Generation and Characterization of Pyrodextrins and Maltodextrins from Pea and Maize Starches

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Abstract

Peas are largely grown for their protein component to produce pea protein concentrate and isolate. After the protein extraction, pea starch is generated as the main co-product. Thus, there is substantial untapped potential in the development of value-added products from pea starch, the leading component in pea grains. The low cost, in combination with the underutilization status of pea starch, fosters research efforts in transforming this polysaccharide into higher-value products, which will allow for increasing growth in this relatively overlooked market. Such development will enable the utilization of the starch in a wider variety of products and provide additional options for valuable health benefits such as increased dietary fiber. Dietary fiber is a high-value food ingredient as it is well documented to have numerous positive effects on health, such as aiding in weight management, glycemic and insulinemic control, and improvement of colon health. One good example of dietary fiber that can be derived from pea starch is pyrodextrin. Similarly, the production of maltodextrin from pea starch will aid in the development of new products because pea-based maltodextrin is expected to have different properties as compared to the more common maize-based maltodextrins. These different properties will not only create new applications for pea starch but also aid in further development of new applications in a large variety of sectors.

For the first study, pea, waxy maize, and normal maize starches were stirred with hydrochloric acid at a pH of 3.0 for 30 min, dewatered, and dried at 40°C to reach a moisture content of 15.0% ± 2.5%. The samples were then heated at 180°C for 1, 2, or 4 h, followed by equilibration under atmospheric conditions and milling with a 0.5-mm sieve. The pea-, waxy maize-, and normal maize-based pyrodextrins had a decrease in amylose content of 35.1% to 1.1%, 2.3% to 0.7%, and 27.7% to 10.8%, respectively. The samples also had an increase in water solubility of 1.1% to 94.1%, 0.2% to 40.7%, and 0.1% to 39.9%, respectively. Additionally, the modification increased the enzymatic resistance of pyrodextrin. The pea-based pyrodextrin had approximately 30% enzymatic digestion resistance, while the normal maize- and waxy maize-based pyrodextrins only had around 10%. Overall, the pea-based pyrodextrin showed greater changes across the tested properties, indicating that pea starch is a better substrate for pyrodextrinization than maize starches.

For the second study, the same pea, waxy maize, and normal maize starches were hydrolyzed using α-amylase with and without ultrasonication to determine if the application of ultrasonication during the enzyme reaction resulted in a greater degree of enzymatic hydrolysis and changes to the

physicochemical properties of the derived maltodextrins. Results showed the enzyme activity was minimally impacted by the addition of ultrasonication. The pea-, waxy maize-, and normal maize-based maltodextrins all had minimal change to their molecular-weight distributions with the addition of ultrasonication. However, notable change was observed in the water solubility of pea-based maltodextrins, while the waxy maize- and normal maize-based samples both showed minimal change in water solubility at room temperature. The transmittance of light through the samples in aqueous medium was also affected by ultrasonication. The pea-based and normal maize-based maltodextrins showed a notable increase in the transmittance while minimal change was observed in the waxy maize-based samples. Overall, the ultrasonication during the α-amylase hydrolysis of pea, waxy maize, and normal maize starches led to notable effects on the physicochemical properties of the produced maltodextrins.

The new findings from this thesis research will be useful for the production of pea-based pyrodextrin and maltodextrin that will have diverse applications in the food system. The gained new knowledge and technologies will enable the pulse processing industry to identify new markets for this main component in pulse grains.

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List of Abbreviations

a^*	Red-green color
b^*	Blue-yellow color
d.b.	Dry basis
DE	Dextrose equivalent
DI water	Deionized water
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DP	Degree of polymerization
DSC	Differential scanning calorimetry
^{1}H NMR	Proton nuclear magnetic resonance
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
L^*	Lightness
pHBH	4-hydroxybenzoic acid hydrazide
RI	Refractive index
T_c	Conclusion temperature
T_o	Onset temperature
T_p	Peak Temperature
U	Enzyme activity unit
w/w	weight/weight
ΔE	Color change
ΔH	Enthalpy change

1 PROJECT OVERVIEW

1.1 Summary

1.1.1 *Study 1 – Development and characterization of pyrodextrins from starches of various botanical origins*

Pyrodextrins are starch derivatives prepared using a combination of heat and acid and are characterized by their higher solubility in cold water, increased dietary fiber content, and transmittance of light in aqueous medium as compared to native starch (Bai *et al*., 2014; Bai & Shi, 2016; Stephen *et al*., 2006). These properties can lead to diverse applications of pyrodextrins in food and beverage products. Food applications of pyrodextrins include binders in confectionaries and coatings in baked goods (Inada *et al*., 1994). In beverages, pyrodextrins are used for the encapsulation of water-insoluble flavorings and oils (Laurentin *et al*., 2003; Stephen *et al*., 2006). In addition to these applications, pyrodextrins can also provide health benefits due to their high dietary fiber content, which makes them a suitable ingredient for producing healthier foods by boosting the fiber level or by replacing fat (Stephen *et al*., 2006). The resistance to enzymatic digestion in combination with their distinct functional properties renders pyrodextrins a valuable starch-based ingredient of significant interest.

Results from the literature show that starches from various botanical sources, such as waxy maize, normal maize, and tapioca, have been modified via the pyrodextrinization process. The modification results in several significant changes to the resulting pyrodextrin, including a reduction of apparent amylose level, an increase in water solubility, and an increase in resistance to enzymatic digestion (Bai *et al*., 2014; Lei *et al*., 2020; Weil *et al*., 2020, 2021). However, such modification has not been performed on pea starch as the substrate. The utilization of pea starch for the pyrodextrinization reaction is of interest as pea starch has several distinct functional properties as compared to the more widely used maize and tapioca starches for this purpose. For instance, the amylose content of pea starch has been documented to be between 33% and 49%, higher than those of normal and waxy maize starches: approximately 30% and 2%, respectively (Fredriksson *et al.*, 1998; J. Li *et al.*, 2021; Shen *et al.*, 2016). This high concentration of amylose in pea starch is anticipated to accelerate the pyrodextrinization process and facilitate the formation

of new glycosidic bonds between amylose and amylopectin chains since amylose is known to be located in amorphous regions of pea starch granules (Han *et al.*, 2018; H. Li *et al.*, 2020; Ren *et al.*, 2021). Furthermore, previous research has shown that pea starch has lower gelatinization temperatures than normal and waxy maize starches (L. Li *et al*., 2019), which may facilitate acid hydrolysis of pea starch in the beginning and subsequent reactions (*e.g.*, transglycosylation and repolymerization) during pyrodextrinization.

Another advantage of pea starch is that it naturally has a lower level of endogenous lipids than maize starch (Doublier, 1987). The lower lipid content results in a change to the granule structure and decreased rheological cohesion of pea starch (Jane, 2009; Lee & Choi, 2021; Pérez *et al*., 2009). In addition, lipid oxidation and subsequent development of associated off-flavors will not be a major concern of pea starch-based pyrodextrin, a favorable property for food applications. It has been reported previously that oxidation of endogenous lipids in cereal starches (*e.g.*, maize and wheat) can lead to rancidity problem in maltodextrin, which shares some similar features with pyrodextrin and will be investigated in Study 2 (McPherson & Seib, 1997). Therefore, it was hypothesized that pea starch will achieve a greater level of modification via the pyrodextrinization process and produce pyrodextrin with more desirable functional attributes when compared with normal or waxy maize starches. This hypothesis was tested in Study 1 through the pyrodextrinization of pea, waxy maize, and normal maize starches under the same conditions. The variables for the study included the botanical source of starch and heating time. These factors were determined to be the key variables for pyrodextrinization based on the preliminary research. The pyrodextrins were characterized using a variety of techniques including amylose content determination, color analysis, scanning electron microscopy (SEM), high performance liquid chromatography, gelatinization and retrogradation properties by differential scanning calorimetry, water solubility testing, transmittance of light measurement and *in vitro* digestibility.

1.1.2 *Study 2 – Development and characterization of maltodextrins generated from simultaneous application of enzymatic hydrolysis and ultrasonication*

Maltodextrin is a starch hydrolysate with a dextrose equivalent (DE) less than 20 produced using either acid or amylolytic enzymes (Hobbs, 2009). The hydrolysis of glycosidic linkages by either acid or enzymes increases the DE and significantly changes the physicochemical properties

of the resultant maltodextrin (Robyt, 2009). Maltodextrins are used in a variety of food products, such as beverages, snack foods, frozen meals, and meal replacement drinks, where native starch ingredients cannot meet the requirements (BeMiller & Whistler, 2009; Gregorio *et al*., 2016; Sajilata & Singhal, 2005).

The use of ultrasonication during enzymatic modifications of starch has been demonstrated to enhance the effectiveness of enzymatic reactions with starch. This synergistic effect has been shown across different classes of enzymes as well as different types of starches (Hao *et al*., 2013; Hu *et al*., 2013; Lu *et al*., 2018; Oliveira *et al*., 2018; Singh *et al*., 2015; Szabo & Csiszar, 2017; D. Wang *et al*., 2017). It has been reported in the literature that the addition of intermittent ultrasonication to the reaction of debranching pullulanase with pea starch enhanced the activity of the enzyme, thus increasing the slowly digestible starch and resistant starch contents of pea starch to a greater extent as compared to the treatment with pullulanase alone (Lu *et al*., 2018). In a different study, the hydrolytic activity of α-amylase was improved with the incorporation of ultrasonication at pH 7.0 and temperatures in the range of 20°C to 80°C (Gaquere-Parker *et al*., 2018).

Compared with starches from other botanical sources, such as waxy maize, tapioca, and potato, pea starch is not as commonly utilized to produce maltodextrin. One potential reason is that some physicochemical properties of pea starch can bring technical challenges for the enzymatic conversion to maltodextrin. More specifically, the high amylose content, high final viscosity after pasting, and strong gelling ability of pea starch can impede hydrolysis by α-amylase and other amylolytic enzymes (L. Li *et al*., 2019, 2020). Therefore, it is of interest to determine whether the employment of ultrasonication can improve enzymatic conversion of pea starch to maltodextrin.

It is herein proposed that the same principle can be applied to thermostable α-amylase to enhance its hydrolysis efficiency for faster enzymatic conversion of starch to maltodextrin. This will allow for the use of less enzyme to achieve the desired degree of hydrolysis as well as potentially result in a different molecular-weight distribution profile of the final product. It was anticipated that the addition of ultrasonication to thermostable α-amylase hydrolysis will break down the granular and molecular structures of pea starch at a faster rate, thus leading to maltodextrin with a higher DE, higher water solubility, and transmittance of light and lower viscosity in water. As two important commercial starches with distinct functional properties, waxy

and normal maize starches were also included for comparison in Study 2. Pea, waxy maize, and normal maize starches were subjected to the same combined treatment of thermostable α-amylase hydrolysis and ultrasonication to produce maltodextrin products, the functional attributes of which were characterized and compared.

1.2 Hypotheses

1.2.1 *Study 1 – Development and characterization of pyrodextrins from starches of various botanical sources*

The hypotheses of this study were:

(1) Pea starch will have a faster reaction rate during pyrodextrinization than waxy and normal maize starches because of the higher amylose content and lower gelatinization temperature of the former.

(2) Under the same modification conditions, pea starch-based pyrodextrin will have higher water solubility and light transmittance, slower retrogradation rate, and greater enzymatic resistance in comparison with pyrodextrins derived from waxy and normal maize starches.

1.2.2 *Study 2 – Development and characterization of maltodextrins generated from simultaneous application of enzymatic hydrolysis and ultrasonication*

The hypotheses of this study were:

(1) For the same starch type, the maltodextrin prepared using α-amylase hydrolysis jointly with ultrasonication will show a higher dextrose equivalent (DE) than the counterpart prepared using α-amylase hydrolysis alone; the former will also have a lower viscosity but higher water solubility and transmittance of light than the latter.

(2) Under the same processing conditions, pea starch-based maltodextrin will show a lower DE, water solubility, and transmittance of light but a higher viscosity than those from waxy and normal maize starches.

1.3 Objectives

1.3.1 *Study 1 - Development and characterization of pyrodextrins from starches of various botanical sources*

The objectives of this study were to:

- Produce pyrodextrins from pea, waxy maize, and normal maize starches after pyrodextrinization for 1, 2, and 4 h;
- Perform physicochemical and nutritional characterization of the pyrodextrins generated from the three starches.

1.3.2 *Study 2 – Development and characterization of maltodextrins generated from simultaneous application of enzymatic hydrolysis and ultrasonication*

The objectives of this study were to:

- Produce maltodextrins from pea, waxy maize, and normal maize starches using thermostable α-amylase hydrolysis with and without simultaneous ultrasonication;
- Determine the physicochemical characterization of the maltodextrins produced from the three starches using both methods.

1.3.3 *Overall objectives*

The overall objectives of this study were to:

- Develop methods for value-added modifications to pea starch.
- Demonstrate the value of modified pea starch.
- Illustrate the advantages of pea starch relative to waxy and normal maize starches.

1.4 Organization of thesis

This thesis is organized into eight parts. Part one provides an overview of the thesis. Part two is the literature review and covers the background information of the research and what has been reported in the existing literature. Parts three and four focus on the two studies completed. In the first study, presented in part three, the development and characterization of pyrodextrins from various botanical sources is reported. In part four, the development and characterization of maltodextrins generated from the simultaneous application of enzymatic hydrolysis and ultrasonication is reported. Part five provides a general discussion of the results and how the two studies relate to one another. Part six discusses avenues for future research to expand upon the work reported herein. Part seven is the references used to create the thesis. Part eight is an appendix that contains the supplementary material.

2 LITERATURE SURVEY

2.1 Pea production in Canada

From 2010 to 2019, the average annual production of peas in western Canada was approximately 3.66 million tonnes (N. Wang, 2020). The mean protein content of peas grown in western Canada in 2020 was 22.9%, while the mean starch content over the same period was 47.0% (N. Wang, 2020). There are numerous varieties of peas grown commercially, which are broadly classified into green peas, yellow peas, and specialty market classes (*e.g.*, Vienna, Austrian, dun peas) (Canadian Grain Commission, 2023). The most common types of peas grown commercially are yellow and green peas (Alberta Pulse Growers, 2023), and yellow peas are more commonly used to produce flour, starch, protein, and other ingredients (Wu *et al.*, 2023).

2.2 Why was pea starch used for the thesis research?

Currently, peas are primarily grown for their protein component to produce pea protein concentrate and isolate. After the protein extraction, pea starch is generated as the main co-product. In the agri-food sector, this co-product has limited utilization and a low value addition, such as use in animal feeds (Drewnowski, 2010; Shelepina, 2020). Thus, there is substantial untapped potential in the development of value-added products from pea starch, the leading component in pea grains. The low cost in combination with the underutilization status of pea starch fosters research efforts in transforming this polysaccharide into high-value products, which will allow for increasing growth in this relatively overlooked market. Such development will enable the utilization of the starch in a wider variety of products and provide additional options for valuable health benefits such as increased dietary fiber. Dietary fiber is a high-value food ingredient as it is well documented to have numerous positive effects on health, such as aiding in weight management, glycemic and insulinemic control, and improvement of colon health (Cao *et al.*, 2018; J. Chen *et al.*, 2020; Kaur *et al.*, 2020). One good example of dietary fiber that can be derived from pea starch is pyrodextrin. Similarly, the production of maltodextrin from pea starch will aid in the development of new products. Pea-based maltodextrin is expected to have alternative properties

as compared to the more common maize-based maltodextrins. These different properties will not only create new applications for pea starch but also aid in further development of new applications in a large variety of sectors.

2.3 Structure of starch

Starch is a polysaccharide synthesized by higher plants for energy storage (Eliasson, 2006). While produced in all plants to some level, certain plants have more starch than others, with many of these plants being bred and cultivated for food production such as maize, pea, wheat and tapioca (BeMiller & Whistler, 2009; Stephen *et al*., 2006). While the structures of starch granules vary among different botanical origins, starch consists of amylose and amylopectin as the main components. Amylose is an essentially linear chain of D-glucose molecules connected by α-1,4-linkages (**Figure 2.1**), whereas amylopectin is a highly "branched" molecule of D-glucose molecules with approximately 5% α-1,6-linkages (**Figure 2.2**) (Cornejo-Ramírez *et al*., 2018; Jane, 2009; Pérez *et al*., 2009). The branched chains of amylopectin contain α-1,4-linkages, and the chains are connected to each other through α-1,6-linkages. In waxy and normal starches (containing < 40% amylose), the adjacent branch chains of amylopectin form double helixes that contribute to the crystalline structure of starch granules, while amylose is mainly present in amorphous regions of granules (**Figure 2.3**) (Gallant *et al*., 1997). Starches from various botanical sources have different ratios of amylose to amylopectin, which affect their granular structure, functional properties, and enzymatic digestion (Srichuwong *et al*., 2005; X. Wang *et al*., 2022).

Double-helical crystallites in native starch granules are organized into different patterns as reflected by X-ray diffraction patterns. There are three common starch crystalline structures known as A-, B-, and C-type (Dome *et al*., 2020; Nakamura, 2015; V. Singh *et al*., 2006). A-type crystalline structure has a monoclinic packing pattern with the double helices tightly packed next to each other (**Figure 2.4A**), such as in native waxy and normal maize, wheat, rice, and other cereal starches. B-type crystalline structure has a hexagonal packing pattern, where the double-helical structures are organized in a less compact manner (**Figure 2.4B**) (Junejo *et al*., 2022). C-type granules are a mixture of A- and B-type polymorphs, and it has been demonstrated that the B-type polymorphs are located in the interior and surrounded by the A-type polymorphs in the periphery of pea starch granules (Bogracheva *et al*., 1998; Cai *et al*., 2014). Additionally, V-type crystals

can be detected as a minor component in the X-ray diffractograms of certain cereal starches, such as high-amylose maize, which is a packing structure of single-helical amylose-lipid complexes (Cheetham & Tao, 1998).

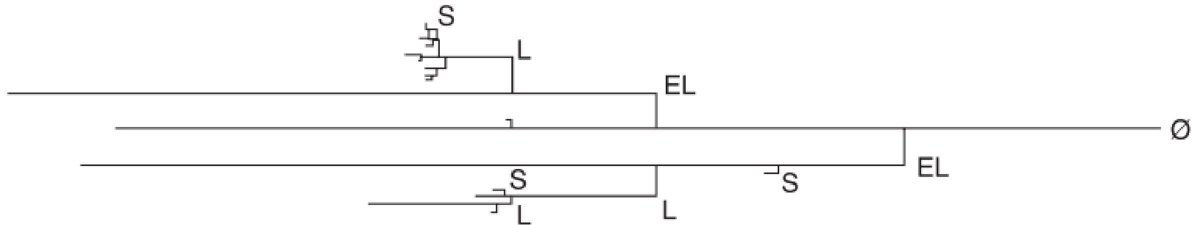


Figure 2.1. Structure of amylose containing extremely long (EL), long (L), and short (S) chains (Takeda *et al*., 1990).

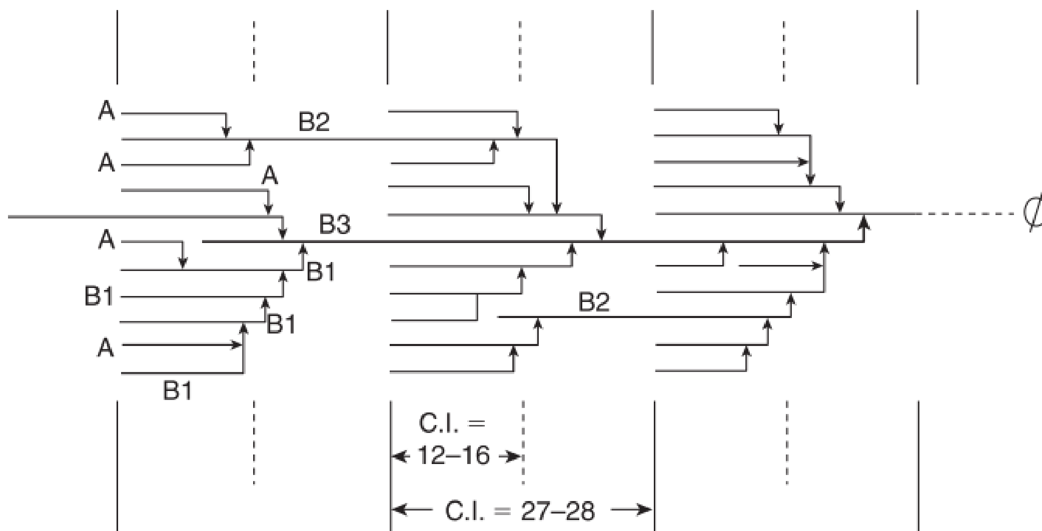


Figure 2.2. Diagram of a section of amylopectin showing the branching pattern of unit α-1-4 chains (A, B1–B3) joined together by α-1,6 linkages (branch points). C.l. = chain length (Manners & Matheson, 1981).

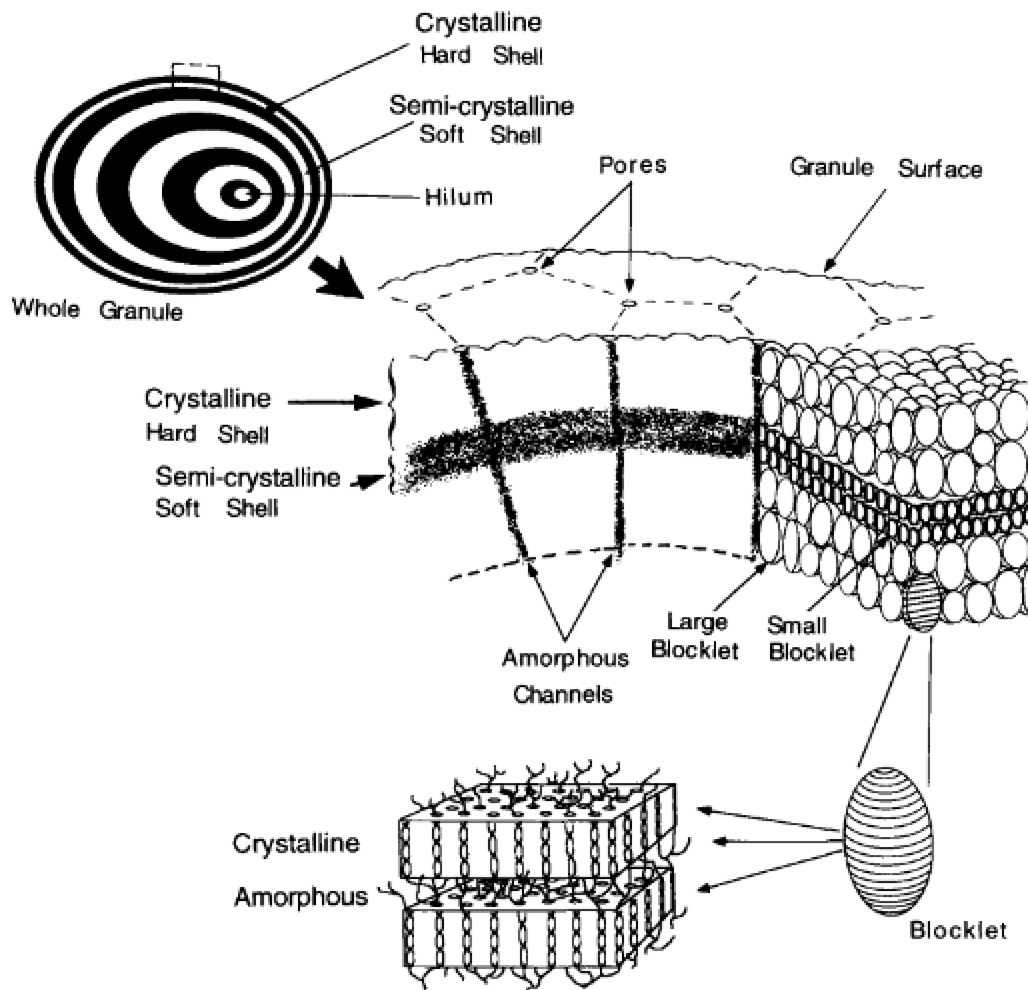
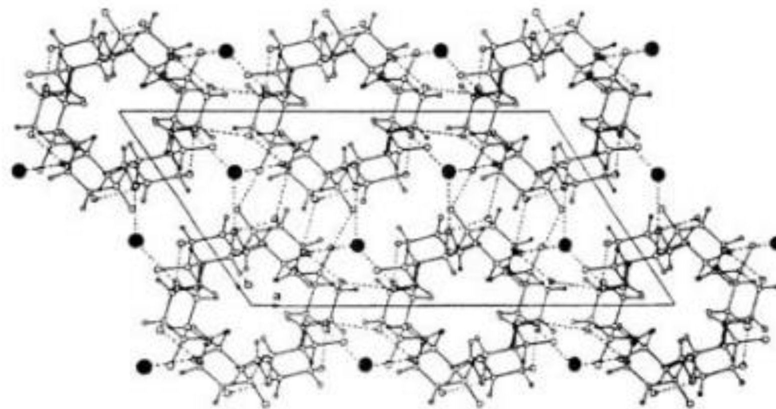


Figure 2.3. Granular structure (upper left) of starch composed of alternating hard crystalline (dark color) and soft semi-crystalline shells (light color). These layers (center) are composed of blocklets with amorphous radial channels that create pores running through them. The individual blocklets (bottom) are composed of crystalline and amorphous lamellae (Gallant *et al*., 1997).



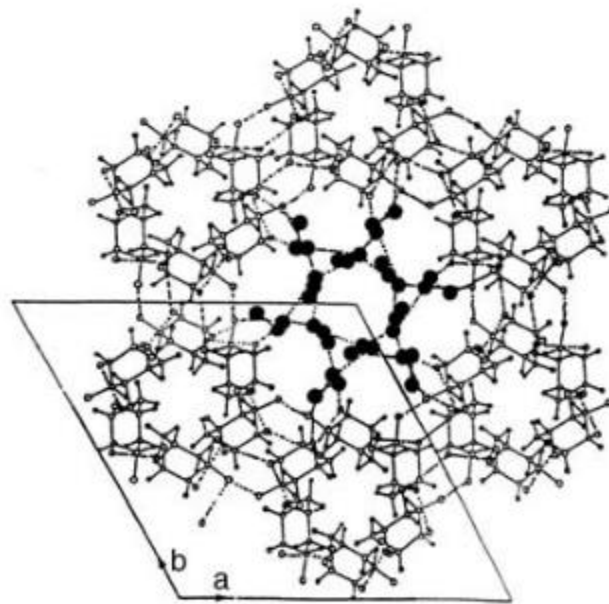


Figure 2.4. A-type (A) and B-type (B) crystalline structures of starch (Imberty & Perez, 1988).

2.4 Differences in starches from various botanical sources

It has been well documented that starches from different botanical sources vary in granular size and amylose content (BeMiller & Whistler, 2009). Waxy and normal maize starches are two of the most commonly used types in the industry. These two types of starches are widely used due to large global production and versatile functionalities (Schwartz & Whistler, 2009). Waxy and normal maize starches generally contain around 0% – 5% and 20% – 30% amylose, respectively (K. Wang *et al*., 2014; S. Wang *et al*., 2014). The high concentration of amylopectin imparts different properties to waxy maize starch, including less iodine binding capacity, reduced syneresis, increased pasting viscosity, and good clarity of cooked paste (Sánchez *et al*., 2010). Compared to waxy and normal maize starches, pea starch has more amylose (33.1% to 48.8%), slightly larger granules, and longer branch chains of amylopectin (BeMiller & Whistler, 2009; L. Li *et al*., 2019; Ratnayake *et al*., 2002; Shen *et al*., 2016). Pea starch has a C-type crystalline structure, while waxy and normal maize starches have an A-type (BeMiller & Whistler, 2009). In terms of functional properties, pea starch has lower gelatinization temperatures, lower peak viscosity but higher final viscosity, greater gel strength, and faster retrogradation than waxy and normal maize starches (L. Li *et al*., 2019; Lu *et al*., 2021).

2.5 Starch modification

Native starch has particular physicochemical properties such as the temperature at which it gelatinizes, viscosity development during pasting, retrogradation during cold storage, and many others that define its applications. However, the intrinsic properties of native starch are sometimes unsuitable for a specific application. The modification of starch allows the alterations of these properties to create ingredients that are better suited to different applications. Chemical, physical, and enzymatic methods have been developed and applied to achieve this purpose (BeMiller & Whistler, 2009; W. Gao *et al*., 2021; L. Guo, 2018; Tang *et al*., 2023). In addition, there are methods, such as pyrodextrinization, that use a combination of chemicals and elevated temperatures to promote the formation of new glycosidic linkages resistant to enzymatic digestion. Such modifications result in significant changes in the physicochemical and nutritional properties of starch. For example, the pyrodextrinization of starch has been shown to increase the dietary fiber content of this carbohydrate (Weil *et al*., 2021).

