Physicochemical properties and *in vitro* digestibility of pulse starches and derivatized type 3 resistant starch

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ABSTRACT

The objective of this thesis was to isolate and characterize pea, lentil and faba bean starches from air-classified flours and to develop a novel type 3 resistant starch (RS3) from the isolated pulse starches. In the pulse processing industry in Canada, the majority of pulse starches are generated in the form of starch-rich flours from air classification. The first study aimed to isolate starches of a high purity from the most common starch-rich pulse flours (*i.e.*, pea, lentil and faba bean) and to determine and compare the molecular structure, functional properties and *in vitro* digestibility of the isolated pulse starches with those of four important commercial starches (*i.e.*, Roquette pea, normal maize, waxy maize and tapioca). The isolated pulse starches showed amylose contents and amylopectin branch-chain-length (BCL) distributions similar to that of Roquette pea starch. Consequently, the granular morphologies, crystalline structure, thermal properties, pasting properties, gelling ability and *in vitro* digestibility of the isolated pulse starches were comparable to those of Roquette pea starch but were distinctively different from those of commercial maize and tapioca starches.

Because the isolated pea, lentil and faba bean starches exhibited similar amylose contents and comparable amylopectin BLC distributions, the isolated pea starch was selected as the representative pulse starch to develop a novel RS3 product through acid thinning, debranching and recrystallization in the second study, with commercial normal maize starch being included for comparison. Starting from the respective native starches, the modification method yielded 68.1% of RS3 from pea and 59.6% from normal maize. *In vitro* starch digestibility assay revealed that pea RS3 (in both uncooked and cooked states) was less digestible to amylolytic enzymes than normal maize RS3. The results obtained could be ascribed to the formation of thermally more stable double-helical crystallites in the former, which consisted of a larger proportion of intermediate chains and a smaller proportion of short chains when compared with normal maize RS3. This study indicated that the developed modification method was effective to increase the enzymatic resistance of pulse starch, which could be employed to prepare low-glycemic food ingredients and expand new markets for the underutilized co-product.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of variance
AT	Acid-thinned
ATPS	8-aminopyrene-1,3,6-trisulfonate
BCL	Branch-chain-length
CLSM	Confocal laser scanning microscopy
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
DSC	Differential scanning calorimetry
FACE	Fluorophore-assisted capillary electrophoresis
GOPOD	Glucose oxidase/peroxidase
HPSEC	High-performance size-exclusion chromatography
LiBr	Lithium bromide
RDS	Rapidly digestible starch
RI	Refractive index
RS	Resistant starch
RS1	Type 1 resistant starch
RS2	Type 2 resistant starch
RS3	Type 3 resistant starch
RS4	Type 4 resistant starch
RS5	Type 5 resistant starch
RVA	Rapid Visco Analyzer
SDS	Slowly digestible starch
SEM	Scanning electron microscopy
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate
T_c	Conclusion gelatinization temperature
T_o	Onset gelatinization temperature
T_p	Peak gelatinization temperature
ΔH	Enthalpy change

1. INTRODUCTION

1.1 Overview

Pulses refer to the dry edible seeds of the *Leguminosae* family, including peas, lentils and faba beans as the most common species in North America (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001). Due to the high nutritional value, pulses have traditionally been consumed as an important component of human diets in many regions of the world (Hoover, Hughes, Chung, & Liu, 2010). However, the hard-to-cook trait and inferior texture and flavor of the whole seeds of pulses limit their use in human foods (Boye, Zare, & Pletch, 2010).

In order to increase the utilization of pulses in the food industry, one fractionation method − dry milling combined with air classification − has been widely used in Canada for the fractionation of pulses into various food ingredients, including pulse protein, fiber and starch, which enhances their utilization in a broader range of food products (Hoover, Hughes, Chung, & Liu, 2010). This processing method is relatively simple and requires lower operation costs and less usage water (Sosulski & Sosulski, 1986).

The derived pulse protein fraction, as a high-value food ingredient, possess functional properties (*e.g.*, solubility, water binding, lipid binding, emulsification, foaming and gelation properties) comparable to soy and whey proteins which are commonly used in the food industry. The functional properties of pulse proteins have been exploited to develop a variety of products such as beverages, sauces, bakery products, extruded products and ready-to-eat snacks (Boye, Zare, & Pletch, 2010; Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). The fiber-rich fraction, primarily obtained from the dehulling of pulse crops, can be used to increase the dietary fiber contents of food products and serves as a functional ingredient (Tosh & Yada, 2010). However, the starch fraction, showing poorer nutritional value and less desirable functionality, is underutilized in the pulse industry. Therefore, industry is exploring new strategies to promote the value-added utilization of this co-product.

Molecular structure, thermal and pasting properties, gel formation and digestibility of pea, lentil and faba bean starches have been investigated in the literature

(Ambigaipalan, Hoover, Donner, Liu, Jaiswal, Chibbar, et al., 2011; Cai, Cai, Man, Zhou, & Wei, 2014; Chung, Liu, & Hoover, 2009; Hoover, Hughes, Chung, & Liu, 2010; Jane, Chen, Lee, McPherson, Wong, Radosavljevic, et al., 1999). Generally, pulse starches exhibit relatively higher amylose contents and longer branch chains of amylopectin compared with starches important for the global starch industry − normal maize, waxy maize and tapioca starches (Biliaderis, Maurice, & Vose, 1980; Li & Yeh, 2001). These structural features of pulse starches could be desirable for the development of starch ingredients rich in resistant starch (RS).

RS, as a novel type of dietary fiber, is defined as a fraction of starch that can survive digestion in the human small intestine and then be passed into the large intestine for microbial fermentation (Englyst & Macfarlane, 1986; Englyst, Wiggins, & Cummings, 1982). In general, RS can be classified into five different types (Englyst, Kingman, & Cummings, 1992; Hasjim, Lee, Hendrich, Setiawan, Ai, & Jane, 2010): RS1 is physically inaccessible to digestion; RS2 is raw granular starch with the B- or C-type crystalline structure; RS3 represents retrograded starch that reforms double-helical crystallites; RS4 is chemically modified starch, in which new covalent bonds are formed between the added chemical derivatives and starch molecules; and RS5 refers to amylose-lipid complex.

Among the five types of RS, RS3 is of particular interest due to its desirable functional attributes (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). Previous studies have shown that RS3 commonly has a small particle size, white appearance, bland flavor, high dissociation temperature, and low water-holding capacity, which can be utilized to produce low-bulk, high-fiber, and low-calorie foods with improved texture and mouthfeel, such as in baked foods and extruded products (Haralampu, 2000; Sajilata, Singhal, & Kulkarni, 2006).

Currently, a limited number of studies on the preparation of RS3 from pulse starches has been reported in the literature. As the majority of pulse starches generated from the dry milling process is in the form of starch-rich flours from air classification in the Canadian pulse processing industry, there is an urgent need to develop an effective method to isolate pulse starches from such flours and to gain a deeper understanding of their physicochemical properties and digestibility. Therefore, in the first study of my thesis project, we aimed to isolate starches of a high purity from the most common starch-rich pulse flours available in the market, including pea, lentil and faba bean. The molecular structure, functional properties and *in vitro* digestibility

of the isolated pulse starches were characterized and compared with those of important commercial starches, including Roquette pea, normal maize, waxy maize and tapioca starches. Results showed that the isolated pea, lentil and faba bean starches exhibited similar amylose contents and amylopectin branch-chain lengths and comparable functional properties and *in vitro* digestibility. Therefore, the isolated pea starch was selected in my second study to develop a novel RS3 through acid thinning, debranching and recrystallization, with commercial normal maize starch being used for comparison. The structures and physicochemical properties of the two obtained RS3 products were characterized and related to their *in vitro* digestibility.

The thesis project not only advanced our understanding of the structure, functional properties and *in vitro* digestibility of native pulse starches but also generated new knowledge and technologies for the development of nutritionally important RS ingredients from pulse starches. The new information will be of great importance for the industry to develop effective strategies to add value and expand new markets for this co-product.

1.2 Objectives

(1) To isolate high-purity starch from starch-rich flours of pea, lentil and faba bean and to determine the molecular structure, functional properties and *in vitro* digestibility of the isolated pulse starches in comparison with commercial Roquette pea, normal maize, waxy maize and tapioca starches;

(2) To produce RS3 from the isolated pea starch and commercial normal maize starch through acid thinning, debranching and recrystallization and to characterize and compare the structural features, physicochemical properties and *in vitro* digestibility of the two RS3 products.

1.3 Hypotheses

(1) Isolated pulse starches – despite being from three different species of pulse crops – will show molecular structure, functional properties and *in vitro* digestibility comparable to those of commercial Roquette pea starch but different from those of commercial normal maize, waxy maize and tapioca starches.

(2) RS3 produced from isolated pea starch through acid thinning, debranching and recrystallization will show better thermal stability and greater enzymatic resistance than RS3 from commercial normal maize starch.

2. LITERATURE REVIEW

2.1 Utilization of pulses in human foods

Pulses are the dry edible seeds of plants that belong to the *Leguminosae* family, with peas, lentils, chickpeas and edible beans being the most common species (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001). Leading producers of pulses in the world include India, Canada, Brazil, China, Myanmar, and the U.S.A. With a vast and varied agricultural land, Canada is the second largest producer of pulses in the world; more specifically, the largest producer of peas and the second of lentils (Hoover, Hughes, Chung, & Liu, 2010). The Canadian pulse industry has grown rapidly in recent years and has become the leader in pulse trade globally (Pulse Canada, 2019).

Pulses are rich in complex carbohydrates (*e.g.*, resistant starch and other dietary fibers), proteins, minerals, vitamins and phytochemicals (*e.g.*, isoflavones, phytosterols and saponins) but relatively low in lipids (except chickpea) (Faye, 2007; Patterson, Maskus, & Dupasquier, 2009; Rochfort & Panozzo, 2007). Furthermore, pulses are gluten-free and thus they can be an alternative food choice for people who are allergic or intolerant to gluten. In addition, studies have shown that the consumption of pulses can provide potential health benefits to humans, such as aiding with weight management, reducing glycemic response, increasing satiety, and lowering the risks of chronic diseases (*e.g.*, type-2 diabetes, cancers and cardiovascular diseases) (Jenkins, Kendall, Augustin, Mitchell, Sahye-Pudaruth, Mejia, et al., 2012; Mudryj, Yu, & Aukema, 2014).

Traditionally, pulses have been consumed as whole or split seeds (*e.g.*, canned pulses) in many parts of the world, especially for low-income people in developing countries (Hoover, Hughes, Chung, & Liu, 2010). However, whole seed is an undesirable form to promote the use of pulses in human foods because of the hard-to-cook trait and unacceptable texture and flavor to some consumers (Boye, Zare, & Pletch, 2010). Mudryj, Yu, Hartman, Mitchell, Lawrence, and Aukema (2012) reported that only 7.9% of the population in the U.S.A. and 13.1% in Canada are

pulse consumers. Therefore, there have been continuous efforts in exploring new strategies to increase the utilization of pulses in the food industry, especially in the market of North America.

Recently, there is growing interest in fractionating pulses into different forms of value-added ingredients, such as starch, protein concentrate and isolate, and hull fiber, which can be utilized in a broader range of food products. Therefore, processing pulses into various high-value ingredients is a promising approach to expanding new markets for these crops in the food industry (Boye, Zare, & Pletch, 2010; Hoover, Hughes, Chung, & Liu, 2010; Tosh & Yada, 2010).

2.2 Fractionation of pulses using wet and dry milling methods

Commercially, the fractionation of pulses can be achieved using two methods: wet and dry (Boye, Zare, & Pletch, 2010; Hoover, Hughes, Chung, & Liu, 2010; Tosh & Yada, 2010). The wet method typically comprises a series of processing steps, such as dehulling, soaking in water, milling, acid-base treatment, filtration and drying, in order to efficiently separate hull fiber, starch and protein (Hoover, Hughes, Chung, & Liu, 2010; Wood & Malcolmson, 2011). Dehulling is the key step for the removal of 97−98% hull fiber, which is mainly water-insoluble dietary fiber that can bind with protein and other nutrients (Dalgetty & Baik, 2003; Wood & Malcolmson, 2011). In addition, removal of the seed coat reduces the contents of anti-nutritional factors (*e.g.*, tannins), thus improving the nutritional quality, texture and palatability of final ingredient products (Wood & Malcolmson, 2011). The dehulled pulse seeds are then soaked and milled, followed by the addition of a base solution (*e.g.*, 1.0 M NaOH solution, pH = 8−11) to solubilize protein. The protein solubilized in the supernatant and insoluble starch fraction can be separated by filtration. The solubilized protein is subsequently precipitated by adjusting the pH of the solution to the protein isoelectric point (~pH 4.5). Finally, protein and starch can be recovered by drying separately (Boye, Zare, & Pletch, 2010; Hoover, Hughes, Chung, & Liu, 2010). Although the wet milling method can generate pulse protein and starch of a high purity, it has some drawbacks such as high processing costs and high water usage.

The dry method includes dehulling, milling, and air classification (Hoover, Hughes, Chung, & Liu, 2010). Hammer, pin or impact milling is applied to grind the dehulled pulse seeds into flours of small particle sizes, which are then subjected to air classification for fractionation. During air classification, finely ground pulse flour is fractionated in a fluidized tower system

with the supply of a spiral air stream to obtain a protein-rich flour (light, fine fraction) and a starch-rich flour (heavy, coarse fraction), which is achieved based on the different densities and particle sizes of the two fractions (Boye, Zare, & Pletch, 2010). This step can be repeated several times to improve the separation efficiency. As an example, Tyler, Youngs, and Sosulski (1981) repeated the air classification step twice to produce starch-rich and protein-rich fractions from eight pulses. After the first classification step, the starch fraction (SI) was re-milled and air classified for a second time, yielding the second starch fraction (SII) and the second protein fraction (PII). Consequently, increased starch contents and decreased protein and crude fiber contents in SI and SII of the pulse samples were obtained after each time of pin milling and air classification. Compared with the wet method, the dry method produces protein and starch fractions of a lower purity and leads to a greater level of starch damage. However, because of the relatively simple process, low operation costs and less use of water (Sosulski & Sosulski, 1986), the dry method is more popular for the fractionation of pulses in Canada.

The derived pulse protein fraction, as a high-value food ingredient, can be used in a broad range of products such as soups, salad dressings, bakery products, extruded products and ready-to-eat snacks due to the desirable functional and nutritional properties (Boye, Zare, & Pletch, 2010; Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). Fiber-rich fraction resulting from the dehulling of pulse crops can be used to enhance the dietary fiber contents of food products (Tosh & Yada, 2010). In contrast, the starch fraction, showing poorer nutritional value and less desirable functionality, is underutilized in the industry. Therefore, the pulse industry is exploring new approaches to increasing the industrial applications of pulse starch, which requires a comprehensive understanding (*e.g.*, molecular structure, physicochemical properties, and digestibility) of this co-product.

2.3 Structure of pulse starch granules

2.3.1 Granular feature of starch and molecular structures of amylose and amylopectin

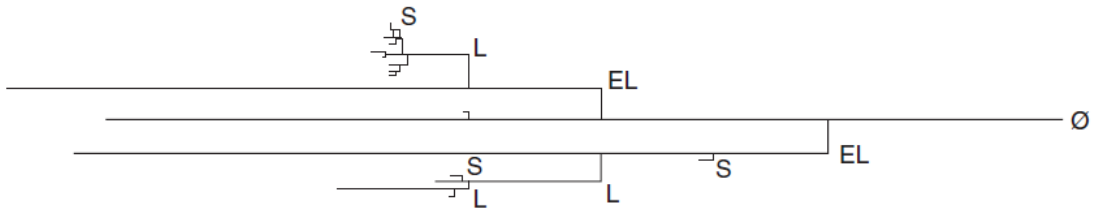
Research efforts have been undertaken to advance our understanding of the granular features and molecular structures of pulse starches (Chung, Liu, Donner, Hoover, Warkentin, & Vandenberg, 2008; Hoover, Hughes, Chung, & Liu, 2010). Pulse starch granules generally have a smooth surface with oval, spherical or irregular shapes. Pea starch granular size ranges from 14 to 32 μm in width and from 15 to 37 μm in length (Hoover & Ratnayake, 2002). The width and

length of lentil starch granules range between 6 and 32 μm and between 6 and 37 μm, respectively. The granular size of faba bean starch has been reported to be 9 to 24 μm in width and 11 to 48 μm in length (Hoover, Hughes, Chung, & Liu, 2010).

Amylose and amylopectin are the two primary components present in pulse starches, similar to other normal and high-amylose starches. Amylose is a primarily linear polymer of α-D-glucopyranose linked by α-1,4 glycosidic linkages with several branches of α-1,6 linkages (Fig. 2.1 A) (Takeda, Shirasaka, & Hizukuri, 1984; Takeda, Shitaozono, & Hizukuri, 1990), whereas amylopectin has a highly branched structure with about 5% α-1,6 branched linkages (Fig. 2.1 B) (Hizukuri, 1986). Amylose content and amylopectin branch-chain-length (BCL) distribution are the two important factors that can determine the granular structure, functionality and digestibility of pulse starches (Hoover, Hughes, Chung, & Liu, 2010). Pea, lentil and faba bean starches isolated from Canadian pulse cultivars have been reported to contain 34.9−37.1%, 36.7−39.0% and 31.38−32.23% amylose contents, respectively (Ambigaipalan, et al., 2011; Chung, Liu, Donner, Hoover, Warkentin, & Vandenberg, 2008). Li and Yeh (2001) reported that the amylose content of pea starch (28.1%) was higher than that of normal maize (25.1%), waxy maize (3.0%) and tapioca starches (19.9%) — three starches popularly used for different industrial applications.

Hizukuri (1986) has classified the branch chains of amylopectin into A, B, and C chains and has proposed that they are organized in a cluster model (Fig. 2.1 B). A chains [degree of polymerization (DP) 6−12] are present within a single cluster and attach to B or C chains via α-1, 6 linkages. B chains carry A chains or other B chains and can be further classified into B1 (DP 13−24), B2 (DP 25−36), and B3 (DP >37) chains, depending on their length and the number of clusters they span. B1, B2 and B3 chains extend through one, two and three clusters, respectively (Hanashiro, Abe, & Hizukuri, 1996). The C chain carries the sole reducing end in the amylopectin molecule. In the study by Chung, Liu, and Hoover (2009), pea and lentil starches have been demonstrated to consist of a smaller proportion (24.1−26.9%) of short branch chains (DP 6−12) but a slightly larger proportion (57.8−59.9%) of long branch chains (DP 13−24) when compared with normal maize starch (31.6 and 55.3%, respectively).

A



B

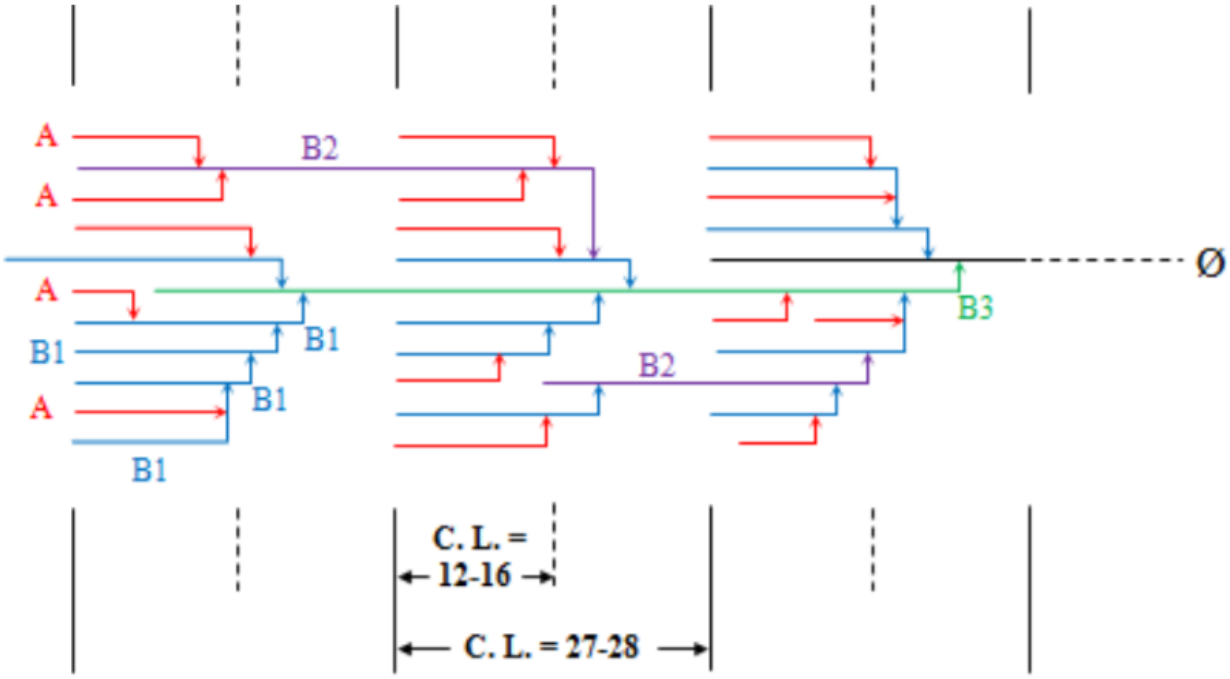


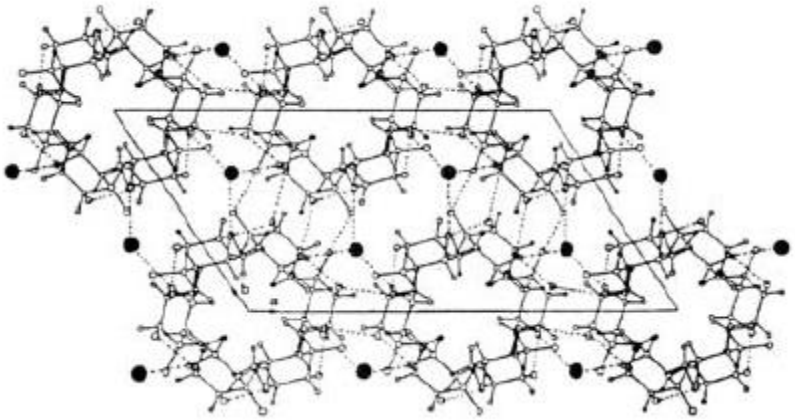
Fig. 2.1 Proposed structure of amylose and amylopectin. (A) Proposed structure of the branched amylose molecule comprising immature clusters. EL, extremely long; L, long; and S, short chains; Ø, reducing end (Takeda, Shitaozono, & Hizukuri, 1990). (B) A proposed cluster model of amylopectin with A (—), B1 (—), B2 (—), and B3 (—) chains. The chain carrying the reducing end (Ø) is the C chain. —, α-1,4-glucan chain; →, α-1,6 linkage (Hizukuri, 1986).

2.3.2 Organization of starch molecules within granules

The external chains of amylopectin have been proposed to form double helices, contributing to the crystalline structure of starch granules (Hizukuri, 1986; Jane, 2009). A-, B- and C-type X-ray diffraction patterns are observed in native starches as a result of the different packing patterns of double-helical crystallites in the granules (Singh, 2011). In general, A-type

starches (*e.g.*, wheat and barley), with a larger proportion of short branch chains (DP 6−12) in the amylopectin, have the double-helical crystallites packed in a monoclinic lattice (Fig. 2.2 A); B-type starches (*e.g.*, potato and high-amylose maize), with a smaller proportion of short branch chains in the amylopectin, have the double-helical crystallites organized in a hexagonal lattice (Fig. 2.2 B) (Hanashiro, Abe, & Hizukuri, 1996; Jane, et al., 1999; Pérez, Baldwin, & Gallant, 2009; Singh, 2011). Pulse starches, including pea, lentil and faba bean, have been illustrated to have the C-type X-ray diffraction pattern, which is a mixture of the A- and B-type polymorphs in varying proportions (Chung, Liu, Donner, Hoover, Warkentin, & Vandenberg, 2008; Gernat, Radosta, Damaschun, & Schierbaum, 1990). The B-type polymorph contents in pea, lentil and faba bean starches have been reported to be in the range of 22.1−28.8, 28.1−36.1 and 20.6−26.3%, respectively (Ambigaipalan, et al., 2011; Hoover, Hughes, Chung, & Liu, 2010). Bogracheva, Morris, Ring, and Hedley (1998) have explicitly demonstrated that the B-type polymorph in pea starch is organized in the center of the granules, whereas the A-type polymorph is located in the peripheral region.

A.



B.

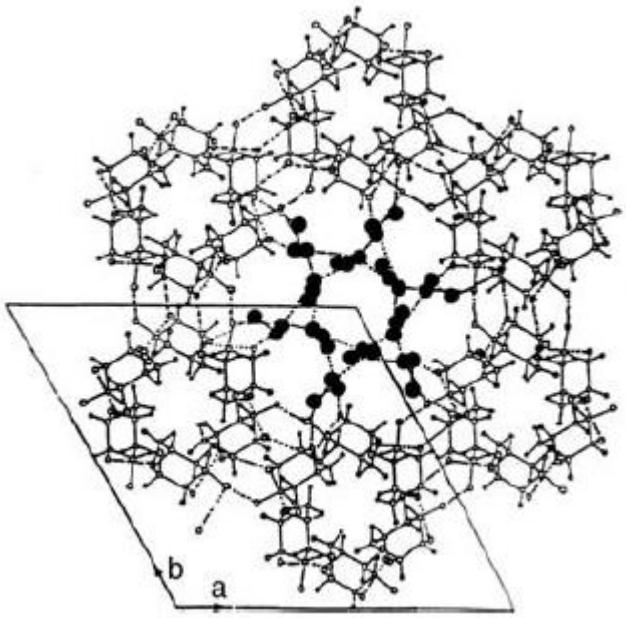


Fig. 2.2 Different packing patterns of double-helical crystallites in A- and B-type polymorphs (Pérez, Baldwin, & Gallant, 2009).

