37°C with shaking (200 strokes/min). Aliquots of hydrolyzed solution (100 μL) were taken at different time intervals and mixed with 1mL of 50% ethanol to terminate the enzymatic reaction. The glucose released in each measurement was determined via a glucose oxidase peroxidase diagnostic kit (from Megazyme) by taking the absorbance of samples against the reagent at 510nm using UV/VIS spectrophotometer. At a digestion time of 20 min, RDS was calculated by multiplying glucose (mg) released by 0.9 and dividing 100mg. The glucose content at 120min was determined by the same method, and SDS was obtained by the difference of digested starch between 20min and 120min. Undigested starch within 120min was considered as RS fraction, which is calculated by the difference between total starch and the sum of RDS and SDS. Each samples was analyzed in triplicates. (Note that all the in vitro digestibility assays in this study were conducted by Elizabeth Donner at Guelph Food Research Centre but interpreted by the author).

3.5.6 Thermal properties

Differential scanning calorimetry (DSC) measurements were conducted using a Q200 differential scanning calorimeter equipped with a refrigerated cooling system (TA Instruments, USA). DSC samples were prepared by adding 1.3g Milli-Q water to 1g dry starch powder, mixed and placed in sealed plastic bags at room temperature for 24 hours. During DSC tests, pea starch sample (5-9mg) was weighted into a Tzero hermetic pan and scanned from 20°C to 120°C at 10°C /min. Each samples was analyzed in duplicates. A nitrogen flow of 50ml/min was used during all DSC tests. The TA Universal Analysis 2000 software was used to analyze the DSC curves and to identify gelatinization temperatures and determine gelatinization enthalpy.

3.5.7 Rapid visco analysis (RAV)

The pasting properties of the pea starch samples were measured using a Rapid Visco Analyzer RVA4 (Perten Instruments Australia Pty. Ltd, Warriewood, NSW, Australia) according to AACC 76-21.01 (1999). The viscosity parameters were recorded in cP units (1 cP = 1 $mPas^{-1}$). Starch (3g, adjusted to 14% moisture basis) and 25 ml Milli-Q water were measured and mixed in the aluminum sample bin. The starch slurry was equilibrated at 50°C for 1 min, heated from 50°C to 95°C at 6°C/min, maintained at 95°C for 5 min, cooled subsequently at 6°C/min to 50°C, and held at 50°C for 2 min. The paddle speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. Each sample was analyzed in duplicates. (Note that all the RVA tests in this study were conducted by Elizabeth Donner at Guelph Food Research Centre but interpreted by the author).

The standard viscogram representing the pasting profile and the RVA parameters is illustrated in Figure 3.4. Values measured from the RVA pasting profile were determined according to RVA4 Installation and Operation Manual (Perten Instruments Australia Pty Ltd., 2010) as follows.

- Peak viscosity: maximum paste viscosity achieved in the heating stage of the profile.
- Trough viscosity: minimum paste viscosity achieved after holding at the maximum temperature.
- Final viscosity: the viscosity at the end of each run.
- Pasting temperature: the onset temperature of rapid increase in viscosity (defined as viscosity increases by ≥24cP within 6 seconds).
- Peak time: the time at which peak viscosity was recorded.
- Breakdown: difference between peak and trough viscosity.
- Setback: difference between final and trough viscosity.

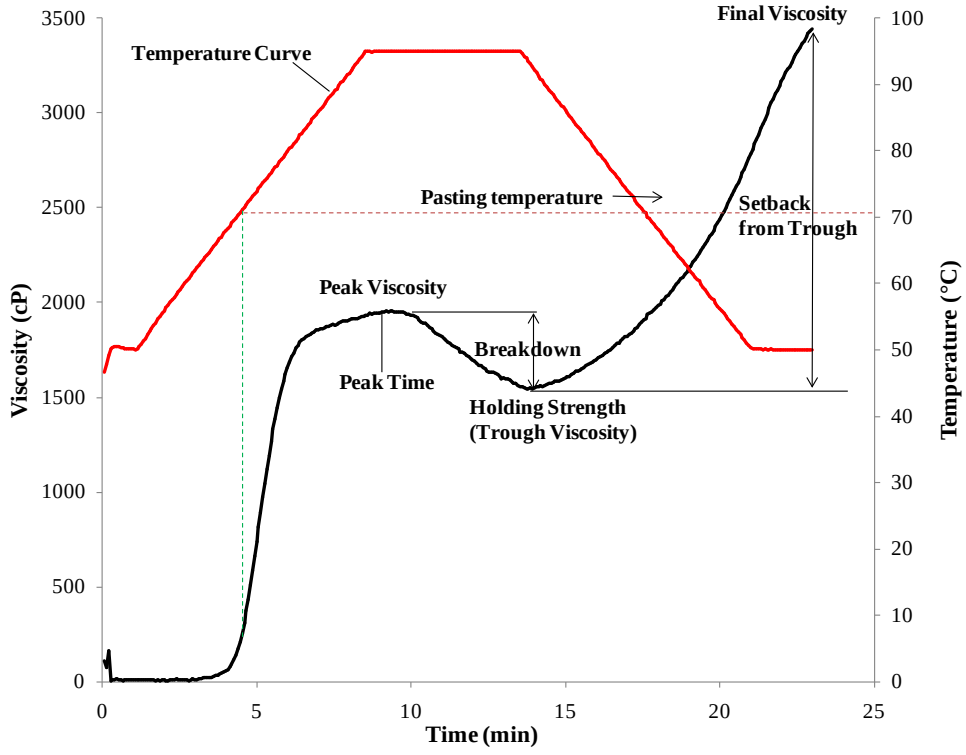

Figure 3.4 Standard RVA profile and the typical RVA parameters. Adapted from Perten Instruments Australia Pty Ltd. (2015)

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 Morphology of pea starches

Pea starch granule morphology was observed by optical microscopy under bright field light and polarized light as well as SEM. Bright field microscopy (Figure 4.1(a-b)) and SEM (Figure 4.2(a-c and j-l)) images showed wide variations, in granule shape (oval, round, elongated, elliptical and multilobed) and size amongst granules of raw NutriPea and Meelunie pea starches. Smaller granules tended to be more circular as compared to those larger ellipsoidal granules. The size distributions of two types of raw pea starch granules were demonstrated by histograms of Feret diameters shown in Figure 4.3 and 4.4. According to statistical analysis on granule optical micrographs (Figure A.1 and Figure A.2 in Appendix A), the pea starch granule size ranges from 12.3 to 47.1μm in Feret diameter for NutriPea and 15.4 to 40.8μm for Meelunie, respectively. The majority of granule size was found between 20 to 35μm for both types of pea starches. Bright field optical images (Figure 4.1(a-b)) indicated the presence of cracks on granules of both raw NutriPea and Meelunie pea starches and to a similar extent of cracking. Cracks have also been observed in other pulse starches (Ambigaipalan et al., 2011; Maaran et al., 2014) and transgenically modified potato starches (Blennow et al., 2003). Cracks have been attributed to low granule integrity stemming from sub-optimal packing of amylopectin double helices (Blennow et al., 2003). The existence of cracks could promote the diffusion of cross-linking reagents into the starch granules during reactive extrusion process.

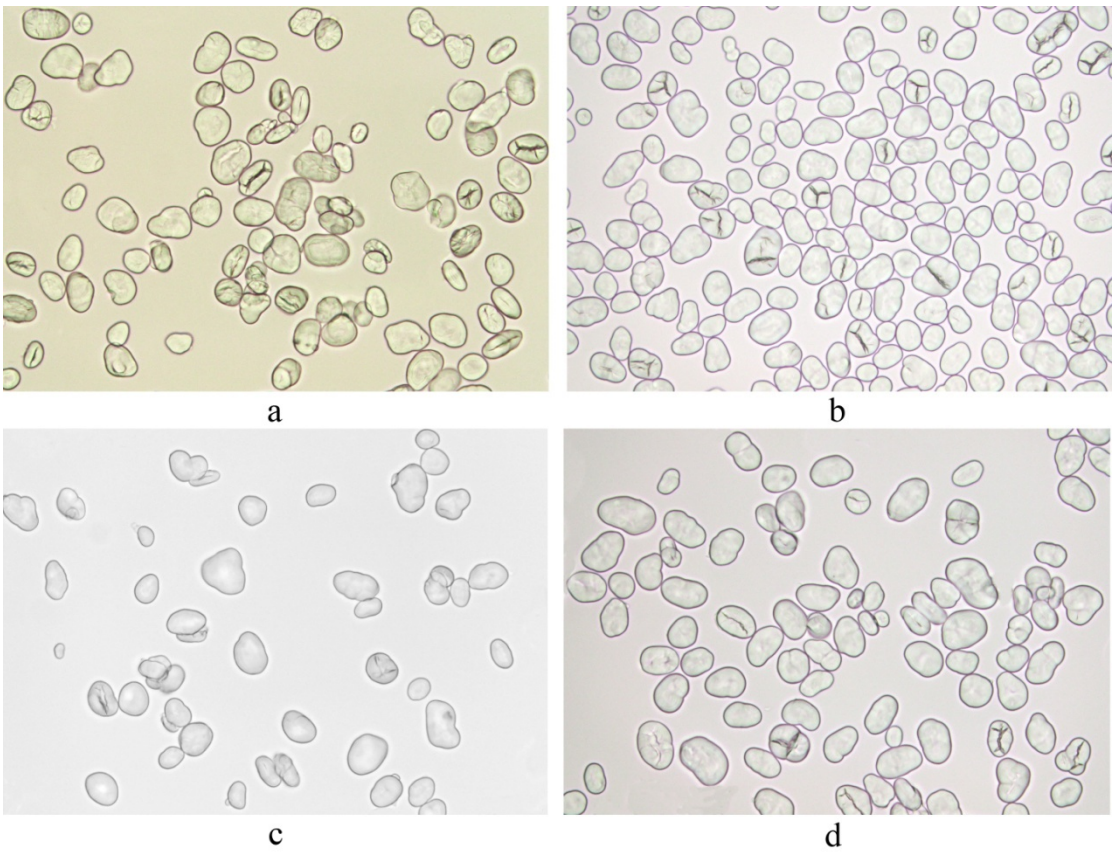

Figure 4.1 Bright field optical micrographs (x600) of raw pea starches and optimally extruded pea starch phosphates (from NutriPea and Meelunie): (a) raw NutriPea pea starch (NPS), (b) raw Meelunie pea starch (MPS), (c) optimally extruded NutriPea pea starch phosphate (NE8), (d) optimally extruded Meelunie pea starch phosphate (ME5).

It can be observed from Figure 4.1 (c: NE8, d: ME5) and Figure 4.2 (d: B2, g-h: NE8, m-o: ME5) that the extruded pea starch phosphates (B2, NE8 and ME5) under optimal conditions largely remain intact with distinct shapes regardless of starch type and processing equipment and as compared to their raw counterparts, NPS (Figure 4.1(a) and Figure 4.2(a-c)) and MPS (Figure 4.1(b) and Figure 4.2(j-l)). This was mainly attributed to the low shear screw design along with the optimized feed formulation and processing conditions of both extruder and batch mixer.

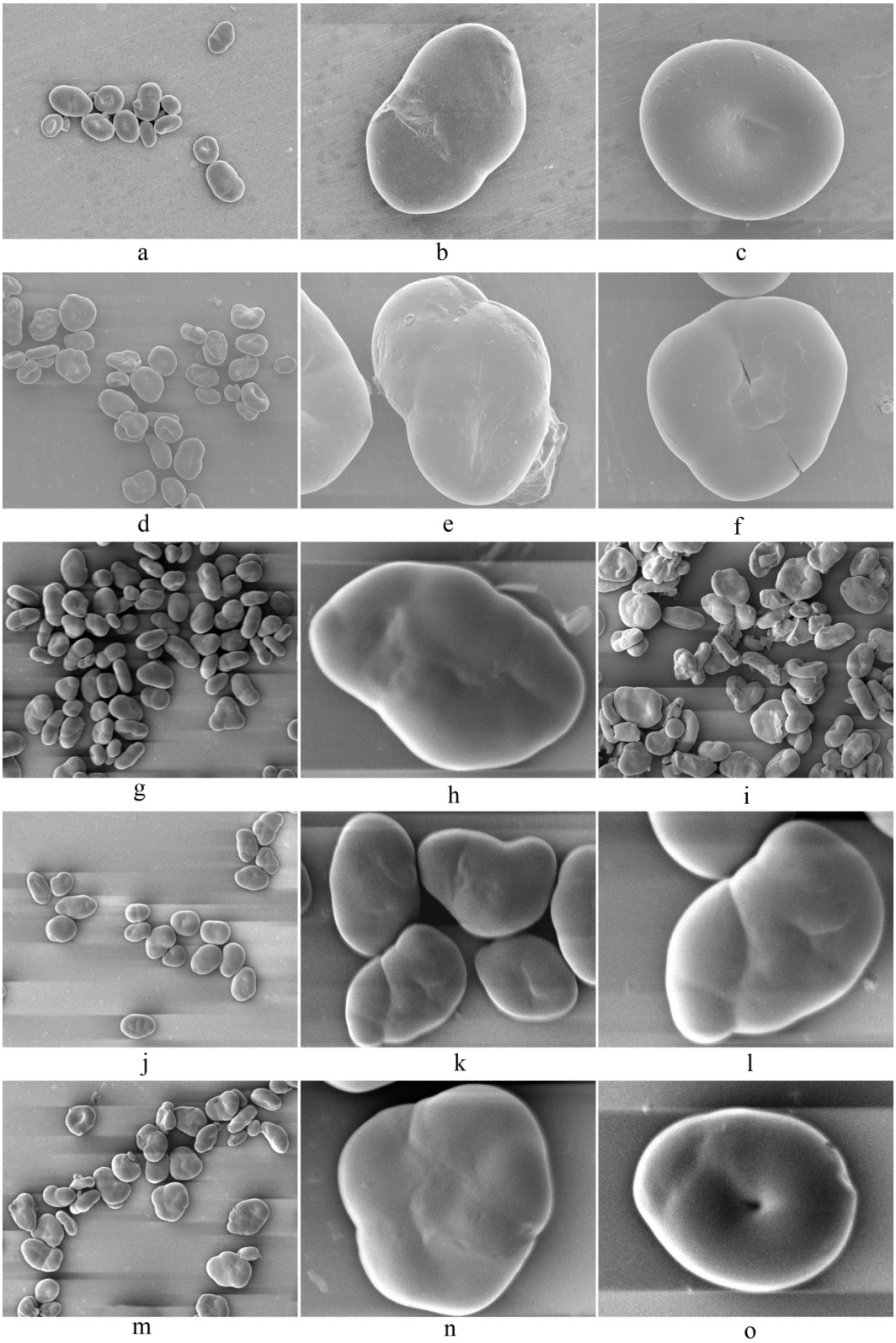

Figure 4.2 Scanning electron micrographs of raw pea starches (NPS and MPS) as well as the pea starch phosphates prepared in Haake batch mixer and extruder: (a-c) NPS (a: x400, b-c: x3000); (d) B2 (x400); (e-f) B1 (x3000); (g-h) NE8 (g: x400, h: x3000); (i) NE26 (x400); (j-l) MPS (j: x400, k: x1500, l: x3000); (m-o) ME5 (m: x400, n-o: x3000)

Table 4.1 Statistical data of granule diameter of raw NutriPea pea starch

Stats	Feret Diameter[A)] (μm)	Major Axis length (μm)	Minor Axis Length (μm)
Mean	28.3	31.8	25.3
Std Dev	6.0	7.4	5.8
Std Error	0.3	0.4	0.3
95% CI	0.6	0.8	0.6
99% CI	0.8	1.0	0.8
Count	356	356	356
Min	12.3	12.3	12.3
Max	47.1	61.5	47.1

A)Assuming all granules are ellipsoidal, $\text{Feret Diameter} = \sqrt{\text{Mjaor Axis Length} \times \text{Minor Axis length}}$;

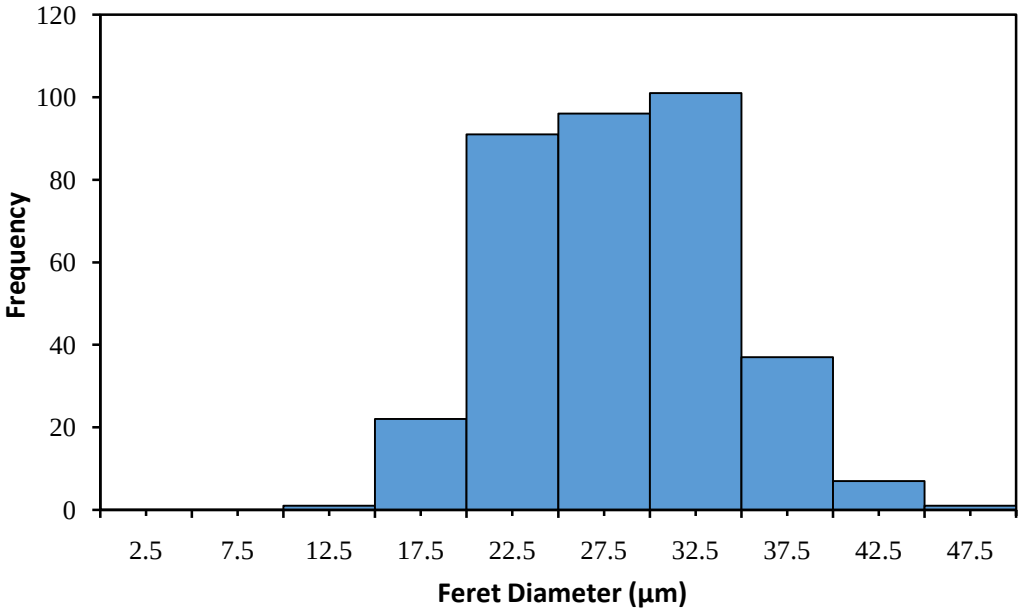

Figure 4.3 Histogram of Feret diameter showing size distribution of raw NutriPea pea starch granules. Frequency refers to number of granules in a given granular size range.