2.6 Pyrodextrin

Dietary fiber is an important part of the human diet and provides several important health benefits, such as aiding in weight management, glycemic and insulinemic control, and improvement of colon health (Cao *et al*., 2018; J. Chen *et al*., 2020; Kaur *et al*., 2020). One type of dietary fiber currently available in the market is pyrodextrin. Previous research has demonstrated that pyrodextrin can provide several desirable properties that are absent in unmodified starch, including increased dietary fiber content, improved solubility, reduced viscosity, and enhanced cold-storage stability (H. Li *et al*., 2020; Weil *et al*., 2020; Zhu *et al*., 2020). The lower digestibility of pyrodextrin renders it an important value-added ingredient for the use in health-oriented foods and beverages. Potential applications of pyrodextrins include bread, ice cream, candy, salad dressings, and beverages (Inada *et al*., 1994; Ohkuma *et al*., 1997).

Pyrodextrins have been developed from botanical sources such as waxy maize, normal maize, and tapioca starches (Bai *et al*., 2014; Laurentin, 2004; Lin *et al*., 2018; Mao, Li, *et al*., 2021; Weil *et al*., 2020, 2021). The reaction conditions for pyrodextrinization have been largely established, which can be used as a starting point for the determination of the viability of pea starch as a raw material for preparing pyrodextrins. The pyrodextrinization process occurs due to the specific combination of heat and acid (Mason, 2009). Firstly, the acid hydrolyzes the amylose and amylopectin chains in starch, producing shorter starch chains with better molecular mobility. The subsequent heating step in the presence of acid provides the needed energy input for those depolymerized starch chains to form new branched linkages, such as α-1,6, β-1,6, α-1,2, and β-1,2 linkages (**Figure 2.5**) (Bai & Shi, 2016). These new linkages and highly branched structure result in the pyrodextrin having a higher resistance against the digestion in the small intestine, which thus is accepted as a dietary fiber (J. Chen *et al*., 2020; Weil *et al*., 2021).

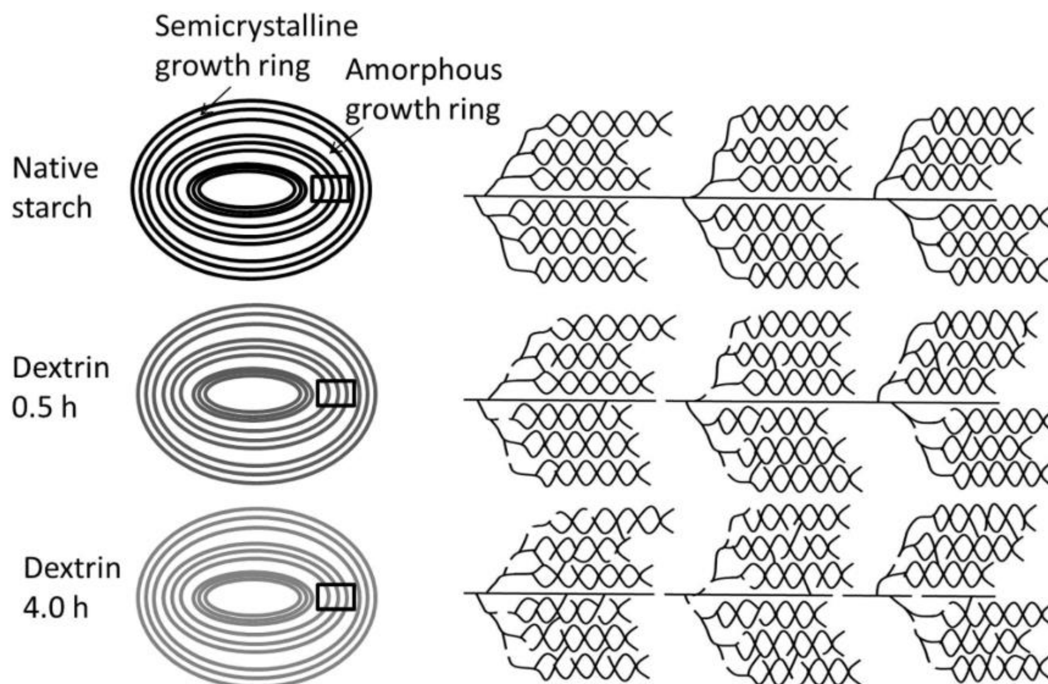


Figure 2.5. Structural changes to starch during pyrodextrinization reaction. The model demonstrates native starch is partially crystalline (left) and shows the cluster model (right) (Bai *et al*., 2014).

The preparation of pyrodextrins from normal and waxy maize starches and the resulting effects on the structures and physicochemical properties have been extensively investigated (Bai *et al.*, 2014; Han *et al.*, 2018; Lei *et al.*, 2020; Lin *et al.*, 2018; Mao, Chen, *et al.*, 2021; Mao, Li, *et al.*, 2021; Sun *et al.*, 2021). By contrast, pea starch has not been utilized as the raw material for producing pyrodextrin in the current literature. Since pea starch has noticeably lower gelatinization temperatures, more amylose, and less endogenous lipids as compared to waxy and normal maize starches (Li *et al*., 2021), it could be more suitable for developing pyrodextrin, which was the main objective of Study 1 of this thesis research.

Furthermore, in Study 1, waxy and normal maize starches were included for comparison. The pyrodextrin samples prepared from the three representative commercial starches were extensively characterized with respect to their structure, functional properties, and nutritional value. The new information will be meaningful for using pea starch to generate pyrodextrins with distinct functional attributes that can expand industrial applications of this soluble dietary fiber, thereby leading to new markets for this underutilized pulse starch.

2.7 Maltodextrin

2.7.1 *Maltodextrin as a starch derivative*

Maltodextrin is a versatile ingredient produced through either enzymatic or acid hydrolysis of starch. The degree of such hydrolysis can be quantified as dextrose equivalent (DE), a measure of the reducing capability of maltodextrin expressed as equivalent to D-dextrose. DE can range from 0 to 100, with a DE of 100 being pure D-dextrose and a DE of 0 being native starch with no determinable reducing capability (Kearsley & Dziedzic, 1995b). To be classified as a maltodextrin, a product typically has a DE of less than 20. If the DE of a product is above 20, it is classified as a syrup rather than a maltodextrin (Food and Drug Administration, 2008).

Maltodextrins are used in a wide range of products, such as snack foods, desserts, beverages, frozen meals, meal replacement drinks, and as fillers in medication. They are used as a flavor carrier, bulking agent and for a multitude of other functions resulting in them being consumed by a large majority of people on a daily basis (BeMiller & Whistler, 2009; Stephen *et al*., 2006). While maltodextrins can theoretically be generated from any starch type, the majority of commercial maltodextrins are produced from maize and potato starches (Stephen *et al*., 2006; Weil *et al*., 2020). Maltodextrins typically are highly soluble in cold water and have a limited sugar content, limited sweetness, and low viscosity when dispersed in water. These properties vary significantly with the specific DE of the maltodextrin (BeMiller & Whistler, 2009; Stephen *et al*., 2006).

The technical definition of dextrose equivalent is the measurement of the content of reducing sugars present in a sample, as represented on a dry basis relative to dextrose (Kearsley & Dziedzic, 1995b). It is calculated according to **Equation 2.1**, where M_w *dextrose* is the molecular weight of dextrose (180 g/mol) and M_n *sample* is the number-average molecular weight of the sample (Corn Refiners Association, 1993; Lane & Eynon, 1934).

$$DE = \frac{M_w\ dextose}{M_n\ sample} * 100 \qquad \textbf{Equation 2.1}$$

Several methods are available for determining the DE of maltodextrins. These methods include osmometry, chromatography, and titration (Akyüz *et al*., 2021; Delheye & Moreels, 1988; Lane & Eynon, 1934; Rong *et al*., 2009). One titration method is the Lane-Eynon method that determines the DE through the reduction of copper (II) to copper (I), specifically Cu^{2+} to Cu_2O, by the aldehyde groups of the reducing sugars in maltodextrin (Lane & Eynon, 1934; Rong *et al*., 2009). The completion of the titration can be determined through the observation of color change in Fehling's reagents. These reagents have a bright blue color initially when there is a surplus of Cu^{2+} ions. As more Cu^{2+} ions are reduced to Cu_2O, the color shifts from blue to red orange. The titration is considered complete once no more blue color remains in the mixture.

Another method for characterizing maltodextrins is through the analysis of the degree of polymerization (DP), typically via chromatography. DP is a measure of the number of D-glucopyranose units of a maltodextrin or starch chain. A sample with DP of 1 is pure D-glucose, while maltose and isomaltose have DP of 2. As the length of the chain increases, the number of branch points and structure of the oligosaccharide becomes more complicated and the determination of an exact structure becomes more difficult (BeMiller & Whistler, 2009). However, even without knowing the exact structure of all the oligosaccharides within a maltodextrin sample, valuable information can be obtained through the determination of the DP profile of one sample because maltodextrins with the same DE can have vastly different DP profiles, which can possess significantly different functional properties, such as sweetness and viscosity (Kapelko-Zeberska *et al*., 2016; Mollan Jr. & Celik, 1996). Therefore, the determination of DP distribution is a valuable tool in the characterization of a maltodextrin.

Since maltodextrins are produced in an aqueous medium, removal of the excess water through proper drying is an important step of the production. The two most common methods are spray drying and lyophilization (BeMiller & Whistler, 2009). Spray drying is a process by which the hot maltodextrin solution is sprayed out at high temperatures and pressures through a small nozzle. This results in the atomization of the sample and the flash evaporation of the water, leaving behind maltodextrin as dry solids (de Melo Ramos *et al*., 2019). This method is commonly used in the production of maltodextrin on an industrial scale due to the more efficient energy usage and the ability for high throughput. One drawback, however, is that a significant quantity of product is typically lost during the process. This is due to the maltodextrin, which is naturally sticky, adhering to the sides of the equipment. Once all the interior sides of the equipment are coated by the dried

maltodextrin, the rate of loss decreases. This makes spray drying a more efficient process for a large scale of maltodextrin production because the initial loss of maltodextrin is insignificant for the whole drying process.

In contrast to spray drying, lyophilization is a process better suited to small-scale processing. Lyophilization is a process where a sample is frozen and then placed in a chamber at very low temperatures (< -40°C) and pressures (< 200 mb), resulting in the sublimation of water from the sample, which leaves behind the solids (Gaidhani *et al*., 2015; Rajeevani *et al*., 2015). This process is more energy intensive than spray drying and therefore is more commonly used on a bench-top scale (de Jesus & Maciel Filho, 2014; F. Gao *et al*., 2013). One advantage of this method is the high sample recovery rate (Koksel *et al*., 2008). A drawback of this approach is the potential for the formation of retrograded starch. Retrogradation is a process where adjacent maltodextrin chains re-form double helices and precipitate from the water phase, which is commonly observed as a white cloudiness in the dispersion (B. Zhang *et al*., 2014). Samples with a lower DE (*i.e.*, a higher average DP) are more prone to retrogradation (Fredriksson *et al*., 1998; S. Wang *et al*., 2015). However, this process can be counteracted through the flash-freezing of a maltodextrin sample in water with liquid nitrogen. The flash freezing process converts the sample from a liquid state into a solid state instantly, which minimizes the retrogradation of maltodextrin chains during the subsequent freeze drying (de Jesus & Maciel Filho, 2014). Due to the hygroscopic property of glucose and other low-DP oligosaccharides, lyophilization does not work well with high-DE samples (DE > 15) (Gaidhani *et al*., 2015; Rajeevani *et al*., 2015). Lyophilization of a high-DE sample often produces a concentrated syrup or hard "candy" crystals. This can be counteracted to a certain degree by drying high-DE samples at a relatively low content of solids (Gabarra & Hartel, 1998; Salfo *et al*., 2018). Therefore, it is important to consider the DE and DP profile of a maltodextrin product before lyophilization.

2.7.2 *Enzymatic modification of starch*

With an increasing demand for clean-label starches from the food industry, enzymatic modification of starch, where starch is modified using starch-acting enzymes to break down or alter the structure of amylose and amylopectin in starch granules, has attracted growing interest. There are several different classes of starch-acting enzymes, including hydrolyzing α-amylases and β-amylases, debranching pullulanases and isoamylases, transferases, and branching enzymes

(Robyt, 2009). These enzymes have different functionalities and modify physicochemical properties of starch following their reaction patterns.

Two types of enzymes, α-amylases and amyloglucosidases, break down the α-1,4 glycosidic linkages in amylose and amylopectin chains. Alpha-amylases are endo-acting enzymes, meaning that they attach and cleave internal α-1,4 glycosidic linkages of amylose and amylopectin molecules (**Figure 2.6**) (Purich & Allison, 2002). The enzyme attacks at one of these linkages and cleaves it, resulting in the production of two or multiple smaller oligosaccharide chains. This mechanism results in a rapid decrease in viscosity and average DP as well as increase in solubility and DE.

Enzymatic hydrolysis of starch leads to a lower average molecule weight of the sample and converts the long linear amylose chains and branched amylopectin chains into shorter oligosaccharides. These smaller molecules can be more easily solubilized in water, which results in the hydrolyzed starch having decreased viscosity, increased solubility, and increased clarity after dispersed in water (Pérez *et al*., 2009). The degree of hydrolysis determines the extent of change in the properties of starch. The degree of hydrolysis can be measured as DE as well as by analyzing the DP profile as indicated above.

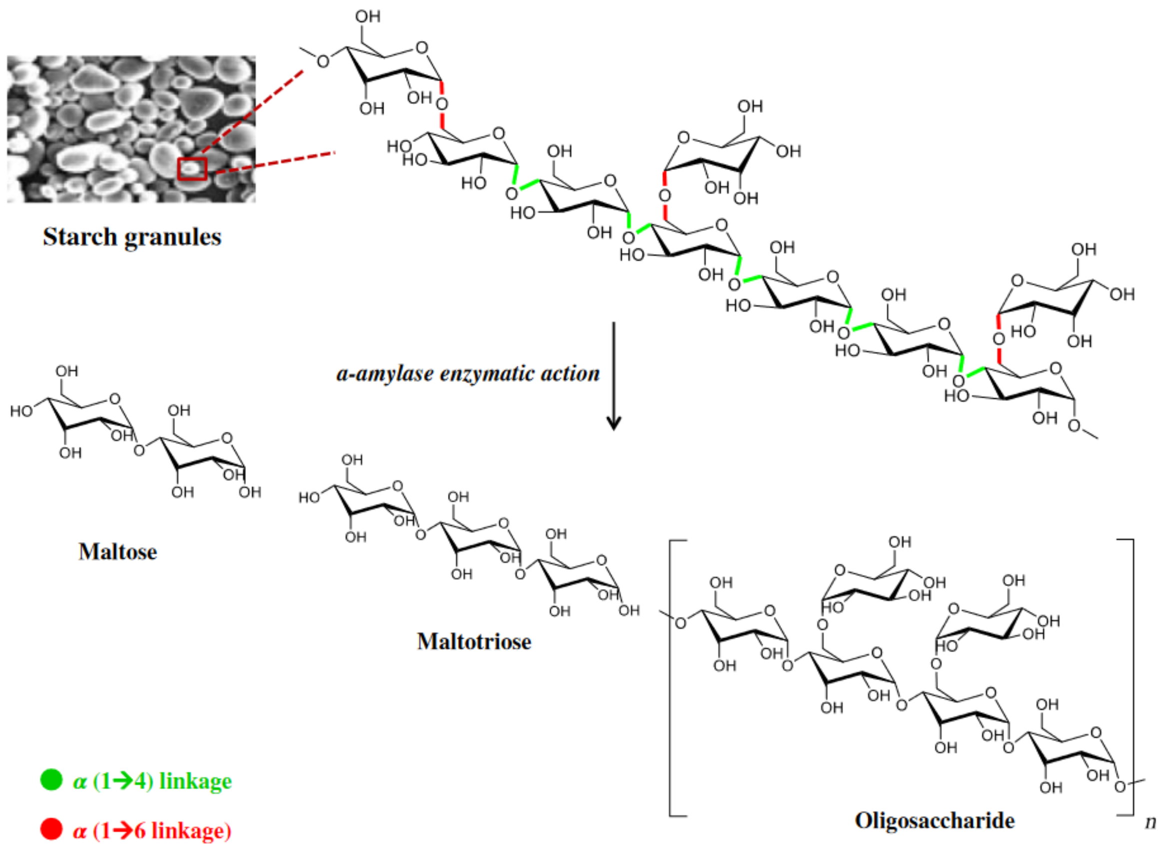


Figure 2.6. Production of oligosaccharides via α-amylase hydrolysis of starch. The α-amylase enzyme hydrolyzes the α-1,4 linkages breaking it into smaller molecules (Visvanathan *et al.*, 2020).

2.8 Use of ultrasonication to modify starch

Ultrasonication is the use of ultrasonic waves (typically >18 kHz in frequency) to agitate a solution (Bermudez-Aguirre, 2017; Jambrak *et al.*, 2010). Ultrasonication has been applied to many different applications, including treatment of biomass, removal of rust, and preparation of emulsions (Cheah *et al*., 2022; Ghamartale *et al*., 2021; Kassim *et al*., 2022). Ultrasonication has also been used in the food industry, particularly within the field of grain science, to alter the physicochemical properties of starch (Rahaman *et al*., 2021). This treatment on starch causes the disruption of granular structure and partial gelatinization (Hu *et al.*, 2013). The disruption of starch granules is attributed to cavitation. Cavitation occurs when the static pressure of a liquid is reduced to less than the vapor pressure of the liquid, which is present as vapor-filled microbubbles from the pressure waves in the ultrasonicator. These bubbles quickly collapse due to the surrounding pressure of the liquid, thereby creating shock waves that damage starch granules and depolymerize amylose and amylopectin molecules (**Figures 2.7 and 2.8**) (Czechowska-Biskup *et al*., 2005; Fuchs, 2015; Rahaman *et al*., 2021; Sreedhar *et al*., 2017; Vernès *et al*., 2020). This leads to changes in the functional properties of starch, including enhanced oil absorption, increased viscosity, and greater susceptibility to saccharification (Bonto *et al*., 2021; Karwasra *et al*., 2020).

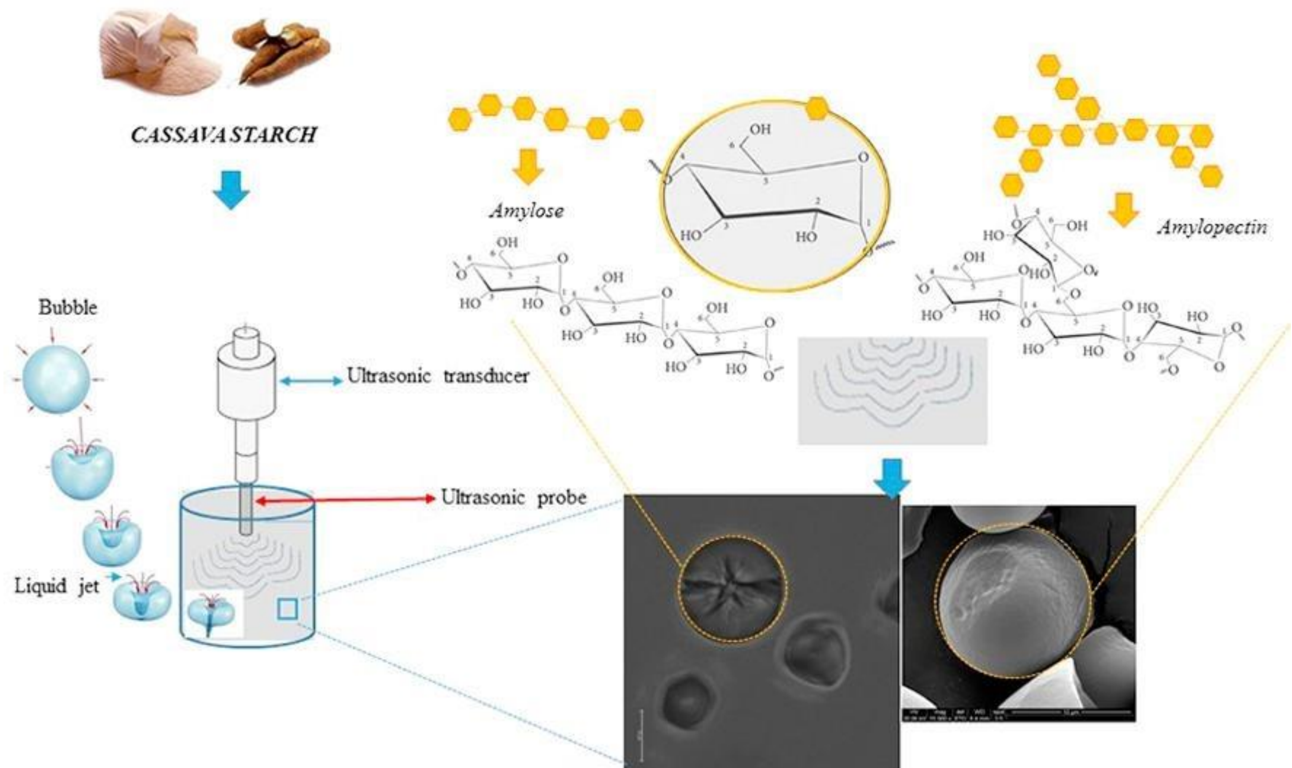


Figure 2.7. A diagram illustrating ultrasonic modification of cassava starch. Ultrasonic waves produced by the transducer form cavitation bubbles that damage the amylose and amylopectin molecules resulting in damage to the starch granules (bottom right images) (Monroy *et al*., 2018).

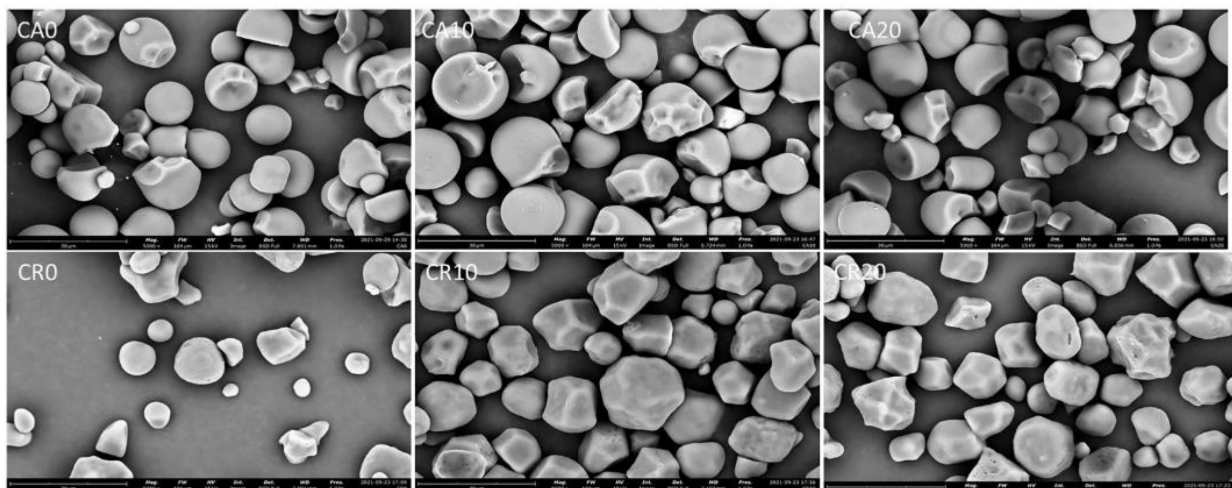


Figure 2.8. Scanning electron microscopy (SEM) images of normal maize (CR) and cassava (CA) starches treated with ultrasound for 0, 10 or 20 min. As the treatment time increased, grooves and notches formed on the granules due to the ultrasonic treatment (Rahaman *et al*., 2021).

An ultrasonic pulse at a frequency of 20 kHz has a wavelength of 7.715 cm in boiling water and of 7.405 cm in water at room temperature. This means that the wavelength of the ultrasonic pulse is substantially greater than the sizes of both starch and starch-acting enzymes, meaning that they cannot be modified directly by the ultrasonic pulse. This is because the smaller objects are unaffected by the frequency as the waves are unable to vibrate the material due to the lack of mass. Thus, the modification effect of ultrasonication on starch comes from the formation of cavitation bubbles within the water (Herceg *et al*., 2010). Although the same effect is anticipated to inactivate enzymes in solution due to the high temperature and pressure of the cavitation, it has been demonstrated to enhance enzymatic reactions with starch instead, with minimal inactivation from the presence of ultrasonic waves (Herceg *et al*., 2010; Lu *et al*., 2018; X. Yu *et al*., 2018).

While ultrasonication alone is very effective in altering starch properties, this physical modification method has also been applied in combination with starch-acting enzymes to enhance their activities. Research has shown that the application of intermittent ultrasonication increased the rate of debranching of pea starch by pullulanase (Hu *et al.*, 2013; *Lu et al.*, 2018). The authors proposed that the enhanced enzyme activity was attributed to various potential factors, including the thermal effects from ultrasonication, increased binding of the enzyme with starch substrate as a result of decreasing local viscosity of the starch dispersion, and/or physical effects such as bubbles or additional shear induced by the ultrasonic waves (Gaquere-Parker *et al*., 2018; Lu *et al*., 2018). Moreover, it has been reported that the hydrolysis of potato starch by α-amylase increased with intermittent ultrasonication (Oliveira *et al*., 2018).

Based on the current understanding of both modification methods, the ultrasonication will be combined with α-amylase hydrolysis to prepare maltodextrins from pea, waxy maize, and normal maize starches in Study 2. This new method is particularly important for effective amylolysis of pea starch because this pulse starch possesses a strong gelling ability, which hinders enzymatic breakdown of the molecules at a high starch concentration (L. Li *et al*., 2020). It is anticipated that the combined modification method can lead to molecular structures and functional properties of maltodextrins distinctively different from those of counterparts prepared using enzymatic hydrolysis alone.

2.9 Summary

Pea starch is an underutilized and low-cost source of starch with physicochemical properties that that lend itself well to the development of value-added modifications. Two such modifications are the conversion of the starch to pyrodextrin and maltodextrin. Pyrodextrin is a dietary fiber produced through the simultaneous application of heat and acid. This process results in the formation of new digestion-resistant linkages that provide the starch-derivative with its digestion resistant properties. This modification results in increased dietary fiber content, improved solubility, reduced viscosity, and enhanced cold-storage stability (H. Li *et al*., 2020; Weil *et al*., 2020; Zhu *et al*., 2020).

Maltodextrin is produced through either the acid or enzymatic hydrolysis of starch. The enzymatic hydrolysis of starch is completed through the utilization of an α-amylase, which hydrolyzes the α-1,4 linkages in amylose and amylopectin. This breaks down the granular starch and results in increased higher water solubility, lower viscosity, and greater sweetness (Plácido Moore *et al*., 2005; Stephen *et al*., 2006; Tsusaki *et al*., 2009). The addition of ultrasonication to the reaction of enzymes with starch has been reported to increase the activity of the enzyme and affect the properties of starch. This results in a more efficient enzymatic reaction thereby both reducing the costs from enzyme use and producing a product with novel physicochemical properties.

3 Development and characterization of pyrodextrins from starches of various botanical origins

3.1 Abstract

Peas are primarily grown for their protein to produce pea protein concentrate and isolate. However, this process results in a substantial fraction of pea starch that remains. This starch is considered a low-value by-product from this process and is primarily used in applications such as animal feeds. Therefore, there is notable interest in the development of value-added modifications to the starch. One such application is pyrodextrinization, a process that increases the water solubility and resistance to enzymatic digestion of the starch. To investigate this, pea, waxy maize, and normal maize starches were slurried in water, followed by pH adjustment to 3.0 using 2.0 M hydrochloric acid solution and magnetic stirring for 30 min. After dewatering and drying at 40°C to reach a moisture content of 15.0% ± 2.5%, the samples were heated at 180°C for 1, 2, or 4 h, followed by equilibration under atmospheric conditions and milling with a 0.5-mm sieve. The pea-, waxy maize-, and normal maize-based pyrodextrins had a decrease in amylose content of 35.1% to 1.1%, 2.3% to 0.7% and 27.7% to 10.8%, respectively. The samples also had an increase in water solubility of 1.1% to 94.1%, 0.2% to 40.7% and 0.1% to 39.9%, respectively. Additionally, the modification increased the enzymatic digestion resistance, which was near zero in the native starches. The pea-based pyrodextrin had approximately 30% enzymatic digestion resistance while the normal maize and waxy maize pyrodextrins only had around 10%. Overall, the pea-based pyrodextrin showed greater change across the tested properties, indicating that pea starch could be a better substrate for pyrodextrinization than maize starches.

3.2 Introduction

Starch is one of the most common plant-based ingredients in the world, with commercial starches sourced from maize, tapioca, potato, and pea. Despite the wide variety of botanical sources for starches, maize starches dominate the market, particularly in North America (BeMiller & Whistler, 2009; Cornejo-Ramírez *et al*., 2018; Hobbs, 2009). Other types of starches have unique functional properties as compared to maize starches, such as pea starch. These benefits include a lower lipid content and gelatinization temperature as well as a higher amylose content (Drewnowski, 2010; Shelepina, 2020). These unique properties offer new potential uses for applications that more common varieties, such as maize starches, are less suitable for. Despite these benefits and the fact that peas are one of the main crops cultivated in Canada, the plant is largely grown for its protein, and starch is generated as a low-value by-product from the fractionation process (Ren *et al*., 2021; N. Wang, 2023). However, peas only comprise 20% to 25% protein, with 40% to 55% starch as the leading component (N. Wang, 2022, 2023). Currently, pea starch is used in low-value applications, such as a carbohydrate source in animal feeds and a thickener, binder, stabilizer, and gelling agent in human foods (Farshi *et al*., 2023; Montero *et al*., 2017). Therefore, the development of value-added applications for pea starch is of significant interest.