Lineback (1984) has proposed a schematic model of the organization of amylose and amylopectin molecules into a starch granule (Fig. 2.3). Hilum is the organic center of a starch granule, around which the granule grows. Amylose and amylopectin are synthesized radially toward the periphery by apposition simultaneously. Amylopectin branch chains are associated into double-helical crystallites within clusters and organized into a structure known as crystalline lamellae; whereas amylose, interspersed and intertwined with amylopectin, is present in the amorphous regions (Jane, 2009). At the granular level, a native starch granule displays a typical Maltese cross when viewed under polarized light microscopy, reflecting a radial orientation of the principal axis of the crystallites (Pérez & Bertoft, 2010).

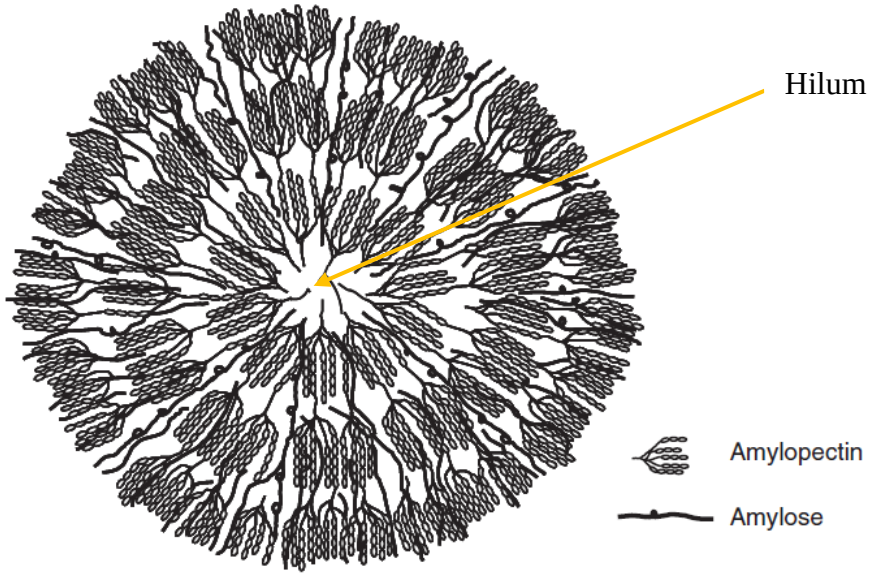


Fig. 2.3 Schematic of the organization of a starch granule (Jane, 2009).

2.4. Thermal properties of pulse starch

2.4.1 Gelatinization properties

After being heated to a certain temperature in the presence of excess water, starch undergoes a thermal transition known as "gelatinization", which results in the melting of ordered crystallites existing in the native starch granules and eventually causes the loss of birefringence (Maltese cross) (Hoover & Ratnayake, 2002). Differential scanning calorimetry (DSC) is the most commonly used technique to measure the gelatinization behaviors of starch because of its high sensitivity and ease of use. Temperatures [T_o (onset), T_p (peak), T_c (conclusion)] and enthalpy change (ΔH) of starch gelatinization are parameters typically used to define the dissociation of ordered chain domains of amylopectin over a temperature range (Biliaderis, 2009). Cooke and Gidley (1992) have suggested that ΔH reflects the amount of energy required to melt double-helical structure during gelatinization. In general, starch consisting of amylopectin with longer branch chains shows higher gelatinization temperatures and a greater enthalpy change because longer branch chains are favorable for the formation of stable double-helical crystallites, and thus more energy is required for the gelatinization to occur (Jane, et al., 1999; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005).

2.4.2 Retrogradation

During storage, the solvated and dispersed starch chains re-associate with each other and reform double helices, resulting in the formation of an aggregated and crystalline structure. The process is defined as "retrogradation" (Hoover, 1995). Both amylose and amylopectin molecules can retrograde. Retrogradation of gelatinized native starch requires several days or even longer, accompanied by the exudation of water (*i.e.*, "syneresis") and the formation of B-type crystallites (Hoover, 1995; Miles, Morris, Orford, & Ring, 1985). Generally, pulse starches retrograde faster and to a greater extent than normal and waxy cereal starches due to their higher amylose content and longer branch chains of amylopectin (Hoover, Hughes, Chung, & Liu, 2010; Lai, Lu, & Lii, 2000; Shi & Seib, 1992).

2.5. Pasting properties

Pasting occurs when starch is further heated and stirred after gelatinization: starch granules swell to provide a high viscosity of the paste and some amylose and amylopectin molecules leach out from the swollen granules (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). Rapid Visco Analyzer (RVA) and Brabender Visco-amylograph are widely used to study the pasting behaviors of starch. A typical pasting curve shows the pasting properties of starch under programmed heating temperature and constant shearing conditions (Fig. 2.4) (Wani, Singh, Shah, Schweiggert-Weisz, Gul, & Wani, 2012). In the initial stage, starch granules absorb water and the viscosity increases during heating. Pasting temperature is defined as the temperature at which starch begins to develop viscosity. After reaching the maximum viscosity (*i.e.*, "peak viscosity"), swollen starch granules start to rupture and break apart into fragments during continuous shearing, showing the "breakdown" in the viscosity. "Setback" in the pasting profile occurs when the starch paste is cooled down: starch molecules re-associate to provide an increased viscosity in the final stage. Most pulse starches exhibit low peak viscosities before disintegration, low breakdown viscosities, but high setback viscosities during cooling due to their higher amylose contents (Hoover, Hughes, Chung, & Liu, 2010).

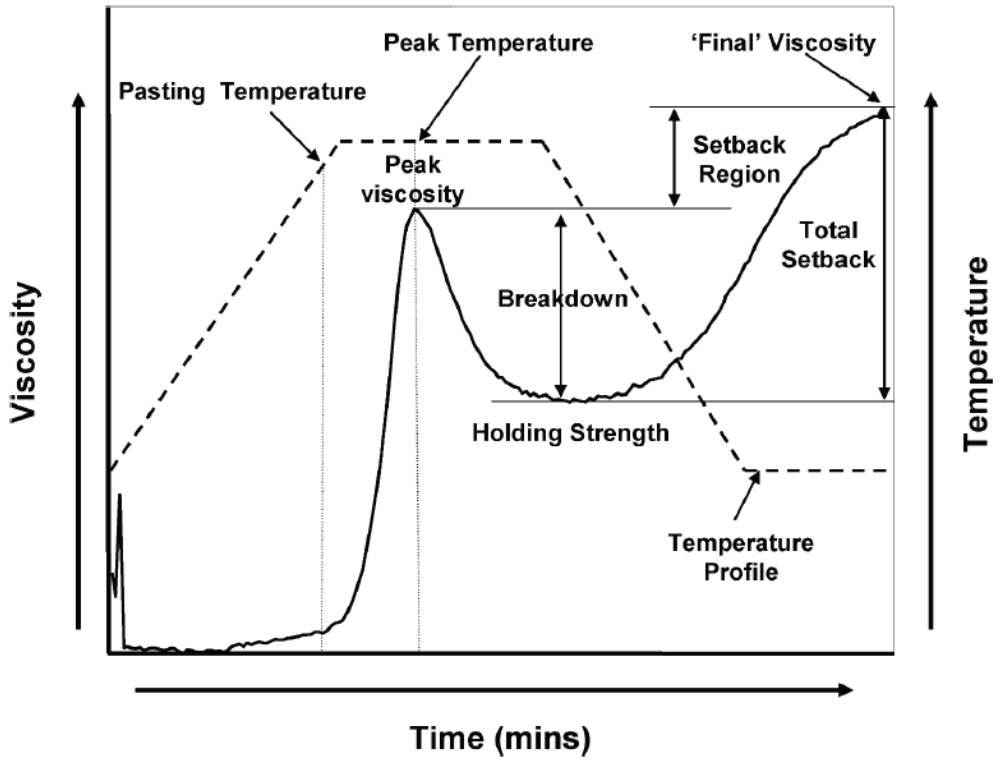


Fig. 2.4 A typical starch pasting curve measured using Rapid Visco Analyzer (RVA) or Brabender Visco-amylograph (Wani, Singh, Shah, Schweiggert-Weisz, Gul, & Wani, 2012).

2.6. Gel formation

Upon cooling and storage, the aforementioned starch paste develops into a gel, a process in which the swollen starch granules fill up the whole volume of the mold and amylose and amylopectin molecules interact with one another to form a network to hold water in the structure (Keetels, Van Vliet, & Walstra, 1996). Generally, gel strength increases with a higher amylose content for normal starches because amylose tends to restrict the swelling and maintain granular integrity of starch (Biliaderis, 2009; Hoover, Hughes, Chung, & Liu, 2010).

2.7. Digestibility of pulse starch

Starch is the main carbohydrate component of human foods. After ingestion, starch can be hydrolyzed by amylolytic enzymes to produce glucose for energy supply in the human body. Enzymes that are involved in the breakdown of starch in the human gastrointestinal tract include salivary α-amylase, pancreatic α-amylase, glucoamylase, maltase, sucrase and isomaltase (Lin, Lee, & Chang, 2016). Digestion rate and extent of starch are influenced by its granular features,

molecular structures, functional properties as well as interactions between starch and other constituents (*e.g.*, lipids, proteins, and fiber) (Hoover, Hughes, Chung, & Liu, 2010).

Previous research has provided evidence that some ingested starch can survive digestion in the human small intestine and then be passed into large intestine for microbial fermentation. This portion of starch is defined as "resistant starch" (RS) (Englyst & Macfarlane, 1986; Englyst, Wiggins, & Cummings, 1982). In a later study of Englyst, Kingman, and Cummings (1992), starch was hydrolyzed by a mixture of porcine pancreatic extract and amyloglucosidase at 37°C for 20 and 120 min to determine the contents of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) *in vitro*. RDS is mainly composed of amorphous and dispersed starch and can cause a rapid rise in blood glucose level after ingestion; SDS is the starch fraction that is hydrolyzed between 20 and 120 min; and RS refers to the portion of starch that cannot be hydrolyzed after 120 min of incubation. Native pulse starches, including pea and lentil starch, are found to be less digestible than normal maize starch, which could be due to the less porous granular structure, higher amylose contents, and larger portions of long amylopectin chains of pulse starches (Ambigaipalan, et al., 2011; Chung, Liu, & Hoover, 2009). The relatively high enzymatic resistance of pulse starches makes them promising raw materials for the preparation of RS for food applications.

2.8. Resistant starch

Resistant starch (RS), as a novel type of dietary fiber, has attracted much research attention in recent years because it can provide various health benefits to humans after consumption, which include decreasing postprandial glycemic/insulinemic responses, improving insulin sensitivity, increasing lipid metabolism and reducing obesity, promoting colon health, and enhancing the absorption of minerals (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Sajilata, Singhal, & Kulkarni, 2006). Research efforts have been made to develop different types of RS from starches of various botanical origins. In general, RS can be classified into five different types (RS1−5) (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Putseys, Lamberts, & Delcour, 2010). In this section, the discussion primarily focuses on the RS generated from pulse starches.

2.8.1 Type 1 resistant starch (RS1)

RS1 is physically inaccessible to digestive enzymes because the starch is embedded in protein and/or cellulose matrices (*e.g.*, intact cells in pulses). RS fractions in whole pulse seeds or coarsely ground pulse flours are good examples of RS1 (Hoover & Zhou, 2003; Vargas-Torres, Osorio-Díaz, Islas-Hernández, Tovar, Paredes-López, & Bello-Pérez, 2004). Chung, Liu, Hoover, Warkentin, and Vandenberg (2008) investigated the RS contents of pea and lentil flours after hydrolyzing the samples for 16 h with porcine pancreatic α-amylase and amyloglucosidase. They concluded that the high RS contents of pea and lentil flours (10.1−14.7% and 14.4−14.9%, respectively) could partly be attributed to the high protein content, which could directly interact with starch to restrict enzymatic breakdown. In addition, Dhital, Bhattarai, Gorham, and Gidley (2016) have explicitly shown that the intactness of cell wall structure was the limiting factor that controlled the rate and extent of hydrolysis of starch trapped inside the pulse cotyledon cells.

2.8.2 Type 2 resistant starch (RS2)

RS2 refers to native, ungelatinized starches with the B- or C-type crystalline structure (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Hoover & Zhou, 2003). Most pulse starches, including pea, lentil and faba bean, display the C-type X-ray diffraction pattern and have high RS contents. Chung, Liu, and Hoover (2009) reported that the RS contents of native pea (10.0%) and lentil (9.1%) starches are greater than normal maize starch (4.6%). Faba bean starch has also been shown to consist of 8.1−15.0% RS after 16 h digestion (Ambigaipalan, et al., 2011).

2.8.3 Type 3 resistant starch (RS3)

RS3 represents retrograded starch that reforms double-helical crystallites with concomitant resistance to enzymatic hydrolysis. When starch granules are suspended in water and heated, some amylose molecules will leach out from the granules into solution (in a random coil conformation) during gelatinization. Upon cooling, RS3 is formed when free amylose chains begin to re-associate with each other to reform double helices that are stabilized by hydrogen bonds (Fig. 2.5) (Haralampu, 2000; Hsien-Chih & Sarko, 1978).

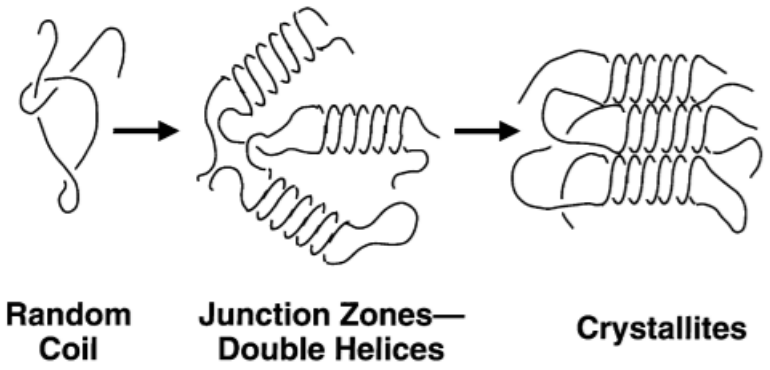


Fig. 2.5 Schematic presentation in the formation of RS3 (Haralampu, 2000).

Amylose molecules are favorable for the preparation of RS3 because of their faster rate of retrogradation and the formation of highly resistant crystallites (Eerlingen & Delcour, 1995; Sievert & Pomeranz, 1989, 1990). To foster the formation of this type of RS, starch can be debranched enzymatically (*e.g.*, isoamylase, pullulanase) which specifically hydrolyzes α-1,6 glycosidic bonds to release a larger proportion of linear glucan chains for enhanced retrogradation (Cai & Shi, 2010; Shi, Chen, Yu, & Gao, 2013). In addition, ultrasonication was shown to have a synergistic debranching effect with pullulanase, which produced 73.5% linear glucans and resulted in 26% RS content from the debranched pea starch (Lu, Belanger, Donner, & Liu, 2018).

Apart from the content of amylose, the degrees of polymerization (DP) of amylose molecules have also been shown to affect RS3 formation: an appropriate chain length (minimum DP of 10, optimum DP of 100) is more desirable for the formation of stable double helices (Eerlingen, Decuninck, & Delcour, 1993; Gidley, Cooke, Darke, Hoffmann, Russell, & Greenwell, 1995). Previous studies have shown that a reduction of chain length either by limited β-amylolysis or α-amylolysis prior to debranching promoted the formation of RS3 from various starches (Luckett & Wang, 2012; Villas-Boas & Franco, 2016; Zhang & Jin, 2011). Mild acid thinning by hydrochloric acid has also been found to increase the RS contents of extruded normal maize starch and annealed pea and lentil starches (Hasjim & Jane, 2009; Vasanthan & Bhatty, 1998). These results can be explained by the fact that partial hydrolysis by enzyme or acid can enhance the molecular mobility of starch chains and generate a greater proportion of linear starch molecules of moderate chain lengths for enhanced retrogradation. Furthermore, physical treatments (*e.g.*, heat-moisture and temperature-cycle treatments) that can promote the

recrystallization of linear starch chains and enhance the tight packing of the crystallites have also been employed to achieve a higher level of RS3 (Mutungi, Rost, Onyango, Jaros, & Rohm, 2009; Zeng, Ma, Gao, Yu, Kong, & Zhu, 2014).

The structure of RS3 from the recrystallized debranched starch can be influenced by several factors. Generally, a higher solids concentration, higher recrystallization temperature, and shorter chains tend to induce the A-type crystalline structure, whereas a lower solids concentration, lower recrystallization temperature, and longer chains favor the formation of B-type crystalline structure (Fig. 2.6) (Liu, Gu, Hong, Cheng, & Li, 2017).

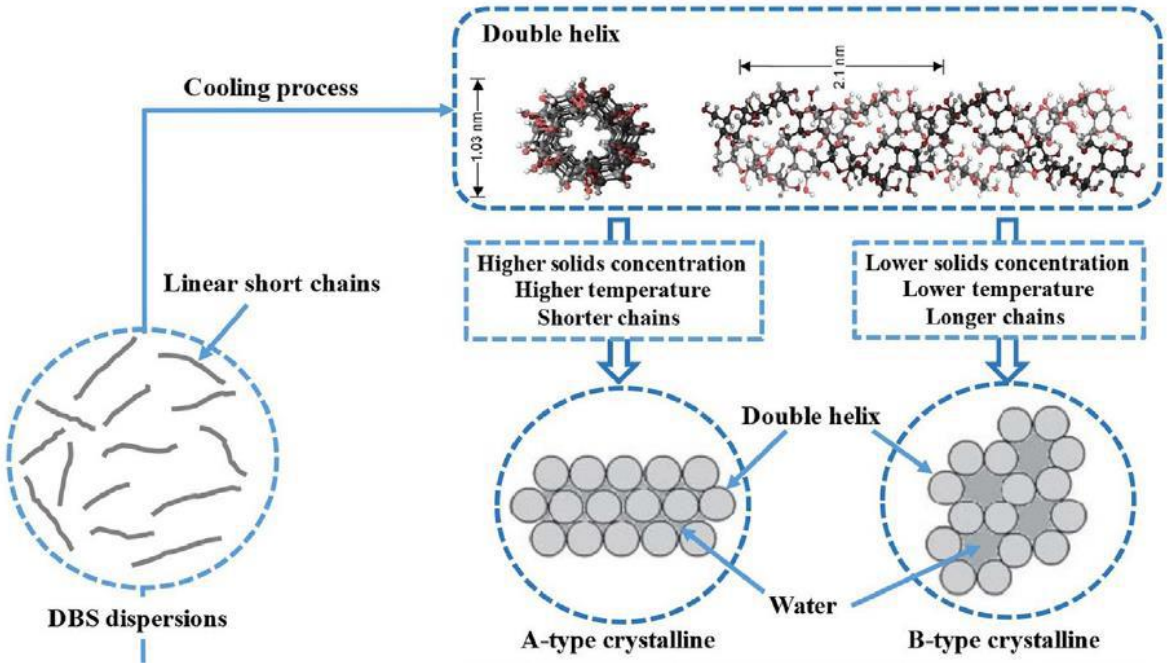


Fig. 2.6 Schematic diagram of the crystalline structure of debranched starch products (Liu, Gu, Hong, Cheng, & Li, 2017).

Recently, new methods have been adopted to produce RS3 in a spherulite form (Fig. 2.7) (Kiatponglarp, Rugmai, Rolland-Sabaté, Buléon, & Tongta, 2016). Spherulites are spherical semi-crystalline structures that exhibit a specific birefringence in the form of a "Maltese" cross when observed under a polarized light microscope (Bassett, 2003). Various studies have shown that spherulites can be formed by heating aqueous amylose dispersions above 170°C (defined as "clearing temperature"), followed by a rapid cooling process to 10°C (Nordmark & Ziegler, 2002; Ziegler, Creek, & Runt, 2005). Creek, Ziegler, and Runt (2006) have proposed that in the heating process double-helical crystallites (10−20 wt% isolated amylose) begin to melt at around

70°C, and fixed network entanglements are thermally dissociated at 130°C to a liquid phase. Continually, the system becomes isotropic at a temperature between 160 and 180°C. Upon cooling, rapid quenching is used to induce phase separation instead of gel formation. Therefore, amylose solution is transformed from the isotropic state into polymer-poor and polymer-rich phases. Double helices formed in the polymer-rich phase can be further rearranged into self-aligned crystallites, which eventually develop spherulites and precipitate from the liquid medium.

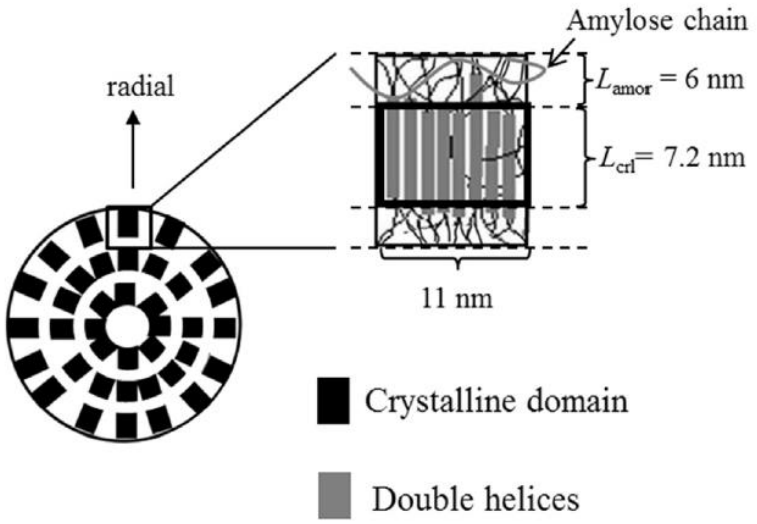


Fig. 2.7 Schematic model of the radical positive spherulites (Kiatponglarp, Rugmai, Rolland-Sabaté, Buléon, & Tongta, 2016).

In the study of Cai and Shi (2013), a novel process combining isoamylase debranching, melting and recrystallization has been developed to produce spherulites from a debranched waxy maize starch solution with a high solids concentration (25%, w/w), which generated 78.1−84.6% RS (Fig. 2.8). Results showed that spherulites recrystallized at lower temperatures (4 and 25°C), exhibited the B-type crystalline structure, had a larger size (5−10 μm), a lower melting temperature (70−110°C), and a lower RS content (78.1−79.6%), whereas spherulites developed at a higher temperature (50°C), displayed the A-type diffraction pattern, had a smaller size (1−5 μm), a higher melting temperature (100−140°C), and a higher RS content (84.6%).

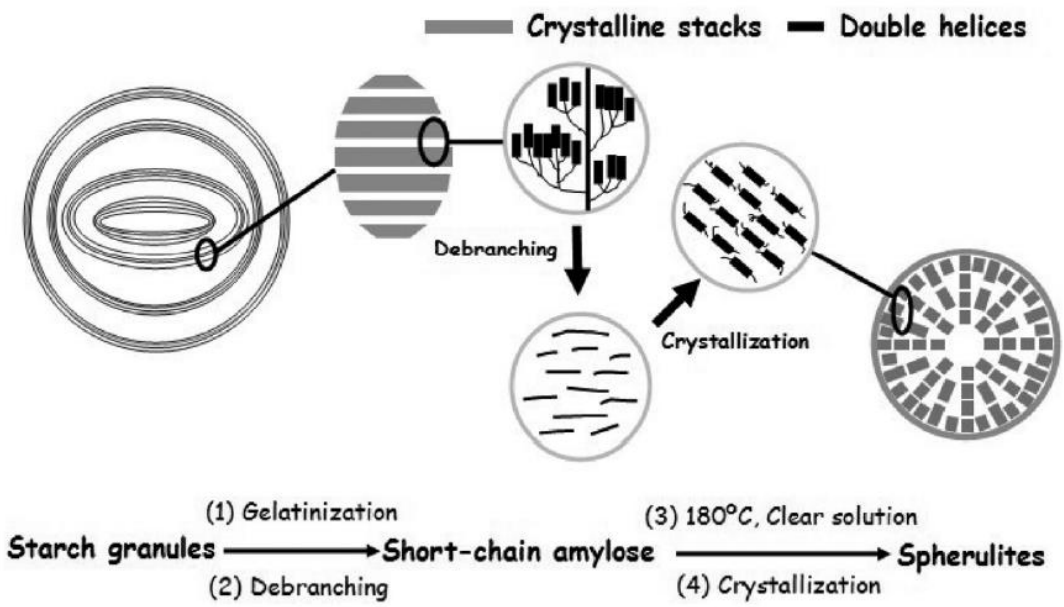


Fig. 2.8 Schematic illustration of transition from debranched waxy maize starch into spherulites (Cai & Shi, 2013).