Table 4.2 Statistical data of granule diameter of raw Meelunie pea starch

Stats	Feret Diameter[A)] (μm)	Major Axis length (μm)	Minor Axis Length (μm)
Mean	26.7	30.8	28.3
Std Dev	5.1	6.5	4.6
Std Error	0.4	0.5	0.3
95% CI	0.7	0.9	0.6
99% CI	1.0	1.2	0.8
Count	193	193	193
Min	15.4	16.4	12.3
Max	40.8	51.3	36.9

A) Assuming all granules are ellipsoidal, $\text{Feret Diameter} = \sqrt{\text{Mjaor Axis Length} \times \text{Minor Axis length}}$;

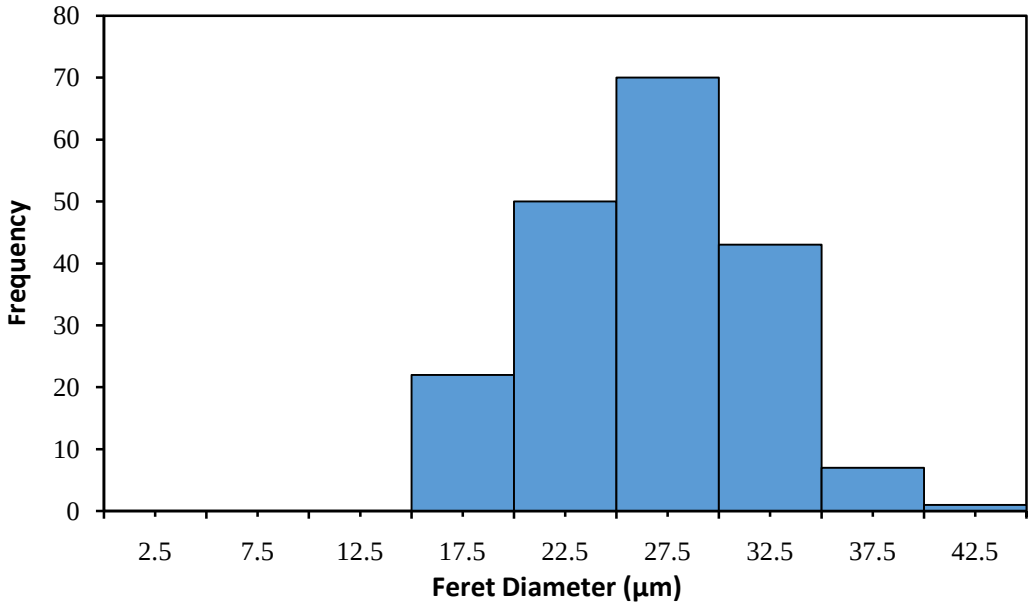

Figure 4.4 Histogram of Feret diameter showing size distribution of raw Meelunie pea starch granules. Frequency refers to number of granules in a given granular size range.

The birefringence patterns of the raw NutriPea pea starches and various cross-linked NutriPea pea starches are presented in Figure 4.5. Compared with raw NutriPea pea starch (Figure 4.5 (a)), the granules of solution cross-linked (Figure 4.5 (b-c)) and optimally extruded NutriPea pea starch phosphates (Figure 4.5 (d and f)) displayed desirable strong birefringence patterns which suggested that the granule integrity was maintained and gelatinization could be inhibited under optimized reactive extrusion conditions just as was found with the solution method. However, pea starch phosphate (B1) produced with 60% feed moisture and 5% STMP/STPP in batch mixer exhibited weak birefringence pattern which indicated minor gelatinization and shear damage, evident in Figure 4.2 (e-f). Because of relatively long time (5min) for bulk processing in the batch mixer, some pea starch granules were found broken (Figure 4.2f) or partly fused to another granule's wall (Figure 4.2e). It was noteworthy that NutriPea pea starch phosphate NE26 (Figure 4.2(i)) prepared

under less-than-desirable extrusion conditions (1.02kg/h, 150rpm and screw design #2) indicated considerable starch damage in the forms of being partly melted or showing broken granules as well as fragmented or distorted granules. The damage was considered to be linked to the excessive mixing with screw design #2. The influence of screw design on the properties of pea starch phosphates will be further discussed in Section 4.3.5.

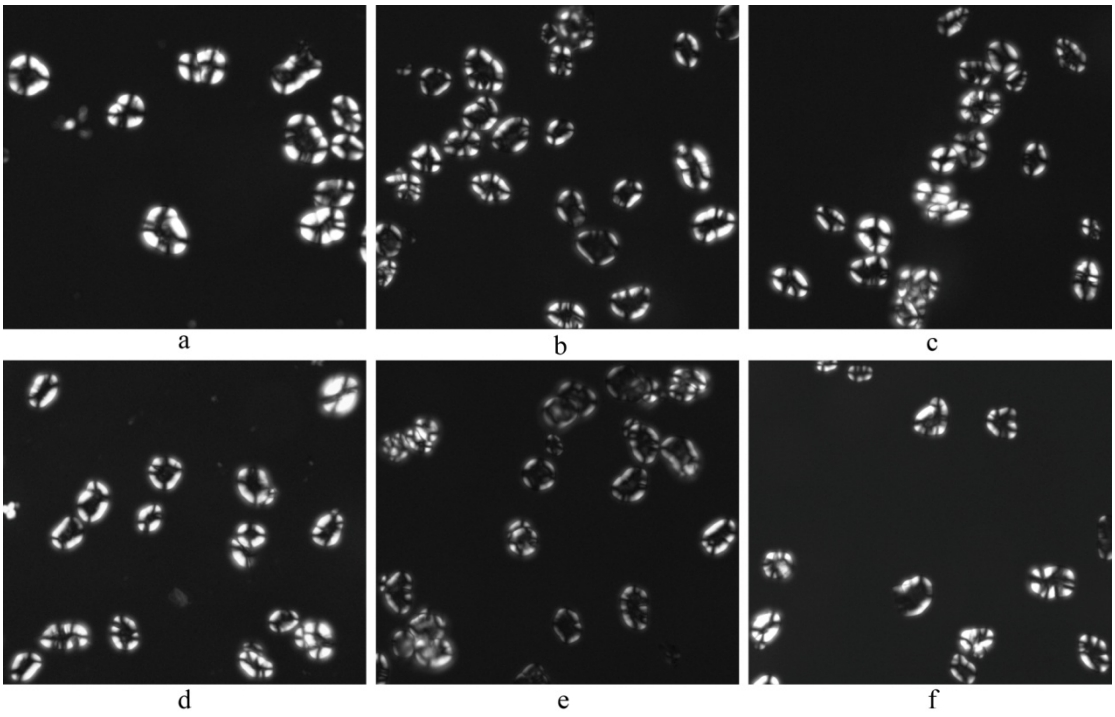

Figure 4.5 Polarized light microscopy (x600) of raw NutriPea pea starch and varied NutriPea pea starch phosphates prepared in aqueous slurry, Haake batch mixer and extruder: (a) NPS, (b) C1, (c) C2, (d) NE8, (e) B1, (f) B2.

4.2 The bound phosphorus content

4.2.1 Effects of feed formulation on phosphorus content

4.2.1.1 Effect of pH on phosphorus content

The phosphorus contents of the NutriPea pea starch samples prepared at two pH levels in Haake batch mixer are presented in Table 4.3. The phosphorus in modified

starch for food use is regulated by the Code of Federal Regulation (CFR, 2001) of the U.S. Food and Drug Administration. If STMP/STPP is chosen to phosphorylate starch for food use, the modified starch cannot contain more than 0.4% phosphorus.

Table 4.3 Phosphorus contents of NutriPea pea starch phosphates prepared at two pH levels in Haake batch mixer

Sample[A)]	99:1 (w/w) STMP/STPP (wt%)	pH of CL Reagents Solution	CL Conditions	Phosphorus[B)] (%)
A1	1	**11.5**	95°C, 20min, 50rpm	**0.013**
A2	3	**11.5**		**0.016**
A3	5	**11.5**		**0.022**
A4	7	**11.5**		**0.032**
A5	10	**11.5**		**0.057**
B1	5	**12.9**	95°C, 5min, 50rpm	**0.264**
B2	10	**12.9**		**0.452**
B3	5	**12.9**	95°C, 20min, 50rpm	**0.269**
B4	10	**12.9**		**0.454**

A). The feed formulations of all the nine samples employed 3% Na_2SO_4;

B). Results are means of duplicates. The average standard deviation was <2% of the mean value.

Two important trends can be observed from Table 4.3. First, bound phosphorus content increased with increased concentration of STMP/STPP which is in agreement with the research of Woo & Seib (2002) and Shi et al. (2013). More importantly, the pH level of cross-linking reagents solution showed a drastic effect on the phosphorus content of pea starch phosphates. Under very high pH (12.9) alkaline condition, the phosphorus content of the pea starches phosphates was multiplied in comparison to that of their counterparts phosporylated under medium high pH (11.5) alkalinity. Hence, using a very high alkalinity (pH 12.9) for bulk phosphorylation of pea starch is critical to achieve high phosphorus incorporation in short reaction time. This

observation is consistent with the findings of Sang et al. (2007) on aqueous solution phosphorylated wheat starch. Specifically, they reported that phosphorylation of wheat starch at pH 10.5 was much slower than phosphorylation at pH 11.5 and 12.5. STMP concentration had to be almost tripled at pH 10.5 in order to prepare wheat starch phosphate with 0.4% phosphorus compared to the reaction done at pH 11.5. At pH 10.5, less than 1% of hydroxyl groups on starch would be ionized compared to 5-10% ionized at pH 11.5. Sang et al. (2007) proposed that ionized hydroxyl groups on starch rather than the protonated hydroxyl groups react more rapidly with STMP.

4.2.1.2 Effect of feed moisture content on phosphorus content

Table 4.4 Phosphorus contents of extruded pea starch phosphates prepared with different feed moisture contents

Sample[A)]	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP(wt%)	Na_2SO_4 (wt%)	Phosphorus[B)] (%)
NE12	**40**	3	1	**0.115**
NE16	**47**	3	1	**0.158**
NE8	**40**	5	1	**0.143**
NE17	**47**	5	1	**0.181**
NE13	**40**	7	1	**0.162**
NE18	**47**	7	1	**0.232**
NE19	**50**	7	1	**0.256**
NE23	**40**	7	0	**0.187**
NE24	**47**	7	0	**0.221**
ME5	**40**	7	1	**0.183**
ME6	**47**	7	1	**0.265**

A). All the samples are extruded at 1.02kg/h and 150rpm with screw design#1.

B). Results are means of duplicates. The average standard deviation was <2% of the mean value.

It can be seen from Table 4.4 that increasing feed moisture content from 40%

to 47% or 50% markedly boosted the phosphorus content of extruded pea starch phosphates for both NutriPea and Meelunie pea starches. This observation is in agreement with the research of Seker & Hanna (2005) on corn starch. In contrast to the excessive amount of water used in conventional phosphorylation in aqueous slurry, the feed moisture content in the reactive twin-screw extrusion process had to be limited typically lower than 50% to favor the preparation of granular starch phosphate extrudates. Hence, higher feed moisture contents are obviously conducive to improve the reaction efficiency of phosphorylating reagents in the bulk state, resulting in higher phosphorus incorporation. However, powder texture was observed to deteriorate at higher feed moisture contents (47% and 50%) especially in the absence of Na_2SO_4 due to the higher extent of starch swelling and damage under high shear twin-screw extrusion environment. The negative influence of higher feed moistures will be further discussed in a later section 4.3.2. A content of 40% was found to be the optimal feed moisture to produce good granular pea starch phosphates.

4.2.1.3 Effect of STMP/STPP level on phosphorus content

The phosphorus contents of extruded NutriPea and Meelunie pea starch phosphates prepared with different STMP/STPP levels are summarized in Table 4.5. Phosphorus contents generally showed an upward trend with increased STMP/STPP levels, regardless of feed moisture contents, Na_2SO_4 levels and the type of pea starches; the ratio of STMP relative to STPP was fixed to 99:1 in this study. Thus, phosphorus incorporation positively correlated with the feed level of STMP/STPP.

Table 4.5 Phosphorus contents of extruded pea starch phosphates prepared with different STMP/STPP levels

Sample[A)]	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Phosphorus[B)] (%)
NE12	40	**3**	1	**0.115**
NE8	40	**5**	1	**0.143**
NE13	40	**7**	1	**0.162**
NE16	47	**3**	1	**0.158**
NE17	47	**5**	1	**0.181**
NE18	47	**7**	1	**0.232**
NE19	50	**7**	1	**0.256**
NE20	50	**10**	1	**0.282**
NE21	40	**3**	0	**0.116**
NE22	40	**5**	0	**0.138**
NE23	40	**7**	0	**0.187**
NE24	47	**7**	0	**0.221**
NE25	47	**10**	0	**0.245**
ME1	40	**3**	1	**0.155**
ME3	40	**5**	1	**0.177**
ME5	40	**7**	1	**0.183**

A). All the samples are extruded at 1.02kg/h and 150rpm with screw design #1.

B). Results are means of duplicates. The average standard deviation was <2% of the mean value.

4.2.1.4 Effect of sodium sulphate (Na_2SO_4) level on phosphorus content

The phosphorus contents of extruded NutriPea and Meelunie pea starch phosphates prepared with different sodium sulphate levels are presented in Table 4.6. As suggested by NE7, NE2, NE10, and NE22, NE8, NE4, and ME2, ME3, ME4, 1% was found to be the optimal Na_2SO_4 level for maximum phosphorus content without compromising good powder quality because Na_2SO_4 appeared to alleviate starch

damage by inhibiting starch gelatinization. Increasing Na_2SO_4 concentration from 1% to 3% or 5% substantially decreased the phosphorus content of extruded starch phosphates. Completely removing Na_2SO_4 from the formulation tended to jeopardize both phosphorus content and powder texture.

Table 4.6 Phosphorus contents of extruded pea starch phosphates prepared with different sodium sulphate levels

Sample	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Extrusion Conditions	Phosphorus[A)] (%)
NE7	40	5	**1**	1.02kg/h, 50rpm	**0.158**
NE2	40	5	**3**	1.02kg/h, 50rpm	**0.145**
NE10	40	5	**5**	1.02kg/h, 50rpm	**0.113**
NE22	40	5	**0**	1.02kg/h, 150rpm	**0.138**
NE8	40	5	**1**	1.02kg/h, 150rpm	**0.143**
NE4	40	5	**3**	1.02kg/h, 150rpm	**0.123**
NE24	47	7	**0**	1.02kg/h, 150rpm	**0.221**
NE18	47	7	**1**	1.02kg/h, 150rpm	**0.232**
ME2	40	5	**0**	1.02kg/h, 150rpm	**0.146**
ME3	40	5	**1**	1.02kg/h, 150rpm	**0.177**
ME4	40	5	**3**	1.02kg/h, 150rpm	**0.162**

A). Results are means of duplicates. The average standard deviation was <2% of the mean value.

The statistical script language, R, was employed to run analysis on the phosphorus data presented in Appendix B (Table B.1) which were extracted from Table 4.4-4.6. The significances of the three major ingredients on phosphorus incorporation are compared in a Pareto plot in Figure 4.6. It can be seen that feed

moisture was the most significant factor on the phosphorus content of extruded pea starch phosphates. Due to the competition for the limited amount of water during bulk phosphorylation, STMP/STPP and sodium sulphate exhibited negative interaction on the phosphorus content of extruded pea starch phosphates. Therefore, raising Na_2SO_4 concentration from 1% to 3% or 5% correspondingly reduced the amount of water available to STMP/STPP, leading to lower phosphorus content.

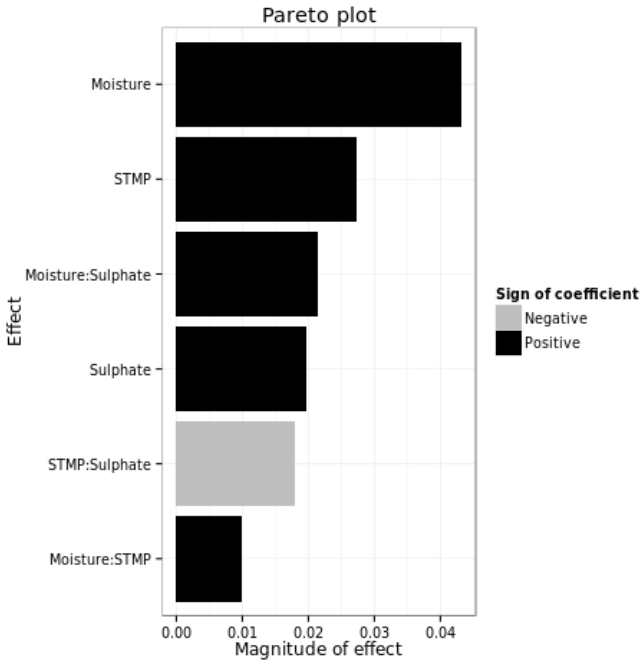

Figure 4.6 Statistical analysis result on ranking the significances of feed moisture content, STMP/STPP and sodium sulphate levels to the phosphorus content (multiple R^2= 0.9713, p=0.001)

4.2.2 Effects of extrusion conditions on phosphorus content

4.2.2.1 Effect of screw speed on phosphorus content

As indicated by NE1-NE5 in Table 4.7 which were extruded under the same feed formulation (40% moisture, 5% SMTP/STPP and 3% Na_2SO_4), increasing screw (#1) speed from 30rpm to 200rpm at a fixed feed rate (1.02kg/h) decreased both minimum and mean residence time, resulting in diminished phosphorus content which

is inconsistent with the previous observations of O'Brien and Wang (2009) on waxy corn and potato starch. Variance showed no correlation with the phosphorus content. The results showed that residence time played a dominant role herein phosphorus incorporation compared to mixing, likely because the screws were highly starved even at the lowest screw speed and the starch could only be dragged forward rather than moved back and forth between screws. NutriPea pea starch phosphates prepared at 30rpm experienced a much longer mean residence time of about 124s versus a mean residence time of approximately 26s for those prepared at 200rpm. Hence, extrusion at 30rpm achieved better phosphorylation efficiency and gave higher phosphorus content than extrusion at 200rpm for NutriPea pea starch samples phosphorylated under the same feed formulation.

Table 4.7 Phosphorus contents and calculated RTD parameters of extruded NutriPea pea starch phosphates (40% moisture, 5% STMP/STPP, 3% Na_2SO_4) prepared at five different screw speeds with screw design #1

Sample	Extrusion Conditions	Phosphorus[A)] (%)	Minimum Residence Time[B)](s)	Mean Residence Time[B)](s)	Variance[B)] (s^2)
NE1	1.02kg/h, **30rpm**	**0.152**	94	**124**	**230**
NE2	1.02kg/h, **50rpm**	**0.145**	58	**78**	**113**
NE3	1.02kg/h, **100rpm**	**0.135**	29	**45**	**137**
NE4	1.02kg/h, **150rpm**	**0.123**	20	**35**	**115**
NE5	1.02kg/h, **200rpm**	**0.098**	14	**26**	**67**

A). Results are means of duplicates. The average standard deviation was <2% of the mean value.

B). Results are means of duplicates. The average standard deviation was <4% of the mean value.

