FUNCTIONALITY OF STARCHES

WITHIN BATTER FORMULATIONS

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ABSTRACT

Industrial batters, which contain starches, are used to coat potato products. It is the mix of different starches and their ability to form films that contribute to the key eating characteristics. The major part of this study was to use commercial starches and batters to ascertain the different roles of the starches whilst the batter formulation rapidly changed from 60% moisture to very low levels. Materials studied included starches of different botanical origin, native or with various chemical and physical modifications, and other materials such as flour, stabilisers and salt. A group of stabilised crosslinked tapioca starches were examined in detail, but it was not possible to align the behaviour in excess water with the given levels of modification.

The theory for film creation was that structured swollen granules were embedded in starch polymers that formed a continuous network with the moisture content being critical for the transition from batter to film. To ascertain the starches' characteristics in solution, a low moisture system, 78% N-methylmorpholine N-oxide (NMMO) - 22% water, was used and this revealed the complexity of modified starches. A major finding was that the NMMO-water system differentiated starches of the same origin (tapioca), which was not readily achieved by other techniques. To mimic film forming, starch formulations were assessed at moderate (10-50%) moisture levels using a "popping" and a hot press method. All the starches and combinations produced non-uniform, popped and interconnected films at 15% moisture (185°C for 4.6 s). This demonstrated sufficient starch polymer solubilisation to allow adhesion between the amorphous granular structures. Sorption isotherms may suggest an interaction between salt levels and processing of the starches. A critical factor for a coating is to act as a moisture barrier to maintain crispiness and therefore water sorption behaviour post processing is a key quality factor.

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LIST OF ABBREVIATION

a_w	water activity	**K**	energy constant (GAB)
BET	Branauer-Emmett-Teller	**LLS**	laser light scattering
C	energy contant (GAB)	**M_0**	monolayer (GAB)
CMC	carboxymethyl cellulose	**MC**	methyl cellulose
Con A	concanavalin A	**Na_2SO_4**	sodium sulfate
CMC	carboxy methylcellulose	**NaCl**	sodium chloride
db	dry basis	**NaOH**	sodium hydroxide
DIC	instantaneous controlled pressure drop	**NMMO**	N-methylmorpholine N-oxide
		NMR	nuclear magnetic resonance
DMA	dynamic mechanical analysis	**OSA**	octenyl succinic anhydride
		PEF	pulsed electric fields
DMSO	dimethyl sulfoxide	**PFTE**	polytetrafluoroethylene
DP	degree of polymerisation	**RVA**	rapid visoc analysis
DS	degree of substitution	**SP**	swelling power
DSC	differential scanning calorimetry	**STMP**	sodium trimetaphosphate
		STPP	sodium tripolyphosphate
ECH	epichlorohydrin	**T_g**	glass transition temperature
FID	free induction decay	**T_{gelat}**	starch gelatinisation temperature
GAB	Guggenheim-Anderson-de Boer	**T_m**	melting temperature
		T_r	minimum temperature for protein thermosetting
GMS	glycerol monostearate		
HPMC	hydroxylpropyl methylcellulose	**wb**	wet basis

CHAPTER 1 INTRODUCTION

This thesis is a product of research on starch formulations and the functionalities of the starches as they are processed. The initial drive was the curiosity towards the behaviour of starches within commercial batters, especially modified starches, and how their functionalities affected the final attributes in the formation of a film coating potato chips. The film formation properties are considered to be particularly important. The objective is to create a film with the desired attributes of potato chips that are linked to consumer preferences. The properties of a coating film can be varied to enable targeting of final product attributes such as crispiness, oil uptake and texture.

In the course of the process from the dry starch formulation (batter mixture) to the final product, starch formulations undergo harsh processes that alter their properties. The changes to the starches impact their film forming properties and these are directly linked to the attributes of the film.

Understanding the processing is important as this impacts the starches and their properties due to the marked changes to moisture content and temperature in the system. Figure 1.1 shows the changes a batter mixture undergoes when processed in a normal procedure for frying of potato chips (this process will be discussed in more detail in section 1.1.1.1). The diagram gives an overview of the changes to the starch formulation, but also points out some of the important features for understanding the functionalities of the starches within the batters. The attributes of the final battered product are emphasised since these characteristics are very relevant to the storage of the products and finally at consumption.

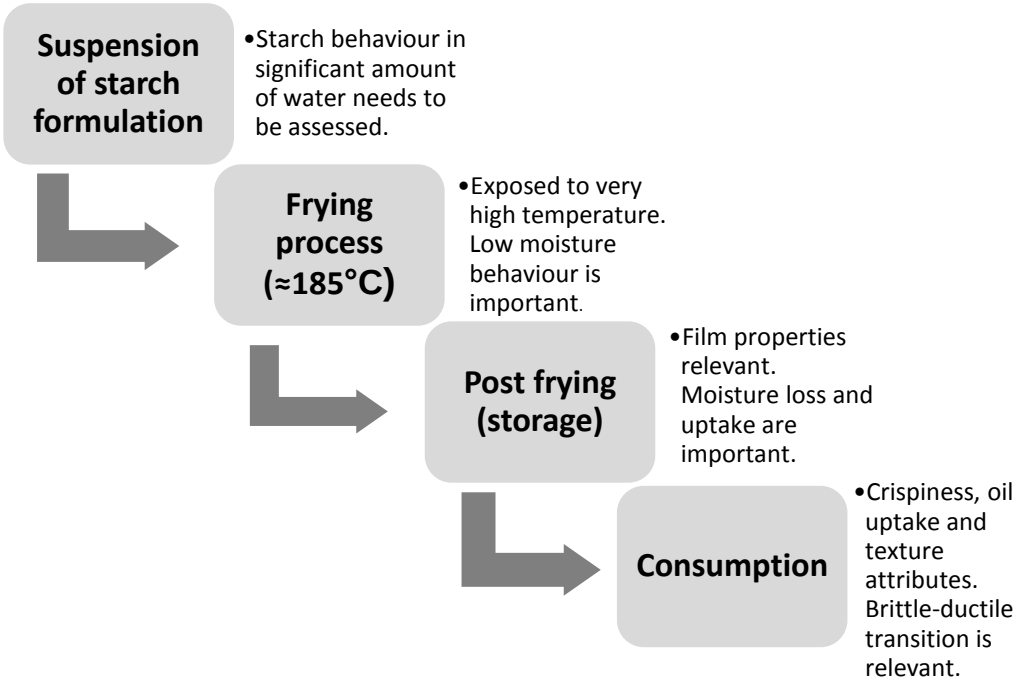


Figure 1.1: Diagram of the main processing steps in frying of a potato chip. The steps shown are relevant for starch formulations and their properties.

The work carried out and reported in this thesis has focused on the first three steps shown in Figure 1.1 and thus no sensory analysis or mechanical tests were done to elucidate the final attributes of the starch formulations. Later in this chapter there is a review of some of the critical literature relevant to the topic and from this a list of aims and objectives for the work has been elucidated. The methods used are given in chapter 2. Chapter 3 covers the characteristics of a set of commercial samples including their functionalities in excess water. The focus of chapter 4 was low moisture systems and information on a group of stabilised and crosslinked starches that were studied in detail. Chapter 5 reports the frying process and some of the final attributes such as wet batter pick up and oil uptake. Furthermore moisture uptake and loss (sorption) is examined. Finally, chapter 6 reviews the film formation properties of starch formulations utilising a novel popping machine and a heating press to assess the films. Conclusions and further work are reported in the final chapter.

1.1 Concept of a batter and its key attributes

A batter is most often defined as a liquid mixture of various ingredients with starchy materials such as flour being the main solids component. It can be used as a coating batter or on its own i.e. a pancake batter. The focus of this study is on batters used for coating potato chips (French fries). The dry coating batter can consist of starch containing ingredients such as flour and starches (native and modified) and other ingredients such as spices, stabilisers and leavening agents. The properties of the batter are directly dependent on the functionalities of the individual ingredients and their interactions. The batter ingredients are usually dry mixed before being suspended in water and applied to a base material (see section 1.1.1.1). The suspension of the dry mixture in water is typically at 35-45% solids, contingent on the properties of the ingredients. Depending on the desired end product, the batter composition will differ to achieve the desired attributes of the batter. Key reasons for applying a batter mixture to a base material are the reduction of oil uptake, increased crispiness and water retention within the base.

1.1.1 Application

The dry batter mixture is suspended in water prior to application onto the base material. The solids to water ratio is very important for the application of the batter suspension since wet batter pickup and the flow of the batter will be affected by the change in solids. Increasing the solids of the batter mixture will also influence the viscosity and therefore the processability and may affect homogeneity within the batter. For battered potato chips the relevant solids content in the batter suspension can differ between 35-45% depending on the batter components including thickeners (stabilisers). The dry batter mixture is suspended in water under continuous shear and at room temperature to avoid gelatinisation of the starch

components in the batters. Wet batter is applied to the base material and an even film is obtained covering the base material. Then a process is initiated to fry or cook the product allowing flash evaporation of moisture from the product resulting in a very rapid formation of the batter film. The formation of a film coating the base material occurs when the heat (from frying or cooking) melts the ordered regions of the starch components within the batter mixture allowing them to form a firm film coating the base material. The end product is either consumed or stored i.e. held under heating lamps or stored in a freezer. These treatments also require specific film properties.

1.1.1.1 A typical batter processing procedure

The processing of a coating batter is briefly described above including the important steps where the properties of the batter components are crucial to the quality of the end product. An example of a typical batter processing procedure is given in key notes below to highlight the processing conditions of a batter mixture. The example is completed for battered potato chips (Hume and Rose, 2013, Sahin and Sumnu, 2008).

- The raw potato is cleaned and cut into regular shapes of specific dimensions.
- The potato pieces are blanched in two different solutions (or in a two stage blancher), i) in water at 85 °C for 15-17 minutes ii) in a solution containing sodium acid pyrophosphate (about 1%) at 65 °C for 60 seconds.
- The potato pieces are dried in a convection oven at 105 °C until they have passed the test of stickiness, a measure of how dry and sticky the potato base is (5 hang test).

- The dry batter mixture is suspended in cold water (between 35-45% solids) at room temperature and sheared to obtain homogeneity of the batter suspension (suspension should ideally be 10-15 °C).
- The potato pieces are coated with the batter suspension and the thickness of the film is adjusted by air flow or by the duration of the potato piece held in the batter suspension. Thickness of the film is measured from the wet batter pickup.
- The battered potato piece is fried in a mixture of oils at 180-185 °C for 60 seconds (for pre-fried potato chips) and then cooled down to room temperature (no more than 10 minutes) before being frozen in a blast freezer (maximum of -18 °C), then packed in bags and stored in a normal freezer.

The frying process allows a rapid escape of moisture from the batter which results in low moisture content in the formed film. However the base material, the potato piece, maintains most of the moisture (final frozen product contains 64-65% moisture) avoiding escape of moisture due to the formed film. The storage conditions of the fried battered potato chips are relevant for the properties required by the batter mixtures to achieve the final desired attributes. Storage conditions can involve the need for good freeze-thaw stability, re-heat properties or long-time storage, hence the batter needs to ensure the integrity of the potato chip including maintaining the texture and crispiness upon final cooking or re-heating.

1.1.1.2 Other processing conditions

The processing conditions described in section 1.1.1.1 are for preparation of pre-fried potato chips that are frozen and often used for the catering industries. The processing of potato chips depends on the desired end products and the desired properties. The potatoes can be of different origin and season and therefore of different texture (hardness), flavour, shape and size. Processing conditions can be altered depending on potato properties e.g. blanching time and temperature as well as drying time to achieve the same stickiness (Hume and Rose, 2013). The processing of the potato prior to coating is crucial to the wet batter pickup and the overall performance of the batter mixture. Some attributes of the batter mixture might only be expressed if the base material is correctly pre-treated e.g. a wet potato piece will lack stickiness hence it is coated with less batter resulting in reduced film formation and possible areas without batter. Further processing conditions such as air flow and duration of the potato base in the batter suspension can all be altered to adjust batter pickup.

The composition of the dry batter mixture and the solids content are important factors in the processing of the battered potato chip and will be covered in section 1.2. Processing conditions are important for the behaviour of the batter mixture and the resulting batter attributes. During processing the batter mixture undergoes harsh treatment including the change from being a dispersion into a melted film, with the flash off of water. The moisture contents of the final batters may be as low as 1.5% moisture to ensure the crispiness of the final product to be eaten.

1.1.1.3 Change of moisture content during processing

The change of moisture content within the batter suspension upon frying results in the formation of a melted film that is enforced by the rapid evaporation of water and the high frying temperature. Thus the film on the potato base forms a thin layer covering the potato base. Some required attributes of this thin film are listed below (Sahin and Sumnu, 2008):

- A layer to provide crisp characteristics
- A layer to control moisture release (and moisture uptake)
- A layer that adheres to the base material (potato)
- A layer that controls fat uptake

These attributes can be prioritised and changed according to the desired end product and they can be controlled by the components of the batters.

1.1.2 Components of the batter mixture

The batter components will be chosen according to the attributes required for the desired product. The major ingredients in a batter for potato chips are starches and starch containing components such as flour. The minor components can be spices, salt, stabilisers and leavening agents. The starches can be applied either in their natural (native) state or after they have undergone physical or chemical modification (see section 1.2.1). Modified starches may contribute with a wide range of properties (section 1.2.1.5) resulting in different attributes of the batter mixture and therefore the batter composition can be very complex with a range of different starch components each with individual specific functionalities.

1.2 Key components of the batter mixtures

Starches or starch based materials are the major component in batter blends (for potato chips) and therefore it is important to understand their individual physicochemical properties for a better prediction of the batter behaviour which is directly related to the individual components of the batters. Extensive knowledge about starches and their functionality is available from various studies e.g. (Murphy et al., 2000, Singh et al., 2007, Zhu, 2015). However their interactions and behaviour across the moisture ranges applicable for batters are less well documented and will be the dominant subject of the study covered in this thesis.

1.2.1 Starches

1.2.1.1 Sources of starches

Starch is a natural storage of energy in various plants and it can be present in many different granular shapes such as spheres, ellipsoids, polygons, platelets, and irregular tubules (Pérez and Bertoft, 2010, Zobel, 1988). The starch granules are mainly isolated from seeds, roots and tubers but they are also present in stems, leaves, fruits and pollen. Granules vary not only in their shapes, but also their sizes are spread in an interval of 0.1 - 200μm (Pérez and Bertoft, 2010). Due to the big variance in shapes and sizes of starch granules, their physicochemical properties differ and hence starches from different sources are targeted for different applications. Starches can be derived from different sources, but other extrinsic conditions such as differences in growing conditions i.e. sowing season, water availability and area, may influence the starch properties as well. Further the isolation or extraction of the starch granules and the subsequent processing (drying and milling) may influence the starch functionalities.

1.2.1.2 Native structures

Starch granules are grown from the hilum and in successive alternating amorphous and semi-crystalline layers (by apposition). The two main carbohydrate components in the starch biopolymer are amylose and amylopectin. Amylose is an essentially linear polysaccharide composed of α-(1-4)-linked D-glucose units (can contain branches) and in most starches amylose constitutes about 15-35% of the granules. Amylopectin is a highly branched polysaccharide which is composed of a backbone of α-(1-4)-linked D-glucose units with α-(1-6)-linked branches (Figure 1.2 modified from (Pérez and Bertoft, 2010)).

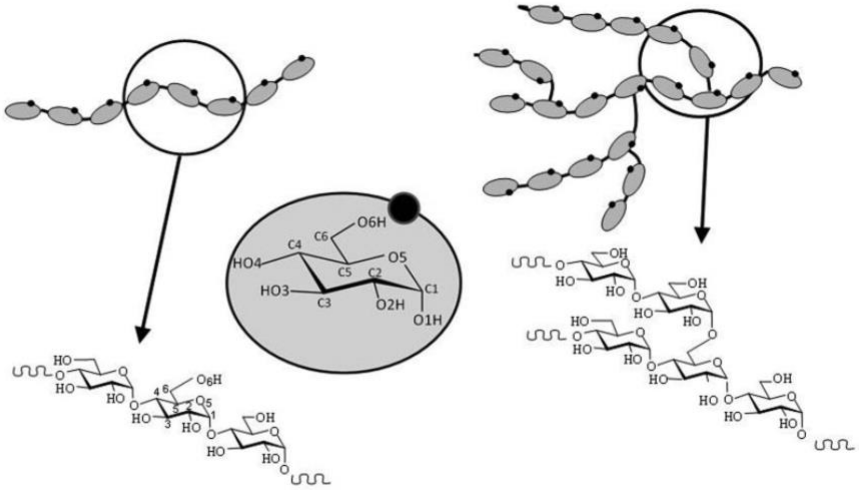


Figure 1.2: The structure of the amylose and amylopectin units and a schematic presentation of the polymers. Figure modified from Pérez and Bertoft (2010).

The two polysaccharides, amylose and amylopectin have distinct individual properties and their presence as well as their molecular structure within the starch granule is important for the understanding of the various starch properties. The functionality of starch can be modified by a biotechnological process which can result in alteration of the main starch components amylose and amylopectin. This may create high amylose content starches (usually around 70%) or waxy starches

with high amount of amylopectin (as high as 99%). However this will not be the focus of the thesis and therefore not covered in detail.

1.2.1.2.1 The cluster model versus the backbone model

There are currently two accepted theories (models) describing the composition of amylose and amylopectin in the granular structure at molecular level, the cluster model (racemose model) and the backbone model (Bertoft, 2013, Pérez and Bertoft, 2010). The traditional and most well-known theory is the cluster model. The cluster concept was initially suggested by Nikuni in a Japanese journal in 1969 (Bertoft, 2013) however unaware of that publication, French presented a similar cluster model in 1972 (French, 1972). A schematic view of the cluster model as described by Jenkins and co-workers is presented in Figure 1.3 (Jenkins and Donald, 1995, Jenkins et al., 1994). Modification to the cluster model and alternative models based on the cluster concept has been established in accordance with new experimental evidence (Bertoft, 2013). The cluster model is mainly based on the experimental work of enzymatic determination of the unit chain profile of amylopectin and modified starch material.

Figure 1.3a) shows the starch granule which consists of concentric rings of alternating amorphous and semi-crystalline compositions/regions built from the hilum. The semi-crystalline growth rings are enlarged in Figure 1.3b) to display the schematic order of the stacks of alternating amorphous and crystalline lamellae. Within the semi-crystalline growth ring (Figure 1.3c) a cluster structure for amylopectin is shown. The cluster structure is formed by A-, B- and C-chains of amylopectin (C-chain not shown in Figure 1.3).

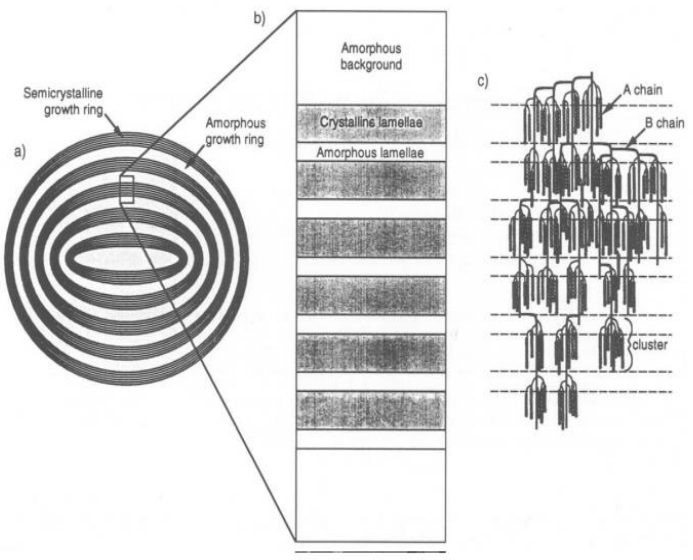


Figure 1.3: Starch granular structure. Reprinted from Jenkins and Donald (1995).

A-chains were initially identified as chains not carrying other chains (unsubstituted) whereas B-chains were carrying other chains (Bertoft, 2013). The A-chains consist of amylopectin in the form of double helices, which constitutes the crystalline lamellae. B-chains of amylopectin are forming the inter-cluster connections and therefore the amorphous lamellae are predominantly formed by the branching points from the A- and B-chains (Jenkins et al., 1994, Jenkins and Donald, 1995). Further every macromolecule contains a single C-chain which is carrying the reducing end group (not shown in Figure 1.3).

Amylose's structural presence in the starch granule is yet to be confirmed, but suggestions have been made that a large portion might be found within the amorphous growth ring/lamellae and hence only small amounts will be associated with the semi-crystalline region. Amylose might also co-crystallise with amylopectin within the crystalline lamellae and will therefore be present in the semi-crystalline region (Jenkins and Donald, 1995). If any lipids are present within the granule (these are not present in all starches) they may form inclusion complexes with amylose, hence binding part of the available amylose.

The sizes and branching of the chains in the amylopectin cluster has been examined extensively by Bertoft and co-workers (Bertoft, 1991, Bertoft, 2004, Bertoft, 2013, Bertoft and Koch, 2000, Bertoft et al., 2008, Pérez and Bertoft, 2010) and the cluster concept has been probed due to new experimental data challenging the cluster model. Based on enzymatic methods for isolation of the cluster unit Bertoft and co-workers suggested the backbone model with building blocks (small branched units) as an alternative model instead of the traditional cluster model (Bertoft, 2013). The backbone model has the same overall granule structure as the cluster model but it suggests another structural role for amylopectin. Introducing the building blocks consisting of amylopectin chains with different chain lengths and various different degree of branching and degree of polymerisation resulted in placing the different blocks in a systematic scheme (according to type, length and DP) with some knowledge about the composition within the backbone model.

A schematic presentation of the backbone model (see Figure 1.4) re-printed from (Bertoft, 2013) gives a semi-detailed indication of the complex structure of the backbone model. The principal idea of the backbone model is that a long chain (C-chain) containing the reducing end (thick red line, Figure 1.4) is forming the backbone (in the amorphous region) with building blocks (internal chain segments) connecting the backbone to the external chain segments forming double helices perpendicular to the backbone. The double helices are bound to the backbone through the building blocks (circled and red shaded on Figure 1.4) which may be attached to side chains in the backbone (thin black lines) containing branches (marked with a little black arrow). The determination and definition of the different building blocks and the external chain segments are not covered further in this thesis.

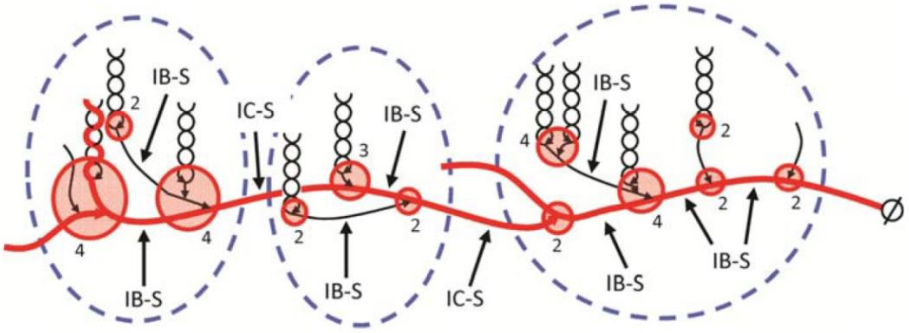


Figure 1.4: A schematic representation of the concept of the backbone model. Adapted and reprinted from Bertoft (2013). IB-S interblock segments (DP<9), IC-S intercluster segments (DP≥9), dashed blue circles indicate units of clusters. Ø marks the reducing end.

It is important to note that both the traditional cluster model and the backbone model are equally possible representations of the structures at this time since both of them are supported by some experimental data, but they are also both missing some direct proofs of existence. An example is that both models would result in the same data collection (lintner, residual of acid-treated starch) if starch is treated with a dilute acid solution (see Figure 1.4, re-printed from Bertoft (2013)).