2.8.4 Type 4 resistant starch (RS4)

RS4 is chemically modified, such as cross-linked starch and substituted starch, in which new covalent bonds are formed between chemical derivatives and starch molecules (Hoover & Sosulski, 1991; Hoover & Zhou, 2003). In the study of Shi, Gu, Wu, Yu, and Gao (2013), higher RS contents of modified pea starch were observed as the degree of cross-linking increased. The largest RS content (64%) was achieved when 12% sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) (cross-linking agents) were used.

2.8.5 Type 5 resistant starch (RS5)

RS5 refers to the amylose-lipid complex. The single-helical complex formed between amylose and lipids can not only restrict the swelling of starch granules but also promote rearrangement into lamellar crystallites, which remarkably enhance the enzymatic resistance of RS5 (Putseys, Lamberts, & Delcour, 2010). In the study of Ai, Hasjim, and Jane (2013), the enzymatic hydrolysis rates of cooked normal maize, tapioca and high-amylose starches were significantly reduced by the addition of 10% (w/w, dry basis) lipids prior to cooking.

3. CHARACTERISTICS OF PEA, LENTIL AND FABA BEAN STARCHES ISOLATED FROM AIR-CLASSIFIED FLOURS IN COMPARISON WITH COMMERCIAL STARCHES[1]

3.1 Abstract

This study aimed to isolate starches of a high purity from starch-rich pea, lentil and faba bean flours and to characterize and compare the isolated starches with important commercial starches. Isolated pea, lentil and faba bean starches had a purity of 94.8–97.9% and contained only 0.9–1.1% damaged starch. The isolated pulse starches showed amylose contents and amylopectin branch-chain-length distributions similar to those of commercial pea starch. Therefore, the granular morphologies, crystalline structure, thermal properties, pasting properties, gelling ability and *in vitro* digestibility of the isolated pulse starches were comparable to those of commercial pea starch but distinctively different from those of commercial maize and tapioca starches. The desirable functionality of the isolated pulse starches (*e.g.*, strong gelling ability) renders them suitable for some specific industrial applications, and further modifications can be utilized to enhance their functionality for broader use. This research provided the fundamental knowledge required for future efforts to promote value-added utilization of pulse starches.

Keywords: starch-rich pulse flour; starch isolation; pulse starch; structure of starch; functionality of starch; *in vitro* starch digestibility

3.2 Introduction

Pulses (or grain legumes) are dried, edible seeds of leguminous crops, such as peas (*Pisum sativum* L.), lentils (*Lens culinaris* L.), faba beans (*Vicia faba* L.), chickpeas (*Cicer*

[1]Li, L., Yuan, T. Z., Setia, R., Raja, R. B., Zhang, B., & Ai, Y. (2019). Characteristics of pea, lentil and faba bean starches isolated from air-classified flours in comparison with commercial starches. *Food Chemistry*, *276*, 599-607.

arietinum L.) and beans (*Phaseolus vulgaris* L.). Pulses are a rich source of nutrients that are important for human health, including complex carbohydrates (*e.g.*, resistant starch and other dietary fibers), proteins, minerals and phytochemicals (*e.g.*, isoflavones, phytosterols and saponins) (Rochfort & Panozzo, 2007; Tosh & Yada, 2010). Epidemiological evidence shows that pulses have lower glycemic impact than other starchy foods in human diets, such as cereal grains and potato (Hutchins, Winham, & Thompson, 2012). Therefore, pulses have been recommended for the prevention of type-2 diabetes and for improved glycemic control in diabetic individuals (Jenkins, et al., 2012). Regular consumption of pulses has also shown promise in the prevention and treatment of other chronic diseases, such as obesity, cardiovascular diseases and cancers (Mudryj, Yu, & Aukema, 2014).

Traditionally, pulses are consumed as canned foods or dry seeds–either in a whole or split form. As examples, dry peas are used in soups, dhals and snack foods. Recent research and development efforts have focused on the fractionation of pulses to produce various food ingredients, including pulse proteins, fibers and starches. This approach can effectively increase the utilization of pulses in various food products. Two methods are commonly employed in the industry to process pulse seeds into different fractions: wet and dry method (Hoover, Hughes, Chung, & Liu, 2010).

For both methods, whole seeds of pulses are typically dehulled first. The removed hull can be ground to pass through a screen to produce hull fiber with the target particle size, which can be added to boost the dietary fiber contents of food products (Dalgetty & Baik, 2003). In the wet method, the dehulled pulse seeds are soaked and then milled in a wet state. The protein in the suspension can be extracted and concentrated using different methods, such as alkaline extraction-isoelectric precipitation, acid extraction, water extraction, salt extraction and ultrafiltration (Boye, Zare, & Pletch, 2010). The starch remaining in the suspension can be purified by additional washing to remove protein residue and filtration through a screen of a certain pore size (*e.g.*, 70 μm) to remove fiber residue, which is followed by recovering and drying to obtain isolated starch. Pulse starch isolated using the wet method has a high purity, containing only 0.04−0.50% protein and 0.01−0.20% ash (Davydova, Leont'Ev, Genin, Sasov, & Bogracheva, 1995; Hoover & Sosulski, 1991). Other commercial starches, such as maize and tapioca starches, are isolated from the respective crops using similar wet methods. In the dry method, the dehulled pulse seeds are milled−using a hammer mill, pin mill or impact mill−to

obtain a pulse flour. The flour is further separated into protein-rich fraction and starch-rich fraction using air classification technology based on their different densities and particle sizes: the protein-rich flour is finer and lighter and the starch-rich flour is coarser and denser (Boye, Zare, & Pletch, 2010). This method can yield protein-rich fraction containing 49.3−75.1% protein and 0.0−4.6% starch and starch-rich fraction containing 71.0−85.9% starch and 4.0−10.4% protein from dehulled pulse seeds of different species (Tyler, Youngs, & Sosulski, 1981). Compared with the wet method, the dry method produces protein and starch fractions of a lower purity. Additionally, the dry method leads to a greater level of starch damage. For example, it has been reported that 1.0−3.2% starch was damaged in pea flour prepared using different dry milling methods (Maskus, Bourré, Fraser, Sarkar, & Malcolmson, 2016; Pelgrom, Vissers, Boom, & Schutyser, 2013), while the pulse starches isolated using the wet method consist of less than 0.1% damaged starch (Maaran, Hoover, Donner, & Liu, 2014). The difference can be attributed to that dehulled seeds are milled in a dry state and a high degree of particle size reduction is required before the separation by air classification in the dry processing (Maskus, Bourré, Fraser, Sarkar, & Malcolmson, 2016; Pelgrom, Vissers, Boom, & Schutyser, 2013). However, because the dry method requires a considerably lower operation cost and less use of water (Sosulski & Sosulski, 1986), it is the method more widely used for the fractionation processing of pulses in Canada. The derived protein-rich flour, having desirable functional and nutritional properties (*i.e.*, a good source of plant protein), is used as a popular ingredient for the formulation of new products in the food industry (Boye, Zare, & Pletch, 2010). In contrast, the starch-rich flour shows poorer nutritional value and less desirable functionality, and thus the pulse processing industry is exploring new approach to increasing industrial utilization of this co-product.

One solution to this problem is to modify pulse starches using chemical, physical and/or enzymatic methods to improve their inherent properties for wider industrial applications. In the starch industry, the modifications are mostly carried out on isolated starches of a high purity, and a comprehensive understanding of the structure and functionality of the native starches is required prior to the modification processes. Currently, the majority of pulse starches generated from the pulse processing industry in Canada is in the form of starch-rich flours from air classification, and there is a lack of knowledge regarding the structure and functionality of the starches from different pulse crops. Therefore, in the present study we aimed to: 1) isolate

starches of a high purity from the most common starch-rich pulse flours available in the market, including pea, lentil and faba bean; and 2) advance our understanding of the structural features and functional properties of the isolated pulse starches by characterizing and comparing them with important commercial starches, including pea, normal maize, waxy maize and tapioca starches that are isolated using wet methods. The acquired new knowledge will answer the following critical research questions: (1) Will the pulse starches isolated from the air-classified starch-rich flours perform similarly to commercial pea starch fractionated using a wet method? (2) Can the pulse starches be used to replace the major industrial starches (*e.g.*, maize and tapioca starches) in some specific applications in the industry? This research also provided the basis required for further modifications of pulse starches for increased industrial utilization.

3.3 Materials and Methods

3.3.1 Materials

Starch-rich flour of pea (Starlite) was obtained from Parrheim Foods (Saskatoon, SK, Canada) and those of lentil (Homecraft® 2103) and faba bean (Homecraft® 3103) were provided by AGT Food and Ingredients (Saskatoon, SK, Canada). All the three starch-rich flours were products from air classification of corresponding dehulled pulse flours. Four commercial starch samples were included in the study: pea starch from Roquette Canada Ltd. (Winnipeg, MB, Canada); normal maize starch (Cargill Gel™ 03420), waxy maize starch (Cargill Gel™ 04230) and tapioca starch (Cream Gel™) from Cargill Inc. (Minneapolis, MN, U.S.A.). All the commercial starches were isolated from the respective crops using wet methods.

Pancreatin from porcine pancreas and amyloglucosidase from *Aspergillus niger* (300 U/mL) were purchased from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Isoamylase (1,000 U/mL), Total Starch Assay Kit, Starch Damage Assay Kit and D-Glucose Assay Kit were procured from Megazyme International Ltd. (Co. Wicklow, Ireland). All the other chemicals used in the study were reagent grade and purchased from Fisher Scientific Company (Ottawa, ON, Canada) or Sigma-Aldrich Canada Co. (Oakville, ON, Canada).

3.3.2 Starch isolation

The starch-rich flour (~300 g) was suspended in 750 mL of distilled water and stirred for 1 h. The suspension was centrifuged at 7,000 *g* for 20 min. After the supernatant was carefully

discarded, the protein layer on the top of the precipitate was scraped off manually using a spatula. This protein removal step was repeated twice in total. The sediment was then re-suspended in 600 mL of distilled water, followed by pH adjustment to 9.5 by adding 0.5 M NaOH dropwise and magnetic stirring for 1 h to solubilize the remaining protein (Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). The suspension was filtered through a nylon cloth with openings of 60 µm to remove the fiber residue, and the obtained filtrate was filtered using a Whatman #1 filter paper to remove the protein solubilized in the alkaline solution. The fiber removal and protein washing steps were repeated three times in total. The isolated starch was re-suspended in 600 mL of distilled water, neutralized using 0.5 M HCl dropwise, recovered by filtration using a Whatman #1 filter paper, washed three times by 100 mL distilled water and three times by 100 mL anhydrous ethanol in the filter funnel, and then dried at 40°C in a convection oven overnight.

3.3.3 Proximate analyses

Proximate analyses were performed on the isolated pulse starches, corresponding starch-rich flours, and commercial starches (including Roquette pea, normal maize, waxy maize and tapioca starches) to determine the purity of the isolated starches and to calculate the yields of starch isolation. Moisture content was determined using AACC Method 44−15.02 (AACC, 2000). Starch content was quantified using Total Starch Assay Kit following AACC Method 76−13.01 (AACC, 2000). In brief, complete dispersion of starch was achieved using 2.0 M KOH in an ice-water bath, the dispersed starch was hydrolyzed into D-glucose using thermostable α-amylase and amyloglucosidase, and the total amount of released D-glucose was quantitated colorimetrically using a glucose oxidase/peroxidase (GOPOD) method. The total amount of generated D-glucose was applied to calculate the starch content of each sample. The yield of starch isolation as described in Section 3.3.2 was calculated as: %yield of starch = 100 × dry mass of isolated starch / (initial dry mass of starch-rich flour × starch content of starch-rich flour on a dry basis). Damaged-starch content was determined using Starch Damage Assay Kit following AACC Method 76−31.01 (AACC, 2000). Nitrogen content was measured by the Dumas combustion method using a Nitrogen/Protein Analyzer (CN628, LECO Corp., St. Joseph, MI, U.S.A.), which was converted to protein content (%N × 6.25) in accordance with AACC Method 46−30.01 (AACC, 2000). Ash content was measured using AACC Method 08−01.01

(AACC, 2000). Starch, protein and ash contents of the starch and flour samples were reported on a dry basis (db).

3.3.4 Amylose content of starch

Iodine affinity of the isolated pulse and commercial starches was determined by potentiometric titration using an autotitrator (Titrando 888, Metrohm AG, Mississauga, ON, Canada) equipped with a Pt-ring electrode and a calomel reference electrode (Song & Jane, 2000). Firstly, starch was dispersed in 90% (v/v) dimethyl sulfoxide in a boiling water bath, precipitated with five volumes of anhydrous ethanol, and centrifuged at 3,000 *g* for 15 min. The supernatant was discarded to remove the dissolved endogenous lipids (Li, Jiang, Campbell, Blanco, & Jane, 2008). The recovered starch was dried at 105ºC overnight. The defatted, dried starch (100.0 mg, db) was autotitrated using an iodine solution (containing 50 mM potassium iodide, 50 mM potassium chloride and 0.8 mM iodine) to measure the iodine affinity, which was converted to amylose content after dividing by a conversion factor of 0.2 (Duan, Donner, Liu, Smith, & Ravenelle, 2012; Takeda, Hizukuri, & Juliano, 1987).

3.3.5 Branch-chain-length distribution of amylopectin[2]

Branch-chain-length (BCL) distributions of amylopectins of the isolated pulse and commercial starches were determined using fluorophore-assisted capillary electrophoresis (FACE) (ProteomeLab PA800, Beckman Coulter Inc., Brea, CA, U.S.A.), following the method of Asare, Jaiswal, Maley, Båga, Sammynaiken, Rossnagel, et al. (2011). Briefly, starch was gelatinized using 50 µL of 2.0 M NaOH and the dispersion was then neutralized with 32 µL of glacial acetic acid, which was followed by the addition of 100 µL of 1.0 M sodium acetate buffer (pH 4.5), 1.0 mL of distilled water, and 10 U of isoamylase (1,000 U/mL) for the debranching reaction. The released linear glucan chains were labelled with 8-aminopyrene-1,3,6-trisulfonate (ATPS). An N-CHO (PVA) capillary was used to separate the labelled glucan chains of different lengths for subsequent quantification. Maltose was used as an internal standard for the analysis.

[2] This experiment was done by Dr. Ramadoss Bharathi Raja at the Department of Plant Sciences, U of S.

3.3.6 Granular morphology of starch

Granular morphology and surface structure of the isolated pulse and commercial starches were examined using a field-emission scanning electron microscope (SEM, SU8010, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada) under the conditions of 3.0 kV and 10 μA. For the sample preparation, starch was sprinkled onto a carbon tape that was affixed on an aluminum stub, and the sample was then coated with gold using a sputtering technique. Images of the starch granules were captured at three magnifications: 500×, 1,500× and 5,000×.

3.3.7 Wide-angle X-ray diffraction of starch[3]

The isolated pulse and commercial starches were equilibrated in a chamber of 100% relative humidity at 25°C for 24 h. The wide-angle X-ray diffraction patterns of the starch samples were analyzed using a D/Max 2200 X-ray diffractometer (Rigaku Corp., Tokyo, Japan) operating at 40 kV and 40 mA with Cu Kα radiation (λ = 1.5406Å). The starch powder was packed tightly in a rectangular glass cell and scanned from the diffraction angle (2θ) of 4° to 40° at a rate of 2°/min at room temperature. The relative crystallinity of the starch was calculated using OriginPro 2018 Software (OriginLab Corp., Northampton, MA, U.S.A.) in accordance with the method of Hayakawa, Tanaka, Nakamura, Endo, and Hoshino (1997).

3.3.8 Thermal properties of starch[4]

Gelatinization and retrogradation properties of the isolated pulse and commercial starches were determined using a differential scanning calorimeter (DSC Q2000, TA Instruments, New Castle, DE, U.S.A.) according to the method of Song and Jane (2000). Starch (2−3 mg) was weighed into a Tzero alodined pan (TA Instruments, New Castle, DE, U.S.A.) and thoroughly hydrated with distilled water (~3 volumes, v/w) using a microsyringe. After sealing the pan and equilibrating the sample at ambient temperature for more than 2 h, the starch was scanned from 10°C to 110°C at a rate of 10°C/min. The gelatinized sample was then stored at 4°C for 7 days and scanned again using the same procedure to examine the melting peak of retrograded starch. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy changes (ΔH) of the thermal transitions were analyzed using Universal Analysis 2000 Software (TA Instruments, New Castle,

[3] This experiment was done by Dr. Bin Zhang's research group at School of Food Science and Engineering, South China University of Technology.
[4] This experiment was done by Rashim Setia at the Department of Food and Bioproduct Sciences, U of S.

DE, U.S.A.). Percentage starch retrogradation was calculated as: % retrogradation (%R) = 100 × ΔH of dissociation of retrograded starch / ΔH of starch gelatinization.

3.3.9 Pasting properties and gelling ability of starch

Rapid Visco-Analyzer (RVA 4500, Perten Instruments, Winnipeg, MB, Canada) was used to measure the pasting properties of the isolated pulse and commercial starches following the method of Song and Jane (2000) with minor modifications. Starch slurry (8% db, 28.0 g total weight) was loaded to the RVA instrument and run using the Standard Method 2 programmed in the Thermocline Software. Briefly, the suspension was equilibrated at 50°C for 1 min, heated to 95°C at a rate of 6°C/min, maintained at 95°C for 5 min, and then cooled at a rate of 6°C/min to reach 50°C. The rotating speed of the paddle was 960 rpm for the initial 10 s and 160 rpm for the rest period in the run.

To prepare starch gel, the starch suspension of the same concentration (8% db, 28.0 g total weight) was heated using the RVA under the same conditions and maintained at 95°C for 5 min, poured into a plastic jar (internal diameter = 33.0 mm, height = 38.0 mm) and covered with a lid, and then stored at room temperature for 2.5 h. The strength of the developed starch gel was measured using a Texture Analyzer TA.XT. Plus (Texture Technologies Corp., South Hamilton, MA, U.S.A.) with Probe TA-10 (diameter = 12.7 mm). After a trigger force of 0.5 g was attained, the probe proceeded to penetrate the gel at a test speed of 0.5 mm/s and at a compression depth of 12.0 mm. The maximum force during the 12.0 mm compression was defined as the strength of starch gel.

3.3.10 *In vitro* digestibility of raw and cooked starch

In vitro digestibility of the isolated pulse and commercial starches, in both raw and cooked forms, was determined using an *in vitro* method of Englyst, Kingman, and Cummings (1992) with slight modifications (Ai, Nelson, Birt, & Jane, 2013). Sample containing 600 mg starch (db) was suspended in 15.0 mL of distilled water. The starch was cooked by incubating the starch suspension in a boiling water bath for 10 min with vigorous shaking every 2 min. Sodium acetate buffer (5.0 mL, pH 5.2, 0.4 M, containing 0.08% sodium azide) was added to the raw and cooked samples. After the equilibration in a water bath of 37°C with shaking (160 rpm) for 15 min, a freshly prepared enzyme mixture (5.0 mL) of porcine pancreatin extract and

amyloglucosidase was added to hydrolyze the starch. Rapidly digestible starch (RDS; digested within 20 min), slowly digestible starch (SDS; digested between 20 and 120 min) and resistant starch (RS; undigested after 120 min) contents of the starch sample were calculated from the amounts of released D-glucose at time intervals of 20 min and 120 min as quantitated using D-Glucose Assay Kit (Englyst, Kingman, & Cummings, 1992).

3.3.11 Confocal laser scanning microscopy[5]

Confocal laser scanning microscopy (CLSM) was performed following the method of Jane, Atichokudomchai, and Suh (2004) to reveal the internal structure of starch granules. Each of the isolated pulse and commercial starches (~4 mg) was incubated in 400 μL of 0.01% (w/v) Rhodamine B solution containing 0.02% (w/v) sodium azide for 24 h at ambient temperature for staining. The stained starch was recovered by centrifugation at 3,000 *g* for 10 min and the supernatant was carefully removed. The pellet was re-suspended in 200 μL of distilled water and centrifuged again to remove the unbound dye, and then suspended in 20 μL of 50% (v/v) glycerol. The prepared starch suspension (1 μL) was mounted on a glass slide using one drop of 80% (v/v) glycerol containing 2% (w/v) agarose and then observed using a confocal laser scanning microscope (TCS SP5, Leica Microsystem, Concord, ON, Canada) equipped with a 63× objective (HCX PL APO 63.0×/1.40 Oil, Leica Microsystem). The stained starch was examined using laser beam with a wavelength of 543 nm, and the emission peak was found between 555 nm and 700 nm. Multiple granules from the same starch were observed to obtain representative images of the sample. The captured images were processed using Fuji Image J Software.

3.3.12 Statistical analysis

All the analyses were performed in triplicate except for wide-angle X-ray diffraction (one replicate). Statistical differences among the results of the isolated pulse and commercial starches were analyzed using one-way ANOVA, Tukey's multiple comparison test at a significance level of 0.05. Correlations between the data of structural characteristics, functional properties and *in*

[5] This experiment was done by Tommy Z. Yuan and Liying Li at the Department of Food and Bioproduct Sciences, U of S.

vitro digestibility of the starches were evaluated using the Pearson correlation test. The statistical analyses were conducted on IBM SPSS Statistics Analysis (Version 24.0).

3.4 Results and Discussion

3.4.1 Chemical compositions of starch-rich pulse flours, isolated pulse and commercial starches, and yields of starch isolation

The starch-rich pea, lentil and faba bean flours obtained from commercial sources consisted of 65.3−77.7% starch, 7.9−20.2% protein and 1.40−2.29% ash, respectively, on a dry basis (Table 3.1), confirming that air classification process was not very effective to separate protein, fiber and minerals from starch (Boye, Zare, & Pletch, 2010; Tyler, Youngs, & Sosulski, 1981). The employed starch isolation method generated pea, lentil and faba bean starches of 94.8−97.9% purity, containing only 0.1−0.2% protein and 0.01−0.03% ash (Table 3.1). The isolated pulse starches showed similar starch and protein contents and even lower ash contents in comparison with commercial Roquette pea, normal maize, waxy maize and tapioca starches (95.8−97.5% starch, 0.0−0.3% protein and 0.07−0.15% ash), which suggested that the starch isolation method was effective to produce pulse starches of a desirable purity from air-classified starch-rich flours for further modifications and applications. The isolation method could extract 62.5%, 82.1% and 84.7% starch from starch-rich pea, lentil and faba bean flours, respectively. The markedly lower yield of starch isolation from pea flour could result from the strong association between starch and protein in the flour. After the pea flour was suspended and stirred in distilled water and then centrifuged at 7,000 *g* for 20 min, the starch was not well separated from the protein layer on the top. Consequently, a large portion of starch was also removed when the protein layer was scraped off manually using a spatula as described in the starch isolation process (Section 3.3.2). It is important to note that the starch-rich flours contained relatively high levels of damaged starch (1.9–3.4%), resulting from the severe milling of the pulse flours in a dry state to achieve the desirable degree of particle size reduction for subsequent air classification (Maskus, Bourré, Fraser, Sarkar, & Malcolmson, 2016; Pelgrom, Vissers, Boom, & Schutyser, 2013). Nevertheless, the damaged-starch contents of the isolated pea, lentil and faba bean starches (0.9–1.3%) were comparable to those of the commercial starches (0.9–1.6%) fractionated using wet methods. The data suggested that large proportions of damaged starch granules in the starch-rich flours were removed during the starch isolation process.

Table 3.1 Chemical composition of starch-rich pulse flours, isolated pulse and commercial starches, and yields of starch isolation[a]

	Chemical composition				Yield of starch (%)[f]
	Starch content (%, dry basis)[b]	Damaged-starch content (%, dry basis)[c]	Protein content (%, dry basis)[d]	Ash content (%, dry basis)[e]	
Starch-rich flour					
Pea	77.7 ± 0.6 c	3.0 ± 0.0 b	7.9 ± 0.1 c	1.40 ± 0.01 c	N.A.[g]
Lentil	72.0 ± 0.2 d	3.4 ± 0.1 a	15.3 ± 0.1 b	1.78 ± 0.01 b	N.A.
Faba bean	65.3 ± 0.5 e	1.9 ± 0.1 c	20.2 ± 0.4 a	2.29 ± 0.01 a	N.A.
Isolated starch					
Pea	94.8 ± 0.2 b	1.1 ± 0.0 f	0.2 ± 0.0 d	0.03 ± 0.00 g	62.5 ± 1.1 b
Lentil	97.9 ± 1.0 a	1.3 ± 0.0 e	0.1 ± 0.0 d	0.03 ± 0.02 fg	82.1 ± 4.2 a
Faba bean	96.5 ± 1.9 ab	0.9 ± 0.0 h	0.2 ± 0.0 d	0.01 ± 0.01 g	84.7 ± 4.3 a
Commercial starch					
Roquette pea	97.2 ± 0.5 ab	1.0 ± 0.0 g	0.2 ± 0.0 d	0.07 ± 0.01 ef	N.A.
Normal maize	95.8 ± 0.3 ab	1.1 ± 0.0 f	0.3 ± 0.0 d	0.08 ± 0.01 e	N.A.
Waxy maize	97.5 ± 1.1 a	1.6 ± 0.0 d	0.1 ± 0.0 d	0.15 ± 0.01 d	N.A.
Tapioca	97.1 ± 0.8 ab	0.9 ± 0.0 gh	0.0 ± 0.0 d	0.09 ± 0.02 e	N.A.