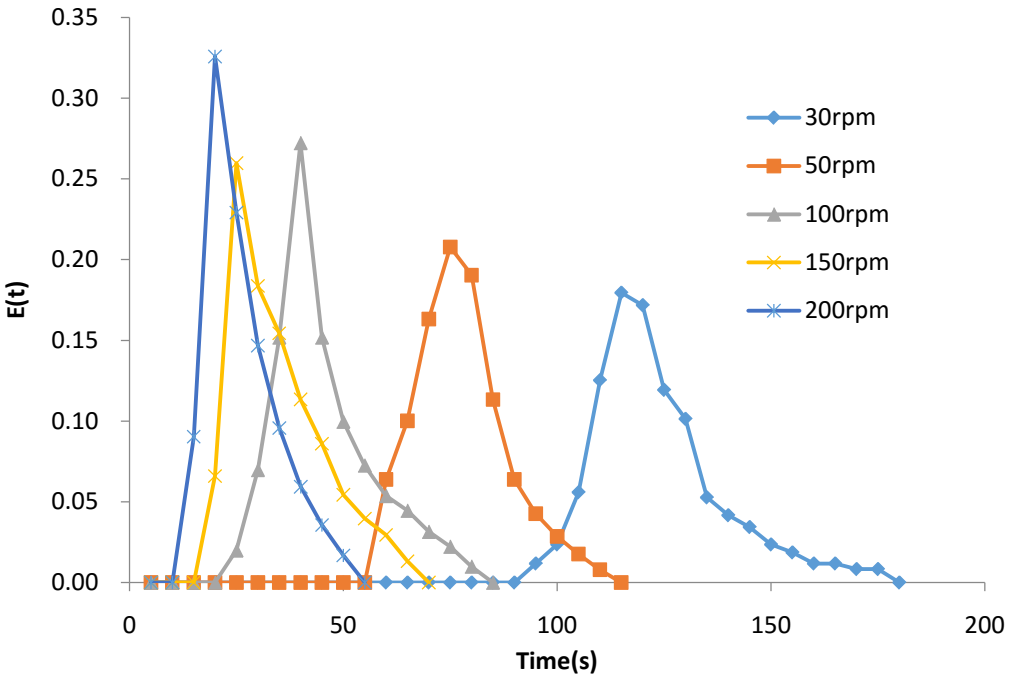

Figure 4.7 RTD of screw design #1 at a fixed feed rate (1.02kg/h) and five different screw speeds (30rpm to 200rpm)

4.2.2.2 Effects of feed rate and screw design on phosphorus content

The phosphorus contents and RTD parameters of extruded NutriPea pea starch phosphates prepared with the same feed formulation (40% moisture, 7%STMP/STPP, and 1% Na_2SO_4) and the same screw speed (150rpm) at three different feed rates with two different screw designs are presented in Table 4.8.

For both screw designs at a fixed screw speed (150rpm), it is interesting to note that high feed rate (3.06kg/h) yielded higher phosphorus content although increasing feed rate decreased both mean residence time and variance. This trend was particularly pronounced for screw design #2. Moreover, screw design #2 gave higher phosphorus contents than screw design #1. The main difference between screw design #1 and #2 is that two spacing rings were moved to zone 7 in screw design #2 (see Figure 3.1 and 3.2), giving rise to a significantly longer mean residence time, greater

time variance and higher phosphorus content. Because of the relatively high extrusion temperature (95°C), feed rate (mass flow rate) had counteracting effects on feed moisture and residence time. Low feed rate tended to bring about more evaporation of water at high temperature due to the lower degree to fill in the barrel. According to Figure 4.6, the feed moisture content was likely to be more important on phosphorus content than residence time. Thus, the starch phosphates extruded at high feed rate lost less water despite the shorter residence time, resulting in a higher phosphorus content.

Table 4.8 Phosphorus contents and calculated RTD parameters of extruded NutriPea pea starch phosphates (40% moisture, 7%STMP/STPP, and 1% Na_2SO_4) prepared at three different feed rates

Sample	Extrusion Conditions	Phosphorus[A] (%)	Minimum Residence Time[B](s)	Mean Residence Time[B](s)	Variance[B] (s^2)
NE13[C]	**1.02kg/h,** 150rpm	**0.162**	21	**50**	**518**
NE14[C]	**2.04kg/h,** 150rpm	**0.161**	21	**41**	**222**
NE15[C]	**3.06kg/h,** 150rpm	**0.177**	20	**33**	**83**
NE26[D]	**1.02kg/h,** 150rpm	**0.186**	25	**94**	**2014**
NE27[D]	**2.04kg/h,** 150rpm	**0.206**	25	**56**	**484**
NE28[D]	**3.06kg/h,** 150rpm	**0.217**	24	**45**	**210**

A). Results are means of duplicates. The average standard deviation was <2% of the mean value.

B). Results are means of duplicates. The average standard deviation was <4% of the mean value.

C). NE13-15 were extruded with screw design #1.

D). NE26-28 were extruded with screw design #2.

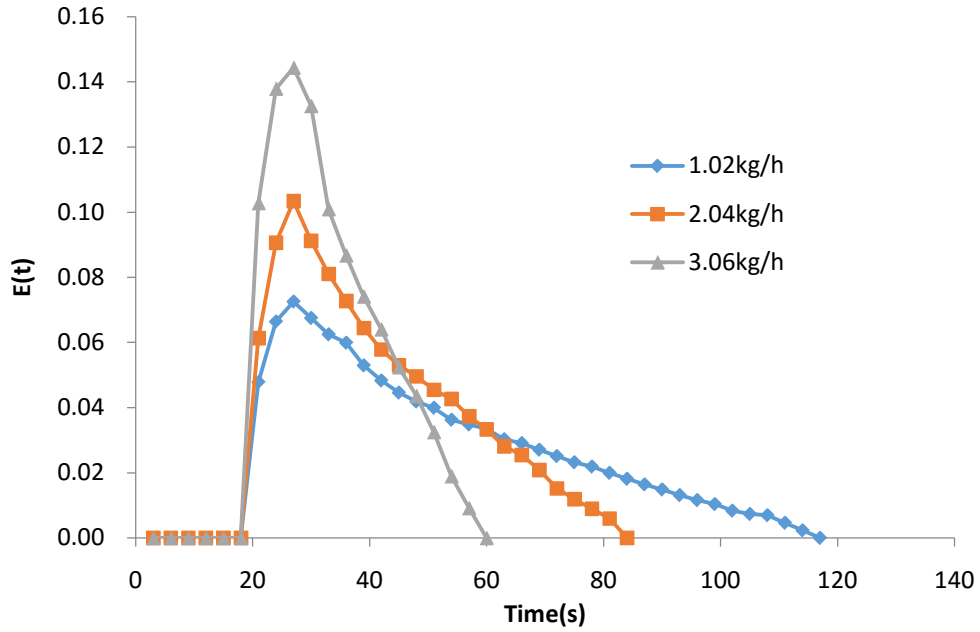

Figure 4.8 RTD of screw design #1 at a fixed screw speed (150rpm) and three different feed rates (1.02kg/h to 3.06kg/h)

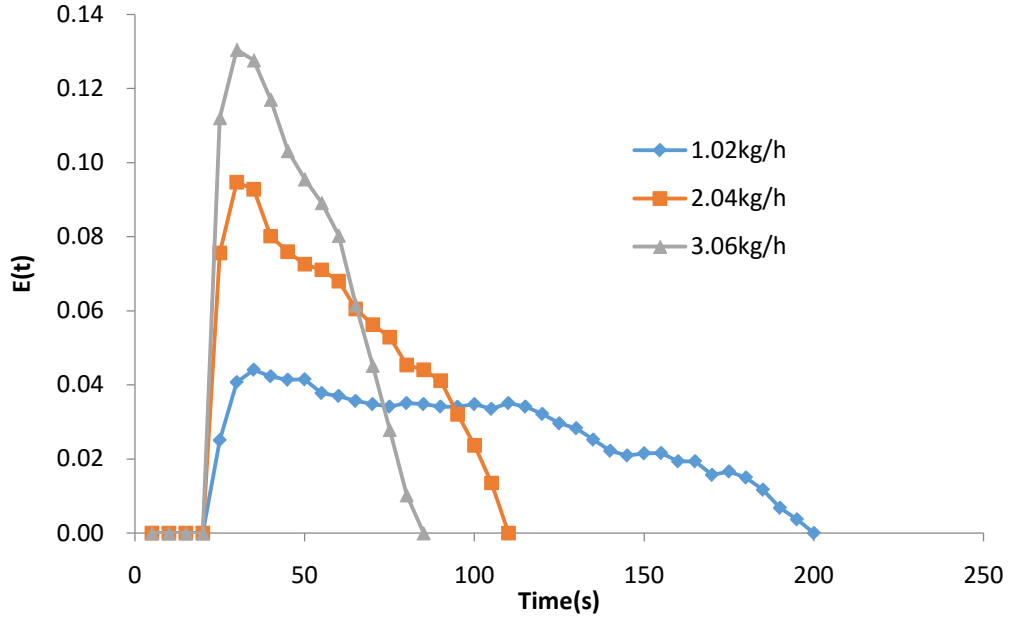

Figure 4.9 RTD of screw design #2 at a fixed screw speed (150rpm) but three different feed rates (1.02kg/h to 3.06kg/h)

4.2.2.3 Effect of foaming injection for in-line addition of CL reagent solution on phosphorus content

The phosphorus contents of the NutriPea pea starch phosphates prepared by foaming extrusion technology are outlined in Table 4.9. After several extrusion trials

on two candidate foaming agent, lecithin was found to be an appropriate food-grade foaming agent in this study. The other candidate foaming agent (hydroxypropyl methylcellulose) turned out to block the syringe pump due to its seemingly high reactivity with STMP. Lecithin was fully dissolved in the cross-linking reagent solution in a sealed glass bottle via vibrant magnetic stirring one night prior to extrusion.

Table 4.9 Phosphorus content of extruded NutriPea pea starch phosphates prepared via foaming injection of cross-linking reagent solution

Sample[A)]	Na_2SO_4 (wt%)[a]	Lecithin (wt%)	Extrusion Conditions[B)]	Flow Rate of CL Solution (mL/min)	Phosphorus[C)] (%)
NEF1	3	0.15	2.04kg/h, 150rpm	8.58	**0.012**
NEF2	3	0.15	2.04kg/h, 50rpm	8.58	**0.021**
NEF3	1	0.15	2.04kg/h, 150rpm	8.48	**0.013**
NEF4	1	0.15	2.04kg/h, 50rpm	8.48	**0.015**
NEF5	1	0.25	2.04kg/h, 150rpm	8.48	**0.014**
NEF6	1	0.25	2.04kg/h, 50rpm	8.48	**0.015**

A). The formulations of all the six samples used 40% feed moisture content and 5% 99:1 (w/w)STMP/STPP level.

B). All the samples were extruded with screw design #1.

C). Results are means of duplicates. The average standard deviation was <2% of the mean value.

As can be seen from Table 4.9, the one-step extrusion process featured by in-line foaming injection of cross-linking solution gave very low level (only one tenth) of phosphorus incorporation compared to the counterparts produced with two-step extrusion process in which CL reagent solution was premixed with pea starch prior to

extrusion. This is probably caused by the very inadequate mixing (in less than one minute) of pea starch and cross-linking reagent solution during foaming injection process. Moreover, the lecithin was likely to create a protective coating over the starch granules, hindering STMP/STPP access and phosphorus incorporation. As suggested by NEF3-NEF6, the concentration of lecithin had no effect on phosphorus content. Among these six samples (NEF1-NEF6), NEF2 exhibited the highest phosphorus content of 0.021% with satisfactory powder texture.

4.3 In vitro digestibility

The Englyst (1992) digestion profiles (in vitro digestibility) of the native pea starches, the non-phosphoryalted starch extrudate controls, as well as the various cross-linked pea starch phosphates prepared in the extruder, the batch mixer and aqueous slurry are summarized in Table 4.10. RDS is defined as the amount of starch digested in the first 20 min of a standard digestion reaction mixture. SDS is defined as the starch that is digested after the RDS but in no longer than 120 min under standard conditions of substrate and enzyme concentration. RS is defined as the starch or products of starch degradation that escapes digestion in the small intestine of healthy individuals and cannot be digested within 120 min, and may be completely or partially fermented in the large intestine as a substrate for the colonic microflora acting as a prebiotic material (Englyst et al., 1992).

RDS was thought to mainly represent amorphous starch fractions that occur in high amounts in freshly cooked or baked starchy foods (e.g., breads). SDS was

believed to be mostly associated with the physically inaccessible amorphous starch fractions as well as the optimal mix of amorphous and semi-crystalline materials in raw starches with A-type or C-type (e.g., pulses starches) crystalline pattern and B-type starches either in granule form or cooked retrograded form. In comparison, RS was believed to be mainly related to the crystalline regions for raw starches (Lehmann & Robin, 2007; Sajilata et al., 2006). In this study, the inherent RS fractions (i.e., amylopectin crystals) in native pea starches could be categorized to RS2. Further, RS4 was formed when pea starches were phosphorylated in bulk state or in aqueous slurry. In general, the α-amylase resistance of starches was thought to be influenced by a complex combination of various factors, including morphological & surface properties of starch granules, the extent of starch damage, as well as the composition (e.g., amylose/amylopectin ratio and protein content) and (molecular and supramolecular) structure of starch granules. At the molecular level, the crystallite structure (packing of amylopectin double helices), the packing of amorphous phase, the unit molecular chain length as well as the amylose-amylopectin interactions affected the enzymatic susceptibility (Lehmann & Robin, 2007; Tester et al., 2004b).

Table 4.10 Digestion profiles of native and cross-linked pea starch phosphates prepared in the extruder, batch mixer and aqueous slurry

Samples	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Cross-linking Conditions	Phosphorus[a] (%)	Digestion Profile[b] (Englyst method)		
						RDS(%)	SDS(%)	RS(%)
NPS[c]	N/A	N/A	N/A	N/A	**0.009**	52.49±0.39	28.84±0.21	**18.67±0.60**
NE0[d]	40	0	0	1.02kg/h, 95°C, 150rpm	**0.009**	79.30±0.58	3.83±0.82	**16.88±0.24**
NE1	40	5	3	1.02kg/h, 95°C, 30rpm	**0.152**	62.39±0.16	19.34±0.50	**18.27±0.66**
NE2	40	5	3	1.02kg/h, 95°C, 50rpm	**0.145**	61.63±0.16	20.66±0.39	**17.71±0.55**
NE3	40	5	3	1.02kg/h, 95°C, 100rpm	**0.135**	59.40±0.34	23.42±0.17	**17.18±0.51**
NE4	40	5	3	1.02kg/h, 95°C, 150rpm	**0.123**	57.23±0.17	24.90±0.03	**17.87±0.20**
NE5	40	5	3	1.02kg/h, 95°C, 200rpm	**0.098**	54.98±0.08	25.80±0.33	**19.22±0.41**
NE7	40	5	1	1.02kg/h, 95°C, 50rpm	**0.158**	65.81±0.16	16.09±0.25	**18.10±0.41**
NE8	40	5	1	1.02kg/h, 95°C, 150rpm	**0.143**	52.10±0.09	25.33±0.02	**22.57±0.11**
NE10	40	5	5	1.02kg/h, 95°C, 50rpm	**0.113**	60.75±0.25	21.69±0.47	**17.56±0.72**
NE11	47	7	3	1.02kg/h, 95°C, 50rpm	**0.182**	69.28±0.08	12.91±0.63	**17.81±0.71**
NE12	40	3	1	1.02kg/hr, 95°C, 150rpm	**0.115**	52.55±0.43	28.32±0.63	**19.12±0.19**
NE13	40	7	1	1.02kg/h, 95°C, 150rpm	**0.162**	51.35±0.14	27.31±0.36	**21.34±0.50**
NE15	40	7	1	3.06kg/h, 95°C, 150rpm	**0.177**	55.30±0.67	24.73±0.44	**19.98±0.23**
NE18	47	7	1	1.02kg/h, 95°C, 150rpm	**0.232**	61.45±0.51	18.14±0.06	**20.41±0.57**

Samples	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Cross-linking Conditions	Phosphorus[a] (%)	Digestion Profile[b] (Englyst method)		
						RDS(%)	SDS(%)	RS(%)
NE19	50	7	1	1.02kg/h, 95°C, 150rpm	**0.256**	71.84±0.39	10.78±0.10	**17.38±0.48**
NE22	40	5	0	1.02kg/h, 95°C, 150rpm	**0.138**	64.91±0.78	18.41±1.03	**16.68±0.25**
NE26	40	7	1	1.02kg/h, 95°C, 150rpm	**0.186**	72.76±0.12	7.52±0.12	**19.73±0.00**
NE28	40	7	1	3.06kg/h, 95°C, 150rpm	**0.217**	63.01±0.37	16.87±0.14	**20.12±0.52**
B1[e]	60	5	3	95°C, 5min, 50rpm	**0.264**	72.88±0.00	8.94±0.17	**18.18±0.17**
B2[e]	60	10	3	95°C, 5min, 50rpm	**0.452**	59.49±0.16	19.88±0.32	**20.63±0.48**
C1[f]	N/A	5	3	45°C, 5 h	**0.042**	53.00±0.08	30.82±0.20	**16.18±0.28**
C2[f]	N/A	10	3	45°C, 5 h	**0.086**	49.47±0.08	33.56±0.02	**16.97±0.10**
C3[f]	N/A	20	20	50°C, 9 h	**0.741**	17.23±0.17	39.77±0.01	**43.00±0.18**
MPS[g]	N/A	N/A	N/A	N/A	**0.009**	11.99±0.40	37.18±1.29	**50.83±1.69**
ME0[h]	40	0	0	1.02kg/h, 95°C, 150rpm	**0.009**	44.44±0.76	36.01±1.46	**19.55±2.22**
ME1	40	3	1	1.02kg/h, 95°C, 150rpm	**0.155**	27.43±0.04	42.63±0.39	**29.94±0.43**
ME3	40	5	1	1.02kg/h, 95°C, 150rpm	**0.177**	32.82±2.00	39.20±0.33	**27.98±2.33**
ME5	40	7	1	1.02kg/h, 95°C, 150rpm	**0.183**	26.42±0.32	42.23±0.22	**31.35±0.55**

a). Results are means of duplicates. The average standard deviation was <2% of the mean value; b). Results are means of triplicates ± standard deviations; c) Native NutriPea pea starch; d). Non-phosphorylated NutriPea pea starch extrudate control; e). Prepared in the Haake batch mixer; f). Prepared by solution cross-linking in aqueous slurry; g). Native Meelunie pea starch; h).Non-phosphorylated Meelunie pea starch extrudate control.

