USE OF IODINE AS A MARKER TO PROBE DIFFERENCES IN GRANULAR ARCHITECTURE OF STARCHES FROM VARIOUS BOTANICAL SOURCES

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ABSTRACT

USE OF IODINE AS A MARKER TO PROBE DIFFERENCES IN GRANULAR ARCHITECTURE OF STARCHES FROM VARIOUS BOTANICAL SOURCES

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The properties and functionality of starches from botanical sources vary widely. Differences are attributed to chain length distribution variations of amylose and amylopectin, to granule size and shape among other things; but also likely these differences stem from the dissimilarity in how constitutive components are organized within granules. The research goal is to further understand the architectural organization of granules by utilizing iodine as a marker. The objectives were to investigate pasting behaviour, granular swelling, polymer leaching, and rheological changes to corn, wheat, potato, and tapioca starch gels formed using different iodine concentrations. Differences were observed in pasting behaviour of A, B and C-type starches upon addition of iodine. Despite increasing iodine concentration, the granules swelled fully suggesting granule stabilization due to complex formation. The firmness and complex moduli of the starch gels decreased as a function of iodine concentration; the extent of decrease varied with the starch suggesting differences in microstructure of starch gels. A specific level of iodine was required to saturate polymers within granules to prevent leaching, to reduce pasting properties, swelling capacity and to affect textural properties. These observations add new perspective to better understand starch granular architecture.
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CHAPTER 1

INTRODUCTION

Starch is a major storage carbohydrate biopolymer found in higher plants. It is deposited in the form of granules of various shapes and sizes based on different botanical sources (Eliasson, 2006; Butrim et al., 2009). Starch forms a major source of metabolic energy for both plants during germination and for humans in food. A steadily increasing demand in food and non-food industry for starches with well-defined properties continues to spur research to better understand the structure-function relationships of starch polymers. Corn, wheat, potato and tapioca starches are commercially important starches around the world (Swinkels, 1985) and were investigated in this study.

Over the last few decades the external and internal surfaces of starch granules have been viewed by employing a number of techniques. The external surface as well as stained samples of gelatinised starches can be very well observed by using light microscopy (Bogracheva, 2002; Jayakody, 2005; Mishra, 2006) and confocal scanning laser microscopy (CSLM) (Gonera, 18; Velde et al., 2002). While the external surface of the starch granules can be scanned up to the nanoscale by using atomic force microscopy (AFM) (Dang, 2006), scanning electron microscopy (SEM) (Gallant et al., 1992, Li et al., 2003; Suh, 2004) or transmission electron microscopy (TEM) (Li et al., 2003; Li et al., 2004) are used for analysing the internal packing as well as external features of starches. Optical microscopy also reveals the shape of the starch granules ranging from small spherical to ellipsoidal and lenticular (Banks and Muir, 1980, Jane et al., 1994, Butrim et
al., 2009). The structural components, i.e. amylose and amylopectin, which are the major starch polymers, have been well analysed using different methods, such as HPLC, GPC and spectroscopy.

Starch is a major component of flour and the specific functional properties of starch required by the food industry are limitless. No other food ingredient is as versatile as starch in providing functionality to food products. The functional properties of starches in food products are thought to be dependent on the size, shape and the amount of starch polymers. But these factors are not all inclusive in explaining the variations among the functional properties of different starches. In order to explain variability, we think that the architecture of the starch granules, i.e. the arrangement of the starch polymers within the granules plays a vital role. While the structural components of the starch granules have been well studied over the years, the architectural aspect still needs to be explored. The term “architecture” of a starch granule refers to the location and organisation of the different glucan polymers including amylose, amylopectin and the intermediate materials that make up the granule. A number of studies are underway currently that are investigating these aspects.

In this study we use one approach by using iodine as a marker to differentiate the architectural attributes of starch. This research investigates the effect of iodine on viscosity and textures of starch gels, in order to interpret the organisation of the polymers within the starch granules from different botanical sources.
CHAPTER 2

GOAL, HYPOTHESES, ASSUMPTIONS AND OBJECTIVES

2.1 Goal

The goal of this study is to understand the role of the architectural organization of granules on the pasting behaviour of starches. The research utilizes iodine as a marker to probe architectural differences based on the ability of iodine to selectively bind with linear segments of glucan polymers and to observe the changes to granular swelling, polymer leaching and the subsequent rheological changes in the gel structure as a function of iodine concentration.

2.2 Hypotheses

1) The pasting properties of starches with different crystalline polymorphs will not differ when heated in the presence of increasing iodine concentrations.

2) The extent of granule swelling and polymer leaching from starch granules with different crystalline polymorphs will not be significantly different in the presence of increasing iodine concentrations.

3) The rheological properties of gels resulting from starches treated with increasing iodine concentrations will not be significantly different.
2.3 Assumptions

1) Upon introduction of water to native starch granules only individual segments of polymers are plasticized; not entire molecules are available due to steric hindrances. At room temperature, starch absorbs about 30% of its weight of water (Seker and Hanna, 2006).

2) Linear segments of starch polymers form a helical complex with iodine and based on the degree of polymerisation (DP) of these segments, absorption maxima can be measured at wavelengths ranging from 490 to 620 nm with increasing DP from 20-105 (Banks et al., 1971).

3) It is not the entire molecule of amylose, amylopectin or intermediate material that binds iodine, but only mobile segments of polymers in native starch granules bind iodine.

4) The complex formed between iodine and linear segments of glucan polymers dissociates only at temperatures higher than 90°C (Patel et al., 2006).

5) Iodine can bind to polymers either on the surface or inside the starch granules in dilute systems.

6) The potassium ions (K⁺) from the potassium iodide (KI) in iodine solution do not have an effect on the pasting behaviour of starches (Jane, 1993; Saibene and Seetharaman, 2008).

2.4 Objectives

1) To investigate the changes in pasting properties of starches from different botanical origins as a function of iodine added at room temperature or at 95°C.
2) To investigate starch granule swelling capacity and polymer leaching upon addition of varying concentrations of iodine.

3) To investigate the textural and rheological properties of starch gels containing varying concentrations of iodine.
CHAPTER 3

LITERATURE REVIEW

3.1 Structural components of starch

Starch consists of two major polymers, amylose and amylopectin, along with some polymers of intermediate lengths and intermediate number of branches. The amount of these structural polymers varies depending on the botanical source of the starch.

3.1.1 Amylose

Amylose is an essentially linear homo-polymer consisting of glucose residues linked majorly via α-(1-4)-linkages and occasionally by α-(1-6)-linkages (Figure 3-1). The molecular weight varies from $5 \times 10^5 - 10 \times 10^5$ Da and the degree of polymerisation (DP) is 100-1000 depending on the botanical source. In contrast to waxy starches that have no or negligibly low amounts of amylose, normal starches contain between 15-30% w/w of amylose (Yuryev, 2007). Amylose behaves like a random coil in dilute neutral aqueous systems. In water, amylose precipitates when present at concentrations lower than 1.5%; above this concentration an elastic gel is formed (Ellis, 1985; Gidley, 1989). The high amylose starches can contain amylose as high as 70% w/w (Parker and Ring, 2001) and according to Li and Yeh (2001), the granule size does not correlate with the amylose content of starches. The potentiometric and iodimetric methods for the quantification of amylose are sensitive to the extremely long amylopectin unit chains,
which are over-estimated by the methods based on branching pattern rather than molecular size (Takeda and Hizukuri, 1987; Song and Jane, 2000).

### 3.1.2 Amylopectin

Amylopectin is a branched starch homo-polymer consisting mainly of α-(1-4) linkages that form the backbone and non-random α-(1-6) – linkages every 24 to 30 glucose units (Fig. 3-2). It constitutes 70-85% w/w of a starch granule and its molecular weight ranges between $10^6$ and $10^9$ Da depending on the source and extraction techniques (Eliasson, 2006). Cameron et al. (1994) showed that like amylose, pure amylopectin forms a gel at 3% concentration. These researchers noted that amylopectin forms a gel more rapidly as compared to high molecular weight amylose; the kinetics of double helix formation by the shorter branches of amylopectin will be easier than by the amylose in which segments of the entire backbone are involved. Moreover, the origin of amylopectin gels is reasoned to be the double helix formation by the outer A- chains. Due to its branched structure, amylopectin has a limited ability toward hydrogen bonding and the gels remain relatively clear and fluid (Moore, 1984). The longer chains of amylopectin facilitate crystal formation with longer double helices, which in turn provide greater restriction to amorphous regions and increased need for hydration (Charles et al., 2005).

### 3.1.3 Intermediate fractions

The intermediate fractions can constitute up to 30% of the total polymer composition of starch granules depending on the starch source. ‘Intermediate fractions’ with structural properties between amylose and amylopectin have been described for some starches (Banks and Greenwood, 1975; Manners, 1985; Koornhuyse, 1996; Tziotis et al., 2004).
The functional properties of these intermediate fractions are different from the amylose and amylopectin fractions in the same source; and might have a significant effect on the cumulative physico-chemical properties. The location and participation of these intermediate fractions in a granule is not known or understood. At present, it is thought to be localised in the amorphous background within the granule.

3.1.4 Additional elements in starch

Minor quantities of lipids (up to 1%) are present in the form of a helical complex in the amorphous parts of the granule (Morrison et al., 1993). The proteins (below 0.5%) are located on the surface (Lowy et al., 1981) or in the interior along with the starch-synthesizing and degrading enzymes. Phosphorous is also found in some starches and its amount varies with the botanical source. According to Tabata and Hizukuri (1971), tuberous starches are rich in covalently bound phosphate (e.g. 1 phosphate ester per 200 units in potato starch), and the long unit chains are more substituted than short ones (Takeda and Hizukuri, 1982; Blennow et al, 1998). The presence of these minor components may have a role in the swelling and gelatinization of starches (Morrison et al., 1993).

3.2 Model of internal structure of starch

A number of different models have been proposed over the past 6 decades for the architecture of the starch granule and amylopectin organization (French, 1972; Nikuni, 1969; Hizukuri, 1986). According to the most recently accepted model based on SEM, TEM, enzyme degradation studies and AFM, the internal structure of a starch granule (Figure 3-3) is believed to consist of large and small blocklets arranged in the crystalline
and semi-crystalline hard shells, respectively (Gallant et al, 1997). The SEM observations have shown the presence of pores (0.05-0.1 μm diameter) which are apparently the openings of the amorphous channels and divide the surface of the granules into polygonal areas of 1 μm diameter. The blocklets consist of crystalline and amorphous lamellae based on the earlier X-ray studies. The ordered double helical amylopectin side chain clusters, constituted by A, B1, B2 and B3 chains (Figure 3-4), are believed to form clusters and constitute the crystalline lamellae. The branch points of these clusters are interspersed with the amorphous lamellae (Gallant et al., 1997). The location of amylose within the internal structure of starches from different sources, however, has not been clearly explained. Studies suggest that amylose is primarily present in the amorphous regions while amylopectin is responsible for the crystalline region in the granule (Shamekh et al., 1998), as evidenced by the greater susceptibility of amylose to acid and enzymatic digestion. Other studies suggest that the amylose participates in the crystalline lamellae but only in some starches (Saibene et al., 2008).

The most recent model by Bertoft (Figure 3-5) shows a number of clusters on a dextrin based on a two-dimensional backbone (Bertoft, 2004). The arrows show places susceptible to endo-attack by α-amylase, which can divide the long internal segment into smaller groups or individual clusters. Multiple endo-attacks can lead to the formation and confirm the presence of very short A-chains and long A- and B-chains. This two-dimensional model explains the presence of very short and large amounts of long chains which were not involved in clusters but spanned the amorphous lamellae as single branches, apart from the large amounts of glucosyl residues at the inter-cluster areas (Bertoft, 2007).
3.3 Classification of starches

3.3.1 Based on constitutive polymer composition

Starches can be categorized into different types based on the amount of amylose present within the granules. Waxy starches contain negligible amounts of amylose, while high amylose starches contain >50% amylose content. The normal or wild type starches contain different proportions of amylose and amylopectin depending on the botanical source (Eliasson, 2006). Also, the degree of branching and length of the constitutive polymers can vary between starches from different botanical origins (Hizukuri, 1986).