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.
[b] Determined using Megazyme Total Starch Assay Kit following AACC Method 76−13.01.
[c] Determined using Megazyme Starch Damage Assay Kit following AACC Method 76−31.01.
[d] Determined using a Nitrogen/Protein Analyzer following AACC Method 46−30.01.
[e] Determined using an electric muffle furnace following AACC Method 08−01.01.
[f] %yield of starch = 100 × dry mass of isolated starch / (initial dry mass of starch-rich flour × starch content of starch-rich flour on a dry basis).
[g] N.A. = Not applicable.

3.4.2 Amylose content and branch-chain-length distribution of amylopectin

Amylose and amylopectin are the two primary components present in starch granules, and amylose content and BCL distribution of amylopectin are critical structural features that determine the functionality and digestibility of starch (Jane, 2006). In the current study, amylose contents of the isolated pulse and Roquette pea starches (38.0−41.1%) were significantly higher than those of the maize and tapioca starches (1.9−31.2%; Table 3.2). Amylopectins of the isolated pulse and Roquette pea starches consisted of significantly smaller proportions (14.7−22.1%) of DP 6−12 short branch chains but larger proportions (55.0−60.4%) of DP 13−24 branch chains than those of the maize and tapioca starches (27.0−34.3% and 47.4−51.9%, respectively; Table 3.2 and Appendix Fig. 1). The average branch-chain lengths of amylopectins of the isolated pulse and Roquette pea starches (DP 20.1−21.3) were slightly higher than those of the maize and tapioca starches (DP 18.4−19.7).

Table 3.2 Amylose contents and branch-chain-length distributions of amylopectins of isolated pulse starches and commercial starches[a]

Starch	Amylose content (%)[b]	Branch-chain-length distributions[c, d]				Average DP[d]
		DP 6−12 (%)	DP 13−24 (%)	DP 25−36 (%)	DP > 36 (%)	
Pea	41.1 ± 0.4 a	22.0 ± 0.4 d	56.1 ± 0.1 b	13.1 ± 0.2 bc	8.8 ± 0.2 ab	20.1 ± 0.1 b
Lentil	38.0 ± 0.9 b	22.1 ± 0.4 d	55.0 ± 0.2 b	13.6 ± 0.1 b	9.4 ± 0.1 ab	20.4 ± 0.1 b
Faba bean	39.9 ± 0.8 ab	21.5 ± 0.7 d	56.0 ± 0.2 b	12.8 ± 0.4 bc	9.7 ± 0.5 a	20.4 ± 0.3 b
Roquette pea	40.7 ± 0.9 ab	14.7 ± 2.0 e	60.4 ± 0.7 a	15.1 ± 0.8 a	9.8 ± 1.0 a	21.3 ± 0.6 a
Normal maize	31.2 ± 1.0 c	27.0 ± 1.4 c	51.5 ± 0.7 c	12.1 ± 0.5 c	9.3 ± 0.2 ab	19.7 ± 0.2 b
Waxy maize	1.9 ± 0.0 d	30.6 ± 0.8 b	51.9 ± 0.8 c	10.4 ± 0.3 d	7.1 ± 0.1 c	18.4 ± 0.1 c
Tapioca	30.6 ± 1.9 c	34.3 ± 0.3 a	47.4 ± 0.6 d	10.0 ± 0.6 d	8.3 ± 0.6 bc	18.6 ± 0.3 c

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Determined using the iodine potentiometric titration method.

[c] Analyzed using fluorophore-assisted capillary electrophoresis (FACE); DP = degree of polymerization.

[d] Molar basis.

3.4.3 Granular morphology of starch

As observed using scanning electron microscopy, granular morphologies of the isolated pulse starches (Fig. 3.1 A−C) resembled that of Roquette pea starch (Fig. 3.1 D) but differed from those of commercial normal maize, waxy maize and tapioca starches (Fig. 3.1 E−G). The granules of the isolated pulse and Roquette pea starches displayed oval, kidney and irregular shapes, which were similar to the shapes of starch granules from other pulse crops as described by Hoover, Hughes, Chung, and Liu (2010). Variations were found in the granule sizes of different pulse starches: the isolated pulse and Roquette pea starches in the present study had granules of 10–45 μm, while starches from other pulse crops could have some granules of smaller (< 10 μm; *e.g.*, cowpea, kidney bean and yam bean) or larger sizes (> 45 μm; *e.g.*, adzuki bean and horse gram) (Hoover, Hughes, Chung, & Liu, 2010). The starches isolated from the air-classified flours of pea, lentil and faba bean showed some broken granules and visible scratches on the surface of some granules, whereas the Roquette pea starch isolated from pea cotyledons using a wet method did not exhibit these features. The observed physical damage to the pea, lentil and faba bean starch granules could be caused by the more severe dry milling of the flours as explained above (Maskus, Bourré, Fraser, Sarkar, & Malcolmson, 2016; Pelgrom, Vissers, Boom, & Schutyser, 2013). However, these visible features appeared to be minor damage to the structure of starch granules because the isolated pulse starches showed damaged-starch contents (0.9–1.3%) similar to those of commercial pea and other starches isolated using wet methods (0.9–1.6%; Table 3.1). The absence of fiber and protein residues in the isolated pulse starches agrees well with the high purity of those samples as illustrated in Table 3.1.

The granules of normal maize and waxy maize starches exhibited polygonal, irregular and spherical shapes with some indentations on the surface (Fig. 3.1 E−F). The sizes of normal maize and waxy maize starch granules varied between 5 μm and 30 μm, noticeably smaller than those of pulse starches. The tapioca starch granules showed the characteristic truncated and spherical shapes with smooth surface (Fig. 3.1 G), and had sizes (5−25 μm) also smaller than those of pulse starches. The granular morphologies of the starches displayed in this study are consistent with the traits previously described by other researchers (Hoover, Hughes, Chung, & Liu, 2010; Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994).

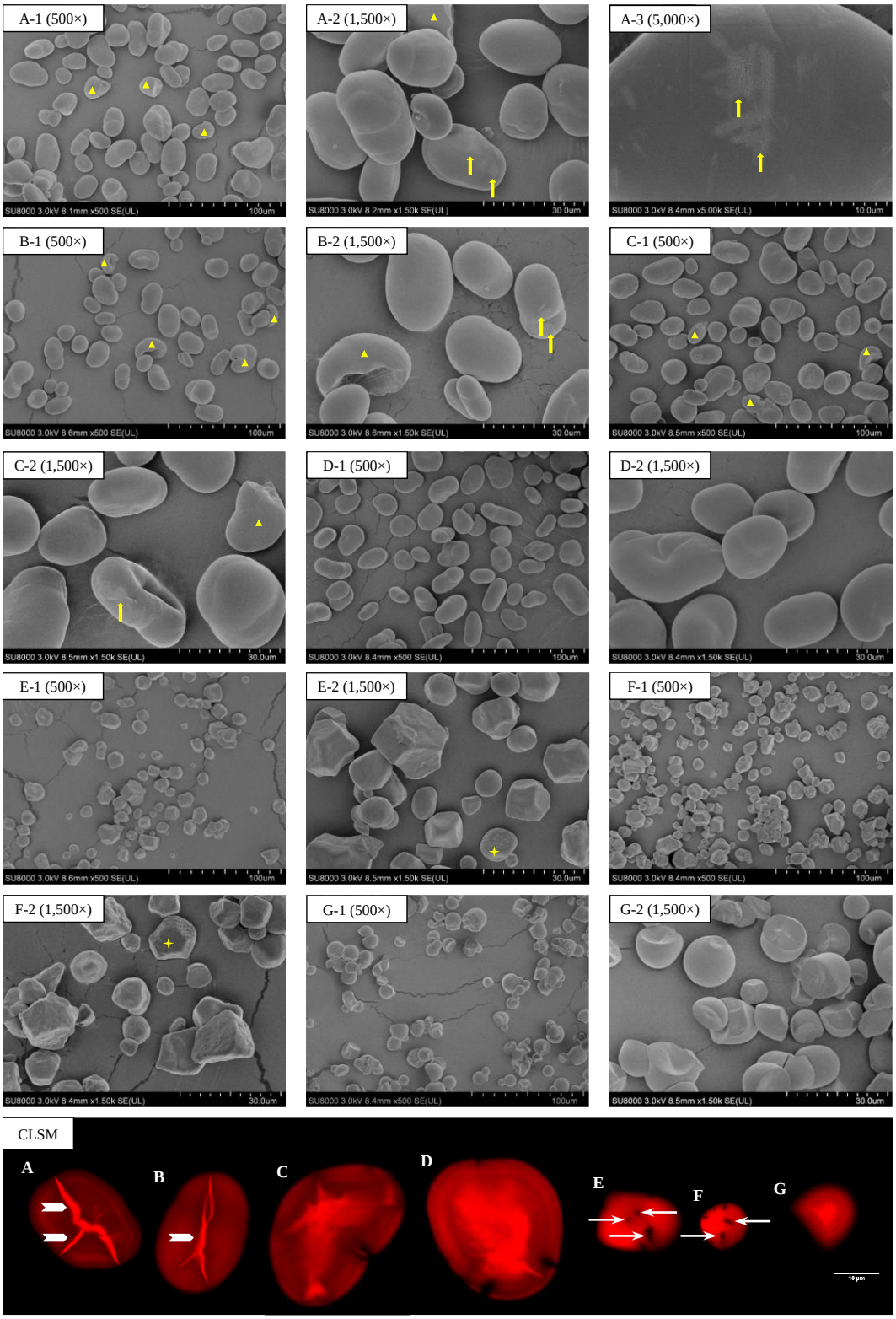


Fig. 3.1 Scanning electron microscopy and confocal laser scanning microscopy (CLSM) images of isolated pulse starches and commercial starches. A: pea; B: lentil; C: faba bean; D: Roquette pea; E: normal maize; F: waxy maize; G: tapioca. For scanning electron microscopy images, the

magnification at which the image was taken is given in parentheses. Triangles mark broken starch granules; vertical arrows mark scratches on the surface of starch granules; stars mark starch granules with indentations on the surface. For CLSM images, horizontal arrows mark internal voids that failed to bind the Rhodamine B dye in starch granules; chevrons mark internal fissures that strongly bound the Rhodamine B dye in starch granules.

3.4.4 Wide-angle X-ray diffraction of starch

Wide-angle X-ray diffraction revealed the C-type pattern of the isolated pulse and Roquette pea starches and the A-type pattern of the maize and tapioca starches (Appendix Fig. 2), corresponding well with previous research (Hoover, Hughes, Chung, & Liu, 2010; Jane, et al., 1999). The percentages crystallinity of the isolated pulse and Roquette pea starches ranged from 25.1% to 30.0% and those of the maize and tapioca starches varied between 29.4% and 34.9%. The percentages crystallinity of the studied starches were negatively correlated ($r = -0.815$, $p = 0.025$) with their amylose contents, which could be attributed to the facts that amylose is present in the amorphous phase of starch granules and only amylopectin is responsible for the formation of double-helical crystalline structure (Jane, 2006).

3.4.5 Thermal properties of starch

The C-type isolated pulse and Roquette pea starches had significantly lower onset (T_o; 55.6−60.1°C) and peak (T_p; 61.6−65.2°C) gelatinization temperatures than the A-type maize and tapioca starches (62.8−66.1°C and 68.2−71.2°C, respectively; Table 3.3), even though the amylopectins of the pulse starches possessed noticeably larger proportions of branch chains with DP >12 (Table 3.2) that are favorable for the formation of thermally more stable crystallites (Shi & Seib, 1995). The lower gelatinization temperatures of the C-type pulse starches could be explained by the organization of different polymorphs in the granules. It is known that the granules of C-type starch have a combination of A- and B-type polymorphs. According to previous research, in the granules of C-type pulse starches, such as pea starch, the B-type polymorphs are located in the center and surrounded by the A-type polymorphs (Bogracheva, Morris, Ring, & Hedley, 1998). During the heating in an aqueous medium, the gelatinization of C-type pulse starch granules is initiated from the central hilum area, and the B-type polymorphs are melted at a substantially lower temperature range than the A-type polymorphs because of the loose packing of the former type (Jane, et al., 1999). Therefore, the four C-type pulse starches displayed considerably lower gelatinization temperatures compared with the three A-type maize and tapioca starches. Gelatinization enthalpy change (ΔH) reflects the amount of energy required to melt double helices in starch during the gelatinization process (Cooke & Gidley, 1992). The gelatinization ΔH values of the pulse starches (12.4−13.5 J/g) were lower than those of the maize and tapioca starches (15.1−18.0 J/g). The differences could be ascribed to the pulse starches

having higher contents of amylose, a component existing in the amorphous phase and not contributing to the formation of double-helical crystalline structure in starch granules as discussed above (Jane, 2006).

When the gelatinized starch samples were stored at 4°C for 7 days, the branch chains of amylopectin reformed double helices between one another to partially restore the crystalline structure of starch, a process defined as starch retrogradation (Hoover, 1995). Scanning of the stored starch samples in accordance with the same procedure as used in gelatinization showed a melting peak of retrograded starch in the DSC thermograms (Table 3.3). The melting peak of the retrograded starches displayed lower temperatures and smaller ΔH than the gelatinization peak of the native counterparts. Percentages retrogradation of the isolated pulse and Roquette pea starch (36.5−52.4%) were greater than those of maize and tapioca starches (7.2−31.5%). Percentages retrogradation of the starches displayed a negative correlation with the proportions of DP 6−12 short branch chains (r = -0.74, p = 0.056) but a positive correlation with the proportions of DP 13−24 branch chains of the amylopectins (r = 0.75, p = 0.054). The data are consistent with previous studies demonstrating that DP 6−12 short branch chains were undesirable, while those of DP 13−24 were favorable for the recrystallization process (Lai, Lu, & Lii, 2000; Shi & Seib, 1992).

Table 3.3 Gelatinization and retrogradation properties of isolated pulse starches and commercial starches[a,b]

Starch	Gelatinization of starch[c]				Melting of retrograded starch[c]				Retrogradation (%)[d]
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	
Pea	58.0 ± 0.1 d	63.5 ± 0.1 e	77.7 ± 0.4 c	13.4 ± 0.9 d	47.1 ± 0.5 ab	58.1 ± 0.4 a	75.0 ± 1.1 a	6.7 ± 0.0 a	50.2 ± 3.4 ab
Lentil	60.1 ± 1.1 c	65.2 ± 0.9 d	72.7 ± 0.4 de	13.5 ± 0.4 cd	46.8 ± 0.3 ab	58.2 ± 0.5 a	72.8 ± 0.1 b	6.2 ± 0.3 b	46.0 ± 2.9 b
Faba bean	58.9 ± 0.1 d	64.2 ± 0.1 de	72.1 ± 0.5 e	12.4 ± 0.3 d	45.8 ± 0.9 bc	58.5 ± 0.3 a	73.8 ± 0.2 ab	6.5 ± 0.2 ab	52.4 ± 2.1 a
Roquette pea	55.6 ± 0.3 e	61.6 ± 0.2 f	73.6 ± 0.7 d	12.4 ± 0.2 d	46.2 ± 0.2 b	58.3 ± 0.5 a	72.7 ± 0.3 b	4.5± 0.2 c	36.5 ± 1.8 c
Normal maize	66.0 ± 0.1 a	70.1 ± 0.2 b	84.0 ± 0.3 a	15.1 ± 0.7 bc	42.5 ± 1.2 d	56.1 ± 0.3 b	69.7 ± 0.5 c	4.7 ± 0.1 c	31.5 ± 1.0 cd
Waxy maize	66.1 ± 0.1 a	71.2 ± 0.1 a	77.1 ± 0.4 c	16.0 ± 0.3 b	44.1 ± 0.6 cd	56.7 ± 0.3 b	69.6 ± 0.2 c	4.4 ± 0.2 c	27.5 ± 1.5 d
Tapioca	62.8 ± 0.0 b	68.2 ± 0.1 c	80.1 ± 0.5 b	18.0 ± 0.8 a	48.3 ± 0.1 a	57.9 ± 0.1 a	68.6 ± 0.5 c	1.3 ± 0.2 d	7.2 ± 1.0 e

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Measured using a differential scanning calorimeter.

[c] T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, and ΔH = enthalpy change.

[d] % retrogradation = 100 × ΔH of dissociation of retrograded starch/ΔH of starch gelatinization.

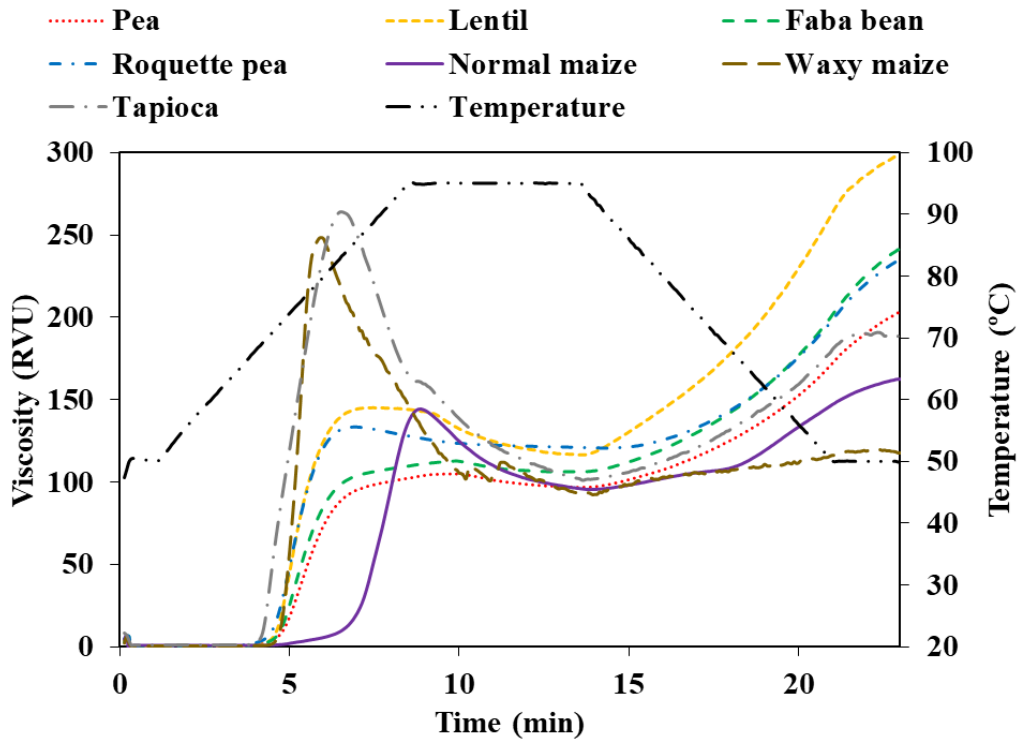
3.4.6 Pasting properties and gelling ability of starch

Pasting profiles of the isolated pulse and Roquette pea starches appeared to be similar, while they were distinctly different from those of maize and tapioca starches (Fig. 3.2 A). The pasting temperatures of the four pulse starches (70.2−72.1°C) were slightly higher than that of tapioca starch (68.4°C), close to that of waxy maize starch (71.3°C), but substantially lower than that of normal maize starch (84.2°C). The remarkably higher pasting temperature of normal maize starch was a result of amylose-lipid complex formation that restricted the swelling of starch granules during the pasting process (Ai, Hasjim, & Jane, 2013). In general, isolated pulse and Roquette pea starches displayed lower peak (105.9−145.4 RVU) and breakdown viscosities (7.5−28.8 RVU) but greater setback (106.0−182.0 RVU) and final viscosities (202.9−298.6 RVU) when compared with the maize and tapioca starches (144.4−264.1, 49.0−163.3, 26.0−87.7 and 117.6−188.5 RVU of peak, breakdown, setback and final viscosities, respectively). Amylose contents of the studied starches were inversely correlated with the peak and breakdown viscosities (r = -0.74, p = 0.057; r = -0.78, p = 0.037, respectively) but positively correlated with the setback and final viscosities (r = 0.77, p = 0.041 and r = 0.77, p = 0.043, respectively). The effects of amylose on the pasting properties of the starches could have the following explanations: (1) On peak viscosity–During the initial heating process from 50°C to 95°C at a rate of 6°C/min, amylose restricted the swelling of starch granules to reduce the peak viscosity, while amylopectin was the primary constituent responsible for viscosity development (Tester & Morrison, 1990). (2) On breakdown viscosity–After the swollen starch granules reached the peak viscosity, amylose could hold the granular integrity and prevent the collapse of the swollen granules, thus leading to a lower breakdown viscosity (Blazek & Copeland, 2008). (3) On setback and final viscosities–During the cooling process from 95°C to 50°C at a rate of 6°C/min, amylose molecules re-associated with each other to form a rigid network, contributing to the increased viscosity in the cooled starch paste (Jane, et al., 1999).

Strength of the starch gels prepared by cooking the starch suspensions using the RVA and storing the samples at ambient temperature for 2.5 h is shown in Fig. 3.2 B. Gel strength of the isolated pea starch (368.2 g) was comparable to that of the Roquette pea starch (328.3 g) but noticeably lower than that of the isolated lentil and faba bean starch (562.1 g and 727.4 g, respectively). The gel strength of normal maize starch (121.0 g) was markedly lower than those of the pulse starches, while waxy maize and tapioca starches failed to develop a gel and

remained as a paste with good fluidity after the 2.5-h storage. The gel strength values of the starches were negatively correlated with the peak and breakdown viscosities (r = -0.77, p = 0.045 and r = -0.80, p = 0.031, respectively) but positively correlated with the setback and final viscosities (r = 0.83, p = 0.021 and r = 0.81, p = 0.028, respectively). The results suggested that factors, including swollen granules with well-maintained integrity (supported by the very small breakdown viscosities of pulse starches) and strong network formation during cooling and storage (supported by the high setback and final viscosities of pulse starches), contributed to the development of strong gels from pulse starches (Ai, Hasjim, & Jane, 2013; Ring, 1985). Waxy maize and tapioca starches failed to form a gel as the other starches did due to the lack of endogenous lipids and/or amylose as reported before (Ai, Hasjim, & Jane, 2013). These two starches swelled to a considerably greater extent and lost the granular integrity after cooking (Fig. 3.2 A). The strong gelling ability of the pulse starches, particularly faba bean and lentil starches, renders them suitable for the preparation of food products that require the formation of firm starch gels, such as glass noodles and jello-type dessert (Wang, Warkentin, Vandenberg, & Bing, 2014).

(A)


Pea
Lentil
Faba bean
Roquette pea
Normal maize
Waxy maize
Tapioca
Temperature
Viscosity (RVU)
Temperature (°C)
Time (min)
300
250
200
150
100
50
0
100
90
80
70
60
50
40
30
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5
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15
20

(B)

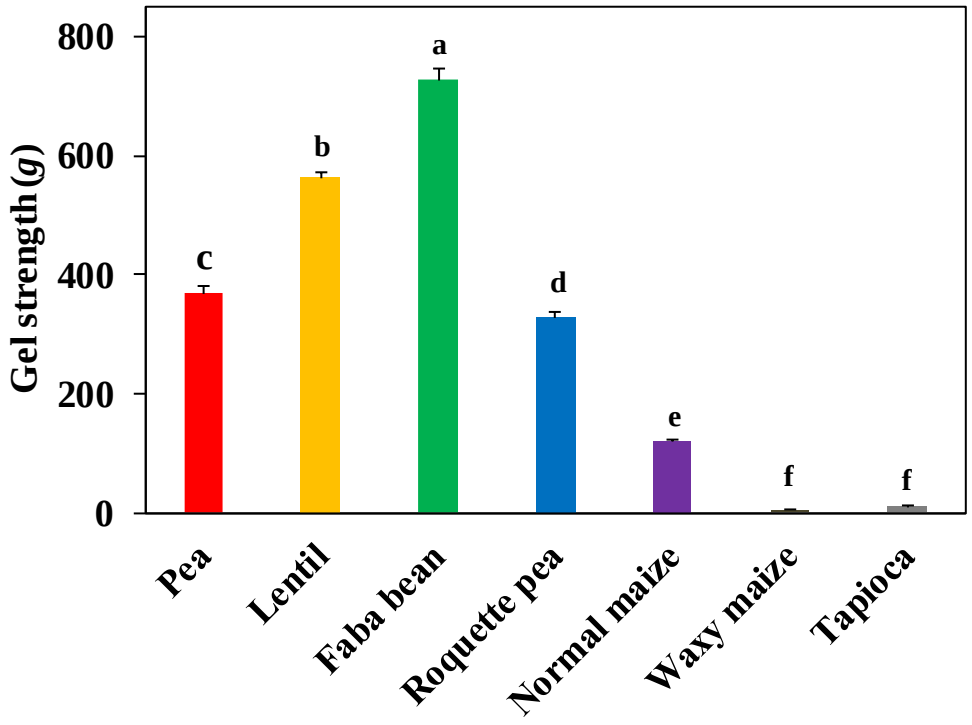

Gel strength (g)
800
600
400
200
0
a
b
c
d
e
f
f
Pea
Lentil
Faba bean
Roquette pea
Normal maize
Waxy maize
Tapioca

Fig. 3.2 Pasting properties and gelling ability of isolated pulse starches and commercial starches. (A) Pasting profiles measured using a Rapid Visco-Analyzer 4500 with 8% (w/w, dry starch basis) starch suspension. (B) Gel strength measured using a Texture Analyzer TA.XT. Plus. Starch suspensions of the same concentration (8% db, 28.0 g total weight) were heated using the Rapid Visco-Analyzer under the same conditions and maintained at 95°C for 5 min, poured into a plastic container and covered with a lid, and then stored at room temperature for 2.5 h for gel formation. For the gel strength bars, the numbers with the same letter are not significantly different at $p < 0.05$.