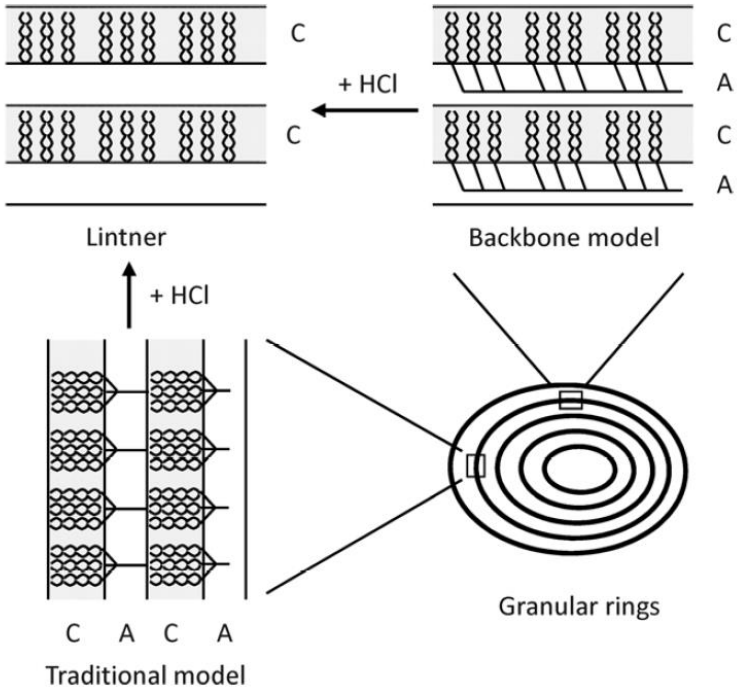


Figure 1.5: A schematic presentation showing the same product after acid treatment regardless of the starch granular structure adopting the cluster model or the backbone model. Reprinted from Bertoft (3013).

1.2.1.2.2 Polymorphs in native starches

Not to be confused with the denotation of the different chains of amylopectin, the packing of the amylose double helices and/or the less branched amylopectin double helices at the molecular level reveal different crystalline arrangements resulting in A- and B-type crystallinity and a combination thereof C-type crystallinity i.e. different crystalline polymorphs (Pérez and Bertoft, 2010, Tester et al., 2004). Figure 1.6(a) displays the left handed and parallel stranded double helices of amylopectin/amylose. The double helices crystallise into a monoclinic lattice packed in a parallel fashion for the A-type polymorph. Four water molecules are located within one unit cell of the A-type crystalline polymorph (Figure 1.6(b)).

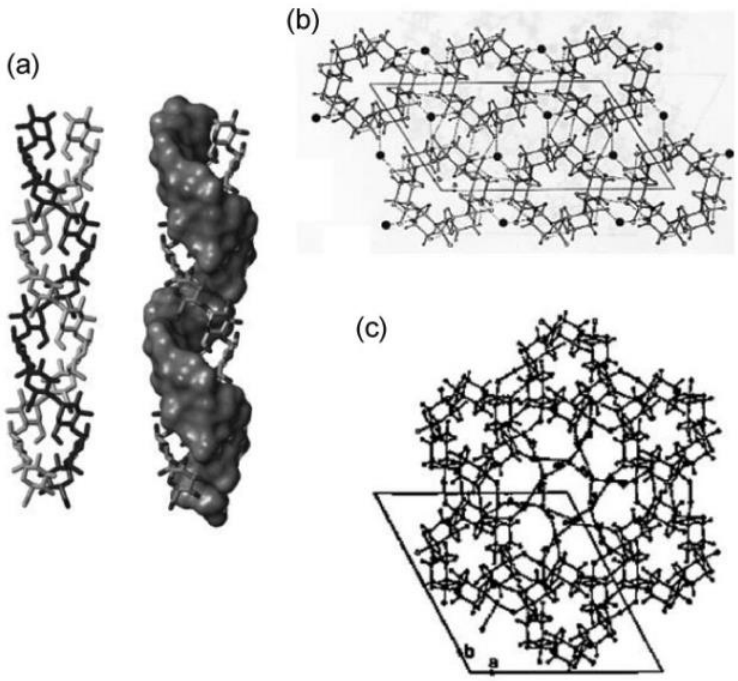


Figure 1.6: (a) The amylose or amylopectin (less branched) forming double helical structure. (b) The A-type polymorph and (c) The B-type polymorph. Reprinted from Pérez and Bertoft (2010).

For the B-type crystalline polymorph the chains (double helices) are crystallised in a hexagonal lattice however the packing is still in a parallel fashion (see Figure 1.6(c)). B-type polymorph is loser packed than the A-type polymorph and thus can hold 36

water molecules where half of them are tightly bound to the double helices and the rest of them form complexes (Pérez and Bertoft, 2010).

The packing of the double helices and consequently their water holding capacity within the crystalline structure does influence the physicochemical behaviour of starches. In addition to the already mentioned crystallinities, another V-type crystalline polymorph can be found in starches containing lipids as part of the granular structure (Pérez and Bertoft, 2010). Most cereal starches such as maize, rice, wheat and barley may contain relatively high amounts of lipids (up to 1.5%) within their granular structure. Two other minor components contained in the starch granular structure are proteins (0.1-0.7% by weight) mainly observed as granular bound proteins and phosphorous present in most starches in various forms i.e. phospholipids and phosphate monoesters (the latter being highly abundant in native potato starch) (Pérez and Bertoft, 2010). The theories presented about the molecular structure of the starch granule are only valid if the starch is in its natural (native) state without any modification or processing.

1.2.1.3 Changes to starches molecular structure as they are processed

In this thesis starches referred to as native starches, are natural starches which have not been processed, altered or modified either physically or chemically (see section 1.2.1.5). The native starches exhibit unique properties as thickeners and stabilisers however these properties are often only conferred upon processing of the starches. Processing may cover a wide range of treatments, but all of them change the native molecular structure. Starches are often used as thickeners and stabilisers when suspended in excess water and heated (with or without stirring) to reach the

gelatinisation point at which the granular structure is disrupted and the viscosity of the system increases.

Starch gelatinisation is well-known as the mechanism for the dissolution of starch in excess water under controlled heating and shear. Ingress of water into the granule (primarily the amorphous regions) results in swelling and minor leaching of amylose into the suspension. The granule continues to swell until it has reached its maximum swelling capacity (peak viscosity) and then the granule starts to break down releasing more polymers into solution and the crystallinity of the starch granule is lost. Finally the starch granule is dissolved with the polymers being free in solution. Upon cooling and storage of the cooked starch, it is energetically favourable for the amylose and amylopectin to realign themselves into ordered structures and hence restore some crystallinity resulting in network formation (formation of gels). This process is called retrogradation and does not necessarily occur for all starch systems thus it will be dependent on the gelatinisation process (harsh or mild) and the storage conditions (temperature and duration).

Retrogradation is an important aspect of the starch functionalities however in the film formed by starches at high frying temperature, retrogradation will be less relevant since the moisture has been flashed off and the conditions for realignment of the biopolymers thus retrogradation is very unlikely to occur. In section 1.1.1.1 where various processing steps are described demonstrate that moisture levels in the batter layer will vary considerably (between 60% and 1.5%). Due to this range it is difficult to predict when retrogradation will occur. Amylose reorganisation or amylose-lipid complexation can be expected but the moisture contents over extended periods required for amylopectin recrystallisation would seem unlikely.

If only limited water is present or the starch is processed in a low water environment, the starch granule will behave differently compared to the gelatinisation behaviour. High temperature processing and limited water content (e.g. frying or extrusion) will melt the starch granules resulting in a phase transition from semi-crystalline to amorphous within the granule (Wang 1993). The melting of the starch granule will limit the swelling or expansion of the granule. Depending on processing conditions a compact extrusion product or a film-type material will be formed.

1.2.1.4 Starch behaviour in different solvent systems

Application of starches to various food formulations requires knowledge about behaviour and interactions within the food matrix. Starch behaviour is typically considered in water, but other solvent systems will alter the starch behaviour. The physicochemical properties of the starches may change just by adding simple salts to the starch suspension. The addition of salt may alter the gelatinisation temperature, swelling properties and gel strength depending on the type and concentration of the salt used (Ahmad and Williams, 1999, Jane, 1993, Lii and Lee, 1993).

Other solvents such as dimethyl sulfoxide (DMSO) have also been used to probe the starch behaviour in alternative systems. DMSO has been utilised to explore the starch molecular structure and interactions with other factors (Perry and Donald, 2000). Another relevant solvent to probe starches' solubility is the organic solvent N-methylmorpholine-N-oxide (NMMO) which is used by industry to create cellulose fibres.

1.2.1.4.1 N-methylmorpholine-N-oxide as a low water content system

The use of NMMO can assist in ascertaining the differences in hydrodynamic properties. The organic solvent NMMO is known for dissolving the starch granule, dependent on concentration (Koganti et al., 2011). Hence at higher concentrations of the solvent the granule swelling and dependence on granular structure will be avoided. NMMO is commonly used as a solvent in the Lyocell process producing cellulose fibres (Borbély, 2008). The use of an organic solvent will therefore enable a better understanding of the differences between granule swelling and polymers in solution (see section 4.1.1). The effect of changing the solvent or adding other chemicals to the starch solutions can help to elucidate the behaviour of starches and their structures at the molecular level.

NMMO is probed as a low water environment due to the difficulties associated with analysing starches at low moisture. The chemical environment achieved by using NMMO is very specific and cannot be directly compared to a low water environment such as film. The use of NMMO was merely to examine the different behaviour and mechanisms associated with low water environment.

In addition to changing the suspending matrix, the starch structure can be changed. Physical or chemical changes to the starch structure can alter the molecular structure of starches and modification has been widely used to improve starch functionalities in food materials.

1.2.1.5 Modification of starches

Starches can be either physically or chemically modified to achieve the desired properties for use in the food industry. The modifications are mainly done to provide

properties such as controlled peak viscosity, improved tolerance to processing conditions, desirable texture and prolonged stability (Light, 1990).

1.2.1.5.1 Physical modification

Starches are typically used in different physical forms i.e. dry or wet and further in these conditions they can be utilised as dry starch powders, dry films, swollen granules or dispersions (Light, 1990). Physical modifications are often implemented to alter the properties of the native starches for easier processing (cooking). Pregelatinisation of starches is a physical modification altering the starch ability to swell fast reaching a high viscosity without or with little cooking hence removing the necessity for cooking. The pregelatinised starches are prepared by complete gelatinisation and drying thus destroying the granular structure leading to complete fragmentation observed by the absence of optical birefringence (Ashogbon and Akintayo, 2014). Pregelatinisation can be obtained by pre-cooking of the starches either by drum drying, extrusion or spray drying. The drum drying process also alters the particle size of the granules, which along with the damage to the granule that inflects the pregelatinisation property, also changes the starch properties in respect of dispersion, smoothness and rate of viscosity development (Murphy et al., 2000). Extrusion and spray drying can also alter these factors depending on the harshness of the pregelatinisation process and the drying/milling conditions.

Other physical modifications include hydrothermal processes which unlike the pregelatinisation process preserve the molecular integrity of the starch granule after modification (Ashogbon and Akintayo, 2014). Annealing and thermal agitation are two thermal processes which utilize holding the starches above the glass transition temperature, but below the onset temperature for gelatinisation with high and low

moisture content respectively to achieve changed properties such as increased crystallinity and granule rigidity by means of vibrational movement (Ashogbon and Akintayo, 2014). However starch origin and the treatment conditions utilised will reflect the physical properties of the heat-moisture treated starch.

Non-thermal physical modification of starches utilise processes such as high hydrostatic pressure, ultrasound, pulsed electric fields treatment and microwave processing to affect the physicochemical properties of the starch (Ashogbon and Akintayo, 2014). Also a range of newly developed methods such as superheated starch, iterated syneresis, thermally inhibited treatment (dry heating), osmotic pressure treatment, multiple deep freezing and thawing, instantaneous controlled pressure drop (DIC) process, mechanical activation with stirring ball mill, micronization in vacuum ball mill, pulsed electric fields (PEF) treatment and corona electrical discharges (Ashogbon and Akintayo, 2014) can be defined as physical modifications. These non-thermal processes and the recently developed processes are not covered in detail within this thesis.

1.2.1.5.2 Chemical modification

Marked changes can be made to the starch granular structure by utilising chemical modification, which often introduces a new functional group into the starch molecules hence altering the physicochemical properties of the starch. Considerable change resulting from chemical modification can include alteration in the proximate composition, gelatinisation, retrogradation and pasting characteristics (Ashogbon and Akintayo, 2014). Chemical modification may also facilitate intra- and intermolecular bonds with random distribution in the starch granule. Figure 1.7

shows a schematic overview of some chemical, enzymatic and biochemical modification of starches (adapted from (Murphy et al., 2000)).

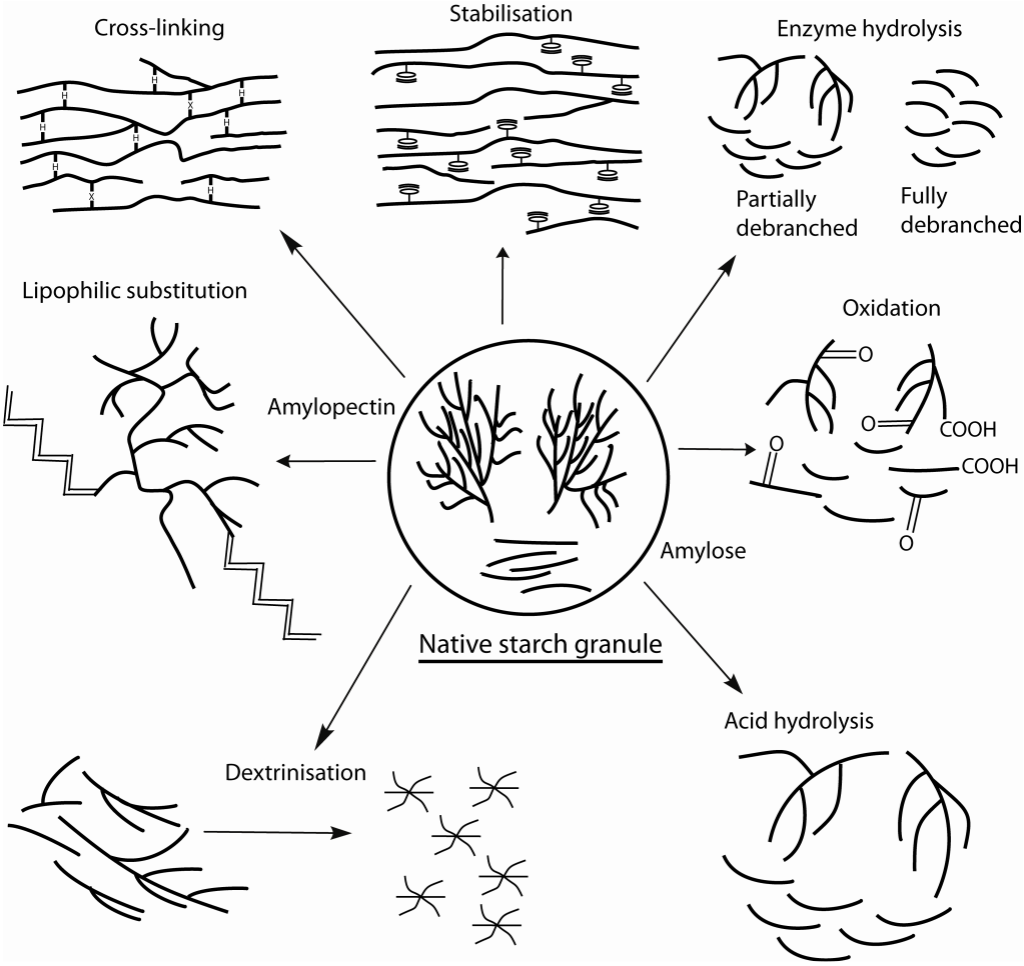


Figure 1.7: A schematic overview of modification of starches. Adapted from Murphy et al. (2000) and reprinted from (Niazi, 2013).

Further Table 1.1 lists the most commonly used chemical modification processes including the corresponding E-numbers of the resulting modified starches. The table also provides information on the chemicals used for modification. The effect and level of modification depends on various factors such as starch source (botanical origin), processing conditions (reactant concentration, pH, duration, presence of catalyst), type of substituent, degree of substitution (DS), degree of polymerisation (DP) as well as the substituents distribution in the starch granule (Ashogbon and Akintayo, 2014). These conditions will be further discussed in the sections below for the selected chemical modifications.

Crosslinking

Implementing crosslinking into the starch granular structure markedly changes the physicochemical properties of starch. Different crosslinking agents such as phosphorous oxychloride ($POCl_3$), sodium tetrametaphosphate (STMP), sodium tripolyphosphate (STPP) and epichlorohydrin (ECH) can be utilised to introduce the crosslinking within the starch granule. The molecular structure and physicochemical properties of the crosslinked starches will depend on the crosslinking agents since they produce different crosslinking systems according to the agent used (Ashogbon and Akintayo, 2014, Carmona-Garcia et al., 2009). Hence care should be taken when analysing and comparing data retrieved from various crosslinked starches due to influence of the crosslinking agent. As seen in Table 1.1 there are three classification types for crosslinked starches: monostarch phosphate (E1410), distarch phosphate (E1412) and a combination of these that is resulting in phosphated distarch phosphate (E1413).

The crosslinking is suggested to strengthen the bonding between starch chains hence resulting in decrease in swelling power (or swelling factor). This is due to the formation of intermolecular bridges (crosslinks) by phosphorous residues introduced by the crosslinking reaction (Ashogbon and Akintayo, 2014, Koo et al., 2010). Koo et al. (2010) reported a marked decrease in swelling factor with increasing level of crosslinking (with STMP/STPP). Further the clarity of the crosslinked starch paste decreased with increasing crosslinking, which was suggested to be linked to the restricted swelling of the granule (Koo et al., 2010).

Table 1.1: Overview of selected starch modifications and the chemicals used (Ashogbon and Akintayo, 2014, FOA/WHO, 2011, Murphy et al., 2000, Zhu, 2015).

Modification	Type	Chemical	E-number
Crosslinking / Esterification	Monostarch phosphate	Ortho-phosphoric acid, sodium or potassium ortho-phosphate, sodium tripolyphosphate	E1410
Crosslinking	Distarch phosphates	Sodium trimetaphosphate or phosphorous oxychloride	E1412
Crosslinking	Phosphated distarch phosphate	Combination of E 1410 and E 1412	E1413
Crosslinking + acetylation	Acetylated distarch phosphate	Sodium trimetaphosphate or phosphorous oxychloride and acetic anhydride or vinyl acetate	E1414
Stabilisation (acetylation)	Acetylated starch	Acetic anhydride / vinyl acetate	E1420 / E1421
	Acetylated distarch adipate	Adepic anhydride and acetic anhydride	E1422
Stabilisation	Hydroxypropyl starch	Propylene oxide	E1440
	Hydroxypropyl distarch phosphate	Propylene oxide and sodium trimetaphosphate or phosphorous oxychloride	E1442
Dextrinisation	Dextrin	Heated process (roasting). Acids may be added	E1400
Conversion Acid hydrolysis	Acid treated starch	Hydrochloric acid, ortho-phosphoric acid or sulfuric acid	E1401
Alkaline conversion	Alkaline treated starch	Sodium hydroxide or potassium hydroxide	E1402
Oxidation	Oxidised starch	Sodium hypochlorite (Hydrogen peroxide, peracetic acid, potassium permanganate, chromic acid and nitrogen dioxide)	E1404
Crosslinking (substitution)	Starch sodium octenyl succinate	Octenylsuccinic anhydride	E1450
Oxidation	Acetylated oxidised starch	Sodium hypochlorite and acetic anhydride	E1451
Crosslinking (substitution)	Starch aluminium octenyl succinate	Octenyl succinic anhydride and aluminium sulphate	E1452

Additionally X-ray diffraction patterns of crosslinked starches versus their native reference samples show little difference thus showing no difference in crystallinity upon crosslinking. This indicates that the crosslinking mainly takes place in the amorphous region of the starch granule (Ashogbon and Akintayo, 2014, Koo et al., 2010). Although the crosslinking is suggested to occur within the amorphous regions of the starch granule, some researches have reported changes to the morphology of the granule (Ashogbon and Akintayo, 2014, Carmona-Garcia et al., 2009).

The level of crosslinking is an important factor that is usually determined by the amount of crosslinking agent used. Koo et al. (2010) determined the degree of crosslinking by measuring the peak viscosity (using a controlled stress rheometer) hence calculated the percentage of crosslinking as the ratio of the difference between the peak viscosity of the reference sample and the crosslinked sample to the reference sample (Koo et al., 2010). For this reference to be useful it is needs to be the actual starting material for the crosslinking. Carmona-Garcia et al. (2009) evaluated the impact on some morphological, physicochemical and functional characteristics of different crosslinking reagents using phosphorous oxychloride, STMP/STPP and ECH. Their results showed dependence on the type of crosslinking agent. However, it needs to be anticipated that the level of modification was different and determined using two different methods (Carmona-Garcia et al., 2009).

It was also suggested that the phosphorous oxychloride and STMP produce the same structure upon crosslinking, but they incorporate different numbers of phosphate groups (STMP incorporating more) and at different locations. STMP introduced crosslinks in the interior of the granule and phosphorous oxychloride on the surface of the granule. ECH was suggested to introduce the crosslinking mainly in the

crystalline areas of the starch granule although the mechanism of crosslinking for STMP and ECH are considered to be similar and through penetration into the inner granule resulting in more crosslinking inside the granular structure (Carmona-Garcia et al., 2009). It should be noted that the crosslinking agents require different reaction conditions as well. For example, crosslinking using $POCl_3$ takes place at alkaline conditions in the presence of salt whereas STMP requires high temperature and hydration of the starch (Singh et al., 2007). Studies by Wattanachant et al. (2003) also suggested that comparing the crosslinking agents $POCl_3$, STMP/STPP and ECH at different concentrations led to different levels of crosslinking measured by phosphorous content. However note should be taken that stabilisation was done prior to crosslinking resulting in marked differences in the levels of substitution.

Stabilisation

Propylene oxide is usually utilised in the etherification of native starches. The bulky groups introduce stability by disrupting the inter- and intra-molecular hydrogen bonds causing weakening of the starch granular structure by steric hindrance thus preventing the easy realignment for retrogradation (Murphy et al., 2000, Singh et al., 2007). The stabilisation with propylene oxide is often conducted in alkaline conditions or with an alkaline catalyst and it is reported that the level of stabilisation is indicated by the amount of propylene oxide added in the preparation of the stabilised starch. Wattanachant et al. (2003) reported that increasing concentration of propylene oxide resulted in increasing molecular substitution. However, they also observed that following stabilisation the crosslinking reaction (same crosslinking agent and concentration) resulted in increased levels of crosslinking (measured by degree of substitution and phosphorous content). They suggested that by increasing the level of stabilisation and therefore introducing the hydrophobic groups the

bonding between starch molecules is weakened. This weakening will facilitate easier access for the crosslinking agents, thus the levels of crosslinking are higher because of the increased level of stabilisation (Wattanachant et al., 2003).

Increased levels of stabilisation (and same amount of crosslinking by concentration) demonstrated different viscosity development and no clear trends were observed. However, all of the modified starches had higher cold paste viscosities compared to the native starch (Wattanachant et al., 2003). Caution should be taken as to whether the data represents the effect of increasing stabilisation alone or if it is the result of dual modification leading to increased levels of crosslinking with increased level of stabilisation.

The dual modification of stabilisation followed by crosslinking is getting more and more attention due to the high demands of specific starches with unique and customised properties. However, no clear correlation has yet been established between increased level of stabilisation and/or crosslinking and observed functionalities which would be interesting to investigate further.

Dextrinisation

The conversion of starches into dextrins is a combination of a physical and chemical modification. Starch is usually degraded under acidic conditions (chemical modification) using a heated process (roasting, physical modification) converting starches into low molecular weight polymers. Dextrinisation is a two stage process thus dextrins are products of either low or high conversion. Low conversion is only the partial depolymerisation of the starches obtained by hydrolysis either by dry roasting (using the natural moisture content of the starch) or with catalytic quantities of acid present. High conversion is obtained if the polymer fractions from

the hydrolysis recombine in a branched manner, thus branched repolymerisation (Murphy et al., 2000). Dextrins are more often classified as low molecular sugar polymers derived from starch than an actual starch.

1.2.1.6 Starches properties in film formation

Starches are the main components of batters and therefore it is relevant to understand the key functionalities of the wide range of available starches. It has been reported that high amylose starch creates strong and flexible films (Liu and Han, 2005, Wolff et al., 1951) whereas amylopectin creates weak amorphous films (Liu and Han, 2005, Rindlav-Westling et al., 1998). Film forming properties have been studied for batters and Altunakar et al. (2004) found that the high amylose containing starch provides more brittle and crispy coatings in fried products compared to waxy maize starch. Furthermore the gelatinisation and film forming was found to play an important role in contributing to the crispiness and texture of the fried products (Altunakar et al., 2004).