3.3.2 Based on X-Ray diffraction pattern

Starches from different botanical origins can be classified into three categories based on their X-ray diffraction patterns; A, B or C type (Zobel, 1988) (Figure 3-6). Granule crystallinity is around 15-45% (Zobel, 1988) and is a result of different packing of the amylopectin side-chain double helices. Most cereal starches exhibit A-type crystalline polymorphism. Potato and other tubers exhibit B-type crystalline polymorph, though there are exceptions. The C-type polymorphs present in some starches consist of a mixture of A and B types in various proportions, and are generally found in rhizomes and bean starches (Eliasson, 2006). The A-type crystalline polymorph consists of a monoclinic unit cell packing with 4 water molecules associated with its structure, while the B-type crystalline polymorph contains 36 water molecules associated with hexagonal unit cells. The C-type polymorphs consist of the B-type crystalline packing in the core surrounded by the A-type crystalline polymorphs (Bogracheva et al., 1998). Vh-type of
crystalline pattern is observed for starches in which amylose complexes with iodine or lipids (Biliaderis, 1998).

3.3.3 Based on distribution of granules

Starches from different sources are known to have either unimodal or bimodal distribution of granules. The cereal starches, e.g., wheat, rye, barley have a bimodal distribution, as they contain two types of granules; the larger A-granules are disc shaped while the smaller B-granules are spherical (Wilson et al., 2006). On the other hand, grains of corn and tapioca starches possess unimodal distribution of similarly starch granules (Butrim et al., 2009). Based on these differences in size, distribution and shapes of starch granules, there is a range of temperatures characterized as the gelatinization temperature.

3.4 Starch Gelatinization

Gelatinization can be defined as the collapse (disruption) of molecular order within the starch granule resulting in a series of irreversible changes in structural properties such as granular swelling, crystallite melting, loss of birefringence, viscosity development and solubilisation of the starch polymers (Eliasson, 2006). The solubilisation of granules and leaching of amylose is believed to increase the viscosity of starch dispersions (Miller et al., 1973) and has been observed for all starches including potato starch and other B-type starches (Gallant et al., 1998).

3.5 Starch pastes/gels

Physico-chemical properties of starch e.g. paste viscosity and stability in solution are likely to be direct functions of the molecular constitution of the polymer including
molecular size, unit chain length distribution, branching pattern, degree of phosphate substitution, and granular size and distribution (Blennow et al., 2003). Cereal starch pastes and gels can be considered as composite systems of swollen granules and extragranular matrix (Doublier et al., 1987). As the starch hydrates, it binds with water and swells in size. Over the years the pasting properties of starches have been studied and applied in the food industry. The increase in the viscosity in particular, upon addition of water and heat has been the most widely used property of starch in formulating various food products.

3.5.1 Factors affecting starch gelation

The solubilisation of amylose, and in case of waxy starches, cross linking, leads to the decreased rigidity of the starch granules. Rigidity of granules and retrogradation of extragranular leachate govern the formation of either starch paste or gel (Tsai et al., 1997). It is known that polymers in starch pastes upon cooling network by interlinking, resulting in the formation of an opaque gel with granule remnants embedded in a macromolecular matrix (Miles et al., 1984). Upon heating of granular starch in excess water, the amorphous phase of the starch granules gets hydrated and the granules undergo swelling (Jenkins and Donald, 1997), followed by a total disruption of the granular structure (Swinkels, 1985). The granule swelling is considered to be a two stage process leading to an increase in the viscosity until about 90°C. This leads to the formation of a transient network of granules closely packed and resulting in granule disruption (Blanshard, 1979) and leaching of amylose and small amylopectin clusters (Eliasson 1986). The increased polymer mobility facilitates rearrangement of the amylose polymers. Realignment of the polymers occurs simultaneously in amorphous and crystalline domains (Ratnayake,
Starches with high amount of amylopectin (long chain polymers) aggregates show higher peak viscosities (Blennow et al., 2001). Figure 3-7 shows a typical starch pasting curve obtained in an RVA. When the concentration of solids increases in a starch-water system, upon heating the swelling of granules is slowed down due to the limited amount of water available and this restricts their solubility as well. The varying size of different starch granules and their unique pattern of swelling are responsible for the contrast in the swelling capacities, which in turn is assumed responsible for the differential leaching of the polymers upon heating.

Higher peak viscosities have been correlated to the amount of amylopectin aggregates in starches, whereas very high amounts of starch bound phosphate or amylose has been suggested to suppress the content of large amylopectin aggregates (Blennow et al, 2001).

3.5.2 Appearance of starch pastes/gels

Starch pastes have been described as a dispersion of swollen particles in a macromolecular medium. Swollen particles are composed of remnants of swollen/collapsed granules and the continuous phase is a solution of macromolecules in the soluble fraction of polymer structure. Microscopic studies on cereal starch granules have shown that the cereal starches swell in two planes and maintain the granular integrity after pasting at 96°C (Bowler et al., 1980, Williams and Bowler, 1982). Waxy starch gels are softer and less sticky than normal starch gels (Corke et al., 1999).

In dilute systems, the viscosity is dependent on the volume fraction of the swollen granules (Steeneken, 1989). As a result of greater hydration, the starches are more swollen with higher light transmittance (Lim and Seib, 1993). Also, the opacity of gels
has been linked to the branching pattern and cross linking at the microstructural level (Kerr and Cleveland, 1959). Outer parts of the amylopectin chains follow the single chain behaviour of linear polymers. Due to a higher degree of branching the hydrogen bonding between the polymer chains will be lower, which ultimately leads to a more opaque gel (Eliasson, 2006).

3.5.3 Effect of starch-bound and guest molecules on pasting properties of starches

The pasting properties of starch are highly dependent on the granule size and the degree of granular swelling. It is known that the viscosity of starch pastes is primarily governed by a combination of the volume fraction of the disperse phase; the concentration and composition of the continuous phase play a vital role as well (Doublier et al., 1987). The presence of cross-linked ionic species (e.g., phosphate group), proteins or lipids that form a clathrate (Figure 3-8) in the native starches has a significant effect on the granular swelling (Debet and Gildy, 2006).

Due to its macrostructure and microstructure, starch readily forms “inclusion complexes” and surface adsorbates (Morrison, 1998). The inclusion complexes can form by the involvement of the inner core of helical polymers, intergranular space or the starch matrix. Starch complexes can result from dipolar interactions, hydrogen bonding and/or clathrate formation. Phosphate can either be covalently linked in the form of esterified phosphates on amylopectin chains or as phospholipids. The significantly higher levels of negatively charged phosphate groups on potato starch (200-1000 ppm) and waxy starches as compared to the cereal starches (less than 20 ppm) have been shown to cause
repulsions among the various polymer chains, hence reducing the inter-chain associations (Tabata et al., 1975; Lim and Seib, 1993; Suzuki et al., 1994).

Cereal starches contain 0.6-1.3% lipids, which is higher than the lipid content of other starches (Morrison, 1988). It has been shown that lipids (in the form of fatty acids) competitively inhibit the binding of iodine to the linear glucan polymers (Muis et al., 1946) as both lipids and iodine form similar helical complexes with the linear portions of glucan polymers. The amylose-lipid complexes exist either in the amorphous zone (melting temperature ~ 96-100°C) or in the semi-crystalline zone (melting temperature ~ 105-125°C). With the addition of 2% lipids to wheat starch in an amylograph, the pasting peak and consistency were shown to increase along with a strong second peak, even though no setback was observed. When lipid was added to the starch paste after it reached 95°C in the amylograph, the peak and the setback were shown to have reduced significantly (Takahashi and Seib 1988). Very high amounts of starch bound phosphate or amylose appeared to suppress the content of large aggregates resulting in low viscosity (Blennow et al., 2001). Considering all these factors we still do not know for sure the reason for the wide range of differences in the functional properties of starches from different botanical sources within the context of the structural components of starch.

3.6 Starch-Iodine Interaction

The glucan polymers in starch are capable of forming a complex with iodine in both solution and vapour form (Rendleman, 2003; Saibene et al., 2006, 2008). It is known that pure amylose has higher iodine binding capacity (IBC) as it adsorbs nearly 20 g iodine per 100 g, and amylopectin adsorbs 1 g iodine per 100 g (Amari and Nakamura, 1976;
Davis et al., 1994). Iodine ions bind the linear polysaccharide polymer chains and form a helical inclusion complex in which the iodine occupies the central cavity of the helical polysaccharide (Rundle and French, 1943). Based on the degree of polymerisation of the glucan polymers, the complex with iodine can show absorbance over a wide range of wavelengths (530-642 nm) (Banks, et al., 1971) resulting in a spectrum of colours ranging from red to blue as the absorbance increases, linearly, with the amount of iodine bound by the polysaccharide polymers (Banks et al.,1974). Bates et al. (1943) suggested that the longer amylose chains get saturated with the poly-iodide ionic chains (Stein and Rundle, 1948) before the shorter chains of amylopectin and other intermediate polymers could bind any. In this research we will be capitalising on the starch-iodine interaction in deciphering the structural and architectural information of the starch granules.

3.7 Retrogradation

The gelatinised starch exists in an amorphous state and over time the thermodynamic equilibrium is disturbed resulting in the formation of a more ordered crystalline state, and this phenomenon is known as retrogradation. Changes in the rheological properties, such as increased firmness or rigidity of the gelatinized starch are observed as a result of retrogradation (Eliasson and Gudmundsson, 2006). A change in the viscoelastic behaviour upon retrogradation has been shown to be reflective of a change at the microstructural level but the molecular mechanisms behind the phenomenon are still not completely understood (Svegmark & Hermansson, 1991). Apart from the above mentioned changes loss of moisture or water holding capacity and restoration of crystallinity are also observed upon aging gelatinized starch, leading to unacceptable texture of food products containing starch (Eliasson and Gudmundsson, 2006). Cross-
linking had no influence on amylopectin retrogradation of potato and amaranth starches, indicating that the cross-links largely occurred in the amorphous region, and that covalent bonds formed by a small degree of cross-linking did not interfere with the reassociation of amylopectin chains (Corke, 2007). Starches from different botanical sources display varied retrogradation tendencies.

Lipids and surfactants are known to interfere with the retrogradation process, which seemingly does not depend on the amylose-to-amylopectin ratios of starches. The water content of the samples along with the storage temperature plays a very important role in controlling the rate and extent of retrogradation (Eliasson and Gudmundsson, 2006). The retrogradation does have a marked effect on the firmness of starch gels but the effect of an inclusion complex with iodine has not been used for investigation of the starch architecture.

3.8 Structural deformation studies

Rheological properties are based on the structural deformation and flow of matter when subjected to stress. Fluid materials which contain significant amount of high molecular weight compounds (polymers) and/or suspended particles exhibit viscoelastic behaviour. Studies on rheology of foods are useful and find important application in quality control, sensory assessment, handling and processing; but can also be used as a tool to understand the composition, especially the microstructure of materials (e.g. dissolved polymers, suspended solids) and structure of products (e.g. gels, emulsions). The term “gel” is defined as a class of systems which show solid-like properties in the presence of excess solvent.
In this study a "paste" may be considered as a composite material with the swollen starch granules dispersed in a solution which may or may not contain polymers; while a "gel" would contain mostly collapsed starch granules entrapped in a polymer matrix formed by the leached polymers. Also, the type of pasting procedure is important for the definition of textural characteristics and this behaviour is not governed by the same rules in all starches (Doublier et al., 1987).

3.8.1 Small deformation

Polymer gels are part elastic (bounce back into shape) and part viscous (flowing). The viscoelastic behaviour of materials can be studied by means of dynamic shear, creep-compliance, and stress relaxation techniques. For studying the viscoelastic properties of materials such as gels, stress relaxation is a convenient method, where the time-dependent shear stress (tangentially applied force vector divided by the area of application) is studied for step increase in strain (Rao, 1999). The rheological behaviour of starch pastes and gels is a result of the combination of parameters including the volume fraction of swollen granules (swelling capacity), their deformability and concentration of solids in the system. There are various levels of heterogeneity due to the different mechanisms of chain association and their distribution throughout the system. Depending on the nature of the mechanism involved in their formation, gels can be categorised into chemical gels and physical gels. Chemical gels arise from the chemical reactions involving cross-linking leading to formation of covalent bonds among the different polymer chains, whereas physical gels involve a number of different physical interactions among chain segments. Chemical gels are characterized by a permanent stability of the network formed as a result of chemical bonds. On the other hand the
different types of chain-chain interactions in a physical gel must act cooperatively to ensure gel stability; portions of the polymer chains are involved in the network formation and many such portions join to form extended junction zones. Therefore, in such physical gels the resistance to deformation and physical modification can extend only over a certain range of applied strain. It is difficult to develop an understanding of the viscoelastic properties and classify these physical gels, especially weak gels based on their structural differences (Lapasin and Pricl, 1995).