One modification that increases the value of starch is pyrodextrinization. Pyrodextrinization is a process where the starch is first held under acidic conditions followed by the adjustment of the moisture content to ~15% and then heating at an elevated temperature, typically 130-190°C (Laurentin *et al*., 2003; Mao, Li, *et al*., 2021). Along with the hydrolysis of existing linkages, this process results in the formation of new α-1,6, β-1,6, α-1,2, and β-1,2 linkages between starch chains as well as possible repolymerization (Bai & Shi, 2016). These reactions lead to several important changes in the physicochemical properties of the resulting pyrodextrin, including lower amylose content, higher water solubility and clarity in cold water, lower enthalpy change of gelatinization, slower rate of retrogradation, and most notably, reduced enzymatic hydrolysis (*i.e.*, increased dietary fiber content) (Bai *et al*., 2014; Lei *et al*., 2020; Weil *et al*., 2020, 2021). The high dietary fiber content of pyrodextrin has attracted significant research interest because the inclusion of dietary fiber in the human diet has been linked to numerous positive health outcomes, including aiding in weight management, attenuating glycemic and insulinemic responses, and enhancing colon health (Bai *et al.*, 2014; Cao *et al.*, 2018; El-Sayed *et al.*, 2005; Han *et al.*, 2018;

Weil *et al.*, 2020). In addition, the changes in the functional properties render pyrodextrin more suitable for various food applications, particularly beverages, due to its easier incorporation into formulations (Laurentin *et al*., 2003; Stephen *et al*., 2006).

In this study, pea, waxy maize and normal maize starches were treated with acid, adjusted to a moisture content of approximately 15%, and then heated at 180°C to produce pyrodextrins. The physicochemical properties of the native starches and pyrodextrins were characterized using a wide variety of techniques, including apparent amylose content, solubility in water, and *in vitro* digestibility. The results demonstrated that pea starch was more suitable than waxy or normal maize starches for producing pyrodextrins under the tested conditions. The combination of the underutilization status of pea starch and the benefits of pyrodextrinization positions pea starch as the preferred substrate for this modification. The findings from this research will help the pulse industry explore new markets for this abundant and affordable polysaccharide.

3.3 Materials and methods

3.3.1 *Materials*

Pea starch (N-735) was donated by Roquette Canada Ltd. (Winnipeg, MB, Canada). Waxy maize starch (Cargill Amylogel™ 03003) and normal maize starch (Cargill Gel™ 03420) were gifts from Cargill Inc. (Minneapolis, MN, U.S.A.). Potato amylose standard (Megazyme P-AMYL) and maize amylopectin standard (Sigma 10120-250G) used for iodine colorimetry to determine amylose content was purchased from Megazyme International Ltd. (Co. Wicklow, Ireland) and Sigma-Aldrich Canada Co. (Oakville, ON, Canada), respectively. All the other chemicals were purchased from Fisher Scientific Company (Waltham, MA, U.S.A.) or Sigma-Aldrich Canada Co. and were of reagent grade purity or higher.

3.3.2 *Preparation of pyrodextrin*

Pea, waxy maize, and normal maize starches were pyrodextrinized using a method developed from previous publications (Mao, Chen, *et al*., 2021; Weil *et al*., 2020, 2021). The starch was suspended in deionized (DI) water [40.0%, w/w, dry basis (d.b.)], followed by pH adjustment to 3.00 ± 0.05 using 2.0 M hydrochloric acid. After magnetic stirring (~400 rpm) for 30 min at

room temperature, the starch suspension was filtered using a 150-mm Buchner funnel with Whatman #1 filter paper. The filtered pea, waxy maize, and normal maize starches had moisture contents of 36-41%, 48-53%, and 45-50%, respectively. The starch cake was crumbled by hand until no large chunks (> 0.5 cm) remained. The sample was spread evenly in a thin layer on a tray, placed in a forced-air oven (Model FD 56, BINDER Inc., Bohemia, NY, U.S.A.) at 40°C, and dried to a moisture level of 12.5-17.5%. The treated starch was transferred to a glass tray and heated in the same forced-air oven at 180 °C for 1, 2, or 4 h. After cooling to room temperature, the sample was ground using a KitchenAid BCG111OB Blade Coffee Grinder (KitchenAid, Benton Harbor, MI, U.S.A.) and then a mortar and pestle to pass through a 125-µm sieve. The collected sample was equilibrated at room temperature for 7 d prior to subsequent characterization.

3.3.3 *Characterization methods*

3.3.3.1 *Apparent amylose content*

Apparent amylose contents of the native starches and pyrodextrins were determined according to the procedure reported by Chrastil (1987) with modifications (J. Li *et al.*, 2021). Each sample was weighed (0.1000 ± 0.0100 g) into a 15-mL centrifuge tube with a magnetic stir bar and then 600 µL of DI water was added. The sample was then mixed for approximately 10 min for proper hydration. Dimethyl sulfoxide (DMSO; 5.40 mL) was added to the centrifuge tube. The sample was mixed using a Fisher brand Analog Vortex Mixer 02-215-414 (Thermo Fisher Scientific, Waltham, MA, U.S.A.) set to the maximum for around 10 s. The sample was then placed into a boiling water bath for 20 min with mild magnetic stirring to achieve full dispersion. The sample was then removed and cooled to room temperature. An aliquot (2.00 mL) of the sample was transferred to a new 15-mL centrifuge tube and 10 mL of 100% ethanol was added to the new tube, which was vortexed to precipitate the sample. The sample was centrifuged at 3000 *g* for 15 min using an Eppendorf Centrifuge 5810 R (Eppendorf Group, Hamburg, Germany). The supernatant was carefully separated, and the centrifuge tube was placed upside down for 15 min to remove almost all the ethanol in the supernatant. Urea-DMSO solution [0.6 M urea in 90% (v/v) DMSO, 6.00 mL] was added along with a magnetic stir bar. The sample was then mixed using a magnetic stir plate with occasional mixing on a vortex mixer until the pellet was dispersed. The sample was placed in a boiling water bath with magnetic stirring for 30 min to ensure the sample

was fully dissolved. After cooling to room temperature, 100 μL of the sample was transferred to a new centrifuge tube. Trichloroacetic acid solution [0.5% (v/v), 5 mL] was added to the tube, followed by 50.0 μL of 0.01 N iodine-potassium iodide (I_2-KI) solution. After vortexing for 30 s, the mixture was kept at room temperature for 30 min, and the absorbance was measured at 620 nm using a Thermo Scientific Genesys 30 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). A standard curve was prepared using the mixtures of commercial potato amylose and maize amylopectin standards at different ratios: 0.0%, 25.0%, 50.0%, 75.0% and 100.0% amylose.

3.3.3.2 *Color measurement*

Color measurement of the native starches and pyrodextrins was carried out based on the method described by Z. Zhang *et al*. (2005). The sample was added to a transparent plastic petri dish to thoroughly and evenly cover the bottom. The color of the sample was measured using a ColorFlex EZ spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, U.S.A.). Reported parameters included lightness (L^*), red-green color (a^*), and blue-yellow color (b^*), which were used to calculate the total change in color (ΔE) relative to the native starch using **Equation 3.1** (International Color Consortium, 2004).

$$\Delta \boldsymbol{E} = \sqrt{(\boldsymbol{L}^*_{\boldsymbol{native}} - \boldsymbol{L}^*_{\boldsymbol{pyro}})^2 + (\boldsymbol{a}^*_{\boldsymbol{native}} - \boldsymbol{a}^*_{\boldsymbol{pyro}})^2 + (\boldsymbol{b}^*_{\boldsymbol{native}} - \boldsymbol{b}^*_{\boldsymbol{pyro}})^2}$$

Equation 3.1

3.3.3.3 *Scanning electron microscopy*

Imaging of the native starches and pyrodextrins was completed using scanning electron microscopy (SEM) based on the work of L. Li *et al*. (2020). Briefly, the sample was sprinkled onto a double-sided tape atop an aluminum stub and then sputter coated with gold. The prepared sample was viewed using a field-emission SEM under the conditions of 3.0 kV acceleration voltage and

10 μA probe current (SEM SU8000, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada). Representative SEM images were captured at different magnifications.

3.3.3.4 *Molecular-weight distribution*

The sample (~20 mg) was added to a 15-mL centrifuge tube, followed by the addition of 5 mL of dimethyl sulfoxide (DMSO) with 0.5% LiBr (w/w) to disperse the sample at a concentration of 5 mg/mL. The sample was then placed in a boiling water bath for 10 min with gentle magnetic stirring. After cooling to room temperature, the sample was filtered through a 5-µm disk filter and loaded onto the HPSEC-RI auto-sampler tray. The sample was run using 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). Both the column compartment and RID temperatures were set to 50°C ± 0.1°C. The flow rate of the eluent for the HPSEC-RI was 0.5 mL/min and an injection volume was 20 μL. DMSO containing 0.5% LiBr was used as the eluent. Standards of glucose, maltotriose, maltohexose, pullulan (average molecular weight = 6,200 g/mol), pullulan (48,800 g/mol), and pullulan (348,000 g/mol) were run at a concentration of 5 mg/mL to calibrate the system. Only the 4-h pyrodextrins were characterized due to the other samples having poor solubility and molecular weights beyond the separation capacity of the system. RI data were collected and then normalized based on the total area under the curve (retention time from 9 to 19.5 min).

3.3.3.5 *Gelatinization and retrogradation properties*

Differential scanning calorimetry of the native starches and pyrodextrins was performed according to the method reported in previous literature (Yuan & Ai, 2022). Each sample (~10 mg) was weighed into a stainless-steel sample pan, followed by the addition of three-fold weight of distilled water to homogeneously wet the powder. The pan was hermetically sealed, and the starch-water mixture was allowed to equilibrate for at least 2 h. The sample was analyzed using a PerkinElmer Differential Scanning Calorimeter 8000 with Intracooler 2 chiller (Perkin Elmer, Woodbridge, ON, Canada). For measurement of gelatinization properties, the sample was scanned at a rate of 10°C/min from 10 to 150°C. For the measurement of retrogradation properties, following the gelatinization scan, the sample was stored at 4°C for 7 d. The sample was then scanned at a rate of 10°C/min from 10 to 150°C. The parameters of the detectable transitions,

including onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy change (ΔH), were calculated.

3.3.3.6 *Water solubility*

Water solubility of the native starches and pyrodextrins was determined based on the method described by Han *et al*. (2018). The sample (1.0000 ± 0.1000 g) was weighed into a 15-mL centrifuge tube and 9.00 mL of DI water was added to the sample tube. The tube was mixed on a Fisher brand Analog Vortex Mixer 02-215-414 (Thermo Fisher Scientific, Waltham, MA, U.S.A.) set to the maximum for 1 min. The sample was then centrifuged at 3,220 g for 15 min using an Eppendorf Centrifuge 5810 R (Eppendorf Group, Hamburg, Germany). All the supernatant was carefully transferred to a weighing pan. The supernatant was dried overnight at 40°C, followed by 110°C for 3 h. The water solubility was calculated according to **Equation 3.2**.

$$\boldsymbol{Water\ solubility}\ (\%) = \frac{\boldsymbol{Total\ weight\ of\ pan\ and\ dried\ solids\ (g) - Weight\ of\ pan\ (g)}}{\boldsymbol{Initial\ dry\ weight\ of\ sample\ (g)}} * \boldsymbol{100\%}$$

Equation 3.2

3.3.3.7 *Transmittance*

The transmittance of light of the native starches and pyrodextrins in water was measured. The sample was added to DI water to reach a total weight of 30.0 g containing 2.0% (w/w) dry solids in a 50-mL centrifuge tube containing a magnetic stir bar. For the measurement without cooking, the tube was placed in a 25°C water bath with shaking at 160 rpm for 20 min, after which the transmittance of was measured at 640 nm using a Thermo Scientific Genesys 30 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). For the measurement with cooking, the tube was placed in a boiling water bath with magnetic stirring, followed by incubation at 25°C with shaking at 160 rpm for 20 min, after which the transmittance was measured at 640 nm. To determine the transmittance after cold storage at 4°C, the sample was prepared at both 25 and 100°C as described above, which was then stored at 4°C for 1, 3, 7, and 10 d. After the storage,

the sample was incubated in a 25°C water bath with shaking at 160 rpm for 5 min prior to the transmittance measurement at 640 nm (Weil *et al*., 2020).

3.3.3.8 *In vitro digestibility*

In vitro digestibility of the 4-h pyrodextrins after cooking was determined using the methods of Cheng *et al*. (2025) and J. Li *et al*. (2021). The sample (600 mg) was weighed into a 50-mL centrifuge tube with a magnetic stir bar and 15.0 mL of DI water was added to the tube. The sample was placed in a boiling water bath with magnetic stirring for 10 min. After cooling to room temperature, 5.0 mL of sodium acetate buffer (pH 5.2, 0.4 M) was added to the tube, followed by the addition of 5.0 mL of an enzyme cocktail containing amyloglucosidase and α-amylase. After 0, 5, 10, 15, 20, 40, 60 and 120 min of hydrolysis, a sample aliquot of 250 μL was transferred to a 15-mL centrifuge tube containing 10.0 mL of 66% ethanol and then mixed using a vortex mixer for 30 s. The sample tube with ethanol was centrifuged at 3000 *g* for 10 min. The glucose concentration in the supernatant was measured using a D-Glucose assay kit (GOPOD Format) from Megazyme (Megazyme Ltd., Wicklow, Ireland). Percent starch digested at a certain time point (*t*) was calculated using **Equation 3.3**.

$$\%\ \textbf{Starch hydrolysis}\ (t) = \frac{\textit{Hydrolyzed starch}\ (t)}{\textit{Initial total starch mass of sample}} * 100\% \qquad \textbf{Equation 3.3}$$

3.3.4 *Statistical analysis*

Pyrodextrins were prepared in two independent batches for each heating time point (N = 2). Each sample was then analyzed in duplicate to achieve N = 4 for data reporting. Comparison of sample means was completed using One-way ANOVA with Tukey HSD adjustment at a level of significance at 0.05. The statistical analysis was carried out using JMP Pro 16 version 16.2.0 (SAS Institute Inc., Cary, NC, U.S.A.).

3.4 Results and discussion

3.4.1 *Amylose content*

The amylose contents of the pea and normal maize starches were noticeably reduced by pyrodextrinization. The amylose contents of waxy maize-based samples also decreased with a longer pyrodextrinization time, but the extent of change was marginal because of the extremely low amylose content of the native sample. Between the two normal starches, pea had a significantly greater reduction in amylose content: the amylose content of the pea sample was reduced from 35.1% to 1.1%, while that of the normal maize sample was reduced from 27.8% to 10.8% (**Figure 3.1**). The greater reduction in the amylose content of pea sample suggested that it was modified to a larger degree than the normal maize starch (Lovera *et al*., 2020; Weil *et al*., 2020).

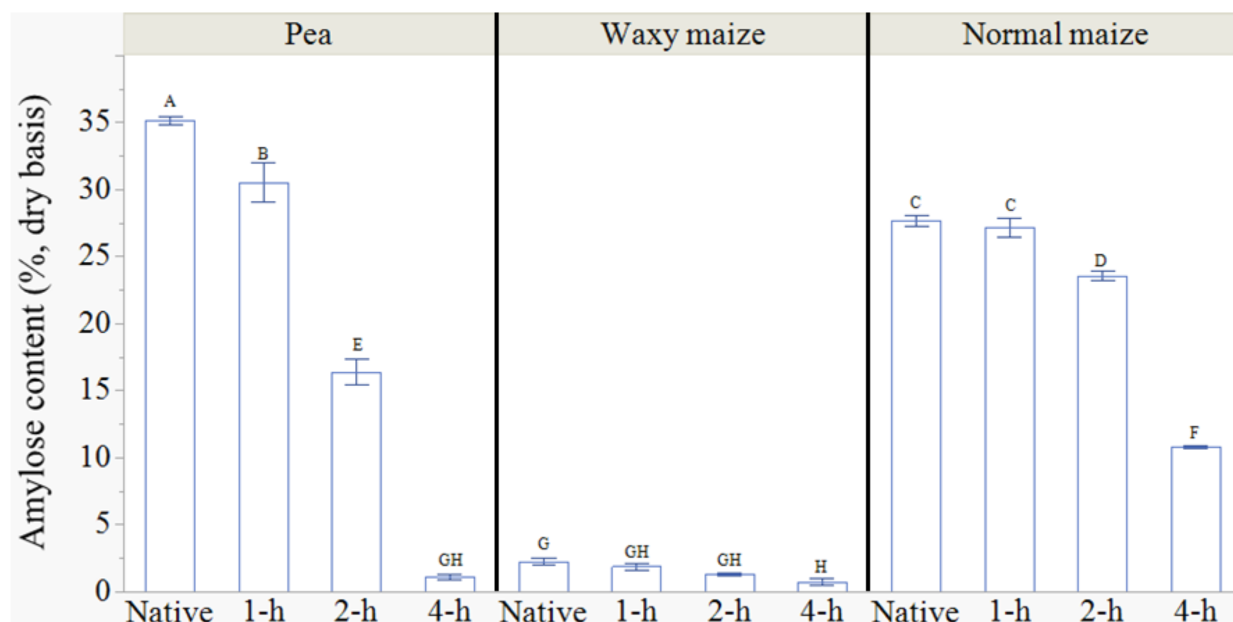


Figure 3.1. Amylose contents of native starches and pyrodextrins from 1-, 2-, 4-h modification. Values are presented as average ± standard deviation (N = 4); values with the same letter above the bars are not significantly different at $p < 0.05$.

3.4.2 *Color parameters*

The pea-based pyrodextrins showed significantly more color development than the normal maize or waxy maize samples (**Table 3.1**). The 4-h pea-based pyrodextrin had an orange-yellow color and the waxy and normal maize samples only showed a slight yellow color (**Figure 3.2**). This was confirmed by the lowest L^*, the highest a^* and b^*, and the greatest ΔE of the 4-h pea pyrodextrin among the three samples (Hill *et al.*, 1997). The larger degree of color development as a result of browning reactions in the pea-based pyrodextrins also indicated that the pea starch had a greater degree of modification than the other two starches. This degree of color development was less than that reported by Weil *et al*. (2020). They measured waxy maize- and normal-maize-based pyrodextrins as having ΔE values of 10.6 and 9.2, respectively. The reduced color development in the current study were attributed to the difference in the reaction conditions and starting starches.

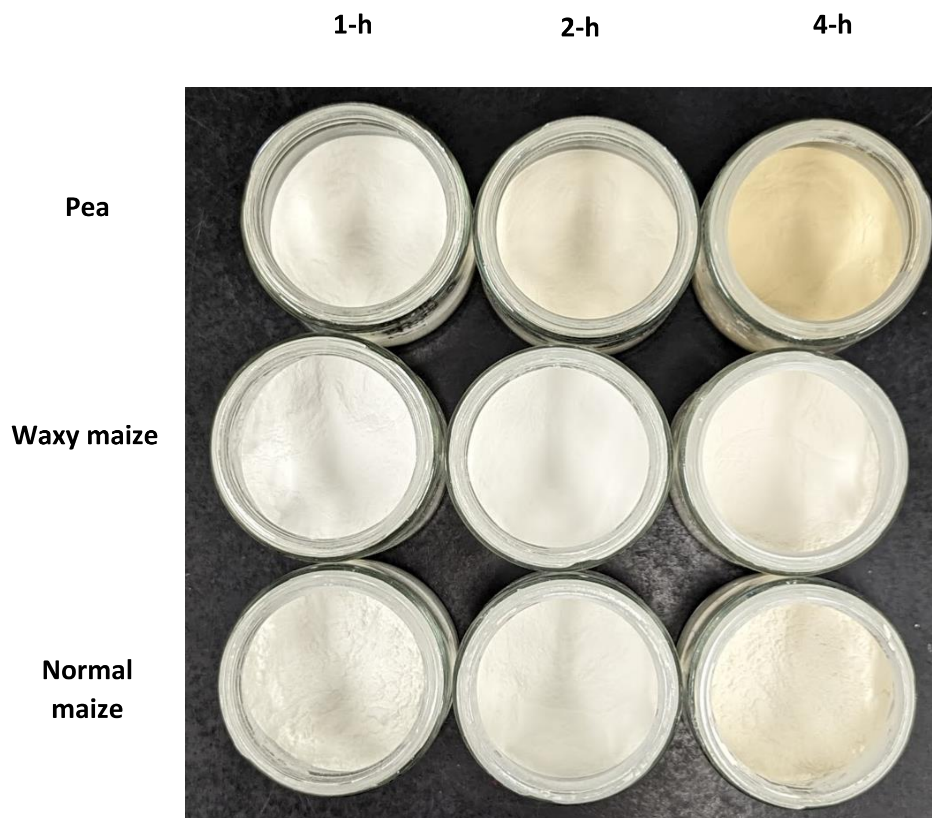


Figure 3.2. Image of pea, waxy maize, and normal maize pyrodextrins from 1-, 2-, 4-h modification.

Table 3.1. Color parameters and water solubility of native starches and pyrodextrins from 1-, 2-, 4-h modification[1].

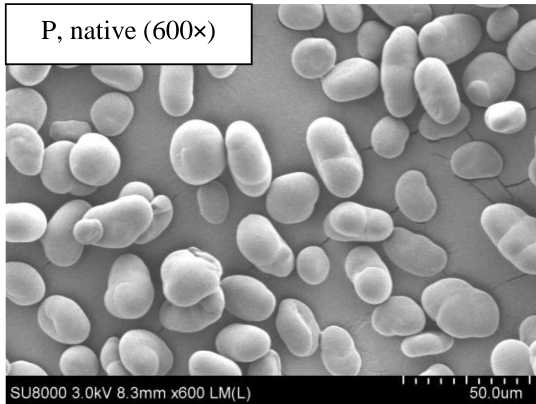
Sample	Color[2]				Water solubility (%)
	L*	***a****	***b****	**ΔE**	
Pea					
Native starch	96.0 ± 0.1[a]	-0.7 ± 0.0[g]	3.6 ± 0.2[g]	-	1.1 ± 0.0[def]
1-h	93.7 ± 0.1[e]	-0.3 ± 0.0[e]	8.0 ± 0.2[d]	5.0 ± 0.2[e]	2.2 ± 0.3[de]
2-h	90.2 ± 0.2[g]	0.9 ± 0.1[b]	14.9 ± 0.9[b]	12.8 ± 0.9[b]	11.9 ± 1.9[c]
4-h	83.9 ± 0.4[h]	3.1 ± 0.2[a]	22.9 ± 0.5[a]	23.0 ± 0.6[a]	94.1 ± 0.1[a]
Waxy maize					
Native starch	95.9 ± 0.3[ab]	-0.1 ± 0.0[d]	2.5 ± 0.1[h]	-	0.2 ± 0.0[f]
1-h	95.4 ± 0.3[bc]	-0.2 ± 0.0[de]	2.8 ± 0.1[h]	0.6 ± 0.3[g]	1.1 ± 0.1[def]
2-h	94.8 ± 0.2[d]	-0.3 ± 0.0[e]	4.5 ± 0.2[f]	2.3 ± 0.1[f]	2.8 ± 0.3[c]
4-h	92.4 ± 0.1[f]	0.3 ± 0.0[c]	8.8 ± 0.3[d]	7.2 ± 0.3[c]	40.7 ± 1.8[b]
Normal maize					
Native starch	95.7 ± 0.2[ab]	-0.6 ± 0.0[f]	5.3 ± 0.1[e]	-	0.1 ± 0.1[f]
1-h	95.1 ± 0.3[cd]	-0.5 ± 0.0[f]	5.0 ± 0.1[ef]	0.7 ± 0.3[g]	0.3 ± 0.0[ef]
2-h	94.7 ± 0.2[d]	-0.5 ± 0.0[f]	5.5 ± 0.1[e]	1.1 ± 0.2[g]	1.5 ± 0.2[def]
4-h	92.2 ± 0.1[f]	0.2 ± 0.0[c]	10.3 ± 0.2[c]	6.1 ± 0.1[d]	39.9 ± 0.7[b]

[1] Values are presented by average ± standard deviation (N = 4); in the same column, values with the same letter are not significantly different at $p < 0.05$.

[2] L^* is lightness. a^* is red-green color, b^* is blue-yellow color, and ΔE is the change in color relative to the native starch calculated using **Equation 3.1**.