3.4.7 *In vitro* digestibility of raw and cooked starch

For the raw starch samples, the isolated pulse and Roquette pea starches generally possessed smaller RDS (15.3−21.0%) and SDS contents (34.5−40.9%) but larger RS contents (35.5−46.7%) than the normal maize and waxy maize starches (20.8−34.7% RDS, 51.9−56.7% SDS and 10.9−18.3% RS, respectively; Table 3.4), indicating that the pulse starches were more resistant to enzymatic hydrolysis. The results could be attributed to their different structural characteristics: (1) The pulse starches contained higher levels of amylose (Table 3.2) that contributed to a less porous granular structure (Jane, 2006); (2) Amylopectins of the pulse starches consisted of smaller proportions of DP 6−12 short branch chains but larger proportions of DP 13−24 branch chains (Table 3.2), which could lead to a more ordered crystalline structure (Perera, Lu, Sell, & Jane, 2001). These important structural features of the pulse starch granules reduced their susceptibility to enzymes during the hydrolysis process when compared with the normal maize and waxy maize starches.

Although the normal maize, waxy maize and tapioca starches showed similar amylose contents (between normal maize and tapioca starches; Table 3.2), comparable BCL distributions of amylopectins (Table 3.2), and the same A-type X-ray diffraction pattern (Appendix Fig. 2), the raw tapioca starch was the most resistant to enzymatic hydrolysis among all the starches (Table 3.4). Previous research has demonstrated that internal structure of starch granules is another critical factor that can significantly influence the digestibility of raw starch (Jane, 2006). Confocal laser scanning microscopy (CLSM) was utilized to elucidate the mechanistic roles of the internal granular structure of the studied starches in their digestibility, and the images are presented in Fig. 3.1–CLSM. The normal maize and waxy maize starches had internal voids that failed to bind the Rhodamine B dye (Fig. 3.1–CLSM, E−F). Those voids, reflecting the porous internal structure of normal maize and waxy maize starch granules, were the weak points for the amylolytic enzymes to initiate the fast hydrolysis of these two starches (Jane, 2006). In contrast, tapioca starch granules had a solid and homogenous internal structure (Fig. 3.1–CLSM, G), which is in accordance with their smooth and nonporous surface structure (Fig. 3.1 G). The absence of porous structure on the surface and internally rendered the tapioca starch least susceptible to amylolysis. Generally, internal voids were not found in the pulse starch granules (Fig. 3.1–CLSM, A−D), which could also explain their greater enzymatic resistance than the normal maize and waxy maize starches (Table 3.4). However, some pulse starch granules (Fig.

3.1–CLSM, A−B) displayed internal fissures, a structure different from the voids as the fissures could strongly bind the dye. This fissure structure corresponds well with the internal feature of pea starch granules as illustrated by polarized light microscopy (Bogracheva, Morris, Ring, & Hedley, 1998). The formation of the fissures and their impact on the enzymatic hydrolysis of raw pulse starch require further investigation.

After cooking, the RDS contents of all the starches noticeably increased, while the SDS and RS contents substantially decreased (Table 3.4), indicating that gelatinization enhanced the digestibility of starch. More importantly, the variations in the digestibility of the raw starches disappeared after they were gelatinized, as the differences in the RDS, SDS and RS contents between the cooked starches were largely insignificant. The data indicated that the different chemical compositions and molecular structures among the studied starches did not affect their digestibility in the cooked form, which could be attributed to that cooking gelatinized the starches and turned the starch molecules into an amorphous state that was readily hydrolyzable to the added enzymes.

Table 3.4 *In vitro* digestibility of raw and cooked isolated pulse starches and commercial starches[a]

Starch	Raw[b]			Cooked[b, c]		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
Pea	21.0 ± 1.9 b	38.3 ± 1.9 cd	35.5 ± 1.0 d	86.0 ± 1.0 b	2.9 ± 0.6 a	5.9 ± 1.0 a
Lentil	19.0 ± 0.9 b	37.6 ± 1.4 cde	41.3 ± 0.6 c	89.3 ± 1.0 a	1.8 ± 1.7 a	6.9 ± 1.3 a
Faba bean	15.3 ± 0.7 c	34.5 ± 0.8 de	46.7 ± 0.4 b	88.1 ± 2.0 ab	2.5 ± 0.3 a	5.9 ± 1.8 a
Roquette pea	18.4 ± 0.9 b	40.9 ± 1.4 c	37.9 ± 0.9 d	88.1 ± 0.2 ab	1.4 ± 1.3 a	7.8 ± 1.2 a
Normal maize	20.8 ± 0.6 b	56.7 ± 1.2 a	18.3 ± 1.6 e	88.1 ± 1.4 ab	2.3 ± 1.5 a	5.4 ± 0.4 a
Waxy maize	34.7 ± 1.2 a	51.9 ± 1.8 b	10.9 ± 0.8 f	90.3 ± 0.9 a	0.6 ± 0.5 a	6.6 ± 0.3 a
Tapioca	11.0 ± 0.6 d	34.4 ± 0.6 e	51.8 ± 1.2 a	90.6 ± 0.7 a	1.5 ± 0.8 a	5.0 ± 1.1 a

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] RDS: rapidly digestible starch, SDS: slowly digestible starch, and RS: resistant starch; values were calculated on dry starch basis.

[c] Starch was cooked in a boiling water bath for 10 min.

3.5 Conclusions

Pea, lentil and faba bean are three different species of pulse crops that are widely grown and utilized for value-added processing in Canada and worldwide. Pea, lentil and faba bean starches of a high purity (94.8–97.9% starch) were isolated from their respective air-classified starch-rich flours. The used starch isolation method was effective to remove large percentages of damaged starch granules from the starch-rich flours, resulting in low damaged-starch contents of the isolated pulse starches (0.9–1.3%); therefore, the observed slight physical damage to the granules of isolated pea, lentil and faba bean starches under SEM did not appear to markedly influence their structure, functional properties and *in vitro* digestibility compared with commercial Roquette pea starch.

The isolated pulse starches, despite being from three different species of pulse crops, showed amylose contents, amylopectin BCL distributions, granular morphologies, and crystalline structure comparable to those of commercial Roquette pea starch but distinctly different from those of commercial normal maize, waxy maize and tapioca starches. Due to the unique organization of A- and B-type polymorphs in the granules, the four C-type pulse starches exhibited lower gelatinization temperatures than those of the A-type maize and tapioca starches. The pulse starches generally showed smaller percentages crystallinity, lower gelatinization enthalpy changes and lower pasting viscosities, but stronger gelling ability compared with those of maize and tapioca starches. In addition to amylose content and amylopectin BCL distribution, the internal structure of starch granules played a critical role in determining the *in vitro* digestibility of raw starches; however, the differences in the digestibility of the pulse, maize and tapioca starches disappeared after they were gelatinized. The structure, functionality and digestibility of pea, lentil and faba bean starches as demonstrated in this study will help the industry explore new markets for this major component of pulse seeds.

3.6 Connection to the Next Study

Among the aforementioned five types of RS, RS3 has attracted wide research interest due to its thermal stability (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Lockyer & Nugent, 2017). RS3 can be used to manufacture low-bulk, high-fiber products with enhanced texture and mouthfeel due to the advantages of having a small particle size, white appearance, bland flavor, high dissociation temperature, and low water-holding

capacity. Additionally, RS3 can act as a functional ingredient to lower the caloric value of different food products (Haralampu, 2000; Sajilata, Singhal, & Kulkarni, 2006).

Currently, minimal research has been conducted on the preparation of RS3 from pulse starches. Based on results reported in Chapter 3, the relatively high amylose content and long branch chains of amylopectin of pulse starches are desirable structural features for the development of RS3 with significant enzymatic resistance. Because isolated pea, lentil and faba bean starches exhibited similar molecular structure, functional properties and *in vitro* digestibility, isolated pea starch was selected for the subsequent study so as to develop a novel RS3. The acquired new knowledge and technology will be valuable to the food industry to expand markets for this co-product with value addition.

4. DEVELOPMENT, STRUCTURE AND *IN VITRO* DIGESTIBILITY OF TYPE 3 RESISTANT STARCH FROM ACID-THINNED AND DEBRANCHED PEA AND NORMAL MAIZE STARCHES[6]

4.1 Abstract

In this research, type 3 resistant starch (RS3) was developed from native pea starch through acid thinning, debranching and recrystallization, and the resultant pea RS3 was then characterized and compared with that generated from native normal maize starch. Starting from the respective native starches, the modification method yielded 68.1% of RS3 from pea and 59.6% from normal maize. The particles of pea and normal maize RS3 showed a coarse surface and irregular shapes and sizes. Both pea and normal maize RS3 displayed the B-type X-ray diffraction pattern, with 41.0% and 37.7% relative crystallinity, respectively. *In vitro* starch digestibility assay revealed that pea RS3 – in both uncooked and cooked states – was less digestible to amylolytic enzymes than normal maize RS3 due to the formation of thermally more stable double-helical crystallites in the former. The information presented in the study is valuable for the development of RS ingredient from pea starch for food applications.

Keywords: Pea starch; Normal maize starch; Type 3 resistant starch; *In vitro* digestibility; Thermal stability

4.2 Introduction

Pea, as a member of the *Leguminosae* family, is an important cool-season crop in the agriculture sector in Canada, the leading producer of dry pea globally (Smýkal, Aubert, Burstin, Coyne, Ellis, Flavell, et al., 2012). Dry pea is a popular grain legume (pulse) that is included in the diets by consumers in different regions in the world. Similar to other pulse crops such as

[6] Li, L., Yuan, T. Z., & Ai, Y. Department of Food and Bioproduct Sciences, University of Saskatchewan. Submitted to *Food Chemistry* (October 1st, 2019).

chickpea, lentil and faba bean, pea is good source of protein, resistant starch (RS) and other dietary fiber, and micronutrients (*e.g.*, minerals, folate and other B-vitamins) (Dahl, Foster, & Tyler, 2012). The health benefits of consuming pea have been well documented in the literature, including reducing glycemic and insulinemic responses (Schäfer, Schenk, Ritzel, Ramadori, & Leonhardt, 2003), and lowering the risks of chronic diseases (*e.g.*, type-2 diabetes, cancers and cardiovascular diseases) (Dahl, Foster, & Tyler, 2012).

Most recently, pea has gained increasing popularity in the food industry because it is a promising source of plant protein. The protein in pea (content ranging from 14.3% to 29.5%) can be fractionated in a concentrate or isolate form, which is the main ingredient for the production of plant-based meat substitutes, beverages, snacks *etc.* (Boye, Zare, & Pletch, 2010; Jha, Tar'an, Diapari, & Warkentin, 2015). The fractionation is typically achieved through two approaches in the industry: dry and wet methods (Hoover, Hughes, Chung, & Liu, 2010). In either process, the predominant constituent of pea seeds – starch (content ranging from 27.5% to 51.0%) – is generated as a co-product that is underutilized (Jha, Tar'an, Diapari, & Warkentin, 2015). While there is a growing demand for pea protein, the pulse processing industry is also facing the challenge of finding new markets for pea starch.

Compared with commercially important starches such as those from waxy maize, normal maize and tapioca, isolated pea starch exhibits similar digestibility after cooking (Chung, Liu, & Hoover, 2009; Li, Yuan, Setia, Raja, Zhang, & Ai, 2019). The data correspond well with the findings from previous studies showing that the intact cell wall structure in cotyledon is the primary factor contributing to the low glycemic effect of pea (and other pulses), rather than the chemical composition and structure of the starch (Dhital, Bhattarai, Gorham, & Gidley, 2016). Nevertheless, pea starch has a higher amylose content and a larger proportion of long branch chains of amylopectin than the aforementioned waxy and normal starches (Li, Yuan, Setia, Raja, Zhang, & Ai, 2019). These structural features render pea starch more suitable for the development of RS, a category of food ingredient that can provide low glycemic/insulinemic benefits to consumers.

RS refers to a fraction of starch that can survive the digestion in human small intestine and pass into large intestine for microbial fermentation (Englyst & Macfarlane, 1986; Englyst, Wiggins, & Cummings, 1982). Based on the mechanisms responsible for the enzymatic resistance, RS has been categorized into five different types: RS1 – physically inaccessible

starch; RS2 – granular, raw starch with the B- or C-type polymorph; RS3 – retrograded starch; RS4 – chemically-modified starch; and RS5 – amylose-lipid complex (Englyst, Kingman, & Cummings, 1992; Hasjim, Lee, Hendrich, Setiawan, Ai, & Jane, 2010). Compared with the other types of RS products, RS3 has better thermal stability and allows for a relatively higher incorporation level without causing a detrimental effect on the quality of certain food products (*e.g.*, extruded foods) (Haralampu, 2000). Thus, continuous research efforts have been undertaken to develop RS3 for food applications (Cai & Shi, 2014; Mutungi, Rost, Onyango, Jaros, & Rohm, 2009).

RS3 is formed from the recrystallization of starch chains driven and stabilized by hydrogen bonding, and the double-helical crystallites can further grow into a tightly packed structure (Haralampu, 2000). In comparison with highly branched amylopectin, linear amylose is generally more favorable for the recrystallization process and thus the development of RS3 (Berry, 1986). To foster the formation of RS3, starch can be debranched using a debranching enzyme (*e.g.*, isoamylase, pullulanase) that specifically hydrolyzes α-1, 6 glycosidic bonds to release a larger proportion of linear glucan chains (Cai & Shi, 2010; Shi, Chen, Yu, & Gao, 2013). In addition to the content of linear amylose, the degrees of polymerization (DP) of amylose molecules have also been shown to affect RS3 formation: a DP range of 10 to 100 is more desirable for the formation of stable double-helical crystallites (Eerlingen, Decuninck, & Delcour, 1993; Gidley, Cooke, Darke, Hoffmann, Russell, & Greenwell, 1995). Previous study has also demonstrated that limited β-amylolysis or α-amylolysis to moderately shorten the starch chain length can promote the formation of RS3 from various starches (Villas-Boas & Franco, 2016). Hasjim and Jane (2009) have reported that mild acid thinning by hydrochloric acid increased the RS content of extruded normal maize starch. These results can be explained by the fact that partial hydrolysis by enzyme or acid can increase the molecular mobility of starch chains and generate a greater proportion of linear starch molecules of moderate chain lengths for enhanced retrogradation. Furthermore, physical treatments that can promote the recrystallization of starch chains and enhance the tight packing of the crystallites, such as heat-moisture and temperature-cycle treatments, can also be employed to achieve a higher level of RS3 (Mutungi, Rost, Onyango, Jaros, & Rohm, 2009).

Recently, new methods have been developed to produce RS3 in a spherulite form from various starches (Cai & Shi, 2013; Kiatponglarp, Rugmai, Rolland-Sabaté, Buléon, & Tongta,

2016). In the study of Cai and Shi (2013), a novel process of combining isoamylase debranching, melting and recrystallization has been reported to produce spherulites from waxy maize starch. As the linear chains released from waxy maize starch have a relatively narrow molecular weight distribution, the resultant double-helical crystallites can be arranged in an ordered pattern to form dense and compact spherulites, contributing to a high RS content of the obtained RS3 product. Also, after the debranching reaction, the waxy maize starch was effectively liquefied, allowing for the use of a high solids concentration (25%, w/w) in the modification process.

In the current literature, few studies have reported the utilization of pea and other pulse starches for the production of RS3. Because of the higher amylose content and a greater proportion of long branch chains of amylopectin than commercial waxy and normal starches as indicated above (Li, Yuan, Setia, Raja, Zhang, & Ai, 2019), native pea starch could be a more promising starting material for the development of RS3. Therefore, in this research pea starch was used to prepare RS3, with normal maize starch being included as the control for comparison. To perform the modification at a high solids content (25%, w/w), the pea and normal maize starches were firstly acid thinned to prevent the formation of a firm gel that could hinder efficient debranching reaction. The acid-thinned (AT) starches were subsequently subjected to isoamylase debranching and recrystallization to generate RS3. The structures and physicochemical properties of the two obtained RS3 products were characterized and related to their *in vitro* digestibility. This study demonstrated the good potential of utilizing pea starch for the development of RS ingredient. The acquired new knowledge and technology can be transferred to the industry to expand the market for this co-product with value addition.

4.3 Materials and Methods

4.3.1 Materials

Pea starch was isolated from an air-classified starch-rich pea flour (Starlite, Parrheim Foods, Saskatoon, SK, Canada) using a wet method as described by Li, Yuan, Setia, Raja, Zhang, and Ai (2019). Commercial normal maize starch (Cargill Gel™ 03420) was obtained from Cargill Inc. (Minneapolis, MN, U.S.A.). Isoamylase (240 U/mg), potato amylose standard, Total Starch Assay Kit, and D-Glucose Assay Kit were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). Maize amylopectin standard, Celatom®, protease from *Bacillus licheniformis*, heat-stable α-amylase, amyloglucosidase from *Aspergillus niger*, and pancreatin

from porcine pancreas were procured from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Six standards that were used for the calibration of high-performance size-exclusion chromatography (HPSEC) system were obtained from different sources: glucose from Alfa Aesar (Ward Hill, MA, U.S.A.); maltotriose and maltohexose from Sigma-Aldrich Canada Co.; and three pullulan standards (6.2, 48.8 and 348 kDa, respectively) from PSS Polymer Standards Service GmbH (Mainz, Germany). All the other chemicals used in the study were of reagent grade.

4.3.2 Preparation of type 3 resistant starch

Type 3 resistant starch (RS3) was prepared from pea and normal maize starches using a three-step process: acid thinning, debranching and recrystallization.

4.3.2.1 Acid thinning of starches

Native pea and normal maize starches were suspended in hydrochloric acid aqueous solution (0.8 M) at 30% concentration [w/w, dry starch basis (dsb)] and then hydrolyzed at 50°C in a water bath with shaking (140 rpm) for 4, 8, 12 and 24 h. At each time interval, 1.0-mL aliquot of the starch suspension was sampled, followed by centrifugation at 3,000 *g* for 10 min. The total carbohydrate content of the supernatant containing the solubilized starch was measured by a phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Percentage of starch hydrolysis was reported as: %hydrolysis = 100 × (total carbohydrate in the supernatant × 0.9) / initial dry mass of starch.

To recover the acid-thinned (AT) starch, the starch suspension was neutralized by adding 4.0 M sodium hydroxide aqueous solution dropwise and then washed three times with 100 mL of distilled water and three times with 100 mL of anhydrous ethanol before drying at 40°C in a convection oven overnight. The yield of AT starch was calculated as: %yield of AT starch = 100 × dry mass of recovered AT starch / initial dry mass of starch.

4.3.2.2 Debranching of AT starches

The resulting AT starch (25.0 g, dsb) was suspended in sodium acetate buffer (0.01 M, pH 4.0) to reach a total suspension weight of 100 g in a pressure tube (Ace Glass Inc., Vineland, NJ, U.S.A.), which was then cooked in a boiling water bath for 30 min with vigorous magnetic

stirring. After storing the cooked starch in a 130°C convection oven for 30 min, the sample was cooled to 50°C, followed by the addition of 0.8% (v/w, dsb) isoamylase (100 U). The starch and enzyme mixture was incubated at 50°C for 24 h for the debranching reaction to occur (Cai & Shi, 2013). Because the AT pea and normal maize starches with an acid hydrolysis duration shorter than 24 h formed firm gels at the concentration used for debranching (*i.e.*, 25%, w/w, dsb), the added isoamylase was unable to efficiently debranch and liquefy the starch under the conditions used for the reaction. Therefore, only 24-h AT pea and normal maize starches were included in the debranching step and subsequent experiments.

4.3.2.3 Recrystallization of debranched AT starches to produce RS3

After the above debranched AT starch was heated and maintained at 130°C for 30 min, the sample was incubated at 30°C for 24 h for recrystallization to form RS3. The generated RS3 was recovered by filtration using Whatman #1 filter paper, washed three times with 100 mL of distilled water, dried at 40°C overnight, and then gently ground by mortar and pestle to pass through a 125-μm sieve. The yield of RS3 was calculated as: %yield of RS3 = 100 × dry mass of obtained RS3 / initial dry mass of AT starch.

4.3.3 Amylose content of starches and RS3

Amylose contents of the native, AT, and debranched AT pea and normal maize starches, as well as their derived RS3 products, were measured according to an iodine colorimetric method reported by Chrastil (1987). Each sample (~100 mg) was accurately weighed and suspended in 6.0 mL of 90% (v/v) dimethyl sulfoxide (DMSO) and boiled for 20 min with mild magnetic stirring. After cooling to room temperature, anhydrous ethanol (10.0 mL) was added to 2.0 mL of the starch-DMSO dispersion, followed by centrifugation at 5,000 *g* for 15 min. The supernatant was carefully discarded, and the defatted sample was dispersed in 6.0 mL of urea-DMSO solution (0.6 M urea in 90% DMSO) by heating in a boiling water bath with mild magnetic stirring for 30 min. After cooling to ambient temperature, an aliquot of 100.0 μL was sampled from the dispersion and immediately mixed with 5.0 mL of 0.5% (v/v) trichloroacetic acid and 50.0 μL of 0.01 M I_2-KI solution. After the color development at ambient temperature for 30 min, the absorbance of the sample at 620 nm was read on a spectrophotometer. A standard

curve was constructed by using the blends of potato amylose and maize amylopectin standards at different ratios (0.0%, 27.9%, 50.2%, 72.5% and 100.0% amylose).

4.3.4 Particle morphology of RS3

Pea and normal maize RS3 samples were sprinkled onto a carbon tape that was affixed on an aluminum stub, coated with gold using a sputtering technique, and then viewed using a field-emission scanning electron microscope (SEM, SU8010, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada) under the conditions of 3.0 kV and 10 μA. Representative SEM images of the RS3 samples were captured at three magnifications: 100×, 500× and 2,500×.

4.3.5 Particle-size distributions of RS3

Particle-size distributions of the pea and normal maize RS3 products were measured using a Mastersizer 2000 (Malvern Instruments, Malvern, U.K.). Each sample (~1 g) was suspended in 10 mL of distilled water and stirred at ~150 rpm for 5 min before the measurement. The refractive indices of the RS3 particles and dispersant (distilled water) were set at 1.5 and 1.33, respectively. The volume weighted mean particle size (D [4,3]) was calculated by the installed software.

4.3.6 Wide-angle X-ray diffraction of RS3

Wide-angle X-ray diffraction patterns of the pea and normal maize RS3 products were determined using an Ultima IV X-ray diffractometer (Rigaku Americas Corp., Woodlands, TX, U.S.A.) with Cu Kα radiation at 40 kV and 44 mA. After the samples were equilibrated in a chamber of 100% relative humidity at 25°C for 24 h, they were scanned from the diffraction angle (2θ) of 3 to 40° at a rate of 1.3°/min at room temperature. The relative crystallinity of each sample was calculated using OriginPro 2019 Software (OriginLab Corp., Northampton, MA, U.S.A.) in accordance with the method of Hayakawa, Tanaka, Nakamura, Endo, and Hoshino (1997).

4.3.7 *In vitro* digestibility and total dietary fiber contents of RS3

In vitro digestibility of the pea and normal maize RS3 – in both uncooked and cooked forms – was determined using a modified Englyst Method (Englyst, Kingman, & Cummings,

1992; Li, Yuan, Setia, Raja, Zhang, & Ai, 2019). The cooking step was carried out by incubating the RS3 suspension in a boiling water bath for 10 min, with vigorous shaking every 2 min. Amounts of glucose released by enzyme digestion at time intervals of 20 and 120 min were quantified using D-Glucose Assay Kit. Contents of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were calculated as reported by Englyst, Kingman, and Cummings (1992). Total dietary fiber contents of native pea and normal maize starches, as well as their derived RS3 products were determined using AOAC Method 991.43 (AOAC, 2012).

4.3.8 Thermal properties of RS3

Thermal properties of the pea and normal maize RS3 samples were determined using a differential scanning calorimeter (DSC 8000, PerkinElmer Inc., Waltham, MA, U.S.A.) according to the method of Hasjim, Lee, Hendrich, Setiawan, Ai, and Jane (2010). RS3 (close to 10 mg) was accurately weighed into a stainless steel pan and thoroughly hydrated with distilled water (~3 volumes, v/w) using a microsyringe. After sealing the pan and equilibrating the sample at room temperature for more than 2 h, the scanning was performed from 10 to 160°C at a rate of 10°C/min. An empty stainless steel pan was used as the reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy change (ΔH) of the thermal transition were calculated from the DSC thermogram using Pyris^{TM} Software Platform Version 11 (PerkinElmer Inc., Waltham, MA, U.S.A.).