The most common variables used in explaining the viscoelastic behaviour are storage modulus (G'), loss modulus (G'') and loss tangent (tan δ). G' represents the stored energy which is recoverable per cycle of deformation, whereas G'' is a measure of energy lost as viscous dissipation per cycle of deformation and tan δ is the ratio of the energy dissipated to that stored per cycle of deformation. Complex modulus (G*), which takes into account the contribution of the independent variables G' and G'' can also be used to express the structural deformation as a function of strain (Rao, 1999).

\[ |G^*| = \sqrt{(G')^2 + (G'')^2} \]

Flory (1953) recognized that some polymer chains may not contribute to the elasticity of a gel network. After a week of storage crystal formation (retrogradation) takes place and phase separation occurs; as a result the amylopectin chain association is no more a gel. The relevant information on the role of polymer chains in the formation of starch gels is sparse and needs to be investigated.
3.8.2 Large deformation

During the reorganization of the molecules, double helices and crystallites may form along existing internal elements which may have numerous terminal linkages along its length as would occur due to $\alpha-(1\rightarrow6)$ covalent branch points in starch. The terminal elements can be individual external chains of a molecule or multi-cluster units of branches. McEvoy et al. (1985) also recognized that terminal elements of gels may be more complex than single linear chains. Unlike rubber, starch chains have the capacity to retrograde along internal elements without the formation of a new network linkage, which would contribute to the $\Delta H$ of the gel but not necessarily to the $G'$ of the gel. Upon ageing, the rheological characteristics of the gels change, amylose gelation takes place within 12 h, whereas amyllopectin takes longer to re-crystallize (Miles et al., 1985). The firmness and elasticity of composite gels are a result of a complicated combination of parameters which involve elasticity of continuous phase, the volume fraction of the dispersed phase, the shape and deformability of swollen granules (Doublier et al., 1987). Wheat starch gels are stiffer than corn starch gels at equivalent concentrations and pasting procedures (Wu et al., 1995). The effect of iodine complexation in starch gels on their retrogradation has not been studied and in this research we will try to understand the effect of architectural differences among various starches upon the rheological changes which occur during retrogradation by using iodine as a marker.
Figure 3-1: Schematic representation of amylose structure

(Reproduced from: http://www.rsc.org/education/teachers/learnnet/cfb/carbohydrates.htm)
Figure 3-2: Schematic representation of amylopectin

(Reproduced from:
http://www.rsc.org/education/teachers/learnnet/cfb/carbohydrates.htm)
Figure 3-3: Graphical representation of the internal structure of starch

(Adapted from Gallant et al., 1997)
Figure 3-4: A cluster model of amylopectin showing the arrangement of A, B1, B2 and B3 chains

(Reproduced from Hizukuri, 1986)
Figure 3-5: Graphical representation of a dextrin containing four amylopectin clusters. $\emptyset$, C and A represent the reducing end, the crystalline and amorphous lamellae, respectively.

(Reproduced from Bertoft, 2007)
Figure 3-6: X-ray diffraction diagrams of A-, B-type starches and Vh-type amylose.

(Reproduced from Buleon et al., 1998)
Figure 3-7: A typical temperature profile and pasting curve in the RVA
Figure 3-8: Molecular modelling representation of amylose–fatty acid complexes showing the inclusion of the aliphatic part (C12) of the fatty acid inside the hydrophobic cavity of the amylose single helix.

(Reproduced from Buleon et al., 1998)
CHAPTER 4

USE OF IODINE IN INVESTIGATING THE DIFFERENCES IN PASTING PROPERTIES OF STARCHES FROM VARIOUS BOTANICAL SOURCES

4.1 Abstract

The chemistry of interaction of iodine with starch has typically been used to determine the amount of glucan polymers, both qualitatively and quantitatively. In this study, we exploit the complexation of iodine with starch polymers to understand the differences in pasting properties of starches from different botanical sources in order to better understand their architectural makeup. Increasing concentrations of iodine were added either at room temperature or at 95°C to 8% starch slurry to understand the difference in the pasting properties of corn (CS), wheat (WS), potato (PS) and tapioca (TS) starches. Iodine had a significant effect on a number of different pasting parameters and the extent of impact on these parameters was different for the starches. The observed differences are attributable to the dissimilarity in the organization of granules to a large extent.

4.2 Introduction

The reaction of iodine with a linear glucan polymer is well established (Rundle and French, 1943; Banks et al., 1971). This reaction has been used extensively to quantify amylose content of starches (McGrance et al., 1998), to visualize phase separation of amylose and amylopectin following different hydrothermal treatments (Svegmark and Hermansson, 1991) and to investigate the extent of starch gelatinization (Beleia et al.,
More recently, Saibene et al. (2008) utilized iodine vapour as a marker to demonstrate segmental mobility of polymers in native starch granules at low moisture contents. These authors also reported on the differential impact of iodine on the crystalline structure of the starches as measured by X-ray crystallography and concluded that the amylose-amylopectin arrangement within a granule was likely different based on the starch crystallinity, i.e., A vs. B crystallomorphs.

Pasting properties of starches are typically measured by using a Rapid Visco Analyser (RVA) or Micro Visco-Amylo-Graph (MVAG). A typical RVA pasting curve is characterized by the pasting temperature at which a viscosity increase is observed, peak viscosity at which granule swelling is at its maximum along with concurrent polymer leaching (Li et al., 2008), breakdown viscosity which represents the subsequent disruption of starch granules as a result of constant heat and mechanical shear (Davies, 1995), and setback viscosity (cooling phase) which marks the strengthening of the network through interactions with the leached polymers upon cooling. Doublier et al. (1987) reported that the viscosity of starch pastes is primarily governed by a combination of the volume fraction of the dispersed phase; and the concentration and composition of the continuous phase also contribute to the viscosity. Starches from different botanical sources, and even within the same genus, have significantly different pasting profiles (Swinkels, 1985; Shibanuma et al., 1996). These differences are typically attributed to differences in granule size (Tian et al. 1991), ratio of amylose to amylopectin (Tziotis et al., 2005), amount of crystallinity and presence of lipids (Tester and Morrison, 1990) or ionic species, like phosphates. However, there has not been much conjecture about the effects of granular architecture on granule swelling, polymer leaching and the resulting
gel network established following cooling. The term “architecture” of a starch granule refers to the location and organization of the different glucan polymers including amylose, amylopectin and the intermediate materials that make up the granule. For example, Saibene et al. (2008) and others (Hoover and Vasanthan, 1992, 1994) reported that amylose chains traverse through several crystalline and amorphous zones in potato starch but not in corn starch. However, it is not clear if the differences in the arrangement of polymers within a granule influence the functional behaviour of starches. In this study we explore one aspect of these differences by investigating the effect of iodine on the pasting behaviour of starches with different structural features.

Addition of iodine produced a varied response in wheat starch, with peak and final viscosities first increasing and then decreasing with increasing concentrations of iodine, i.e. upto 0.2% and 0.5% iodine concentration, respectively (Saibene and Seetharaman, 2008). The pasting properties of wheat starch have also been investigated by adding iodine at room temperature or at 95°C in order to study starch granule swelling and polymer leaching during pasting (Saibene and Seetharaman, 2008). In a previous study Patel et al. (2006) show that addition of iodine caused a restriction and delay in the swelling of corn and wheat starches, with no swelling at higher iodine concentrations. It was shown that the amount of iodine required to inhibit granule swelling was higher than that required to inhibit amylose leaching from the granule. Increasing the level of iodine up to 0.33% has been shown to cause an increase in peak, trough and breakdown viscosity of kidney bean starch (Singh et al., 2009). The objective of this study was to investigate the changes in pasting properties of starches from different botanical origins as a function of iodine added either at room temperature or at 95°C.
4.3 Materials

Commercial starches from common corn (Melojel) [CS], common tapioca (Tapioca Starch, TS) were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). A commercial starch from potato (Pencook 10) [PS] was obtained from Penford Food Ingredients Co., Colorado, and wheat starch [WS] was provided by MGP Ingredients Inc., Kansas. Potassium iodide (ACS reagent) was purchased from Fisher Scientific, Nepean, Ontario and iodine crystals were purchased from Sigma Aldrich, St. Louis. The percentage of amylose in CS, WS, PS and TS was 21.2 ± 0.7, 20.5 ± 0.1, 16.7 ± 0.2 and 17 ± 0.5, respectively.

4.4 Methods

In the first set of pasting experiments, iodine solution (0.1% to 1.1% dry starch basis) was added to the starches (8% dry basis, db) at room temperature, with a sample containing distilled water instead of iodine solution used as control. At the lowest level (0.1%) of I₂-KI solution, there was 4.30 x 10⁻⁶ mol I₂/ g starch and 6.59 x 10⁻⁵ mol KI/ g starch; corresponding to 0.07 mM total ionic concentration of iodine in solution. At the highest level of iodine (1.1%), there was 4.75 x 10⁻⁵ mol I₂/ g starch and 7.27 x 10⁻⁴ mol KI/ g starch; corresponding to 0.77 mM total ionic concentration of iodine in solution. The starch-iodine solution mixture (28 g) was heated in a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) equipped with Thermocline for Windows (TCW3) software, from 25°C to 95°C at a rate of 10°C/min with 160 rpm shear. The system was held at 95°C for 3 min before cooling to 25°C at same rate. The temperature was held constant at 25°C before withdrawing the sample.
In the second set of experiments, starch (8% db) and water were mixed in an RVA canister to make up a total of 28 g. The mixture was heated in the RVA from 25°C to 95°C at a rate of 10°C/min. Upon reaching 95°C an additional 5.5 ml of distilled water or iodine solution (0.1% to 4%) was added to the starch slurry bringing the final percentage of solids in the system to 6.7%. The starch slurry was then held at 95°C for 3 min. before cooling to 25°C at same rate and holding stable for 3 minutes. The values for pasting temperature, peak viscosity and temperature, breakdown and setback were recorded. The pH of the samples (7.0 ± 0.1) before and after the experiment was not controlled.

The experimental analyses were conducted at least in duplicate and average values are reported. All statistical analyses were performed using StatGraphics Centurian Data Analysis and Statistical Software (StatPoint Technologies, Inc., Warrenton, VA).

4.5 Results

4.5.1 Pasting properties of starches following addition of iodine at room temperature

The pasting properties of the four starches with iodine concentrations ranging from 0.1-1.1% added at room temperature are listed in the table 4.1. The pasting temperature increased as iodine concentration increased for most starches. The lowest concentration of iodine at which the increase in pasting temperature was observed was different for the different starches. The pasting temperature of CS, WS and PS exhibited an increase at the lowest iodine concentration (0.1%), while the iodine concentration at which TS exhibited an increase in the pasting temperature was 0.2%. The iodine concentration at which no pasting temperature was recorded due to the RVA profile being flat, indicating no
increase in viscosity was also different for the different starches. WS recorded no pasting temperature at an iodine concentration of 0.5%, compared to 1.1% in the case of CS and TS.

The peak times of CS, WS and PS gradually increased with increasing levels of iodine. For TS a delay in the peak time was observed at 0.5% iodine levels or greater. The peak viscosity of PS showed the greatest decrease with the addition of lowest amount of iodine solution (0.1%) as compared to the other starches. With further increase in iodine concentration, the peak viscosity of PS was reduced but the change was not as pronounced. The peak viscosity of TS showed a decrease as well, though the decrease in the peak viscosity was not as high as in PS. The peak viscosity of CS and WS showed a slight increase until the addition of 0.5 or 0.2% iodine, respectively, and the peak viscosity decreased at higher iodine concentrations. The level of iodine at which no viscosity development was observed was different for the two starches.

The trough viscosity of PS decreased with the addition of 0.1% iodine solution but an increase in the concentration of iodine did not bring about any significant change. In the case of TS, 0.2% iodine resulted in a higher trough viscosity as compared to the control and 0.1% iodine sample. For CS and WS there was a decrease in the trough viscosity upon addition of 0.2 and 0.5% iodine solutions, respectively.

The breakdown viscosity of starches decreased upon increasing the concentration of iodine. In CS and WS, the breakdown viscosity first increased and then decreased even though the trough viscosity did not show any significant change (table 4.1). Therefore,
the initial increase in the breakdown viscosity was a result of the increased peak viscosity.

In case of CS and WS, there was a gradual decrease in the final viscosities upon the addition of iodine. On the other hand, for PS and TS, the decrease in final viscosity was not apparent until the addition of 0.5 and 1.1% iodine solution, respectively. While the setback viscosities of CS and WS gradually decreased by up to 80% with the increasing iodine concentrations, an increase was observed for PS. In TS it was only with the addition of 0.5% iodine that a decrease in the setback was recorded.

4.5.2 Pasting properties of starches upon addition of iodine at 95°C

The pasting properties of starches treated with iodine at 95°C and concentrations ranging from 0.1-4% are listed in table 4.2. The peak viscosity of CS did not change until the addition of 0.5% iodine at which there was a significant reduction in the peak viscosity, while there was a slight increase in the peak viscosity of WS with the addition of 0.1% iodine. Thereafter the peak viscosity of WS gradually decreased. Since the peak viscosity for PS and TS was attained before 95°C, there was no effect of iodine on peak viscosities of TS and PS.