4.3.1 Effect of twin-screw extrusion cooking on in vitro digestibility

As suggested by Table 4.10 and Figure 4.10, twin-screw extrusion had significant negative impact on the SDS and RS content of native pea starches. Non-phosphorylated pea starch extrudates (NE0 and ME0) showed dramatically higher RDS content but lower SDS or RS content than their native counterparts (NPS and MPS). A substantial portion of initial SDS (around 25% in NPS) or RS (about 30% in MPS) was converted into RDS by extrusion cooking which is consistent with the recent observations of Sharma et al. (2015) on pulses starches and Sarawong et al. (2014) on green banana flour, respectively. Therefore, the increase in RDS content was the result of corresponding decrease in SDS and/or RS content of pea starches due to the shear damage during high temperature extrusion cooking. The drop in SDS or RS content could be attributed to the disruption of inaccessible amorphous fraction and crystalline structure (i.e., ordering and packing of double helices) induced by the relatively high shear and high heat-moisture (Chung et al., 2009) extrusion environments. The high initial resistance (RS2) of native pea starches was associated with restricted mobility of the molecule chains or individual glucose units being locked into a specific configuration. However, RS2 is vulnerable to be destroyed by thermal processing (Menezes et al., 2011). Also, the disrupted crystalline and blockets structures (Gallant et al., 1992) as well as the deteriorated surface morphologies (e.g., cracks and pores) and properties of starch granules could account for the decreased starch resistance after extrusion processing. Thus, non-reactive twin-screw extrusion process increased the enzymatic susceptibility of pea starch granules.

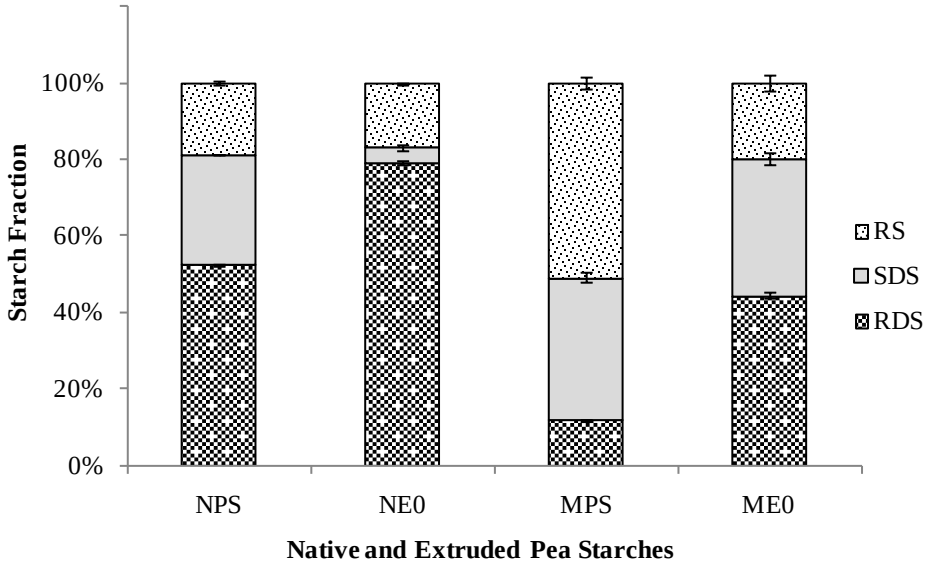

Figure 4.10 Effect of twin-screw extrusion (95°C, 1.02kg/, 150rpm) cooking on the digestion profiles of two types of native pea starches (NutriPea and Meelunie)

4.3.2 Effect of feed moisture content on in vitro digestibility

The effect of feed moisture content on digestion profiles of extruded NutriPea pea starch phosphates is presented in Figure 4.11. It was observed that lower feed moisture content (40%) gave significantly lower RDS content, higher SDS content and higher level of RS content compared with higher feed moisture content (50%). Hence, the lower feed moisture content (40%) is favorable for improving the SDS and RS content of extruded pea starch phosphates despite that it yielded lower phosphorus content compared to higher feed moisture content (50%). During bulk phosphorylation of pea starch in the extruder, feed moisture had complex effects on cross-linking and starch resistance. On one hand, increasing feed moisture boosted phosphorylation extent thus enhanced the formation of RS4. However, raising feed moisture increased the extent of starch gelatinization and degradation, lowering SDS and RS2 content. Due to the high level feed moistures used in this study, the negative effect of feed moisture on starch resistance was predominant. In comparison, Sharma et al. (2015) reported that increasing feed moisture at low levels (from 20% to 24%) did not lower the RS content of non-reactively twin-screw extruded field pea and

kidney bean starches. Sarawong et al. (2014) observed that extrusion cooking at high feed moisture (50%) increased the amylose content, which was positively correlated with RS content though at very low levels.

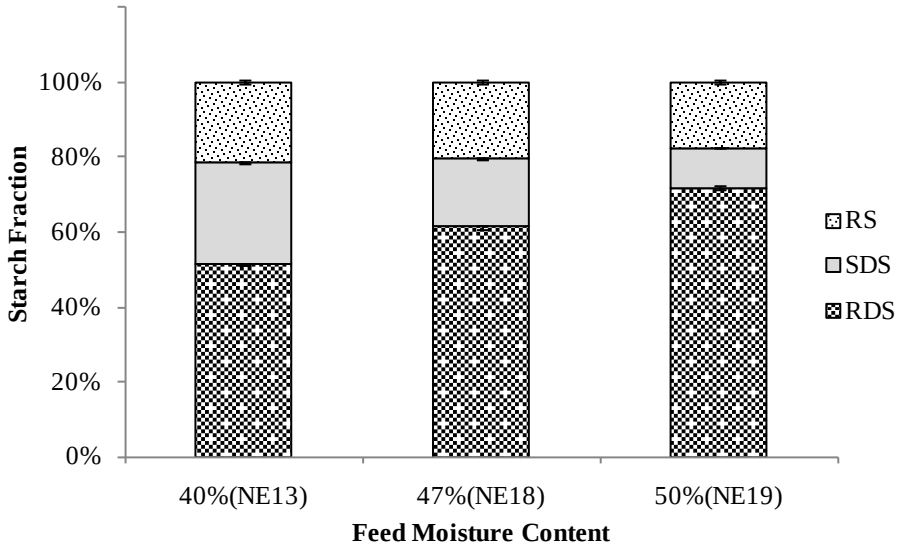

Figure 4.11 Effect of feed moisture content (40-50%) on the digestion profiles of extruded NutriPea pea starch phoshphates

4.3.3 Effect of STMP/STPP levels on in vitro digestibility

The digestion profiles of native pea starch, non-phosphorylated pea starch extrudate control and two phosphorylated pea starch extrudates were shown in Figure 4.12 (NutriPea) and Figure 4.13 (Meelunie). It was observed that the three phosphorylated NutriPea pea starch extrudates (NE12, NE8 and NE13) exhibited noticeably enhanced SDS and RS content compared with non-phosphorylated NutriPea pea starch extrudate control (NE0) (SDS: 3.83±0.82%; RS: 16.88±0.24%). However, NE8 and NE13 only displayed marginally improved RS content if compared to native NutriPea pea starch (SDS: 28.84±0.21%; RS: 18.67±0.60%) due to the counteracting effects of extrusion and phosphorylation. Furthermore, no significant positive correlation between the phosphorus content and RS content was observed for twin-screw extruded NutriPea pea starch phosphates. NE8 exhibited the highest RS content (22.57±0.11%) among all the twin-screw extruded NutriPea pea starch phosphates, followed by NE13 (21.34±0.50%). On the other hand, NE13 (RDS:

51.35±0.14%; SDS: 27.31±0.36%) exhibited a slightly better balanced digestion profile in comparison to NE8 (RDS: 52.10±0.09%; SDS: 25.33±0.02%) when RDS and SDS contents are taken into consideration as well.

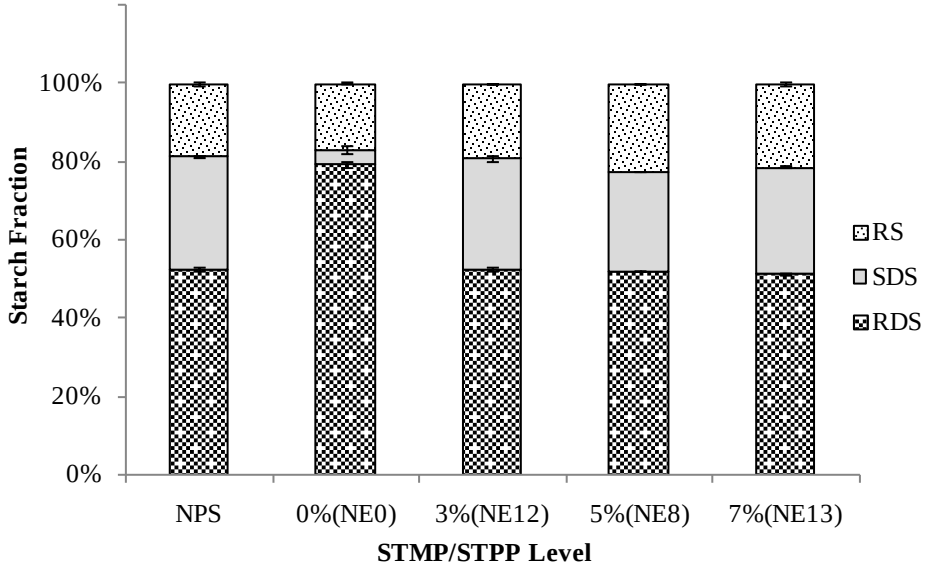

Figure 4.12 Effect of STMP/STPP levels (0-7%) on the digestion profiles of extruded NutriPea pea starch phoshphates

Similar to NutriPea, it can be seen that the three phosphorylated Meelunie pea starch extrudates (ME1, ME3 and ME5) exhibited significantly higher SDS and RS contents compared with non-phosphorylated pea starch extrudate control (ME0) (SDS: 36.01±1.46%; RS: 19.55±2.22%). However, all of them displayed diminished RS content if compared to native Meelunie pea starch (RS: 50.83±1.69%) which means the negative effect of extrusion was predominant here. ME5 exhibited the highest RS content (31.35±0.55%) among the three twin-screw extruded Meelunie pea starch phosphates. On one hand, ME5 displayed significantly higher SDS (42.23±0.22%) and greater RS (31.35±0.55%) contents as compared to non-phosphorylated extrudate control. On the other hand, ME5 displayed substantially diminished RS content but moderately higher level of SDS content if compared to native Meelunie pea starch (SDS: 37.18±1.29%; RS:50.83±1.69%).

Clearly, optimized bulk phosphorylation of pea starches via twin-screw

extrusion could achieve a significant but moderate increase either in RS content (from 18.67% to 22.57% for NutriPea) or in SDS content (from 37.18% to 42.23% for Meelunie) compared to their native counterparts. However, RS and SDS content could not be augmented simultaneously, which was associated with limited levels of phosphorylation reagents as well as the inevitable disruption of granular structure during extrusion cooking. The substantially dropped RS content of Meelunie pea starch suggested that the bulk phosphorylation by twin-screw extrusion could not lead to an increased level of RS for this type of native pea starch that exhibited high initial RS content.

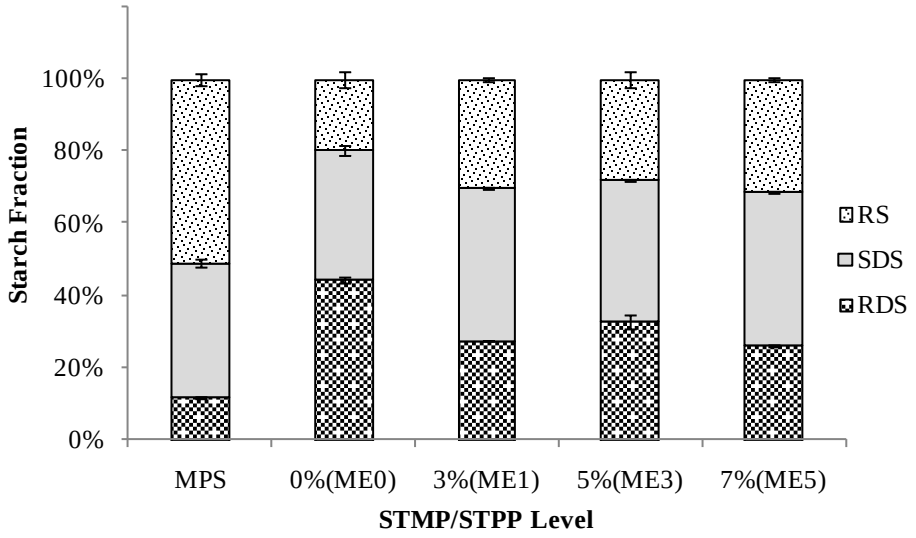

Figure 4.13 Effect of STMP/STPP levels (0-7%) on the digestion profiles of extruded Meelunie pea starch phoshphates

For comparison purpose, the digestion profiles of NutriPea pea starch phosphates prepared in Haake batch mixer and aqueous slurry with similar formulations (5% & 10% STMP/STPP, and 3% Na_2SO_4) are presented in Figure 4.14 and Figure 4.15, respectively. It was found that the pea starch phosphates prepared in Haake batch mixer exhibited lower RS content (B1: 18.18±0.17% and B2: 20.63±0.48%) and SDS content (B1: 8.94±0.17% and B2: 19.88±0.32%) compared to NE8 (RS content: 22.57±0.11%; SDS content: 25.33±0.02%) prepared in twin-screw extruder. This was attributed to the high feed moisture (60%) and long residence time

(5min) for the pea starch phosphates prepared with batch mixer. Moderately solution cross-linked pea starch phosphates displayed lower RS contents (C1: 16.18±0.28% and C2: 16.97±0.10%) but higher SDS contents (C1: 30.82±0.20% and C2: 33.56±0.02%) compared with the extruded sample (NE8) obtained under optimal conditions. This suggested that optimized reactive twin-screw extrusion could produce pea starch phosphate (NE8) with the higher level of RS but lower level of SDS in a very short reaction time (less than 1min) as compared to a lengthy solution cross-linking process. Only the most heavily solution cross-linked pea starch phosphate C3 exhibited both high SDS content (39.77±0.01%) and high RS content (43.00±0.18%) but at the costs of extremely high STMP/STPP and Na_2SO_4 concentrations as well as unpleasantly high phosphorus content (0.741%). Furthermore, as can be seen from Table 4.10 and Figure 4.15, there exists significant positive correlation between the SDS and RS content and phosphorus content of pea starch phosphates prepared in aqueous slurry, which is in agreement with the observations of Woo & Seib (2002) and Shi et al. (2013). This is because starch granules can really maintain intact when cross-linked in shearless aqueous slurry at relatively lower temperature (45°C or 50°C) over hours (5hours or longer).

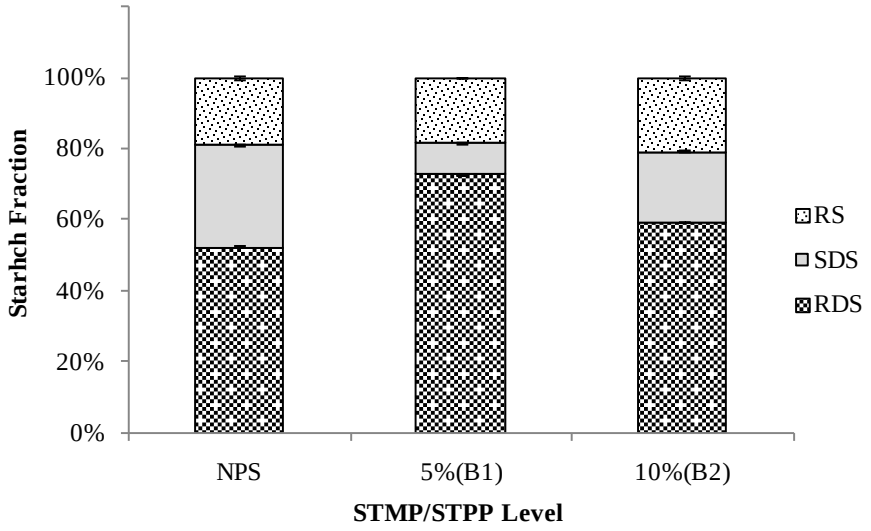

Figure 4.14 Effect of STMP/STPP levels (5-10%) on the digestion profiles of NutriPea pea starches phosphates prepared in batch mixer (95°C, 5min, 50rpm)

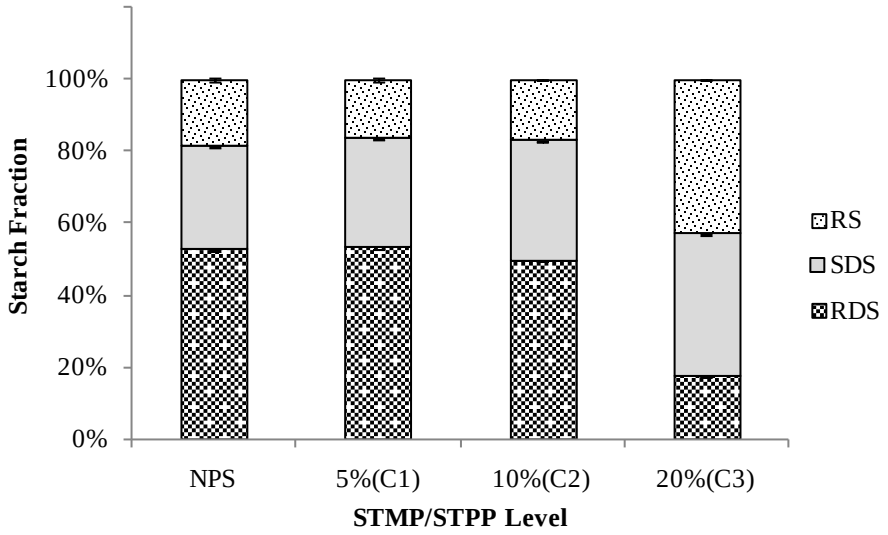

Figure 4.15 Effect of STMP/STPP levels (5-20%) on the digestion profiles of NutriPea pea starches phosphates prepared in aqueous slurry

4.3.4 Effect of Na_2SO_4 levels on in vitro digestibility

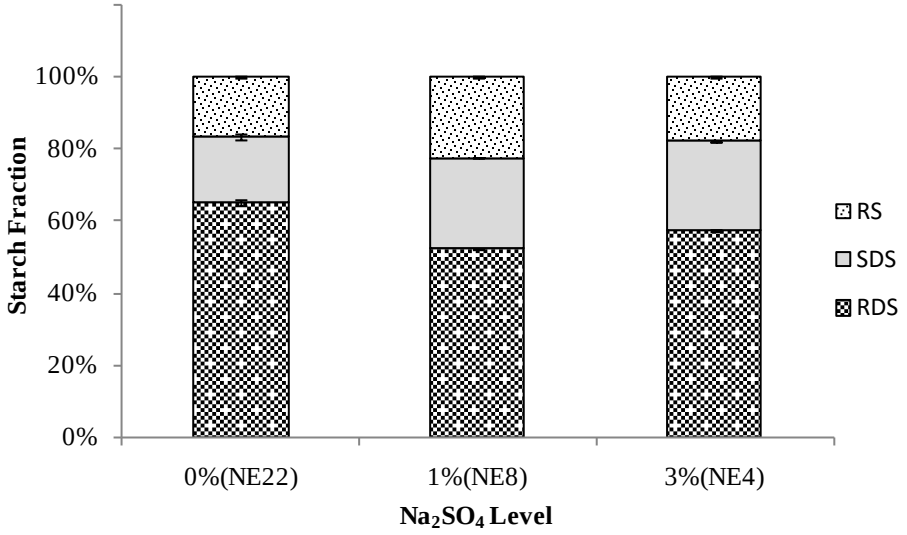

Figure 4.16 Effect of Na_2SO_4 levels (0-3%) on the digestion profiles of extruded NutriPea pea starch phosphates

The effect of Na_2SO_4 levels on digestion profiles of extruded NutriPea pea starch phosphates is shown in Figure 4.16. It can be seen that appropriately lower Na_2SO_4 level (1%) gave more desired digestion profile, i.e. decreased RDS content but increased RS content. Hence, the RS contents of NE8 and NE4 positively correlated to their phosphorus contents. Under 40% feed moisture and 5%

STMP/STPP, 1% appeared to be the optimal level of Na_2SO_4 to achieve the synergistic effect for maximal bulk phosphorylation efficiency and minimal swelling and gelatinization, bringing about an enhanced RS content.