Compared to batter formulations without starches, the addition of starches appeared to enhance gas entrapment inside a fried product hence increasing the volume of the product (Altunakar et al., 2004). Mohamed et al. (1998) discussed the influence of amylose/amylopectin on crispy frying batter and suggested that hardness of a fried batter was influenced by the degree of polysaccharide-polysaccharide, polysaccharide-water, polysaccharide-oil and polysaccharide-protein interactions. They concluded that an increase in amylose levels would increase polysaccharide-polysaccharide interaction, hence resulting in a more crispy batter and less oil uptake. On the other hand high amylopectin levels would result in a soggy batter due to the ability of the branched amylopectin structure to hold and

interact strongly with water (Mohamed et al., 1998). The use of modified starches in batters to achieve better film forming properties have been investigated showing that pregelatinised tapioca starch retained moisture more efficiently (Altunakar et al., 2004) and crosslinked tapioca starch improved crispiness (Mohamed et al., 1998).

Film forming capacity of starches within batters is considered relevant due to the formation of a thin film introduced by coating with a batter. López at al. (2008 and 2010) studied the film forming capacity of modified corn starches by casting and found that substituted corn starches (acetylated, acetylated crosslinked and hydroxypropylated crosslinked) formed films, whereas the acid modified starch did not (López et al., 2008, López et al., 2010). More work focusing on film forming capabilities and the involvement of modified starches in film formation needs to be carried out to obtain a better understanding of modified starches within batters.

1.2.2 Starchy materials

Usually the major components of a dry batter mixture are flours either of one type or a combination of types. Wheat flour is often used as the major component and its functionality depends on the major components within the flour i.e. starch and protein (gluten). In flours the main component is starch, but the starch can be damaged as a result of the processing of the flours. Due to the levels of starch damage and gluten's efficient water-binding capacity, the difference in water required to obtain similar viscosities between flours with high or low protein content (hard wheat flours and soft wheat flours respectively) are marked. Other flours used in batter mixtures are rice flour and maize flour as well as barley flour and soy flour (Sahin and Sumnu, 2008). Rice flour can be used as an alternative to wheat flour

although possessing different functionalities. This is due to rice flour being gluten-free and therefore containing more starch within the flour relative to wheat flour. Maize flour is primarily used as a viscosity controller enabled by the milling process of the flour which can provide a wide range of viscosities with similar solids content (Sahin and Sumnu, 2008).

1.2.3 Other components

Minor components within a dry batter mixture may have a major effect in the final properties of the batter. These components are briefly described below.

1.2.3.1 Proteins

Gluten is contained in some flours and therefore can be present in batters in relatively high quantities. However other proteins such as dried eggs (or egg albumen) or soy proteins may be added to the dry batter mixture as a protein source. The proteins are added to increase consistency of the batter mixture and to strengthen the protein structure which is seen as an increase in viscosity (Sahin and Sumnu, 2008).

1.2.3.2 Spices, salts and leavening agents

Spices are mainly added to provide flavour to the final battered product and less for functional reasons. The spices might also add colour to the batter mixture depending on the type of spice. Salt (NaCl) can be considered as one of the spices due to its flavour. However, salt also has an impact on the functionalities of the starch and starchy materials. This can be observed as increased gelatinisation temperature after addition of salt to different flours (Sahin and Sumnu, 2008, Xue and Ngadi, 2006). The functionalities of starches also changes in the presence of salt

or salt solutions where both the concentration and the type of salt can be reflected in the change in functionality (Jane, 1993, Wang et al., 1998). Salt can be a key factor causing change in the batter matrix since salt concentration can vary from 1-10% of the dry batter mixture. Other chemical components can be present in the batter due to their ability to act as leavening agents, releasing carbon dioxide and producing salt as a final product. An example of a leavening system is sodium bicarbonate ($NaHCO_3$) which is combined with disodium pyrophosphate ($Na_2H_2P_2O_7$) to produce gaseous carbon dioxide (CO_2), water and the salt ($Na_3HP_2O_7$).

1.2.3.3 Hydrocolloids

The use of hydrocolloids (or gums) in a batter mixture is mainly to increase the viscosity of the batter to obtain a higher batter pick up and also often to stabilise the mixture hence avoiding precipitation and keeping the solids in suspension (Sahin and Sumnu, 2008). The hydrocolloids commonly used are cold soluble in water and include the cellulose derivatives CMC, MC and HPMC as well as xanthan gum and guar gum. Other hydrocolloids such as carrageenan, alginate and gellan gum have also been used (Sahin and Sumnu, 2008). Better adhesion of the batter to the food base resulting in higher batter pick up can also be provided by some hydrocolloids. However the functionalities of the hydrocolloids are highly dependent on concentration and type and can also be temperature dependent (especially for development of viscosity) for some hydrocolloids (Sahin and Sumnu, 2008).

The hydrocolloids are not the main component (usually 0.1-1%) in batter mixtures and yet they may have a key role in the surface properties of the resulting film (Varela and Fiszman, 2011). Hydrocolloids may contribute with functional properties such as film forming properties, viscosity control, improved adhesion properties,

pick up control, better mechanical resistance of the outer crust and freeze-thaw stability (Varela and Fiszman, 2011).

The batters used for the work in this thesis are predominantly starch based by weight. It is recognised that phase separation occur between starch macromolecules and hydrocolloids. This phase separation can have marked effect on the texture and permeability of the film (Annable et al., 1994, Closs et al., 1999).

1.3 Physical attributes of batters

As described in the introduction to this chapter and in section 1.1.1.1, the dry batter mixture undergoes some crucial changes during processing to achieve the desired final fried product. The transitions imposed on the ingredients within the batter formulation are affecting the resulting physical attributes of the batter. This is due to the fact that the batter ingredients are exposed to different moisture levels and different heating/cooling conditions.

1.3.1 High moisture batters

Hydration of the dry batter mixture is the first major change in moisture levels of the batter mixture. The dry batter powder is suspended in water at low shear and room temperature at a concentration of about 40% solids (see section 1.1.1.1). Although the suspension is in significant amounts of water, the individual ingredients may still experience limited water access. Thus hydration might be incomplete due to high volume of the flours and starches and slow diffusion of water into the particulates.

1.3.1.1 The suspension and water absorption

Due to the suspension being high in solids, the hydration of the individual ingredients may be slow and thus requires a relatively long time. Initially the

temperature of the water is close to zero to enable the cold solubility, without too much clumping, which is required by some ingredients (see section 1.2.3.3). This also avoids the temperature of the total batter suspension reaching above room temperature upon mixing. This is important to maintain the integrity of the temperature sensitive ingredients, to control the viscosity of the suspension and to keep the microbial levels low. The water absorption of the individual ingredients will be different depending on the stage of suspension. Pregelatinised starches and some hydrocolloids will interact with the cold water and start to swell and solubilise and the suspension will increase in viscosity. The water absorption is temperature and ingredient dependent and therefore the processing conditions need to be consistent for the manufacture of reproducible products that have the desired physical attributes associated with the use of batters.

1.3.1.2 The state of the starch in suspension

It is reasonable to suggest that the starches in the batter formulations can be in different physical states upon suspension at high moisture levels. Hydration or moisture absorption into the starches might differ due to the ingredients competing for the water. This may result in various hydration levels, packing of the (swollen) granules and differences in the melting of the starches when high temperature is applied. The level of hydration of the starches may therefore be reflected in the film formation, thus impact the final attributes of the batters.

The ordered structures within native starch granules restrict their swelling in cold water. There will be some water uptake, mostly into the amorphous areas of the starch granules, but hydration levels are likely to be below 50% of the weight of the dry starch. The volume occupancy of the native starches, even when hydrated, are

therefore low and their impact on the viscosity of the cold water batter will be limited. Upon application of the batter to the base material, the temperature of the batter mixture has increased slightly (due to generic production of heat caused by the processing equipment), but the temperature will not reach the gelatinisation temperature of the starches and therefore no marked change in viscosity is seen.

Once applied to the base material (potato), the viscosity of the batter mixture can be slightly affected by increasing temperature, but the starches within the batters are only expected to change drastically upon the next processing step. This involves frying of the battered product at high temperature resulting in rapid flash off of moisture and concludes with formation of a thin film (with low moisture) covering the base material. Rapid transition from high to low moisture content at high temperature power the transition in the starches from hydrated (or semi-hydrated) starches that are semi-crystalline, into highly viscous fluids and then into melted amorphous glassy starches forming a film around the base material.

1.3.2 Low moisture film

Once the film is formed, the moisture still left within the film is limited and the surrounding moisture therefore becomes an important factor. The film is directly exposed to the potato surface (base material) and usually also in direct contact with the surrounding air thus exposed to the moisture contained in the air. Moisture content of raw potatoes is about 80-82% and therefore relatively high (Sahin and Sumnu, 2008) thus making the base material a critical factor in the level of moisture retention or sorption of the film.

In industrial potato chip production (French fries) the potato chips are not fully fried but par-fried instead which is essentially a pre-frying process to facilitate an easier

final frying process in the catering business or at home. The frying time is decreased from about 3 minutes (full frying) to 1 minute and then frozen immediately after frying to yield an approximate water content of 64-65% and oil content of 14-18% (Sahin and Sumnu, 2008). This frying process thus allows a relatively high amount of moisture left in the base material that requires the batter coating to be able to retain the moisture, but also to maintain the properties that are required for the final attributes such as crispiness and texture. The moisture content of the formed film must be relatively low due to pre-frying conditions, but an exact value is unknown.

Different batter properties are required upon full frying where the moisture content of the potato batter decreases further resulting in a moisture content of approximately 1.5%. With the total moisture content of the French fries at about 55%, there is a large difference in the moisture content of the film and the internal matrix. The importance of the barrier properties of the film becomes crucial for the water uptake or loss of the fried potato chip. The ability of the film to minimise water uptake and loss from the internal potato core also becomes crucial if the potato chips are to be stored under heat lamps prior to serving and water migration plays a major impact for the final sensory attributes of the chips when eaten.

As described briefly in the introduction to this chapter, the batter mixture undergoes many transitions before reaching a final product which is preferred by the consumers. Figure 1.8 shows a more detailed overview of the extrinsic conditions affecting the starch film after frying including the relevant parameters to take into account when choosing the suitable starches for the batter mixture. As temperature decreases after frying, the starches undergo a transition from the rubbery state to the glass state and thus become crispy. The commercial potato

chips undergo a first fry and then are frozen. The freezing procedures are designed to maintain the low water content of the film during blast freezing and then storage. However it is important to attain good freeze-thaw stability so the products do not change upon freeze storage and subsequent reheating. The potato chips are usually used directly from frozen and therefore the final frying imposes a very large temperature change from frozen (about -20 °C) to approximately 185 °C within a short time interval. The final frying enforces further flash off of more moisture (from 65% to 55%) thus arriving at the final product for consumption.

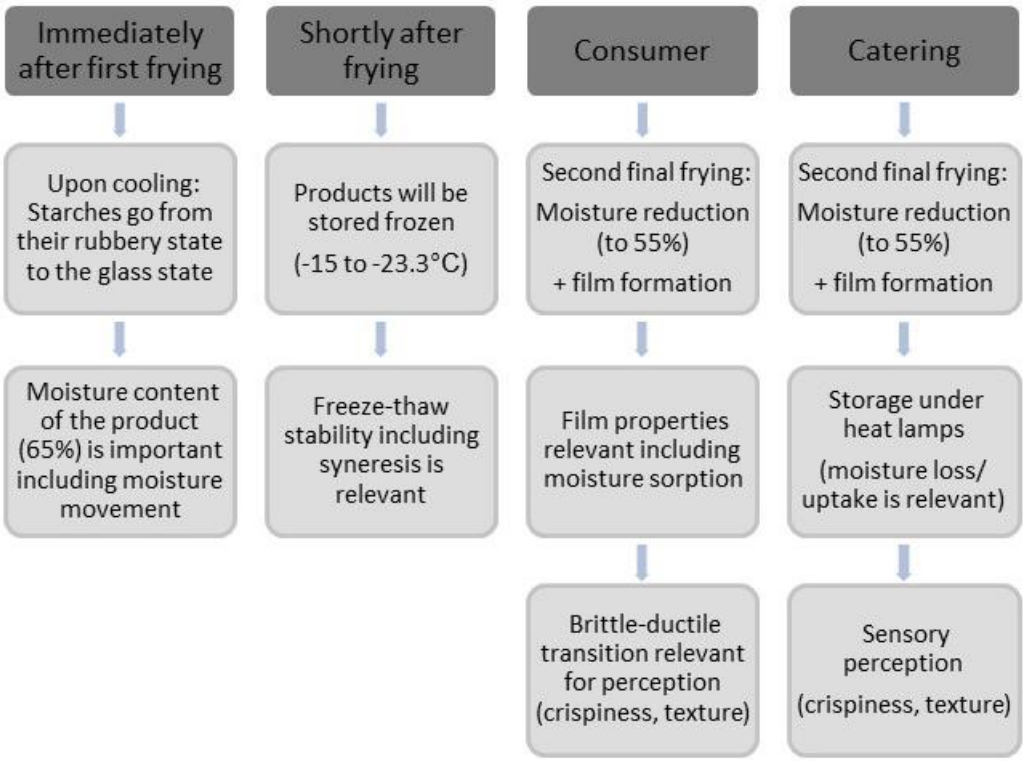


Figure 1.8: Flow chart of the finals steps of the potato chip processing.

The potato chips are either eaten immediately after frying or kept under a heating lamp for later consumption. At this stage of the process, it is important that the product retains its sensory properties, such as crispiness and texture, and these properties can be tested by either sensory testing (panellists) or mechanical tests. Every step in the processing of the potato chip will affect the functionalities of the batter formulation that eventually will determine the final attributes of the product.

The properties of the starch formulation allow the ideal film to form a crispy surface and soft and high moisture interior (potato base) and to create a barrier to minimise the oil uptake. The variation in moisture content of the base material (par-fried/ fully fried) and the change in moisture of the batter mixture may influence the functional properties of the starches within the batter and this needs to be considered when selecting the starches contained in the batters. With regards to moisture movements both during and after frying the moisture in the potato base needs to be considered. Furthermore the surrounding humidity (moisture contained in the ambient air) may be significant and therefore also needs to be controlled by the starch film (batter). Section 1.3.2.1-1.3.2.2 will focus on the movement of moisture in low moisture systems based on starch materials.

1.3.2.1 Changes to starches in the transition from high to low moisture

Upon creation of starch films the starches move from a high water environment (60%) to a low moisture layer located between a matrix of higher moisture (potato base) and ambient air (often at high humidity). These moisture levels associated with the starch are critical for the transitions undergone by the macromolecules as the batter formulations are heated, frozen and stored. During the transition from the native starch granule through melting of starches to form the thin film the starches convert from a semi-crystalline mixture to an amorphous glassy mass. Loss of crystallinity has been discussed previously so the focus of this section is to look at the glass and the amorphous starch.

The reversible glass transition is defined by the transition of an amorphous glassy state into a rubbery state with increasing temperature (Slade and Levine, 1993). However after the frying process, which causes the creation of amorphous starch

and loss of moisture, the decrease in temperature induces the starch film, still flexible when hot, to undergo the transition from the rubbery state to the glassy state. This change in state occurs when the temperature decreases below the glass transition temperature (T_g) and this temperature is moisture dependent. As the batter formulations are often mixtures of starches from different origins (maybe physically or chemically modified) and most likely include other ingredients such as flour, spices and gums (see section 1.2), the batter system may behave in an abnormal way and possibly have multiple glass transitions aligned to the individual ingredients or a mixture thereof. However, there is likely to be a dominant T_g which governs the matrix into which the other components are embedded.

The role of water, as a plasticiser in food systems, is to induce flexibility of solid matrices. Plasticising of the food matrix may be relevant to stability, storage and functional properties. Water is added to the batter mixture system not only to dilute the dry batter mix, but also to aid in the alteration of the properties of the starches (and other ingredients). The lowering of T_g and T_m (melting temperature) in the batter system plays an important role in the microstructure formation within the batter and thus can affect the moisture loss, oil absorption and textural properties (Blanshard and Lillford, 1993, Thanatuksorn et al., 2010).

As described above, water acts as a plasticiser and the addition of moisture to starch has a distinct effect on the glass transition temperature due to its ability to interact with the polymeric material and mobilise the backbone of the large molecules. The inclusion of a low molecular weight material results in a decrease in T_g due to the increase in free volume, defined by being the volume of solution or suspension which is not occupied by molecules (Ferry, 1980). This theory explains why an

undiluted polymer has a much higher glass transition temperature than a diluted polymer solution or a low molecular weight material.

Starches in batter formulations undergo two transitions within the frying process. The first is a phase transition where the semi-crystalline starches melt into an amorphous material/film facilitated by heating (frying at high temperature). The starch formulation (batter mixture) starts with more water than solids prior to heating, so it could be expected that on first entering the fryer, the starches gelatinise and lose their crystallinity and immediately after melt. The fast evaporation of moisture creates a film from the amorphous starchy material. Upon relatively fast cooling (from about 185 °C) to room temperature ($T<T_g$), the amorphous film probably transforms from the rubbery state back into the glassy solid state. This reverse glass transition process is called vitrification (see section 1.3.2.1.1) (Blanshard and Lillford, 1993). The glass transition is dependent on several factors and the glass dynamics cover the time- and temperature-dependence found in the relationships among structure, composition, thermomechanical properties and functional behaviour (Slade and Levine, 1993).

1.3.2.1.1 Vitrification

Vitrification is defined as the solidification process at which a liquid (which ideally exists with a disordered molecular structure) is cooled to a temperature below its T_m (crystalline melting temperature) or its freezing point, at a high enough cooling rate to avoid crystallisation and the liquid then enters its glassy state (Blanshard and Lillford, 1993). Vitrification leads to immobilisation (*freezing in*) of the disordered structure (liquid state) and results in a glassy solid, which is spatially homogenous but lacking a long range lattice order and also is not capable of exhibiting long range,

cooperative relaxation behaviour (WLF kinetics, not discussed) hence not returning to the rubbery state (within a practical time scale) (Blanshard and Lillford, 1993).

In starch systems i.e. in mixtures containing multiple different starches, the glass transition and the vitrification process becomes complex. Shah and Shall (2006) have examined a multi component systems and found that several equations are suitable for calculating theoretical values for an overall T_g of a system, but these calculations require the individual T_g values of the pure components and for some equations the volumetric data as well. The system analysed was at a multi component system containing water, glycerol, salts and PEG and hence is not the model of a starch based system (Shah and Schall, 2006). Mitrus (2005) has reported that amylose and amylopectin have slightly different T_g values, the value for amylopectin being slightly lower (Mitrus, 2005). Also, other choices of plasticisers (e.g. glycerol) can influence the T_g by causing phase separation and multiple T_g values (Mitrus, 2005).

More studies (Bikiaris et al., 2004, Roudaut and Wallecan, 2015) have shown the importance of the interpretation of individual glass transitions in multi component systems. The compatibility between individual components is relevant for the determination of multiple glass transitions temperatures and thus will be reflected in a multi component system. A batter formulation can contain multiple starches, flours, salts, leavening agents and other ingredients (see section 1.2), therefore to understand the T_g value(s) for such a system is not trivial and research covering this area has not yet been undertaken.

Another relevant factor to assess for the batters, which is often related to the glass transition temperature, is the brittle-ductile transition (Barbosa-Cãnovas et al., 2008). This transition is often tested by either mechanical testing or sensory analysis

and several studies have examined the relation between the brittle-ductile transition and sound (Barbosa-Cãnovas et al., 2008, Roudaut et al., 1998). The transition is a measure of the change in texture, thus a change in fracture mechanism from brittle to ductile, and is often used as a measure of crispiness of food and the transition when the food material goes from crispy (brittle) to soft/soggy (ductile).

The glass transition from the rubbery state to the glassy material and thereby creation of surface crispiness is crucial for a potato chip product. However, it is equally important that the crispiness is retained in the potato chip upon consumption but also if the potato chips are kept under heat lamps for later consumption (see Figure 1.8). Thus the retention of crispiness and therefore the brittle-ductile transition is an important factor to address when assessing batter formulations for potato chips. Moreover the relevance of moisture in the food material becomes even more significant since the brittle-ductile transition is indeed dependent on the water movement in food materials as well as the composition of the material (Barbosa-Cãnovas et al., 2008).

Crispiness is a complex textural property and the structure-texture relationship in crispy products can be described using different phenomena. The crispiness can be achieved if the batter is forming a network and capturing bubbles or creating voids within the network resulting in small air bubbles covering the surface of the food product. The brittle material is characterized by a cellular, lamellar or puffed structure (Roudaut et al., 2002). This is also defined as a two phased system, a discontinuous phase (gaseous) which is made of air bubbles created upon fermentation and vaporization of water upon baking or frying and a continuous solid phase supporting the sample weight (Roudaut et al., 2002).

Crispiness is a very important factor in coated potato product but its understanding and assessment is not a major goal for the work described in this thesis.

1.3.2.2 Starches in low moisture (position and relationships)

Levels of moisture are clearly critical for the transformations undergone by the starches that form the bulk components of the batters used to create the desired eating properties of French fries. Moisture levels of starches on dry storage or of the freshly fried film will trend towards equilibrium with their environment. In low moisture systems, sorption behaviour (pick up of water) or desorption (loss of water) is dominated by the chemical nature and molecular structure of the materials.

Sorption isotherms describe the relationship between water activity (a_w) and the moisture content at a constant temperature. The moisture content of a food may not reflect its potential for chemical or physicochemical behaviour, whereas the water activity often better describes this conduct. The relationship between moisture content and water activity often exhibits sigmoidal form, as shown in Figure 1.9. This figure also shows examples of the relationships between moisture content and water activity for different reactions related to food materials.

Film formation occur upon frying when the high temperature causes flash off of moisture. The sudden flash off of moisture may only be controlled by altering the temperature of the frying oil, the speed at which the chips are immersed into the oil and pickup of the batter mixture (controlled by viscosity of the batter). These parameters should be constant in a production line and most variation would occur in the batter pickup thus affecting the thickness of the film. It is difficult to directly measure the moisture in the film as the film will start absorbing moisture as soon as

it leaves the fryer. Thus the moisture/oil barrier properties may be evaluated by other techniques such as dynamic vapour sorption of processed batter mixtures (see section 5.4).

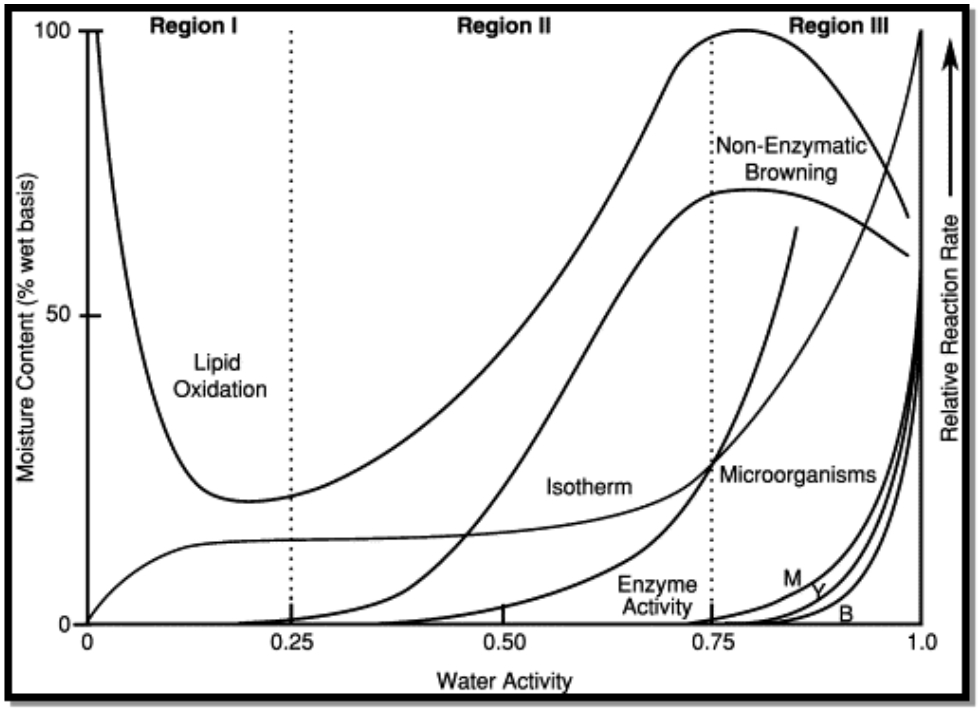


Figure 1.9: The relationship between moisture content and the water activity for different reactions related to food materials. Reprinted from Surface Measurement systems.

Starches are commonly thought to show the general sigmodial isotherm shape, but Figure 1.10 shows the different shapes of curves and the types I – VI which describe the different materials. The type of materials exhibit the different isotherms where type I is a microporous material, type II and III are macroporous adsorbents (like starch), type IV and V are isotherms with hysteresis and finally type VI is defining a step-wise sorption pattern (Sangwichien et al., 2002, Sing, 1985).