The trough viscosity for CS decreased gradually upon the addition of increasing levels of iodine. For WS, a slight increase in trough viscosity was observed at 0.1 and 0.2% iodine levels. The trough viscosity of PS reduced with the addition of iodine, but did not change significantly at higher concentrations of iodine. There was no significant effect of iodine on the trough viscosity of TS.
When iodine was added to the starch-water system at 95°C, there was a gradual decrease in the breakdown viscosities of CS and WS upon addition of increasing levels of iodine. PS and TS had already undergone breakdown in the viscosity before 95°C, therefore addition of iodine at 95°C had minimal effect on their breakdown.

There was a slight increase in the final viscosity of CS upon addition of 0.1% iodine, but a gradual decrease in the final viscosities with subsequent increase in iodine concentrations. In case of TS the trend was opposite to CS as there was a slight decrease in the final viscosity at 0.1% iodine but upon increasing the iodine concentration an increase in the final viscosities was observed. For WS, a gradual decrease in the final viscosities was observed with increasing iodine levels. The final viscosity of PS decreased with 0.1% iodine concentration, but eventually increased with higher levels of iodine.

There was a slight increase in the setback of CS at 0.1% while the setback for WS had the maximum reduction as compared to the other starches. There was no significant change in the setback of PS even with the addition of 2% iodine. In general, more iodine was needed to precipitate the starch when iodine was added at 95°C to all starches in contrast to its addition at room temperature.
4.6 Discussion

This paper reports on the changes to pasting properties of starches following the addition of iodine. It is assumed that it is the iodine-polymer complex that is reflected in the data and that there is negligible influence of the potassium ion in this study. This has been demonstrated in a previous paper by Saibene and Seetharaman (2008). The pasting properties of the control starches are different, as expected. The pasting temperatures of the control starches had the following order: WS>CS>TS>PS; and the peak viscosity followed the following order: PS>TS>CS>WS. The correlation existing between these pasting properties of given starches is possibly an indicative of the differences in their architecture.

Upon the addition of iodine at room temperature, significant differences are observed in the pasting profiles for all the starches. When iodine was added to the starches, it immediately formed a complex with the polymers within the granules. While the color of the granules for most starches was blue, WS and TS exhibited lighter shades of color. However, it still cannot be assumed that it is either amylose or amylopectin, per se, that is complexing with iodine; rather that certain segments of linear chains formed a complex and the length of the linear chain is greater than 100 dp. Upon heating this starch-iodine slurry, a lag in the time required to reach pasting temperature is observed depending on the concentration of iodine added.

In general, the starches exhibited a lag in their pasting properties upon addition of iodine at room temperature. The increase in pasting temperature exhibited by the starches upon the addition of iodine suggests either a hindrance to granule swelling and/or reduced
leaching of polymers into the extragranular spaces. Only TS required a higher concentration of iodine (0.2%) before exhibiting an increased pasting temperature, while the other starches exhibited this change at just 0.1% iodine. Therefore, while the influence of amylose in granular swelling and viscosity development appears to be important, there seem to be other factors that influence the viscosity increase.

The delay in pasting temperature and attainment of peak time of the starches in the presence of iodine added at room temperature suggest that the granular swelling was restricted by the polymer-iodine complex formed. The delay in the pasting temperature upon addition of iodine is in agreement with previous studies (Saibene and Seetharaman, 2008; Singh et al., 2009). The restriction in granular swelling is further supported by the observation that there is a gradual decrease in the peak viscosity of starches upon increasing the concentration of iodine, suggesting a lower amount of leached polymers that would increase viscosity. There were a few exceptions where the peak viscosity increased up to a certain iodine concentration; the initial 30% increase in the peak viscosity of WS upon addition of 0.1% iodine and the increase in the peak viscosity of CS up to 0.2% iodine level (Figure 4-1). This can likely be explained by the repulsion between the high charge density tri-iodide (I⁻³) ions and polymer chains in starch leading to an increase in the granule swelling (Jane, 1993), hence the increased peak viscosities in case of the A-type starches (CS and WS). However, this was not the case in the PS (B-type starch) or TS (C-type starch). The addition of iodine caused a gradual decrease in their peak viscosities (Figure 4-1). The addition of the lowest concentration (0.1%) of iodine caused over 50% reduction in the peak viscosity of PS while the decrease in TS was relatively lower. PS contains longer chain polymers (Jane et al., 1995) compared to
the other starches in this study and the least amount of iodine (0.1%) was sufficient to inhibit the development of peak viscosity. Based on previous studies (Jenkins, Donald, 1997) it could be reasoned that the granule did not swell and/or the polymers did not leach out of the granules.

The breakdown viscosity is regarded as a measure of the degree of disintegration of granules or paste/gel stability (Noda, 2007). With the progressive increase of iodine, the breakdown viscosities of these starches (Figure 4-4) followed a similar trend as with the peak viscosities (Figure 4-1). In the control samples where no iodine is added, the polymers would leach out following granular disruption and result in a breakdown in the viscosity. Therefore, the lowering of breakdown viscosity upon addition of iodine was likely a composite effect of the reduction in the swelling of starch granules and/or a consequent decrease in granular disruption. Upon cooling, these polymers form a network and cause a rise in viscosity also known as setback viscosity. It is a result of reassociation of the polymers leached from the swollen starch granules, and reveals the gelling ability or the retrogradation tendency of the polymers (Noda, 2007). The increase in the setback viscosity of PS (Figure 4-2) upon addition of iodine at room temperature suggests that there was either an increased network formation upon increasing the iodine concentration or an increase in final viscosity. But from the results it is evident the final viscosity of PS did not change significantly until the addition of 1.1% iodine (Figure 4-3). This suggests an increased resistance against the RVA paddle measured as increasing setback viscosity could possibly be due to 1) an increased network formation by the leached polymers and/or 2) close packing of the highly swollen/rigid starch granules in the extragranular polymer matrix, if present.
When iodine was added at 95°C, the polymers had already leached out of the granules at this point and reacted with the iodine in a different manner as compared to the addition of iodine at room temperature. It should be noted that the comparisons among the breakdown and setback viscosities have been made among the samples treated with iodine at 95°C and not with the ones treated with iodine at room temperature. The repulsion caused by iodine in the form of tri-iodide (negative charge) inside the granules before heating was eliminated; hence there was no apparent change in the pasting temperature, peak and breakdown viscosities. Unlike other starches, the A-type starches (CS, WS) had not reached the peak when iodine was added at 95°C; therefore the breakdown was significantly different from the control under similar conditions and has been reported (Figure 4-6). The setback viscosities of the starches treated with iodine at 95°C were different from those at the room temperature addition. Upon addition to the starch-water slurry at 95°C, the iodine possibly reacted with the polymers that had leached out of the granules during gelatinization. The formation of complexes between iodine and the linear polymers inhibited them from forming a network, hence lowering the setback viscosity. Upon adding higher concentrations of iodine, more polymers formed a complex and were unable to re-associate to form a gel network. Since the setback for WS and CS decreased after addition of 0.1 and 0.5% iodine concentration, respectively (Figure 4-7), it can be said that these concentrations of iodine were sufficient to complex with all the leached polymers, hence preventing them from forming a network upon cooling. On the other hand, when iodine was added to PS (B-type) and TS (C-type) at 95°C, there was no significant change in the setback viscosities of these starches, therefore it is likely that the addition of iodine at this stage did not have any effect on the
polymer chains which had already formed a network by then since these starches attained peak viscosity at a much lower temperature. From the experimental observations it became clear that a specific level of iodine was required to saturate the polymers within the starch granules in order to prevent them from leaching out of the granules. Moreover, this specific amount of iodine was found to vary in the starches of different crystalline types; addition of 0.5% to WS and 1.1% iodine to CS, PS, and TS prevented the majority of polymers to leach out of the granules, thereby ceasing the development of viscosity. Moreover there was a difference in the concentration of iodine required by the starches at which the polymer-iodine complex precipitated. This is supported by the study conducted by Pfannemullar et al. (1971) that reported polymers of intermediate length (DP of about 80) tend to be more unstable and precipitate rapidly when complexed with iodine, whereas the short and long polymers were more stable in solutions; the longer chains form helical coils and associate with the shorter chains to prevent them from precipitating.

4.7 Conclusion

The pasting responses of starches of different crystalline polymorphs and from different botanical sources to varying concentrations of iodine were significantly different. The differences in the pasting properties of the two A-type polymorphs, corn and wheat, suggest that location and arrangement of polymers within the granule complexing with iodine were different, resulting in the different responses; 1) amount of iodine required for change in peak viscosity, trough and final viscosities, 2) greater decrease in final and setback viscosities of WS vs. CS. The B- and C-crystalline type starches also exhibited differences; pasting properties of PS reflected a much greater effect of iodine than any of
the other starches. By using iodine as a marker to monitor changes to starch pasting
properties allows us to differentiate the differential organization of the polymers within a
granule.
Table 4.1: Pasting properties of starches treated with iodine at room temperature

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<th>Iod</th>
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<th>PV</th>
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<th>FV</th>
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<tr>
<td>0.0</td>
<td>72.49a</td>
<td>7.94a</td>
<td>1223.00b</td>
<td>1053.00b</td>
<td>2341.00d</td>
<td>170.00b</td>
<td>1288.00c</td>
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Iod = Iodine (%), PT = Pasting Temperature (°C), Pt = Peak time (min), PV = Peak Viscosity (cP), TV = Trough Viscosity (cP), FV = Final Viscosity (cP), BD = Breakdown (cP), SB = Setback (cP); Letters after the values indicate degree significance at 95% confidence interval within each other for each starch.
Table 4.2: Pasting properties of starches treated with iodine at 95°C

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Iod= Iodine (%), PV = Peak Viscosity (cP), TV = Trough Viscosity (cP), BD = Breakdown (cP); Letters after the values indicate degree significance at 95% confidence interval within each other for each starch.

* Values have been shown only till the highest level of iodine concentration at which respective pasting properties were observed.
Figure 4-1: Change in peak viscosity of starches upon addition of iodine at room temperature
Figure 4-2: Change in setback viscosity of starches upon addition of iodine at room temperature
Figure 4-3: Change in the final viscosity of starches upon addition of iodine at room temperature
Figure 4-4: Change in breakdown viscosity of starches upon addition of iodine at room temperature
Figure 4-5: Change in peak viscosity of corn and wheat starch upon addition of iodine at 95°C
Figure 4-6: Change in breakdown viscosity of corn and wheat starch upon addition of iodine at 95°C
Figure 4-7: Change in setback viscosity of starches upon addition of iodine at 95°C
CHAPTER 5

INVESTIGATING THE SWELLING OF STARCH GRANULES FROM DIFFERENT BOTANICAL SOURCES USING IODINE AS A MARKER

5.1 Abstract

The chemical nature of the glucan polymers and their arrangement within the starch granules determines their binding with iodine. In previous studies, this complex formation between iodine and glucan polymers has been shown to alter the pasting properties of starches from different botanical origins. In this study, the effect of iodine on the swelling capacities of corn (CS), wheat (WS), potato (PS) and tapioca (TS) starches was investigated by heating 8% starch slurry with different levels of iodine in a Rapid Visco Analyser (RVA). Increasing concentrations of iodine had an inverse effect on the swelling capacities of starches even though the granules were highly swollen, as observed in the micrographs. Furthermore, the composition of the leachate was different for the starches, suggesting that the swelling capacity was influenced largely by an increase or decrease in the volume of leachate and the absorption spectra of the leachate. The same amounts of iodine resulted in widely different swelling capacity values for the different starches, likely suggesting differences in their granular architecture.

5.2 Introduction

The pasting behaviour of starches has been extensively investigated using a Rapid Visco-Analyzer (RVA) or a Micro Visco-Amylograph (MVAG). The starch granules swell and
increase in size upon heating in excess water with the subsequent exudation of polymers resulting in an increase in viscosity (Li et al., 2008). Swelling of starch granules has been measured and expressed by using a number of parameters including, swelling capacity (Bagley and Christianson, 1982; Toyokawa et al., 1989), swelling factor (Tester and Morrison, 1990; Tester and Karkalas, 1996; Wang and Seib, 1996; Chung et al., 2009), swelling power (Leach et al., 1959; Li and Yeh, 2001; Noosuk et al., 2003) and swelling volume (Crosbie, 1991). Mostly, the differences in the swelling parameters of starches with increasing temperatures have been related to the amylose content of the starches or to amylopectin structure (Tester and Morrison, 1990, 1992; Tester et al., 1991; Tester and Karkalas, 1996). Moreover, the differences in the values of these swelling parameters have been shown to be dependent on the testing procedures and parameters including starch concentration, temperature, and shear (Wang and Seib, 1996). The location and arrangement of glucan polymers that determine the swelling of starch granules and the concomitant changes, occurring during gelatinization is still not fully understood.