4.3.5 Effects of reactive twin-screw extrusion conditions on in vitro digestibility

The effects of screw speed, feed rate and screw design on the digestion profiles of extruded NutriPea pea starch phosphates are illustrated in Figures 4.17 and 4.18. As displayed in Figure 4.17, RDS of extruded pea starch phosphates decreased with the increasing screw speeds with screw design #1 whereas SDS content showed the opposite trend, resulting in a statistically constant RS content from 30rpm to 200rpm. According to the RTD parameters in Table 4.7, the residence time and mixing both decreased with the increased screw speeds. The decreasing amount of particles fill the channel of the screw (i.e. low degree of channel fill) for higher screw speeds at a fixed flow rate gives the particles more freedom to roll and avalanche, minimizing any forces which would press them against the barrel and thus leading to diminished shear damaging to the starch granules. Thus, higher screw speeds gave higher SDS content; conversely, higher speeds corresponded with lower phosphorus content. As revealed in Figure 4.18, feed rates affected the digestion profiles differently between the two different screw designs. Low feed rate (1.02kg/h) gave relatively lower RDS content, lower phosphorus content and higher SDS & RS content with screw design #1; the opposite trend of phosphorus content versus SDS & RS is consistent whether testing screw speed or flow rate on screw design #1. Low feed rate (mass flow rate) means low degree of fill (just as higher screw speed did above) and longer residence time which led to more moisture loss, accounting for higher SDS and RS content due

to alleviated negative influence of feed moisture on starch resistance. Hence, low feed rate is desirable for improving SDS & RS content with screw design #1. By contrast, high feed rate (3.06kg/h) gave relatively lower RDS content, higher phosphorus content and higher SDS & RS content with screw design #2; this is contrary to screw design #1 as now SDS & RS are positively correlated with phosphorus content. The cause could be attributed to excessive mixing (i.e., shear damaging) at the ring section (zone 7) at low feed rate as indicated by the variance value (2014 s^2) in Table 4.8. At low feed rate, the extensive starch damage (NE26) caused by screw design #2 was confirmed by optical and SEM images shown in Figure 4.19. It can be seen that many starch granules of NE26 were distorted, fragmented or damaged very badly. For screw design #2, the negative influences of mixing and shear damaging seemed to be more important than the influence of feed moisture. Therefore, although screw design #2 gave higher phosphorus content, screw design #1 yielded higher SDS & RS content, preferably at low feed rate (1.02kg/h) and high screw speed (≥150rpm).

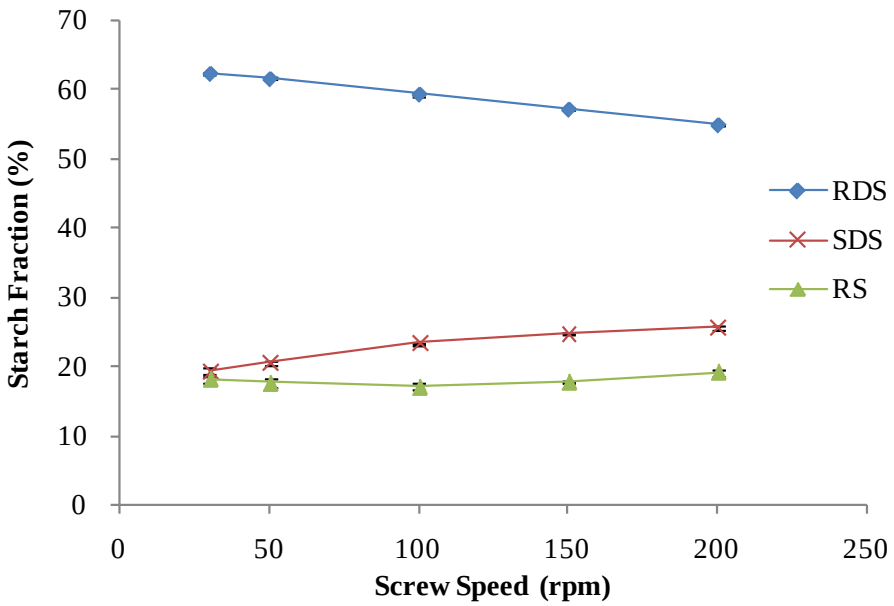

Figure 4.17 Effect of screw speeds (30-200rpm) on the digestion profiles of extruded NutriPea pea starch phosphates (NE1, NE2, NE3, NE4 and NE5)

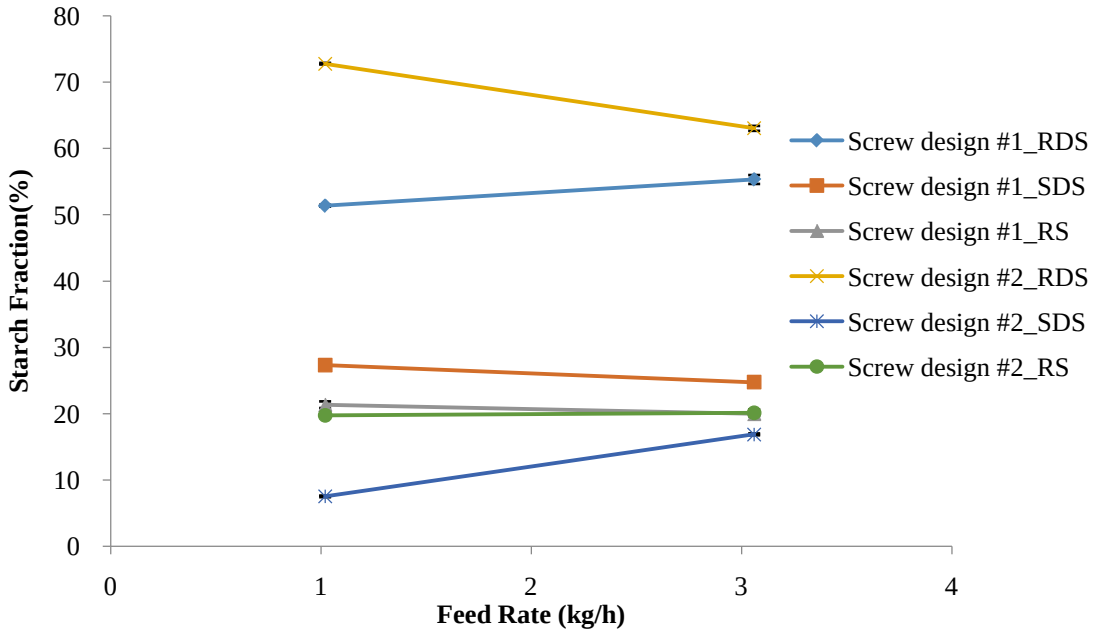

Figure 4.18 Effect of feed rates (1.02-3.06kg/h) on the digestion profiles of extruded NutriPea pea starch phosphates (NE13, NE15, NE26, and NE28)

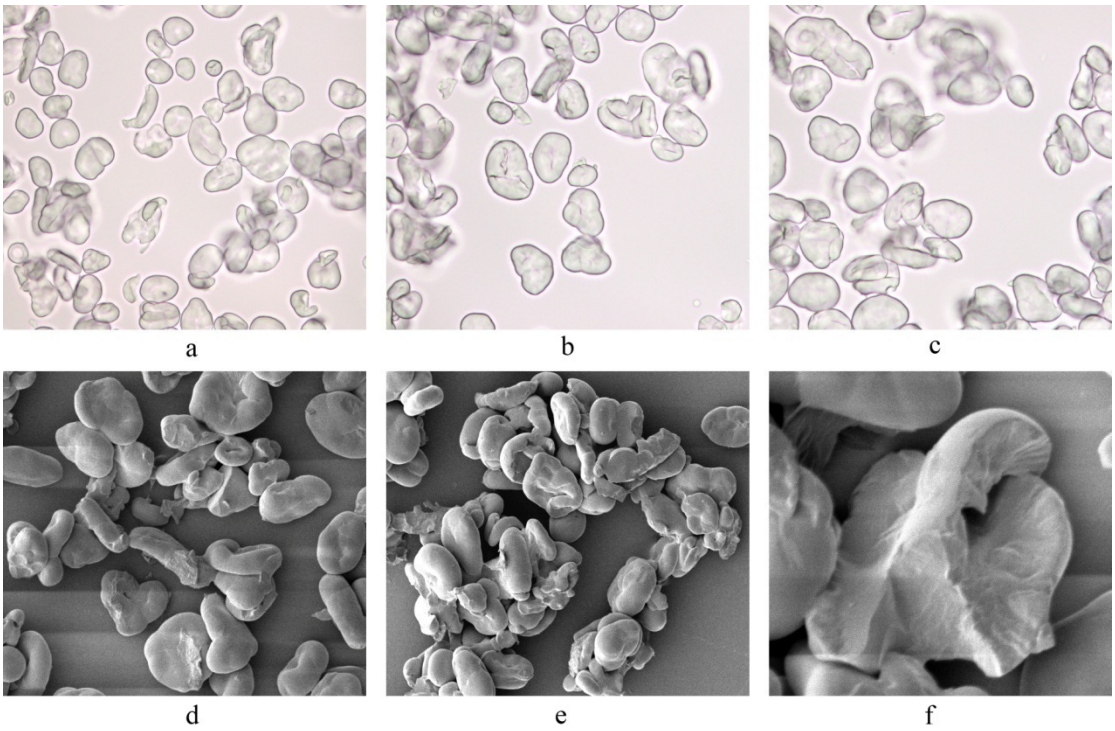

Figure 4.19 Bright field optical micrographs (a-c: x600) and scanning electron micrographs (d-e: x400, f: x3000) of most badly damaged NutriPea pea starch granules (NE26) due to the excessive mixing of screw design #2

The moisture losing rate of two types of pea starch materials (both with 40% feed moisture) in the extruder was simulated and determined at 95°C using the

Mettler-Toledo HG63 moisture analyzer. The linear regression results are illustrated for NutriPea and Meelunie in Figure 4.20 and Figure 4.21, respectively. As shown in the following two figures , the pea starch moisture in this study may decline 4.4-4.5% at 95°C in one minute. On one hand, pea starch material appears to lose moisture a bit faster in the moisture analyzer due to radiant heating. On the other hand, rolling and tumbling of pea starch material in the extruder would gently elevate heat transfer rates by conduction. Therefore, the water desorption rate of starch materials in moisture analyzer could largely reflect their real situation in the extruder. This means that extrusion conditions could affect the digestion profiles of extruded pea starch phosphates due to the change in RTD and consequent change in material moisture. In future studies, the end moisture of starch extrudates should be measured in order to verify the actual influence of various extrusion conditions on moisture losing rates of pea starch phosphates.

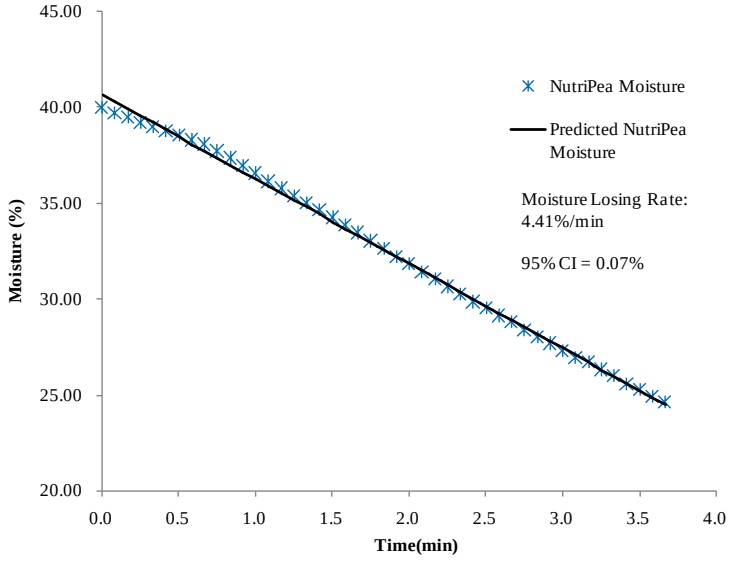

Figure 4.20 Regression line of moisture losing profile at 95°C in moisture analyzer for native NutriPea pea starch with 40% feed moisture

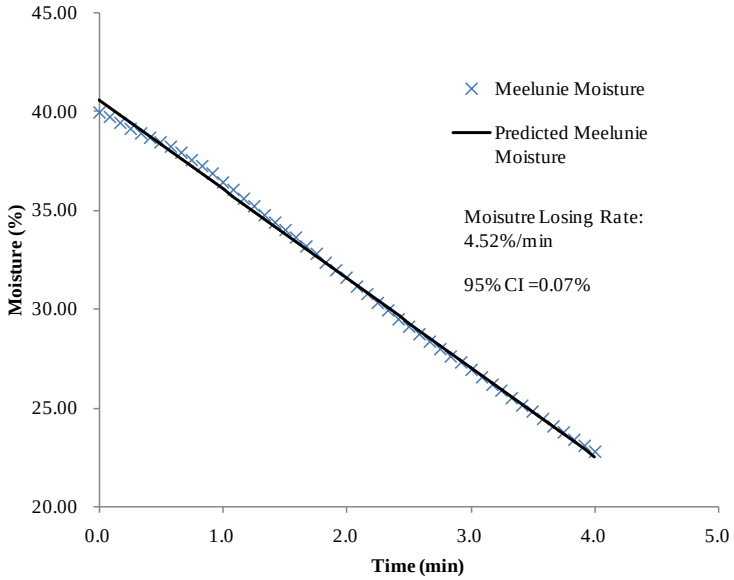

Figure 4.21 Regression line of moisture losing profile at 95°C in moisture analyzer for native Meelunie pea starch with 40% feed moisture

4.4 Thermal properties analysis

Table C.1 in Appendix C summarizes the gelatinization properties of all the pea starch samples in this study, including native pea starches, various pea starch phosphates produced by various methods, and non phosphorylated pea starch extrudate controls. Figure C.1 in Appendix C is an example DSC thermogram (NE5) which shows the typical gelatinization transition as well as the gelatinization parameters determined by DSC.

4.4.1 Effect of twin-screw extrusion cooking on gelatinization parameters

As indicated in Table 4.11, Meelunie native pea starch exhibited higher peak gelatinization temperature but same enthalpy value compared to NutriPea pea starch. It was interesting to note that the native pea starches in this research have generally shown a much smaller gelatinization enthalpy compared with many pulse starches reported by other researchers (Hoover et al., 2010; Maaran et al., 2014). When compared to native pea starches, the non-phosphorylated pea starch extrudates (NE0 and ME0) from NutriPea and Meelunie both revealed a shifting of the gelatinization

temperatures (T_o, T_p, and T_c) toward higher levels and a reduced transition enthalpy (ΔH). The increased gelatinization temperatures can be explained by the heat-moisture effect that the pea starch granules experienced during extrusion cooking. The heat-moisture effect was attributed to either re-crystallization of the small crystalline regions of the granules or new crystallization, because the presence of moisture and the elevated temperature would make polymer chains more mobile and boost the replacement of interchain hydrogen bonds by water molecules, giving rise to the growth of some crystalline regions. Such growth of crystalline regions will make imbibition of water more difficult and will raise the gelatinization temperature range. The diminished heat of gelatinization was caused by the shear damage of starch granules in the extruder (Lai & Kokini, 1991). This was particularly evidenced by the lowest enthalpy (0.47J/g) (Table C.1) and high digestibility (RDS: 72.76±0.12%, SDS: 7.52±0.12%) (Table 4.10) of NE26 which was found to have undergone the highest extent of granule damage due to extreme mixing (Table 4.8 and Figure 4.19).

Table 4.11 Effect of extrusion processing on gelatinization parameters

Sample	Feed moisture (wt%)	Extrusion Conditions[A)]	Gelatinization Parameters[B)]	
			T_p (°C)	ΔH (J/g)
NPS	N/A	N/A	**59.1**	**1.87**
NE0	40	1.02kg/h, 95°C, 150rpm	**64.6**	**0.64**
MPS	N/A	N/A	**64.3**	**1.84**
ME0	40	1.02kg/h, 95°C, 150rpm	**69.4**	**0.65**

A) NE0 and ME0 were prepared with screw design #1.
B) Results are means of duplicates. The average standard deviation of T_p was <1% of the mean value. The average standard deviation of ΔH was <5% of the mean value.

4.4.2 Effect of bulk phosphorylation (reactive twin-screw extrusion) and solution phosphorylation on gelatinization parameters

Table 4.12 outlined the gelatinization properties of the extruded pea starch phosphates prepared with the same feed moisture (40%) under the same extrusion conditions. Although the gelatinization parameters of the extruded pea starch phosphates were not all significantly different, they generally demonstrated

significantly higher transition enthalpy (ΔH) values as compared to their non-phosphorylated starch extrudate controls (NE0 and ME0). An increase in enthalpy was ascribed to a reduced molecular mobility within granule amorphous regions due to increasing numbers of molecular cross-links of phosphorylated pea starch phosphates (Bertolini, 2009).