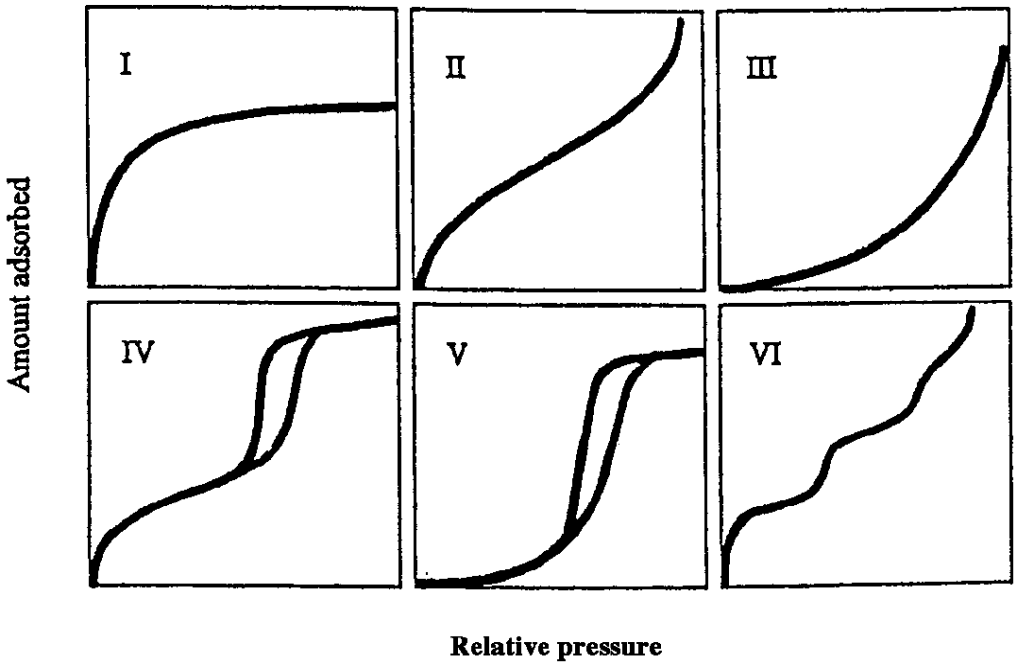


Figure 1.10: The six general types of sorption isotherm exhibited by different materials. Reprinted from Sangwichien et al. (2002).

Different models of absorption have been created to explain the commonly observed sigmoidal form of isotherms and these use various parameters and constants so that each provides a possible explanation of the relationship between low moisture and biopolymers. Most frequently used models for starches are the BET equation and the GAB equation. Both can estimate the monolayer adsorption and subsequent adsorption layers and hence reveal information about the location of the water (Lillford, 1988, Van den Berg, 1985). The GAB equation is often applied to sorption data of food materials and the theory and experimental data modelled using this equation are covered in section 5.4.4.

The interpretation of sorption isotherms may allow an estimation of the amount of water present and the location of water when the system is in equilibrium. However the water movement into the matrix and the rate at which the equilibrium state is achieved can also be described by diffusivity. Diffusivity is dependent on both temperature and moisture contents of the starch sample and describes the migration of water through the sample (Leslie et al., 1991). Leslie and co-workers

found that the effective diffusivity depended on the moisture content in a complex way. At relatively high moisture contents the main water movement was by liquid diffusion. As the starch became drier, leading to increase in porosity, the main water movement was vapour phase diffusion and for dry samples (<10% moisture content) the effective diffusivity fell significantly (Leslie et al., 1991). Furthermore both hydrated starches and gelatinised starches (high amylose and waxy) were tested and showed differences in diffusivity, especially for the waxy sample indicating that starch gelatinisation also impacted the water movement (Leslie et al., 1991).

The kinetics involved in the water-starch interactions are not yet fully understood, especially not for the phase transition at increasing temperature where both pseudo zero order and 1st order reaction kinetics have been shown (Wang et al., 1989, Yeh and Li, 1996). However, the kinetics was shown to be dependent on several factors such as time, temperature, moisture content and type of starch (Wang et al., 1989). For starch films the moisture sorption will also be dependent of the thickness of the sample (Slavutsky et al., 2012). Batters on the potatoes are expected to start as glassy films that are subjected to increasing moisture due to their position on a base of higher moisture. Water uptake affected by diffusion of water onto the film and either uptake or loss to the surrounding air may well change the texture of the film. The question is whether the combination of starches used in the batters can delay the loss of crispiness.

1.3.2.3 Starches in batters

The commercial importance of prepared potato products has continued to accelerate over the last years. The combination of the requirement for rapidly produced home meals, quick service restaurants and consumer liking of fried type

starchy products but with low fat, have made battered potato products an important food item. The mixture of the starches used in the batters has become more and more complex in the desire to achieve the desired eating quality. From an understanding of the stages of the processing required for the potato product and the current knowledge of how starches may behave in such conditions, it is evident that there is much fundamental knowledge lacking. More information and experimental work are required before links can be made between starch incorporation into a batter and the batter's performance. These factors and the requirements of the commercial sponsors helped define this thesis.

1.4 Aims and objectives

Understanding of starches and their complex behaviour in food materials are of great importance in the development of food products. Throughout this study the emphasis was to use commercial starches and starch mixtures, especially those known to provide different quality attributes of batters used to coat potato chips. Modification of starches (section 1.2.1.5) can alter their functionalities e.g. improved stability, cold solubility or increased swelling power.

One objective of this study was to elucidate the functionalities of modified starches applied in batter formulations i.e. in limited moisture environment. The behaviour of starches in limited water content is not a new approach, however only limited literature is available especially for modified starches. The behaviour of modified starches is a key element to the understanding of commercial batter formulations and will directly indicate the final attributes of the battered product.

A second objective was to examine the formation of films and the film forming capabilities of starch blends. The hypothesis was that the ability of starches to form films was due to their melting at high temperature and subsequent flashing off moisture. The film forming abilities of starch formulations were to be illustrated in order to increase understanding and knowledge of the process including their film forming properties and the processing variables of moisture and temperature.

A third objective was to relate the processed starches (i.e. cooked to mimic the film formation process) with the water movement within starch formulations. The starches' sorption profiles were thought to be a key parameter to reveal the ability of the film barrier to control water movement. The moisture sorption (absorption and desorption) may reflect the starches potential as good film formers.

CHAPTER 2 MATERIALS AND METHODS

2.1 General chemicals and solutions

2.1.1 General chemicals

The chemicals used for standard experiments are listed in Table 2.1. These chemicals were used without further purification. When water was used in an experiment as a solvent or diluent, it was always purified water from an Ondeo Purite Select (equipped with a Puripac PP8) unless stated differently. This was due to possible interaction between salts/minerals in the tap water and the starches to be analysed.

Table 2.1: The general chemical used for experiments and their manufacturer

General chemicals	Manufacturer
Amylose/Amylopectin Assay Procedure Megazyme	Megazyme International Ireland Limited, Bray Business Park, Bray, Co. Wicklow, K-Amyl 04/06
Calcium chloride dehydrate	Fisher Scientific, catalogue no. C/1500/50
Dimethyl sulfoxide (DMSO)	Sigma Aldrich, catalogue no. D5879
Ethanol absolute	Fisher Scientific, catalogue no. E/0650DF/17
Glacial acetic acid	Fisher Scientific, catalogue no. A/0400/PB15
Hydrochloric acid 2M	Fisher Scientific, catalogue no. J/4250/17
Iodine 0.05M	Fisher Scientific, catalogue no. J/4410/15
Magnesium chloride hexahydrate	Sigma Aldrich, catalogue no. M2670
Manganese chloride tetra hydrate	Sigma Aldrich, catalogue no. M3634
MOPS sodium salt	Sigma Aldrich, catalogue no. M9381
N-Methyl-Morpholine N-Oxide (NMMO)	BASF, Ludwigshafen, Germany
Sodium acetate, anhydrous	Fisher Scientific, catalogue no. S/2120/53
Sodium azide	Sigma Aldrich, catalogue no. 71290
Sodium chloride	Fisher Scientific, catalogue no. S/3160/60
Sodium hydroxide	Fisher Scientific, catalogue no. S/6600/53
Sodium sulfate anhydrous	Fisher Scientific, catalogue no. J/7620/15
Total Starch Assay Procedure Megazyme	Megazyme International Ireland Limited, Bray Business Park, Bray, Co. Wicklow, K-Tsta 04/2009

2.1.1 N-methylmorpholine N-oxide (NMMO)

The N-methylmorpholine N-oxide solution was supplied from BASF Germany and was a 60% NMMO/ 40% water solution.

Concentration procedure

In order to use the solvent as the required 78% NMMO/ 22% water solution, water was carefully evaporated from the 60% NMMO/ 40% water solution. This was carried out by using a vacuum oven at 40 °C (Gallenkamp, Fistreem International Ltd., Loughborough, UK). Propyl gallate (0.01%) was added to the NMMO solution to decrease of oxidative reactions during the evaporation process. The solution was placed in an uncapped Duran bottle (250 ml) in an oven (Sanyo Convection oven MOV-112F, Sanyo Electric Biomedical Co., Ltd. Japan) at 50 °C and the refractive index (RI) was measured after a total of 22 hours using a pocket refractometer (Pal-1, Atago, Tokyo, Japan). After drying the RI was at 72 and indicated that the water content was at 22% (compared to a known reference value).

2.2 Megazyme Assay Procedures

The two Megazyme assay procedures are adopted from McCleary *et al.* (McCleary, 1997) for the total starch assay procedure and from Yun and Matheson (Yun and Matheson, 1990) for the amylose/amylopectin assay procedure.

2.2.1 Solutions

2.2.1.1 Sodium acetate buffer solution (100mM, pH 4.5)

Glacial acetic acid (5.9 ml) was added to 900 ml water. The pH was adjusted to pH 4.5 using 1M NaOH (approximately 30 ml) and 0.2 g sodium azide was added to the solution once the pH had been confirmed. The solution was adjusted to 1 l with water and stored at 4°C.

2.2.1.2 Concentrated concanavalin A solvent (600mM, pH 6.4 sodium acetate buffer)

The following chemicals were dissolved in 900 ml water: 49.2 g anhydrous sodium acetate, 175.5 g sodium chloride, 0.5 g calcium chloride di-hydrate, 0.7 g magnesium chloride hexa-hydrate and 0.7g manganese chloride tetra-hydrate. Once dissolved the pH was adjusted to pH 6.4 by adding dropwise glacial acetic acid. The solution was adjusted to 1 l with water and stored at 4°C. The working concentration of Con A solvent had to be used on the same day. It was prepared by diluting 30 ml of concentrated Con A solvent to 100 ml with water.

2.2.1.3 Ethanol solutions 80 and 95% (v/v)

Absolute ethanol was used and diluted to the required solutions (80% and 95%).

2.2.1.4 MOPS buffer (3-(N-morpholino)propanesulfonic acid, 50mM, pH 7.0)

MOPS (sodium salt) (11.55g) was dissolved in 900 ml water and adjusted to pH 7.0 using 1M hydrochloric acid (approximately 17 ml). Then 0.74 g calcium chloride di-hydrate and 0.2 g sodium azide were added and dissolved. The volume was adjusted to 1 l with water.

2.2.2 Materials provided within the Megazyme Assay kits, their preparation and the procedures

2.2.2.1 Total starch assay kit and their preparation

Bottle 1: Thermostable α-amylase (3,000 U/ml on Ceralpha reagent at pH 6.5 and 40 °C or 1600 U/ml on Ceralpha reagent at pH 5.0 and 40 °C).

The working concentration was made by diluting 1.0 ml of the contents of bottle 1 to 30 ml with sodium acetate buffer (see 2.2.1.1).

Bottle 2: Amyloglucosidase (3300 U/ml on soluble starch at pH 4.5 and 40 °C)

The content of bottle 2 was used as supplied.

Bottle 3: GOPOD reagent buffer containing potassium phosphate buffer (0.26M, pH 7.4), p-hydroxybenzoic acid (0.22M) and sodium azide (0.4 w/v).

The working concentration was made by diluting the contents of bottle 3 (initial solution ≈ 500 ml) and mixing with bottle 4 and finally diluting to one litre. The final solution was used immediately after preparation.

Bottle 4: GOPOD reagent enzymes (glucose oxidase (> 12,000 U) plus peroxidase (> 650 U) and 1-aminoantipyrine (80 mg)).

Bottle 4 was mixed with 20 ml of the initial solution made from bottle 3 and quantitatively transferred to the flask.

Bottle 5: D-glucose standard solution.

Bottle 6: Standard maize control starch.

Bottle 5 and 6 were used as supplied.

2.2.2.2 Total starch Assay Procedure

The principle for the total starch assay was to use a three step procedure to finally utilise the released hydrogen peroxide to produce a quinoneimine dye detectable by colorimetric analysis.

If the starch sample contained resistant starch, it was dissolved by treating the sample with hot DMSO before adding the thermostable α-amylase which hydrolysed the starch material into branched and unbranched maltodextrins. The maltodextrins were then quantitatively hydrolysed to D-glucose by amyloglucosidase. An oxidation of D-glucose utilising glucose oxidase was releasing hydrogen peroxide and produced D-gluconate as an end product. The hydrogen peroxide was used as a reagent together with p-hydroxybenzoic acid and 4-aminoantipyrine to produce the detectable quinoneimine dye.

Procedure

The starch sample was weighed to 100 mg ± 1 mg into 15 ml screw-capped polypropylene tubes (Sarstedt AG and Co, Nümbrecht, Germany) and in duplicates. The sample was wetted with 0.2 ml 80% ethanol to aid dispersion and 2 ml hot DMSO (70 °C) was added and mixed thoroughly on a vortex mixer (Grant-bio PV-1, Grant Instruments Ltd., Cambridge, UK). The solution was placed in a boiling water bath as quickly as possible to avoid lump formation. The sample was mixed frequently for 10 minutes during the gelatinisation process. Then 3 ml α-amylase (in MOPS buffer, see section 2.2.2.1) was added and incubated in the boiling water bath for 12 minutes while mixing vigorously after 4, 8 and 12 minutes. The tube was placed in a 50 °C closed incubator (Shaking incubator, The Mickle laboratory Engineering Co. Ltd., Surrey, UK) and 4 ml sodium acetate buffer followed with 0.1 ml amyloglucosidase were added to the solution and then mixed. The solution was incubated for 30 minutes at 50 °C and then the content of the tube was transferred to a 100 ml volumetric flask with water using a funnel to aid transfer. It was important to ensure that all the content was transferred by rinsing the tube several times before filling the flask to 100 ml. After thorough mixing aliquots of 1 ml were transferred to 1.5 ml microtubes (Sarstedt AG and Co, Nümbrecht, Germany) and centrifuged on a bench top centrifuge (Heraeus Fresco 21, Thermo Electron Corporation, Osterode, Germany) at 3000 g for 10 minutes at 20 °C.

From this step the total starch content could be measured using two different methods, either the colorimetric method utilising the released hydrogen peroxide (Megazyme total starch standard method) or the glucose analyser (Analox GM9, Analox Instruments Ltd, London, UK). For the colorimetric analysis, 0.1 ml of the supernatant was transferred in duplicates to 15 ml glass tubes and 3 ml of GOPOD reagent was added and mixed immediately on a vortex mixer prior to incubation for 20 minutes at 50 °C. Together with the starch samples, a blank (0.1 ml water and 3

ml GODPOD reagent) and two glucose controls (0.1 ml D-glucose standard and 3 ml GODPOD reagent) were incubated. After incubation the absorbance was read at 510 nm against the blank sample on a CARY 50 Probe UV-visible Spectrophotometer (Varian, Palo Alto, USA). The calculation of total starch content was done using the equation provided by Megazyme Ireland International Ltd.

Equation 1

$$\text{Starch } \% \frac{\text{w}}{\text{w}}(as\ is) = \Delta A\ \times F\ \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{1}{W} \times \frac{162}{180}$$
$$= \Delta A\ \times F\ \times \frac{FV}{W} \times FV \times 0.9$$

Where

$\Delta A =$ Absorbance (reaction)read against the reagent blank

$F = \dfrac{100\ (\mu g\ of\ D-glucose)}{absorbance\ for\ 100\ \mu g\ of\ glucose}$ (conversion from absorbance to µg)

$FV =$ final volume (100ml)

0.1= volume of sample analysed

$\dfrac{1}{1000} =$ conversion from µg to mg

$\dfrac{100}{W} =$ factor to express starch as a percentage of flour weight

$W =$ the weight in mg ("as is" basis)of the flour analysed

$\dfrac{162}{180} =$ adjustment from free D − glucose to anhydro D − glucose

(as occurs in starch)

This results in the following equation:

Equation 2

$$\text{Starch } \% \frac{\text{w}}{\text{w}}(dry\ wet\ basis)$$
$$= Starch\ \% \frac{w}{w}(as\ is) \times \frac{100}{100 - moisture\ content\ (\% \frac{w}{w})}$$

When the glucose analyser was used, D-glucose content was determined from the clear filtrate. About 10 µl was inserted into the glucose analyser with a Miroman

positive displacement pipette (Gilson S.A.S., Villiers-le-Bel, France) and a D-glucose value was obtained. Three washing cycles were done before inserting a new sample. The glucose reagent (Analox Instruments, London, UK) was used to wash between cycles and the pipette was rinsed with water three times. The glucose content was calculated using the same equation as derived by Megazyme Ireland Ltd., but modified since the value of D-glucose is given in mg/dl hence no dilution factors are required. Therefore

Equation 3

$$\text{Starch } \%\frac{\text{w}}{\text{w}}(dry\ wet\ basis) = Starch_c\ \%\frac{w}{w}(as\ is) \times \frac{100}{100 - moisture\ content\ (\%\frac{w}{w})}$$

Where

$$\text{Starch}_c\ \%\ \text{w/w}\ (as\ is) = c_{glucose} \times \frac{100}{W} \times \frac{162}{180} = c_{glucose} \times \frac{100}{W} \times 0.9$$

With similar factors as Equation 1 and

$$c_{glucose} = \text{concentration of D} - \text{glucose in mg/dl}$$

2.2.2.3 Amylose/Amylopectin Assay Procedure kit and their preparation

Bottle 1: Freeze dried Con A.

The working concentration was made by dissolving the contents of bottle 1 (Con A) in 50 ml of Con A solvent (see 2.2.1.2).

Bottle 2: Amyglucosidase (3300 U on starch at pH 4.5 at 40 °C) plus fungal α-amylase (500 U on Ceralpha reagent at pH 5.0 and 40 °C).

The working concentration was made by dissolving the contents of bottle 2 in 20 ml of sodium acetate buffer (100mM, pH 4.5) (see 2.2.1.1).

Bottle 3: GOPOD reagent buffer containing potassium phosphate buffer (1M, pH 7.4), p-hydroxybenzoic acid (0.22M) and sodium azide (0.02 w/w).

The working concentration was made by diluting the contents of bottle 3 (initial solution ≈ 500 ml), mixing with bottle 4 and finally diluting to one litre. The final solution was used immediately after preparation.

Bottle 4: GOPOD reagent enzymes (glucose oxidase (> 12,000 U) plus peroxidase (> 650 U) and 1-aminoantipyrine (80 mg)).

Bottle 4 was mixed with 20 ml of the initial solution made from bottle 3 and quantitatively transferred to the flask.

Bottle 5: D-glucose standard solution.

Bottle 6: Starch reference sample with specified content of amylose (66%).

Bottle 5 and 6 were used as supplied.

2.2.2.4 Amylose/Amylopectin Assay Procedure

The principle of the amylose/amylopectin assay procedure was similar to the principles of the total starch assay procedure. The starch sample was fully dispersed in hot DMSO and lipids were removed by ethanol precipitation of the starch. The amylopectin was precipitated by addition of the lectin concanavalin A, which was specifically bound in a complex with amylopectin using an acetate/salt buffer as solvent. The precipitate was removed by centrifugation and an aliquot of the supernatant containing amylose was enzymatically hydrolysed to D-glucose by the glucose oxidase/peroxidase reagent. For the determination of total starch another aliquot of the solution still containing the amylopectin-Con A complex was hydrolysed to D-glucose using the oxidase-peroxidase reagent. The concentration of amylose was determined from the ratio of the GOPOD absorbance of the supernatant of the Con A precipitated sample to the GOPOD absorbance of the total starch.

Procedure

Duplicate starch samples were weighed, 25 mg ± 1 mg, into 15 ml polypropylene screw-capped tubes (Sarstedt AG and Co, Nümbrecht, Germany). Two starch controls, with known amounts of amylose, were used as references. The sample dispersed in 1 ml hot DMSO (70 °C) was added and mixed thoroughly on a vortex mixer (Grant-bio PV-1, Grant Instruments Ltd., Cambridge, UK). The solution was placed in a boiling water bath as quickly as possible to avoid lump formation. The sample was mixed frequently for 15 minutes during the gelatinisation process. The solution was allowed to cool down for 5 minutes at room temperature before adding 2 ml ethanol and the solution was mixed gently. Further 4 ml ethanol was added and mixed carefully on a vortex mixer. The tube was capped with parafilm (Parafilm M, Bemis flexible packaging, Wisconsin, USA) and left for at least 15 minutes or preferably overnight.

The supernatant was removed by centrifugation at 2000 g for 5 minutes (Multifunction centrifuge, Jouran CR31i, Thermo Electron Industries S.A.S., Chateau-Gontier, France) and then discarded. The tube was drained by turning the tubes upside down on tissue paper and left for 10 minutes. The precipitate was dissolved in 2 ml DMSO and thoroughly mixed before the solution was left in a boiling water bath for 15 minutes and mixed frequently to avoid lumps. On removal of the tube from the boiling water bath, immediately 4 ml Con A solvent was added and mixed. The content was transferred using Con A solvent into a 25 ml volumetric flask, rinsed several times and mixed. An aliquot of 1 ml was transferred to 1.5 ml micro tubes (Sarstedt AG and Co, Nümbrecht, Germany) where 0.5 ml Con A was added and the sample gently mixed by repeated inversion. The solution was left for 1 hour at room temperature and then centrifuged at 14000 g for 10 minutes at room temperature. From the supernatant 1 ml was transferred to a 15 ml centrifuge tube and 3 ml of

sodium acetate buffer (100mM) was added. The solution was mixed carefully on a vortex mixer and heated in a boiling water bath for 5 minutes in order to denature the Con A.

To equilibrate the solution it was placed in a 40 °C water bath for 5 minutes before adding the amyloglucosidase/α-amylase enzyme mixture (see section 2.2.2.3). The solution was incubated at 40 °C for 30 min following centrifugation for 5 minutes at 2000 g. For the colorimetric analysis 1.0 ml of the supernatant was transferred in duplicates to 15 ml glass tubes and 4 ml of GOPOD reagent was added. The solution was mixed immediately on a vortex mixer prior to incubation for 20 minutes at 40 °C. Together with the starch samples a blank (1.0 ml sodium acetate buffer and 4 ml GODPOD reagent) and two glucose controls (0.1 ml D-glucose standard, 0.9 ml sodium acetate buffer and 4 ml GODPOD reagent) were incubated. Concurrently the total starch solution was incubated, which was prepared by taking out aliquots of 0.5 ml of the solution from the volumetric flask and transferring them into 15 ml glass tubes. Then 4 ml of sodium acetate buffer was added and the solution was mixed before adding 0.1 ml amyloglucosidase/ α-amylase enzyme reagent to the tube and incubating for 10 minutes at 40 °C. After incubation 1 ml was transferred to a glass tube and 4 ml GOPOD reagent was added and mixed. This solution was then co-incubated for 20 minutes at 40 °C with the solution only containing amylose. After incubation the absorbance was read at 510 nm against the blank sample.

The calculation of amylose/amylopectin ratio was done using the equation provided by Megazyme.

Equation 4

$$\text{Amylose \%}\left(\frac{\text{w}}{\text{w}}\right) = \frac{Absorbance\ (con\ A\ supernatant)}{Absorbance\ (Total\ starch\ aliquot)} \times \frac{6.15}{9.2} \times \frac{1}{100}$$

$$= \frac{Absorbance\ (con\ A\ supernatant)}{Absorbance\ (Total\ starch\ aliquot)} \times 66.8$$

Where 6.15 is the dilution factor for Con A and 9.2 is the dilution factor for the total starch extracts.