Iodine, binds to linear glucan chains to form helical inclusion complexes which can show absorbance over a wide range of wavelengths (Banks et al., 1971). This complex formation results in a spectrum of colours ranging from red to blue as the absorbance increases. The length of the glucan polymers primarily determines the extent of their binding with iodine. The brownish-violet stain in the outer zone of the starch granules with the binding of iodine has been linked to a greater proportion of amylopectin towards the periphery (Conde-Petit et al., 1998). In more recent research, the effect of iodine on the swelling of starch granules has been studied for wheat and corn starch (Patel et al., 2006). These authors reported that swelling of wheat and corn starches was completely
restricted at high concentrations of iodine (0.2%). In another study, the effect of increasing concentrations of iodine on the pasting behaviour of wheat starch was investigated (Saibene and Seetharaman, 2008). In the previous chapter, the differences in the pasting profiles of different starches treated with varying levels of iodine were discussed (Ch. 4). We hypothesized that the differences observed in that study were likely due to the dissimilarity in their granular architecture, i.e., the changes are not only due to size or shape of granules, or the amylose/amylopectin properties, but were more likely due to how these polymers are organized within the granules in starches from different botanical sources. The objective of this study was to investigate starch granule swelling capacity and polymer leaching upon addition of varying concentrations of iodine.

5.3 Materials

Commercial starches from common corn endosperm (Melojel) [CS], common tapioca endosperm (Tapioca Starch, TS) were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). A commercial starch from potato (Pencook 10) [PS] was obtained from Penford Food Ingredients Co., Colorado, and wheat starch [WS] was from MGP Ingredients Inc., Kansas. Potassium iodide (ACS reagent) was purchased from Fisher Scientific, Nepean, Ontario and iodine crystals were supplied by Sigma Aldrich, St. Louis. The percentage of amylose in CS, WS, PS and TS was 21.2 ± 0.7, 20.5 ± 0.1, 16.7 ± 0.2 and 17 ± 0.5, respectively.
5.4 Methods

5.4.1 Sample Preparation

In the pasting experiments, iodine solution (0% to 1.1% dry starch basis) was added to the starches (8% dry basis) at room temperature. At the lowest level (0.1%) of I₂-KI solution, there was $4.30 \times 10^{-6}$ mol I₂/g starch and $6.59 \times 10^{-5}$ mol KI/g starch; corresponding to 0.07 mM total ionic concentration of iodine in solution. At the highest level of iodine addition (1.1%), there was $4.75 \times 10^{-5}$ mol I₂/g starch and $7.27 \times 10^{-4}$ mol KI/g starch; corresponding to 0.77 mM total ionic concentration of iodine in solution. The starch-iodine solution mixture (28 g) was heated in a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) equipped with Thermocline for Windows (TCW3) software, from 25°C to 95°C at a rate of 10°C/min with 160 rpm shear. The system was held at 95°C for 3 min before cooling to 25°C at 10°C/min. The temperature was held constant at 25°C before withdrawing the sample for subsequent analyses. pH of the samples (7.0 ± 0.1) before and after the experiment was not controlled. The experimental analyses were conducted at least in duplicate and average values are reported. All statistical analyses were performed using StatGraphics Centurian Data Analysis and Statistical Software (StatPoint Technologies, Inc., Warrenton, VA).

5.4.2 Swelling Capacity (Q)

The starch slurry from the RVA was withdrawn every 10°C from 50°C to 90°C and centrifuged at a speed of 1,500 x g for 5 min. The weight of sediment and volume of supernatant were recorded. Swelling capacity (Q) was reported as the ratio of the sediment weight to the dry weight of starch (Bagley and Christianson, 1982).
5.4.3 Spectroscopic analysis and total carbohydrate content of supernatant

A wavelength scan of the supernatant separated from the gels/pastes was performed using a Konica Minolta CM-3500d Spectrophotometer (Konica Minolta Sensing, Inc., New Jersey, USA) equipped with SpectraMagic NX CM-S100 software. The soluble carbohydrates in the supernatant were measured by the phenol-sulphuric acid method (Dubois et al., 1956).

5.4.4 Light Microscopy

The bright-field light images of the starch pastes/gels obtained from the RVA were viewed under 40x magnification and imaged by using an Olympus BX60 Microscope (Olympus Optical Co. Ltd., Japan) fitted with a photographic camera. The micrographs were analysed using the ImageProPlus software (Media Cybernetics, Inc., Bethesda, MD, USA). The control sample was stained with 0.2% iodine solution, while the samples treated with iodine during experimentation did not require any further staining.

5.5 Results

5.5.1 Swelling Capacity (Q)

Starch swelling capacity (Q) data are presented in Figure 5. The Q values of control starches generally increased with increasing temperature, as expected, and had different maximum values (Q_{max}) for different starches. Control CS attained Q_{max} at 75°C (Fig. 5-1a), while PS (Fig. 5-1c) and TS (Fig. 5-1d) attained Q_{max} at 70°C. Furthermore, Q values for CS was lower than that observed for PS or TS (8 vs 13 or 12). However, WS did not reach Q_{max} even at 80°C (Fig. 5-1b), but its Q value was similar to CS. The
temperatures at which an initial increase in Q value was observed was also dependent on the type of starch as has been reported earlier (Bagley and Christianson, 1982; Lagariggue et al., 2008). However, differences were observed in Q values following the addition of iodine to the different starches. At temperatures lower than 45°C the addition of iodine to the starches did not exhibit any significant differences in Q. However, above 45°C, the effect of iodine concentration was different for the various starches.

For CS, the differences in swelling capacity were evident at a temperature above 55°C; and Q of CS was inversely related to the iodine content above that temperature (Figure 5-1a). For WS, the addition of the lowest amount of iodine resulted in a significant decrease in the Q value at 55°C (Figure 5-1b). Further increases in iodine concentration reduced Q and the effect was more pronounced at temperature higher than 65°C. Iodine addition to PS had a small but significant reduction in Q at 55°C (Figure 5-1c). At higher temperatures, the curve plateaued out after 70°C, suggesting there was no further swelling in PS; however, there was decrease in the value of Q at higher iodine concentrations. The swelling capacity of TS was greatly reduced at 70°C, but only at iodine concentrations greater than 0.2% (Figure 5-1d).

5.5.2 Volume, absorption spectra and total carbohydrate content of supernatant

The volumes of supernatant separated from the starch material at the end of pasting experiment are reported in Figure 5-1 (secondary y-axis) and in Figure 5-2. In general, the volume of supernatant decreased as the starch samples were heated (Figure 5-2). Furthermore, the volume of supernatant increased with increasing iodine concentration, though the temperature at which the change was observed was different for the starches.
The inverse effect of iodine concentration on the supernatant volume obtained for CS (Figure 5-2a) and PS (Figure 5-2c) was clearly noticeable by 70°C, while the same effect was observed at 60°C for WS (Figure 5-2b) and TS (Figure 5-2d). The volume of supernatant in PS was reduced dramatically at 70°C and was negligible at higher temperatures; a similar effect was observed for TS but unlike PS, the addition of increasing levels of iodine resulted in comparatively higher volumes of supernatant in TS even at higher temperatures.

The wavelength scans of supernatants from CS, WS, PS and TS are shown in figures 5-3a and 5-3b. The supernatants from CS and WS were sampled for wavelength scan following heating up to 80°C in the RVA (Figure 5-3a); whereas supernatants were only scanned until 60°C for PS and TS (Figure 5-3b) since there was no supernatant at higher temperatures. The two concentrations of iodine reported for each starch (Figure 5-3a, 5-3b) correspond to the final levels of iodine at which gel formation still occurred and the iodine level where there was no gel formation. The absorbance was higher for the latter, which contained greater concentration of iodine. The absorbance maxima for CS occurred at a higher wavelength as compared to WS, both of which were lower than the absorbance maxima for PS and TS.

The total carbohydrate content of the supernatant was determined for the control samples of CS and WS (Figure 5-1a, b) and it increased with increasing temperature. CS control supernatant had higher carbohydrate content (2.14 mg) at 45°C as compared to WS (0.85 mg). The total carbohydrate content of the samples containing iodine was not determined due to the interference of iodine with the assay. Considerable volume of supernatant could not be obtained for PS and TS gels after 60°C (Figure 5-2c, d); therefore the values
of total carbohydrate content of these starches could not be determined for the control
samples of these starches.

5.5.3 Light Microscopy

The microscopic images show the material obtained after the pasting experiment (Figure
5-4a-l). The three images for the individual starches show the control sample, the highest
level of iodine used where gel formation still occurred and the iodine level at which no
gel formation occurred. It should be noted that for the control starches, iodine was added
during slide preparation (staining) for microscopy while the treated starches exhibit color
from the iodine added during the experiment. The remnants of collapsed granules
dispersed in the extragranular polymer matrix are clearly visible in all control starches,
but more evident in CS and WS controls (Figure 5-4a, d) as compared to PS or TS
(Figure 5-4g, j). The highest concentration of iodine at which gel formation was observed
for CS and PS was 0.5%; the granules clearly seem to have collapsed in CS (Figure 5-
4b), whereas fully swollen granules were still visible in PS (Figure 5-4h). At 1.1% iodine
level, CS and PS granules were both highly swollen but there was no gel formation
(Figure 5-4c, i). On the other hand, the addition of 0.5% iodine to WS and TS (Figure 5-
4e, l) inhibited gel formation even though the granules seem to have collapsed.

5.6 Discussion

The swelling capacity is a measure of the cumulative effect of swollen granules (Jacquier
et al., 2006) and the leached polymers (Nunez-Sanitago et al., 2004) in relation to the dry
starch weight. Therefore any change in swelling capacity is indicative of changes to the
extent of granule swelling and/or the amount of leached polymers. When iodine is added
to starch at room temperature, the changes in swelling capacity are then a consequence of iodine interaction with the granular starch polymers that either restricts granule swelling or reduces the amount and/or composition of the extragranular leachate. Data reported in this study demonstrates that the increase in Q-value of starches was either delayed, for example, TS at 0.5 % iodine addition, or reduced with increasing levels of iodine addition (Figure 5-1d). According to Bagley and Christianson (1982) values of cQ< 1 (c, starch concentration and Q, swelling capacity) suggest the existence of excess solvent between particles, whereas cQ> 1 would mean that all the solvent had been absorbed by the particles. This explains why the PS, TS and the waxy starches (data not shown) at 8% dry starch concentration could not be separated into sediment and supernatant at higher temperatures. CS and WS, on the other hand are known to exhibit granule ghosts upon gelatinization (Stark and Lynn, 1992) and the granule ghosts in WS have been shown to have a greater thickness when compared to those of CS (Glenn et al., 2008). These granule ghosts/remnants are believed to be held together through weak interactions between amylopectin chains rather than with proteins or lipids (Atkin et al., 1998; Rockland et al., 1977). These observations were in agreement with those of Li and Yeh (2001) using a much lower starch concentration of 1%. For CS and WS, cQ was less than 1 at all temperatures, which suggests the presence of excess solvent between the starch particles. However, it should be noted that the swelling capacity may be overestimated due to entrapment of interstitial water in the extragranular polymer matrix in PS and TS, even though it does offer a fair approximation of the volume fraction occupied by the starch granules and the leached polymers (Nayouf et al., 2003, Lagarrigue et al., 2008).
CS and WS are both A-type crystalline polymorphs based on X-Ray crystallography. However, the effect of iodine was more pronounced in WS as compared to CS. Therefore, the differences observed in the swelling capacity of these two starches are likely due to differences in 1) the shape of the granule and the volume occupied by the swollen granules and/or 2) the amount of polymers that leached into the extragranular matrix. WS has a bimodal granule size distribution with small, spherical B-granules that make up about 30% starch content; and disc-shaped large A-granules that amount to 70% content by weight of starch (Soulaka and Morrison, 1985). Iodine should likely have a greater effect on the swelling of large granules thus resulting in significantly decreased swelling capacity observed at the 0.1% iodine level. However, microscopic images reveal that the granules are swollen and folded even at 0.2% iodine concentration (Figure 5-4e). Thus, iodine does not appear to affect granule swelling, but rather polymer leaching from the granules contributing to the decreased swelling capacity. However, for control starches, the increase in the carbohydrate content in supernatant of WS was twice as much as for CS by 75°C (58.5% increase vs. 29.5% increase, respectively). Therefore, even though there was more extragranular leachate in control WS, the volume of leachate decreased significantly even with the lowest amount of iodine addition. Granule swelling was not affected even at 0.5% iodine concentration but the slurry does not form a gel at this concentration, further confirming that the iodine interaction with granular starch altered polymer leaching into the extragranular space.