Table 4.12 Effect of bulk phosphorylation (reactive twin-screw extrusion) on gelatinization parameters

Sample[A)]	Feed moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Gelatinization Parameters[B)]	
				T_p (°C)	ΔH (J/g)
NPS	N/A	N/A	N/A	**59.1**	**1.87**
NE0	40	0	0	**64.6**	**0.64**
NE4	40	5	3	**63.9**	**1.18**
NE12	40	3	1	**65.8**	**1.51**
NE8	40	5	1	**64.8**	**1.49**
NE13	40	7	1	**64.6**	**1.86**
NE21	40	3	0	**66.5**	**1.28**
NE22	40	5	0	**66.3**	**0.97**
NE23	40	7	0	**65.9**	**1.47**
MPS	N/A	N/A	N/A	**64.3**	**1.84**
ME0	40	0	0	**69.4**	**0.65**
ME1	40	3	1	**69.0**	**0.90**
ME3	40	5	1	**67.3**	**0.86**
ME5	40	7	1	**66.5**	**1.58**

A) All the extruded pea starch samples were prepared with the same feed moisture (40%) under the same extrusion conditions (95°C, 1.02kg/h and 150rpm).
B) Results are means of duplicates. The average standard deviation of T_p was <1%. The average standard deviation of T_o, T_c and ΔH were <5%. T_o, T_p, and T_c indicate the temperature of onset, peak and conclusion of gelatinization, respectively. ΔH= enthalpy of gelatinization (J/g of dry matter).

Table 4.13 Effect of solution phosphorylation on gelatinization parameters

Sample	99:1 (w/w) STMP/STPP (wt%)	Cross-Linking Conditions[A)]	Gelatinization Parameters[A)]	
			T_p (°C)	ΔH (J/g)
NPS	N/A	N/A	**59.1**	**1.87**
C1	5	45°C, 5 h	**64.7**	**1.21**
C2	10	45°C, 5 h	**63.9**	**0.90**
C3	20	50°C, 9 h	**62.6**	**2.03**

A) Results are means of duplicates. The average standard deviation of T_p was <1%. The average standard deviation of T_o, T_c and ΔH were <5%. T_o, T_p, and T_c indicate the temperature of onset, peak and conclusion of gelatinization, respectively. ΔH= enthalpy of gelatinization(J/g of dry matter).

As displayed in Table 4.13, the solution cross-linked pea starch phosphates (C1, C2 and C3) generally exhibited higher peak gelatinization temperature in comparison to their native counterpart. Cross-linking could impede cooperative melting of crystals in starch granules, which could be responsible for some of the increase in gelatinization temperature. This trend in gelatinization behavior is consistent with the research of Woo & Seib (2002) and Shi et al (2013).

4.4.3 Effect of STMP/STPP levels on gelatinization parameters

Table 4.14 Effect of STMP/STPP levels on peak gelatinization temperature

Sample[A)]	99:1 (w/w) STMP/STPP (wt%)	Phosphorus (%)	Gelatinization Parameters[B)]	
			T_p (°C)	ΔH (J/g)
A1	**1**	0.013	**65.7**	1.40
A3	**5**	0.022	**68.4**	0.81
A5	**10**	0.057	**70.8**	1.08
B1	**5**	0.264	**70.2**	1.21
B2	**10**	0.452	**62.8**	1.09
C2	**10**	0.086	**63.9**	0.90
C3	**20**	0.741	**62.6**	2.03
NE12	**3**	0.115	**65.8**	1.51
NE14	**7**	0.162	**64.6**	1.86
ME1	**3**	0.155	**69.0**	0.90
ME5	**7**	0.183	**66.5**	1.58

A) A1-A 5, B1-B2, C2-C3, NE12-NE14, and ME1-ME 5 were prepared under the same conditions, respectively, only differing by STMP/STPP levels.
B) Results are means of duplicates. The average standard deviation of T_p was <1% of the mean value. The average standard deviation of ΔH was <5% of the mean value.

As indicated in Table 4.14, peak gelatinization temperature increased with the increased level of STMP/STPP for lightly cross-linked pea starch phosphates (A1, A3 and A5) with low phosphorus content (<0.06%) which was prepared at pH 11.5 in batch mixer. Conversely, the peak gelatinization temperature declined with increased level of STMP/STPP for moderately and heavily cross-linked pea starch phosphates prepared in batch mixer (B1 and B2), in extruder (NE12, NE14, ME1, and ME5) and

in aqueous slurry (C2 and C3) which is inconsistent with the observation of Shi et al. (2013) on solution cross-linked pea starch. In general, there have been fairly conflicting observations in the literature about the influence of phosphorylation (cross-linking) on the thermal properties of starches. Gelatinization temperatures and/or enthalpies may remain unchanged or increase or decrease upon cross-linking, mainly depending on botanical sources of starches, cross-linking conditions, the degree of cross-linking as well as the sample preparation and testing method of DSC (Bertolini, 2009; Jyothi et al., 2006; Landerito & Wang, 2005; O'Brien et al., 2009; Shi et al, 2013; Woo & Seib, 2002).

4.4.4 Effect of Na_2SO_4 level on gelatinization parameters

As displayed in Table 4.15, extrusion sample (NE22) phosphorylated with the absence of Na_2SO_4 gave slightly higher peak gelatinization temperature compared with the counterpart (NE4) phosphorylated with 3% Na_2SO_4 which might be associated with the more pronounced heat-moisture effect of NE22.

Table 4.15 Effect of Na_2SO_4 levels on peak gelatinization temperature

Sample[A)]	Na_2SO_4 (wt%)	Phosphorus[B)] (%)	Gelatinization Parameters[C)]	
			T_p(°C)	ΔH (J/g)
NE22	**0**	0.138	**66.3**	0.97
NE8	**1**	0.143	**64.8**	1.49
NE4	**3**	0.123	**63.9**	1.18

A). All these three samples were prepared with the same feed moisture (40%) and STMP/STPP level (5%) under the same extrusion conditions (95°C, 1.02kg/h and 150rpm).
B). Results are means of duplicates. The average standard deviation was <2% of the mean value.
C). Results are means of duplicates. The average standard deviation of T_p was <1% of the mean value. The average standard deviation of ΔH was <5% of the mean value.

4.4.5 Effect of screw speeds on gelatinization parameters

As suggested by Table 4.16, high screw speed (NE5) gave a slightly lower peak gelatinization temperature compared to the very low screw speed (NE1), probably owing to the less pronounced heat-moisture effect at high screw speed (short

residence time).

Table 4.16 Effect of screw speeds on peak gelatinization temperature

Sample[A)]	Screw Speed (rpm)	Phosphorus[B)] (%)	Gelatinization Parameters[C)]	
			T_p(°C)	ΔH (J/g)
NE1	**30**	0.152	**65.0**	1.30
NE2	**50**	0.145	**64.2**	1.30
NE3	**100**	0.135	**64.1**	1.53
NE4	**150**	0.123	**63.9**	1.18
NE5	**200**	0.098	**63.1**	1.53

A). All these five samples were prepared with the same formulation (40% feed moisture, 5% STMP/STPP and 3% Na_2SO_4) under the same temperature (95°C) and feed rate (1.02kg/h).
B). Results are means of duplicates. The average standard deviation was <2% of the mean value.
C). Results are means of duplicates. The average standard deviation of T_p was <1% of the mean value. The average standard deviation of ΔH was <5% of the mean value.

4.5 RVA pasting profiles

The RVA pasting parameters and profiles of native NutriPea pea starch and extruded NutriPea pea starch phosphates are presented in Table 4.17 and Figure 4.22, respectively. It can be observed that non-phosphorylated NutriPea pea starch extrudate (NE0) showed much lower peak viscosity (PV), final viscosity (FV), breakdown viscosity (BDV) and setback viscosity (SBV) as compared with its native counterpart (NPS) which is in agreement with the result of Sharma et al. (2015). The reduced PV of extruded starch (NE0) compared to intact starch (NPS) could be attributed to partial degradation and gelatinization of starch during extrusion cooking. Lower BDV and FV of extruded starch might be related to the reduction of their re-crystallization tendency. However, most phosphorylated NutriPea pea starch extrudates (NE8, NE13, and NE15) exhibited higher PV, FV, and SBV in comparison to their non-phosphorylated control (NE0) which was attributed to the formation of intermolecular bonding due to extensive phosphorylation. Although not all the RVA parameters are significantly different, all non-phosphorylated or phosphorylated

NutriPea pea starch extrudates generally exhibited decreased BDV and increased pasting temperature, reflecting higher shear and thermal stability, which is consistent with the of results of Seker et al. (2003a, 2003b, 2005, 2006) and Sharma et al. (2015).

Table 4.17 RVA parameters of native NutriPea pea starch and extruded NutriPea pea starch phosphates

Sample	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Pasting Temp (°C)
NPS	1908±69	1594±71	315±141	3413±47	1820±118	9.5±0.4	69.0±0.8
NE0	1103±42	1099±38	4±4	1900±75	801±37	13.0±0.0	73.7.0±0.3
NE12	949±40	952±39	-3±1	1479±53	527±14	13.0±0.0	91.2±0.2
NE8	1833±16	1828±16	5±1	2908±12	1080±28	12.3±0.9	73.4±0.8
NE13	1699±1	1689±7	10±6	2756±6	1067±13	11.9±1.3	72.6±0.9
NE15	1603±9	1599±8	4±1	2548±8	949±17	12.7±0.4	73.3±0.2
NE18	1181±87	1178±93	3±6	1886±143	709±50	12.8±0.0	76.8±3.1
NE26	1708±11	1670±40	38±28	2763±18	1093±21	11.2±0.9	71.5±0.1
NE28	1654±0	1651±2	4±2	2733±8	1083±11	12.7±0.0	73.0±0.2

Note: All RVA results are means of duplicates ± standard deviations.

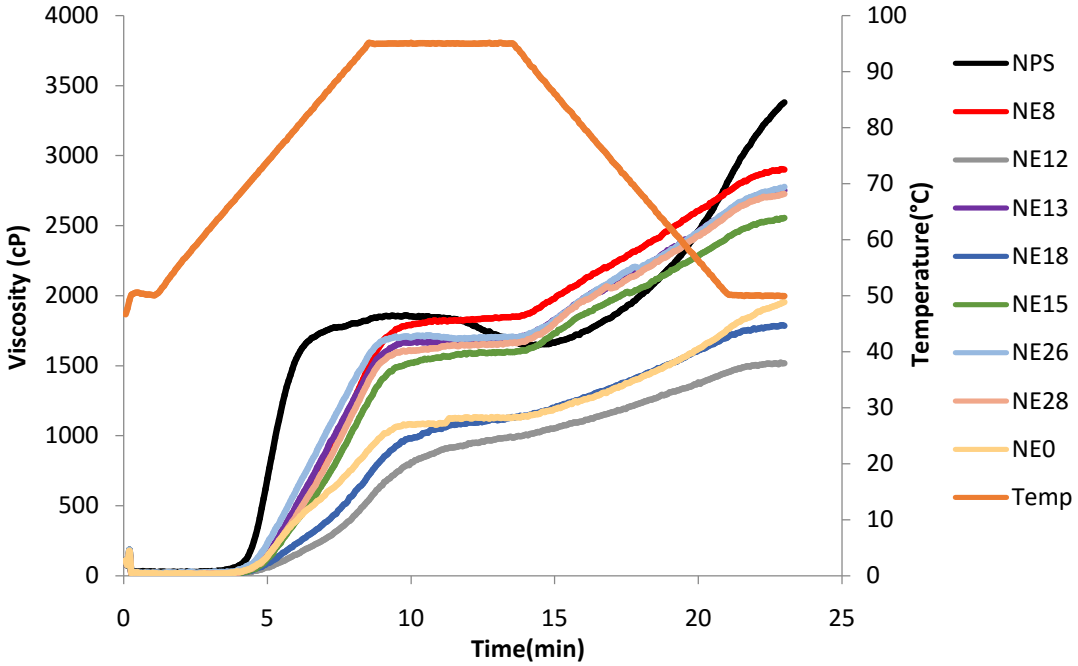

Figure 4.22 The RVA curves of native pea starch, non-phosphorylated pea starch extrudate control and extruded pea starch phosphates (all from NutriPea)

CHAPTER 5 CONCLUSIONS

5.1 Conclusions

This thesis explored the potential to produce granular pea starch phosphates with increased RS and/or SDS content using rapid and continuous reactive extrusion process. The bulk phosphorylation of pea starches (NutriPea and Meelunie) was investigated using a Haake batch mixer and Leistritz co-rotating intermeshing twin screw extruder. The effects of feed formulations and extrusion conditions on morphology, physicochemical and functional properties of bulk cross-linked pea starch phosphates were examined.

To establish optimal reactive extrusion conditions, the bulk phosphorylation of NutriPea pea starches was first studied in a Haake batch mixer. Using a strong alkaline (pH 12.9) cross-linking reagent solution was found to be critical to achieving a rapid (5min) and high phosphorus incorporation in bulk phosphorylation process (at 95°C). It was found that the feed moisture had to be limited to 60% to favor the production of granular material in the batch mixer. However, the most heavily (10% STMP/STPP) cross-linked pea starch phosphate prepared in the batch mixer showed only a slightly increased RS content from 18.67±0.60% to 20.63±0.48%% but significantly decreased SDS content from 28.84±0.21% to 19.88±0.32% compared to native NutriPea counterpart.

Based on the preliminary study in the batch mixer, the bulk phosphorylations of pea starches (NutriPea and Meelunie) were investigated in a Leistritz twin-screw extruder though emphasis was placed on NutriPea due to its good flowability. For comparison purpose, NutriPea pea starch was also phosphorylated in aqueous slurry. The effects of feed formulations and extrusion conditions on phosphorus

incorporation and Englyst digestion profiles were examined intensively. The phosphorus content and in vitro digestion profiles of the extruded pea starch phosphates were highly dependent on feed moisture content. Furthermore, enzyme resistance did not positively correlate with phosphorus content for extruded pea starch phosphates in contrast to their counterparts prepared in aqueous slurry. This was considered to be because extrusion cooking process markedly increased the susceptibility of pea starch granules to enzymatic digestion. Lower feed moisture content (40%) gave lower phosphorus content, significantly lower RDS content, and higher SDS and/or RS content. Bulk phosphorylation in the extruder resulted in decreased RS2 content but increased RS4 content. Screw design #1 was better than # 2 in terms of preparing resistant starch that matched the desire for highest RS & SDS content. High screw speeds (150rpm and 200rpm) and low feed rate (1.02kg/h) brought about higher yields of SDS and RS in spite of lower phosphorus incorporation.

As compared with non-phosphorylated NutriPea pea starch extrudate control (SDS: 3.83±0.82%; RS: 16.88±0.24%), 40% feed moisture, 5% STMP/STPP and 1% Na_2SO_4 were found to be the optimal formulation for NutriPea to give significantly higher SDS content (25.33±0.02%) and greater RS content (22.57±0.11%) when extruded at 1.02kg/h and 150rpm. However, the optimal NutrPea extrusion product displayed moderately improved RS content but marginally lower level of SDS content if compared to native NutriPea pea starch (SDS: 28.84±0.21%; RS: 18.67±0.60%) due to the counteracting effects of extrusion and phosphorylation. The optical and SEM micrographs confirmed that the granule integrity was largely retained after optimized reactive extrusion process. In comparison, 40% feed moisture, 7% STMP/STPP and 1% Na_2SO_4 were found to be the optimal reaction conditions for Meelunie, yielding

significantly higher SDS (42.23±0.22%) and greater RS (31.35±0.55%) contents as compared to non-phosphorylated extrudate control (SDS: 36.01±1.46%; RS: 19.55±2.22%). On the other hand, the optimal Meelunie extrusion product displayed substantially diminished RS content but moderately higher level of SDS content if compared to native Meelunie pea starch (SDS: 37.18±1.29%; RS:50.83±1.69%). Clearly, optimized bulk phosphorylation of pea starches via twin-screw extrusion could achieve a significant but moderate increase either in RS content (for NutriPea) or in SDS content (for Meelunie) compared to their native counterparts. However, RS and SDS content could not be augmented simultaneously, which was associated with limited levels of phosphorylating reagents as well as the inevitable disruption of granular structure during extrusion cooking. The substantially dropped RS content of Meelunie pea starch suggested that the bulk phosphorylation by twin-screw extrusion could not lead to an increased levels of RS for this type of native pea starch that exhibited high initial RS content

DSC was carried out to reveal the factors affecting the gelatinization properties of varied pea starch phosphates or extrudates. Extrusion cooking gave rise to increased gelatinization temperatures and decreased gelatinization enthalpy for both types of pea starches due to heat-moisture effect and shear damage, respectively. The peak gelatinization temperature indicated a positive relationship with STMP/STPP levels for lightly cross-linked pea starch phosphates but a negative relationship for moderately and heavily cross-linked pea starch phosphates. Higher screw speed or higher concentration of Na_2SO_4 resulted in lower peak gelatinization temperature due to less pronounced heat-moisture effect. No significant correlation was observed between gelatinization characteristics and Englyst digestion profiles. Evidenced by

RVA pasting profiles, NutriPea pea starch extrudates exhibited enhanced thermal and shear stability in comparison to their native counterpart.

A novel foaming injection technology of cross-linking reagents solution was pioneeringly introduced in order to uniformly coat all starch particles at the lowest moisture level possible during the continuous production of granular NutriPea pea starch phosphates. Yet, the resulting phosphorus incorporation was much lower than expected and would require further studies.

5.2 Recommendations

The effectiveness of using rapid bulk phosphorylation by twin-screw extrusion process to increase enzyme resistance of pea starches was relatively small. This could be associated with the complex counteractive influences of extrusion cooking and bulk phosphorylation. One suggestion about future work would be lowering the feed moisture to 30% to favor the formation of SDS and RS. This can be done by drying up the raw pea starch before mixing with cross-linking reagent solution to maximize the amount of additional water required for dissolving desired levels of STMP/STPP and Na_2SO_4. Meanwhile, end moisture of the starch extrudates should be monitored to keep track of the moisture loss in various reactive extrusion conditions. Also, non-intermeshing twin-screw extruder or single screw extruder equipped with very low-shear screw geometry could be utilized to minimize the shear damage on starch granules. Another suggestion would be to employ more characterization methods, such as XRD, NMR, alternative in vitro method like Prosky method (AOAC 991.43), swelling power test, and freeze-thaw stability test to better reveal and understand the relationships between the structure and functional properties of extruded pea starch phosphates.

REFERENCES

Akerberg AKE, Liljeberg HGM, Granfeldt YE, Drews AW & Bjorck IME (1998). An *in vitro* method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fiber. *Journal of Nutrition* 128, 651–660.

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., ... & Seetharaman, K. (2011). Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. *Food Research International*, 44(9), 2962-2974.

American Association of Cereal Chemist (1999). AACC Method 76-21.01 General Pasting Method for Wheat or Rye Flour or Starch Using the Rapid Visco Analyser.

American Association of Cereal Chemists (2000). Approved Methods of the AACC, 10th ed. The Association: St Paul, MN. Method 32-40.01

American Association of Cereal Chemists (2001). Approved Methods of the AACC, 10th ed. The Association: St Paul, MN. Method 40-75.01. Determination of Minerals by Inductively Coupled Plasma Spectroscopy

Association of Official Analytical Chemists (2000). Approved Methods of the AOAC, 17th ed. Method 991.43. Total dietary fiber enzymatic gravimetric method. First action. 2000. The Association: Gaithersburg, MD.