2.3 Starches and batters

A feature of the work carried out for the studies described in this thesis are the large number of starch containing samples investigated. These starches and blends of materials were of commercial relevance and the majority of starch materials were sourced and provided by Newly Weds Food Limited, in cooperation with McCain Foods Limited. Details of the original suppliers and some other information on the starchy materials were not made available for the purposes of this thesis. To ease in tracking the starches and their use in blends, and due to different batches of starchy materials being supplied at different times, the starchy materials and blends have been coded by whether they are a single material XXX, or a blend XXXX and according to year of study e.g. 10x for 1st year samples, 20X for 2nd year etc. Previous studies have been carried out by Rajiv Perumal (Perumal, 2013) who investigated the individual behaviour of natural starches from a range of botanical sources and therefore the focus of the work reported in this thesis was based on modified starches and blends of materials used to make batters.

2.3.1 Modified starches

The modified starches were chemically and/or physically modified. Table 2.3 presents the different starches and the details given by the suppliers about their

botanical source and the modification type and chemical used for modification. All of the samples were free-flowing powders, but did vary in colour and particle size.

2.3.2 Native starches

The behaviour of the modified starches needed to be compared with the properties of the native starches of the same origin. A few native samples were therefore collected and used as references. These are listed in Table 2.2.

Table 2.2: Native starches used as references

Samples	Thesis reference number
Maize starch	121
Wheat starch	122
Rice starch	123
Potato starch	124
Sago starch	125
Waxy rice starch	126
Waxy maize starch	127
Pea starch	128
High Amylose maize starch	129
Tapioca starch A	130
Tapioca starch B	131
Tapioca starch C	132

Table 2.3: Modified starches with the details of their botanical source, chemical used for modification and E-number.

Botanical Source	Modification Type	Chemical	E-Number	Reference no.
Waxy Maize	Sodium octenyl succinate	Hydrophobic cyclic anhydride	E1450	101
Maize (high amylose)	Stabilised	Stabilised with acetic anhydride	E1420	102
Pea	Di starch phosphate	Phosphorous oxychloride	E1412	103
Waxy Maize	Di starch phosphate	Phosphorous oxychloride	E1412	104
Tapioca	Hydroxyl propylated di starch phosphate	Stabilised with propylene oxide and crosslinked with phosphorous oxychloride	E1442	106
Pea	Dextrinisation	Low conversion (white)	Dextrin	107
Pea	Dextrinisation	Highly converted (yellow)	Dextrin	108
Maize	Fluidity starch -acid treated	Hydrochloric acid	None	109
Tapioca	Di starch phosphate	Phosphorous oxychloride	E1412	110
Tapioca	Hydroxyl propylated di starch phosphate	Stabilised with propylene oxide and crosslinked with phosphorous oxychloride	E1442	111
Tapioca	Stabilised	Stabilised with propylene oxide	E1440	112
Sago	Fluidity starch -acid treated	Hydrochloric acid	None	113
Tapioca	Di starch phosphate	Phosphorous oxychloride	E1412	114
Tapioca	Hydroxyl propylated di starch phosphate	Stabilised with propylene oxide and crosslinked with phosphorous oxychloride	E1442	115
Tapioca	Hydroxyl propylated di starch phosphate	Stabilised with propylene oxide and crosslinked with phosphorous oxychloride	E1442	116

2.3.3 Batters

Various materials, normally high in carbohydrate, are blended together to make batters with different characteristics in order to provide a suitable batter for a specific food application. The composition of a batter is dependent on the final application and whether it serves as a texturing agent or a flavour additive or both. Two sets of individual ingredients and their batters have been used in this study. The details and compositions are given in Table 2.4 - Table 2.7.

Table 2.4: Individual ingredients for batter 2001-2010 (Table 2.5)

Samples	Thesis reference number
Wheat flour low protein	201
Wheat flour heat treated	202
Rice flour	203
Potato native starch	204
Maize flour low viscosity	205
Maize starch native	206
High amylose starch maize	207
Waxy maize	208
High amylose starch modified maize	209
Pea dextrin	210
Potato dextrin	211
Tapioca starch	212
Tapioca flour	213
Xanthan gum	214
Wheat starch	215
Pea dextrin 2	216
Pea starch	217
Sago starch	218
Tapioca dextrin	219
Tapioca dextrin + maize high amylose	220

Table 2.5: Batters 2001-2010 including the starch materials utilised in these batters

Ingredients Utilised	Thesis reference number
Modified potato starch, Wheat starch, Rice flour, Modified maize, Maize starch, Tapioca dextrin	2001
Modified potato starch, Rice flour, Pea dextrin, Pea fibre	2002
Modified Potato starches, Rice flour, Potato Dextrin, Tapioca dextrin	2003
Modified Maize, Modified Potato, Waxy rice, Tapioca dextrin, Pea fibre	2004
Wheat flour, Rice flour, Modified Maize, High Amylose Maize, Tapioca dextrin	2005
Modified Tapioca , High amylose maize, Rice flour	2006
Wheat flour (heat treated and non-heat treated), Wheat starch	2007
Maize flour, Rice flour, Modified maize, High amylose maize, Tapioca dextrin	2008
Wheat flour, Modified tapioca starch, Native tapioca, Tapioca dextrin	2009
Modified potato starch, Potato dextrin, Xanthan Gum	2010

Table 2.6: Individual ingredients for batters 3001-3003 (Table 2.7)

Samples	Contained in batter	Code
Potato modified starch	3001	301
Wheat starch native	3001	302
Rice flour	3001	303
Modified maize + tapioca dextrin	3001	304
Modified maize starch	3001	305
Tapioca starch	3001	306
Rice flour	3002	307
Modified potato starch	3002, 3003	308
Pea dextrin (b)	3002	309
Pea fibre	3002, 3003	310
Pea dextrin (a)	3002	311
Modified potato starch (b)	3002	312
Dextrin tapioca	3003	313
Modified maize waxy	3003	314
Modified maize waxy (b)	3003	315
Waxy rice	3003	316
Potato dextrin		317

Table 2.7: Specific batters chosen for further investigation.

Samples	Thesis Reference number
Batter 1	3001
Batter 2	3002
Batter 3	3003

2.4 Starch characterisation

Due to their complex macromolecular structures and their different properties starches require consistent and detailed methods for their characterisation. Often starches are characterised at high water levels because many applications, such as sauces and dressings, are created using high temperatures and plenty of water. Therefore for this current work starches have been examined using a wide range of techniques.

2.4.1 Visual evaluation and moisture content

The visual evaluation of the samples was done prior to any experiment or when samples changed significantly. The following visual characteristics were evaluated: colour, particle size (apparent when looking with the naked eye), homogeneity including any lumps or ingredients separating from the rest of the sample, stickiness, dustiness and odour.

The moisture content of every powder sample will vary due to differences in storage, packaging and processing. Therefore it is important to determine and understand the moisture content of the samples. Moisture contents can be quoted on a wet or dry weight basis. Typically moisture contents are quoted on wet weight basis (wb) in this thesis.

2.4.1.1 Moisture content determination

A foil cup was dried overnight at 105 °C and the initial weight was noted. A sample of 3.0 g ± 0.010 g was weighed into the cup and the sample was dried in a 105 °C oven for 24 hours. The samples were cooled in a desiccator for 2 minutes and the total weight (cup and sample) were obtained. Three replicates were made for each sample.

2.4.2 Swelling power

The swelling power of the starches shows their capability to swell during a controlled heating and mixing process. The procedure of the swelling power is adapted and modified from (Leach et al., 1959), (Crosbie, 1991) and (McCormick et al., 1991).

2.4.2.1 Swelling power procedure

A starch sample of 30 mg ± 1 mg (1.0 % wet basis) was weighed into a pre-weighed 50 ml conical based, screw-capped test tube in 3 replicates (Sarstedt AG & Co, Nümbrecht, Germany). Water (30ml) was added using an Eppendorf® Multipipette® Xstream rapid dispensing pipette. The tubes were capped and the contents were mixed by inversion twice immediately to avoid lump formation and then transferred to a water bath 95 ± 2 °C for incubation. During the 30 minutes incubation and until the samples had gelatinised, mixing was done at intervals to avoid lumping. For the initial 3 minutes the mixing was carried out at 20 seconds intervals, followed by mixing every 30 seconds for 2 minutes, every 1 minute for 5 minutes and then every 5 minute to a total of 30 minutes. After the full 30 minutes the tubes were placed immediately into a 20 °C water bath for 5 minutes to cool down. The samples were centrifuged at 1000g (Multifunction centrifuge, Jouran CR31i, Thermo Electron Industries S.A.S., Chateau-Gontier, France) for 20 minutes at 15 °C and the volumes

of the swollen granules were recorded by weight. The supernatant was removed using a disposable Pasteur pipette (or by gently pouring) and weighed before the residual pellet was dried overnight in an oven at 105 °C. The pellet and the tube were weighed to determine the residue left in the tube.

The swelling power (SP) was determined as the ratio of the swollen granules (by weight) to the dry weight of the total starch material. The determination of swelling power was corrected for leaching amylose as well as other soluble matters such as salt, dextrins, colours or leavening agents due to the supernatant being removed from dry weight material. The starch sample was corrected for loss of material into the supernatant.

$$SP = \frac{mass_{swollen,wet\ weight}(g)}{mass_{dry\ weight}(g)}$$

2.4.3 Iodine staining

For the modified starches the Megazyme Amylose/Amylopectin Assay Procedures were difficult to interpret due to the complex structure of the modified starches and another iodine staining method adopted from (Knutson, 1986) was probed. It is well-known that amylose forms a complex with iodine when iodine is added to a solution of starch. Thus the amylose content can be estimated from reading the absorbance of the developed colour and comparing with starch controls with known contents of amylose.

2.4.3.1 Iodine staining procedure

A starch sample of 5.0 mg was weighed into a 15 ml Sarstedt tube (Sarstedt AG & Co, Nümbrecht, Germany) with screw cab. The sample was dissolved in 10 ml 90 % DMSO containing 0.0045 M iodine (90 ml DMSO with 10 ml 0.05 M I_2). The samples were dissolved at 50 °C in a water bath for 1 hour and mixed occasionally. 1 ml of

the solution was diluted with 8 ml of water and mixed thoroughly. The iodine-amylose complexes (solutions) were left for 30 minutes in order to stabilise to maximum absorbance. The absorbance was read at 600 nm immediately after the 30 minutes. To probe the experiment samples were also left overnight at room temperature and the absorbance was measured again. This did not show any differences in the results. Two reference samples with known amylose contents were used to determine a standard curve to calculate the amylose levels.

2.4.4 Rapid visco analysis (RVA)

Rapid visco analysis is a well-established method used for the measurement of the pasting and apparent viscosity properties of starches. Data points of peak viscosity, end viscosity and pasting temperature can be collected from the pasting profiles where temperature and stirring rates are controlled (see Figure 2.1).

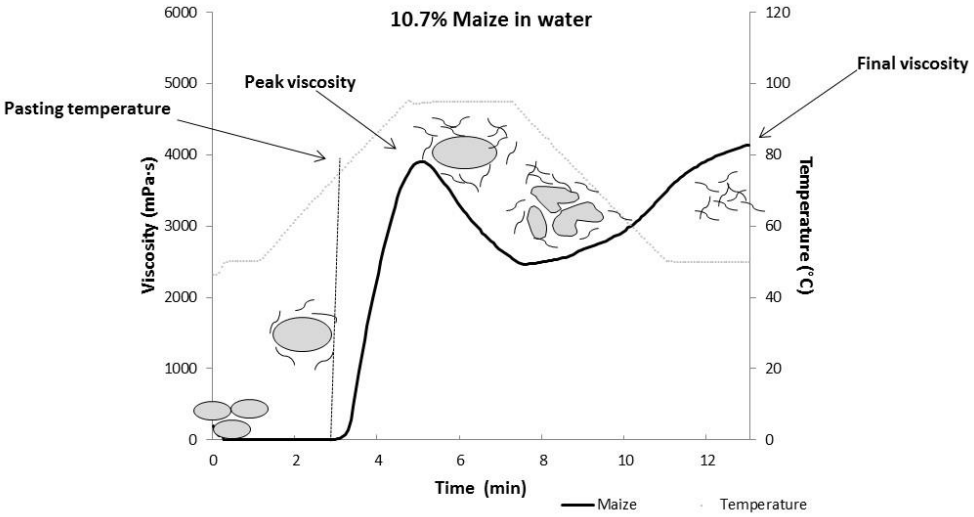


Figure 2.1: A schematic presentation of the theoretic dissolution of the starch granule in water. The pasting characteristics as pasting temperature, peak viscosity and final viscosity are shown. Normal native maize (10.7%, 121) is used as an example.

The work reported in this thesis utilising RVA analysis was carried out as follows:

- With the standard set-up: starch mixed with water at a solids content of 12.5%.
- Low solids content: water solvent.

- NMMO solvent set-up: solvent was changed from water to N-methylmorpholine N-oxide (NMMO) to explore the solubilisation of the starches. Background is that NMMO is known to solubilize the starch granule from the outside or layer by layer, whereas water as a standard plasticiser solubilises by penetration into the granule structure, concomitant swelling and break down.

2.4.4.1 Standard Procedure

A solution of 12.5% (wb) starch or batter in water was mixed briefly rotating the standard plastic spindle in the aluminium RVA canister and loaded to the Rapid Visco Analyser (RVA) Super 4 (Newport Scientific Pty. Ltd., Warriewood, Australia). The standard solution in 78% NMMO - 22% water was 5% (wb) starch or batter. Further addition of materials/solvents such as iodine, GMS or salt were dry mixed (if solids) or added to the solution prior to mixing. The standard temperature profiles for the RVA are given in Figure 2.2 and Table 2.8. The NMMO solution was preheated to 50 °C before adding to the starch, and no extra materials where added when using NMMO. For all experiment the standard software Thermocline for Windows (version 2.4, Newport Scientific Pty. Ltd., Warriewood, Australia) was used to obtain data which was recorded every 4 seconds or every 8 seconds for water and NMMO solution respectively.

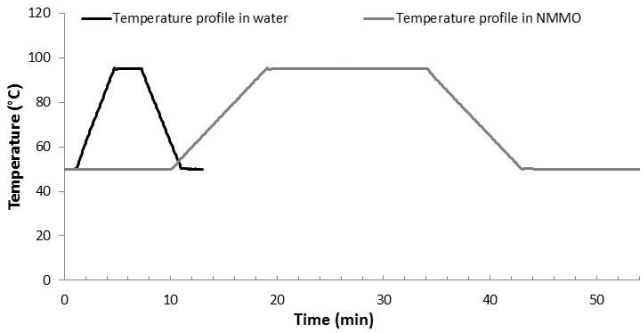


Figure 2.2: RVA temperature profiles for water and NMMO

Table 2.8: The set-up for two different RVA temperature profiles using either water or NMMO as a solvent

RVA in water	RVA in NMMO
• Rotates at 960 rpm for the initial 10 seconds. Then maintains a speed of 160 rpm throughout the 13 minutes test. • Start temperature 50 °C for 1 minute. • Gradual heating to 95 °C for 4 minutes. • Temperature held for 2 minutes at 95 °C. • Then cooled down to 50 °C over 4 minutes. • Temperature held for 2 minutes at 50 °C.	• Maintains a speed of 160 rpm throughout the 54 minutes test. • Start temperature 50 °C for 10 minutes. • Gradual heating to 95 °C for 10 minutes. • Temperature held for 14 minutes at 95 °C. • Then cooled down to 50 °C over 10 minutes. • Temperature held for 10 minutes at 50 °C.

2.4.5 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measures the heat flow when materials are heated and is often used to follow the change of state of the materials, for example when there is a loss in crystallinity. DSC is a thermoanalytical technique which is measuring the differences in the amount of heat required to change the temperature. Hence the changes in one target sample are compared to a reference (often an empty pan) and these are compared and measured as a function of temperature. The DSC method can be used to obtain a wide range of characteristic properties for a sample e.g. information about fusion (melting of starch crystallites), crystallisation and the glass transition temperature (T_g). Therefore the set-up for DSC has to be targeted towards the aim of the data collection. It is possible to change many factors including the heating rate, solvent system, concentration etc.

Two solvent systems were used to explore the difference upon starch solubilisation. Water was used as the standard plasticiser and normal media for investigating starch granule structure. N-methylmorpholine N-oxide (NMMO) was used to

investigate the solubilisation of the starch with the expectation that the granule morphology would not dominate the behaviour.

2.4.5.1 Procedure

A Mettler-Toledo DSC with an automatic robot sampler (DSC-823^e, Mettler-Toledo, Leicester, UK) was used for all experiments. The concentrations of the samples were either in what has been termed high or low moisture content. High moisture samples had a starch:water ratio of 1:2, while low moisture content samples had a starch:water ratio of 1:0.6, which correspond to 33.3% and 62.5% (wb) starch material respectively. The samples were prepared 24 hours prior to the DSC experiment. The powder was hydrated by weighing 0.5 g of starchy material into a 7 ml Bijou container (Thermo Fisher Scientific, Newport, UK) and adding the desired amount of water. The sample was then mixed vigorously and continued mixing overnight on a bench-top roller mixer (Denley Spiramix, Thermo Electron Corporation, Osterode, Germany). Between 20-50 mg of the hydrated sample was transferred to a stainless steel pan prior to starting the experiment and an empty pan was used as a reference. For the samples containing 78% NMMO-22% water as a solvent, 5 mg ± 1 mg starchy material was weighed directly into the stainless steel pan. The NMMO solution (including 0.01% propyl gallate) was added in a 1:9 starch:solvent ratio (10 % starch material) due to the rheological properties of the NMMO solvent. The sample was left overnight on a bench-top roller mixer.

The heating rate of 10 °C/min was the standard setting for the experiments with water as a solvent and run for most experiments, but an increased heating rate of 40 °C/min was also used. The samples were rerun after 1 week (storage at 4 °C) to investigate retrogradation. For DSC experiments using the NMMO solvent a heating rate of 5 °C/min was used.

The melting point and enthalpy of transition of indium were used for temperature and enthalpic calibration of the DSC traces respectively. The enthalpic values obtained from the DSC traces were corrected for the moisture content of the samples.

2.4.6 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was used to determine moisture content in addition to method described in section 2.4.1. The thermogravimetric analysis is a method measuring the change in weight of the sample as a function of increasing temperature.

Measuring the moisture content using the TGA method varies from the method described in section 2.4.1. This is due to smaller sample size, higher heating temperature and controlled heating rate (see section 2.4.1).

2.4.6.1 Procedure

A Mettler-Toledo TGA/SDTA851[e] was used to measure the moisture content of the samples. A sample of 10-15 mg ± 1 mg was weighed into an aluminium TGA pan (100µl, Mettler-Toledo, Leicester, UK) and the lid was sealed using a manual press. A sealed empty pan was used as a reference. The temperature range was set-up dependent on the sample and was modified in order to improve the measurement. The settings were either 25-250 °C at a 10 °C/min heating rate or 25-200°C with a heating rate of 5 °C/min.

2.4.7 Texture analyser

A TA.HD Plus Stable Microsystems texture analyser was used to measure the gel strengths of the starch solutions. The sample preparation used the standard RVA method as described in section 2.4.4.1 with 12.5 % starch in water. Upon completion of the RVA experiment the sample was removed immediately, the spindle taken out

of the solution carefully and the surface of the paste levelled to an even surface. The paste was allowed to cool down and the canister was covered with parafilm and stored at 4 °C for 24 hours. The texture analyser was set to measure the breaking force (first maximum) of the gel and displaying the curve while other parameters could be calculated from the raw data. The parafilm was removed and the canister placed below a 40 mm long cylindrical probe with a diameter of 10 mm and a flat probe surface. The measurement used a pre-test speed of 1 mm/sec and a 5 g trigger force. The test speed was 2 mm/sec and the distance into the gel/paste was set to 15 mm. The samples were measured in triplicate.

2.4.8 Microscopy

Simple light microscopy was used to visualize the size of the starch granules to compare the different types of origin and to some extent modification. Using polarized light microscopy provided information about the crystallinity of the starch granules and whether the Maltese crosses were still observable, thus indicating the integrity of the starches, e.g. after modification. The hot stage microscopy was used to identify the temperature upon melting of helices and disruption of the starch granules (gelatinisation) as well as observing the dissolution patterns of the starches in different solvents. Scanning electron microscopy (SEM) was used to explore the details on the starch granule outer structure. The impact of the modification on the granules could in some cases be visualised by using the SEM technique.

2.4.8.1 Simple light microscopy

The light microscope used for viewing the samples was a Leitz Diaplan light microscope (Ernst Leitz Wetzlar GmbH, Germany) coupled with a PixeLink PL-A662 digital camera (Pixelink, Ottawa, Canada), which was attached to the ocular lens of

the microscope. The Linksys 32 software was used to capture the data and images were taken using a 10x magnification to visualise the granules sizes and shapes.

A small amount of sample (≈1 mg) was placed on a glass slide. Using a disposable Pasteur pipette one drop of water was added to the sample and a glass cover slip (18 mm x18 mm) was carefully placed on the top of the dispersion. No air should be between the glass slide and cover slip to prevent disruption of the visual interpretation of the granules. The examination of the sample was done immediately after their preparation. When taking polarised images a circular polarising filter was attached to the microscope and the light intensity was increased in order to see the birefringence of the sample. The camera settings were constant at size 640x480, exposure 89.0 ms, white balance: middle towards warm, saturation 0.41 and gamma 0.45.

2.4.8.2 Hot stage microscopy

For hot stage microscopy a different set-up and sample preparation were required. A Linkam THMS600 variable temperature stage (Linkam Scientific Instruments, Surrey, UK) was coupled to the Leitz microscope and connected to the computer using the Linksys 32 software. A sample was prepared by using the RVA method for the NMMO solvent (5% starch, section 2.4.4) to dissolve the sample. A small amount of sample (1-2 drops) was placed on a circular glass slide (16 mm in diameter) using a disposable Pasteur pipette. Glue was added around the edge of the glass cover slip (16 mm in diameter) and it was carefully placed on the top of the NMMO-starch dispersion avoiding trapping any air inside. The glue was allowed to harden (15 minutes) before the sample was inserted into the hot stage probe holder and the experiment started. The initial heating rate was 10 °C/min until heated to 40 °C (hold for 3 minutes) and then 5 °C/min up to a temperature of 94 °C. The

temperature was constant at 94 °C for 3 minutes and then increased to 95 °C at 10 °C/min. The temperature was held for 30 minutes at 95 °C to observe the granules behaviour. Images were taken every 30 seconds up to 95 °C and then every 2 minutes or when changes occurred.

2.4.8.3 Scanning electron microscopy

The equipment used for SEM measurements was a JEOL JSM-6060LV (JEOL Ltd., UK) operated at 10 kV and 12 kV. A small amount of sample was placed on a double sided carbon tape and excess material carefully removed by controlled air blow. The sample was gold coated with a gold spotter and inserted into the microscope. Images were taken at different magnifications. Details are given in the bottom of every image along with a scale bar.

2.4.9 Particle size distribution

The particle size distribution can be measured either in suspension or in dry powder. The particle size distribution can be described in different ways. The most commonly used descriptions are the differential distribution and the cumulative distribution based on diameter.

Differential distribution will often form a bell shaped curve with a maximum and give the values for mean diameter and modal diameter. The differential distribution can show the relative “amount” by number, surface area, volume, mass or intensity of scattered light at each particle size. The cumulative distribution shows the relative “amount” at or below a particular size. The cumulative distribution can be used to read the percentage between any diameters or the median diameter, e.g. d_{50}.

2.4.9.1 Procedure

The Beckman Coulter Particle Sizer (LS 13 230, Beckman Coulter Ltd., High Wycombe, UK) controlled by the LS 13 320 software was used to measure the

particle size distribution and collect the data. The Tornado Dry Powder System including vacuum supply was used as only powder samples were analysed. Calibration with Coulter LS Control G35D was done following cleaning with Tornado Cleaner Garnet Abrasive (both product supplied by Beckman Coulter Ltd., High Wycombe, UK).

The sample was run in triplicate with cleaning between different samples (not between triplicate samples) and the background was measured before every sample to make sure the particle sizer was clean. The optical model Fraunhofer ifr 78d was used for calculating and plotting the data.

2.4.10 Wide angle X-ray diffraction

X-ray crystallography was used to determine the crystallinity of the starch granules and to explore whether crystallinity changed with different treatment of the starches. The crystallinity calculation was based on the procedure of Hermans and Weidinger (Hermans and Weidinger, 1948) where the crystalline and amorphous areas on the diffractogram were used to calculate an average crystallinity value.

2.4.10.1 Procedure

A starch sample in the form of a randomly oriented powder was packed into a disc shaped plastic sample holder which was loaded into a detachable rotating cell. This was mounted in a Bruker D5005 X-ray diffractometer set up in a slit focus reflection geometry mode. Copper K alpha (Cu K_α) radiation of wavelength 1.5418 Å was generated by an x-ray tube at voltage and current settings of 40kV and 40 mA. Wide angle measurements were carried out in the range 3 to 38° 2θ. Data was recorded for 2.5 s in angular steps of 0.05°. Further randomisation was achieved by sample rotation at a speed of 60 rpm. The total time for data collection was 29 minutes.