CS granules, on the other hand, are lenticular in shape and have a unimodal size distribution (Buttrim et al., 2009). When iodine was added to CS, the swelling capacity decreased only slightly until 0.5% iodine level. A significant decrease in Q was observed
only at 1.1% iodine concentration at 90°C; but the extent of reduction is significantly lower than that observed for WS. Again, the microscopic images reveal swollen and folded granules at 0.5% iodine concentration and fully swollen granules at 1.1% iodine concentration. Therefore, iodine did not seem to influence granule swelling but rather affected polymer leaching. However, the concentration of iodine required to reduce polymer leaching and prevent gel formation was higher for CS (0.5%) as compared to WS (0.2%). These observations suggest that even though both CS and WS are A-type crystalline polymorphs, the arrangement of the polymers within the granules are likely different between these two starches. It is also interesting to observe the differences in the color of the microscopic images of CS and WS with 0.5% iodine addition (Figure 5-4b vs. Figure 5-4f). CS exhibited a bright blue color but WS exhibited light pinkish purple color; suggesting differences in the chain length of the polymers that complexed with iodine. This is further supported by the wavelengths of maximum absorption in case of CS and WS supernatants containing the leached polymers (Figure 5-3a). The supernatant separated from WS treated with 0.5% iodine solution has absorbance maxima at lower wavelengths as compared to CS, thus indicating the presence of shorter chains in the leachate. These results, based on the differences in the polymer chain lengths, are in agreement with the findings of Jane et al. (1999), but the small difference in the percentage distribution of short and long polymer chains does not explain the wide differences observed in the present study.

PS is a B-type crystalline polymorph (McPherson and Jane, 1999), and has granules that are oval in shape with a larger average granule size compared to other starches (Butrim et al., 2009). It is therefore expected that PS will have a higher Q-value compared to WS or
Unlike with CS and WS, the swelling capacity of PS was delayed slightly at 60°C for all levels of iodine addition, and reached a maximum at 70°C and did not change after that. The extent of reduction in swelling capacity and the overall changes with increasing iodine concentration were also very small compared to other starches. The volume fraction (in dilute or concentrated systems) determines the ability of starch granules to swell to their maximum (Steeneken, 1989); and the c* values (c*=100/Swelling capacity) of PS is reported to be 0.25% db (Eerlingen et al., 1997), a value much lower than the concentration used in this study. At 8% db, the concentration of PS is magnitudes higher than close packing concentration, the concentration in a suspension at which starch granules are swollen to equilibrium and fill up the available space (Vandeputte et al., 2003). Therefore, it is quite likely that the minimal effects on the swelling capacity (Figure 5-1c) are due to the high concentrations of starch used in the study. Even if there was a reduction in granule swelling, the magnitude of reduction was likely smaller and not evidenced in the swelling capacity data. In comparison with CS, the microscopic images also reveal swollen and crumpled granules at 0.5% iodine concentration and fully intact swollen granules at 1.1% iodine concentration (Figure 5-4h, i). However, the absence of gel formation at 1.1% iodine concentration suggests the absence of extragranular leachate.

TS is a C-type crystalline polymorph with a unimodal size distribution (Butrim et al., 2009) consisting of spherical indented granules that are larger than WS and CS (4-19 μm) but smaller than PS (8-60 μm). C-type crystalline polymorphs are a combination of A-type crystalline polymorph on the outside of the granule and a B-type polymorph in the centre of the granule (Bogracheva et al., 1998). The swelling capacity values for control
TS are similar to those of PS (Figure 5-1c, d). The swelling capacity of TS with iodine was similar to PS at iodine concentration below 0.5% and was similar to CS and WS at higher iodine concentrations; and its gel forming ability was similar to WS with no gel formation at 0.5% iodine concentration. The micrographs show granule swelling at 0.5% iodine level where no gel formation took place confirming the proposition that leached polymers are required to form a gel. Interestingly, unlike PS, the volume of supernatant from TS increased upon addition of higher concentrations of iodine, suggesting that the iodine-polymer complex formed prevented them from leaching into the extragranular matrix. The comparatively higher volume of supernatant from 1.1% iodine samples (Figure 5-2d) confirms the inhibition of polymer leaching and gel formation with lower cQ due to polymer-iodine complex formation. However, this was not the case with PS (Figure 5-2c), suggesting that PS has either, 1) higher proportion of longer chain polymers less susceptible to leaching into the extragranular areas and/or, 2) a different arrangement of polymers inside the granules limiting their mobility, when compared to TS in this case, and rest of the starches, in general. Observations from this study suggest that more than the difference in the percentage distribution of short or long chains, it is the arrangement of these polymers which governs the swelling of the starches and leaching of the polymers.

5.7 Conclusion

In this study, the ability of iodine to complex with linear polymers was used as a marker to understand starch granule swelling and polymer leaching. Increasing concentration of iodine stabilized the polymer layout within the granules by forming complexes, thereby preventing the granule swelling and leaching of polymers. The data reported in this study
supports the argument that starch granules from different botanical origins are packed differently as the same volume of iodine results in differential swelling and folding of granules.
Figure 5-la: Swelling capacity of corn starch as a function of temperature and concentration of iodine. The value in brackets, above the volume of supernatant in control sample (represented by secondary y-axis), indicates total carbohydrate content (mg in supernatant) for the control sample at that temperature.
Figure 5-1b: Swelling capacity of wheat starch as a function of temperature and concentration of iodine. The value in brackets, above the volume of supernatant in control sample (represented by secondary y-axis), indicates total carbohydrate content (mg in supernatant) for the control sample at that temperature.
Figure 5-1c: Swelling capacity of potato starch as a function of temperature and concentration of iodine. The secondary y-axis represents volume of supernatant in control sample.
Figure 5-1d: Swelling capacity of tapioca starch as a function of temperature and concentration of iodine. The secondary y-axis represents volume of supernatant in control sample.
Figure 5-2a: Volume of supernatants obtained from corn starch slurries as a function of temperature and concentration of iodine
Figure 5-2b: Volume of supernatants obtained from wheat starch slurries as a function of temperature and concentration of iodine
Figure 5-2c: Volume of supernatants obtained from potato starch slurries as a function of temperature and concentration of iodine
Figure 5-2d: Volume of supernatants obtained from tapioca starch slurries as a function of temperature and concentration of iodine
Figure 5-3a: Wavelength scans of CS and WS supernatants after treatment with iodine and heating in the RVA up to 80°C
Figure 5-3b: Wavelength scans of PS and TS supernatants after treatment with iodine and heating in the RVA up to 60°C
Figure 5-4: Light micrographs of corn starch treated with a) 0, b) 0.5 and c) 1.1% I₂-KI solution
Figure 5-4: Light micrographs of corn starch treated with a) 0, b) 0.5 and c) 1.1% I₂-KI solution
Figure 5-4: Light micrographs of wheat starch treated with d) 0, e) 0.2 and f) 0.5% I₂-KI solution
Figure 5-4: Light micrographs of potato starch treated with g) 0, h) 0.5 and i) 1.1% I\textsubscript{2}-KI solution
CHAPTER 6

TEXTURE AND RHEOLOGY OF STARCH GELS CONTAINING IODINE

6.1 Abstract

The objective of this study was to investigate the textural and rheological properties of starch gels containing varying concentrations of iodine. Corn (CS), wheat (WS), potato (PS) and tapioca (TS) starch gels containing varying concentrations of iodine were prepared in a Rapid Visco Analyser (RVA). The gels were analyzed by using a texture analyzer for large deformation properties following storage for 1 or 7 days and also by using a rheometer for small deformation properties. With increasing concentration of iodine, the firmness of gels decreased and the extent of decrease varied for the different starches. The extent of change in complex modulus (G*) of the gels was highest in the A-type starch, CS, but was different from WS, which is also an A-type. The G* for PS gels (B-type) decreased with the addition of 0.1% iodine but there was no significant difference upon increasing the iodine concentration. The G* for the C-type TS gels was lower than 15 Pa. The observations suggest a difference in the microstructure of the starch gels formed resulting from differential leaching of polymers bound with iodine.

6.2 Introduction

Starch plays an important role in providing texture to various food products. Based on the type of starch and processing conditions, different interactions occur between the polymers and/or added food ingredients, such as lipids or salts. During the process of
gelatinization, the starch granules absorb water and change in shape and size due to swelling and crystal melting (Atwell et al., 1988, Jenkins and Donald, 1997). There is concomitant leaching of glucan polymers (amylose and amylopectin) from the swollen granules, which form a gel network upon cooling (Eliasson, 1986). Depending on their structural origins (Rao et al., 1997), chemical nature of the polymers and the dispersed granule remnants (Rockland et al., 1977; Stark and Lynn, 1992; Atkin et al., 1998; Glenn et al., 2008), starch gels from different botanical origins exhibit a wide variety of textural properties (Thebaudin et al., 1998).

The textural properties of starch are usually studied by either measuring the flow behaviour of the starch dispersions or the structural deformation of the gelatinized samples. In a low frequency set, the rheological parameters, such as elastic and loss moduli (G'/G'”) depend only on the dimensionless strain; as the oscillatory strain is increased the samples maintain their resistance to deformation until they reach a critical range of strain values, whereupon they yield to undergo a rapid transition from a high to low modulus. The plateau or the linear viscoelastic region reveals the significant differences in the at-rest resistance to deformation i.e., the stiffness - of the products. The network formed by the leached polymers determines the strength of the gel and a strong interaction between the polymers is required to obtain gels with higher strength (Liu and Lelievre, 1992).

The gel obtained after cooking the starches in excess water is an amalgamation of starch granules and the leached polymers; the ratio of which is thought to impact the rheological characteristics of the gels (Inaba et al., 1995). In the previous studies, the effect of iodine on granular swelling (Patel et al., 2006, Chapter 5) and pasting properties of starch were
studied (Saibene et al. 2008, Chapter 4). It was shown that apart from restricting granular swelling, iodine complexes with and inhibits the leaching of polymers. The objective of this study was to investigate the effect of iodine on the textural and rheological properties of starch gels.

6.3 Materials

Commercial starches from common corn endosperm (Melojel) [CS], common tapioca endosperm (Tapioca Starch, TS) were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). A commercial starch from potato (Pencook 10) [PS] was obtained from Penford Food Ingredients Co., Colorado, and wheat starch [WS] was from MGP Ingredients Inc., Kansas. Potassium iodide (ACS reagent) was purchased from Fisher Scientific, Nepean, Ontario and iodine crystals were supplied by Sigma Aldrich, St. Louis. The percentage of amylose in CS, WS, PS and TS was 21.2 ± 0.7, 20.5 ± 0.1, 16.7 ± 0.2 and 17 ± 0.5, respectively.

6.4 Methods

6.4.1 Sample Preparation

In the first set of pasting experiments, iodine solution (0.1% to 1.1% dry starch basis) was added to the starches (8% dry basis) at room temperature, with a sample containing distilled water instead of iodine solution used as control. At the lowest level (0.1%) of I₂-KI solution, there was 4.30 x 10⁻⁶ mol I₂/ g starch and 6.59 x 10⁻⁵ mol KI/ g starch; corresponding to 0.07 mM total ionic concentration of iodine in solution. Whereas at the highest level of iodine addition (1.1%), there was 4.75 x 10⁻⁵ mol I₂/ g starch and 7.27 x 10⁻⁴ mol KI/ g starch; corresponding to 0.77 mM total ionic concentration of iodine in
The starch-iodine solution mixture (28 g) was heated in a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) equipped with Thermocline for Windows (TCW3) software, from 25°C to 95°C at a rate of 10°C/min with 160 rpm shear. The system was held at 95°C for 3 min before cooling to 25°C at the same rate. The temperature was held constant at 25°C before withdrawing the sample.

In the second set of experiments, starch (8% db) and water were mixed in an RVA canister to make up a total of 28 g. The mixture was heated in the RVA from 25°C to 95°C at a rate of 10°C/min. Upon reaching 95°C an additional 5.5 ml of distilled water or iodine solution (0.1% to 4%) was added to the starch slurry adjusting the final percentage of solids in the system to 6.7%. The starch slurry was then held at 95°C for 3 min. before cooling to 25°C at the same rate and holding stable for 3 minutes. The pH of the samples (7.0 ± 0.1) before and after the experiment was not controlled.