3.4.3 *Particle morphology*

SEM imaging of the pyrodextrins revealed several notable features. Firstly, despite the harsh pyrodextrinization conditions, all the pyrodextrin samples largely retained the granular structure of native starches. The waxy maize-based pyrodextrins showed the greatest degree of damage to the granules (marked by blue arrows) overall, followed by normal maize and pea (**Figure 3.3**). The greatest degree of damage found in the waxy maize starch was attributed to its lowest amylose content (**Figure 3.1**). Linear amylose is known to enhance granular integrity of starch during processing (Lin *et al.*, 2018). Due to the virtual absence of amylose, the waxy maize starch showed the highest fragility during pyrodextrinization (Hsieh *et al.*, 2019). Pea-based pyrodextrins also had more obvious aggregation among granules (marked by red circles) caused by the modification.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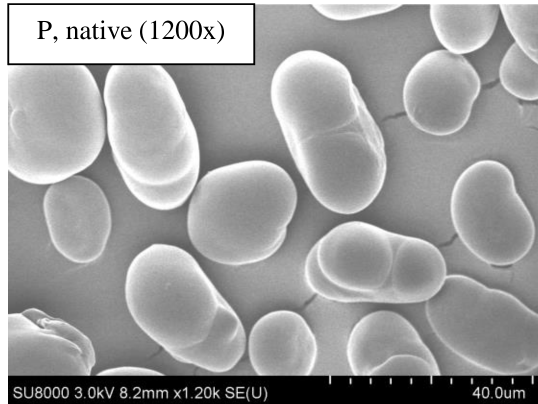
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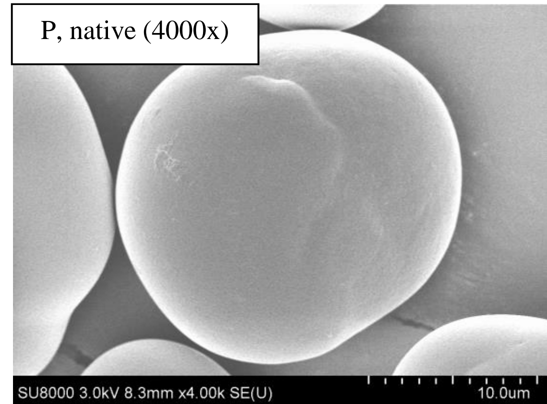
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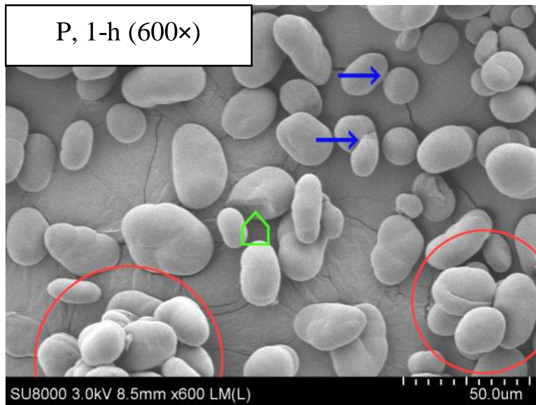
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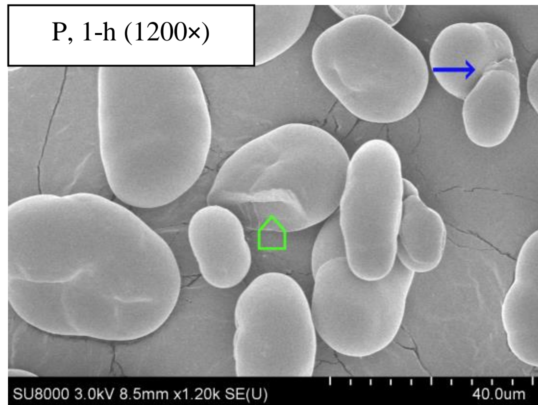
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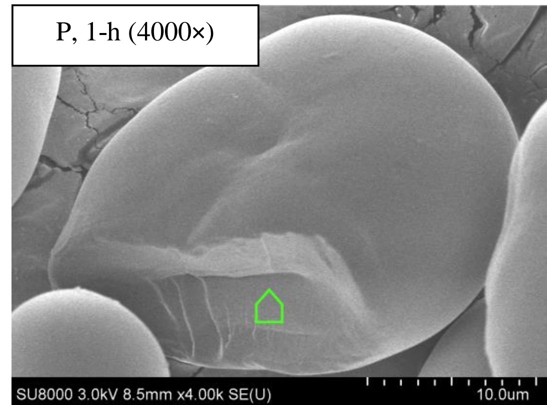
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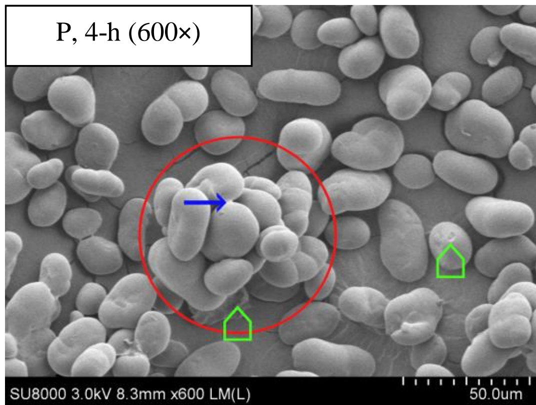
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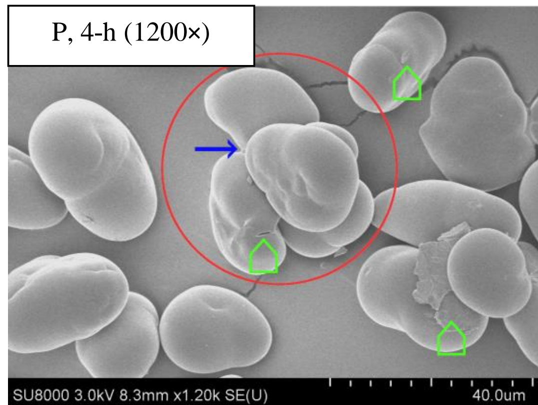
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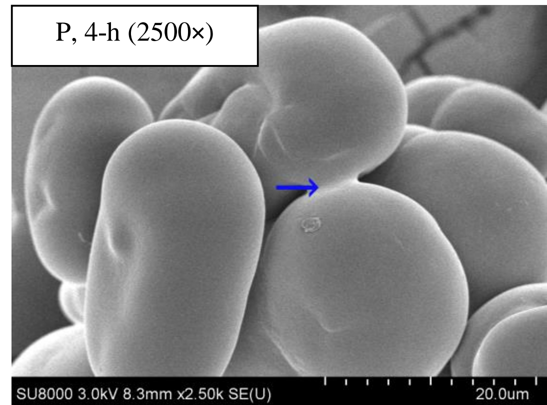
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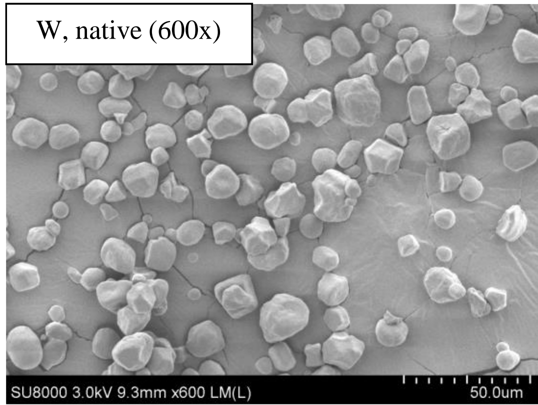
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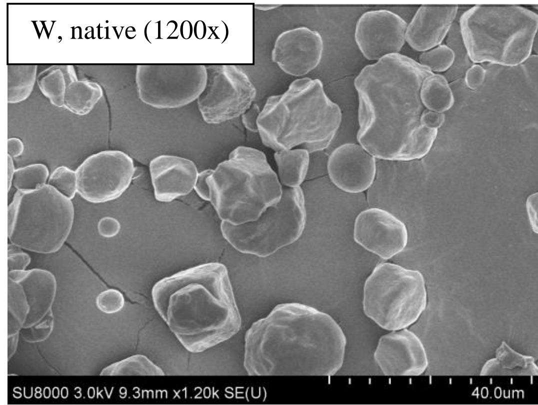
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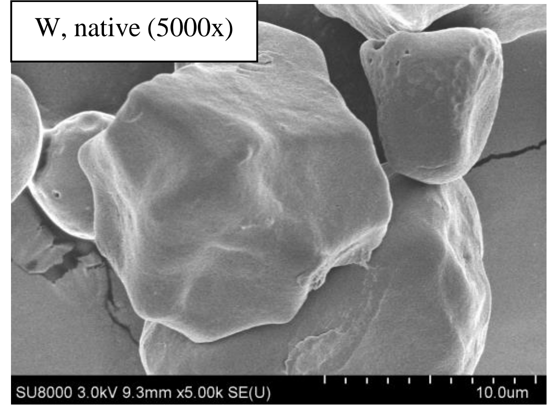
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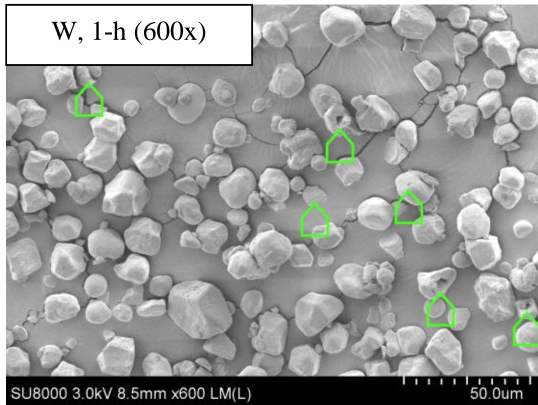
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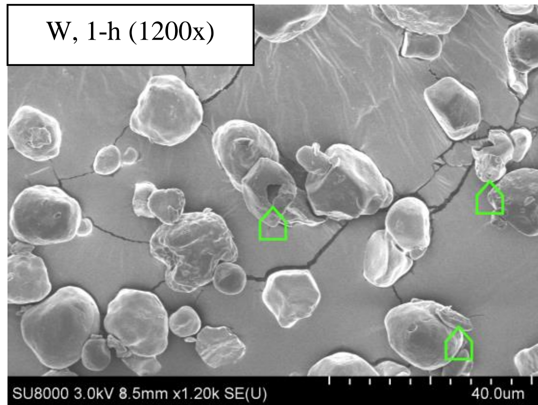
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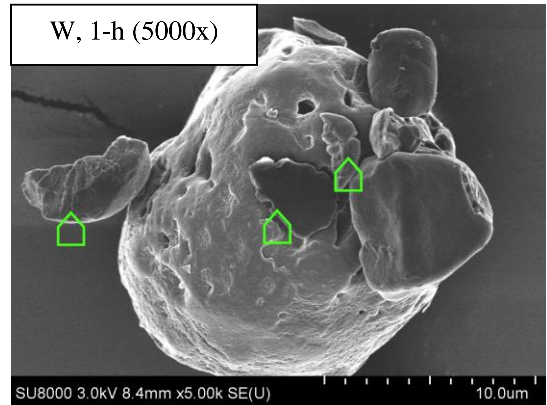
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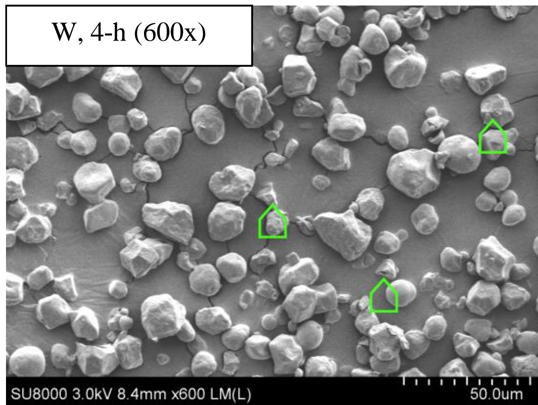
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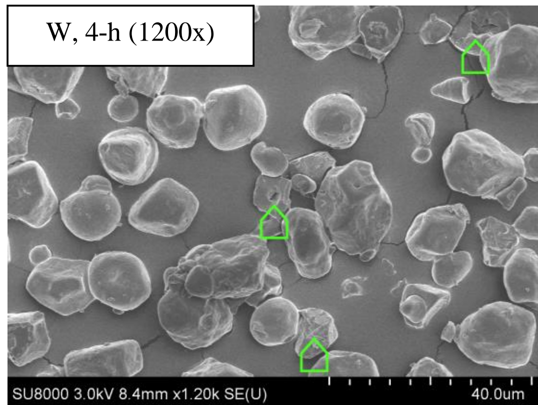
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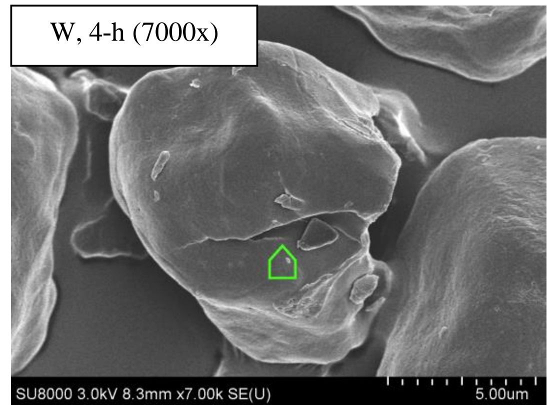
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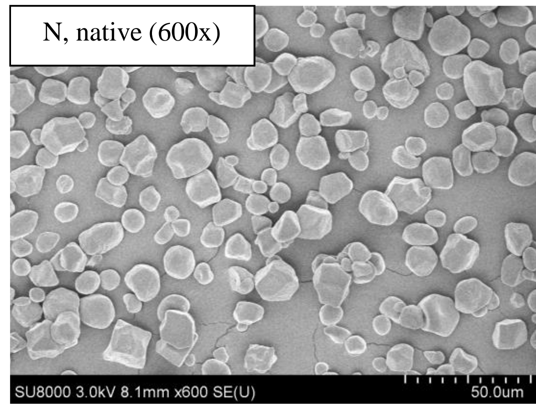
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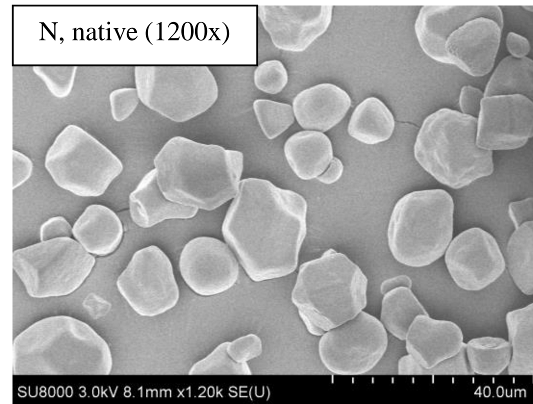
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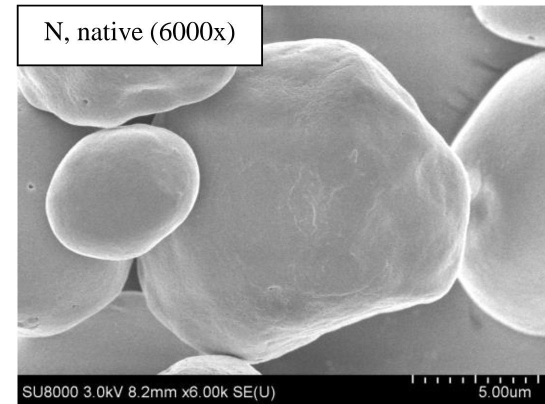
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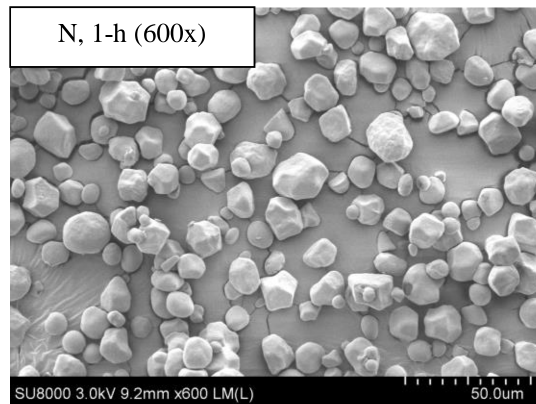
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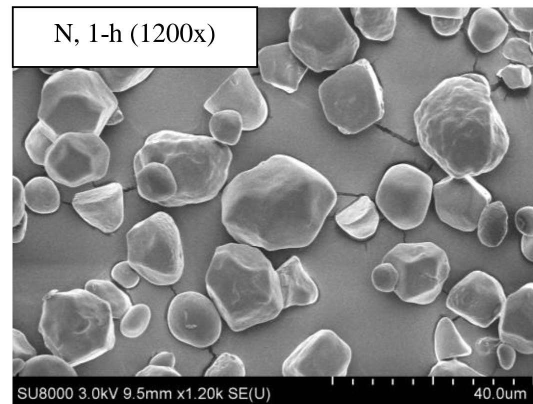
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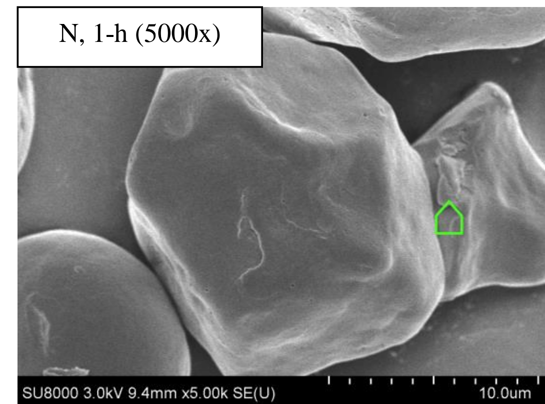
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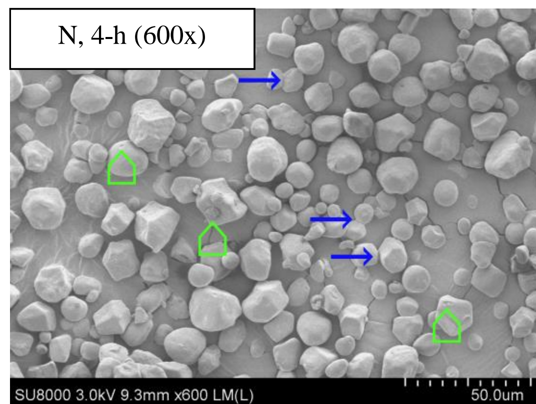
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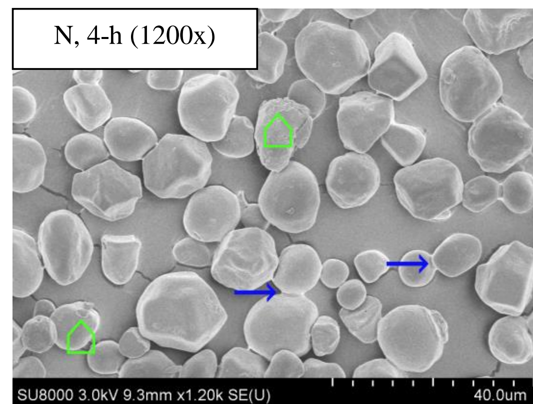

N, 4-h (1200x)
SU8000 3.0kV 9.3mm x1.20k SE(U)
40.0um

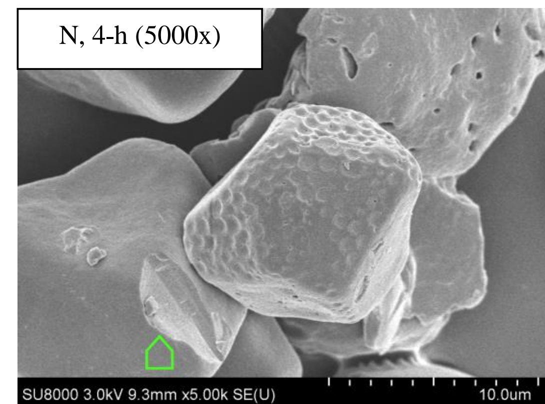

N, 4-h (5000x)
SU8000 3.0kV 9.3mm x5.00k SE(U)
10.0um

Figure 3.3. Scanning electron microscopy images of pea, waxy maize, and normal maize starches and pyrodextrins. P = pea, W = waxy maize, N = normal maize; native = native starch, “1-h”, “2-h”, and “4-h” = pyrodextrins from 1-, 2-, 4-h modification. Green pentagons indicate granule damage; blue arrows indicate visible aggregation between granules; and red circles indicate aggregated granule clusters.

3.4.4 *Molecular-weight distribution*

Due to the limitations of the HPSEC-RI system as indicated in *Section 3.3.3*, only the 4-h pyrodextrins were analyzed. The HPSEC curve of the 4-h pea-based pyrodextrin showed the least proportion of large molecules and the highest proportion of small molecules among all the three samples (**Figure 3.4**), indicating the lowest average molecular weight of this sample. The observation likely resulted from the greatest degree of hydrolysis of pea starch by pyrodextrinization (L. Li *et al*., 2020). The molecular-weight distributions of the three 4-h pyrodextrins were largely similar to those of pyrodextrins prepared in other studies (Mao, Li, *et al*., 2021; Weil *et al*., 2021). Compared with native starches, pyrodextrins have considerably smaller molecular weights, which is ascribed to the depolymerization of starch chains by a combination of acid and heat (Yoo & Jane, 2002). The substantially lower molecular weights of pyrodextrins impart special functional properties to this type of starch derivatives as discussed below.

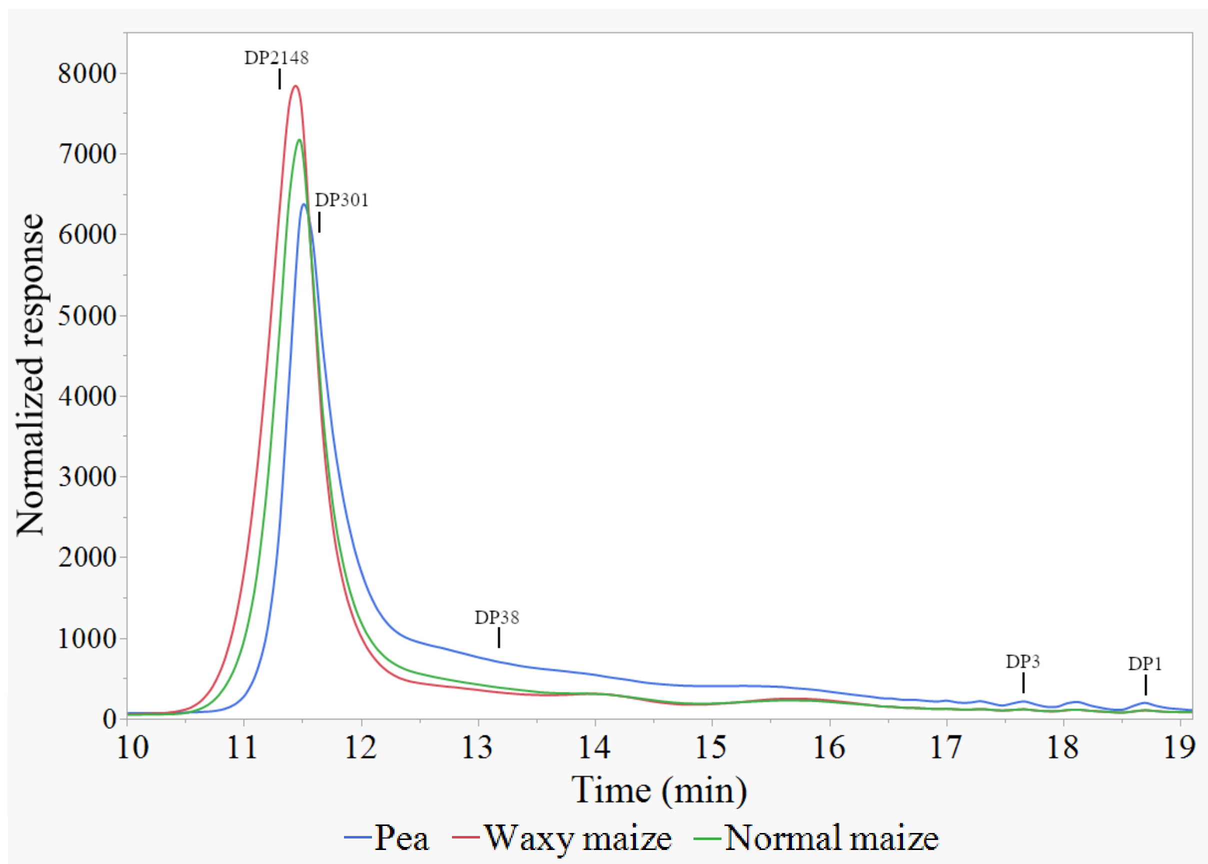


Figure 3.4. Normalized (by area) high-performance size-exclusion chromatograms of 4-h pea, waxy maize, and normal maize-based pyrodextrins. Values on the top of the graph indicate elution times of the standards with different degrees of polymerization (DP) applied to calibrate the system.

3.4.5 *Gelatinization and retrogradation properties*

Native starches have high gelatinization and retrogradation rates do the structure of the starch. The level varies based on the type of starch and the concentration of amylose and amylopectin (Lawton, 2015; Pan & Jane, 2000; Schirmer *et al.*, 2015). The pea starch had slightly lower gelatinization T_o and T_p than the waxy maize and normal maize starches. Gelatinization ΔH exhibited a descending order of waxy maize, normal maize, pea, consistent with their different levels of amylopectin (**Figure 3.1**) (Ai & Jane, 2015). The pyrodextrinization from 1 to 4 h gradually lowered the gelatinization temperatures and diminished the gelatinization ΔH, indicating that the modification destroyed the crystalline structure of starch. The most obvious effect was found with pea starch. The 4-h pea-based

pyrodextrin only had ΔH of 0.7 J/g. The observation was generally consistent with the existing literature reporting that more significant changes in the gelatinization properties of starches were noted after pyrodextrinization for 3 to 5 h (Mao, Li, *et al*., 2021; Weil *et al*., 2020).

Across all the three starches, only slight changes were found in T_o, T_p, and T_c of the melting of retrograded starch as the pyrodextrinization time was increased from 0 to 2 h. When the modification time reached 2 h, ΔH of this thermal transition of all the three starches decreased in comparison with the respective native counterparts; when the modification time reached 4 h, the melting peak of retrograded starch disappeared (**Table 3.2**). The data suggested that pyrodextrinization, particularly 4-h modification, inhibited starch retrogradation, which could be attributed to depolymerization and transglycosylation of starch chains as mentioned previously. Overall, the results were in good agreement with those reported in other studies (Han *et al.*, 2018; H. Li *et al.*, 2020; Weil *et al.*, 2020). The absence of starch retrogradation in the 4-h pyrodextrins after 7-d storage at 4°C indicated that this type of starch derivative had pronounced stability during cold storage.

The retrogradation of the samples was also affected by the modification. For all three starches, the 4-h pyrodextrin had no measurable ΔH due to the pyrodextrinization. The pea-based samples had a decrease in the ΔH from 11.3 to 5.7 J/g between the native starch and 2-h pyrodextrin (and subsequent decrease to 0.0 at 4 h). This indicated the modification was notable in the 1-h and 2-h samples. This was partly attributed to the higher amylose content of the starch. In contrast, the waxy maize- and normal maize-based samples did not show a significant decrease in between the native starches and the 1-h and 2-h samples (**Table 3.2**). This was indicative of the less extensive reaction in the samples. However, as no peak was measured in the 4-h pyrodextrins. Thus, it was evident the process did occur, only to a lesser degree.

Table 3.2. Gelatinization and retrogradation properties of native starches and pyrodextrins from 1-, 2-, 4-h modification[1].

Sample	Gelatinization of native starch[2]				Melting of retrograded starch[2, 3]			
	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								
Native starch	66.8 ± 0.5[bc]	72.9 ± 0.3[ab]	81.4 ± 1.1[cd]	16.9 ± 0.5[b]	42.0 ± 0.6[bc]	61.9 ± 0.7[a]	73.7 ± 0.7[a]	11.3 ± 1.1[a]
1-h	63.3 ± 0.5[de]	74.4 ± 0.5[a]	83.6 ± 0.8[bc]	15.6 ± 0.7[bc]	42.9 ± 0.1[ab]	57.3 ± 0.3[cd]	71.3 ± 0.6[b]	6.9 ± 0.2[b]
2-h	52.3 ± 0.6[g]	64.9 ± 3.2[d]	86.1 ± 3.0[b]	10.5 ± 0.4[d]	42.8 ± 0.1[ab]	59.1 ± 1.7[bc]	74.2 ± 1.6[a]	5.7 ± 0.3[cd]
4-h	54.9 ± 2.3[f]	59.7 ± 1.7[e]	68.6 ± 0.8[g]	0.7 ± 0.1[f]	------------------------ No peak --------------------------			
Waxy maize								
Native starch	67.7 ± 0.2[ab]	74.9 ± 0.2[a]	82.0 ± 0.4[cd]	19.2 ± 0.8[a]	43.4 ± 0.3[a]	57.2 ± 1.0[cd]	64.2 ± 0.6[c]	1.3 ± 0.3[f]
1-h	61.4 ± 0.4[e]	74.0 ± 0.4[a]	89.7 ± 0.9[a]	16.9 ± 0.7[b]	43.2 ± 0.2[a]	59.4 ± 0.8[abc]	65.4 ± 0.7[c]	2.8 ± 0.3[e]
2-h	50.7 ± 0.3[g]	67.8 ± 0.5[c]	84.8 ± 0.5[b]	14.8 ± 0.4[c]	43.2 ± 0.9[a]	60.6 ± 1.0[ab]	64.7 ± 0.8[c]	0.8 ± 0.1[f]
4-h	47.8 ± 1.4[h]	53.5 ± 0.9[f]	60.1 ± 1.4[h]	3.2 ± 0.4[e]	------------------------ No peak --------------------------			
Normal maize								
Native starch	69.2 ± 0.2[a]	73.3 ± 0.2[ab]	79.5 ± 0.5[de]	17.1 ± 0.5[b]	41.8 ± 0.3[c]	56.1 ± 0.3[de]	66.1 ± 0.1[c]	6.4 ± 0.3[bc]
1-h	65.3 ± 1.1[cd]	71.0 ± 1.1[b]	78.2 ± 0.3[ef]	14.7 ± 0.6[c]	41.7 ± 0.4[c]	54.1 ± 1.7[e]	64.5 ± 0.8[c]	6.4 ± 0.2[bc]
2-h	55.5 ± 0.4[f]	66.9 ± 0.4c[d]	76.1 ± 0.2[f]	11.6 ± 1.1[d]	42.0 ± 0.3[bc]	53.7 ± 0.8[e]	64.0 ± 1.3[c]	5.3 ± 0.1[d]
4-h	50.7 ± 1.0[g]	55.2 ± 1.0[f]	60.1 ± 0.3[h]	1.2 ± 0.3[f]	------------------------ No peak --------------------------			

[1] Values are presented by average ± standard deviation (N = 4); in the same column, values with the same letter are not significantly different at $p < 0.05$.

[2] Determined using differential scanning calorimetry scan at a rate of 10$^{\circ C}$/min from 10 to 150°C.

[3] Retrogradation samples run following seven days of storage at 4°C.

3.4.6 *Water solubility and transmittance*

Pea, waxy maize and normal maize starches were all insoluble in water at 25°C. For the same type of starch, as the pyrodextrinization time increased, the solubility of the resultant pyrodextrins gradually increased. The pea-based pyrodextrins showed a significantly larger increase in the water solubility over the maize-based counterparts. With water solubility of 94.1%, the 4-h pea pyrodextrin was almost completely soluble while the waxy and normal maize pyrodextrins were only 39.8% and 39.9% soluble, respectively (**Table 3.3**). The higher solubility of the pea-based pyrodextrins was attributed to the greater decrease in long starch chains and increase in branched linkages formed during pyrodextrinization (**Figure 3.4**) (Guo *et al*., 2017). These structural changes allowed for easier hydration and dissolution of the pyrodextrin in water (BeMiller & Whistler, 2009; Lin *et al*., 2018). The higher water solubility also corresponded to greater transmittance in water.

At room temperature, the pea-based samples had minimal increase in the transmittance of the sample up to 2 h. This indicated the effect of the pyrodextrinization was limited up to that time. However, a significant increase was observed in the 4 h samples, indicating a greater change. Over the 10-d storage, the native pea starch and all pyrodextrins showed minimal change (**Table 3.3**). The waxy maize- and normal maize- samples had a similar trend to the pea-based samples but the 4-h pyrodextrin did not show an increase in transmittance like the 4-h pea-based pyrodextrin. This was attributed to the more limited pyrodextrinization reaction in the samples.