Association of Official Analytical Chemists (2002). Approved Methods of the AOAC, 17th ed. Method 2002.02. Total dietary fiber enzymatic gravimetric method. First action. 2002. The Association: Gaithersburg, MD.

Baldwin, P. M., Adler, J., Davies, M. C., & Melia, C. D. (1998). High resolution imaging of starch granule surfaces by atomic force microscopy. *Journal of Cereal Science*, 27(3), 255-265.

Belitz, H., Grosch, W., Schieberle, P., & SpringerLink (Online service). (2009). *Food Chemistry*, (4.). Berlin, Heidelberg: Springer Berlin Heidelberg.

BeMiller, J. N., & Whistler, R. L. (Eds.). (2009). *Starch: chemistry and technology*. Academic Press.

Bertolini, A. C. (2010). *Starches: Characterization, properties, and applications*. Boca Raton: Taylor & Francis.

Bertoft, E., Manelius, R., & Quin, Z. (1993). Studies on the structure of pea starches. Part 2. α-Amylolysis of granular wrinkled pea starch. *Starch*, 45, 258–263.

Bertoft, E. (2004). On the nature of categories of chains in amylopectin and their connection to the super helix model. *Carbohydrate Polymers*, 57, 211-224.

Bertolini, A. (Ed.). (2009). Starches: characterization, properties, and applications. CRC Press.

Blanshard, J. M. V. (1987). Starch granule structure and function: a physicochemical approach, in Starch: Properties and Potentials, Critical Reports on Applied Chemistry, Vol.13, ed. T. Galliard. John Wiley and Sons, Chichester, U.K., pp. 1654.

Blazek, J., & Gilbert, E. P. (2011). Application of small-angle X-ray and neutron scattering techniques to the characterization of starch structure: A review. *Carbohydrate Polymers*, 85(2), 281-293.

Blennow, A., Hansen, M., Schulz, A., Jørgensen, K., Donald, A. M., & Sanderson, J. (2003). The molecular deposition of transgenically modified starch in the starch granule as imaged by functional microscopy. *Journal of structural biology*, 143(3), 229-241.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International journal of biological macromolecules*, 23(2), 85-112.

CFR (Code of Federal Regulations) (2001). Food starch modified. In Food Additives Permitted in Food for Human Consumption, Title 21, Chapter 1, Part 172, section 172.892. US Government Printing Office, Washington.

Champ M, Martin L, Noah L & Gratas M (1999). Analytical methods for resistant starch. In *Complex Carbohydrates in Foods*, 169–187 [SS Cho, L Prosky and M Dreher, editors]. New York, NY: Marcel Dekker Inc.

Champ, M., Langkilde, A. M., Brouns, F., Kettlitz, B., & Bail-Collet, Y. L. (2003). Advances in dietary fibre characterisation. 2. Consumption, chemistry, physiology and measurement of resistant starch; implications for health and food labelling. *Nutrition research reviews*, 16(02), 143-161.

Chung, H. J., Liu, Q., Donner, E., Hoover, R., Warkentin, T. D., & Vandenberg, B. (2008). Composition, molecular structure, properties and in vitro digestibility of starches from newly released Canadian pulse cultivars. *Cereal Chemistry*, 85, 471–479.

Chung, H. J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, 75(3), 436-447.

Chung, H. J., Liu, Q., & Hoover, R. (2010). Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches. *Food research international*, 43(2), 501-508.

Cummings, J. H., & Englyst, H. N. (1995). Gastrointestinal effects of food carbohydrate. *The American journal of clinical nutrition*, 61(4), 938S-945S.

De Vries JW (2004) Dietary fibre: the influence of definition on analysis and regulation. *Journal of the Association of Official Analytical Chemists International*, 87 (3): 682–706.

Delval, F., Crini, G., Bertini, S., Morin-Crini, N., Badot, P. M., Vebrel, J., & Torri, G. (2004). Characterization of crosslinked starch materials with spectroscopic techniques. *Journal of applied polymer science*, 93(6), 2650-2663.

Dupuis, J. H., Liu, Q., & Yada, R. Y. (2014). Methodologies for increasing the resistant starch content of food starches: A review. Comprehensive R*eviews in Food Science and Food Safety*, 13(6), 1219-1234.

EEC (2000). Official Journal of European Communities 30.10.2000, L277, 52–59.

Eerling, R.C., Crombez, M., & Delcour, J.A. (1993). Enzyme-resistant starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chem*, *70*(3), 339-344.

Englyst, H. N., & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *The American journal of clinical nutrition*, 42(5), 778-787.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46, S33–S50.

Fannon, J. E., Gray, J. A., Gunawan, N., Huber, K. C., & BeMiller, J. N. (2004). Heterogeneity of starch granules and the effect of granule channelization on starch modification. *Cellulose*, 11(2), 247-254.

Fausto, F. D., Kacchi, A. I., & Mehta, D. (1997). Starch products in confectionery. *Beverage & Food World*, 24(4), 4–16.

Francis, F.J. and Clydesdale, F.M. (1975). Food Colorimetry: Theory and Applications, Van Nostrand Reinhold/AVI, New York

French, D.(1972). Fine structure of starch and its relationship to the organization of starch granules. *Denpun Kaguku* 19(1), 8–25.

French, D. (1984). Organization of the starch granules, in Starch: Chemistry and Technology, ed. R. L. Whistler, J. N. BeMiller and J. F. Paschall. Academic Press, Orlando, 183-247.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M. J., Sánchez-Zapata, E., & Pérez-Álvarez, J. A. (2010). Resistant starch as functional ingredient: A review. *Food Research International*, 43(4), 931-942.

Gallant, D. J., Bouchet, B., Bulion, A. and Perez, S. (1992). Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition*, 46, S3-S 16.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydrate Polymers*, 32(3), 177-191.

Goni I, Manas E, Garcia-Diz L, Saura-Calixto F (1996) Analysis of resistant starch: a method for food and food products. *Food Chemistry*, 56, 445–449.

Guerrero, L. A. C., & Betancur, A. D. (1998). Cross-linkage of canavalia ensiformis starch with adipic acid: Chemical and functional properties. *Journal of Agriculture and Food Chemistry*, 46, 2087–2091.

Hasjim, J., & Jane, J. L. (2009). Production of Resistant Starch by Extrusion Cooking of Acid-Modified Normal-Maize Starch. *Journal of Food Science*, 74(7), C556-C562.

Hasjim, J., Ai, Y., & Jane, J. L. (2013). Novel Applications of Amylose-Lipid Complex as Resistant Starch Type 5. *Resistant starch sources, applications and health benefits*, 79-94.

Helbert, W., & Chanzy, H. (1996). The ultrastructure of starch from ultrathin sectioning in melamine resin. *Starch/Stärke*, 48(5), 185-188.

Hizukuri, S., Kaneko, T., & Takeda, Y. (1983). Measurement of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochimica et Biophysica Acta (BBA)-General Subjects*,760(1), 188-191.

Hoover, R., & Manuel, H. (1996). Effect of heat–moisture treatment on the structure and physicochemical properties of legume starches. *Food Research International*,

29, 731–750

Hoover, R., & Ratnayake, W. S. (2002). Starch characteristics of black bean, chickpea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry*, 78, 489–498.

Hoover, R., & Sosulski, F. W. (1986). Effect of cross-linking on functional properties of legume starches. *Starch*, 38, 149–155.

Hoover, R., & Sosulski, F. W. (1991). Composition, structure, functionality and chemical modification of legume starches: A review. *Canadian Journal of Physiology and Pharmacology*, 69, 79–92.

Hoover, R., Hughes, T., Chung, H., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International*, 43, 399–413.

Hoover, R., & Zhou, Y. (2003). In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes – A review. *Carbohydrate Polymers*, 54, 401–417.

Huber, K. C., & BeMiller, J. N. (2001). Location of Sites of Reaction Within Starch Granules. *Cereal Chemistry*, 78(2), 173-180.

Huth, M., Dongowski, G., Gebhardt, E., & Flamme, W. (2000). Functional properties of dietary fibre enriched extrudates from barley. *Journal of Cereal Science*, 32(2), 115-128.

Jones, J.M .(2000). Update on defining dietary fiber. *Cereal Foods World*, 45, 219–220.

Juan, Z., & ZhengWu, W. (2009). Optimization of reaction conditions for resistant Canna edulis Ker starch phosphorylation and its structural characterization. *Industrial Crops and Products*, 30(1), 105-113.

Jyothi, A. N., Moorthy, S. N., & Rajasekharan, K. N. (2006). Effect of Cross-linking with Epichlorohydrin on the Properties of Cassava (Manihot esculenta Crantz) Starch. *Starch/Stärke*, 58(6), 292-299.

Kasemsuwan, T. and Jane, J.-L. (1994). Location of amylose in normal starch granules. II. Locations of phosphodiester cross-linking revealed by phosphorus-31 nuclear magnetic resonance. *Cereal Chem.* 71:282–287.

Kaur, L., Singh, J., and Singh, N. (2006). Effect of cross-linking on some properties of potato (*Solanum tuberosum* L.) starches. *J. Sci. Food Agric.* 86:1945–1954.

Kim, C. T., Shih, F. F., Champagne, E. T., & Daigle, K. (1999). Effects of phosphorylating salts and temperature on the preparation of rice starch phosphates by extrusion. *Starch/Stärke*, 51(8‐9), 280-286.

Kumar, A., Ganjyal, G. M., Jones, D. D., & Hanna, M. A. (2006). Digital image processing for measurement of residence time distribution in a laboratory extruder. *Journal of Food Engineering*, 75(2), 237-244.

Lai, L. S., & Kokini, J. L. (1991). Physicochemical changes and rheological properties of starch during extrusion.(A review). *Biotechnology progress*, 7(3), 251-266.

Landerito, N. A., & Wang, Y. J. (2005). Preparation and properties of starch phosphates using waxy, common, and high-amylose corn starches. II. Reactive extrusion method. *Cereal chemistry*, 82(3), 271-276.

Lee, S. Y., & McCarthy, K. L. (1996). Effect of screw configuration and speed on RTD and expansion of rice extrudate. *Journal of food process engineering*, 19(2), 153-170.

Lehmann, U., & Robin, F. (2007). Slowly digestible starch–its structure and health implications: a review. *Trends in Food Science & Technology*, 18(7), 346-355.

Li, H., Thompson, M. R., & O'Donnell, K. P. (2014). Understanding wet granulation in the kneading block of twin screw extruders. *Chemical Engineering Science*, 113, 11-21.

Lim, S., & Seib, P. A. (1993). Preparation and pasting properties of wheat and corn starch phosphates. *Cereal Chemistry*, 70, 137-144.

Liu, T. Y., Ma, Y., Yu, S. F., Shi, J., & Xue, S. (2011). The effect of ball milling treatment on structure and porosity of maize starch granule. *Innovative Food Science & Emerging Technologies*, 12(4), 586-593.

Lu, Z. H., Yada, R. Y., Liu, Q., Bizimungu, B., Murphy, A., De Koeyer, D., ... & Pinhero, R. G. (2011). Correlation of physicochemical and nutritional properties of dry matter and starch in potatoes grown in different locations. *Food Chemistry*, 126(3), 1246-1253.

Lu, Z. H., Donner, E., Yada, R. Y., & Liu, Q. (2012). The synergistic effects of amylose and phosphorus on rheological, thermal and nutritional properties of potato starch and gel. *Food Chemistry*, 133(4), 1214-1221.

Maaran, S., Hoover, R., Donner, E., & Liu, Q. (2014). Composition, structure,

morphology and physicochemical properties of lablab bean, navy bean, rice bean, tepary bean and velvet bean starches. *Food chemistry*, 152, 491-499.

Maningat, C. C., Seib, P. A., & Bassi, S. D. (2013). Dietary Fiber Content of Cross-linked Phosphorylated Resistant Starch (RS4) Determined by the Prosky and McCleary Methods. Part II. Comparison of Assay Data 1, 2. *Cereal Foods World*, 58(5), 252-263.

Martin, C., & Smith, A. M. (1995). Starch biosynthesis. *Plant Cell*, 7, 971-985.

McCleary BV & Monaghan DA (2002). Measurement of resistant starch. *Journal of the Association of Official Analytical Chemists International*, 85: 665–75.

McCleary BV & Rossiter P (2004). Measurement of novel dietary fibres. *Journal of the Association of Official Analytical Chemists International*, 87 (3): 707–17.

Menezes, E. W., Tadini, C. C., Tribess, T. B., Zuleta, A., Binaghi, J., Pak, N., & Lajolo, F. M. (2011). Chemical composition and nutritional value of unripe banana flour (Musa acuminata, var. Nanicão). *Plant foods for human nutrition*, 66(3), 231-237.

Mu, B., & Thompson, M. R. (2012). Examining the mechanics of granulation with a hot melt binder in a twin-screw extruder. *Chemical engineering science*, 81, 46-56.

Muir JG & O'Dea K (1992). Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. *American Journal of Clinical Nutrition*, 56, 123–127.

Muir JG & O'Dea K (1993). Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. *American Journal of Clinical Nutrition*, 57, 540–546.

Murua-Pagola, B., Beristain-Guevara, C. I., & Martinez-Bustos, F. (2009). Preparation of starch derivatives using reactive extrusion and evaluation of modified starches as shell materials for encapsulation of flavoring agents by spray drying. *Journal of Food Engineering*, 91, 380-386.

Nabeshima, E. H., & Grossmann, M. V. E. (2001). Functional properties of pregelatinized and cross-linked cassava starch obtained by extrusion with sodium trimetaphosphate. *Carbohydrate Polymers*, 45, 347-353.

Nugent, A. P. (2005). Health properties of resistant starch. *British Nutrition Foundation, Nutrition Bulletin*, 30, 27–54.

O'Brien, S., Wang, Y. J., Vervaet, C., & Remon, J. P. (2009). Starch phosphates

prepared by reactive extrusion as a sustained release agent. *Carbohydrate polymers*, *76*(4), 557-566.

O'Brien, S., & Wang, Y. J. (2009). Effects of shear and pH on starch phosphates prepared by reactive extrusion as a sustained release agent. Carbohydrate polymers, 77(3), 464-471.

Paltridge, N. G., Palmer, L. J., Milham, P. J., Guild, G. E., & Stangoulis, J. C. (2012). Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant and soil*, 361(1-2), 251-260.

Pérez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Stärke*,62(8), 389-420.

Perten Instruments Australia Pty Ltd. (2010). RVA4 Installation and Operation Manual. Warriewood NSW, Australia.

Perten Instruments Australia Pty Ltd. (2015). Rapid visco analyzer application & method. Warriewood NSW, Australia.

Prosky L, Asp NG, Furada I et al. (1985). Determination of total dietary fiber in foods and food products: collaborative study. *Journal of the Association of Official Analytical Chemists International*, 68: 677–9.

Robin, J. P. (1974). Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chemistry*, 51, 389-406.

Safford, R., Jobling, S. A., Sidebottom, C. M., Westcott, R. J., Cooke, D., Tober, K. J., ... & Gidley, M. J. (1998). Consequences of antisense RNA inhibition of starch branching enzyme activity on properties of potato starch. *Carbohydrate Polymers*, 35(3), 155-168.

Sajilata, M. G., & Singhal, R. S. (2005). Specialty starches for snack foods. *Carbohydrate polymers*, 59, 131–151.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch – A review. *Comprehensive Reviews in Food Science and Food Safety*, 5, 1–17.

Sang, Y., & Seib, P. A. (2006). Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation. *Carbohydrate Polymers*, 63, 167–175.

Sang, Y., Prakash, O., & Seib, P. A. (2007). Characterization of phosphorylated cross-linked resistant starch by ^{31} P nuclear magnetic resonance (^{31} P NMR)

spectroscopy. *Carbohydrate polymers*, 67(2), 201-212.

Sankhon, A., Yao, W. R., Amadou, I., Wang, H., Qian, H., & Sangare, M. (2013). Effect of Pyrodextrinization, Crosslinking and Heat-Moisture Treatment on In vitro Formation and Digestibility of Resistant Starch from African Locust Bean (Parkia biglobosa). *Tropical Journal of Pharmaceutical Research*, 12(2), 173-179.

Sarawong, C., Schoenlechner, R., Sekiguchi, K., Berghofer, E., & Ng, P. K. (2014). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food chemistry*, 143, 33-39.

Seker, M., Sadikoglu, H., Ozdemir, M., and Hanna, M.A. (2003a). Cross-linking of starch with reactive extrusion and expansion of extrudates. *Int. J. Food Prop.,* 6, 473–480.

Seker, M., Sadikoglu, H., and Hanna, M.A. (2003b). Phosphorus binding to starch during extrusion in both single- and twin-screw extruder with and without a mixing element. *Journal of Food Engineering.* 59, 355-360.

Seker, M., Sadikoglu, H., and Hanna, M.A. (2004). Properties of cross-linked starch produced in a single screw extruder with and without a mixing element. *J. Food Proc. Eng.* 27, 47–63.

Seker, M., & Hanna, M. A. (2005). Cross-linking starch at various moisture contents by phosphate substitution in an extruder. *Carbohydrate Polymers*, 59, 541-544.

Seker, M. and Hanna, M.A. (2006). Sodium hydroxide and trimetaphosphate levels affect properties of starch extrudates. *Ind. Crop. Prod.* 23, 249–255.

Shalviri, A., Liu, Q., Abdekhodaie, M. J., & Wu, X. Y. (2010). Novel modified starch–xanthan gum hydrogels for controlled drug delivery: Synthesis and characterization. *Carbohydrate Polymers*, 79(4), 898-907.

Sharma, S., Singh, N., & Singh, B. (2015). Effect of extrusion on morphology, structural, functional properties and in vitro digestibility of corn, field pea and kidney bean starches. *Starch/Stärke*, 67(9-10), 721-728.

Shi, M., Gu, F., Wu, J., Yu, S., & Gao, Q. (2013). Preparation, physicochemical properties, and in vitro digestibility of cross-linked resistant starch from pea starch. *Starch/Stärke*, *65*(11-12), 947-953.

Singh, N., Sandhu, K. S., & Kaur, M. (2004). Characterization of starches separated from Indian chickpea (Cicer arietinum L.) cultivars. *Journal of Food*

Engineering, 63, 441–449.

Singh, J., Kaur, L., & McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review. *Food hydrocolloids*, 21(1), 1-22.

Smith, R. J., & Caruso, J. L. (1964). Determination of phosphorus. In R. L. Whistler (Ed.), *Methods in carbohydrate chemistry* (p. 42). New York: Academic Press, 42.