6.4.2 Rheology (Small Deformation)

The small-deformation rheological measurements of the starch systems were performed by controlled stress dynamic oscillatory rheometer (AR-2000 Rheometer, TA Instruments, New Castle, DE, USA) equipped with a parallel plate system (20 mm diameter). The material obtained at the end of the pasting experiment in the RVA was poured into moulds (20 mm x 3 mm) and stored overnight at room temperature. The samples, in the form of discs, were subjected to a strain-sweep test with the strain varying from 0.1 to 1000%, at an angular frequency of 1 rad/s (0.16 Hz) and a set temperature of 25°C. The strain-sweep tests were conducted to determine the linear viscoelastic region of the samples along with various rheological parameters such as elastic modulus, loss
modulus, phase angle and critical strain at fracture (strain at which the gel is disrupted and there is a dramatic decrease in the value of $G'$).

6.4.3 Texture Analysis (Large Deformation)

The starch pastes/gels obtained from the RVA were poured into petri dishes (35 x 10 mm); aluminum foil was used to raise the walls of the dishes and cover the top. The samples were then stored at room temperature for 1 day and at 4°C for 7 days. After removing the aluminum foil, the excess sample above the rim of each petri dish was removed with a dental floss and the freshly cut surface of the gel was analysed for the textural changes. The stored samples were analysed using a TAXTplus texture analyser (Texture Technologies, Inc.); the gels were measured for their compressibility using a spherical compression probe (TA-23, 0.25 inch diameter). The pre-test, test and post-test speeds were set at 1, 0.2 and 0.2 mm/s, respectively. A force of 5 g was used to compress the 10 mm gels through a depth of 4 mm. The maximum force at 4 mm depth exerted by the sample against the probe was recorded.

6.4.4 Statistical Analysis

The experimental analyses were conducted at least in duplicate and average values are reported. All statistical analyses were performed using StatGraphics Centurian Data Analysis and Statistical Software (StatPoint Technologies, Inc., Warrenton, VA).
6.5 Results

6.5.1 Rheology

Figure 6-1 to 6-4 show the behaviour of complex moduli of 8% starch gels treated with varying concentrations of iodine as a function of strain. An oscillation strain sweep test revealed significant differences in the behaviour of starch gels containing different levels of iodine. Complex modulus ($G^*$), which is a combination of the elastic ($G'$) and loss ($G''$) moduli, is a measure of the resistance to deformation by the gels. When gels containing increasing levels of iodine were subjected to small deformation strains, a decrease in the $G^*$ and LVR was observed for CS. The addition of 0.1% iodine to CS did not result in any significant difference as compared to the control sample. CS gels containing 0.2% and 0.5% iodine underwent a reduction in $G^*$ by 23% and 46%, respectively. The LVR was also observed to shorten with increasing iodine concentration (Figure 6-2). There was no significant difference between the WS control and 0.1% iodine containing WS gels. At 0.2% iodine level a 10% decrease in the $G^*$ was observed (Figure 6-3). With PS, the addition of lowest concentration of iodine (0.1%) led to gels with reduced $G^*$, but no further reductions were observed in gels with increasing levels of iodine (Figure 6-4). The $G^*$ for TS and waxy starches were below 15 Pa for control and at all levels of iodine (results not shown), and no significant differences were observed.

6.5.2 Texture Analysis

Table 6-1 shows the firmness of the starch gels treated with iodine at room temperature. The firmness of the starch gels was determined after 1 day of storage at room temperature
and 7 days of storage under refrigeration. All starch gels treated with iodine decreased in firmness as a function of increasing iodine concentration irrespective of the temperature at which it was introduced into the system (RT vs. 95°C) or the age of gel (1 day vs. 7 day). Furthermore, the highest level of iodine at which different starches did not form a gel was different. Firmness of 1 day old CS gel did not show a significant difference from the control upon addition of 0.1% iodine; further addition of iodine resulted in softer gels. WS, PS and TS gels treated with increasing levels of iodine exhibited a gradual decrease in firmness. The firmness of the 7 day old starch gels as compared to 1-day old gels, both in control gels and in the presence of iodine was not significantly different for WS and TS; while the control for CS and 0.1% iodine-treated samples of CS and PS hardened upon retrogradation. The firmness of TS control gel, waxy corn starch (WCS) and waxy potato starch (WPS) gels at all levels could not be determined as the gels were too soft.

The firmness of starch gels treated with iodine at 95°C is reported in table 6-2. Upon addition of iodine at 95°C, the gels formed were softer as compared to the ones in which iodine was added at room temperature. It should be noted that in the latter case, an additional 5.5 ml of water was involved, and was largely responsible for the softness of the starch gels when compared to the ones in which iodine was added at room temperature. Firmness of the gels treated with iodine at 95°C decreased as a function of increasing concentration for all starches. PS was the only starch to still form a gel even at 4% iodine concentration, but the gels did not show any significant retrogradation upon storage after 7 days, except at 0.5 and 1.1% iodine level. A concentration of 1.1% iodine was enough to inhibit gel formation in rest of the starches.
6.6 Discussion

6.6.1 Rheology

The rheological behaviour of starch pastes and gels is a result of the combination of various parameters such as volume fraction of swollen granules (swelling capacity), their deformability, and concentration of solids in the system. This behaviour is not governed by the same rules in all starches (Doublier et al., 1987). In this study a paste/gel may be considered as a composite material with the swollen/collapsed starch granules entrapped in a polymer solution/matrix.

The technique used for rheological measurement involved dynamic oscillations as a function of varying strain, keeping the frequency constant. The gradual disruption of the three-dimensional gel network can be detected only under very small deformation conditions (Lapasin and Pricl, 1995); therefore, extremely low frequency was used to determine the transition strain between gel and sol state. The angular frequency was chosen to be 1 rad/sec (0.16 Hz.) as it was important to observe a net differentiation between the gel and the second state following the phase transition. The “second” state in this set up of experiments is achieved when recoverable strain, the critical strain at which an elastic solid-like material phase transforms into a viscous state due to shear deformity response, is exceeded. Overall, the complex modulus ($G^*$) of the composite gels is a resultant of a combination of parameters involving elasticity of the continuous phase, the volume fraction of the dispersed phase and the shape and deformability of the swollen particles. A difference in yield stresses (the applied stress, which must be exceeded in order to make a structured fluid flow) was observed for the samples containing different
levels of iodine; with increasing concentration having an inverse effect on the relative yield stress. This suggests that the adhesion between the granule remnants and the extragranular matrix is weakened by the addition of iodine and the application of stress led to a premature failure of the gel phase. The slope of the curve upon disruption of the gel also increased, suggesting a reduced elastic behaviour of gels containing higher levels of iodine. Therefore, it is reasonable to expect that a gel with lower G* will be relatively much weaker.

CS and WS have higher G* in comparison with the other starches, but giveaway to deforming strains much earlier, thereby suggesting a weaker network. Since the granules of PS, TS, WCS and WPS were considerably more solubilised during the pasting experiment, the elasticity (stiffness) of the gels was much less pronounced. PS, TS and the waxy starches have low G*, which would suggest that the network formed by the polymers is loose, but well-knit (interconnected) since it required a relatively higher strain to disrupt the gels as compared to CS and WS gels. Therefore, the lower values of elasticity in these starches could likely be due to the lack of rigid, swollen granules, possibly, with the polymers inside as in case of CS and WS. Klucinec and Thompson (2002) suggested that formation of physical junction zones (PJZ) between amylose and external chains of amylopectin molecules could possibly occur, hence providing further stability to the gel. Formation of complexes with iodine would lead to formation of lesser such PJZ and also decreased interactions between the internal elements, hence lowering the rate and extent of retrogradation apart from having a significant impact on the gel strength. Since WS contains shorter chains, and PS contains longer chains, one would expect complexation with iodine to have a higher impact on the PJZ formation in PS.
This is reflected in the lowering of the G* for PS, while the same quantity of iodine did not produce a significant effect on WS. As mentioned earlier (Chapter 4), the effect of iodine was observed only above a critical concentration characteristic for the individual starches, and below this concentration there was no appreciable variation in the G* values of the starch gels. In some cases this was the highest level of iodine at which a gel was formed. This hypothesis is based on the postulation that the polymer-iodine complex alters the behaviour of gels from normal control gels, and should change the elasticity (solid-like response) of the gel.

6.6.2 Texture Analysis

The ratio between intact granules and granule remnants have been related to the textural properties of the starch pastes (Blennow et al., 2003). The granules of waxy starches and potato starches are known to form sticky gels upon disruption and solubilisation. The texture analysis of the opaque gels of CS and WS was performed after 24 h to allow the retrogradation of amylose (Miles et al., 1985; Sievert and Wursch, 1993) and after 7 days to allow the recrystallization (retrogradation) of amylopectin chains (Eliasson, 1985). CS seemed to form the firmest gels as compared to the other starches, which was contrary to the results of Takahashi and Seib (1988), who stated that at concentrations higher than 6% one day old WS gels were harder than CS. The starches behave differently on cooling; while the cereal starch pastes exhibit a sharp transition upon cooling due to the formation of strong gels, PS gels are less affected by cooling. The presence of distinguishable granular remnants and portions of polymer clusters made the CS, WS gels heterogeneous based on visual inspection, whereas in PS, TS, WCS and WPS gels, the resulting network was more homogenous. This was true in the control gels and those
containing low concentrations of iodine where long range ordered sections of polymer chains were present. Gel firmness had an inverse relationship with iodine concentration for all starches, with a few exceptions [because of destabilization of starch granules during gelatinization due to increased charge density of iodide ions (Jane, 1993)]. It is known that for all polysaccharide polyelectrolytes the presence of low ionic strength leads to stabilization of the system and in some cases promotes the formation of ordered helical conformation by screening the repulsion between the charges of the polyion. (Jane, 1993). It can be observed that the retrogradation patterns (gel firmness after 1 day vs. 7 day) were different for the starches in the presence of increasing iodine concentrations.

The presence of large numbers of swollen granules with minimal amount of polymer leachate in the extragranular matrix was observed in cases where no gel formation took place (Figure 5-4c). In WS, the formation of a paste instead of a physical gel at 0.5% iodine level in spite of the presence of polymer leachate in the extragranular material (Section 5.5.2) suggests that the presence of the large number of granule remnants inhibited the polymer association. In other words, gel formation was inhibited in such a disperse system. However, the physical gels can be categorized into strong and weak gels on the basis of their rheological properties. The results reaffirm that the chain lengths of the polymers in WS are shorter as compared to the other starches that were studied. On the other hand PS, TS and waxy starches contain longer chain length polymers with a greater number of long range ordered sections, possibly containing interconnections.

The results from the rheological measurements do not allow much speculation on the microscopic or molecular scale within the same starch treated with different levels of
iodine. However, the gels from the different starches show a magnitude of difference in their elasticity. The critical concentration of iodine required for saturating the polymers depends on the nature of the polymers involved, i.e. their length, branching patterns and interconnections with guest molecules.