The transmittance of the pea-, waxy maize- and normal maize-based pyrodextrins markedly increased after boiling in water. The changes were the greatest in the native starches and became less as the pyrodextrin time increased. This was due to the boiling of the samples allowing gelatinization and greater hydration. However, this increased transmittance quickly decreased as the samples were held over 10 d (**Table 3.3**). This was due to retrogradation. The degree of the decrease varied based on the sample reaction time and starch. For the pea-based samples, the native starch, 1-h and 2-h samples all had a decrease to levels comparable to that in the unboiled sample after 10 d. The 4-h pea pyrodextrin did not show the same trend and the transmittance remained constant because of the high solubility of the sample. For the boiled waxy-maize based samples, the increase in transmittance from boiling was greater than that of the pea-based samples. Additionally, the waxy-maize based samples showed a lesser decrease

in transmittance. This was due to the high degree of branching in waxy maize limiting retrogradation. The 4-h waxy maize-based pyrodextrin also did not show a decrease. The normal maize-based samples resembled the trend of the pea-based samples with the transmittance increasing at 0 d and then decreasing over the 10-d storage. The degree of decrease was similar to the pea-based samples for the native starch, 1-h and 4-h pyrodextrins. The 2-h normal maize-based pyrodextrin showed a lesser decrease than the normal maize starch and 1-h pyrodextrin but less than the 4-h pyrodextrin. This was different from what was observed in the 2-h pea-based pyrodextrin.

Table 3.3. Transmittance of native starches and pyrodextrins from 1-, 2-, 4-h modification at 640 nm after heating at 25° or 100°C and subsequent storage at 4°C for 0 to 10 d[1].

Sample	Preparation temperature (°C) [2]	Transmittance (%) after storage at 4°C				
		0 d	1 d	3 d	7 d	10 d
Pea						
Native starch	25	0.9 ± 0.0$^{i, A}$	1.0 ± 0.1$^{h, A}$	1.0 ± 0.2$^{h, A}$	1.0 ± 0.1$^{h, A}$	1.0 ± 0.2$^{f, A}$
1-h		1.3 ± 0.5$^{i, A}$	1.3 ±0.5$^{h, A}$	1.4 ± 0.6$^{h, A}$	1.4 ± 0.6$^{h, A}$	1.4 ± 0.6$^{f, A}$
2-h		0.8 ± 0.0$^{i, A}$	0.8 ± 0.0$^{h, A}$	0.8 ± 0.0$^{h, A}$	0.8 ± 0.0$^{h, A}$	0.8 ± 0.0$^{f, A}$
4-h		77.9 ± 3.2$^{b, B}$	81.0 ± 0.8$^{b, AB}$	81.8 ± 1.2$^{b, AB}$	81.6 ± 1.5$^{c, AB}$	82.3 ± 1.3$^{b, A}$
Native starch	100	17.1 ± 1.6$^{h, A}$	1.4 ± 0.3$^{h, B}$	1.1 ± 0.2$^{h, B}$	1.0 ± 0.2$^{h, B}$	1.0 ± 0.2$^{f, B}$
1-h		25.7 ± 2.0$^{g, A}$	5.6 ± 0.2$^{g, B}$	1.7 ± 0.1$^{h, C}$	0.9 ± 0.1$^{h, C}$	0.8 ± 0.0$^{f, C}$
2-h		80.9 ± 2.0$^{b, A}$	57.7 ± 6.9$^{d, B}$	31.9 ± 5.6$^{f, C}$	8.6 ± 1.5$^{g, D}$	4.3 ± 0.4$^{e, D}$
4-h		89.5 ± 1.1$^{a, A}$	88.9 ± 1.0$^{a, A}$	89.3 ± 1.1$^{a, A}$	88.9 ± 1.1$^{a, A}$	89.5 ± 1.1$^{a, A}$
Waxy maize						
Native starch	25	0.3 ± 0.0$^{i, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, B}$	0.3 ± 0.0$^{h, B}$	0.3 ± 0.0$^{f, AB}$
1-h		0.3 ± 0.0$^{i, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{f, A}$
2-h		0.3 ± 0.0$^{i, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{f, A}$
4-h		1.4 ± 0.3$^{i, A}$	1.6 ± 0.4$^{gh, A}$	1.6 ± 0.4$^{h, A}$	1.6 ± 0.4$^{h, A}$	1.5 ± 0.3$^{f, A}$
Native starch	100	54.6 ± 2.6$^{e, A}$	44.1 ± 2.1$^{e, B}$	40.5 ± 1.8$^{e, B}$	34.8 ± 1.5$^{f, C}$	32.4 ± 1.9$^{d, C}$
1-h		74.8 ± 0.7$^{c, A}$	65.5 ± 0.4$^{c, B}$	62.2 ± 1.0$^{c, C}$	57.2 ± 0.8$^{d, D}$	51.1 ± 0.4$^{c, E}$
2-h		87.2 ± 0.9$^{a, A}$	85.6 ± 1.5$^{a, A}$	84.5 ± 1.6$^{b, A}$	84.0 ± 2.1$^{b, A}$	83.4 ± 3.2$^{b, A}$
4-h		89.7 ± 0.7$^{a, A}$	89.5 ± 0.3$^{a, A}$	89.0 ± 0.5$^{a, A}$	89.4 ± 0.2$^{a, A}$	89.6 ± 0.5$^{a, A}$
Normal maize						
Native starch	25	0.3 ± 0.0$^{i, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{f, A}$
1-h		0.3 ± 0.0$^{i, A}$	0.3 ±0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{f, A}$
2-h		0.3 ± 0.0$^{i, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{h, A}$	0.3 ± 0.0$^{f, A}$
4-h		2.0 ± 0.3$^{i, A}$	2.4 ± 0.4$^{gh, A}$	2.4 ± 0.4$^{h, A}$	2.4 ± 0.4$^{h, A}$	2.4 ± 0.4$^{ef, A}$
Native starch	100	14.8 ± 0.4$^{h, A}$	2.4 ± 0.0$^{gh, B}$	1.6 ± 0.1$^{h, C}$	1.2 ± 0.1$^{h, CD}$	1.0 ± 0.0$^{f, CD}$
1-h		30.6 ± 0.1$^{f, A}$	20.2 ±0.3$^{f, B}$	13.6 ± 0.8$^{g, C}$	6.4 ± 0.9$^{g, D}$	4.0 ± 0.6$^{e, E}$
2-h		65.0 ± 1.3$^{d, A}$	54.9 ± 1.1$^{d, B}$	49.1 ± 0.7$^{d, C}$	38.3 ± 1.4$^{e, D}$	31.7 ± 1.4$^{d, E}$
4-h		89.7 ± 0.4$^{a, A}$	89.6 ± 0.4$^{a, A}$	89.7 ± 0.3$^{a, A}$	88.8 ± 0.7$^{a, A}$	88.7 ± 0.4$^{a, A}$

[1] Values are presented by average ± standard deviation (N = 4). Lowercase letters indicate statistical significance in the same column and uppercase letters indicate statistical significance in the same row; values with the same letter are not significantly different at $p < 0.05$.

[2] Samples were incubated at 25 or 100ºC for 20 min for the preparation. The samples boiled at 100ºC were cooled to 25ºC prior to the measurement of transmittance.

3.4.7 *In vitro digestibility*

After boiling in water, the native pea, waxy maize, and normal starches became highly digestible, and the majority of starch was hydrolyzed into D-glucose after the first 20 min (**Figure 3.5**) (Cheng *et al*., 2025). At the end of the 2-h enzymatic hydrolysis, 68.2%, 87.5%, and 82.5% of the cooked pea, waxy maize, and normal starches were hydrolyzed. The 4-h pyrodextrinization remarkably enhanced the enzymatic resistance of the starches, with the greatest extent of reduction noted for pea-based sample, followed by normal maize- and waxy maize-based samples. At the end of the 4-h enzymatic hydrolysis, the percentage of starch hydrolysis of cooked native pea starch was decreased from 95.2% to 68.2%, that of waxy maize was decreased from 97.3% to 87.5%, and that of normal maize starch was decreased from 95.6% to 82.5%. The reduced enzymatic digestibility of the pyrodextrin samples was primarily attributed to the formation of new glycosidic linkages between starch chains, such as β-1,6, α-1,2, and β-1,2 linkages, that are resistant to enzymatic hydrolysis by α-amylase and amyloglucosidase (Bai & Shi, 2016; Mao, Chen, *et al*., 2021; Mao, Li, *et al*., 2021). The highest enzymatic resistance of 4-h pea-based pyrodextrin was in good accordance with the greatest extent of pyrodextrinization of this pulse starch according to other structural and functional characterization, as presented in previous sections.

The greater enzymatic resistance of the pea-based pyrodextrin compared to the maize-based counterparts will also enable this new ingredient to offer greater nutritional value when added to food products (Kazuhiro *et al.*, 1991). However, in comparison with the results reported in the literature, the resistance to enzymatic digestion of the pyrodextrin samples prepared in this study was comparatively lower (Kapusniak & Jane, 2007; Lin *et al*., 2018). This lower enzymatic resistance of the current study could be explained by the specific types of starches used as well as variations in equipment and reaction conditions. For example, the evaporation of moisture and HCl during the heating at 180°C could effectively influence the extent of starch pyrodextrinization in different studies.

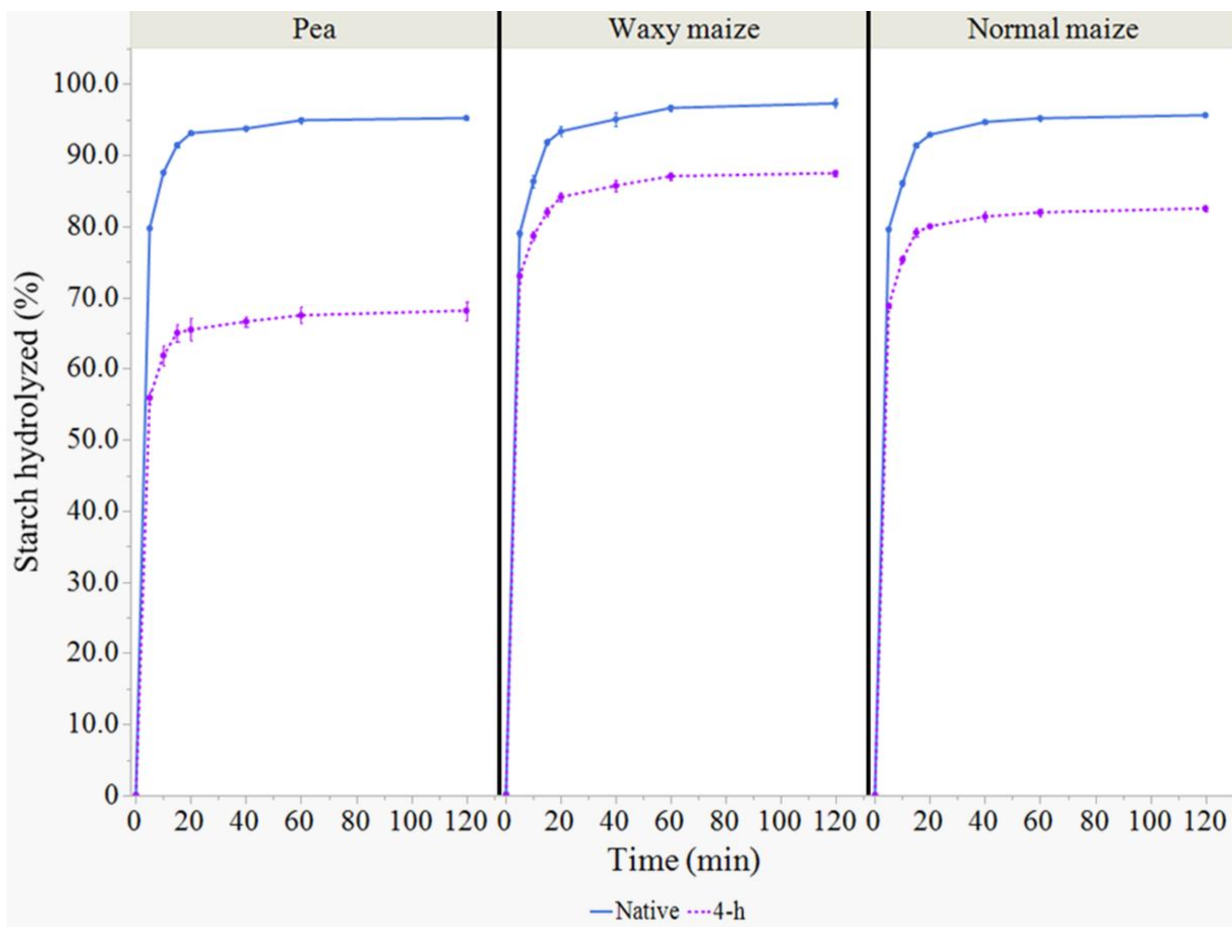


Figure 3.5. Enzymatic hydrolysis of water-boiled native starches and pyrodextrins from 4-h modification. Values are presented by average ± standard deviation (N = 4).

3.5 Conclusions

The pyrodextrinization of pea, waxy maize, and normal maize starches resulted in decreased amylose content, increased color development, alterations to the particle morphology, a reduction in the average molecular weight, a reduction in the degree of gelatinization and retrogradation, an increased in transmittance, a reduction in water solubility, and a decrease in the *in vitro* digestibility. Of the three pyrodextrins, pea-based pyrodextrin showed the greatest extents of changes from the pyrodextrinization process followed by normal maize, with waxy maize being the least affected. This highest degree of modification in the pea-based pyrodextrins was confirmed across all the performed analyses. Notably, the pea-based pyrodextrin had significantly greater water solubility and transmittance and higher enzymatic resistance than the maize-based

pyrodextrins. These desirable properties position the pea-based pyrodextrin as the preferred choice for the use in food products and dietary supplements, particularly beverages (Cao *et al.*, 2018). The reported method to transform native pea starch into pyrodextrin and the favorable functional attributes and high enzymatic resistance of pea-based pyrodextrin will have great potential to increase food application of pea starch, the main co-product from the fractionation of pea seeds.

3.6 Connection to Study 2

As demonstrated in this study, the modification of starch using a combination of heat and acid was used to produce pyrodextrins, a value-added food ingredient. The modification imparted several favorable changes to the techno-functional attributes of the starch derivative, including increased resistance to enzymatic hydrolysis, enhanced water solubility and transmittance, and no detectable retrogradation after storage at 4°C for 7 d. However, this modification method is not the only one for altering the functionality of starch. Another common method to convert native starch into value-added ingredients is the hydrolysis of starch using an α-amylase to produce maltodextrin. Similar to pyrodextrinization, the conversion of starch into maltodextrin involves heat. To achieve fast production of maltodextrin from a native starch, a thermostable α-amylase is commonly used, which has an optimum temperature range of 85° to 90°C, notably lower than 160° to 180°C typically applied in pyrodextrinization (Mao, Chen, *et al.*, 2021; Mao, Li, *et al.*, 2021; McPherson & Seib, 1997; Tsusaki *et al.*, 2009).

The dietary fiber contents of maltodextrins and pyrodextrins also vary substantially. Pyrodextrins are a known source of dietary fiber, meaning that they can retain good enzymatic resistance in the human gastrointestinal tract after consumption (Lovera *et al.*, 2020; Ohkuma *et al.*, 1997; Zhu *et al.*, 2020). By contrast, maltodextrins virtually contain no dietary fiber. This difference in digestibility leads to different food applications of these two ingredients. Maltodextrins are typically used as a flavor carrier, bulking agent, or coating in food products and as a bulking agent in supplements (Lalasa *et al.*, 2013; Punia Bangar *et al.*, 2022; Stephen *et al.*, 2006). Pyrodextrins, in contrast, while also used in basic foods, are added as a source of fiber to items such as beverages and health foods. Both pyrodextrins and maltodextrins are used in supplements. However, unlike maltodextrin used a low-value bulking agent, pyrodextrin is added as a good source of fiber. Additionally, the differences in properties, such as viscosity, water

solubility and the transmittance of light, affect the applications the ingredients (Stephen *et al*., 2006; Weil *et al*., 2020).

Therefore, in the second study, pea starch was utilized to produce maltodextrin through α-amylase hydrolysis. Alpha-amylase breaks down α-1,4 glycosidic linkages in amylose and amylopectin chains. Alpha-amylases are endo-acting enzymes, meaning that they attach and cleave internal α-1,4 glycosidic linkages of amylose and amylopectin molecules (**Figure 2.6**) (Purich & Allison, 2002). Briefly, the enzyme attacks at one of these linkages and cleaves it, resulting in the production of two or multiple smaller oligosaccharide chains. This mechanism results in a rapid decrease in viscosity and average DP as well as increase in solubility and DE. Ultrasonication is a method that can be used to alter the enzymatic reaction. Research has shown the application of intermittent ultrasonication increased the rate of debranching of pea starch by pullulanase (Hu *et al.*, 2013; Lu *et al.*, 2018). The authors proposed that the enhanced enzyme activity was attributed to various potential factors, including the thermal effects from ultrasonication, increased binding of the enzyme with starch substrate as a result of decreasing local viscosity of the starch dispersion, and/or physical effects such as bubbles or additional shear induced by the ultrasonic waves (Gaquere-Parker *et al*., 2018; Lu *et al*., 2018). Moreover, it has been reported that the hydrolysis of potato starch by α-amylase increased with intermittent ultrasonication (Oliveira *et al*., 2018). Based on the current understanding of both modification methods, ultrasonication will be combined with α-amylase hydrolysis to prepare maltodextrins from pea, waxy maize, and normal maize starches in Study 2. This new method is particularly important for effective amylolysis of pea starch because this pulse starch possesses a strong gelling ability, which hinders enzymatic breakdown of the molecules at a high starch concentration (L. Li *et al*., 2020). It is anticipated that the combined modification method can lead to molecular structures and functional properties of maltodextrins distinctively different from those of counterparts prepared using enzymatic hydrolysis alone. Finally most research on maltodextrins has focused on starches such as normal maize, waxy maize and tapioca with limited research on pea-based maltodextrins (Pycial, 2017; Weil *et al*., 2020). Therefore, it is of importance to investigate pea-based maltodextrins in the next study.

4 Development and characterization of maltodextrins generated from simultaneous application of enzymatic hydrolysis and ultrasonication

4.1 Abstract

Peas are primarily grown for their protein, with starch being generated as an underutilized by-product. Pea starch is considered a by-product due to difficulties in processing and therefore it is primarily used in low-value applications such as animal feeds. Ultrasonication has shown potential to ease the modification of starch. To investigate this, pea, waxy maize, and normal maize starches were hydrolyzed using α-amylase with and without ultrasonication. The structural features and functional properties of the resulting maltodextrins were analyzed. Results showed that the enzyme activity was minimally impacted by the addition of ultrasonication. The incorporation of ultrasonication had minimal effects on the molecular-weight distributions and dextrose equivalent values of the derived maltodextrins. However, notable changes were observed in the water solubility, viscosity, and transmittance of the samples at room temperature. The water solubility of pea-based maltodextrins was increased by 59.1% to 14.7% for reaction times ranging from 5 to 20 min. Comparatively, waxy maize- and normal maize-based maltodextrins had changes of 4.0% to 0.0%. The transmittance of light through the samples in aqueous medium was also affected by the addition of ultrasonication to the enzymatic reaction. The pea-based and normal maize-based maltodextrins showed notable increases in transmittance (≤ 48.9% in pea and ≤ 20.2% in normal maize), while minimal change was observed in the waxy maize-based samples (≤ 4.8%). Overall, the addition of ultrasonication to the reaction of thermostable α-amylase with pea, waxy maize, and normal maize starches resulted in notable effects on the physicochemical properties of the generated maltodextrins and a more limited impact on the molecular structure.

4.2 Introduction

The demand for new food products and ingredients, such as maltodextrins, continues to grow with the expansion of consumer markets (Eggleston *et al.*, 2021; B. Yu *et al.*, 2021) . Maltodextrin is starch hydrolysate made through the enzymatic hydrolysis of starch into shorter oligosaccharide chains. They are widely used in a products ranging from beverages, to snack foods, to meal replacement drinks, to frozen meals and more (BeMiller & Whistler, 2009; Gregorio *et al.*, 2016; Sajilata & Singhal, 2005). The conversion of starch to maltodextrin results in notable changes to the physicochemical properties of the carbohydrate, including higher water solubility, lower viscosity, and greater sweetness (Plácido Moore *et al.*, 2005; Stephen *et al.*, 2006; Tsusaki *et al.*, 2009). One important characteristic of maltodextrin is dextrose equivalent (DE). DE is a measure of the level of reducing ends within a sample expressed as relative to dextrose on a scale of 0-100 (Kanyuck *et al.*, 2022; Kearsley & Dziedzic, 1995a). A sample with a DE of 0 is starch, while a sample with a DE of 100 is dextrose. A maltodextrin is considered a sample with a DE less than 20, while a sample with a DE greater than or equal to 20 is considered a syrup (Food and Drug Administration, 2008; McPherson & Seib, 1997). This system provides an effective method to measure the degree of hydrolysis of a sample, with higher DE indicating a greater degree of hydrolysis.

Among the many different starches used commercially for the production of maltodextrin, pea starch is less utilized (Drewnowski, 2010; Shelepina, 2020). This is due to the physicochemical properties of the starch. These properties include a high amylose content, high final viscosity after pasting, and strong gelling ability (BeMiller & Whistler, 2009; L. Li *et al.*, 2019; Ratnayake *et al.*, 2002; Shen *et al.*, 2016). This results in the starch being more difficult to handle and less favorable for producing maltodextrin. However, the addition of ultrasonication to the reaction can potentially alter the functional properties of the starch during the enzymatic hydrolysis (Ekaette & Saldaña, 2021; Gaquere-Parker *et al.*, 2018; S. Singh *et al.*, 2015).

Ultrasonication is the use of ultrasonic waves (typically > 18 kHz) to agitate a solution (Bermudez-Aguirre, 2017; Jambrak *et al.*, 2010). Due to the high energy output during ultrasonication, the rapid formation and collapse of vapor-filled bubbles occur, which is also known as cavitation. This process creates shockwaves that damage starch, resulting in the breakdown of the granular structure (Czechowska-Biskup *et al.*, 2005; Fuchs, 2015; Rahaman *et al.*, 2021; Sreedhar *et al.*, 2017; Vernès *et al.*, 2020). Applying this treatment during the enzymatic

hydrolysis of starch increases the rate of enzymatic modification. This increase is due to the accelerated breakdown of starch, providing the enzyme greater access to the granule through a reduction of steric hinderance (Hao *et al*., 2013; Hu *et al*., 2013; Lu *et al*., 2018; Singh *et al*., 2015; Szabo & Csiszar, 2017). This reduces processing costs through a variety of factors, including reduced enzyme requirements and processing time (Oliveira *et al*., 2018; Szabo & Csiszar, 2017). It therefore is of importance to investigate the effects of the addition of ultrasonication on the enzymatic conversion of starch to maltodextrins as well as on the physicochemical properties of the derived maltodextrins.

4.3 Materials and methods

4.3.1 *Materials*

Pea starch was supplied by Roquette Canada Ltd. (Winnipeg, MB, Canada). Waxy maize and normal maize starches were supplied by Cargill Inc. (Minneapolis, MN, U.S.A.). All three starches were used to produce maltodextrins. Thermostable α-amylase (MilliporeSigma A3403/Termamyl 120L) and soluble potato starch (S2004-1KG) were sourced from MilliporeSigma (Oakville, ON, Canada). All other chemicals were sourced from Fisher Scientific Company (Ottawa, ON, Canada).

4.3.2 *Production of maltodextrin with and without ultrasonication*

Deionized (DI) water (160 mL) was added to a 500-mL metal reaction vessel. A straight mixing impeller and hot plate temperature probe were added to the vessel, which was then covered with an aluminum foil to minimize water evaporation. The DI water was heated to 95°C with stirring using a Carframo 2010 Reversing Digital overhead stirrer (Carfamo Ltd., Georgian Bluffs, ON, Canada). Thirty grams [dry basis (d.b.)] of starch and 41.0 g DI H_2O were weighed and homogeneously mixed in a 150-mL beaker. Calcium chloride dihydrate ($CaCl_2{\bullet}2H_2O$) was weighed (0.14 g) and added to the starch mixture. The pH of the slurry was adjusted to 6.25 ± 0.05 with 0.5 M NaOH. After the water in the vessel reached 95°C, 0.20% (weight/weight) of Termamyl 120L enzyme was added to the water, followed by the prepared starch slurry. For samples with ultrasonication, the sample was ultrasonicated using a Hielscher UP400St probe type sonicator with a S24d14D sonotrode (14 mm diameter) at 100% amplitude (99 μm), operating at 24 kHz and

a maximum power output of 400 W (Hielscher Ultrasonics GmbH, Teltow, Germany). The hot plate was adjusted as needed to maintain a reaction temperature of 90.0°C ± 5.0°C throughout the reaction. The reaction proceeded for 5, 10, 15, or 20 min. At the end of the reaction time, the ultrasonicator was shut off and the pH of the solution was adjusted to 3.00 ± 0.10 with 2.0 M hydrochloric acid (HCl) to inactivate the enzyme. The vessel was removed from the hot plate and stirred for 15 min using a magnetic stir bar and stirrer to inactivate the enzyme and cool the sample. The pH of the solution was then adjusted to 6.00 ± 0.10. The sample was transferred to an aluminum pan and flash frozen with liquid nitrogen. The frozen sample was lyophilized using LabConco Stoppering Tray Dryer with an 18-L collection coil (LabConco, Kansas City, MO, U.S.A.). The shelf freezer was set to -20°C for the first 2 h of lyophilization, at which point the temperature of the shelf freezer was elevated to -5°C to promote the sublimation of water ice. Once dry, the sample was ground using a combination of a KitchenAid KFC3516 Food Chopper (KitchenAid, Mississauga, ON, Canada) and a mortar and pestle. The ground sample was passed through a 250-µm sieve. The powdered sample was collected in a plastic jar, with the lid open for seven days to for moisture equilibration.

4.3.3 *Characterization of maltodextrins from different starches*

4.3.3.1 *Dextrose equivalent*

Dextrose equivalent (DE) of the maltodextrins was determined based on the method described by Lane & Eynon (1934). Sample (2.5 – 6.0 g) was weighed into a 50-mL centrifuge tube according to the estimated DE of the sample. DI water (40 mL) was added to the tube along with a magnetic stir bar. The tube was placed in a boiling water bath on a hot plate with magnetic stirring and boiled for 15 min. The tube was then placed in a water bath to cool to room temperature. The sample was then volumetrically transferred to a 50 mL volumetric and brought up to volume. The solution was transferred to a 50-mL burette and 20 mL was titrated into 20 mL of Fehling's solution (a mixture of 10 mL Fehling's A and 10 mL Fehling's B) in an Erlenmeyer flask on a hot plate. The solution was boiled for 1 min. Four drops of 1% methylene blue solution were added to the flask as the indicator. The solution was further titrated until the blue color disappeared.

4.3.3.2 *Molecular-weight distribution*

Molecular-weight distributions of the maltodextrins were completed via high-performance size exclusion chromatography (HPSEC) according to the methods described by L. Li *et al*., (2020) and Peng & Yao (2018). Replicate samples were mixed at an equal ratio to form a composite sample. The composite sample (25 mg) was added to a 15-mL centrifuge tube, followed by the addition of 5 mL of 0.5% LiBr (w/w) in dimethyl sulfoxide (DMSO) to disperse the sample. The sample was placed in a boiling water bath for 10 min with gentle stirring for complete solubilization. The sample was then filtered through a 5-µm disk filter into an HPSEC sample vial and loaded onto the auto-sampler tray. The samples were run using 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 refractive index (RI) detector. Both the column compartment and RID temperature were set to 50°C. DMSO containing 0.5% LiBr was used as the eluent at a flow rate of 0.5 mL/min. The injection volume of the samples was set at 20 µL. Each composite sample was run in duplicate. Standards of glucose, maltotriose, maltohexose, pullulan (6200 Da), pullulan (48,800 Da), and pullulan (348,000 Da) were run at a concentration of 5 g/mL to calibrate the system. The collected RI data were normalized based on the total area under the curve (retention time from 9.0 to 19.5 min).

4.3.3.3 *Percentage of α-1,6 branch linkages of starch*

Percentages of α-1,4 linkages and α-1,6 linkages in the native starches and 5- and 20-min maltodextrins were determined according to the method described by Li *et al*. (2022). Briefly, three replicates were combined at equal weights to create a composite sample for one replicate measurement. The composite sample (10.00 ± 0.05 mg) was weighed into a 1.5-mL microcentrifuge tube, followed by the addition of 1.0 mL deuterated dimethyl sulfoxide (DMSO-d_6). The sample was mixed thoroughly using a Fisher brand Analog Vortex Mixer 02-215-414 (Thermo Fisher Scientific, Waltham, MA, U.S.A.) set to maximum and then placed in a water bath set to 80°C with shaking at 120 rpm overnight to solubilize the sample. The sample was removed from the water bath and cooled to room temperature. After reaching the room temperature, 0.5 mL of sample was added to an NMR tube, followed by 5.66 µL of deuterated triflouroacetic acid (TFA-d_1). The sample was analyzed using proton nuclear magnetic resonance (^{1}H NMR) with spectra collection at 70°C, 600.17 MHz and 12.5 µs 30° pulse. The repetition time was 6.6 s, the

acquisition time was 5.45 s, the relaxation delay was 1.15 s, and 300 scans were completed. The degree of branching was calculated using **Equation 4.1**, where, A = integrated intensity of peak at 4.80 ppm corresponding to α-1,6 glycosidic bonds and B = integrated intensity of peak at 5.10 ppm corresponding to α-1,4 glycosidic bonds (Gidley, 1985; Schmitz *et al.*, 2009; Tizzotti *et al.*, 2011).

$$\boldsymbol{Branching\ degree}\ (\%) = \frac{\mathbf{A}}{\mathbf{A+B}} * \mathbf{100}\% \qquad \textbf{Equation 4.1}$$

4.3.3.4 *Viscosity*

Viscosity of the native starches and maltodextrins was characterized using a TA Instruments Discovery Hybrid rheometer HR20 (TA Instruments, New Castle, DE) (van der Sman *et al.*, 2022). The sample (2.00 g, d.b.) was weighed into a 50-mL centrifuge tube. The tube was then brought up to a weight of 25.00 ± 0.05 g using DI water. The tube was placed in a boiling water bath for 15 min to ensure complete dispersion of the sample. The sample was cooled to room temperature in a water bath and then transferred to a rheometer sample cup. The cup was placed in the rheometer and analyzed using a starch-pasting HSPC rotor 547052.901 from TA Instruments. The sample was firstly equilibrated at a rotor speed of 955 rpm and 23°C for 10 s. The viscosity of the sample was measured at a rotor speed of 160 rpm for 5 min at 23°C. The viscosity (mPa·s) of the sample was determined by averaging the viscosity reading over the 5 min measurement.