Tamaki, S., Hisamatsu, M., Teranishi, K., & Yamada, T. (1997). Structural Change of Potato Starch Granules by Ball‐mill Treatment. *Starch/Stärke*,*49*(11), 431-438.

Tester, R. F., Karkalas, J., & Qi, X. (2004a). Starch-composition, fine structure and architecture. *Journal of Cereal Science*, 39(2), 151-165.

Tester, R. F., Karkalas, J., & Qi, X. (2004b). Starch structure and digestibility enzyme-substrate relationship. *World's Poultry Science Journal*, 60(02), 186-195.

Thompson, D. B. (2000). Strategies for the manufacture of resistant starch. *Trends in Food Science & Technology*, 11, 245–253.

Thompson, L. U., Maningat, C. C., Woo, K., & Seib, P. A. (2011). In vitro digestion of RS4-type resistant wheat and potato starches, and fermentation of indigestible fractions. *Cereal chemistry*, 88(1), 72-79.

Tomasik, P., & Schilling, C. H. (2004). Chemical modification of starch. *Advances in Carbohydrate Chemistry and Biochemistry*, 59, 175-403.

White, J. L., Szydlowski, W., Min, K., & Kim, M. H. (1987). Twin screw extruders; development of technology and analysis of flow. *Advances in Polymer Technology*, 7(3), 295-332.

Wongsagonsup, R., Shobsngob, S., Oonkhanond, B., & Varavinit, S. (2005). Zeta potential (ζ) and pasting properties of phosphorylated or crosslinked rice starches. *Starch/Stärke*, 57(1), 32-37.

Woo, K., & Seib, P. A. (1997). Cross-linking of wheat starch and hydroxypropylated wheat starch in alkaline slurry with sodium trimetaphosphate. *Carbohydrate Polymers*, 33, 263-271.

Woo, K. S., & Seib, P. A. (2002). Cross-linked resistant starch: Preparation and properties. *Cereal Chemistry*, 79, 819–825.

Wu, Y., & Seib, A. (1990). Acetylated and hydroxypropylated and distarchphosphates from waxy barly: Paste Properties and Free-Thaw Stability. *Cereal Chemistry*, 67(2), 202-208.

Xie, F., Yu, L., Liu, H., & Chen, L. (2006). Starch modification using reactive extrusion. *Starch/Stärke*, 58(3-4), 131-139.

Yue, P., & Waring, S. (1998). Functionality of resistant starch in food applications. *Food Australia*, 50(12), 615-621.

Zhou, Y., Hoover, R., & Liu, Q. (2004). Relationship between a-amylase degradation and the structure and physicochemical properties of legume starches. *Carbohydrate Polymers*, 57, 299–317.

APPENDICES

Appendix A. Bright field optical images of raw pea starches

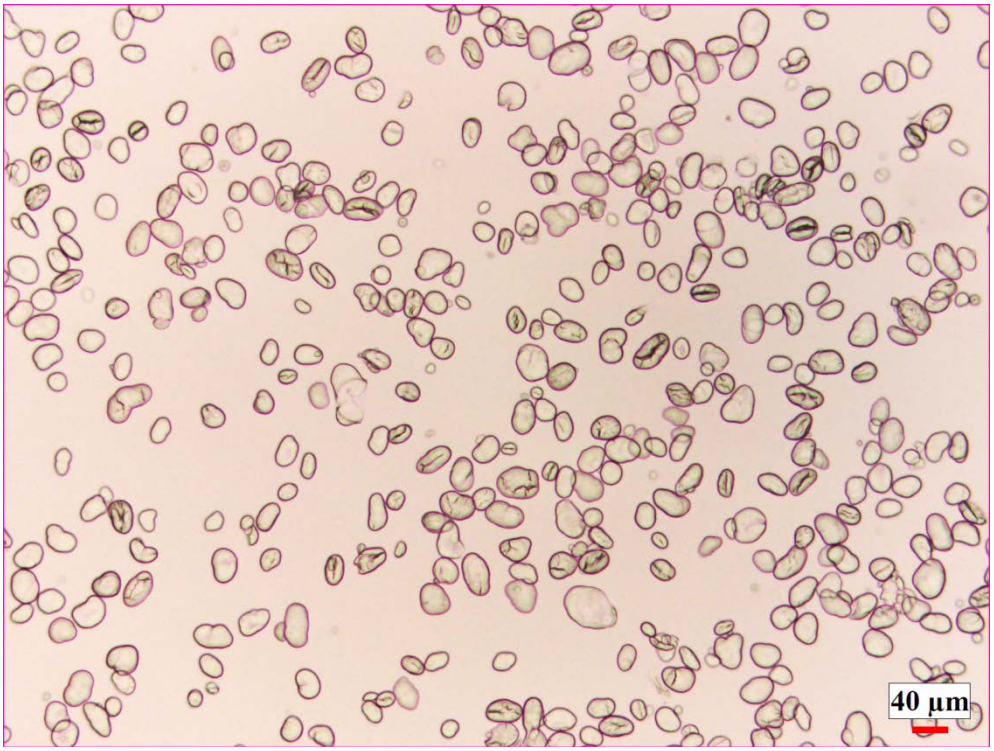

Figure A.1 Bright field optical micrograph (x300) of raw NutriPea pea starch

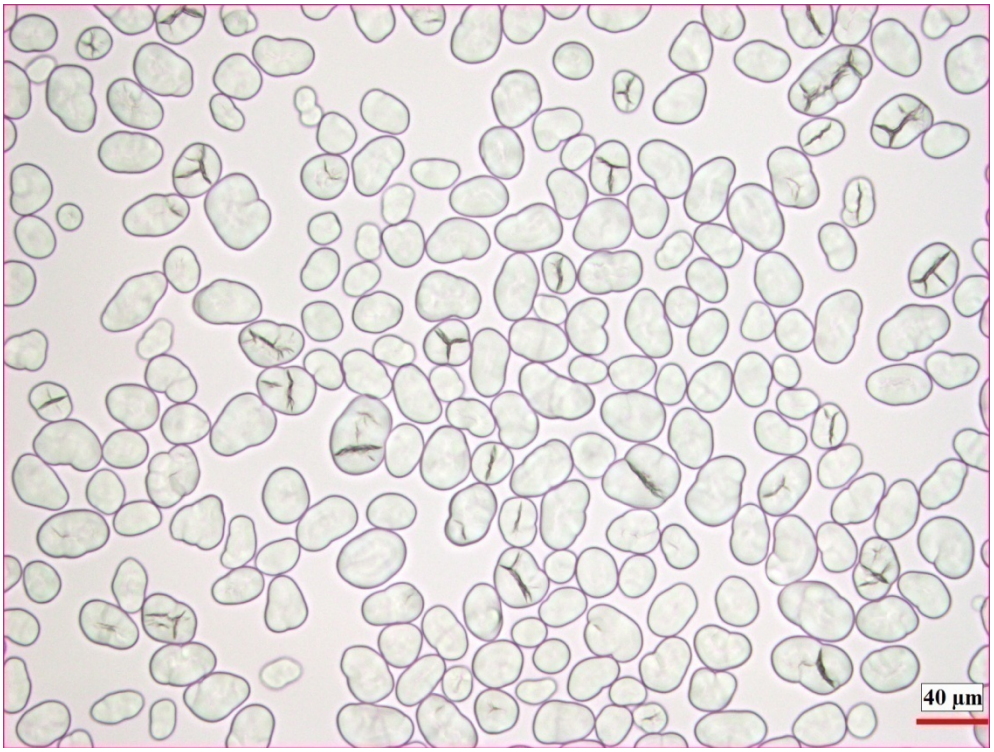

Figure A.2 Bright field optical micrograph (x600) of raw Meelunie pea starch

Appendix B. Phosphorus data of NutriPea samples prepared with different feed compositions for multi-variable statistical analysis

Table B.1 Phosphorus contents of selected NutriPea samples prepared with different coded (actual) levels of ingredients for multi-variable statistical analysis

No.	Sample	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Phosphorus (%)
1	**NE4**	-1 (40)	0 (5)	1 (3)	**0.123**
2	**NE8**	-1 (40)	0 (5)	-0.33 (1)	**0.143**
3	**NE12**	-1 (40)	-1 (3)	-0.33 (1)	**0.115**
4	**NE13**	-1 (40)	1 (7)	-0.33 (1)	**0.162**
5	**NE16**	0.4 (47)	-1 (3)	-0.33 (1)	**0.158**
6	**NE17**	0.4 (47)	0 (5)	-0.33 (1)	**0.181**
7	**NE18**	0.4 (47)	1 (7)	-0.33 (1)	**0.232**
8	**NE21**	-1 (40)	-1 (3)	-1 (0)	**0.116**
9	**NE22**	-1 (40)	0 (5)	-1 (0)	**0.138**
10	**NE23**	-1 (40)	1 (7)	-1 (0)	**0.187**
11	**NE24**	0.4 (47)	1 (7)	-1 (0)	**0.221**
12	**NE19**	1 (50)	1 (7)	-0.33 (1)	**0.256**

Appendix C. Gelatinization properties of all the pea starch samples

Table C.1 Gelatinization properties and phosphorus content of native and various cross-linked pea starch phosphates in 130wt% water determined by DSC

Sample	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%[j])	Na_2SO_4 (wt%)	Cross-linking Conditions	Phosphorus[a] (%)	Gelatinization Parameters[b]			
						T_o(°C)	T_p(°C)	T_c(°C)	ΔH (J/g)
NPS[c]	N/A	N/A	N/A	N/A	**0.009**	53.3	**59.1**	66.7	**1.87**
NE0	40	0	0	1.02kg/h, 95°C, 150rpm	**0.009**	59.9	**64.6**	72.2	**0.64**
MPS[d]	N/A	N/A	N/A	N/A	**0.009**	58.2	**64.3**	74.6	**1.84**
ME0	40	0	0	1.02kg/h, 95°C, 150rpm	**0.009**	64.9	**69.4**	77.4	**0.65**
ME1	40	3	1	1.02kg/h, 95°C, 150rpm	**0.155**	64.1	**69.0**	76.8	**0.90**
ME3	40	5	1	1.02kg/h, 95°C, 150rpm	**0.177**	63.7	**67.3**	75.3	**0.86**
ME5	40	7	1	1.02kg/h, 95°C, 150rpm	**0.183**	61.0	**66.5**	79.8	**1.58**
NE1	40	5	3	1.02kg/h, 95°C, 30rpm	**0.152**	61.6	**65.0**	76.5	**1.30**
NE2	40	5	3	1.02kg/h, 95°C, 50rpm	**0.145**	60.4	**64.2**	73.5	**1.30**
NE3	40	5	3	1.02kg/h, 95°C, 100rpm	**0.135**	60.3	**64.1**	72.3	**1.53**
NE4	40	5	3	1.02kg/h, 95°C, 150rpm	**0.123**	60.2	**63.9**	74.2	**1.18**
NE5	40	5	3	1.02kg/h, 95°C, 200rpm	**0.098**	59.3	**63.1**	72.8	**1.53**
NE7	40	5	1	1.02kg/h, 95°C, 50rpm	**0.158**	61.3	**65.1**	75.0	**1.23**
NE8	40	5	1	1.02kg/h, 95°C, 150rpm	**0.143**	60.9	**64.8**	73.0	**1.49**
NE10	40	5	5	1.02kg/h, 95°C, 50rpm	**0.113**	60.6	**64.1**	74.7	**1.10**
NE11	47	7	3	1.02kg/h, 95°C, 50rpm	**0.182**	61.6	**65.2**	70.8	**1.44**
NE12	40	3	1	1.02kg/h, 95°C, 150rpm	**0.115**	61.2	**65.8**	74.9	**1.51**
NE13	40	7	1	1.02kg/h, 95°C, 150rpm	**0.162**	60.6	**64.6**	73.5	**1.86**
NE14	40	7	1	2.04kg/h, 95°C, 150rpm	**0.161**	62.1	**65.6**	73.2	**0.93**
NE15	40	7	1	3.06kg/h, 95°C, 150rpm	**0.177**	61.6	**65.1**	74.4	**1.61**
NE16	47	3	1	1.02kg/h, 95°C, 150rpm	**0.158**	61.3	**65.6**	74.4	**1.28**

Sample	Feed Moisture (wt%)	99:1 (w/w) STMP/STPP (wt%)	Na_2SO_4 (wt%)	Cross-linking Conditions	Phosphorus[a] (%)	Gelatinization Parameters[b]			
						T_o(°C)	T_p(°C)	T_c(°C)	ΔH (J/g)
NE17	47	5	1	1.02kg/h, 95°C, 150rpm	**0.181**	63.5	**67.0**	72.3	**1.22**
NE18	47	7	1	1.02kg/h, 95°C, 150rpm	**0.232**	62.5	**65.9**	72.9	**1.58**
NE19	50	7	1	1.02kg/h, 95°C, 150rpm	**0.256**	60.8	**65.9**	76.7	**1.22**
NE20	50	10	1	1.02kg/h, 95°C, 150rpm	**0.282**	61.8	**65.4**	73.0	**0.74**
NE21	40	3	0	1.02kg/h, 95°C, 150rpm	**0.116**	63.0	**66.5**	75.2	**1.28**
NE22	40	5	0	1.02kg/h, 95°C, 150rpm	**0.138**	62.8	**66.3**	74.3	**0.97**
NE23	40	7	0	1.02kg/h, 95°C, 150rpm	**0.187**	61.8	**65.9**	74.8	**1.47**
NE24	47	7	0	1.02kg/h, 95°C, 150rpm	**0.221**	62.3	**66.8**	75.6	**0.81**
NE25	47	10	0	1.02kg/h, 95°C, 150rpm	**0.245**	61.1	**65.5**	74.7	**1.35**
NE26[e]	40	7	1	1.02kg/h, 95°C, 150rpm	**0.186**	61.0	**67.0**	75.5	**0.47**
NE27[e]	40	7	1	2.04kg/h, 95°C, 150rpm	**0.206**	62.1	**66.1**	77.5	**1.47**
NE28[e]	40	7	1	3.06kg/h, 95°C, 150rpm	**0.217**	65.7	**66.1**	73.5	**1.34**
A1[f]	60	1	3	95°C, 20min, 50rpm	**0.013**	62.1	**65.7**	72.3	**1.40**
A2[f]	60	3	3	95°C, 20min, 50rpm	**0.016**	61.3	**68.0**	74.3	**1.24**
A3[f]	60	5	3	95°C, 20min, 50rpm	**0.022**	64.6	**68.4**	74.5	**0.81**
A4[f]	60	7	3	95°C, 20min, 50rpm	**0.032**	66.4	**68.8**	73.6	**0.81**
A5[f]	60	10	3	95°C, 20min, 50rpm	**0.057**	67.5	**70.8**	76.0	**1.08**
B1[f]	60	5	3	95°C, 5min, 50rpm	**0.264**	66.4	**70.2**	81.4	**1.21**
B2[f]	60	10	3	95°C, 5min, 50rpm	**0.452**	58.0	**62.8**	70.9	**1.09**
C1[g]	N/A	5	3	45°C, 5 h	**0.042**	60.1	**64.7**	73.8	**1.21**
C2[g]	N/A	10	3	45°C, 5 h	**0.086**	59.2	**63.9**	71.4	**0.90**
C3[g]	N/A	20	20	50°C, 9 h	**0.741**	54.7	**62.6**	74.1	**2.03**

a). Results are means of duplicates. The average standard deviation was <2%; b) Results are means of duplicates. The average standard deviation of T_p was <1%. The average standard deviation of T_o, T_c and ΔH were <5%. T_o, T_p, and T_c indicate the temperature of onset, peak and conclusion of gelatinization, respectively. ΔH= enthalpy of gelatinization(J/g of dry matter); c). Native NutriPea pea starch; d).Native Meelunie pea starch; e). NE26, 27& 28 were prepared with screw design #2. All the rest extruded samples were prepared with screw design #1; f). Prepared in the Haake batch mixer; g). Prepared by solution cross-linking in aqueous slurry.

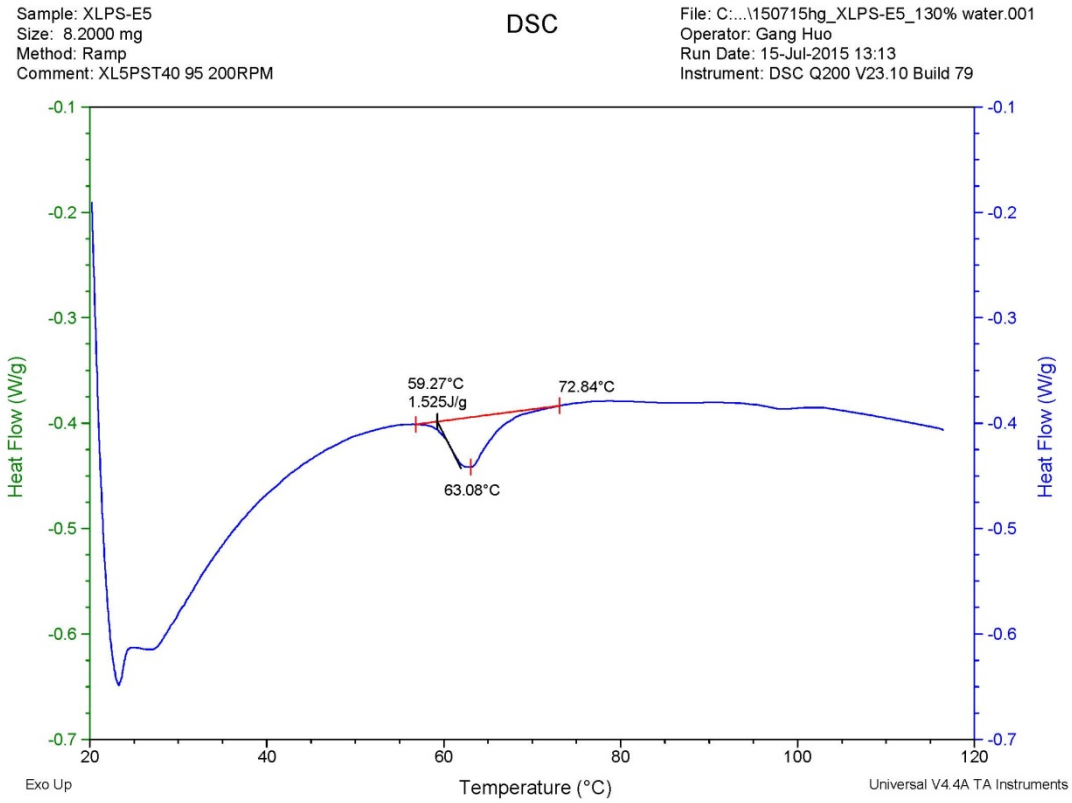

Figure C.1 DSC thermogram of extruded NutriPea pea starch phosphate NE5 containing 130wt% water