4.3.9 Molecular-weight distributions of debranched AT starches and RS3[7]

To determine the molecular-weight distributions, debranched AT pea and normal maize starches were freeze dried immediately after the debranching step as described in Section 4.3.2.2. The freeze-dried samples and RS3 (from Section 4.3.2.3) from both starches (~20 mg each) were dispersed in 2.0 mL of 90% (v/v) DMSO and heated in a boiling water bath with mild magnetic stirring for 20 min. After the precipitation with anhydrous ethanol (40.0 mL) and the centrifugation at 5,000 *g* for 15 min, the supernatant was discarded and the tube was drained at 90° angle for 10 min on a paper towel to remove the excess ethanol. The pellet was then re-

[7] This experiment was done by Tommy Z. Yuan and Liying Li at the Department of Food and Bioproduct Sciences, U of S.

dispersed in 4.0 mL of DMSO containing 0.5% (w/w) lithium bromide (LiBr) in a boiling water bath with mild magnetic stirring for 10 min, followed by filtration through a 5-μm disk filter prior to sample injection.

The molecular-weight distributions of the above prepared debranched AT starch and RS3 samples were determined using a high-performance size-exclusion chromatography instrument equipped with a refractive index detector (HPSEC-RI system) following the method of Peng and Yao (2018). Briefly, an aliquot of 20.0 μL was injected by an autosampler into a 1260 Infinity II LC system (Agilent Technologies Canada Inc., Mississauga, ON, Canada) installed with two connected Zorbax gel PSM 60-S columns (6.2 mm × 250 mm) and equipped with a RI detector. DMSO containing 0.5% LiBr was used as the eluent, and the flow rate was set at 0.5 mL/min. The temperatures of column compartment and RI were controlled at 30°C and 35°C, respectively. Glucose, maltotriose, maltohexose, and three pullulan standards (6.2, 48.8 and 348 kDa) were used for column calibration. RI data were collected and then exported to OriginPro 2019 Software (OriginLab Corp., Northampton, MA, U.S.A.), followed by normalization based on the total area under the curve (retention time from 0 to 19 min).

4.3.10 Statistical analysis

The preparation of pea and normal maize RS3 (Section 4.3.2) was performed in triplicate (*i.e.*, three independent batches for each treatment). The different experiments (except for the wide-angle X-ray diffraction and total dietary fiber content for normal maize RS3) were conducted in duplicate for each batch, and the average of the duplicate measurements was used for the calculation of mean and standard deviation of the sample (*i.e.*, n = 3). Amylose of the native pea and normal maize starches were measured in triplicate for robust statistical analysis (n = 3). Independent-samples t-test was applied to compare the means between the data of pea and normal maize RS3 products, as well as the corresponding yields of acid-thinned starches. Statistical differences among the amylose contents and the molecular-weight distributions of the starch samples at different modified stages were assessed using one-way ANOVA, Tukey's multiple comparison test. The statistical analyses were conducted at a significance level of 0.05 on IBM SPSS Statistics (Version 24.0).

4.4 Results and Discussion

4.4.1 Yields of AT pea and normal maize starches and their corresponding RS3

Under the conditions applied for acid thinning, percentages of hydrolysis of pea and normal maize starches steadily increased, which reached 18.3% and 20.8% at 24 h, respectively (Appendix Fig. 3). In the initial stage (0-12 h), pea starch displayed a faster hydrolysis rate, but it became comparatively slower after 12 h. According to our preliminary tests, acid thinning for 24 h was the minimum period required for the prevention of gelation of these two starches in the follow-up debranching step at 25% concentration (w/w, dsb; Section 4.3.2.2), and acid thinning for a longer period (*e.g.*, 36 and 48 h) did not further elevate the RS contents of the prepared RS3 products as analyzed by the Englyst Method in Section 4.3.7 (data not shown). Thus, 24-h AT pea and normal maize starches were chosen for the subsequent modification and characterization. The yields of 24-h AT pea and normal maize starches were 80.5% and 78.6% (Table 4.1), respectively, which are in good agreement with the percentages of hydrolysis at 24 h as illustrated in Appendix Fig. 3.

After debranching by isoamylase and recrystallization, AT pea and normal maize starches yielded 84.6% and 75.8% RS3, respectively, with the former being significantly higher than the latter (Table 4.1). By calculating from the initial dry mass of native starches, the yields of pea and normal maize RS3 were 68.1% and 59.6%, respectively. The yield values presented in the current study are lower than those reported by Cai and Shi (2013, 2014), who achieved 72.0% and 88.0% yields of RS3 from debranched waxy maize starch using different preparation methods; the values, however, are still considerably higher than the data reported for RS3 prepared from dry bean starches (14.9-29.7%) (Simons, Hall III, & Vatansever, 2018). The use of a high solids content for recrystallization (25%, w/w, dsb) and the moderate yields of RS3 in this study suggested that our modification method could be meaningful for large-scale production of RS3 from normal starches.

Table 4.1 Yields of acid-thinned pea and normal maize starches and their corresponding RS3[a]

	Yield (%, dry basis)	
	Acid-thinned starch[b]	**RS3[c]**
Pea	80.5 ± 2.4 a	84.6 ± 0.7 a
Normal maize	78.6 ± 1.5 a	75.8 ± 0.3 b

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] %yield of acid-thinned starch = 100 × dry mass of recovered acid-thinned starch / initial dry mass of starch.

[c] %yield of RS3 = 100 × dry mass of obtained RS3 / initial dry mass of acid-thinned starch.

4.4.2 Amylose contents of starches and RS3

The amylose content of native pea starch (38.4%) was noticeably higher than that of native normal maize starch (30.3%; Table 4.2), consistent with the data reported before (Li, Yuan, Setia, Raja, Zhang, & Ai, 2019). Upon acid thinning at 50°C for 24 h, the amylose contents substantially decreased to 19.2% and 17.3% for the two AT starches, which could be attributed to the effective breakdown of amylose and long branch chains of amylopectin primarily present in the amorphous regions of the starch granules (Wang & Wang, 2001). After being further debranched, amylose contents of the samples increased to 23.9% and 21.7%, respectively, resulting from the release of more linear chains from starch molecules by isoamylase. Upon recrystallization at 30°C for 24 h, the amylose content of pea RS3 decreased to 21.6%, while that of normal maize RS3 slightly increased to 22.1%.

Table 4.2 Amylose contents of native, acid-thinned, and debranched acid-thinned pea and normal maize starches and their derived RS3[a]

	Amylose content (%)[b]
Native starch	
Pea	38.4 ± 0.4 a
Normal maize	30.3 ± 0.5 b
Acid-thinned starch	
Pea	19.2 ± 0.2 e
Normal maize	17.3 ± 0.3 f
Debranched acid-thinned starch	
Pea	23.9 ± 0.5 c
Normal maize	21.7 ± 0.3 d
RS3	
Pea	21.6 ± 0.2 d
Normal maize	22.1 ± 0.3 d

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.
[b] Determined using an iodine colorimetric method (Chrastil, 1987).

4.4.3 Particle morphology and particle-size distributions of RS3

Pea and normal maize RS3 particles exhibited aggregates having a rough surface and irregular shapes and sizes (Fig. 4.1), which were apparently different from the morphological features of their native counterparts (Li, Yuan, Setia, Raja, Zhang, & Ai, 2019). The RS3 particles also showed morphology obviously different from that of spherulites produced from debranched waxy maize starch (Cai & Shi, 2013). The distinct morphology of RS3 samples in this study could be attributed to the heterogeneous compositions of the debranched AT starches prior to recrystallization: they contained both amylose (partially hydrolyzed by acid) and amylopectin branch chains of various lengths (more details revealed in Section 4.4.6), which was unfavorable for the formation of spherulites having a smoother surface and more regular shapes and sizes (Cai & Shi, 2013). Overall, normal maize RS3 appeared to have larger particle sizes than pea RS3.

Particle size analysis revealed the wide size distributions of RS3 particles, both ranging approximately from 2 to 417 μm (Appendix Fig. 4). The D [4,3] of pea RS3 (45.9 μm) was significantly smaller than that of the normal maize RS3 (73.9 μm), which agrees well with the observation in SEM images (Fig. 4.1).

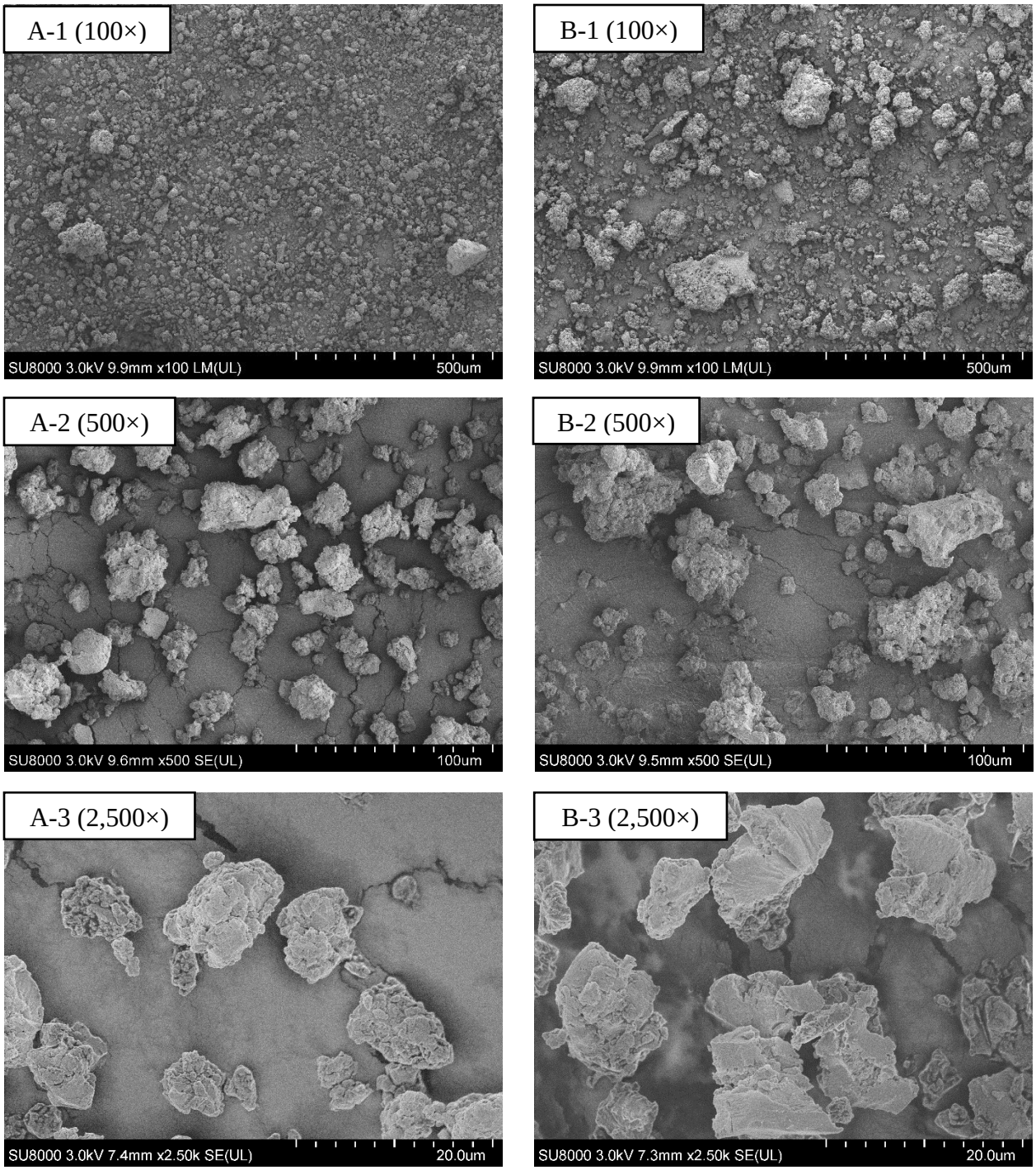


Fig. 4.1 Scanning electron microscopy (SEM) images of pea and normal maize RS3. A: pea RS3; B: normal maize RS3. The magnification at which the image was captured is provided in parentheses.

4.4.4 Wide-angle X-ray diffraction of RS3

The resulting pea and normal maize RS3 both displayed the B-type X-ray diffraction pattern (Appendix Fig. 5), which was distinctively different from that of the native pea (C-type) and normal maize starch (A-type) (Hoover, Hughes, Chung, & Liu, 2010; Jane, et al., 1999). The result corresponds well with previous findings that a low recrystallization temperature and long starch chains typically favored the formation of B-type polymorphs (Buléon, Véronèse, & Putaux, 2007; Kiatponglarp, Tongta, Rolland-Sabaté, & Buléon, 2015). Percentage crystallinity of the pea RS3 (41.0%) was higher than that of normal maize RS3 (37.7%), suggesting the formation of more double-helical crystallites in the former. In addition, the percentages crystallinity of both RS3 were considerably greater than those of their native starches (30.0% and 29.4% of pea and normal maize, respectively) (Li, Yuan, Setia, Raja, Zhang, & Ai, 2019).

4.4.5 *In vitro* digestibility and total dietary fiber contents of RS3

Data of the *in vitro* digestibility of the uncooked and cooked pea and normal maize RS3 are summarized in Table 4.3. For the uncooked samples, pea RS3 showed significantly less RDS (20.1%) and SDS (21.8%) but more RS (52.2%) than normal maize RS3 (31.3% RDS, 26.9% SDS and 36.4% RS, respectively), indicating the greater enzymatic resistance of uncooked pea RS3. The difference in the enzymatic susceptibility between these two RS3, however, could not be explained by their comparable amylose contents (Table 4.2) and the smaller particle size of pea RS3 (Fig. 4.1 and Appendix Fig. 4).

After heating in a boiling water bath for 10 min, the RDS contents of pea and normal maize RS3 markedly increased, whereas their SDS and RS contents noticeably decreased (Table 4.3), which could be attributed to that the cooking step dissociated the double-helical crystallites and thus made the starch chains more susceptible to amylolysis (Wandee, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012). Cooked pea RS3 exhibited a significantly lower RDS content (78.7% of pea *versus* 81.8% of normal maize) and slightly higher SDS (2.9% *versus* 2.1%) and RS (12.4% *versus* 10.7%) than cooked normal maize RS3, suggesting that the former was still more resistant to amylolytic enzymes than the latter to some extent in a cooked form. In addition, it is noteworthy that the RS contents of pea and normal maize RS3 were substantially higher than those of their native counterparts before and after cooking: (1) In an uncooked state, 52.2% RS (pea RS3) *versus* 35.5% RS (native pea starch) and 36.4% RS (normal

maize RS3) *versus* 18.3% RS (native normal maize starch); and (2) In a cooked state, 12.4% *versus* 5.9% and 10.7% *versus* 5.4% for pea and normal maize samples, respectively (Li, Yuan, Setia, Raja, Zhang, & Ai, 2019).

Total dietary fiber content of pea RS3 (5.6%) was significantly higher than that of normal maize RS3 (4.1%; Table 4.3), which is consistent with the difference in their RS contents. In addition, both RS3 products had markedly larger total dietary fiber contents as compared to their native counterparts (1.9% and 0.8% for native pea and normal maize starches, respectively; data not shown). The results indicated that the modification method developed in the current study was an effective approach to enhancing the enzymatic resistance of starch for increased RS and dietary fiber contents, which could be employed to prepare low-glycemic food ingredients for health-promoting purpose (Haralampu, 2000).

Table 4.3 *In vitro* digestibility and total dietary fiber contents of pea and normal maize RS3[a]

RS3	Uncooked[b]			Cooked[b, c]			Total dietary fiber (%)[d]
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)	
Pea	20.1 ± 0.3 b	21.8 ± 1.2 b	52.2 ± 0.6 a	78.7 ± 0.7 b	2.9 ± 0.8 a	12.4 ± 1.1 a	5.6 ± 0.2 a
Normal maize	31.3 ± 1.4 a	26.9 ± 1.5 a	36.4 ± 1.0 b	81.8 ± 0.7 a	2.1 ± 0.8 a	10.7 ± 0.4 a	4.1 ± 0.1 b

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] RDS: rapidly digestible starch, SDS: slowly digestible starch, and RS: resistant starch; values were calculated on a dry starch basis.

[c] RS3 was cooked in a boiling water bath for 10 min.

[d] Determined using AOAC Method 991.43. The measurement was performed in one replicate for each batch of normal maize RS3 due to limited amounts of the samples.

4.4.6 Thermal properties of RS3 and molecular-weight distributions of RS3 before and after recrystallization

To elucidate the mechanisms responsible for the difference in the enzymatic digestibility between pea and normal maize RS3, their thermal properties and molecular-weight distributions before and after recrystallization were determined (Table 4.4, Fig. 4.2 and Appendix Table 1). Pea RS3 showed significantly higher dissociation temperatures (69.7-100.2°C) and larger ΔH (19.5 J/g) than normal maize RS3 (65.0-96.0°C and 17.1 J/g, respectively; Table 4.4), suggesting that in general the double-helical crystallites in the former were thermally more stable and required more energy for dissociation. The higher dissociation temperatures and larger percentage crystallinity of pea RS3 than those of normal maize RS3 (Table 4.4 and Appendix Fig. 5) indicated the development of double-helical crystallites of a more compact structure in the former product. The data corresponded well to the differences in the molecular-weight distributions of these two RS3 before and after recrystallization (Fig. 4.2 and Appendix Table 1). Prior to recrystallization, debranched AT pea starch consisted of a larger proportion of intermediate chains (DP 23-101 of Area 2; 25.6% of pea *versus* 18.5% of normal maize) and a smaller proportion of short chains (DP 6-23 of Area 3; 46.6% *versus* 54.4%) in comparison with the debranched AT normal maize starch; while their contents of amylose (partially hydrolyzed by acid) and long amylopectin branch chains (DP 101-1354 of Area 1) were comparable (27.8% of pea and 27.0% of normal maize). According to previous research, the intermediate chains (DP 23-101) were generally favorable but the short chains (DP 6-23) were unfavorable for the formation of thermally stable crystallites during recrystallization (Gidley & Bulpin, 1987). Consequently, after the recrystallization step, the percentages of short chains decreased but the percentages of intermediate chains proportionally increased in both RS3. The short chains, particularly those with DP <10, could not effectively form crystallites and thus remained soluble in the supernatant (Pfannemüller, 1987), which were partly lost during the recovery process by filtration (Section 4.3.2.3). Due to the existence of more short chains in the debranched AT normal maize starch (54.4% of normal maize *versus* 46.6% of pea), the yield of normal maize RS3 (75.8%) was markedly lower than that of pea RS3 (84.6%; Table 4.1).

On the other hand, at such a high solids content (25%, w/w, dsb), amylose and long amylopectin branch chains – as the minor component in the debranched AT starches (accounting for 27.8% and 27.0% in pea and normal maize) – were more likely to be involved in the

following two processes instead of recrystallizing between each other to yield stable lamellar crystallites: (1) entanglement with each other to form gel networks in local regions (Buléon, Véronèse, & Putaux, 2007); and (2) co-crystallization with intermediate/short chains (Pfannemüller, 1987). Thus, the endothermic dissociation of pea and normal maize RS3 concluded at a temperature of 100.2 and 96.0°C, respectively (Table 4.4), substantially lower than the endothermic transition temperature of amylose crystallites (ranging from 120 to 165°C) (Sievert, 1989). After the recrystallization step, the percentage of Area 1 decreased from 27.8% to 23.1% in pea RS3, whereas that of normal maize RS3 increased from 27.0% to 29.4% (Fig. 4.2 and Appendix Table 1). Further research is required to fully understand how the linear starch molecules of such a broad range of chain lengths interacted with each other to recrystallize for the formation of RS3 products.

After the recrystallization, the resultant pea RS3 contained a noticeably larger proportion of intermediate chains (32.6% of pea *versus* 22.5% of normal maize) and a smaller proportion of short chains (44.3% *versus* 48.1%; Fig. 4.2 and Appendix Table 1) when compared with normal maize RS3, which explained the larger percentage crystallinity (Appendix Fig. 5), better thermal stability and larger dissociation ΔH of the former (Table 4.4). With the denser crystalline structure, uncooked pea RS3 was more resistant to enzyme digestion than the normal maize counterpart as shown in Table 4.3 (Cai & Shi, 2014). Moreover, with such a high T_c (100.2°C) of pea RS3, it was possible that a larger proportion of double-helical crystallites remained undissociated during cooking and that the linear starch chains in this RS3 could re-associate more readily during cooling and prior to the addition of enzyme mixture in the Englyst Assay when compared with cooked normal maize RS3 (Ishiguro, Noda, Kitahara, & Yamakawa, 2000). Therefore, cooked pea RS3 also showed slightly greater enzymatic resistance than the cooked normal maize RS3 (Table 4.3). Furthermore, the greater percentage crystallinity, higher dissociation temperatures and larger ΔH of pea and normal maize RS3 than those of their respective native starches could contribute to the greater enzymatic resistance of the two RS3 products in both uncooked and cooked forms as indicated in Section 4.4.5.

Table 4.4 Thermal properties of pea and normal maize RS3[a,b,c]

RS3	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Pea	69.7 ± 1.5 a	88.5 ± 0.1 a	100.2 ± 0.3 a	19.5 ± 1.1 a
Normal maize	65.0 ± 1.1 b	82.9 ± 0.1 b	96.0 ± 0.4 b	17.1 ± 0.1 b

[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Measured using a differential scanning calorimeter.

[c] T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, and ΔH = enthalpy change.

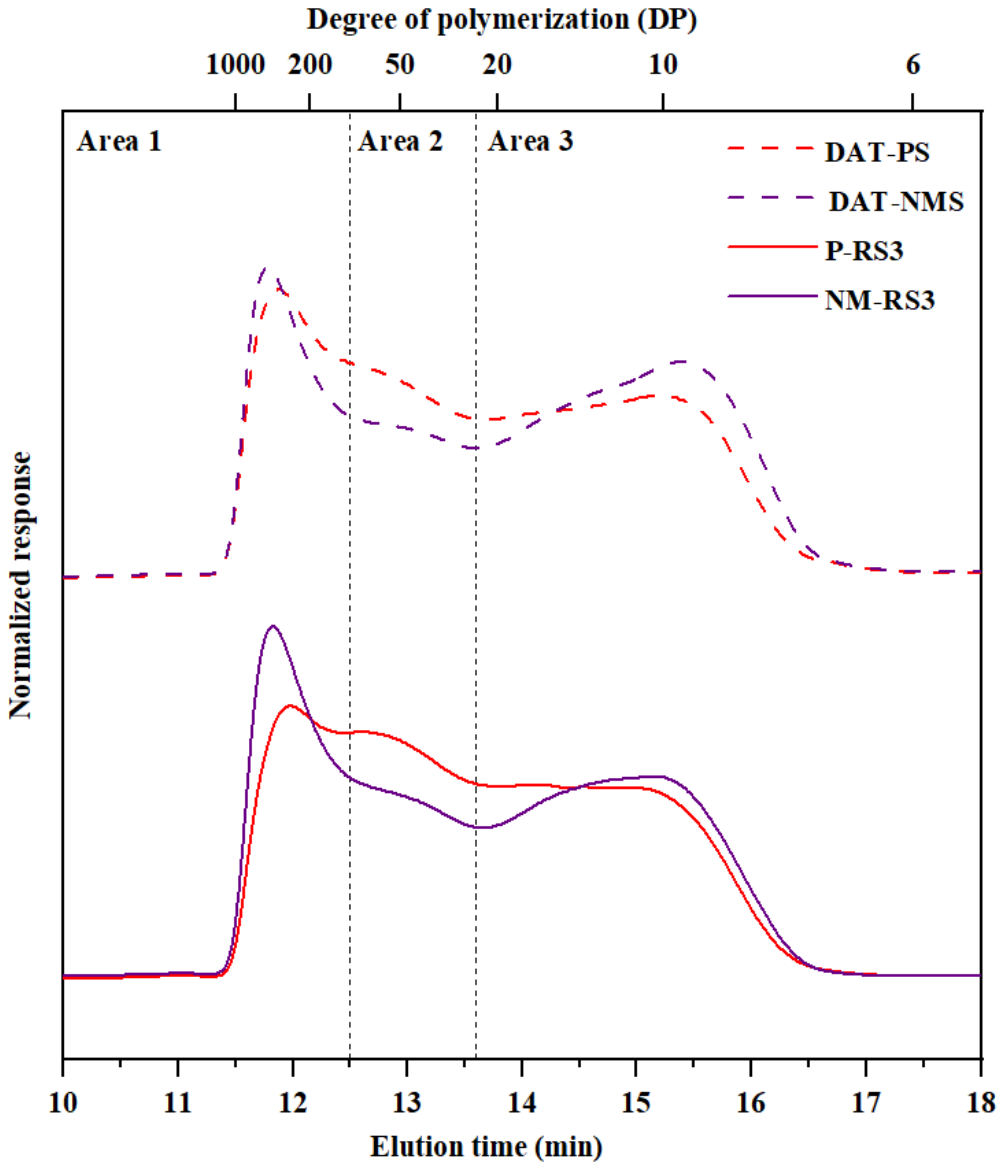


Fig. 4.2 Normalized molecular-weight distribution curves of debranched acid-thinned pea and normal maize starches and their corresponding RS3. DAT-PS: debranched acid-thinned pea starch; DAT-NMS: debranched acid-thinned normal maize starch; P-RS3: pea RS3; NM-RS3: normal maize RS3. The division of Areas 1-3 is indicated by the black dash lines. Area 1: amylose (partially hydrolyzed by acid) and long branch chains of amylopectin based on the retention time of 11.4-12.5 min (corresponding to DP 101-1354); Area 2: intermediate chains based on the retention time of 12.5-13.6 min (corresponding to DP 23-101); and Area 3: short chains based on the retention time of 13.6-17.4 min (corresponding to DP 6-23).