4.3.3.5 *Water solubility*

Water solubility determination of the native starches and maltodextrins was performed based on the method described by Han *et al*. (2018). Sample (1.0000 ± 0.1000 g) was weighed into a pre-weighed 15-mL centrifuge tube, and 9.00 mL of DI water was added to the sample tube. The sample was mixed on a Fisher brand Analog Vortex Mixer 02-215-414 set to maximum for 1 min. The sample was then centrifuged at 3,220 *g* for 15 min using an Eppendorf Centrifuge 5810 R (Eppendorf Group, Hamburg, Germany). The supernatant was carefully transferred to a new centrifuge tube, which was dried overnight at 40°C and then 110°C for 3 h. The centrifuge tube

containing the dry solids was weighed. The water solubility of the sample was calculated according to **Equation 4.2.**

$$\boldsymbol{Water\ solubility}\ (\%) = \frac{\boldsymbol{Total\ weight\ of\ tube\ and\ dried\ solids\ (g) - Weight\ of\ tube\ (g)}}{\boldsymbol{Initial\ dry\ weight\ of\ sample\ (g)}} * \mathbf{100}\%$$

Equation 4.2

4.3.3.6 *Transmittance*

Maltodextrin or native starch (0.50 g, d.b.) was weighed into a 50-mL centrifuge tube. The sample was then brought up to a final weight of 25.00 g with DI water. For the measurement of the transmittance of light as is, the tube was placed in a 25°C water bath with shaking at 160 rpm for 20 min, after which the transmittance of the sample was measured at 640 nm using a Thermo Scientific Genesys 30 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). For the measurement of transmittance of light after heating, the sample was placed in a boiling water bath with magnetic stirring for 20 min. The samples were then placed in a 25°C water bath with shaking at 160 rpm for 20 min, after which the transmittance of the sample was measured at 640 nm against a water blank. Both the as-is and boiled samples were stored at 4°C for 10 days. After 1, 3, 7, and 10-day storage, the samples were removed from the 4°C storage and warmed in a 25°C water bath with shaking at 160 rpm for 5 min. The transmittance of the sample was then measured at 640 nm (Weil *et al*., 2020).

4.3.3.7 *Enzyme activity*

Enzyme activity of thermostable α-amylase with and without ultrasonication was determined based on the method of Moretti & Thorson (2008). As the substrate, soluble potato starch was prepared and reacted by following the same procedure used to prepare the maltodextrin samples as described in *Section 4.3.2* with a reaction time of 10 min. Upon the completion of the reaction, the hydrolysate was diluted by 10000× using DI water. The diluted sample (10 µL) was transferred to a 1.5-mL microcentrifuge tube, followed by the addition of 1 mL of 5% (v/v) para-hydrobenzoic acid hydrazide (pHBH) in 0.5 M HCl. The mixture was heated in a boiling water

bath for 5 min and then cooled to room temperature in a water bath. The absorbance of the developed color was measured at 410 nm against a water blank using a Thermo Scientific Genesys 30 Visible Spectrophotometer. A standard curve was produced using samples with known maltose concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL. One unit of enzyme activity (1 U) was defined as 1 μmol of maltose equivalent reducing sugar released per min from soluble potato starch.

4.3.4 *Statistical analysis*

Maltodextrins were prepared in three independent batches for each enzyme hydrolyzing time. Each native starch was also analyzed in triplicate to obtain an N=3 for valid statistical analysis. Comparison of samples was completed using one-way ANOVA and comparison of means using Tukey HSD at a level of significance at 0.05. The statistical analysis was completed using JMP Pro 17 version 17.2.0 (SAS Institute Inc., Cary, NC, U.S.A.).

4.4 Results and discussion

4.4.1 *Dextrose equivalent*

The addition of ultrasonication to the thermostable α-amylase hydrolysis did not have a significant impact on the ability of the enzyme to hydrolyze the starches. The lack of negative impact was expected, as the inability of ultrasonication to denature proteins has been widely reported (Chorfa *et al*., 2022; Lu *et al*., 2018). However, the lack of a positive impact on the rate of hydrolysis was not anticipated. It was expected that the ultrasonication could result in damage to the granular structure of the starch, resulting in a more active sites for faster enzymatic hydrolysis (Chorfa *et al*., 2022; Lu *et al*., 2018; X. Zhang *et al*., 2022). The incorporation of ultrasonication did not show a clear trend on DE values of the maltodextrin samples from the three starches at different hydrolysis time (**Table 4.1**). Overall, the inclusion of ultrasonication showed marginal influence on DE values of the maltodextrin samples.

Table 4.1. Dextrose equivalent of maltodextrins prepared with and without ultrasonication[1].

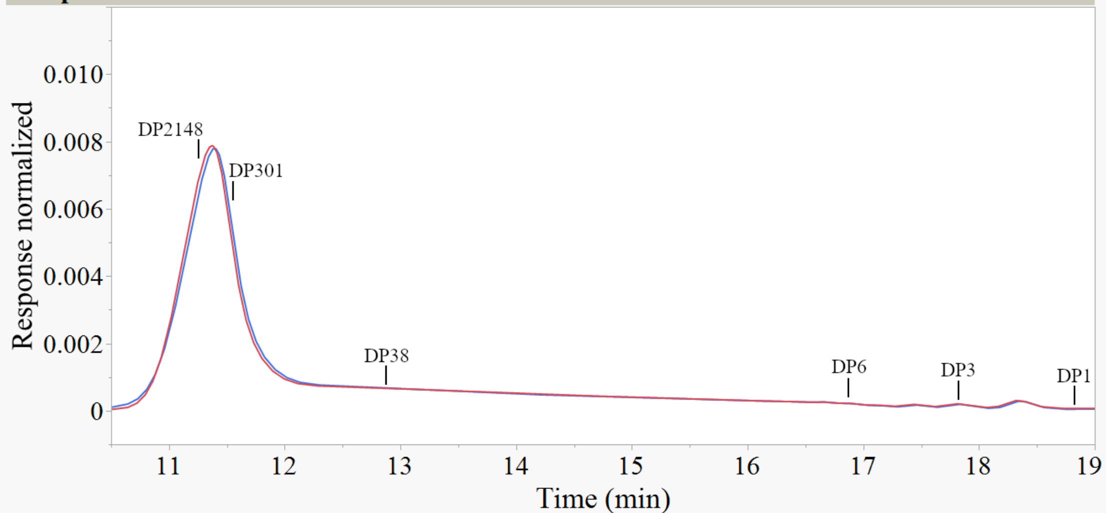
Sample	Ultrasonication	Reaction time (min)	DE
Pea	No	5	3.05 ± 0.09^{FG}
		10	4.09 ± 0.22^{E}
		15	5.29 ± 0.37^{CD}
		20	6.85 ± 0.19^{A}
	Yes	5	2.21 ± 0.06^{H}
		10	4.00 ± 0.13^{E}
		15	5.00 ± 0.10^{D}
		20	7.21 ± 0.22^{A}
Waxy maize	No	5	2.02 ± 0.33^{H}
		10	3.72 ± 0.26^{EF}
		15	5.06 ± 0.50^{D}
		20	6.88 ± 0.23^{A}
	Yes	5	2.33 ± 0.15^{GH}
		10	3.56 ± 0.28^{EF}
		15	5.11 ± 0.12^{D}
		20	6.03 ± 0.23^{B}
Normal maize	No	5	2.24 ± 0.23^{H}
		10	3.54 ± 0.09^{EF}
		15	4.87 ± 0.31^{D}
		20	5.97 ± 0.24^{BC}
	Yes	5	2.38 ± 0.08^{GH}
		10	3.83 ± 0.09^{E}
		15	5.14 ± 0.09^{D}
		20	6.83 ± 0.24^{A}

[1] Values are presented by average ± standard deviation (N=3). Values in the same column with the same letter are not statistically different at $p < 0.05$.

4.4.2 *Molecular-weight distribution*

HPSEC results indicated the addition of ultrasonication to the enzymatic reaction resulted in a slight decrease in the main peak corresponding to large maltodextrin molecules and a slight increase in the smaller peaks corresponding to small maltodextrin molecules (**Figure 3.4**). Less difference was observed between the chromatograms of the samples developed with and without ultrasonication for 5 min than the ones reacted for 20 min, particularly in the main peak of large maltodextrin molecules. A marginal increase in the smaller molecular weight peaks (DP< 38) was

observed in the waxy maize and normal maize-based samples but not the pea-based samples in the 5-min samples. This indicated the benefits from the addition of ultrasonication to the enzymatic reaction were less obvious in the early stage of hydrolysis. However, as the enzymatic reaction time was extended, the pea-based maltodextrins resembled the trend of the maize-based maltodextrins.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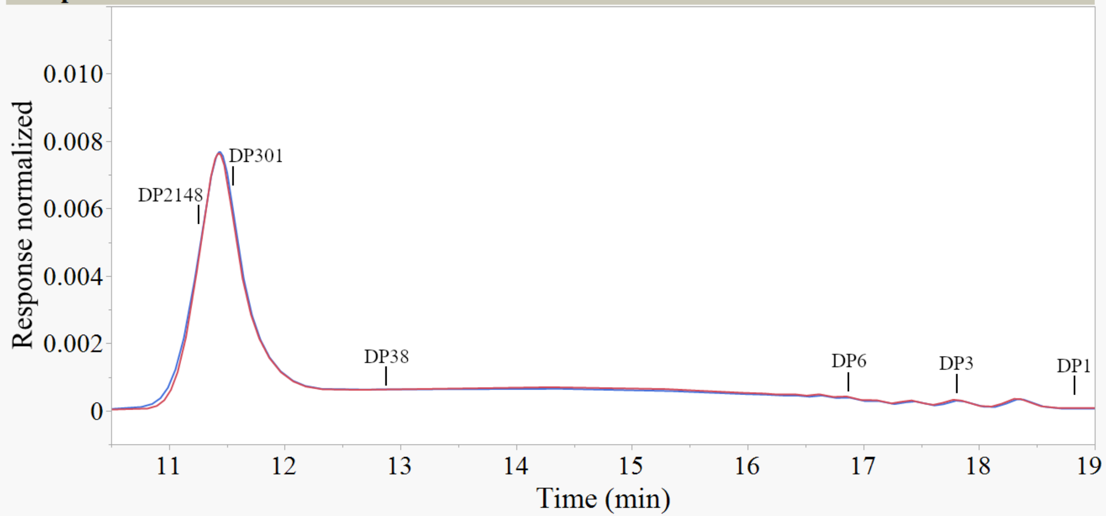
0.006
0.004
0.002
0
DP2148
DP301
DP38
DP6
DP3
DP1
11
12
13
14
15
16
17
18
19
Time (min)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0.006
0.004
0.002
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DP301
DP2148
DP38
DP6
DP3
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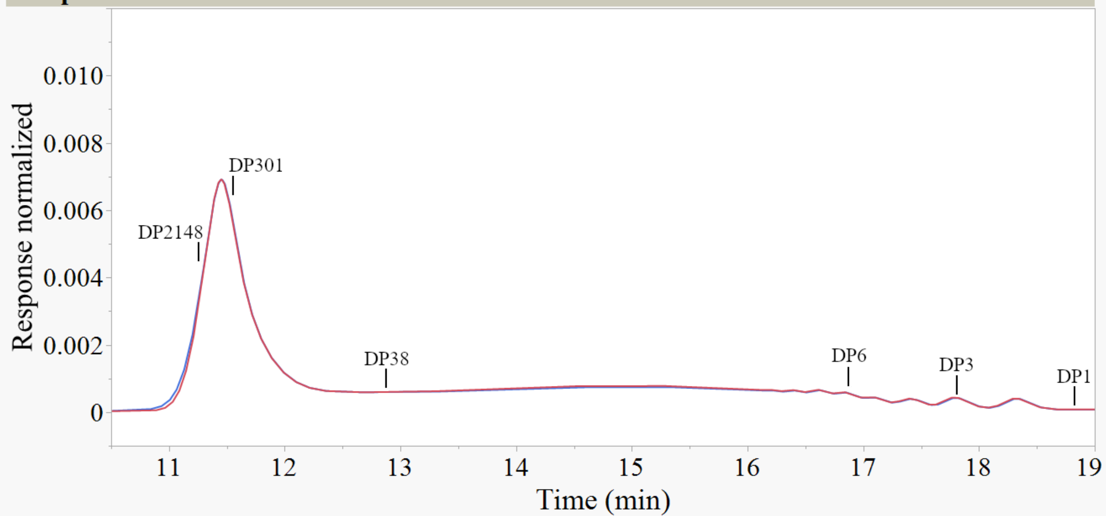
12
13
14
15
16
17
18
19
Time (min)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0.004
0.002
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DP301
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DP38
DP6
DP3
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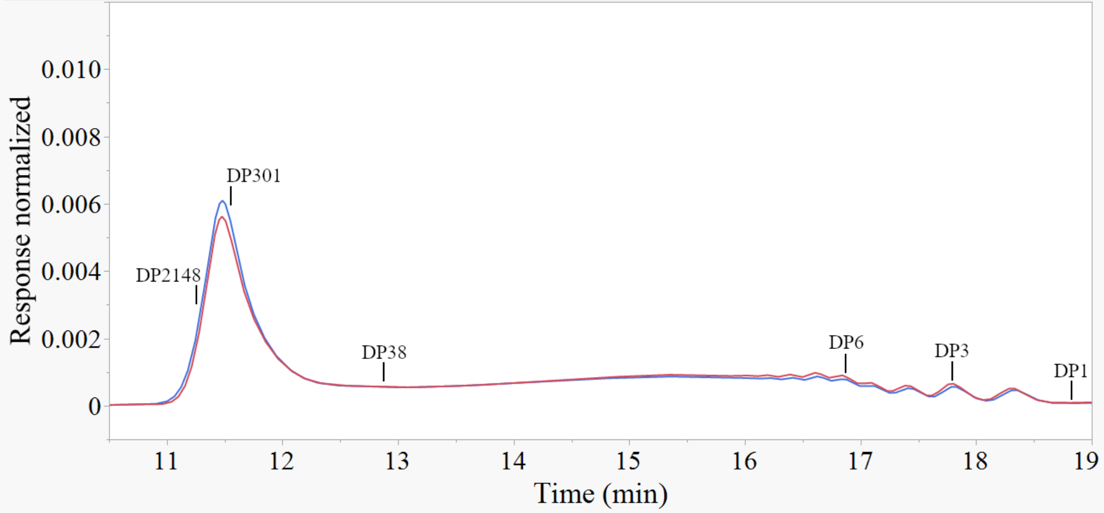
12
13
14
15
16
17
18
19
Time (min)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0.004
0.002
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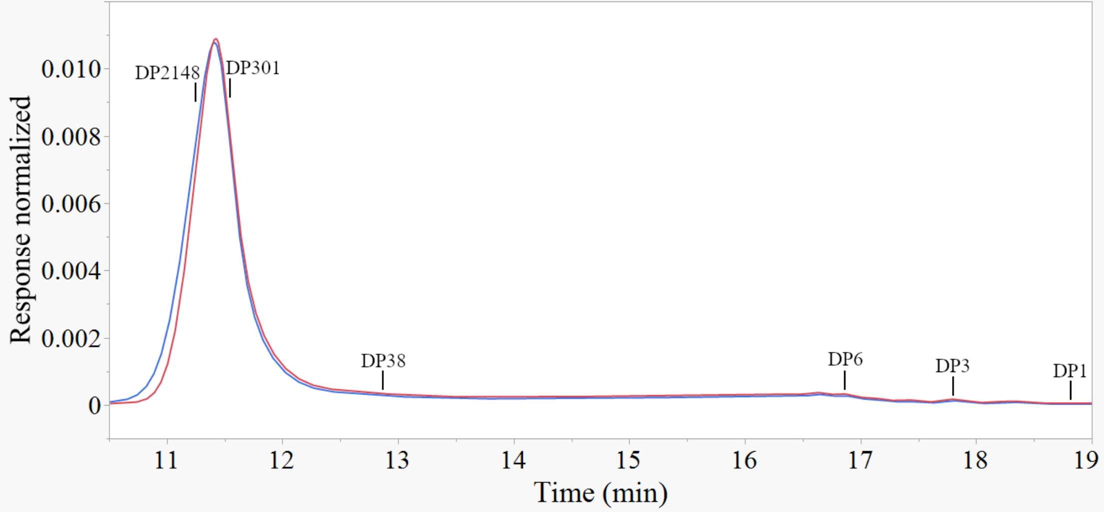
DP38
DP6
DP3
DP1
11
12
13
14
15
16
17
18
19
Time (min)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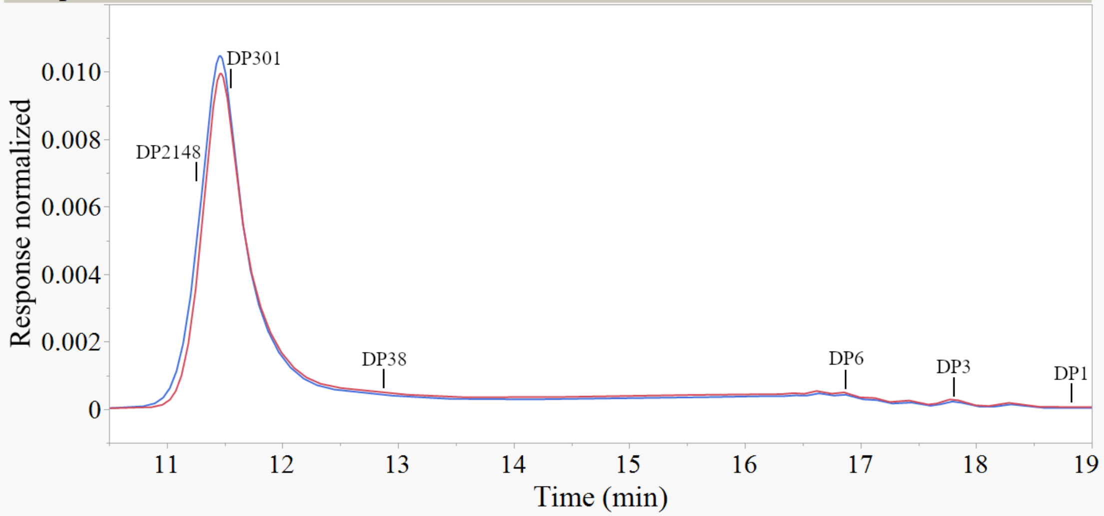
DP1
11
12
13
14
15
16
17
18
19
Time (min)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13
14
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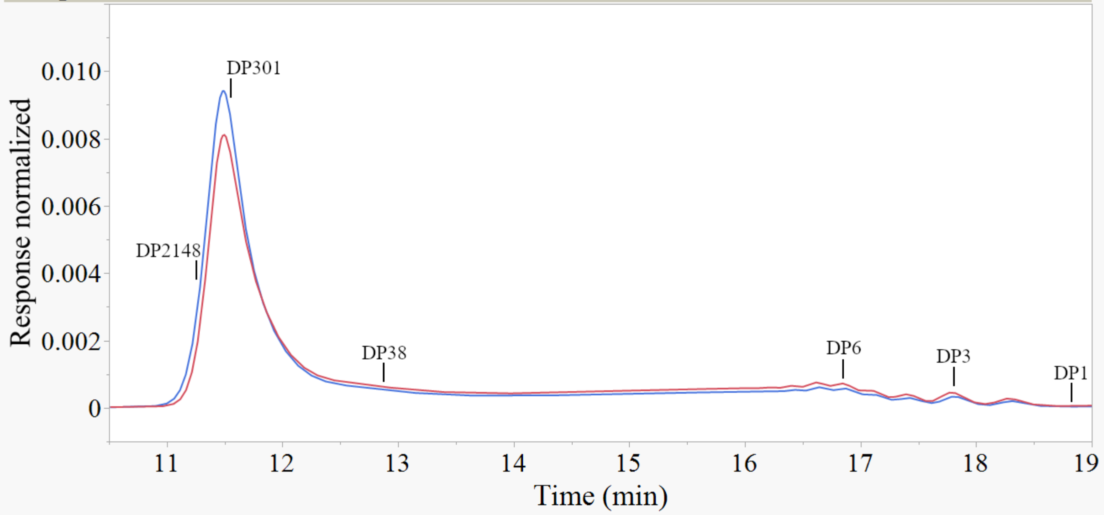
16
17
18
19
Time (min)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DP1
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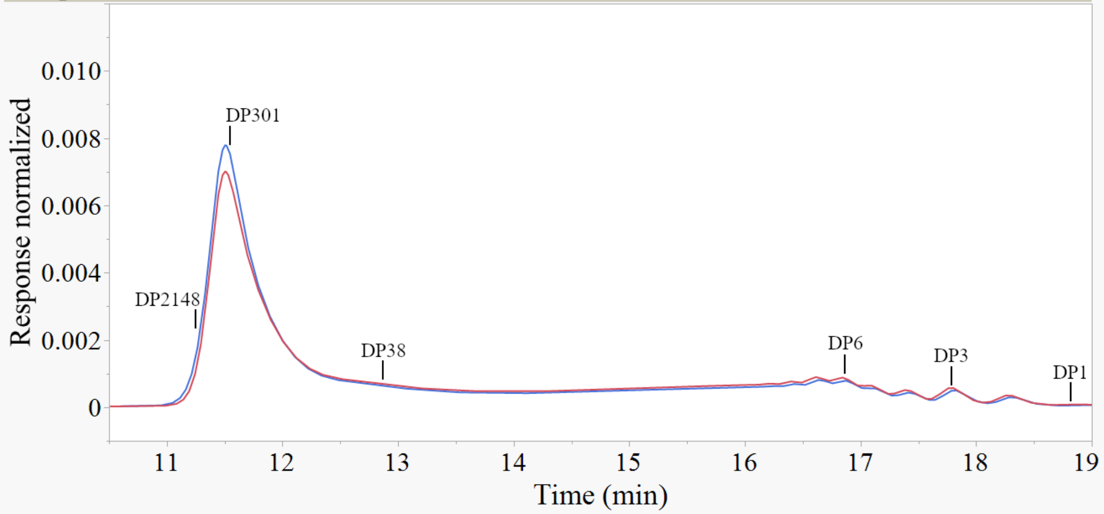
12
13
14
15
16
17
18
19
Time (min)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0.002
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DP6
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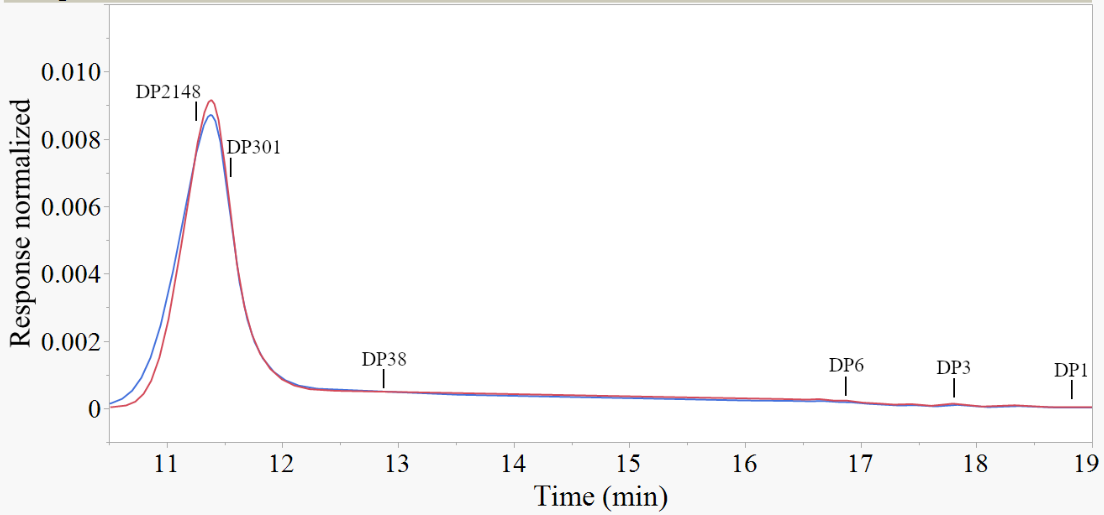
DP1
11
12
13
14
15
16
17
18
19
Time (min)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0.008
0.006
0.004
0.002
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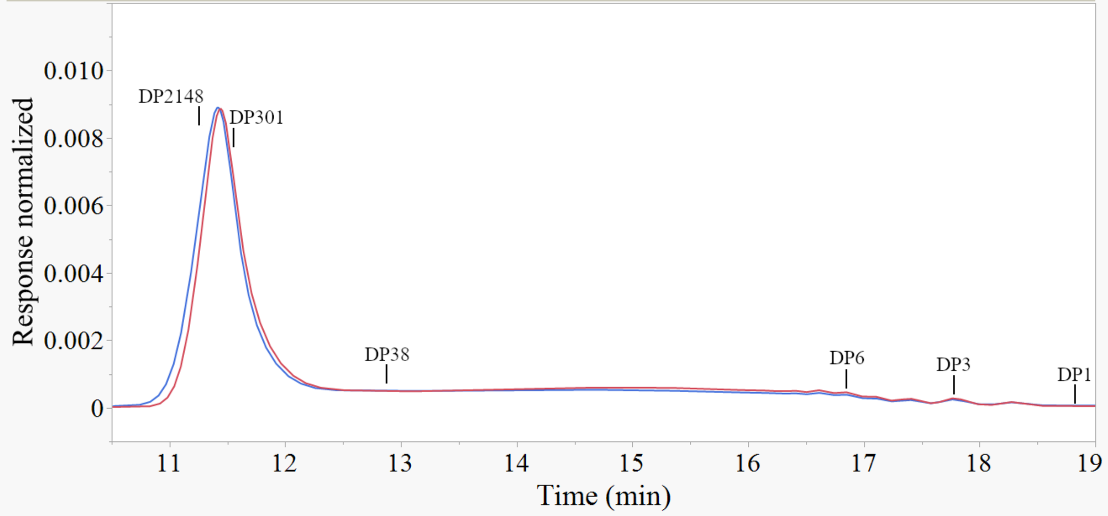
DP2148
DP301
DP38
DP6
DP3
DP1
11
12
13
14
15
16
17
18
19
Time (min)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Response normalized
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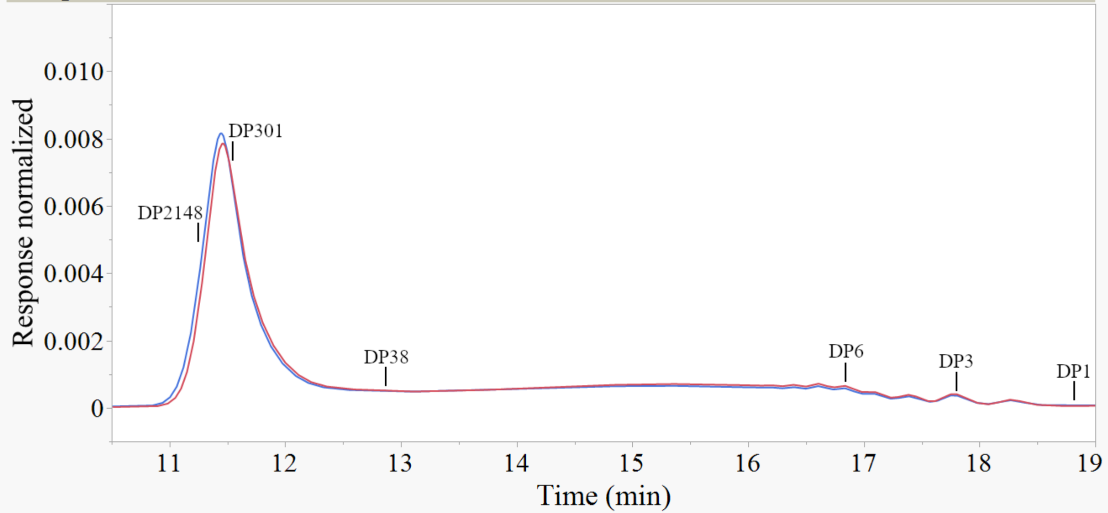
0.008
0.006
0.004
0.002
0
DP2148
DP301
DP38
DP6
DP3
DP1
11
12
13
14
15
16
17
18
19
Time (min)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0.004
0.002
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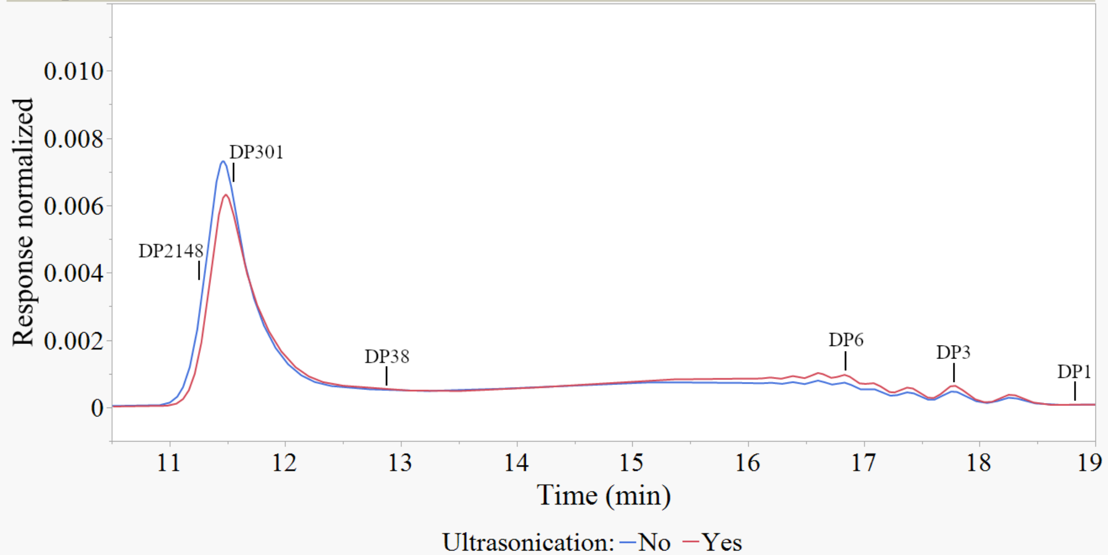
DP301
DP2148
DP38
DP6
DP3
DP1
11
12
13
14
15
16
17
18
19
Time (min)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DP301
DP2148
DP38
DP6
DP3
DP1
11
12
13
14
15
16
17
18
19
Time (min)
Ultrasonication: —No —Yes

Figure 4.1: High-performance size-exclusion chromatograms (HPSEC) of pea- (P), waxy maize- (W), and normal maize- (N) based maltodextrins made with (red) and without (blue) ultrasonication for 5, 10, 15, and 20 min. Labels indicate elution times of standards by degree of polymerization (DP). Results were the average of two replicates of a composite sample of three different maltodextrins made under the same conditions.