2.4.11 Solid state ^{13}C- and ^{31}P-NMR spectroscopy

NMR spectroscopy is an excellent method for determining the structure of compounds or verifying the composition of an unknown molecule. Solid state ^{13}C-NMR and ^{31}P-NMR spectroscopy was used in this study to investigate if the level of cross-linking and stabilisation could be established. The solid state ^{31}P-NMR spectroscopy was used to explore the level of crosslinking (phosphorus oxychloride crosslinking) using potato starch as a reference.

2.4.11.1 ^{13}C- NMR Procedure

Carbon-13 cross polarisation magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectra were recorded on a Bruker (Karlsruhe Germany) AVANCE 600 NMR Spectrometer with narrow bore magnet and 4mm triple resonance probe. The parameters and conditions used in CPMAS experiments are listed as follows. The Proton 90º pulse length was 3 µs. The field strength of the proton and spin locking fields during the contact period was 83 kHz. Samples were packed into 4 mm rotors and spun at 10 kHz. Ppm scales were referenced to the high field peak of adamantane (29.5 ppm) run as an external standard under identical conditions to the samples.

Proton decoupling was provided by a Spinal 64 sequence and the proton power levels during the contact time and decoupling stage could be varied independently to provide optimum signal to noise levels. The highest intensity signal for all types of bonded carbons in these carbohydrate materials lay between a contact time of 1 and 2 milliseconds hence for all CPMAS experiments a value of 2msec was used. All experiments were run at room temperature.

Approximately 5K data points were normally recorded. On data processing this data set was zero filled by at least a factor of 2. 15 Hz of Lorentzian line broadening was

then applied. The data set was Fourier transformed and phased with 0 and 1st order corrections. Baseline fitting routines were consistently applied to all spectra.

Commercial samples (see Table 2.3) with different levels of cross-linking and stabilisation were used. The samples were measured at two different moisture contents "as-is" samples with moisture content between 10% and 12% and hydrated samples with a moisture content of 20-25%. The hydrated samples were created by being kept over water until they reached the desired moisture content.

2.4.11.2 ^{31}P- NMR Procedure

The potato starch sample was used without any further preparation at a moisture content of about 17%. Similarly the other sample containing stabilised and crosslinked starch was used without modification (sample 115, 11.5% moisture). Experiments were carried out on a Bruker (Karlsruhe, Germany) Avance III 600MHz spectrometer.

A 2.5 mm magic angle spinning probe operating at a frequency of 242.9 MHz and a spinning speed of 15 kHz was used for the phosphorous measurements. A simple single 90° pulse experiment was used initially with a pulse duration of 3.4 µs and a relaxation delay of 5 seconds. 2048 scans were recorded and summed for the simple single pulse experiment. A dwell time of 12 µs was used with 2048 points being recorded in the FID. Zero filling to 4096 points was applied before Fourier transformation and a line broadening of 150 Hz was applied. Peaks were centred at 0 ppm with no internal or external referencing.

2.4.12 Rheology

The RVA method is designed for samples changing in viscosity as they are heated and cooled and can be subjected to high shear. The results are empirical and depend

on the methods used. An oscillatory test on the starch samples provides more detailed information about the starch solutions under controlled and known conditions, e.g. shear and strain, and the possibility to look at storage and loss modulus.

2.4.12.1 Procedure

A Modular Compact Rheometer (Anton Paar MCR-301, Austria) was operated with parallel plate geometry with a diameter of 50 mm (PP50) or cone-plate geometry with a diameter of 50 mm and 2° angle (CP50/2). The samples were prepared using a standard RVA measurement at various concentrations depending on the solvent (see section 2.4.4). Samples were run in duplicates. A strain sweep between 0.001 and 100% strain was used to test the samples and the linear range was found at a strain of 1%. Immediately after the strain sweep, a frequency sweep was performed between 0.1 and 100 rad/s angular frequency. The sample was kept at 50°C prior to the experiment and loaded at 50 °C. The rheometer was equipped with a temperature cover, which was used to maintain the temperature at 50°C for the duration of the experiments. Mineral oil was used to seal the sample to avoid evaporation.

2.5 Film formation and characterisation

The individual starches characterised with the methods presented in section 2.4 are often used in a combination for various food applications. The focus of this section is to present the methods used to test these blends of starches when they are used in batters. The purpose of batters is to form a film coating a food item.

2.5.1 Film formation

The formation of film from a combination of starches is not trivial and many factors can affect the behaviour of the final film. The individual starches will add their own

properties to the film. The interaction between starches and other components (salt, leavening agents and stabilisers) could further modify the film properties. To try and elucidate the process of film formation several different techniques were tried. These techniques had features in common with the frying process in which a film is formed upon coating a potato chip.

2.5.1.1 Popping machine

To investigate the performance of starches in limited water conditions and at high temperatures a piece of equipment that will be called the popping machine was used (see Figure 2.3). This equipment was designed to allow samples to be heated at controlled temperatures and pressures and to form a film as the starches gelatinise. At the end of the heating cycle the top plate is elevated allowing the sample to expand, i.e. to pop upon release of pressure at high temperatures and the resultant flash off of the water vapour. Both individual starches and batters were tested using this method with the expectation that the individual film forming properties of the starches and the starch blends would differ. The experimental set-up was developed step by step (see chapter 6) and only the final method is described below.

Figure 2.3: The popping machine is able to press samples at about 400 kPa and various temperatures (changeable for upper and lower plate). The machine is manually operated and pressure and pressing time can be adjusted.

2.5.1.1.1 Procedure

A powder sample of 1.5 g ± 1mg was weighed (not corrected for moisture). Moisture of either 10%, 15% or 20% water (i.e. 10% added moisture equals 0.15 g water) was added to a Whatman filter paper (grade 1, diameter 42.5 mm) and placed in a pre-shaped foil-cup. The powder sample was immediately added on top of the moisturised filter paper and a silicon paper (Lakeland, 42.5 mm in diameter) was placed on top. The sample was quickly inserted into the pre-heated probe holder and popped immediately. The settings for the popping machine were as to mimic the frying process during industrial processing. The upper and lower heating blocks were set to a temperature of 185 °C and the pressure was 400-410 kPa. The popping machine was set to automatically release pressure after t = 4.6 seconds and the sample will pop due to water evaporation and starch gelatinisation. The weight of the sample was taken and the sample was evaluated visually. Different parameters were evaluated such as film formation, crumbliness, flow (creep up), thickness and noise/sizzling during the popping process. The sample was stored in a desiccator to control moisture movement.

2.5.1.2 Hot press

In addition to the popping machine sample films were prepared using a hot press. A major advantage of this system would be that multiple samples could be prepared at the same time. Film formation using a hot press will control the temperature at which the film is formed. The hot press also makes it possible to control the cooling rate and maintain the pressure during cooling. A film is therefore formed with the water level initially incorporated.

2.5.1.2.1 Procedure

A hand-operated Moore hydraulic press with heated platens (see Figure 2.5 (De Focatiis and Buckley, 2008)) was used to mould the film under compression. A special mould plate containing nine separate moulds of 55 mm x 55 mm x 0.7 mm were used to create triplicates of films. A 50 % solution of batter in water was prepared prior to the experiment. The final mould set-up consisted of a “sandwich set-up” with an aluminium carrier plate 287 mm x 370 mm x 2.6 mm, an aluminium support plate 225 mm x 225 mm x 2.6 mm, a 225 mm x 225 mm x 0.15 mm aluminium foil (Multifoil Ltd.), a PTFE coated liner 225 mm x 225 x (Magic non-stick liner, Lakeland, Windermere, UK), the mould plate 225 mm x 225 mm x 0.7 mm but with 9 squared holes in them of 55 mm x 55 mm, a Whatman filter paper (grade 1, cut to 55 mm x 55 mm), sample, a PTFE coated liner, the aluminium foil, the support plate and the carrier plate (see Figure 2.4). The 50% starch suspension (4 g ± 0.1 g) was added to the mould and covered by the top layer as described above. The mould was placed in between the pre-heated (185 °C) platens and pressure of 50 bars was applied manually to the platens. The samples were held for 1 minute before cooling down to room temperature at a rate of 10 °C/min by forcing cold water to run through the press platens. The pressure was released and the mould removed when the platens reached a temperature of 20-50 °C.

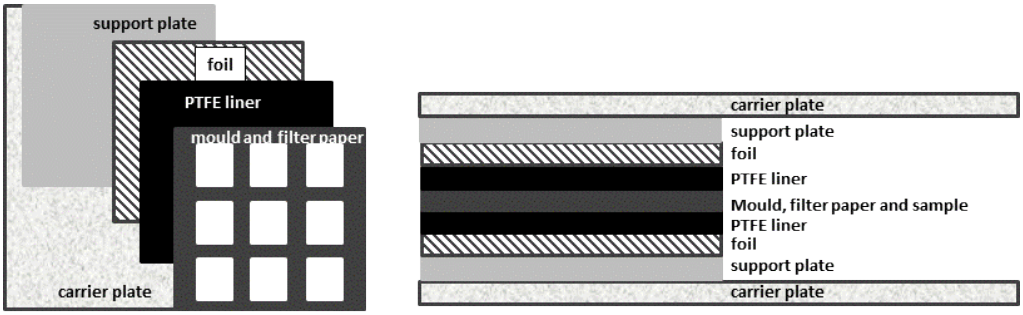


Figure 2.4: A schematic diagram of the "sandwich set-up" used for the hot press experiments. (The measures are not in accordance with the real plates etc.)

Figure 2.5: The Moore hydraulic press based at University Park Campus (University of Nottingham)

2.5.1.3 Frying batters coated on filter paper

The performance of a batter can also be evaluated using a frying process designed by Perumal (Perumal, 2013) to mimic an industrial frying process for battered potato chips. The potato chip is replaced with a filter paper as a base in order to only look at the batter properties, i.e. exclude any variation in the potato chip (e.g. type, moisture content, pre-treatment, composition). Different factors can be determined to evaluate batter properties such as stacking height, wet batter pick up and oil uptake which are defined by the equations given in Table 2.9.

Table 2.9: Equations for batter solid uptake and oil uptake calculated on the basis of the frying process

Equation 1
$$\boldsymbol{Batter\ solid\ uptake}\ (\boldsymbol{g}) = \boldsymbol{Pick\ up}_{\boldsymbol{wet\ batter}}(\boldsymbol{g}) \times \boldsymbol{concentration}_{\boldsymbol{batter\ solution}}(\%) \times \mathbf{0.01}$$

Where $\boldsymbol{Pick\ up_{wet\ batter}(g) = weight\ before_{batter\ solution}(g) - weight\ after_{batter\ solution}(g)}$

Equation 2

$$\boldsymbol{Oil\ uptake\ (g) = weight_{fried\ filter\ paper}(g) - weight_{dry\ filter\ paper}(g) - batter\ solid\ uptake}$$

2.5.1.3.1 Procedure

A 36:64 batter:water (without accounting for the ingredients' moisture) suspension was prepared in a 250 ml pre-weighed glass beaker. The batter solution was mixed vigorously with a plastic spatula and sieved to avoid lumps in the final batter solution. The batter was then stirred using a magnetic stirrer (IKA RCT standard, LLG labware, Germany) for 5 minutes at 800-1000 rpm depending on thickness of the mixture. The dry weight ("as is") of the filter paper was noted before the start of the experiment. The filter paper was wetted with 0.38 ml water using an Eppendorf® Multipipette® Xstream pipette prior to dunking the filter paper into the batter mixture. The filter paper was taken out vertically and allowed to drip for 10 seconds. The battered filter paper was dumped directly into the 180°C deep fryer (Magimix Deep Fryer Pro500 PCR, Magimix UK ltd., Surrey, UK) for 90 seconds. The oil used was standard supermarket sunflower oil (Sainsbury's London, UK). The temperature of the deep fryer was checked regularly with a thermocouple (Elektron Technology, Digitron Instrumentation Ltd., Devon, UK) and the lid was kept on when the fryer was not in use in order to keep the temperature constant at 180°C. Immediately after frying, the weight of the battered filter paper was noted and it was left on blue roll towels to drain. The weight of the batter used was noted (decrease of the weight of beaker and solution) for every sample. After 5 filter papers, the batter solution was stirred with a plastic spatula (and weighed). For each batter 10

replicates were made. After frying the 10 battered filter papers, the papers were stacked in a 50 mm diameter beaker and height was measured. This was repeated 3 times for random orders of the filter paper.

2.5.2 Film Characterisation

The popped films created using the popping machine were mainly evaluated visually, whereas other techniques were used to evaluate the films formed by using the hot press. The films formed using the hot press were similar in thickness (0.7 mm) to the films used for coating potato chips (0.7-0.9 mm). The advantage of being able to control the thickness of the films was that the various thicknesses may be handled and mounted into different equipment for assessment and values should be comparable.

2.5.2.1 Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis (DMA) was carried out on a Dynamic Mechanical Analyser DMA 8000 (Perkin Elmer, USA) equipped with a standard oven for the measurement of temperature scans. The measurement was done at frequencies of 0.1 Hz, 1 Hz, 10 Hz and 30 Hz over a temperature range of -70 °C to 180 °C and at a heating rate of 3 °C/min. Powders were measured using the material pockets technique (Paes, 2010, Raschip et al., 2008, Royall et al., 2005, Mahlin et al., 2009) in single cantilever geometry. The powders were adjusted to the desired moisture content by holding them over a saturated sodium chloride solution (RH is 75.6% at 30.25 °C (Wexler, 1954)) for at least 7 days at 30°C. The powder was distributed evenly within the pocket (Mettler-Toledo, Leicester, UK) and the pocket folded shut and secured by bending over the clips on the pocket. The use of the powder pockets was required in order to determine the glass transition temperature of the samples due to difficulties in forming a well-defined geometrical shape of the material. The

aluminium plates forming the pockets allowed the material to be sheared and hence transition temperatures could be measured at different frequencies. However the sample moduli (E_0) were only relative due to the dominant contribution from the stiff plates forming the pocket.

2.5.2.2 Differential Scanning Calorimetry (glass transition)

The glass transition temperature can also be measured using differential scanning calorimetry (section 2.4.5). A DSC 823^e (Mettler-Toledo, Leicester, UK) was used to measure the glass transition temperature over the temperature range -50 °C to 200 °C. The first heating scan was used to measure the melting of the starch helices. The second run (immediately after the first run) provide information on the glass transition temperature. The samples were prepared concomitant with the samples for DMA holding them over saturated sodium chloride solution (RH 75.6%) for at least 7 days at 30°C. Approximately 10 mg ± 1mg sample was weighed into a stainless steel DSC pan and sealed using a manual press. The Mettler-Toledo Star^e software was used to calculate the enthalpies and glass transition temperatures (T_g).

2.5.2.3 Moisture content and water activity (a_w)

The preparation of samples for the DMTA and DSC analysis lead to an investigation of moisture content using two different methods; a manual method where the sample was placed over a saturated sodium chloride solution (same water activity) and by using dynamic vapour sorption to determine the absorption and desorption profiles (section 2.5.2.4).

2.5.2.3.1 Procedure

The samples were kept over the saturated sodium chloride solution (RH 75.6% at 30.25 °C) in a closed plastic container in order to create an environment with the

same water activity for all samples. The samples were stored for 7 days prior to DSC analysis and TGA, which was carried out on the same day. The moisture content in the DSC pans was determined by drying the samples in an oven set at 105 °C and weighing the samples after 24 hours, 96 hours and 168 hours. The values for moisture contents measured by TGA and after DSC were similar. However the moisture contents for the samples were different at the same water activity (see chapter 5). This was probed by evaluating individual ingredients over saturated sodium chloride and by weighing the samples before and after storage for 7 days.

2.5.2.4 Dynamic vapour sorption (DVS)

The dynamic vapour sorption method can be used to examine either absorptive or desorptive water transfer in materials i.e. moisture uptake or moisture loss. Moisture content and transfer are crucial in many food applications as they determine product crispness and the maintenance of crispness in many foods, such as potato chips.

A Dynamic Vapour Sorption instrument (Model 1, Surface Measurement Systems UK Ltd., London, UK) using an integral Cahn digital recording balance D-200 (Cahn Instruments Inc., California, USA) was used to obtain sorption profiles over a range of relative humidity values from 0% to 95% RH in steps of 10% RH at a constant temperature of 25 °C. The method used to collect the data was a DVS sorption automatic operation (SAO) which was set up to the desired stepwise relative humidity profile. An RH step was considered to have reached equilibrium when either a specified time had been reached or the rate of change of mass had fallen below a specified value. A sample of around 10 ± 1 mg material was pre-weighed and placed carefully in a quartz round-bottomed DVS sample pan and inserted into the temperature-controlled air chamber. The method was set up to initially dry the

sample hence reaching a relative humidity of 0% RH with an equilibrium criterion of a change in mass over time (d_m/d_t) of > 0.005 % min^{-1} for at least 5 consecutive minutes or after 300 minutes. When the equilibrium criterion was fulfilled, a stepwise increase in relative humidity was initiated going from 0% RH to 95% RH increasing in steps of 10% RH, the final step being from 80% RH to 95% RH. After reaching 95% RH the relative humidity was stepwise decreased in steps of 10% RH with the d_m/d_t criterion being 0.005% min^{-1} for 5 consecutive minutes or alternatively after 300 minutes. The data points were collected automatically every 20 seconds and samples were run in duplicates. The total gas flow (N_2) was kept constant at 200 cm^3min^{-1} for all experiments. Using the data collected, the moisture content (change in mass) was plotted against relative humidity and the isotherm profiles obtained. The data was also fitted using the Guggenheim-Anderson-de Boer (GAB) equation (see chapter 5) but due to limitation of the data set, no statistical analysis was conducted.

CHAPTER 3 CHARACTERISATION OF MODIFIED STARCHES WITH REGARD TO THEIR POTENTIAL AS FILM FORMERS

3.1 Starches as potential film formers

Although the primary structures of starch are based on simple glucose units, the higher orders of structure, minor non-carbohydrate components associated with the starch granule and the incorporation of functional groups onto the starch macromolecules can lead to starches having different functionalities. The choice of starch will be dependent on the desired property. Nature has created a range of starches that vary in their granule morphology, growth patterns, amylose/amylopectin ratio, lipid and protein contents therefore any individual native starch possesses unique properties. In addition to these native starches modified starches are available to the industry. The modified starches are native starches which have been physically or chemically altered from their native origin. The modification will depend on the desired property and a broad spectrum of modified starches is readily available for the food industry today. Information on the structures of the starch and the starch modifications permitted for food applications are given in section 1.2.1.2 and 1.2.1.5 respectively.

The work described in this thesis will focus on commercial starches that have been identified as being useful in blends of materials to be used as film formers in food. Understanding the behaviour of the blend of starches contained in a batter is a very complex task due to possible interactions between starches (or carbohydrate components) and other components such as salts, leavening agents and stabilisers if present. The approach for this study was to examine the individual starches and their properties in order to map their behaviour as potential film formers. The

objective was to use this knowledge to enable predictions of the quality of the coatings when formed from a mixture of starches.

The properties of native starches for batters was investigated by Rajiv Perumal in his thesis *Mechanistic understanding of starch components used in potato chip batters* (Perumal, 2013). Therefore in this chapter the modified starches were addressed. Although the key feature of the batters may be their behaviour at low water contents, an understanding of the starches' behaviour in excess water can indicate their potential as film formers. For the current work therefore a range of modified starches (Table 2.3, see also fold-out-table after the bibliography) were characterised in excess water to assess their properties. A group of commercially available stabilised tapioca starches, with reported increasing levels of cross linking, were used to try to clarify the importance of the level of modification on the starches' functionality.

3.2 Standard characterisation of starches

Characterisation of starches can involve various measures that reflect different aspects of the chemical and physicochemical behaviour. A small range of techniques were used to bench mark the behaviour of 15 starch based materials supplied by a company who had been using these in the formulation of batters.

3.2.1 Rapid visco analysis

The pasting properties of starches, which reflect gelatinisation, swelling and breakdown of the starches, is said to act as a fingerprint for these materials. To investigate the starches in a typical high water and high temperature environment, the starches were pasted in a RVA (Rapid Visco Analyser). Details of the equipment and method are given in 2.4.4. The pasting profiles at 14.3% (wb) starch in water are given in Figure 3.1 to Figure 3.3, note that the viscosity scales are not the same for

all the graphs. The pasting profiles were generated in excess water and if a native starch was pasted using the standard profile (see section 2.4.4) then a maximum peak viscosity, a pasting temperature and an end viscosity could be determined as values to explain the pasting properties of the starches. In Figure 3.1 to Figure 3.3 it is seen that not all the starches give the characteristic native starch pasting profile. Samples 107 and 108 showed low viscosity values with almost a horizontal line keeping the same viscosity throughout the pasting profile and sample 101 only showed a very low peak viscosity. It would appear that these starches were modified so heavily that the swelling capacity of the starch granule was lost. As a result the starch granule could have lost its swelling power and no longer has the pasting properties that are characteristic of native starches with typical amylopectin levels.

For other starches such as 104, 106 and 110-111 the starches swelled to a high level resulting in a very high peak and end viscosity. The viscosity curves were often jagged as the samples were in the cooling phase of the profile. This indicated inconsistencies in the paste as the paddle tried to cut through the thick gelling paste. Modification of the starches could lead to different results for the pasting profiles and the difference in pasting properties can indicate if the starch granules were still intact after modification. If the granule integrity was still intact, including the crystalline regions within the starch granule, but pores had been opened up due to enzyme attack (Gallant et al., 1992, Rocha et al., 2010) or other chemical treatment such as acid treatment (Marlida et al., 2007, Yiu et al., 2008), it would have been easier for the water to enter the starch granule. This would facilitate swelling and allowing the amylose/amylopectin to leach which would result in higher viscosities. However if the amylose/amylopectin components were hydrolysed into sugars, e.g. dextrin, very low viscosity (Figure 3.1- Figure 3.2, sample 107 and 108 respectively)

or decreased viscosity would develop upon pasting (Kaur et al., 2011). Every modification would change the pasting behaviour of the starches, but some to a higher extent than others. Stabilisation with propylene oxide disrupted inter- and intramolecular hydrogen bonds in the starch polymers resulting in weakening of the granular structure and therefore increased the acceptability of the starch to water (increased hydration). This most often led to decrease in the pasting temperature and increase in the peak viscosity, however the viscosity development was said to be dependent on the degree of stabilisation (Pal et al., 2002).

Crosslinking of the starch with different reagents e.g. phosphorous oxychloride or mixtures of sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) (Wongsagonsup et al., 2014) could lead to increase in the peak viscosity compared to the native starch. This is due to crosslinking between the amylose/amylopectin polymers which strengthens and stabilises the starch granular structure (Jyothi et al., 2006, Liu et al., 1999, Wongsagonsup et al., 2014). The modified starches 101-104 and 106-116 were all modified differently or to a different level, so they were not directly comparable. However samples 112, 116, 106, 111 and 115 were reported to be stabilised with propylene oxide (to the same level) and 116, 106, 111 and 115 had been crosslinked with phosphorous oxychloride at increasing levels.

Figure 3.5 and Figure 3.6 compare the pasting profiles for the group of tapioca starches pasted at two different concentrations (13.4% and 7.1% respectively). The order of the peak and end viscosities surprisingly did not comply with the order of increasing crosslinking (112< 116< 106< 111< 115) for either of the concentrations. Further the rank order of the peak viscosities for the starches also changed when decreasing the concentration of starch being 130< 112< 115< 116< 116< 111 and 111< 106< 130< 111< 112< 116 for 14.3% and 7.1% starch respectively.