4.5 Conclusions

In this study, pea and normal maize RS3 were prepared from their respective native starches through acid thinning, debranching and recrystallization, with a final yield of 68.1% and 59.6%. The two RS3 products were irregular-shaped aggregates showing a coarse surface. The double-helical crystallites in both RS3 were packed in the B-type X-ray diffraction pattern. Pea RS3 consisted of a larger proportion of intermediate chains (DP of 23-101; 32.6% *versus* 22.5%) and a smaller proportion of short chains (DP of 6-23; 44.3% *versus* 48.1%) when compared with normal maize RS3. Consequently, pea RS3 showed a larger percentage crystallinity, higher dissociation temperatures, and larger ΔH than normal maize counterpart. The formation of more compact double-helical crystallites in the former RS3 was the primary factor responsible for its lower digestibility by amylolytic enzymes before and after cooking. Interestingly, the derived two RS3 products showed noticeably greater enzymatic resistance than their corresponding native starches. The combined process of acid thinning, debranching and recrystallization can be employed to generate pea-based RS ingredient for the preparation of low-glycemic foods, which thus will help the pulse processing industry find new uses of pea starch with value addition.

5. GENERAL DISCUSSION

Results from this thesis project demonstrated the potential of utilizing pulse starches for the development of RS3, which will help the pulse processing industry expand markets for this co-product with value addition. As the majority of pulse starches generated from fractionation processing in Canada is in the form of starch-rich flours from air classification, three of the most common starch-rich pulse flours, pea, lentil and faba bean, were selected to isolate high-purity starches in Study 1. During the isolation process, protein was removed through centrifugation followed by solubilization in an alkaline condition (pH = 9.5), and fiber was separated from starch via filtration using a cloth with openings of 60 µm. The resultant pea, lentil and faba bean starches exhibited high purity (94.8–97.9%; Table 3.1), which were comparable to those of the four important commercial starches isolated using wet methods, including Roquette pea, normal maize, waxy maize and tapioca starches (95.8−97.5%; Table 3.1). Isolated pea, lentil and faba bean starches, as well as Roquette pea starch, possessed significantly higher amylose contents (38.0−41.1%) and consisted of significantly smaller proportions (14.7−22.1%) of DP 6−12 short branch chains of amylopectin but larger proportions (55.0−60.4%) of DP 13−24 amylopectin branch chains than those of the maize and tapioca starches (1.9−31.2% amylose, 27.0−34.3% of DP 6−12 and 47.4−51.9% of DP 13−24, respectively; Table 3.2 and Appendix Fig. 1). Therefore, the granular morphologies, crystalline structure, thermal and pasting properties, gelling ability and *in vitro* digestibility of the isolated pulse starches were comparable to those of Roquette pea starch but were different from those of commercial maize and tapioca starches. The SEM images (Fig. 3.1) showed that all of the isolated pulse and Roquette pea starches displayed oval, kidney and irregular shapes, which were similar to the pulse starch granules published by Hoover, Hughes, Chung, and Liu (2010). In contrast, commercial maize starches exhibited polygonal and irregular-shaped granules, while tapioca starch displayed truncated and spherical shapes. Wide-angle X-ray diffraction revealed the C-type pattern of the isolated pulse and Roquette pea starches and the A-type pattern of the maize and tapioca starches (Appendix Fig. 2). Due to the unique organization of A- and B-type polymorphs in the granules

(Jane, et al., 1999), the four C-type pulse starches exhibited significantly lower onset (T_o; 55.6−60.1°C) and peak (T_p; 61.6−65.2°C) gelatinization temperatures than those of the A-type maize and tapioca starches (62.8−66.1°C for T_o and 68.2−71.2°C for T_p, respectively; Table 3.3). Overall, pulse starches showed smaller percentages crystallinity (25.1−30.0%), lower gelatinization enthalpy changes (ΔH; 12.4−13.5 J/g) and lower pasting viscosities (105.9−145.4 RVU), but greater percentages retrogradation (36.5−52.4%) and stronger gelling ability (328.3−727.4 g) compared with those of maize and tapioca starches (29.4−34.9%, 15.1−18.0 J/g, 144.4−264.1 RVU, 7.2−31.5% and 4.8−121.0 g, respectively; Appendix Fig. 2, Table 3.3 and Fig. 3.2). For raw starch samples, the isolated pulse and Roquette pea starches generally possessed smaller RDS (15.3−21.0%) and SDS contents (34.5−40.9%) but larger RS content (35.5−46.7%) than the normal maize and waxy maize starches (20.8−34.7% RDS, 51.9−56.7% SDS and 10.9−18.3% RS, respectively; Table 3.4). In addition to amylose content and amylopectin BCL distribution, the internal structure of starch granules played a critical role in determining the *in vitro* digestibility of raw starches; where tapioca starch, with a solid and homogenous internal structure (Fig. 3.1-CLSM, G), exhibiting the highest RS content (51.8%; Table 3.4) among the seven starch samples analyzed. However, *in vitro* digestibility differences of the pulse, maize and tapioca starches disappeared following gelatinization.

As shown in Study 1, the relatively high amylose contents and long amylopectin branch chains of pulse starches are desirable structural features for the development of RS3 with significant enzymatic resistance. Because the isolated pea, lentil and faba bean starches exhibited similar molecular structure, functional properties and *in vitro* digestibility, the isolated pea starch was selected in Study 2 to investigate the development of a novel RS3, with commercial normal maize starch being included for comparison. The pea and normal maize starches were acid thinned for 24 h to prevent the formation of a firm gel that could hinder the follow-up debranching reaction. The acid-thinned (AT) starches, with a high solids content of 25% (w/w), were subsequently subjected to isoamylase debranching and recrystallization to generate RS3. This developed method yielded 68.1% of RS3 from the isolated pea starch and 59.6% from the commercial normal maize starch (Section 4.4.1). Both RS3 products exhibited aggregates with a rough surface, and irregular shapes and sizes (Fig. 4.1). Particle size analysis revealed that the D[4,3] of pea RS3 (45.9 μm) was significantly smaller than that of the normal maize RS3 (73.9 μm; Appendix Fig. 4). The resulting pea and normal maize RS3 both displayed the B-type X-ray

diffraction pattern (Appendix Fig. 5), which was distinctively different from that of the native pea (C-type) and normal maize starch (A-type) (Appendix Fig. 2). Pea RS3 possessed a similar amylose content (21.6%), but consisted of a significantly larger proportion (32.6%) of intermediate chains (DP of 23−101) and a smaller proportion (44.3%) of short chains (DP of 6−23) when compared with normal maize RS3 (22.1% amylose, 22.5% of intermediate chains and 48.1% of short chains, respectively; Table 4.2, Fig. 4.2 and Appendix Table 1). Consequently, pea RS3 exhibited significantly larger percentage crystallinity (41.0%), higher dissociation temperatures (T_o; 69.7°C, T_p ;88.5°C and T_c; 100.2°C) and larger ΔH (19.5 J/g) than the normal maize counterpart (37.7% for percentage crystallinity, 65.0°C for T_o, 82.9°C for T_p, 96.0°C for T_c and 17.1 J/g for ΔH, respectively; Appendix Fig. 5 and Table 4.4). With the denser crystalline structure, uncooked pea RS3 showed significantly less RDS (20.1%) and SDS (21.8%) but more RS (52.2%) than normal maize RS3 (31.3% RDS, 26.9% SDS and 36.4% RS, respectively; Table 4.3). In addition, *in vitro* digestibility also revealed that cooked pea RS3 exhibited a significantly lower RDS content (78.7%) and slightly higher SDS (2.9%) and RS (12.4%) than the cooked normal maize RS3 (81.8% RDS, 2.1% SDS and 10.7% RS, respectively), indicating that the pea RS3 after cooking was still less digestible to amylolytic enzymes than the normal maize counterpart (Table 4.3). Total dietary fiber content of pea RS3 (5.6%) was significantly higher than that of normal maize RS3 (4.1%), which is in good agreement with their RS contents (Table 4.3). Interestingly, the two derived RS3 products showed noticeably greater enzymatic resistance than their corresponding native starches before and after cooking: (1) In an uncooked state, 52.2% RS (pea RS3) *versus* 35.5% RS (native pea starch) and 36.4% RS (normal maize RS3) *versus* 18.3% RS (native normal maize starch); and (2) In a cooked state, 12.4% *versus* 5.9% and 10.7% *versus* 5.4% for pea and normal maize samples, respectively (Table 3.4 and Table 4.3). These results show that the modification method developed in this research project was effective in enhancing the enzymatic resistance of isolated pea, lentil and faba bean starches, which could be employed to generate pulse-based RS ingredients for the preparation of low-glycemic foods. The new findings as reported in this thesis research will be meaningful for the pulse processing industry to find new applications of the starch fraction for value-added utilization.

6. GENERAL CONCLUSIONS AND FUTURE STUDIES

The overarching goal of this thesis project was to isolate and characterize pea, lentil and faba bean starches from air-classified flours and to generate a novel RS3 product from the isolated pulse starches. In Study 1, three most common starch-rich pulse flours available in the market (*e.g.*, pea, lentil and faba bean) were selected to isolate starches with 94.8–97.9% total starch contents. Molecular structure, functional properties and *in vitro* digestibility of the isolated pulse starches were determined and compared with those of four important commercial starches, including Roquette pea, normal maize, waxy maize and tapioca starches. Results showed that the isolated pea, lentil and faba bean starches, as well as Roquette pea starch, possessed significantly higher amylose contents and consisted of significantly smaller proportions of DP 6−12 branch chains but larger proportions of DP 13−24 branch chains in their amylopectins when compared with the maize and tapioca starches. Consequently, the isolated pulse starches exhibited granular morphologies, crystalline structure, thermal properties, pasting properties, gelling ability and *in vitro* digestibility comparable to those of Roquette pea starch but obviously different from those of normal maize, waxy maize and tapioca starches. In addition to amylose content and amylopectin BCL distribution, the internal structure of starch granules played a vital role in determining the *in vitro* digestibility of raw, granular starches. Nevertheless, the variations in the digestibility of the pulse, maize and tapioca starches disappeared upon cooking.

According to Study 1, the relatively high amylose contents and long branch chains of amylopectins of pulse starches could render them more desirable for the development of RS3 with great enzymatic resistance. Due to the similar molecular structure, functional properties and *in vitro* digestibility of isolated pea, lentil and faba bean starches as revealed in Study 1, the isolated pea starch was used as the representative pulse starch in Study 2 to develop RS3 through acid thinning, debranching and recrystallization, and the derived RS3 product was characterized and compared with that produced from commercial normal maize starch. The modification method yielded 68.1% of RS3 from the isolated pea starch and 59.6% from the normal maize starch. Pea RS3 consisted of a larger proportion of intermediate chains and a smaller proportion

of short chains in comparison with normal maize RS3. Consequently, pea RS3 showed larger percentage crystallinity, higher dissociation temperatures and a larger ΔH than normal maize counterpart. The better thermal stability of pea RS3 was the primary factor responsible for its less susceptibility to amylolytic enzymes before and after cooking. In addition, the derived two RS3 products showed considerably greater enzymatic resistance than their corresponding native starches. These results suggested that the modification method demonstrated in the current project was effective to increase the enzymatic resistance of pulse starch, which could be employed to generate pulse-based RS ingredient for the preparation of low-glycemic foods.

Although Study 1 of this research showed that the isolated pea, lentil and faba bean starches, as well as Roquette pea starch, possessed comparable amylose contents, amylopectin BCL distributions, granular morphologies and crystalline structure, strength values of their corresponding starch gels were obviously different: with isolated faba bean being the highest, followed by the isolated lentil, pea and Roquette pea. At the current stage, our data cannot explain this phenomenon and more studies need to be carried out to reveal the mechanisms responsible for the different gelling capability of the pulse starches. In addition, each of the pea, lentil and faba bean starches was isolated from only one pulse variety, which might not be representative for starches from different pulse crops. It will be valuable to conduct genotype by environment (G × E) studies to advance our understanding of the structure, physicochemical properties and digestibility of starches from a broad range of pulse crops. This research will be particularly important for pulses that have gained increasing interest from the industry, such as faba bean. In general, pulse starches exhibited significantly stronger gelling ability than maize and tapioca starches. Future studies can examine the application value of various pulse starches for the preparation of food products that require the formation of firm starch gels, such as glass noodles and jello-type dessert.

Because the RS contents of the pea and normal maize RS3 decreased substantially after cooking as shown in Study 2, future research can be performed to investigate whether physical treatments, such as temperature-cycle and heat-moisture treatments, can further improve the RS and total dietary fiber contents of the RS3 products. The food application value of the RS3 ingredients should also be evaluated, especially in food products that do not require high-temperature processing, such as beverages and salad dressing. In addition, RS3 generated from this research does not meet the requirements of “clean label” and the modification method is

time-consuming. Further studies can be carried out to refine the modification process for industrial production.

7. REFERENCES

AACC. (2000). Approved methods of the AACC (10th ed.). St. Paul, MN: American Association of Cereal Chemists.

Ai, Y., Hasjim, J., & Jane, J. (2013). Effects of lipids on enzymatic hydrolysis and physical properties of starch. *Carbohydrate Polymers, 92*(1), 120-127.

Ai, Y., Nelson, B., Birt, D. F., & Jane, J. (2013). In vitro and in vivo digestion of octenyl succinic starch. *Carbohydrate Polymers, 98*(2), 1266-1271.

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., Nantanga, K., & 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.

AOAC. (2012). Official methods of analysis of AOAC international (19th ed.). Association of Official Analytical Chemists International.

Asare, E. K., Jaiswal, S., Maley, J., Båga, M., Sammynaiken, R., Rossnagel, B. G., & Chibbar, R. N. (2011). Barley grain constituents, starch composition, and structure affect starch in vitro enzymatic hydrolysis. *Journal of Agricultural and Food Chemistry, 59*(9), 4743-4754.

Atwell, W., Hood, L., Lineback, D., Varriano-Marston, E., & Zobel, H. (1988). The terminology and methodology associated with basic starch phenomena. *Cereal Foods World (USA), 33*, 306-311.

Bassett, D. (2003). Polymer spherulites: a modern assessment. *Journal of Macromolecular Science, Part B, 42*(2), 227-256.

Berry, C. (1986). Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *Journal of Cereal Science, 4*(4), 301-314.

Biliaderis, C. (2009). Structural transitions and related physical properties of starch. In J. N. BeMiller & R. L. Whistler (Eds.), *Starch: Chemistry and Technology* 3rd ed., (pp. 293-372): Academic Press.

Biliaderis, C., Maurice, T., & Vose, J. (1980). Starch gelatinization phenomena studied by differential scanning calorimetry. *Journal of Food Science, 45*(6), 1669-1674.

Blazek, J., & Copeland, L. (2008). Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydrate Polymers, 71*(3), 380-387.

Bogracheva, T. Y., Morris, V., Ring, S., & Hedley, C. (1998). The granular structure of C-type pea starch and its role in gelatinization. *Biopolymers, 45*(4), 323-332.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414-431.

Buléon, A., Véronèse, G., & Putaux, J.-L. (2007). Self-association and crystallization of amylose. *Australian Journal of Chemistry, 60*(10), 706-718.

Cai, J., Cai, C., Man, J., Zhou, W., & Wei, C. (2014). Structural and functional properties of C-type starches. *Carbohydrate Polymers, 101*, 289-300.

Cai, L., & Shi, Y.-C. (2010). Structure and digestibility of crystalline short-chain amylose from debranched waxy wheat, waxy maize, and waxy potato starches. *Carbohydrate Polymers, 79*(4), 1117-1123.

Cai, L., & Shi, Y.-C. (2013). Self-assembly of short linear chains to A-and B-type starch spherulites and their enzymatic digestibility. *Journal of Agricultural and Food Chemistry, 61*(45), 10787-10797.

Cai, L., & Shi, Y.-C. (2014). Preparation, structure, and digestibility of crystalline A-and B-type aggregates from debranched waxy starches. *Carbohydrate Polymers, 105*, 341-350.

Chrastil, J. (1987). Improved colorimetric determination of amylose in starches or flours. *Carbohydrate Research, 159*(1), 154-158.

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*(4), 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., Warkentin, T. D., & Vandenberg, B. (2008). In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chemistry, 111*(2), 316-321.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydrate Research, 227*, 103-112.

Creek, J. A., Ziegler, G. R., & Runt, J. (2006). Amylose crystallization from concentrated aqueous solution. *Biomacromolecules, 7*(3), 761-770.

Dahl, W. J., Foster, L. M., & Tyler, R. T. (2012). Review of the health benefits of peas (Pisum sativum L.). *British Journal of Nutrition, 108*(S1), S3-S10.

Dalgetty, D. D., & Baik, B.-K. (2003). Isolation and characterization of cotyledon fibers from peas, lentils, and chickpeas. *Cereal Chemistry, 80*(3), 310-315.

Davydova, N., Leont'Ev, S., Genin, Y. V., Sasov, A. Y., & Bogracheva, T. Y. (1995). Some physico-chemical properties of smooth pea starches. *Carbohydrate Polymers, 27*(2), 109-115.

Dhital, S., Bhattarai, R. R., Gorham, J., & Gidley, M. J. (2016). Intactness of cell wall structure controls the in vitro digestion of starch in legumes. *Food & Function, 7*(3), 1367-1379.

Duan, D. X., Donner, E., Liu, Q., Smith, D. C., & Ravenelle, F. (2012). Potentiometric titration for determination of amylose content of starch–A comparison with colorimetric method. *Food Chemistry, 130*(4), 1142-1145.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. t., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350-356.

Eerlingen, R., Decuninck, M., & Delcour, J. (1993). Enzyme resistant starch 11. Influence of amylose chain length on resistant starch and formation. *Cereal Chemistry, 70*(3), 345-350.

Eerlingen, R., & Delcour, J. (1995). Formation, analysis, structure and properties of type III enzyme resistant starch. *Journal of Cereal Science, 22*(2), 129-138.

Englyst, H. N., Kingman, S., & Cummings, J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, S33-50.

Englyst, H. N., & Macfarlane, G. T. (1986). Breakdown of resistant and readily digestible starch by human gut bacteria. *Journal of the Science of Food and Agriculture, 37*(7), 699-706.

Englyst, H. N., Wiggins, H., & Cummings, J. (1982). Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst, 107*(1272), 307-318.

Faye, S. (2007). *The pulse industry in western Canada*: Economics and Competitive Division, Economics Unit.

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

Gernat, C., Radosta, S., Damaschun, G., & Schierbaum, F. (1990). Supramolecular structure of legume starches revealed by X-ray scattering. *Starch-Stärke, 42*(5), 175-178.

Gidley, M. J., & Bulpin, P. V. (1987). Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: minimum chain-length requirement for the formation of double helices. *Carbohydrate Research, 161*(2), 291-300.

Gidley, M. J., Cooke, D., Darke, A., Hoffmann, R., Russell, A., & Greenwell, P. (1995). Molecular order and structure in enzyme-resistant retrograded starch. *Carbohydrate Polymers, 28*(1), 23-31.

Hanashiro, I., Abe, J.-i., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research, 283*, 151-159.

Haralampu, S. (2000). Resistant starch—a review of the physical properties and biological impact of RS 3. *Carbohydrate Polymers, 41*(3), 285-292.

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., Lee, S. O., Hendrich, S., Setiawan, S., Ai, Y., & Jane, J. l. (2010). Characterization of a novel resistant‐starch and its effects on postprandial plasma glucose and insulin responses. *Cereal Chemistry, 87*(4), 257-262.

Hayakawa, K., Tanaka, K., Nakamura, T., Endo, S., & Hoshino, T. (1997). Quality characteristics of waxy hexaploid wheat (Triticum aestivum L.): Properties of starch gelatinization and retrogradation. *Cereal Chemistry, 74*(5), 576-580.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research, 147*(2), 342-347.

Hoover, R. (1995). Starch retrogradation. *Food Reviews International, 11*(2), 331-346.

Hoover, R., Hughes, T., Chung, H., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International, 43*(2), 399-413.

Hoover, R., & Ratnayake, W. (2002). Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry, 78*(4), 489-498.

Hoover, R., & Sosulski, F. (1991). Composition, structure, functionality, and chemical modification of legume starches: A review. *Canadian Journal of Physiology and Pharmacology, 69*(1), 79-92.

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*(4), 401-417.

Hsien-Chih, H. W., & Sarko, A. (1978). The double-helical molecular structure of crystalline A-amylose. *Carbohydrate Research, 61*(1), 27-40.

Hutchins, A. M., Winham, D. M., & Thompson, S. V. (2012). Phaseolus beans: impact on glycaemic response and chronic disease risk in human subjects. *British Journal of Nutrition, 108*(S1), S52-S65.

Ishiguro, K., Noda, T., Kitahara, K., & Yamakawa, O. (2000). Retrogradation of sweetpotato starch. *Starch - Stärke, 52*(1), 13-17.

Jane, J. (2006). Current understanding on starch granule structures. *Journal of Applied Glycoscience, 53*(3), 205-213.

Jane, J. (2009). Structural features of starch granules II. In J. N. BeMiller & R. L. Whistler (Eds.), *Starch: Chemistry and Technology* 3rd ed., (pp. 193-236): Academic Press.

Jane, J., Atichokudomchai, N., & Suh, D.-S. (2004). Internal structures of starch granules revealed by confocal laser-light scanning microscopy. *Starch: Progress in Structural Studies, Modifications and Applications*, 147-156.

Jane, J., Chen, Y., Lee, L., McPherson, A., Wong, K., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch 1. *Cereal Chemistry, 76*(5), 629-637.

Jane, J., Kasemsuwan, T., Leas, S., Zobel, H., & Robyt, J. F. (1994). Anthology of starch granule morphology by scanning electron microscopy. *Starch-Stärke, 46*(4), 121-129.

Jenkins, D. J., Kendall, C. W., Augustin, L. S., Mitchell, S., Sahye-Pudaruth, S., Mejia, S. B., Chiavaroli, L., Mirrahimi, A., Ireland, C., & Bashyam, B. (2012). Effect of legumes as part of a low glycemic index diet on glycemic control and cardiovascular risk factors in type 2 diabetes mellitus: a randomized controlled trial. *Archives of Internal Medicine, 172*(21), 1653-1660.

Jha, A. B., Tar'an, B., Diapari, M., & Warkentin, T. D. (2015). SNP variation within genes associated with amylose, total starch and crude protein concentration in field pea. *Euphytica, 206*(2), 459-471.

Keetels, C., Van Vliet, T., & Walstra, P. (1996). Gelation and retrogradation of concentrated starch systems: 2. Retrogradation. *Food Hydrocolloids, 10*(3), 355-362.

Kiatponglarp, W., Rugmai, S., Rolland-Sabaté, A., Buléon, A., & Tongta, S. (2016). Spherulitic self-assembly of debranched starch from aqueous solution and its effect on enzyme digestibility. *Food Hydrocolloids, 55*, 235-243.

Kiatponglarp, W., Tongta, S., Rolland-Sabaté, A., & Buléon, A. (2015). Crystallization and chain reorganization of debranched rice starches in relation to resistant starch formation. *Carbohydrate Polymers, 122*, 108-114.

Lai, V. M. F., Lu, S., & Lii, C. y. (2000). Molecular characteristics influencing retrogradation kinetics of rice amylopectins. *Cereal Chemistry, 77*(3), 272-278.

Li, J.-Y., & Yeh, A.-I. (2001). Relationships between thermal, rheological characteristics and swelling power for various starches. *Journal of Food Engineering, 50*(3), 141-148.

Li, L., Jiang, H., Campbell, M., Blanco, M., & Jane, J. (2008). Characterization of maize amylose-extender (ae) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydrate Polymers, 74*(3), 396-404.

Li, L., Yuan, T. Z., Setia, R., Raja, R. B., Zhang, B., & Ai, Y. (2019). Characteristics of pea, lentil and faba bean starches isolated from air-classified flours in comparison with commercial starches. *Food Chemistry, 276*, 599-607.

Lin, A. H.-M., Lee, B.-H., & Chang, W.-J. (2016). Small intestine mucosal α-glucosidase: A missing feature of in vitro starch digestibility. *Food Hydrocolloids, 53*, 163-171.

Lineback, D. (1984). The starch granule: organization and properties. *Bakers Digest (USA)*.

Liu, G., Gu, Z., Hong, Y., Cheng, L., & Li, C. (2017). Structure, functionality and applications of debranched starch: A review. *Trends in Food Science & Technology, 63*, 70-79.

Lockyer, S., & Nugent, A. (2017). Health effects of resistant starch. *Nutrition Bulletin, 42*(1), 10-41.