4.4.3 *Percentage of α-1,6 branch linkages of starch*

The measurement of the ratio of α-1,4 to α-1,6 linkages provided an indicator of the degree of hydrolysis and the progression of the enzymatic reaction. This was because the α-amylase selectively hydrolyzes the α-1,4 linkages while leaving the α-1,6 bonds intact (Svensson, 1994; Zhai *et al*., 2022). Results from ^{1}H NMR characterization of the native starches revealed the pea, waxy maize and normal maize starches had α-1,6 linkage percentages of 2.67%, 4.22%, and 3.24%, respectively (**Table 4.2**), corresponding well with their different amylose contents. The percentage of α-1,6 linkages increased in the samples reacted for 5 min and further increased in those reacted for 20 min. Both the pea- and waxy maize-based maltodextrins had a decrease in the percentage of α-1,6 linkages with the addition of ultrasonication while the normal maize-based maltodextrins had an increase. This suggested a less extensive reaction than the conditions with no ultrasonication for the pea and waxy maize starches (Zhai *et al*., 2022). One potential explanation of this difference was that the addition of ultrasonication to the reaction resulted in an alteration to the enzyme action pattern with the pea and waxy maize starches (Chorfa *et al*., 2022; Z. L. Yu *et al*., 2014; X. Zhang *et al*., 2022). Further research is required to elucidate the associated mechanisms.

Table 4.2. Percentages of α-1,4 and α-1,6 linkages in pea, waxy maize and normal maize starches and maltodextrins prepared with and without ultrasonication[1].

Sample	Sample type	Ultrasonication	Reaction time (min)	α-1,4 linkages (%)	α-1,6 linkages (%)
Pea	Native starch	-	0	97.33	2.67
	Maltodextrin	No	5	97.01	2.99
			20	96.59	3.41
		Yes	5	97.13	2.87
			20	96.96	3.04
Waxy maize	Native starch	-	0	95.78	4.22
	Maltodextrin	No	5	94.38	5.62
			20	94.30	5.70
		Yes	5	94.88	5.12
			20	94.70	5.30
Normal maize	Native starch	-	0	96.76	3.24
	Maltodextrin	No	5	96.48	3.52
			20	96.23	3.77
		Yes	5	96.39	3.61
			20	95.99	4.01

[1] Maltodextrin samples (5- and 20-min samples) were a composite of three different repeats all produced under the same reaction conditions. Each composite was run once (N=1).

4.4.4 *Viscosity*

With a longer enzymatic hydrolysis time, the viscosity of the generated maltodextrin samples continued to decline, in good agreement with the reduced molecular weights as (Ai & Jane, 2015)**.** The resulted also revealed that the addition of ultrasonication to the enzymatic hydrolysis resulted in a decrease of the viscosity of the maltodextrins up to 1.2 mPa·s (**Figure 4.2)**. The greatest reduction was observed in the 5-min samples, and the level of reduction was less obvious for a longer hydrolysis time. The decrease in the viscosity of maltodextrins caused by ultrasonication was probably due to better dispersion and solubilization of the samples after the treatment, which will be further discussed below. Because the 5-min samples had the lowest level of enzymatic breakdown and the poorest level of dispersion and solubilization (**Figure 4.2**), the ultrasonication treatment was able to reduce the viscosity to the highest extent.

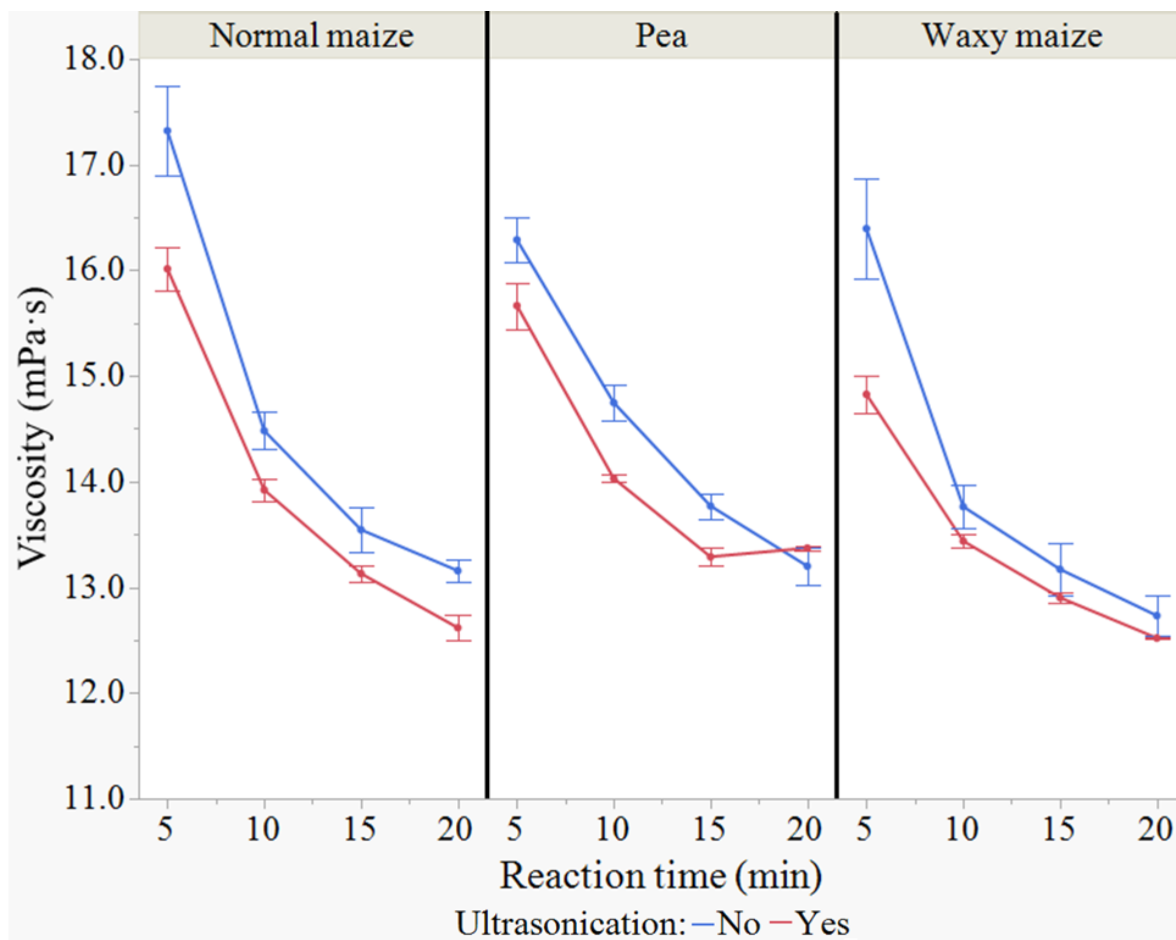


Figure 4.2. Viscosity of maltodextrins made with (red) and without (blue) ultrasonication. Values are presented by average ± standard deviation (N=3).

4.4.5 *Water solubility*

Water solubility results indicated the addition of ultrasonication to the enzymatic reaction increased the water solubility of the samples to varying degrees. The non-ultrasonicated pea maltodextrins were notably less soluble than the maize starches, which was attributed to its highest amylose content and strongest associations between starch molecules (L. Li *et al.*, 2019, 2020). The addition of ultrasonication led to a significant increase in the water solubility of the pea-based samples. This increased water solubility was attributed to the ultrasonication disrupting the granular structure and weakening molecular association of the starch to a greater extent, thereby allowing for better hydration of the molecules (McPherson & Seib, 1997). In contrast, the waxy maize samples had minimal change. This was due to the non-ultrasonicated samples having a high water solubility, thereby limiting the degree of change resulting from the addition of

ultrasonication. This high water solubility was due to the high amylopectin content of the waxy maize starch, which had noticeably less tendency to re-associate and retrograde (Bai & Shi, 2016; Lumdubwong & Seib, 2001). The normal maize samples had a slight increase in water solubility from the addition of ultrasonication, which could be partly explained by the intermediate amylose content of the native starch (**Figure 4.3**).

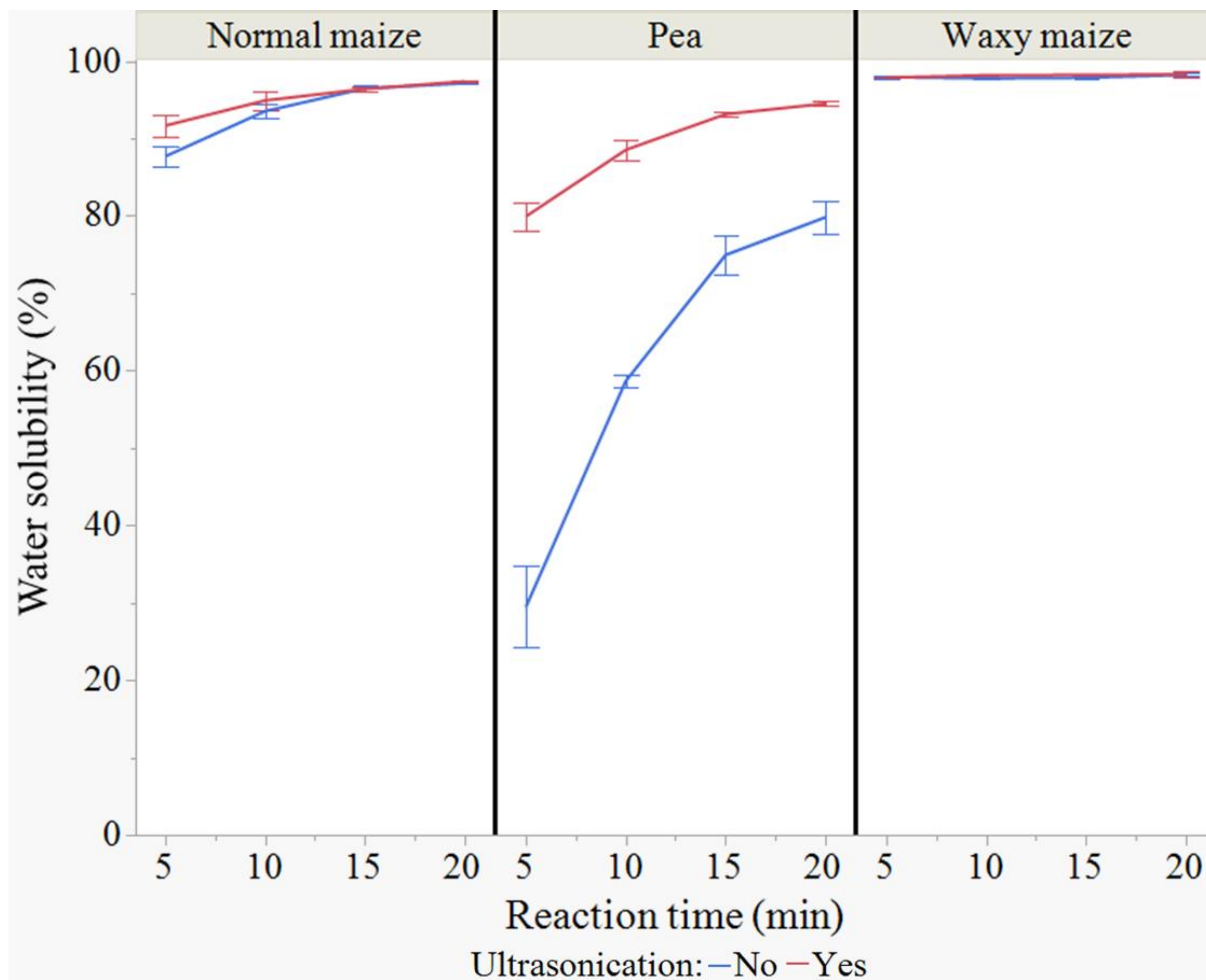


Figure 4.3. Water solubility of maltodextrins made with (red) and without (blue) ultrasonication. Values are presented by average ± standard deviation (N=3).

4.4.6 *Transmittance*

The maltodextrin samples generated from all the three starches showed a consistent trend of increasing transmittance as the reaction time increased. This was as expected, as the longer

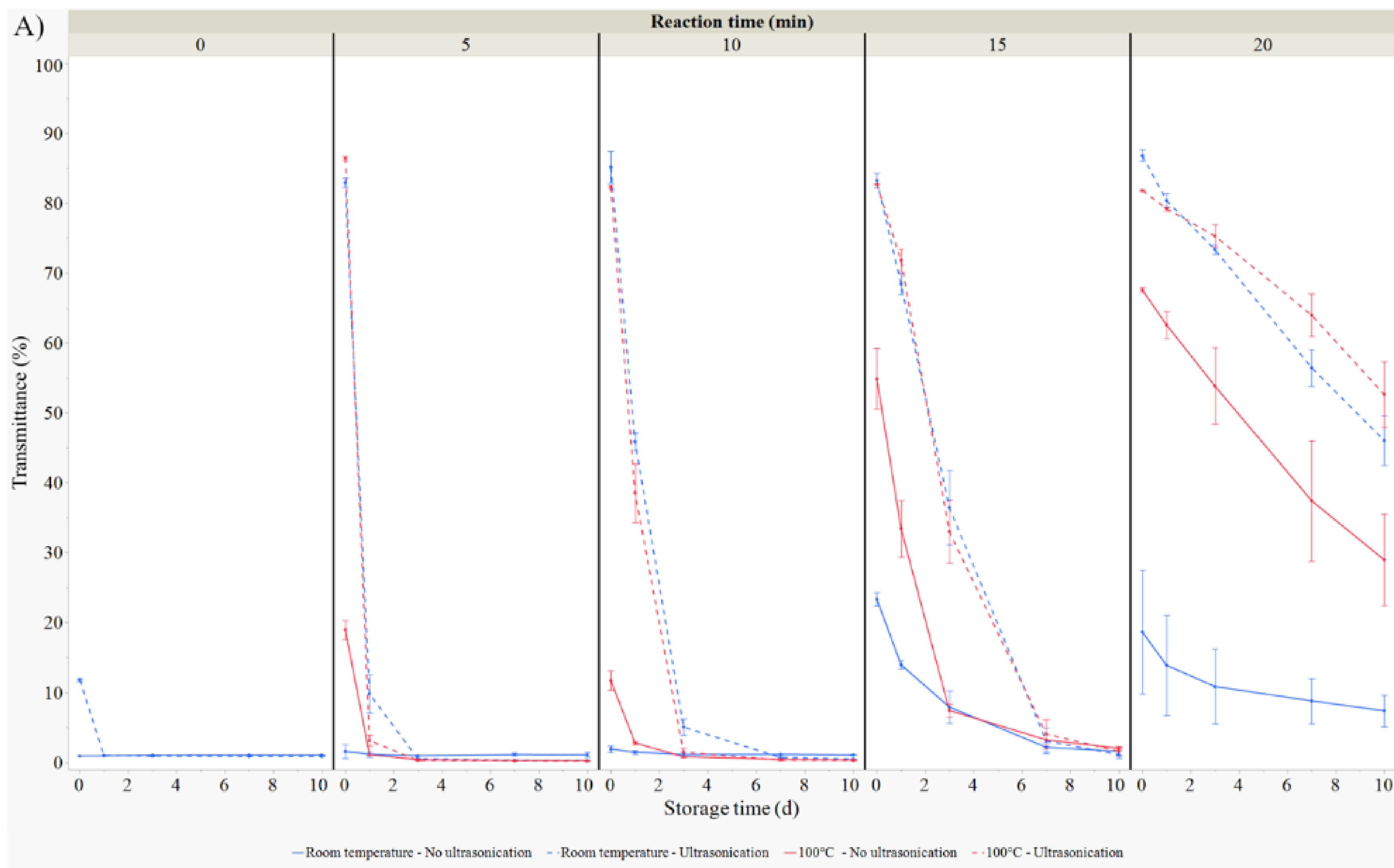
reaction time meant more hydrolysis of the starch. This resulted in a breakdown of the long amylose chains and branched amylopectin into shorter saccharides (**Figures 4.1**) (de Jesus & Maciel Filho, 2014). These shorter saccharides were more soluble and less likely to retrograde (S. Wang *et al*., 2015). Another expected result observed across all three starches was the samples that were boiled at 100°C for 30 min had a higher transmittance than the samples that were dissolved at room temperature. This result was due to the boiling of the maltodextrins allowing for better hydration and dispersion of the samples (S. Wang & Guo, 2020).

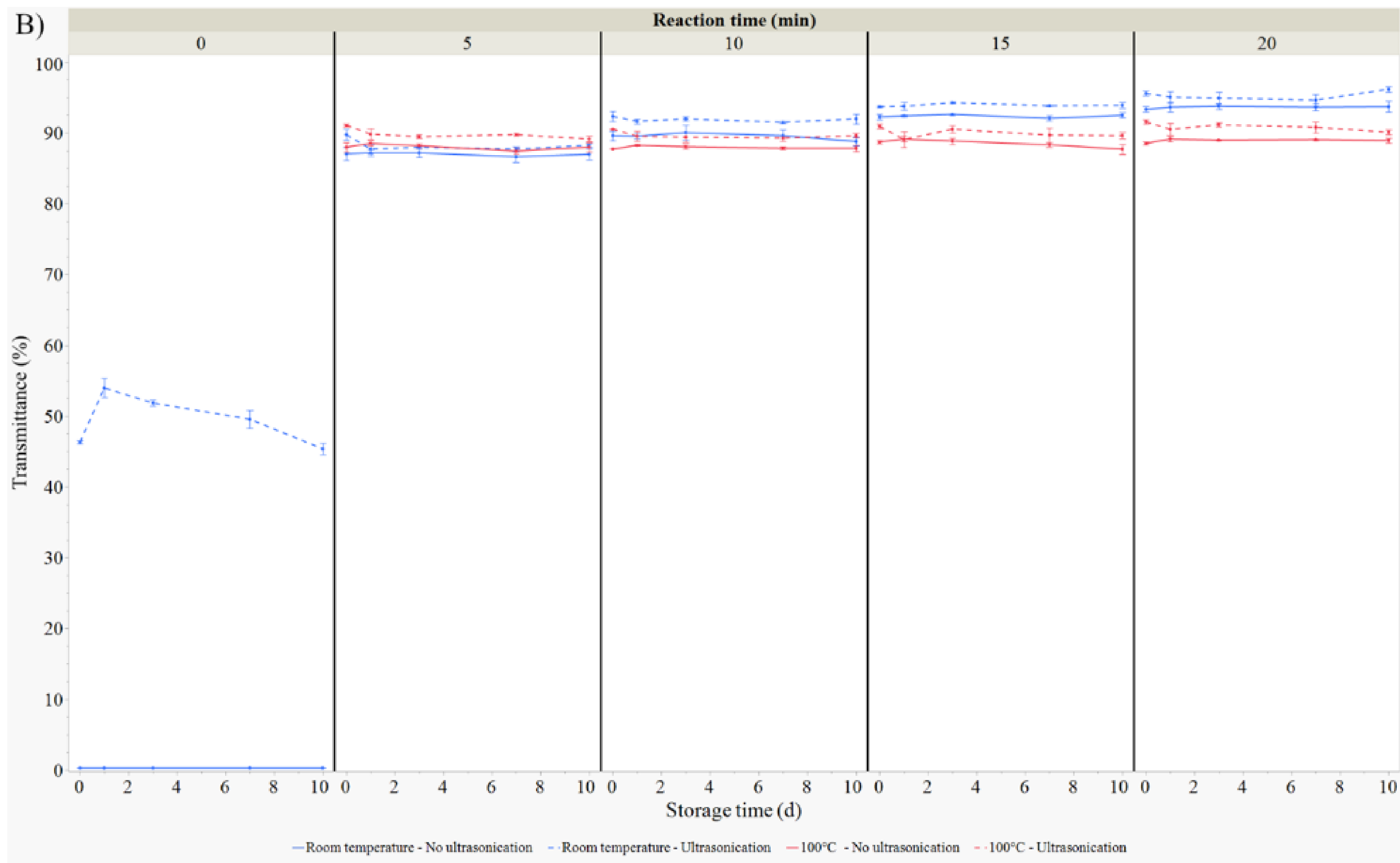
The measurement of the transmittance of the samples revealed the ultrasonicated normal maize had greater transmittance than the non-ultrasonicated samples. This was true for the samples both at room temperature and after being boiled for 30 min for all the time points from 5 to 20 min. This greater transmittance remained even after being held for 10 d at 4°C. Thus, these results indicated the addition of the ultrasonication to the reaction of the α-amylase with normal maize starch had a positive effect on the ability of the enzyme to hydrolyze the linear chains in the sample that were prone to retrogradation.

Similarly, the pea-based maltodextrins showed greater transmittance with the ultrasonicated samples than the non-ultrasonicated samples. However, unlike the normal maize samples, the increased transmittance was no longer observed in most samples after being held at 4°C for just one day. The exception to this were the 15- and 20-min non-ultrasonicated pea-based maltodextrins prepared at room temperature (**Figure 4.4**). The 15-min ultrasonicated pea-based maltodextrin prepared at room temperature retained higher transmittance up until 3-daystorage as compared to the non-ultrasonicated counterpart, while the 20-min ultrasonicated pea-based maltodextrin prepared at room temperature maintained higher transmittance up to 10-day storage as compared to the non-ultrasonicated counterpart. However, this increased transmittance was not observed in the samples after boiling. The data suggested the addition of ultrasonication to the reaction of the α-amylase with the pea starch resulted in disruption of the granular structure and weakening of molecular association (Chorfa *et al*., 2022), which allowed for the better hydration and dispersion of maltodextrin molecules. After boiling in water, the maltodextrin samples from the same starch had similar levels of hydration and dispersion, thus showing comparable transmittance (BeMiller & Whistler, 2009; S. Wang *et al*., 2015; S. Wang & Guo, 2020). However, this trend was not observed in the transmittance of across all the samples and time points. For all reaction times of the pea-based maltodextrins, the boiled samples showed an increased

transmittance over the non-boiled samples at early time points, with the level of increased transmittance decreasing as the time held at 4°C increased, which was attributable to retrogradation of starch chains. This trend was observed in both the native starch and the maltodextrin samples, with the effect being more significant in the maltodextrins. Furthermore, the degree of decrease in the difference became less prevalent as the reaction time of the α-amylase with the pea starch increased, with the samples reacted for 20 min that were boiled maintaining a higher transmittance even after being held for 10 d at 4°C. These results suggested the boiling of the pea-based maltodextrins led to better hydration of the samples as well as a reduced rate of retrogradation (S. Wang *et al.*, 2015). Since the transmittance of the boiled samples eventually reached the same degree as the non-boiled samples in all cases except for the samples hydrolyzed for 20 min by the α-amylase, it can be inferred that the boiling of the samples only slowed the rate of retrogradation rather than fully preventing it. This effect was only observed in the pea-based samples, but not the waxy-maize or normal maize-based samples. This was attributed to the higher amylose concentration and long branch chains of amylopectin in pea starch than the maize starches, which led to a faster retrogradation rate of pea starch (L. Li *et al.*, 2019) The exception to this trend in the pea-based samples was the maltodextrins made with 20-min reaction time. In these samples, while the gap between the boiled and non-boiled samples decreased over the 10-day storage, the boiled samples did not decrease to the same level of transmittance as the non-boiled samples. This slower decrease was likely due to the higher solubility and better dispersibility of the 20-min samples (**Figure 4.4**), which retrograded at a slower rate (Zhong *et al.*, 2021). Therefore, over the 10-day storage, the degree of retrogradation of the boiled maltodextrins were lower than that of the non-boiled counterpart.

Finally, unlike the normal maize-based and pea-based maltodextrins, the waxy maize-based maltodextrins only showed marginal increases in the transmittance with the addition of ultrasonication in the samples hydrolyzed by the thermostable α-amylase for 5-20 min under the conditions of room temperature and boiling. Non-ultrasonicated waxy maize-based maltodextrin dispersed in water at room temperature already showed a high transmittance because it virtually contained no amylose (**Figure 4.4**) and had the lowest viscosity and highest water solubility (**Figures 4.2** and **4.3**). The addition of ultrasonication to the enzymatic conversion process enhanced the transmittance of the samples to a limited level.