6.7 Conclusion

While in CS and PS gels, the presence of iodine had an apparent effect on G*, in WS the differences could not be detected. It is therefore likely that a critical concentration the iodine molecules saturate the polymer chains by complex formation, thereby preventing them from forming associations in the form of a gel. Below this critical concentration of iodine the differences among the gels are very well defined by large deformation studies, i.e. measuring firmness using large force compression of gels in a texture analyser but not through small deformation experiments of varying strain to measure elasticity under a rheometer).
Figure 6-1: Complex moduli \((G^*)\) of control starch gels (8% dry basis)
Figure 6-2: Complex moduli of corn starch gels containing varying levels of iodine
Figure 6-3: Complex moduli of wheat starch gels containing varying levels of iodine
Figure 6-4: Complex moduli of potato starch gels containing varying levels of iodine
Table 6-1: Firmness (in Newton, N) of starches treated with iodine at room temperature

<table>
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<tr>
<th>Iod</th>
<th>CS 1d</th>
<th>CS 7d</th>
<th>WS 1d</th>
<th>WS 7d</th>
<th>PS 1d</th>
<th>PS 7d</th>
<th>TS 1d</th>
<th>TS 7d</th>
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<td>1.03</td>
<td>0.69</td>
<td>0.70</td>
<td>0.56</td>
<td>0.71</td>
<td>nd</td>
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<td>0.83</td>
<td>0.53</td>
<td>0.62</td>
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<td>0.48</td>
<td>0.37</td>
<td>0.38</td>
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<tr>
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<td>0.35</td>
<td>0.50</td>
<td>0.16</td>
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<td>0.44</td>
<td>0.52</td>
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<td>0.37</td>
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<td>0.38</td>
<td>0.08</td>
<td>0.13</td>
</tr>
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<td>Nd</td>
<td>nd</td>
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<td>nd</td>
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</table>

CS = Corn starch, WS = Wheat starch, PS = Potato starch, TS = Tapioca starch, 1d = 1 day, 7d = 7 day;

*values with the same letter (abcd) are not significantly different (p=0.05) for the same starch but different levels of iodine,

*values with the same letter (ABCD) are not significantly different (p=0.05) for the same iodine level in different starches,

*values with the same letter (XY) are not significantly different (p=0.05) for the same level of iodine, same starch but different days, 1 and 7 day.
Table 6-2: Firmness (in Newton, N) of starches treated with iodine at 95°C

<table>
<thead>
<tr>
<th>Iod</th>
<th>CS 1d</th>
<th>CS 7d</th>
<th>WS 1d</th>
<th>WS 7d</th>
<th>PS 1d</th>
<th>PS 7d</th>
<th>TS 1d</th>
<th>TS 7d</th>
</tr>
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<tbody>
<tr>
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<td>0.33</td>
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CS = Corn starch, WS = Wheat starch, PS = Potato starch, TS = Tapioca starch, 1d = 1 day, 7d = 7 day;

*values with the same letter (abcd) are not significantly different (p=0.05) for the same starch but different levels of iodine,

*values with the same letter (ABCD) are not significantly different (p=0.05) for the same iodine level in different starches,

*values with the same letter (XY) are not significantly different (p=0.05) for the same level, same starch but different days, 1 and 7 day.
CHAPTER 7

CONCLUSIONS

The chemistry of the interaction between iodine and linear glucan polymers has been studied extensively over the years; and has been used to understand starch polymer properties, both qualitatively and quantitatively. The basic premise of the interaction relies on the degree of polymerisation (DP) of the polymer reacting with iodine; the higher the DP of the polymer, the more the amount of iodine bound and the complex exhibits absorbance at a higher wavelength. Furthermore, the color of the complex also changes with increasing DP ranging from pink/orange to purple/black with increasing DP. This project utilizes this chemistry to further explore the architectural differences in starch granules from different botanical sources. In a native granule, the numbers of iodine binding sites are limited; and it is likely that the numbers of sites are different between granules based on the organization of the polymers within each granule. Therefore, by exposing the granules to iodine and then monitoring the changes following heating and cooling, we can diagnostically infer information about the organization of the polymers within granules. The goal of the study was to probe the architectural organization of granules by investigating the effects of iodine on granule pasting properties.

Overall, significant differences were observed in the pasting properties of starches with different crystalline polymorphs in the presence of iodine. Following the addition of same amount of iodine at room temperature, the responses following heating and cooling was different for the starches. These differences did not follow the same trend even among the starches with same crystalline types; for example, wheat starch (WS) and corn starch
(CS), both A-type crystalline polymorphs, exhibited differences in granular swelling, pasting and rheological characteristics following addition of same amounts of iodine.

Differences in the pasting properties of starches from various botanical sources are typically understood based on a number of factors including granule size, shape, ratio of constitutive polymers (amylose and amylopectin), chain length profiles of the constitutive polymers, and amount of crystallinity or presence of lipids/proteins/ionic components. However, in this research we propose that in addition to these factors, the organization of the constitutive polymers within the granules, i.e., architecture, is equally, if not more important, in defining and differentiating the properties of the starches.

When iodine is added at room temperature, linear segments of amylose, amylopectin and/or intermediate materials bind iodine prior to heating of starch. It is important to note that only accessible segments of the polymers bind iodine rather than entire molecules of any of the polymers due to the steric hindrances within the native granule. When iodine forms a complex with the glucan polymers, the movement or leaching of polymers out of the granules and into the extragranular matrix is inhibited. The extent of this binding and inhibition to leaching is likely to be affected by the location and organization of the polymers within the starch granules. So the addition of iodine would have a greater effect on glucan polymers arranged in a certain way in one starch compared to another in which iodine cannot bind as easily. While a shorter chain would not be able to move freely after complex formation with iodine, segments of longer polymer chains will be able to bind iodine and still possess mobility; and this difference in the mobility of polymers within starch granules, due to their length and distribution in addition to interaction with the other polymer chains (organization) will define the differences in their functional
properties. Iodine is an excellent diagnostic tool to understand this difference in the architecture of starch granules from various sources. In this study, the pasting, swelling and leaching of polymers into the extragranular matrix and the rheological properties of the resulting gels reflected the differential organization of the polymers within a granule as evidenced by the differences in properties at similar iodine levels.

The peak viscosities of B- and C-type starches decreased with the addition of iodine, while those of the A-type starches showed an initial increase at low iodine concentrations (≤0.2% iodine). The setback viscosity of potato starch (PS) increased with increasing iodine concentrations while there was a gradual decrease in setback for the rest of the starches treated under similar conditions. Based on previous studies, the increase in pasting temperature and lack of viscosity development at higher iodine concentrations (Chapter 4) would generally have been attributed to the lack of granular swelling but the microscopy images (Chapter 5) presented in this study disprove this theory. Therefore, viscosity development in a Rapid Visco Analyser (RVA) is more due to the lack of polymer leaching into the extragranular matrix than to granule swelling. Also, the increasing setback for PS samples treated with increasing iodine concentrations was thought to be a result of either an increasing network formation by the leached polymers or the close packing of the highly swollen granules in the extragranular polymer matrix. The microscopic observations from Chapter 5 suggest that it was the swollen granules which provided resistance to the movement of the paddle that was registered as setback viscosity. This is further supported by the textural and rheological tests (Chapter 6) where the gels formed were weaker due to the absence of a polymer network, hence less retrogradation. At 95°C, iodine bound the polymers in a different manner; in this case,
the granule swelling had occurred and polymers had leached out before iodine was added; therefore, the iodine directly complexed with the leached amylose and/or amylopectin molecules. As a consequence, the amount of iodine required at room temperature or at 95°C to fully saturate the polymer-iodine complexes within the starch granules in order to prevent them from leaching out (at room temperature) or in the extragranular matrix (at 95°C) was different for the starches.

The arrangement of polymers in B-type PS was different from A-type CS and is supported by the results in this study. The addition of iodine brought about a greater depression in the pasting properties of PS when compared to the rest of the starches. The binding of iodine to the longer chain segments which likely participate in both, the crystalline and amorphous zones in PS, prevented their leaching to an extent greater than in CS, where the linear glucan polymers are likely mostly present in the amorphous zone. The complex formation stabilized the polymers within the granules; the presence of fully swollen granules, with addition of higher concentrations of iodine, resulted in granule swelling but inhibition of collapse and leaching of polymers. The absence of leached polymers is reflected in the lack of gel formation. The findings in this study are contrary to the earlier understanding that related swelling of granules to the development of viscosity.

The differences between WS and CS further support the notion of differences in granular architecture; although the amount and chain length distribution of leachate from WS was also lower and of smaller chain length, respectively, compared to CS. Furthermore, WS polymers were saturated at a lower iodine concentration compared to the other starches. Tapioca starch (TS), a C-type crystalline polymorph showed similarities to both A and B-
type starches. Like PS, the polymer chains in TS were longer and exhibited absorbance at 620 nm, but were saturated at lower iodine concentration like WS.

The data presented here set the stage for further experimentation to pursue the exploration of the architectural differences among starches and consequent impact on starch functionality. A better understanding of these differences could provide direction to possibly altering starches either through genetics or through other physico-chemical modifications to deliver improved functionality.
REFERENCES


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M. Seker, M. A. Hanna: Sodium hydroxide and trimetaphosphate levels affect properties of starch exudates. *Industrial Crops and Products* 2006, 23, 249-255.


Figure A-1: Pasting profile of corn starch treated with iodine at room temperature
Figure A-2: Pasting profile of wheat starch treated with iodine at room temperature
Figure A-3: Pasting profile of potato starch treated with iodine at room temperature
Figure A-4: Pasting profile of tapioca starch treated with iodine at room temperature
Figure A-5: Pasting profile of waxy corn starch treated with iodine at room temperature
Figure A-6: Pasting profile of waxy potato starch treated with iodine at room temperature
Figure A-7: Pasting temperature of starch samples treated with iodine at room temperature
Figure A-8: Peak Viscosity of starch samples treated with iodine at room temperature.
Figure A-9: Trough Viscosity of starch samples treated with iodine at room temperature
Figure A-10: Change in the trough viscosity of starches upon addition of iodine at room temperature
Figure A-11: Final Viscosity of starch samples treated with iodine at room temperature
Table A-1: Correlation between various pasting parameters and swelling capacity of corn starch

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CS = Corn starch, PV = Peak viscosity, TV = Trough viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity, SC = Swelling capacity, FRM = Firmness, PVC = Change in peak viscosity, BVC = Change in breakdown viscosity, SVC = Change in setback viscosity, DPT = Delay in pasting temperature.

*Significant at 0.05 level.
Table A-2: Correlation between various pasting parameters and swelling capacity of wheat starch

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WS = Wheat starch, PV = Peak viscosity, TV = Trough viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity, SC = Swelling capacity, FRM = Firmness, PVC = Change in peak viscosity, BVC = Change in breakdown viscosity, SVC = Change in setback viscosity, DPT = Delay in pasting temperature.

*Significant at 0.05 level.
Table A-3: Correlation between various pasting parameters and swelling capacity of potato starch

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<th>TV</th>
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</table>

PS = Potato starch, PV = Peak viscosity, TV = Trough viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity, SC = Swelling capacity, FRM = Firmness, PVC = Change in peak viscosity, BVC = Change in breakdown viscosity, SVC = Change in setback viscosity, DPT = Delay in pasting temperature.

*Significant at 0.05 level.
Table A-4: Correlation between various pasting parameters and swelling capacity of waxy corn starch

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<th>BVC</th>
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</table>

WCS = Waxy corn starch, PV = Peak viscosity, TV = Trough viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity, SC = Swelling capacity, FRM = Firmness, PVC = Change in peak viscosity, BVC = Change in breakdown viscosity, SVC = Change in setback viscosity, DPT = Delay in pasting temperature.

*Significant at 0.05 level.
Table A-5: Correlation between various pasting parameters and swelling capacity of waxy potato starch

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<th>SV</th>
<th>PVC</th>
<th>BVC</th>
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WPS = Waxy potato starch, PV = Peak viscosity, TV = Trough viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity, SC = Swelling capacity, FRM = Firmness, PVC = Change in peak viscosity, BVC = Change in breakdown viscosity, SVC = Change in setback viscosity, DPT = Delay in pasting temperature.

*Significant at 0.05 level.
Table A-6: Correlation between various pasting parameters and swelling capacity of tapioca starch

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TS = Tapioca starch, PV = Peak viscosity, TV = Trough viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity, SC = Swelling capacity, FRM = Firmness, PVC = Change in peak viscosity, BVC = Change in breakdown viscosity, SVC = Change in setback viscosity, DPT = Delay in pasting temperature.

*Significant at 0.05 level.
Table A-7: Calculations of molar concentration of iodine and potassium iodide

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<th>B</th>
<th>C</th>
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<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
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<td>KI (s)</td>
<td>I2 (wt)</td>
<td>KI (wt)</td>
<td>I2 (mol)</td>
<td>K (mol)</td>
<td>I (mol)</td>
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</table>

A- Level of iodine used (%)
B- Volume of 2% I2-KI solution pipetted (in millilitre)
C- Amount of iodine in stock solution (%)
D- Amount of potassium iodide in stock solution (%)
E- Amount of iodine in volume pipetted (in gram); E= B*C
F- Amount of potassium iodide in volume pipetted (in gram); F= B*D
G- Amount of iodine in volume pipetted (in mole); G= E/ 253.8; Weight of I2= 253.8
H- Amount of potassium in volume pipetted (in mole); H= F/165.9; Weight of KI= 165.9
I- Amount of iodide in volume pipetted (in mole); I=H
Table A-7 continued: Calculations of molar concentration of iodine and potassium iodide

<table>
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<tr>
<th>I₂-</th>
<th>I₂ (mol)</th>
<th>K (mol)</th>
<th>Ι (mol)</th>
<th>I₂ (mM)</th>
<th>K (mM)</th>
<th>Ι (mM)</th>
<th>Ι⁻ (mM)</th>
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</table>

J- Amount of iodide per gram starch (in mole); J= G/2.24; Weight equivalent to 8% starch in RVA= 2.24 g

K- Amount of potassium from KI per gram starch (in mole); K= H/ 2.24

L- Amount of iodide from KI per gram starch (in mole); L= I/2.24

M- Amount of iodide from I₂ per gram starch (in millimole); M= J*1000

N- Amount of potassium from KI per gram starch (in millimole); N= K*1000

O- Amount of iodide from KI per gram starch (in millimole); O= L*1000

P- Amount of tri-iodide ions from I₂ and Ι per gram starch (in millimole); P= M+O