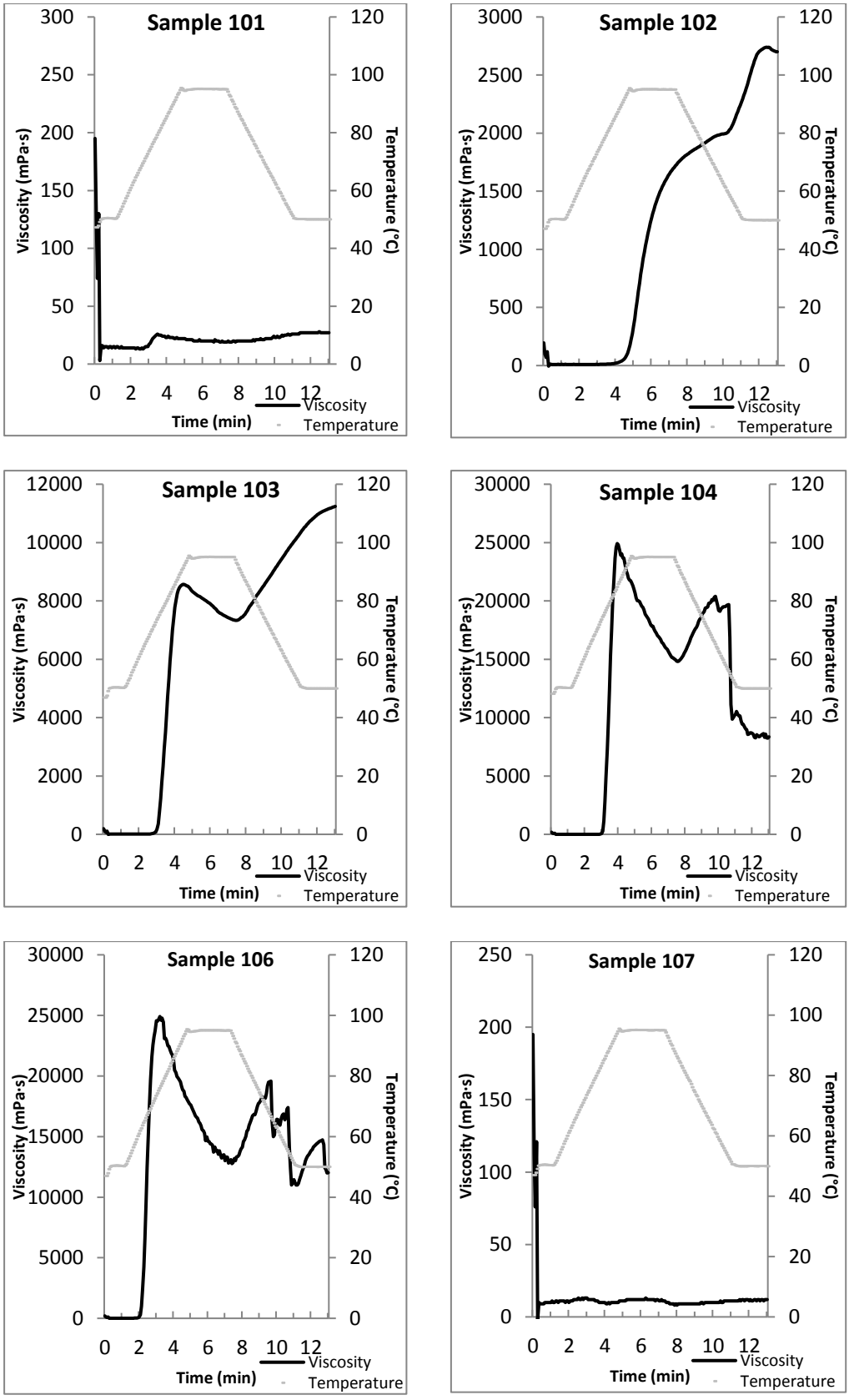


Figure 3.1: The RVA curves for sample 101-104 and 106-107 at a concentration of 14.3% starch in water.

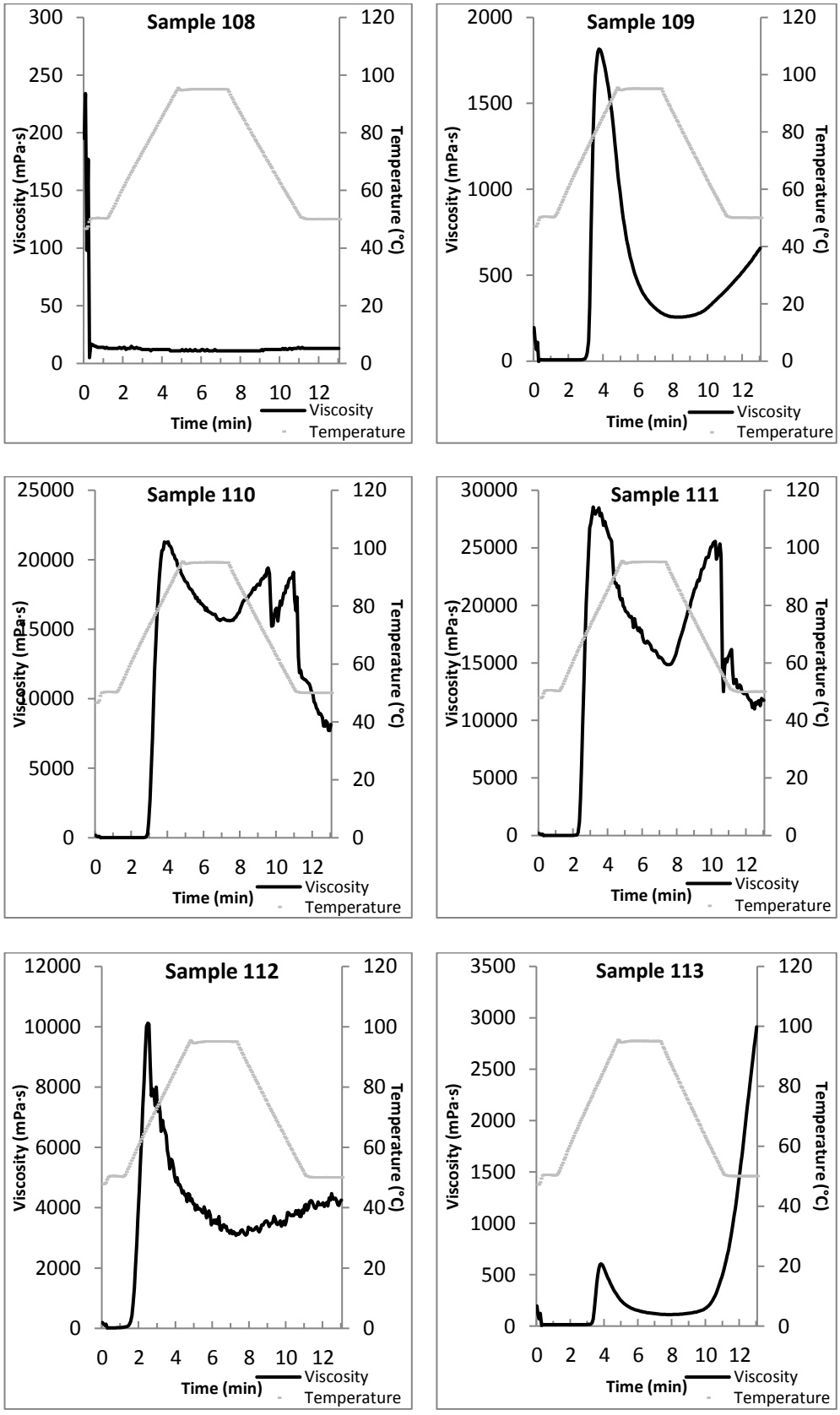


Figure 3.2: The RVA curves for sample 108-113 at a concentration of 14.3% starch in water.

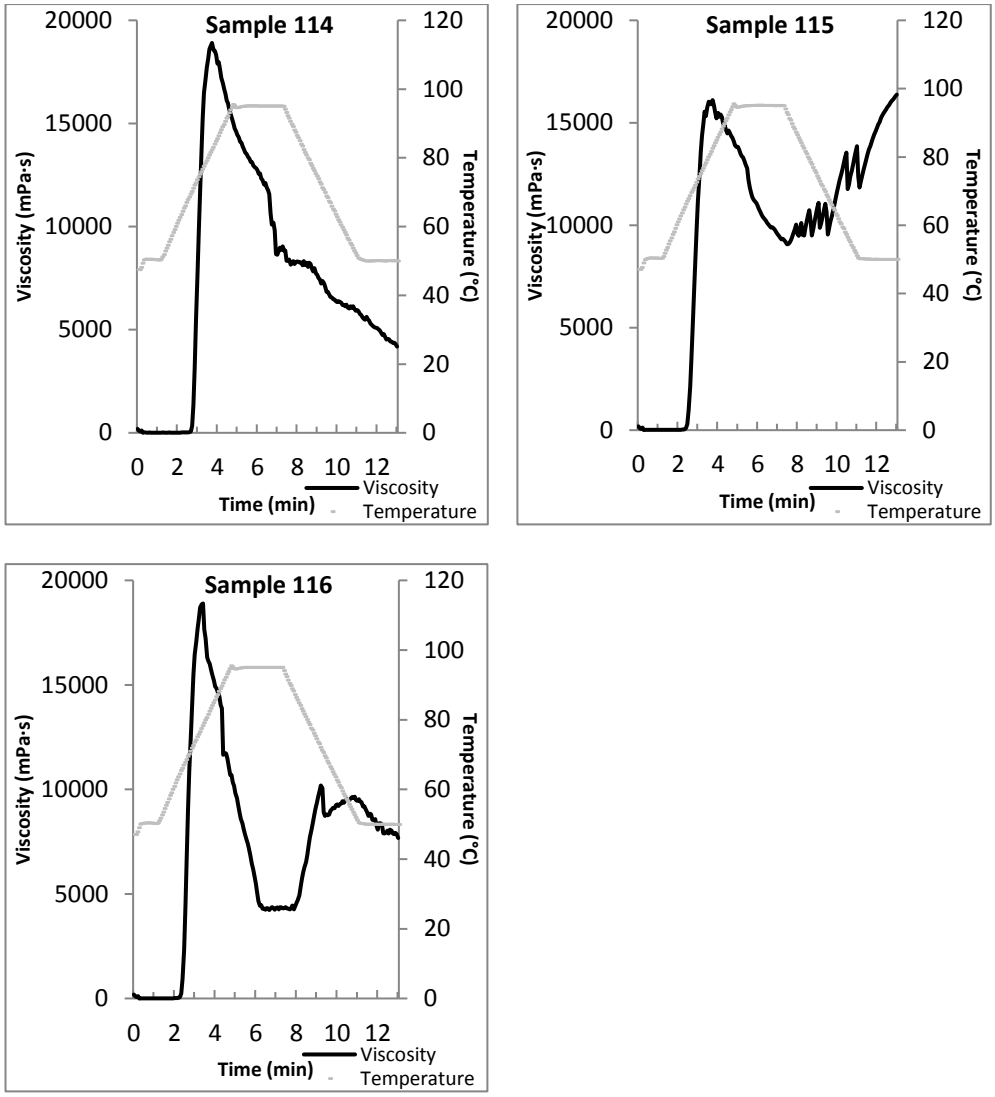


Figure 3.3: The RVA curves for sample 114-116 at a concentration of 14.3% starch in water.

The results observed show the complex nature of the relationships between the different facets of the pasting curve and the problems of understanding the modified starches better. The pasting curve may be dominated by the swelling and disruption of the granules and therefore probing their behaviour in a low water content system, which is covered in chapter 4, may show very different findings. The pasting profiles can be better discussed when other characteristics of the starches are known and therefore they will be discussed in more details at the end of this chapter.

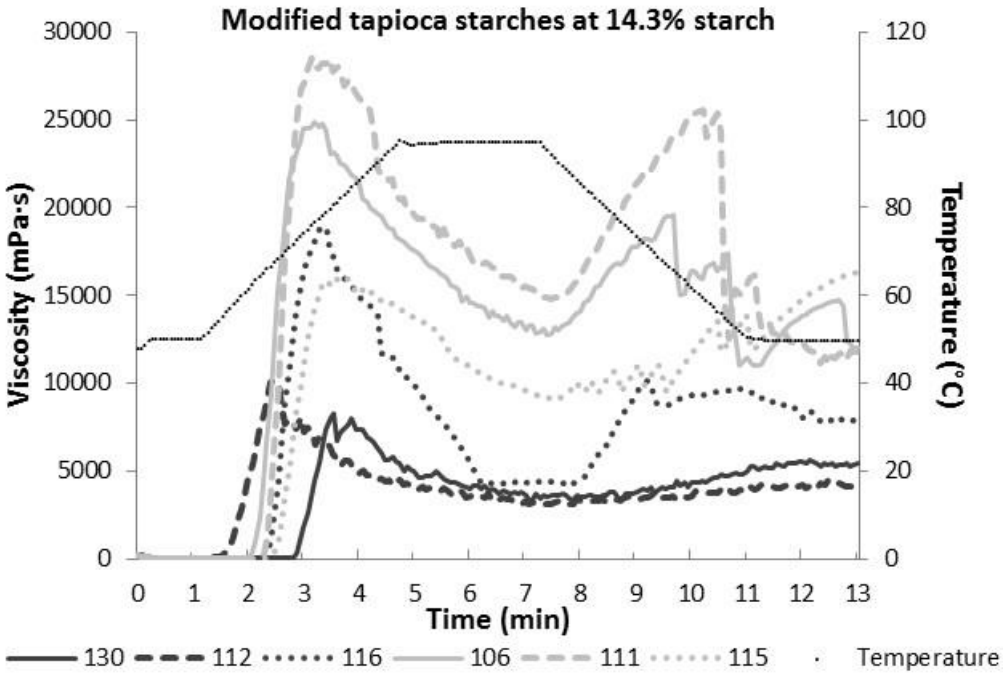


Figure 3.4: The RVA curves for the tapioca group at a concentration of 14.3% starch in water.

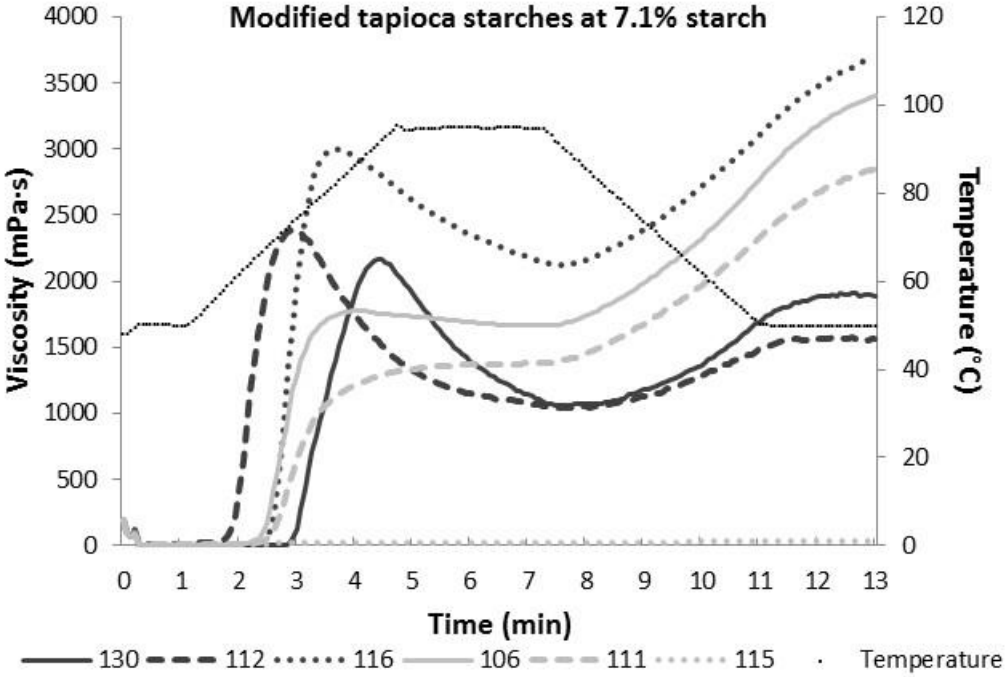


Figure 3.5: The RVA curves for the tapioca group at a concentration of 7.1% starch in water.

3.2.2 Swelling power

The granules ability to swell during heating and limited shearing was tested using the swelling power assay (see section 2.4.2.1). Figure 3.6 shows the swelling power

for the modified starches (101-104, 106-116). Five samples (101-103, 107-108) obtained low swelling power values in the range of 4.4 $g \cdot g^{-1}$ - 10.9 $g \cdot g^{-1}$ where the lowest values were for the two dextrins (107-108) and the crosslinked OSA waxy maize starch (101).

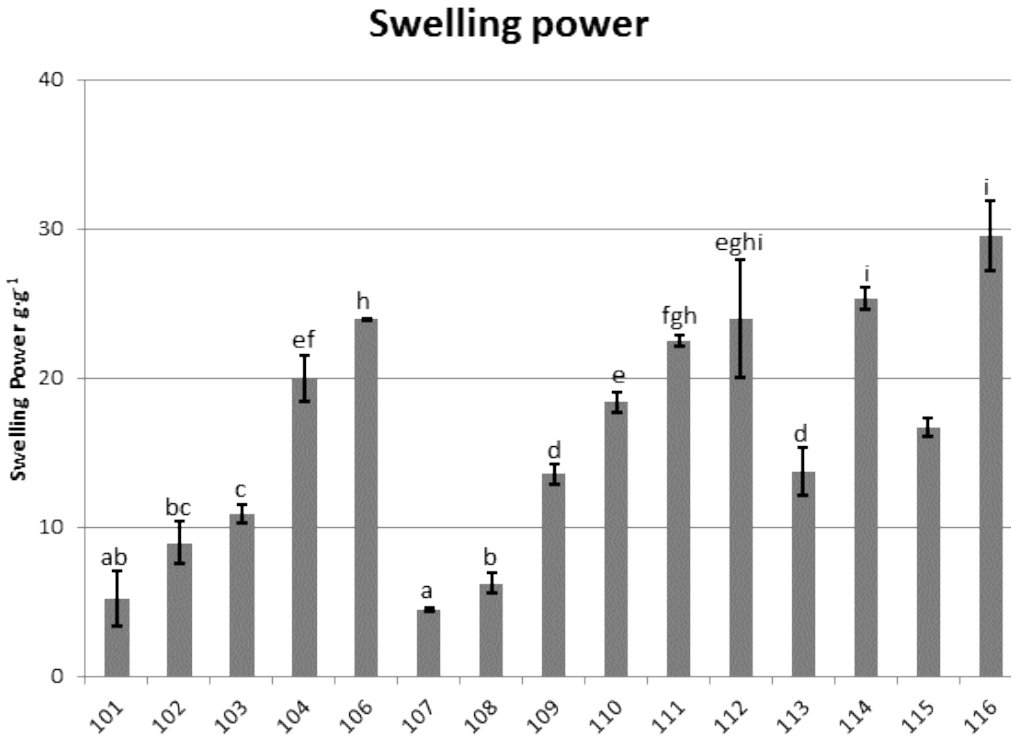


Figure 3.6: The average swelling power of samples 101-104 and 106-116. The error bars represent the standard deviation of three replicates (n=3). The column marked with the same letter showed no significant difference (P>0.05) in t-test.

The higher swelling power values between 20.0 $g \cdot g^{-1}$ - 29.5 $g \cdot g^{-1}$ were observed mainly for the modified tapioca starches (106, 111-112, 114 and 116) as well as the crosslinked waxy maize starch (104). The stabilised and highly crosslinked tapioca starch (115) obtained a slightly lower swelling power of 16.6 $\cdot g^{-1}$ compared to the other modified tapioca starches. The acid treated maize and sago starches (109 and 113 respectively) had similar swelling power values of 13.5 $g \cdot g^{-1}$ and 13.7 $g \cdot g^{-1}$ respectively.

Stirred and non- sheared swelling could be compared directly by plotting the RVA peak viscosities against the swelling power (see Figure 3.7). As seen in Figure 3.7 there was a weak correlation between the swelling power and the RVA peak viscosities. Samples with very low swelling power (<15 $g \cdot g^{-1}$) consistently developed a low peak viscosity (<1820 mPa·s, except sample 103 with a peak viscosity of 8566 mPa·s). At higher swelling power (>15 $g \cdot g^{-1}$) the peak viscosities were very variable between 10000 and 30000 mPa·s. The main difference in methods between measurements of the swelling power and RVA was the shearing of the sample (see section 2.4.2 and 2.4.4). Thus the constant shearing in the RVA measurement (160 rpm) was important to develop viscosity especially for the tapioca starches (111-112, 114-116, see Figure 3.7) and this was not transferable to the non-sheared swelling power.

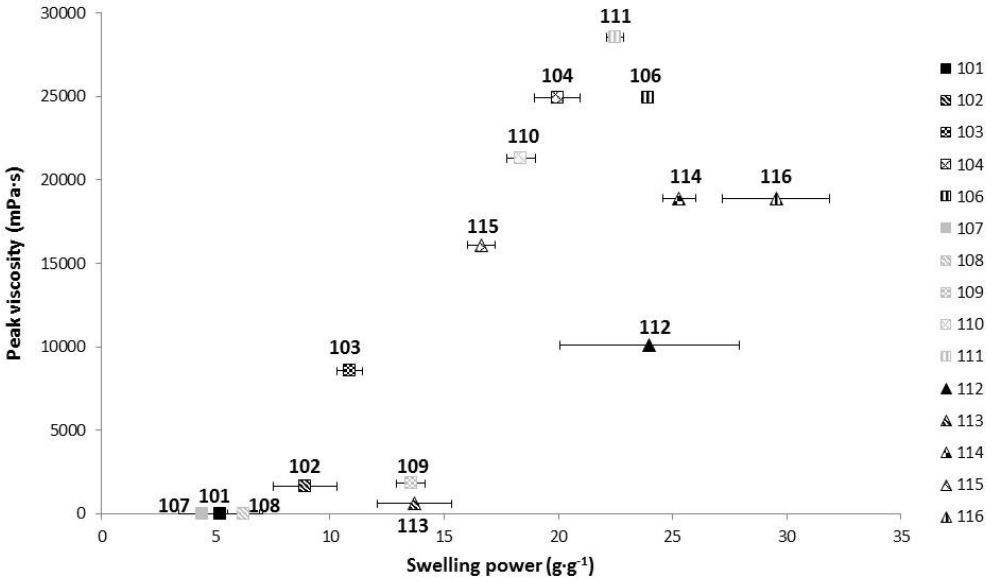
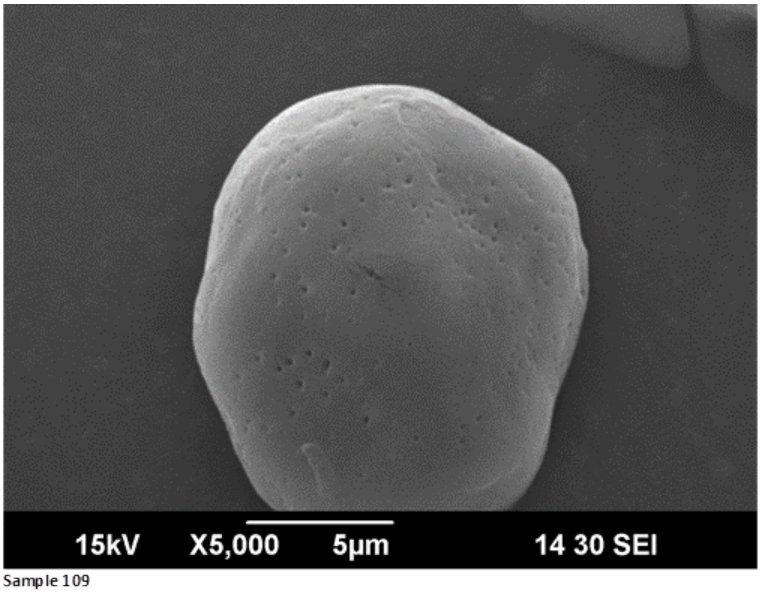


Figure 3.7: The swelling power versus the peak viscosity (RVA) of samples 101-104 + 106-116. Error bars on swelling power denote the standard deviation of three replicates (n = 3).

3.2.3 Scanning electron microscopy

The technique of scanning electron microscopy can visualise the condition of the starch granular structure. Damage or alteration on the outside of the granule can be seen. Figure 3.9 shows SEM images on a x500 magnification of the starch granules.

Whereas Figure 3.10 shows a x2500 magnification in order to see the details of the effect on the starch granule which may be caused by the modification treatment. Starch samples 101-102, 104 and 109 were all modified maize starch samples and hence the sizes of the granules were similar. Samples 101 and 104 appeared to have more squared granule shapes whereas samples 102 and 109 had more circular shapes of the granule. There was some damage on the surface of the granules that was most pronounced for samples 101 and 104. The granules of samples 101-102 and 109 seemed to cluster together. Samples 101 and 104 were OSA waxy maize and crosslinked waxy maize respectively, whereas sample 102 was stabilised high amylose maize and sample 109 acid treated maize. For the acid treated maize (109) high magnification images (x5000, see Figure 3.8) showed punctures in the granule surface. This indicated that the harsh acid treatment had formed pores or channels in the granular surface.