A)
Reaction time (min)
0
5
10
15
20
Transmittance (%)
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10
Storage time (d)
Room temperature - No ultrasonication
Room temperature - Ultrasonication
100°C - No ultrasonication
100°C - Ultrasonication


B)
Reaction time (min)
0
5
10
15
20
Transmittance (%)
Storage time (d)
Room temperature - No ultrasonication
Room temperature - Ultrasonication
100°C - No ultrasonication
100°C - Ultrasonication

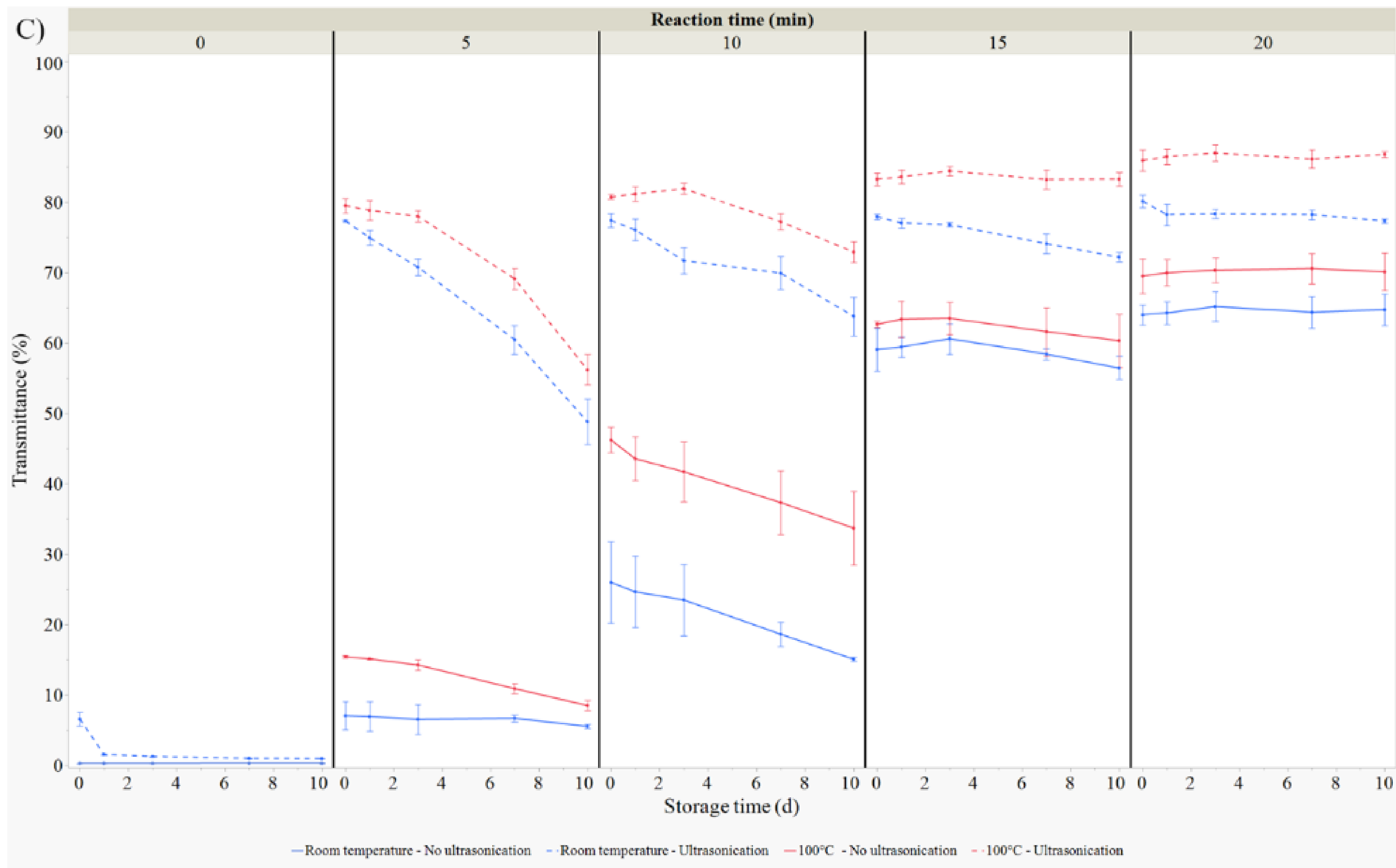

C)
Reaction time (min)
0
5
10
15
20
Transmittance (%)
Storage time (d)
Room temperature - No ultrasonication
Room temperature - Ultrasonication
100°C - No ultrasonication
100°C - Ultrasonication

Figure 4.4. Transmittance at 640 nm after heating at room temperature (solid line) or 100°C (dotted line) and subsequent storage at 4°C for 0-10 d of native starches and maltodextrins made with (red) and without (blue) ultrasonication. Values are presented by average ± standard deviation (N=3). A = Pea-based maltodextrins; B = Waxy maize-based maltodextrins; C = Normal maize-based maltodextrins.

4.4.7 *Enzyme activity*

Analysis of the activity of the α-amylase activity with and without ultrasonication revealed the addition of ultrasonication to the reaction had no significant effect on the activity of the enzyme: the enzyme had an activity of 1349 ± 111 U without ultrasonication present and an activity of 1298 ± 34 U with ultrasonication present. This finding was in good accordance with the reported literature, which indicated that ultrasonication did not have a negative impact on the ability of an enzyme to hydrolyze starch (Chorfa *et al*., 2022; Lu *et al*., 2018; X. Zhang *et al*., 2022). The observation was also in good accordance with the lack of significant changes in the molecular structures of pea, waxy maize and normal maize-based maltodextrins caused by the incorporation of ultrasonication to the enzymatic conversion by thermostable α-amylase as discussed in previous sections of this chapter.

4.5 Conclusions

The addition of ultrasonication to the reaction of the Termamyl 120L α-amylase with pea, waxy maize and normal maize starches to produce maltodextrin led to limited changes in the molecular structures of the maltodextrins and more significant changes to the physicochemical properties. Overall, the normal maize-based maltodextrins showed a greater change in DE and percentage of α-1,6 linkages than either the pea or waxy maize-based maltodextrins. This indicated that the enzyme had a relatively greater increase in the degree of hydrolysis of the normal maize-based maltodextrins with the aid of ultrasonication. Results also showed that the pea-based maltodextrins had a greater increase in water solubility and transmittance in water at room temperature compared to either the waxy maize- or normal maize-based maltodextrins. Therefore, despite the greater degree of hydrolysis, the normal maize was not as impacted in these properties. This lesser impact compared to the pea-based maltodextrins was due to the normal maize-based maltodextrins having a higher water solubility and transmittance in water at room temperature as compared to the pea-based samples. This was supported by the analysis of the activity of the enzyme both with and without ultrasonication, which showed no significant difference in the activity of the enzyme from the addition of ultrasonication to the reaction. Overall, this study found the addition of ultrasonication the reaction of α-amylase with starch had minimal effect on the

activity of the enzyme. The results also showed the more significant effects on the functional properties of the starches, with the pea-based maltodextrins showing the greatest extents of changes, particularly in water solubility.

5 General discussion and conclusions

Every year, approximately 3.66 million tonnes of peas are grown in western Canada (N. Wang, 2020). The main components of the peas are protein and starch, with the protein being the primary product from the plant. The starch is utilized in low-value applications, such as animal feed and filler (Wu *et al*., 2023). Despite this, peas are approximately 45% – 50% starch and 20% – 30% protein, meaning much starch is produced but not utilized to its full potential (N. Wang, 2020). The research of this thesis aimed to develop value-added modifications for pea starch to better utilize this under-valued co-product from the production of pea protein as well as to demonstrate their functionality compared to waxy and normal maize starches. Two different modifications were investigated: pyrodextrinization and the production of maltodextrin in the presence of ultrasonication. For both modifications, it was found that pea starch was better suited for the modification under the tested conditions.

5.1 Comparison of pyrodextrins and maltodextrins

While both pyrodextrins and maltodextrins are derivatives from the hydrolysis of starch, there are several key differences between the two products. Firstly, the pyrodextrin production process involves not only the hydrolysis of existing linkages but also the formation of new branched linkages. This hydrolysis by acid (*e.g.*, HCl) cleaves both α-1,4 and α-1,6 glycosidic bonds of amylose and amylopectin randomly (BeMiller & Whistler, 2009; Moore *et al*., 2015). The maltodextrin production process, in contrast, only involves the hydrolysis of glycosidic bonds, without the formation of new bonds. Additionally, the hydrolysis by α-amylases cleaves α-1,4 bonds only, leaving α-1,6 bonds intact (P. Chen *et al*., 2017; Liang *et al*., 2024; Zuo *et al*., 2017). This leads to a significantly different distribution of glycosidic linkages as compared to the acid hydrolysis process. In addition, the different preparation methods lead to different molecular-weight distributions of the pyrodextrins and maltodextrins (**Figures 3.4 and 4.1**). These structural differences were responsible for the different physicochemical properties and digestibility of the two starch derivatives.

Another notable difference between pyrodextrins and maltodextrins is the color of the products, in both solid and dissolved states. Maltodextrins typically have a white color as a dry powder. After dispersed in water, a high-DE maltodextrin will produce a solution with good clarity, while a low-DE product will have a hazy white color mainly due to poor solubility and dispersibility. In contrast, pyrodextrins have a yellow-orange color as a powder (**Figure 3.2**) and in solution, resulting from browning reactions during the modification (Ajandouz *et al*., 2001; Englyst *et al*., 1992; Kitaoka & Suzu, 1967).

Lastly, pyrodextrins and maltodextrins showed different resistance to enzymatic hydrolysis. Pea, waxy maize, and normal maize-based pyrodextrins had enzymatic hydrolysis rates of 68.2%, 87.5%, and 82.5%, respectively (**Figure 3.5**). This was significantly lower than that of maltodextrins, which is known to be almost completely digestible by amylolytic enzymes (BeMiller & Whistler, 2009; Lumdubwong & Seib, 2001). This difference in resistance to enzymatic hydrolysis affects the applications of the starch products. Pyrodextrins are preferably used in applications in health-oriented foods and beverages. Potential applications of pyrodextrins include bread, ice cream, candy, salad dressings, and beverages (Inada *et al*., 1994; Ohkuma *et al*., 1997). In contrast, maltodextrins are not added for their health benefits, but rather their physicochemical properties. They are used in are used in a wide range of products, such as snack foods, desserts, beverages, frozen meals, meal replacement drinks, and as fillers in medication. They are consumed by a large majority of people on a daily basis (BeMiller & Whistler, 2009; Stephen *et al*., 2006).

5.2 Conclusions

Pea, waxy maize, and normal maize starches were all used to produce pyrodextrins. The starch samples were treated with acid, then dewatered and dried to a target moisture content of ~15%, and finally heated in an oven at 180°C for up to 4 h. Results from this process showed that the pea starch was much better suited to the pyrodextrinization process as compared to both the waxy and normal maize starches. In general, the pea-based pyrodextrins, relative to the waxy and normal maize-based pyrodextrins, had a greater reduction in amylose content, and *in vitro* digestibility as well as greater color development, change in molecular weight distribution, and increases in water solubility and transmittance. These results explicitly demonstrated the pea starch

was better suited for pyrodextrinization than both waxy and normal maize starches under the tested conditions. These results also revealed the untapped potential benefits of pea starch and support the overall goal of creating value-added products from this underutilized co-product from pea protein extraction.

In the second study, pea, waxy maize, and normal maize starches were slurried and then hydrolyzed using a thermostable α-amylase at 90.0°C ± 5.0°C with and without ultrasonication to produce maltodextrin. The goal of this study was to determine the effect ultrasonication had on the enzymatic reaction and to determine any changes to the resultant maltodextrins. Results from this process indicated the addition ultrasonication to the reaction had minimal impact on the enzyme. This was in contrast with previous findings in reported literature and attributed to the production scale representative reaction temperature of 90°C used in this study, which minimized the impact of the ultrasonication. While no significant change was observed in the enzymatic effects on the starch, there were notable changes to the physiochemical properties of the starches. Specifically, the decreased viscosity as well as increased water solubility and transmittance in the samples generated with ultrasonication compared to those generated without were noteworthy. This suggested the ultrasonication disrupted the granular structure, allowing for better hydration and dispersion of the maltodextrins. This effect was more prominent in the pea-based maltodextrins compared to the maize-based maltodextrins. The greater changes in the pea-based maltodextrins were due to the greater gelling ability of the pulse starch. Overall, the addition of ultrasonication to the enzymatic hydrolysis of starch via α-amylase was less effective than hypothesized and additional research is needed to further improve the technique before scale-up and commercialization of the process is feasible.

6 Future studies

While extensive work was completed in the completion of this research, not all possible avenues of interest were able to be investigated. This section aims to highlight potential avenues to expand upon the existing research and provide guidance on possible next steps. These future experiments will provide help elucidate areas still under question from the current research and allow for the continued expansion of the scientific process completed herein.

6.1 Future studies for Study 1

Firstly, while the 95% water solubility of the 4-h pea-based pyrodextrin was significant, it was not close to the 100% solubility that has been reported in the literature for other starches (Bai *et al*., 2014; Weil *et al*., 2020). Therefore, it will be of interest to investigate methods to increase the water solubility of the pea-based pyrodextrins to 100%. One potential method to achieve this goal will be to increase the heating time of the sample at 180°C. As the increase in the heating time of the sample were shown to positively correlate with an increase in the degree of pyrodextrinization and subsequently the water solubility. Increased heating times of 6 h and 8 h will help to determine the ideal heating time required to achieve complete solubility of the pea-based pyrodextrin. However, the longer heating times may also have negative effects on the samples, such as stronger color development.

Another aspect for future research to expand on the pyrodextrinization research is the controlling of the moisture content of the samples during heating at 180°C. It is well documented in existing literature and also confirmed in the research presented here that a moisture content of around 15% is the ideal concentration of water for pyrodextrinization (Bai *et al.*, 2014; Bai & Shi, 2016; Han *et al.*, 2018). During the heating step, most – if not all – moisture is evaporated. A method that can monitor the moisture and pH change during pyrodextrinization can help us better control the pyrodextrin process.

6.2 Future studies for Study 2

One next step for this project will be to investigate why the addition of ultrasonication to the reaction only had a minimal impact on the ability of the enzyme to break down the starch rather than the more obvious positive impact that has been reported in the literature. One potential explanation for the absence of significant improvement from this physical treatment was that the temperature of 90°C used for the reaction. While this temperature was in line with what typically is used when producing maltodextrin at a larger scale, it is higher than what has been reported in the existing literature (Abedi *et al*., 2022; Rahaman *et al*., 2021). Additionally, it is widely reported that the effects of ultrasonication decrease as the solution temperature increases due to the reduced solution viscosity making it more difficult to form cavitation bubbles. Since these bubbles are the key factor to disrupt starch structures at both granular and molecular levels for faster enzymatic breakdown, a reduced level of cavitation will result in the ultrasonication being less beneficial. To overcome this difficultly, future work can investigate running the reaction at a lower temperature, such as 70°-80°C, while still maintaining reaction conditions favorable to scaling up the process.

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8 APPENDIX

8.1 Tables

Table A.1. Transmittance at 640 nm after heating at room temperature or 100°C and subsequent storage at 4°C for 0 to 10 d of native starches and maltodextrins prepared with and without ultrasonication[1].

Sample	Ultrasonication	Assay preparation temperature (°C)[2]	Rxn time (min)	Transmittance (%) after storage at 4°C				
				0 d	1 d	3 d	7 d	10 d
Pea	No	Room temperature	0	0.88 ± 0.01^{b,UV}	0.92 ± 0.02^{b,WX}	1.03 ± 0.02^{a,TU}	1.05 ± 0.03^{a,RS}	1.04 ± 0.04^{a,S}
			5	1.55 ± 0.99^{a,K}	1.15 ± 0.39^{a,IJK}	0.94 ± 0.05^{a,JKL}	1.11 ± 0.21^{a,KL}	1.09 ± 0.35^{a,MN}
			10	1.88 ± 0.47^{a,K}	1.44 ± 0.24^{ab,HIJK}	1.11 ± 0.26^{b,IJK}	1.14 ± 0.04^{b,HIJ}	1.06 ± 0.07^{b,J}
			15	23.35 ± 0.99^{a,K}	13.95 ± 0.58^{b,HIJ}	7.9 ± 2.33^{c,GHIJ}	2.14 ± 0.64^{d,GH}	1.56 ± 0.56^{d,GH}
			20	18.63 ± 8.87^{a,IJK}	13.88 ± 7.16^{a,GHI}	10.84 ± 5.33^{a,FGH}	8.78 ± 3.23^{a,FG}	7.37 ± 2.27^{a,FG}
		100 °C	0	11.80 ± 0.18^{a,UV}	0.99 ± 0.01^{b,WX}	0.87 ± 0.01^{b,TU}	0.84 ± 0.01^{b,RS}	0.83 ± 0.02^{b,S}
			5	82.92 ± 0.61^{a,JK}	9.79 ± 2.75^{b,GHI}	0.51 ± 0.07^{c,FGHI}	0.28 ± 0.00^{c,HIJ}	0.24 ± 0.00^{c,KL}
			10	85.13 ± 2.27^{a,HIJK}	45.86 ± 1.27^{b,EFGH}	5.04 ± 1.16^{c,EFG}	0.68 ± 0.09^{d,FG}	0.46 ± 0.04^{d,GH}
			15	83.24 ± 1.05^{a,FGHIJK}	68.4 ± 1.51^{b,DEFG}	36.44 ± 5.26^{c,DEF}	3.06 ± 1.83^{d,EF}	1.09 ± 0.56^{d,EF}
			20	86.77 ± 0.81^{a,DEFGHI}	80.36 ± 0.96C^{b,DEF}	73.34 ± 0.70^{c,CDE}	56.45 ± 2.66^{d,DE}	46.04 ± 3.53^{e,DE}
	Yes	Room temperature	0	0.88 ± 0.01^{b,UV}	0.92 ± 0.02^{b,WX}	1.03 ± 0.02^{a,TU}	1.05 ± 0.03^{a,RS}	1.04 ± 0.04^{a,S}
			5	18.97 ± 1.31^{a,CDEFG}	1.1 ± 0.06^{b,CDE}	0.36 ± 0.01^{b,CDE}	0.24 ± 0.01^{b,DE}	0.21 ± 0.01^{b,DE}
			10	11.69 ± 1.40^{a,ABCDE}	2.76 ± 0.23^{b,ABCD}	0.79 ± 0.14^{c,ABCD}	0.41 ± 0.03^{c,ABCD}	0.33 ± 0.03^{c,BCDE}
			15	54.86 ± 4.36^{a,ABC}	33.42 ± 4.07^{b,ABC}	7.42 ± 0.92^{c,ABC}	3.20 ± 0.82^{c,ABCD}	2.00 ± 0.10^{c,ABCD}
			20	67.56 ± 0.39^{a,AB}	62.59 ± 1.97^{a,AB}	53.84 ± 5.45^{a,AB}	37.39 ± 8.63^{b,ABC}	28.96 ± 6.50^{b,ABC}
		100 °C	0	11.80 ± 0.18^{a,UV}	0.99 ± 0.01^{b,WX}	0.87 ± 0.01^{b,TU}	0.84 ± 0.01^{b,RS}	0.83 ± 0.02^{b,S}
			5	86.43 ± 0.30^{a,BCDEFG}	3.12 ± 0.77^{b,BCD}	0.27 ± 0.02^{c,BCDE}	0.19 ± 0.01^{c,CDE}	0.16 ± 0.00^{c,BCDE}
			10	82.29 ± 0.29^{a,BCDEFG}	38.52 ± 4.18^{b,BCD}	1.43 ± 0.56^{c,BCDE}	0.32 ± 0.07^{c,BCDE}	0.24 ± 0.04^{c,CDE}
			15	82.67 ± 0.11^{a,BCDEF}	71.79 ± 1.58^{b,ABCD}	33 ± 4.52^{c,ABCD}	4.03 ± 2.08^{d,BCDE}	1.57 ± 0.7^{d,CDE}
			20	81.78 ± 0.11^{a,BCDEF}	79.19 ± 0.28^{a,ABCD}	75.29 ± 1.60^{a,ABCD}	63.97 ± 3.06^{b,ABCDE}	52.63 ± 4.76^{c,BCDE}

Waxy maize	No	Room temperature	0	$0.29 \pm 0.00^{a,VW}$	$0.29 \pm 0.00^{a,WX}$	$0.29 \pm 0.01^{a,TU}$	$0.3 \pm 0.01^{a,RS}$	$0.29 \pm 0.01^{a,S}$
			5	$87.03 \pm 0.95^{a,VW}$	$87.16 \pm 0.46^{a,WX}$	$87.16 \pm 0.65^{a,U}$	$86.63 \pm 0.76^{a,RS}$	$86.97 \pm 0.81^{a,S}$
			10	$89.61 \pm 0.66^{a,VW}$	$89.54 \pm 0.41^{a,WX}$	$90.02 \pm 1.04^{a,TU}$	$89.61 \pm 0.83^{a,RS}$	$88.79 \pm 0.62^{a,S}$
			15	$92.26 \pm 0.37^{a,QR}$	$92.40 \pm 0.12^{a,U}$	$92.61 \pm 0.12^{a,RS}$	$92.05 \pm 0.37^{a,RS}$	$92.47 \pm 0.37^{a,RS}$
			20	$93.33 \pm 0.37^{a,RS}$	$93.61 \pm 0.66^{a,U}$	$93.76 \pm 0.43^{a,RS}$	$93.61 \pm 0.50^{a,PQ}$	$93.69 \pm 0.76^{a,R}$
		100 °C	0	$46.31 \pm 0.22^{c,VW}$	$53.96 \pm 1.39^{a,WX}$	$51.84 \pm 0.38^{ab,TU}$	$49.56 \pm 1.28^{b,RS}$	$45.36 \pm 0.78^{c,S}$
			5	$89.74 \pm 0.75^{a,RS}$	$87.70 \pm 0.70^{b,WX}$	$87.97 \pm 0.12^{b,U}$	$87.70 \pm 0.35^{b,S}$	$88.24 \pm 0.23^{b,S}$
			10	$92.33 \pm 0.68^{a,TU}$	$91.62 \pm 0.37^{a,WX}$	$91.97 \pm 0.32^{a,U}$	$91.48 \pm 0.12^{a,S}$	$91.98 \pm 0.68^{a,S}$
			15	$93.68 \pm 0.12^{a,O}$	$93.76 \pm 0.57^{a,S}$	$94.26 \pm 0.13^{a,ST}$	$93.83 \pm 0.12^{a,QRS}$	$93.90 \pm 0.45^{a,RS}$
			20	$95.57 \pm 0.34^{a,LM}$	$95.06 \pm 0.76^{a,NO}$	$94.92 \pm 0.83^{a,N}$	$94.63 \pm 0.78^{a,N}$	$96.16 \pm 0.38^{a,O}$
	Yes	Room temperature	0	$0.29 \pm 0.00^{a,VW}$	$0.29 \pm 0.00^{a,WX}$	$0.29 \pm 0.01^{a,TU}$	$0.3 \pm 0.01^{a,RS}$	$0.29 \pm 0.01^{a,S}$
			5	$87.97 \pm 0.71^{a,ABCDE}$	$88.51 \pm 0.4^{a,BCD}$	$88.17 \pm 0.31^{a,BCDE}$	$87.43 \pm 0.42^{a,BCDE}$	$87.97 \pm 0.65^{a,BCDE}$
			10	$87.70 \pm 0.00^{a,ABC}$	$88.24 \pm 0.12^{a,ABC}$	$88.04 \pm 0.31^{a,ABC}$	$87.84 \pm 0.23^{a,ABCD}$	$87.84 \pm 0.42^{a,ABCD}$
			15	$88.72 \pm 0.20^{ab,AB}$	$89.13 \pm 0.41^{a,AB}$	$88.85 \pm 0.43^{ab,AB}$	$88.31 \pm 0.35^{ab,AB}$	$87.70 \pm 0.73^{b,AB}$
			20	$88.51 \pm 0.20^{a,A}$	$89.13 \pm 0.36^{a,A}$	$88.99 \pm 0.12^{a,A}$	$89.06 \pm 0.12^{a,A}$	$88.92 \pm 0.36^{a,A}$
		100 °C	0	$46.31 \pm 0.22^{c,VW}$	$53.96 \pm 1.39^{a,WX}$	$51.84 \pm 0.38^{ab,TU}$	$49.56 \pm 1.28^{b,RS}$	$45.36 \pm 0.78^{c,S}$
			5	$91.06 \pm 0.24^{a,ABCDE}$	$89.81 \pm 0.78^{b,ABC}$	$89.47 \pm 0.24^{b,ABCD}$	$89.74 \pm 0.21^{b,ABCD}$	$89.13 \pm 0.41^{b,BCDE}$
			10	$90.50 \pm 0.12^{a,ABCDE}$	$89.54 \pm 0.74^{a,ABCD}$	$89.4 \pm 0.72^{a,ABCD}$	$89.26 \pm 0.43^{a,ABCDE}$	$89.61 \pm 0.32^{a,BCD}$
			15	$90.92 \pm 0.32^{a,ABCDE}$	$89.06 \pm 1.13^{a,ABCD}$	$90.5 \pm 0.48^{a,ABCD}$	$89.68 \pm 1.04^{a,ABCD}$	$89.61 \pm 0.48^{a,BCD}$
			20	$91.55 \pm 0.32^{a,ABCD}$	$90.51 \pm 0.84^{a,ABC}$	$91.13 \pm 0.32^{a,ABC}$	$90.78 \pm 0.75^{a,ABCD}$	$90.09 \pm 0.32^{a,BCD}$

Normal maize	No	Room temperature	0	$0.26 \pm 0.00^{c,W}$	$0.26 \pm 0.00^{bc,X}$	$0.26 \pm 0.01^{abc,U}$	$0.27 \pm 0.00^{a,S}$	$0.27 \pm 0.00^{ab,S}$
			5	$7.03 \pm 1.97^{a,UV}$	$6.90 \pm 2.12^{a,VW}$	$6.53 \pm 2.12^{a,STU}$	$6.67 \pm 0.51^{a,PQR}$	$5.51 \pm 0.30^{a,QRS}$
			10	$25.95 \pm 5.83^{a,Q}$	$24.63 \pm 5.08^{a,T}$	$23.47 \pm 5.07^{a,Q}$	$18.59 \pm 1.75^{a,O}$	$15.04 \pm 0.23^{a,P}$
			15	$59.03 \pm 3.05^{a,NO}$	$59.40 \pm 1.45^{a,OP}$	$60.56 \pm 2.16^{a,M}$	$58.39 \pm 0.76^{a,KL}$	$56.42 \pm 1.65^{a,KL}$
			20	$63.98 \pm 1.43^{a,LMN}$	$64.23 \pm 1.59^{a,MNO}$	$65.14 \pm 2.1^{a,LM}$	$64.34 \pm 2.23^{a,IJK}$	$64.69 \pm 2.26^{a,IJ}$
		100 °C	0	$6.56 \pm 0.99^{a,W}$	$1.52 \pm 0.08^{b,X}$	$1.23 \pm 0.04^{b,U}$	$0.97 \pm 0.03^{b,S}$	$0.93 \pm 0.02^{b,S}$
			5	$77.33 \pm 0.10^{a,ST}$	$74.94 \pm 1.05^{ab,KLM}$	$70.75 \pm 1.18^{b,R}$	$60.42 \pm 2.04^{c,P}$	$48.79 \pm 3.24^{d,Q}$
			10	$77.39 \pm 0.98^{a,P}$	$76.04 \pm 1.52^{ab,QR}$	$71.69 \pm 1.89^{bc,O}$	$69.90 \pm 2.34^{c,N}$	$63.77 \pm 2.74^{d,O}$
			15	$77.92 \pm 0.37^{a,MN}$	$77.03 \pm 0.67^{a,NO}$	$76.80 \pm 0.27^{a,M}$	$74.08 \pm 1.4^{b,KL}$	$72.17 \pm 0.67^{b,JK}$
			20	$80.11 \pm 0.91^{a,L}$	$78.23 \pm 1.52^{ab,U}$	$78.34 \pm 0.65^{ab,KL}$	$78.22 \pm 0.68^{ab,HI}$	$77.33 \pm 0.27^{b,HI}$
	Yes	Room temperature	0	$0.26 \pm 0.00^{c,W}$	$0.26 \pm 0.00^{bc,X}$	$0.26 \pm 0.01^{abc,U}$	$0.27 \pm 0.00^{a,S}$	$0.27 \pm 0.00^{ab,S}$
			5	$15.39 \pm 0.18^{a,FGHIJK}$	$15.08 \pm 0.09^{a,UV}$	$14.21 \pm 0.73^{a,U}$	$10.85 \pm 0.70^{b,S}$	$8.48 \pm 0.73^{c,S}$
			10	$46.19 \pm 1.81^{a,EFGHIJ}$	$43.53 \pm 3.16^{ab,Q}$	$41.67 \pm 4.2^{ab,STU}$	$37.29 \pm 4.54^{ab,RS}$	$33.68 \pm 5.21^{b,S}$
			15	$62.61 \pm 0.44^{a,FGHIJK}$	$63.33 \pm 2.63^{a,LMN}$	$63.46 \pm 2.27^{a,OP}$	$61.58 \pm 3.43^{a,QRS}$	$60.29 \pm 3.76^{a,S}$
			20	$69.48 \pm 2.43^{a,CDEFGH}$	$69.95 \pm 1.87^{a,FGHI}$	$70.32 \pm 1.78^{a,HIJK}$	$70.55 \pm 2.16^{a,L}$	$70.07 \pm 2.68^{a,N}$
		100 °C	0	$6.56 \pm 0.99^{a,W}$	$1.52 \pm 0.08^{b,X}$	$1.23 \pm 0.04^{b,U}$	$0.97 \pm 0.03^{b,S}$	$0.93 \pm 0.02^{b,S}$
			5	$79.50 \pm 10^{a,CDEFGH}$	$78.83 \pm 1.37^{a,WX}$	$77.99 \pm 0.78^{a,U}$	$69.09 \pm 1.45^{b,S}$	$56.18 \pm 2.16^{c,S}$
			10	$80.72 \pm 0.32^{a,GHIJK}$	$81.16 \pm 1.03^{a,RS}$	$81.91 \pm 0.76^{a,TU}$	$77.21 \pm 1.15^{b,S}$	$72.90 \pm 1.52^{b,S}$
			15	$83.24 \pm 0.90^{a,FGHIJK}$	$83.56 \pm 0.96^{a,JKL}$	$84.40 \pm 0.68^{a,P}$	$83.18 \pm 1.34^{a,QRS}$	$83.24 \pm 0.95^{a,RS}$
			20	$85.91 \pm 1.55^{a,GHIJK}$	$86.44 \pm 1.10^{a,GHI}$	$86.97 \pm 1.18^{a,HIJK}$	$86.11 \pm 1.30^{a,JK}$	$86.76 \pm 0.42^{a,LM}$

[1] Values are presented by average ± standard deviation (N = 3). Lowercase letters indicate statistical significance in the same row and uppercase letters indicate statistical significance in the same column; values with the same letter are not significantly different at $p < 0.05$.

[2] Samples were incubated at 25 or 100°C for 20 min for the preparation. The samples boiled at 100°C were cooled to 25°C prior to the measurement of transmittance.

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