Lu, Z.-H., Belanger, N., Donner, E., & Liu, Q. (2018). Debranching of pea starch using pullulanase and ultrasonication synergistically to enhance slowly digestible and resistant starch. *Food Chemistry, 268*, 533-541.

Luckett, C. R., & Wang, Y.-J. (2012). Effects of β-amylolysis on the resistant starch formation of debranched corn starches. *Journal of Agricultural and Food Chemistry, 60*(18), 4751-4757.

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.

Maskus, H., Bourré, L., Fraser, S., Sarkar, A., & Malcolmson, L. (2016). Effects of Grinding Method on the Compositional, Physical, and Functional Properties of Whole and Split Yellow Pea Flours. *Cereal Foods World, 61*(2), 59-64.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research, 135*(2), 271-281.

Mudryj, A. N., Yu, N., & Aukema, H. M. (2014). Nutritional and health benefits of pulses. *Applied Physiology, Nutrition, and Metabolism, 39*(11), 1197-1204.

Mudryj, A. N., Yu, N., Hartman, T. J., Mitchell, D. C., Lawrence, F. R., & Aukema, H. M. (2012). Pulse consumption in Canadian adults influences nutrient intakes. *British Journal of Nutrition, 108*(S1), S27-S36.

Mutungi, C., Rost, F., Onyango, C., Jaros, D., & Rohm, H. (2009). Crystallinity, thermal and morphological characteristics of resistant starch type III produced by hydrothermal treatment of debranched cassava starch. *Starch-Stärke, 61*(11), 634-645.

Nordmark, T. S., & Ziegler, G. R. (2002). Spherulitic crystallization of gelatinized maize starch and its fractions. *Carbohydrate Polymers, 49*(4), 439-448.

Patterson, C., Maskus, H., & Dupasquier, C. (2009). Pulse crops for health. *Cereal Foods World (CFW), 54*(3), 108.

Pelgrom, P. J., Vissers, A. M., Boom, R. M., & Schutyser, M. A. (2013). Dry fractionation for production of functional pea protein concentrates. *Food Research International, 53*(1), 232-239.

Peng, X., & Yao, Y. (2018). Small-granule starches from sweet corn and cow cockle: Physical properties and amylopectin branching pattern. *Food Hydrocolloids, 74*, 349-357.

Perera, C., Lu, Z., Sell, J., & Jane, J. (2001). Comparison of physicochemical properties and structures of sugary-2 cornstarch with normal and waxy cultivars. *Cereal Chemistry, 78*(3), 249-256.

Pérez, S., Baldwin, P. M., & Gallant, D. J. (2009). Structural features of starch granules I. In J. N. BeMiller & R. L. Whistler (Eds.), *Starch: Chemistry and Technology* 3rd ed., (pp. 149-192): Academic Press.

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.

Pfannemüller, B. (1987). Influence of chain length of short monodisperse amyloses on the formation of A-and B-type X-ray diffraction patterns. *International Journal of Biological Macromolecules, 9*(2), 105-108.

Pulse Canada. (2019) *Growing regions. Available from: http://www.pulsecanada.com/about-pulse-canada/growing-regions/. Accessed 2019 October 21.*).

Putseys, J., Lamberts, L., & Delcour, J. (2010). Amylose-inclusion complexes: Formation, identity and physico-chemical properties. *Journal of Cereal Science, 51*(3), 238-247.

Ratnayake, W., Hoover, R., Shahidi, F., Perera, C., & Jane, J. (2001). Composition, molecular structure, and physicochemical properties of starches from four field pea (Pisum sativum L.) cultivars. *Food Chemistry, 74*(2), 189-202.

Ring, S. (1985). Some studies on starch gelation. *Starch-Stärke, 37*(3), 80-83.

Rochfort, S., & Panozzo, J. (2007). Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry, 55*(20), 7981-7994.

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

Schäfer, G., Schenk, U., Ritzel, U., Ramadori, G., & Leonhardt, U. (2003). Comparison of the effects of dried peas with those of potatoes in mixed meals on postprandial glucose and insulin concentrations in patients with type 2 diabetes. *The American Journal of Clinical Nutrition, 78*(1), 99-103.

Shi, M., Chen, Y., Yu, S., & Gao, Q. (2013). Preparation and properties of RS III from waxy maize starch with pullulanase. *Food Hydrocolloids, 33*(1), 19-25.

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.

Shi, Y., & Seib, P. A. (1992). The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydrate Research, 227*, 131-145.

Shi, Y., & Seib, P. A. (1995). Fine structure of maize starches from four wx-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. *Carbohydrate Polymers, 26*(2), 141-147.

Sievert, D. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chemistry, 66*, 342-347.

Sievert, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chemistry, 66*, 342-347.

Sievert, D., & Pomeranz, Y. (1990). Enzyme-resistant starch. II. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chemistry, 67*(3), 217-221.

Simons, C. W., Hall III, C., & Vatansever, S. (2018). Production of resistant starch (RS3) from edible bean starches. *Journal of Food Processing and Preservation, 42*(4), e13587.

Singh, N. (2011). Functional and physicochemical properties of pulse starch. In B. K. Tiwari, A. Gowen & B. McKenna (Eds.), *Pulse Foods: Processing, Quality and Nutraceutical Application* 1st ed., (pp. 91-119): Academic Press.

Smýkal, P., Aubert, G., Burstin, J., Coyne, C. J., Ellis, N. T., Flavell, A. J., Ford, R., Hýbl, M., Macas, J., & Neumann, P. (2012). Pea (Pisum sativum L.) in the genomic era. *Agronomy, 2*(2), 74-115.

Song, Y., & Jane, J. (2000). Characterization of barley starches of waxy, normal, and high amylose varieties. *Carbohydrate Polymers, 41*(4), 365-377.

Sosulski, F., & Sosulski, K. (1986). Composition and functionality of protein, starch, and fiber from wet and dry processing of grain legumes. In, (pp. 176-189): ACS Publications.

Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N., & Hisamatsu, M. (2005). Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. *Carbohydrate Polymers, 62*(1), 25-34.

Stone, A. K., Karalash, A., Tyler, R. T., Warkentin, T. D., & Nickerson, M. T. (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International, 76*, 31-38.

Takeda, Y., Hizukuri, S., & Juliano, B. O. (1987). Structures of rice amylopectins with low and high affinities for iodine. *Carbohydrate Research, 168*(1), 79-88.

Takeda, Y., Shirasaka, K., & Hizukuri, S. (1984). Examination of the purity and structure of amylose by gel-permeation chromatography. *Carbohydrate Research, 132*(1), 83-92.

Takeda, Y., Shitaozono, T., & Hizukuri, S. (1990). Structures of sub-fractions of corn amylose. *Carbohydrate Research, 199*(2), 207-214.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry, 67*(6), 551-557.

Tosh, S. M., & Yada, S. (2010). Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications. *Food Research International, 43*(2), 450-460.

Tyler, R., Youngs, C., & Sosulski, F. (1981). Air classification of legumes. I. Separation efficiency, yield, and composition of the starch and protein fractions. *Cereal Chemistry, 58*(2), 144-148.

Vargas-Torres, A., Osorio-Díaz, P., Islas-Hernández, J. J., Tovar, J., Paredes-López, O., & Bello-Pérez, L. A. (2004). Starch digestibility of five cooked black bean (Phaseolus vulgaris L.) varieties. *Journal of Food Composition and Analysis, 17*(5), 605-612.

Vasanthan, T., & Bhatty, R. (1998). Enhancement of resistant starch (RS3) in amylomaize, barley, field pea and lentil starches. *Starch-Stärke, 50*(7), 286-291.

Villas-Boas, F., & Franco, C. M. (2016). Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches. *Food Hydrocolloids, 52*, 795-803.

Wandee, Y., Puttanlek, C., Rungsardthong, V., Puncha-arnon, S., & Uttapap, D. (2012). Effects of gelatinization and gel storage conditions on the formation of canna resistant starch. *Food and Bioprocess Technology, 5*(7), 2665-2673.

Wang, L., & Wang, Y. J. (2001). Structures and physicochemical properties of acid‐thinned corn, potato and rice starches. *Starch-Stärke, 53*(11), 570-576.

Wang, N., Warkentin, T. D., Vandenberg, B., & Bing, D. J. (2014). Physicochemical properties of starches from various pea and lentil varieties, and characteristics of their noodles prepared by high temperature extrusion. *Food Research International, 55*, 119-127.

Wani, A. A., Singh, P., Shah, M. A., Schweiggert-Weisz, U., Gul, K., & Wani, I. A. (2012). Rice starch diversity: Effects on structural, morphological, thermal, and physicochemical properties—A review. *Comprehensive Reviews in Food Science and Food Safety, 11*(5), 417-436.

Wood, J., & Malcolmson, L. (2011). Pulse milling technologies. In B. K. Tiwari, A. Gowen & B. McKenna (Eds.), *Pulse Foods: Processing, Quality and Nutraceutical Application* 1st ed., (pp. 193-222): Academic Press.

Zeng, F., Ma, F., Gao, Q., Yu, S., Kong, F., & Zhu, S. (2014). Debranching and temperature-cycled crystallization of waxy rice starch and their digestibility. *Carbohydrate Polymers, 113*, 91-96.

Zhang, H., & Jin, Z. (2011). Preparation of products rich in resistant starch from maize starch by an enzymatic method. *Carbohydrate Polymers, 86*(4), 1610-1614.

Ziegler, G. R., Creek, J. A., & Runt, J. (2005). Spherulitic crystallization in starch as a model for starch granule initiation. *Biomacromolecules, 6*(3), 1547-1554.

8. APPENDICES

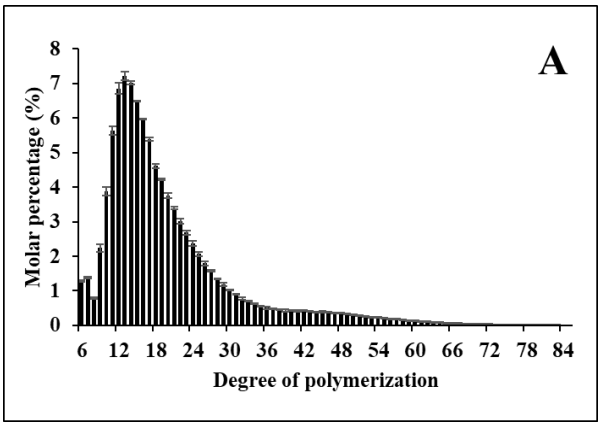
8.1 List of Tables

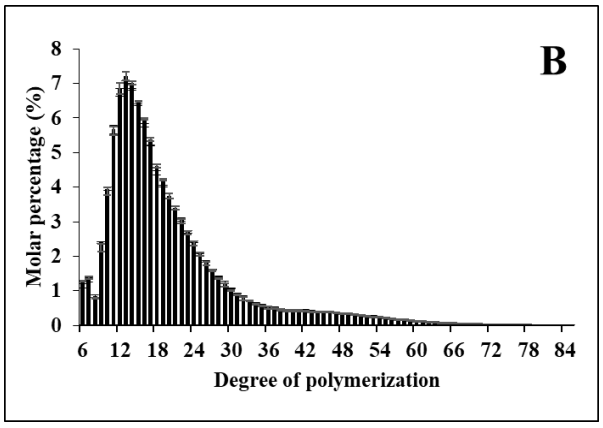
Table 1 Molecular-weight distributions of debranched acid-thinned pea and normal maize starches and their corresponding RS3[a,b,c]

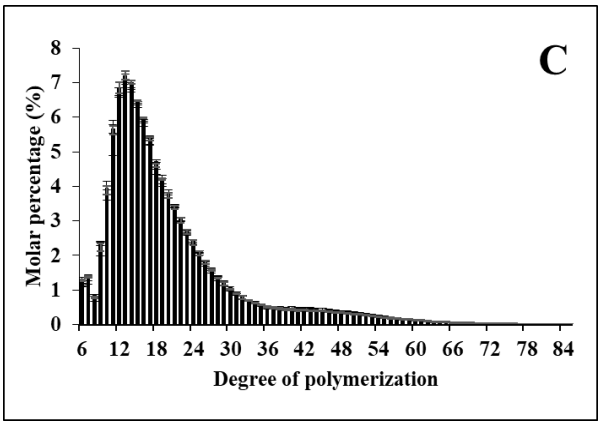
	Area 1 (%)	Area 2 (%)	Area 3 (%)
Debranched acid-thinned starch			
Pea	27.8 ± 0.1 b	25.6 ± 0.2 b	46.6 ± 0.1 c
Normal maize	27.0 ± 0.0 b	18.5 ± 0.1 d	54.4 ± 0.1 a
RS3			
Pea	23.1 ± 0.6 c	32.6 ± 0.3 a	44.3 ± 0.7 d
Normal maize	29.4 ± 0.3 a	22.5 ± 0.4 c	48.1 ± 0.6 b

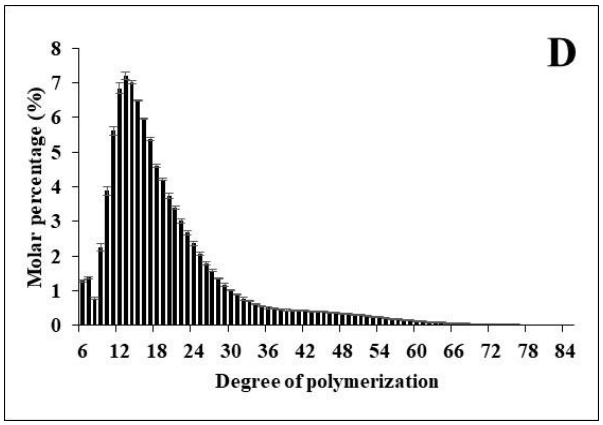
[a] Values are presented as average ± standard deviation (n = 3); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.
[b] Measured using a high-performance size-exclusion chromatography (HPSEC) instrument equipped with a refractive index (RI) detector.
[c] Percentages of Areas 1-3 were calculated as the respective fraction areas divided by the total area under the curve as shown in Fig. 4.2.

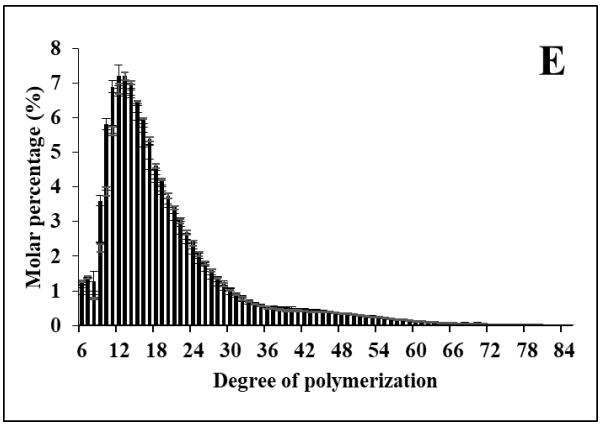
8.2 List of Figures

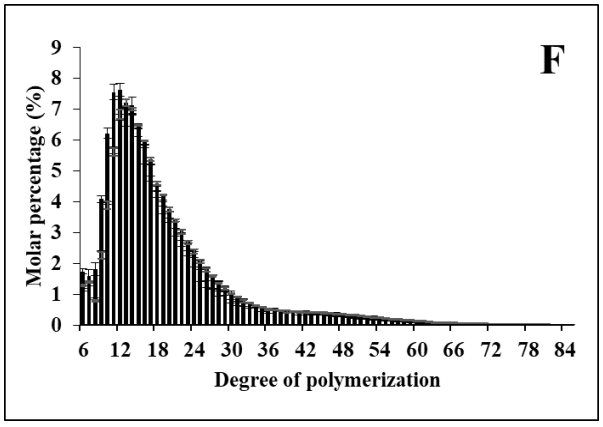












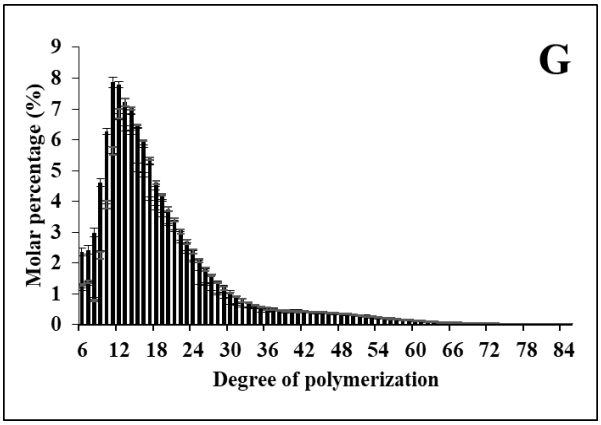


Fig. 1 Branch-chain-length distributions of amylopectins of isolated pulse starches and commercial starches analyzed using fluorophore-assisted capillary electrophoresis (FACE). A: pea; B: lentil; C: faba bean; D: Roquette pea; E: normal maize; F: waxy maize; G: tapioca

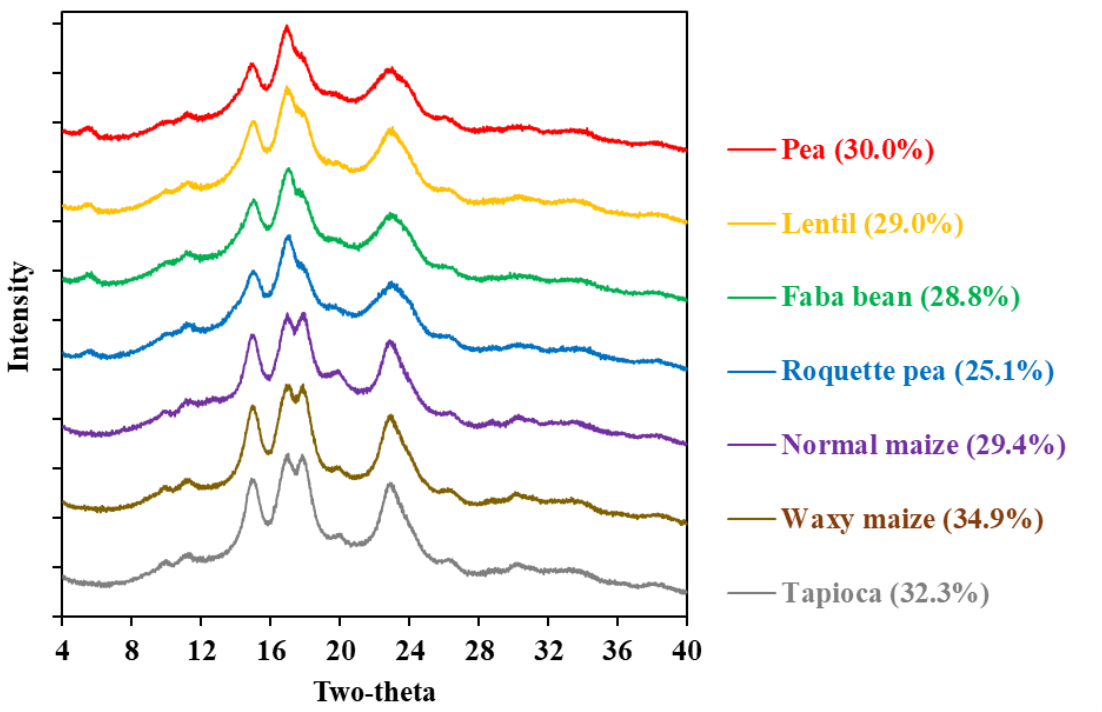


Fig. 2 X-ray diffraction patterns of isolated pulse starches and commercial starches. Percentage crystallinity is given in parentheses.

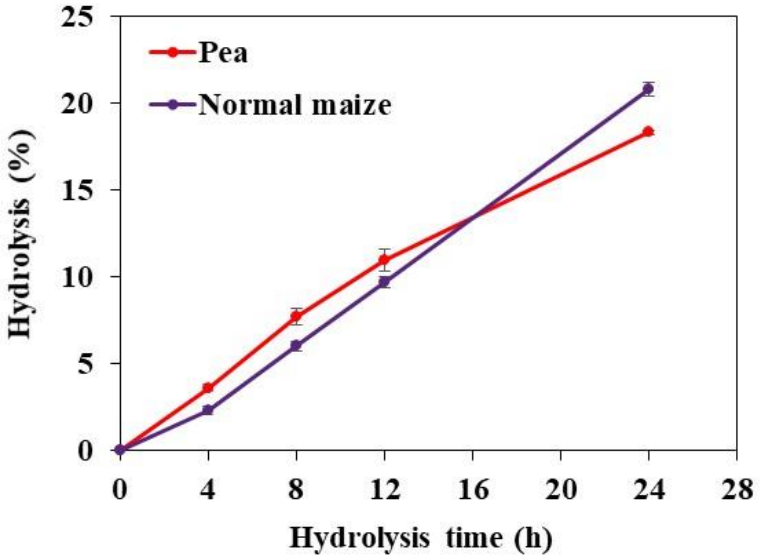


Fig. 3 Hydrolysis rates of native pea and normal maize starches during acid thinning with 0.8 M hydrochloric acid at 50°C. %Hydrolysis = 100 × (total carbohydrate in the supernatant × 0.9) / initial dry mass of starch.

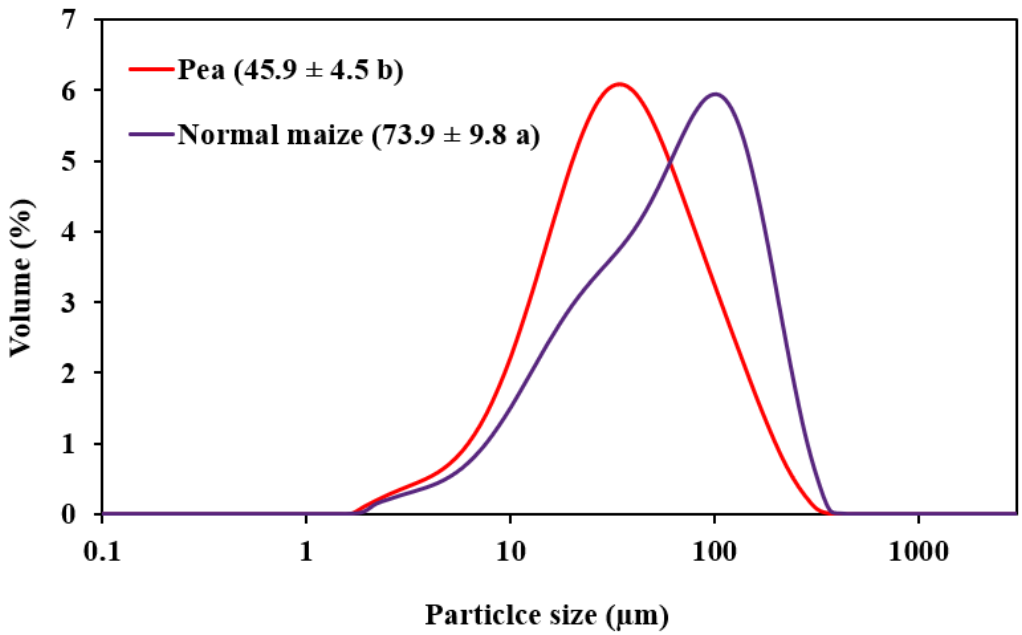


Fig. 4 Particle-size distributions of pea and normal maize RS3. The volume weighted mean particle size (D [4,3]) is given in parentheses. The two numbers are significantly different at $p < 0.05$.

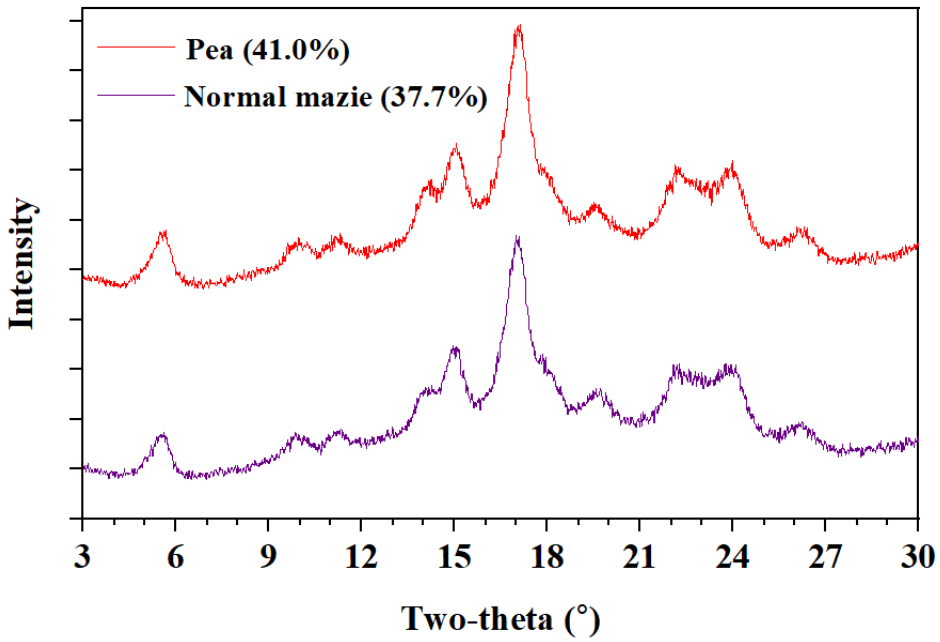


Fig. 5 X-ray diffraction patterns of pea and normal maize RS3. Percentage crystallinity is indicated in parentheses.

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