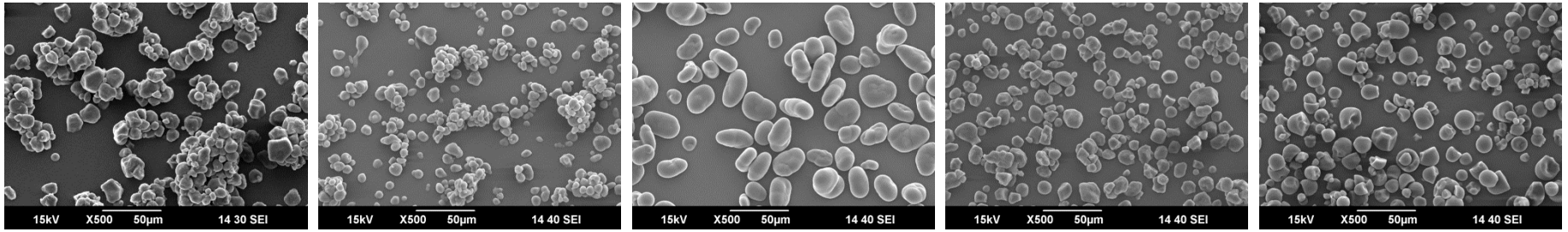
Sample 109

Figure 3.8: Scanning electron images of the modified starch samples 109 (x5000)

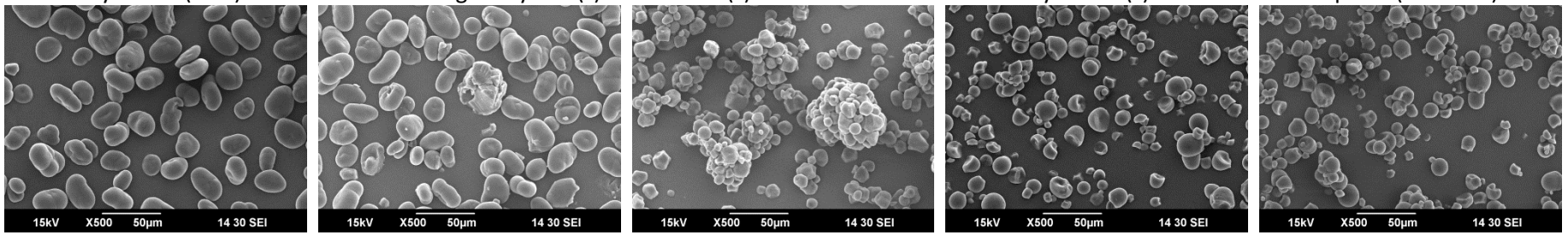
The three samples 103, 107 and 108 were all pea starch samples, where sample 103 was crosslinked with phosphorous oxychloride and samples 107 and 108 were low and high converted dextrinised starch respectively. It is known that the morphology

of the pea granules will vary depending on the genetic origin of the pea starch (Ratnayake et al., 2002). From the SEM images (see Figure 3.10) all pea samples (103, 107-108) did resemble the wild pea type morphology. The average size of the particles looked larger than some of the other starches. The SEM image for sample 113 (acid treated sago starch) showed larger elongated granules with some interruption leading to sharp edges or cavities in the granule caused by the acid treatment. Starch samples 106, 110-112 and 114-116 were all tapioca starches and further they were crosslinked with phosphorous oxychloride, but not all of them were stabilised. The SEM images of the modified tapioca starches were similar visualising truncated granules which has previously been reported for native tapioca starches (Zhu, 2015).

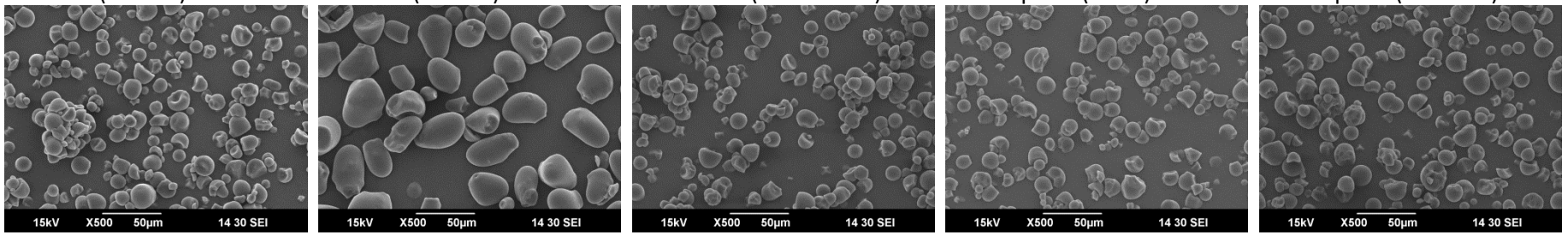
As visualised by the SEM images, the granular sizes of the various starches were different dependent on their type of origin which can also be determined by particle size distribution.



101: Waxy maize (OSA) | 102: Maize high amylose (s) | 103: Pea (s) | 104: Waxy maize (s) | 106: Tapioca (s + x-link)

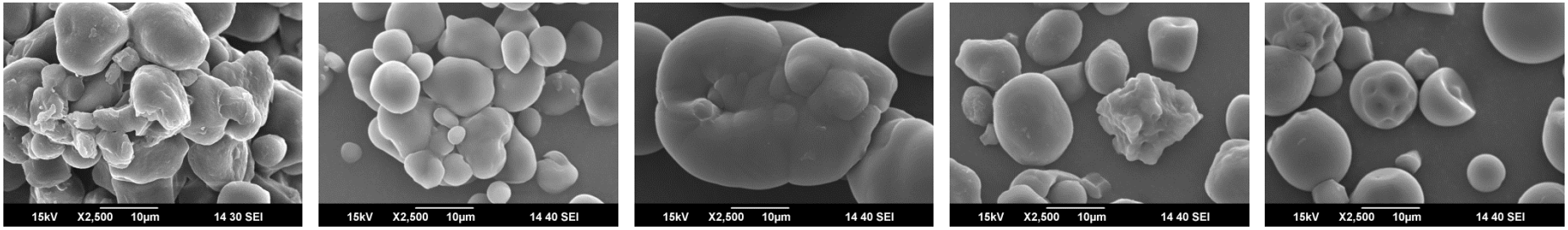


107: Pea (dextrin) | 108: Pea (dextrin) | 109: Maize (HCl treated) | 110: Tapioca (x-link) | 111: Tapioca (s + x-link)



112: Tapioca (s) | 113: Sago (HCl treated) | 114: Tapioca (x-link) | 115: Tapioca (s + x-link) | 116: Tapioca (s + x-link)

Figure 3.9: Scanning electron microscopy images of the modified starch samples 101-104 and 106-116 (x500). s denotes stabilised and x-link denotes crosslinked starches (for more details, see fold-out table after the bibliography).



101: Waxy maize (OSA) | 102: Maize high amylose (s) | 103: Pea (s) | 104: Waxy maize (s) | 106: Tapioca (s + x-link)

107: Pea (dextrin) | 108: Pea (dextrin) | 109: Maize (HCl treated) | 110: Tapioca (x-link) | 111: Tapioca (s + x-link)

112: Tapioca (s) | 113: Sago (HCl treated) | 114: Tapioca (x-link) | 115: Tapioca (s + x-link) | 116: Tapioca (s + x-link)

Figure 3.10: Scanning electron microscopy images of the modified starch samples 101-104 and 106-116 (x2.500). s denotes stabilised and x-link denotes crosslinked starches (for more details, see fold-out table after the bibliography).

3.2.4 Particle size distribution

Table 3.1 shows the mean values and percentage distributions of the particle sizes for the starches (for method see section 2.4.9). The modified maize samples (101-102, 104 and 109) had a fairly wide range of mean diameters from 18-68 µm. The OSA crosslinked waxy maize (101) was very different from the others with a large mean diameter of 67.8 ± 0.7 µm. The other crosslinked waxy maize (104) had a mean diameter of 18.74 ± 0.04 µm and the stabilised high amylose maize (102) and acid treated maize (109) had mean values of 27.6 ± 0.1 µm and 27.3 ± 0.3 µm respectively.

The high mean diameter for the OSA crosslinked maize starch (101) could have been caused by clustering of the granules, which was also seen on the SEM images (Figure 3.9). For the modified pea starch samples 103, 107-108 the two dextrins (107 and 108) both had high mean values of 67.2 ± 0.6 µm and 59.8 ± 0.8 µm respectively whereas the crosslinked pea starch only had a mean value of 26.4 ± 0.1 µm. The mean value for the yellow pea starch has previously been reported to be 33.8 µm (Huang et al., 2007), which suggests that the dextrins cluster together, even in the dry state, and therefore resulted in a high mean diameter (volume) for the particles formed. The acid treated sago starch (113) had a volume mean diameter of 31.9 ± 0.2 µm, which was similar to non-modified sago starch at different growth stages 26.8-33.3 µm (Uthumporn et al., 2014) thus suggesting that the mild treatment with acid did not have a marked impact on the starch morphology.

The modified tapioca starches (106, 110-112, 114-116) had a volume mean value in the range of 14.9-19.4 µm which indicated that the stabilisation and crosslinking with phosphorous oxychloride did not affect the size of the starch granule (Zhu, 2015). The morphology of the modified tapioca starches also appeared to be

unaffected by the stabilisation and crosslinking treatment since it has previously been reported that tapioca granules can possess a somewhat truncated and sharp edged granular type structure (Zhu, 2015).

Table 3.1: The data for the average mean value, mode value (maximum peak in the distribution) and the diameter where 10%, 50% and 90% of the population are below this value (d(10), d(50) and d(90) respectively). SD standard deviation.

Sample	Mean (µm)	S.D (µm)	Mode (µm)	d(10) (µm)	d(50) (µm)	d(90) (µm)
Sample 101 av	67.75	51.44	55.14	15.28	53.24	144.78
SD average	0.73	0.83	0.00	0.08	0.57	1.11
Sample 102 av	27.58	27.03	10.29	7.12	18.39	57.12
SD average	0.14	0.09	0.00	0.05	0.13	0.48
Sample 103 av	26.35	9.41	26.15	16.01	24.98	37.99
SD average	0.11	0.09	0.00	0.03	0.08	0.39
Sample 104 av	18.74	11.10	13.61	8.83	15.24	34.84
SD average	0.04	0.03	0.00	0.05	0.03	0.06
Sample 106 av	15.55	8.04	14.94	7.15	14.71	25.23
SD average	0.59	0.98	0.00	0.20	0.15	1.14
Sample 107 av	75.44	100.67	26.15	15.93	27.73	233.70
SD average	14.37	31.67	0.00	0.07	0.50	32.82
Sample 107 (1-2)	67.15	82.39	26.15	15.91	27.45	214.76
SD average	0.62	0.38	0.00	0.07	0.18	1.42
Sample 108 av	59.840	71.216	26.145	15.582	27.175	183.264
SD average	0.797	0.908	0.000	0.042	0.081	3.415
Sample 109 av	27.29	21.27	14.94	10.22	19.99	51.26
SD average	0.32	0.58	0.00	0.10	0.35	0.41
Sample 110 av	14.97	7.30	14.94	7.25	14.32	23.68
SD average	0.42	0.67	0.00	0.12	0.16	0.87
Sample 111 av	14.88	7.56	14.94	7.01	14.10	23.84
SD average	0.20	0.03	0.00	0.36	0.12	0.12
Sample 112 av	19.42	11.22	16.40	8.66	16.66	35.12
SD average	0.20	0.07	0.00	0.15	0.15	0.50
Sample 113 av	31.91	8.00	34.59	21.92	31.87	42.51
SD average	0.15	0.05	0.00	0.25	0.13	0.10
Sample 114 av	17.55	9.93	14.94	8.36	15.43	30.48
SD average	0.21	0.09	0.00	0.17	0.12	0.37
Sample 115 av	14.96	6.86	14.94	7.53	14.51	23.11
SD average	0.24	0.39	0.00	0.11	0.10	0.40
Sample 116 av	15.20	6.44	16.40	7.73	15.17	23.28
SD average	0.22	0.06	0.00	0.38	0.16	0.36

Another method to explore granular integrity is by using polarised light microscopy and observing the Maltese cross, which indicates crystallinity in the starch granule due to the polymer packing causing birefringence.

3.2.5 Simple and polarised light microscopy

Figure 3.11 toFigure 3.14 present the microscopic images using simple and polarised light to observe the modified starches. All modified maize samples (101-102, 104 and 109) showed the Maltese cross and hence their crystalline regions were still intact despite the modifications. For the modified pea starches (103, 107-108), the crosslinked pea starch (103) and the low converted dextrin (107), the images showed damaged/altered Maltese crosses. For the high converted pea dextrin (108) there were only opaque particles observable by light microscope and polarised light only indicated some crystallinity but no pattern. Hence the integrity of the pea starch had been lost during the high conversion of the pea starch into the pea dextrin (108). The acid treated sago starch (113) did retain the crystallinity as well thus showing the Maltese cross using polarised light.

For the modified tapioca starches (106, 110-112 and 114-116) the characteristic Maltese crosses were observed for them all. Therefore they were thought of as having retained the crystalline pattern that occurred in the native samples. Having observed that the modification process could change some important features of the starch, such as the starch granular structure, it was relevant to determine if the total starch levels, as assessed by commonly used methods, in these modified starches were influenced by the modification.

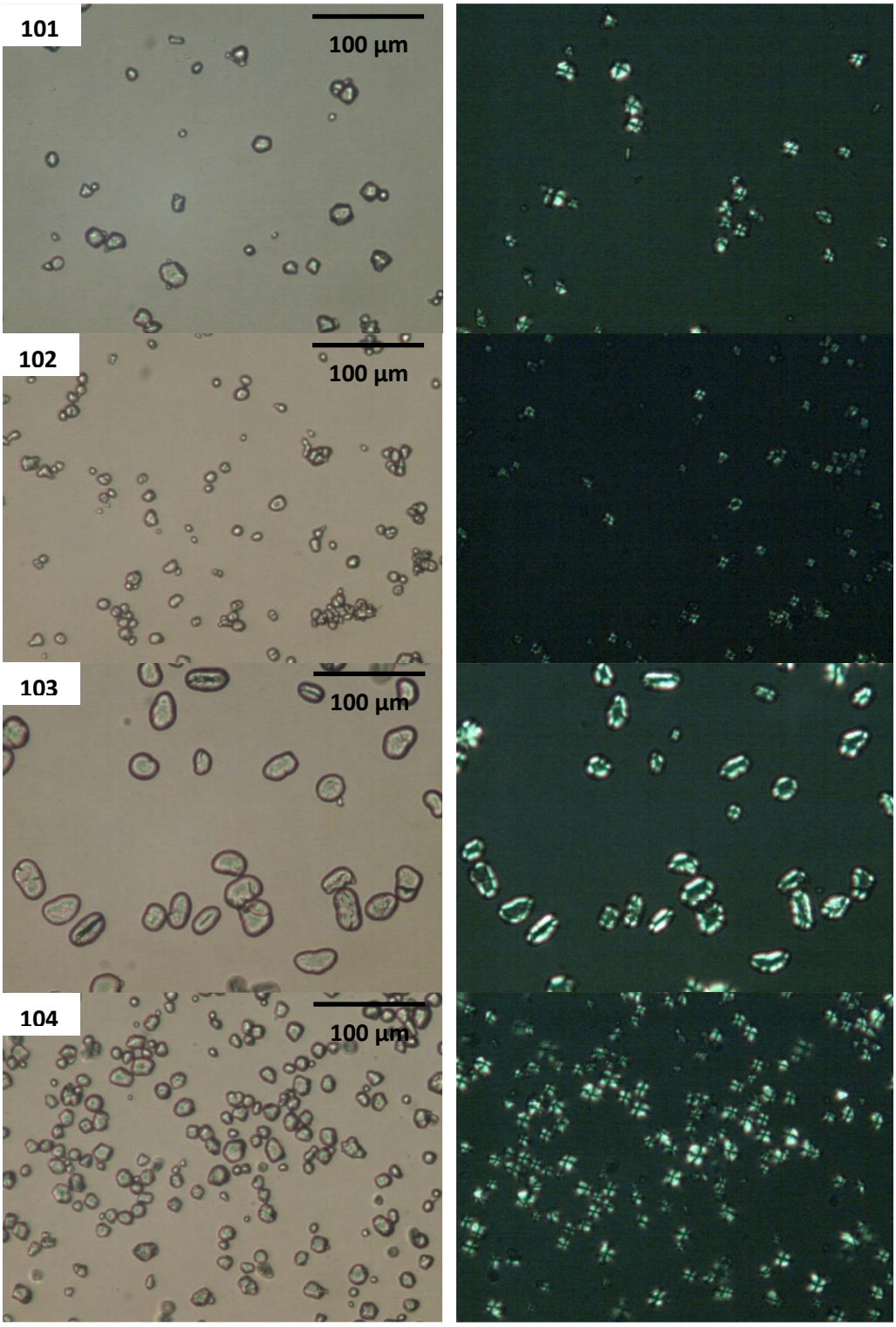


Figure 3.11: Simple light microscopy images for samples 101-104. Right hand side shows the polarised pictures indicating the Maltese crosses.

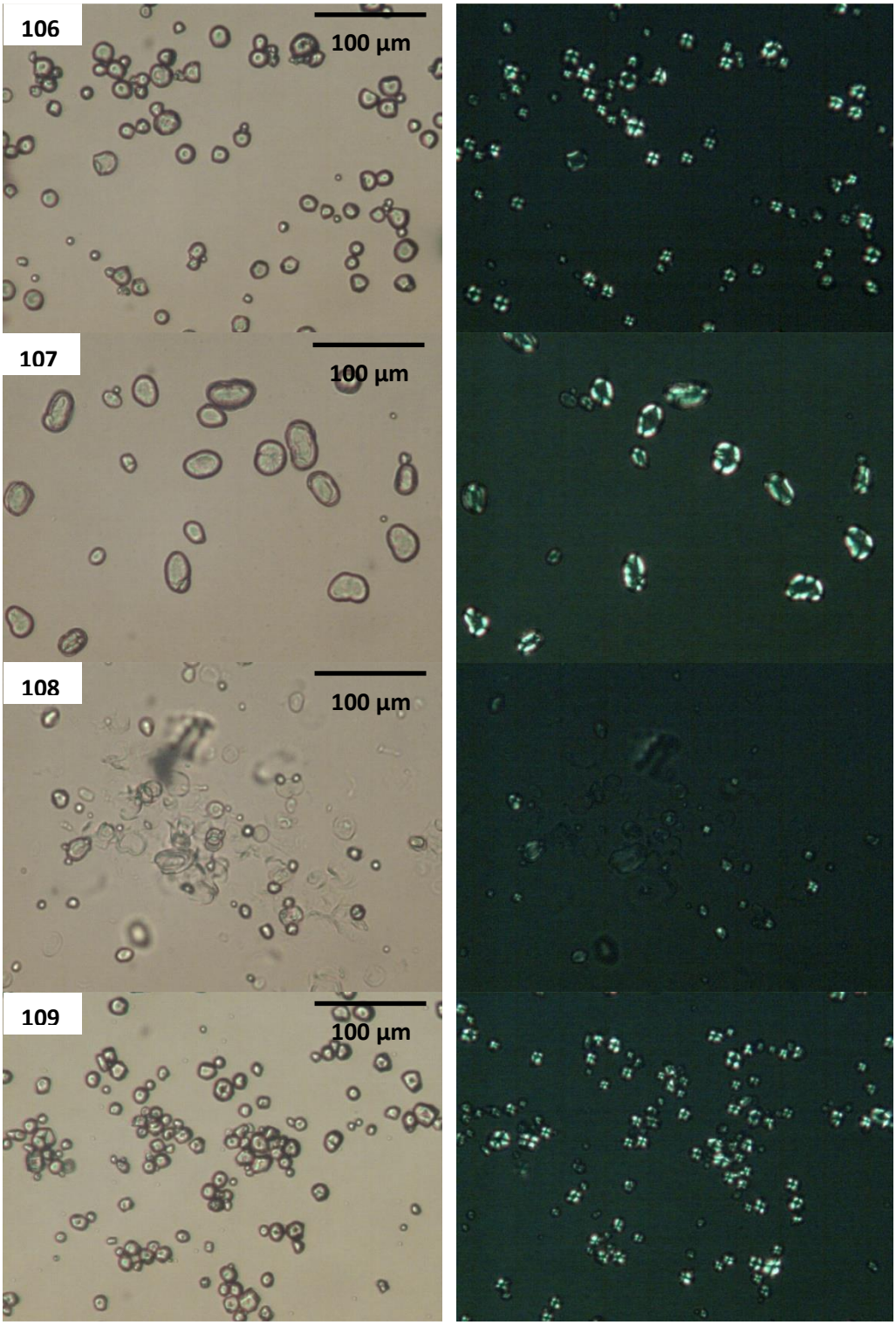


Figure 3.12: Simple light microscopy images for samples 106-109. Right hand side shows the polarised pictures indicating the Maltese crosses.

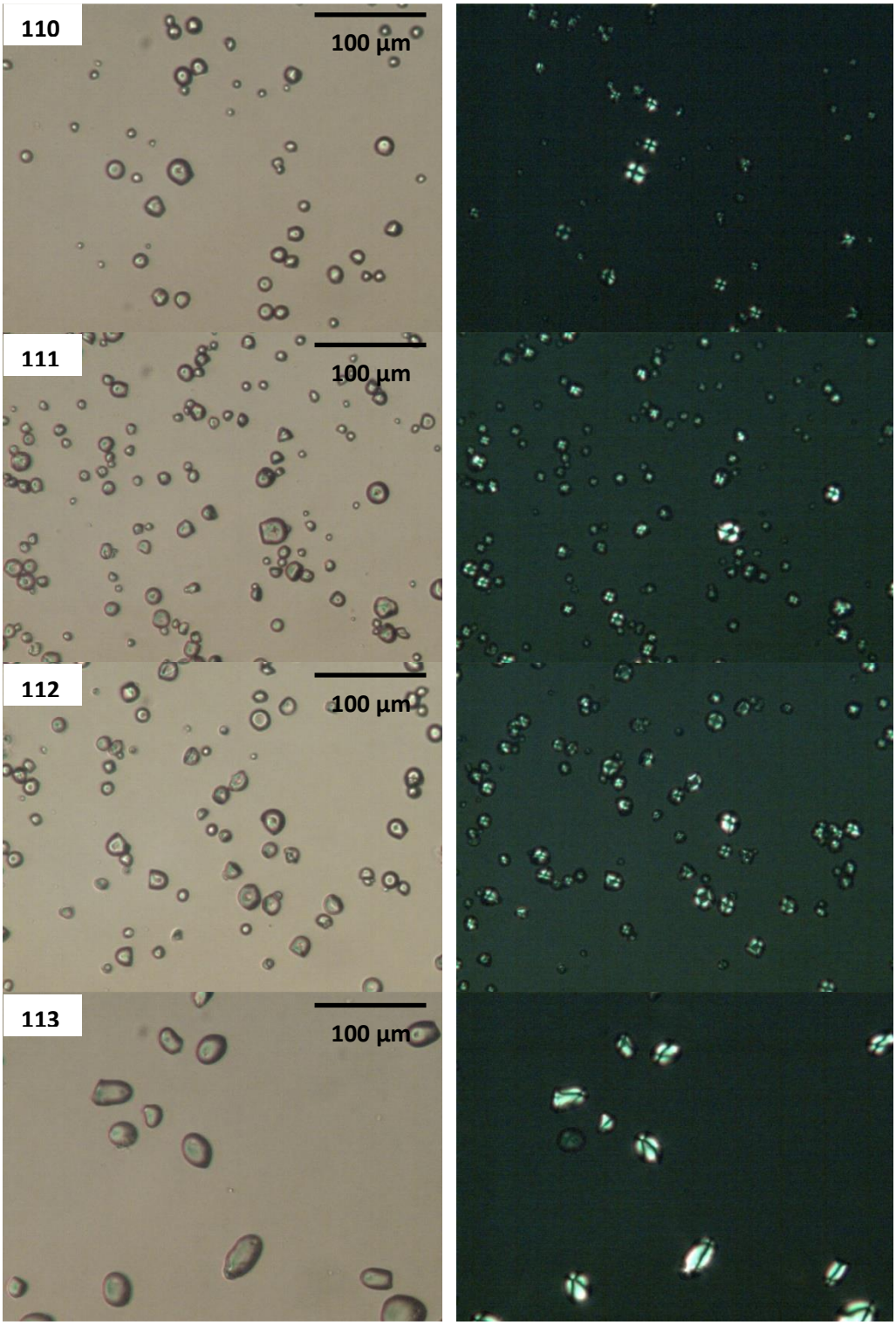


Figure 3.13: Simple light microscopy images for samples 110-113. Right hand side shows the polarised pictures indicating the Maltese crosses.

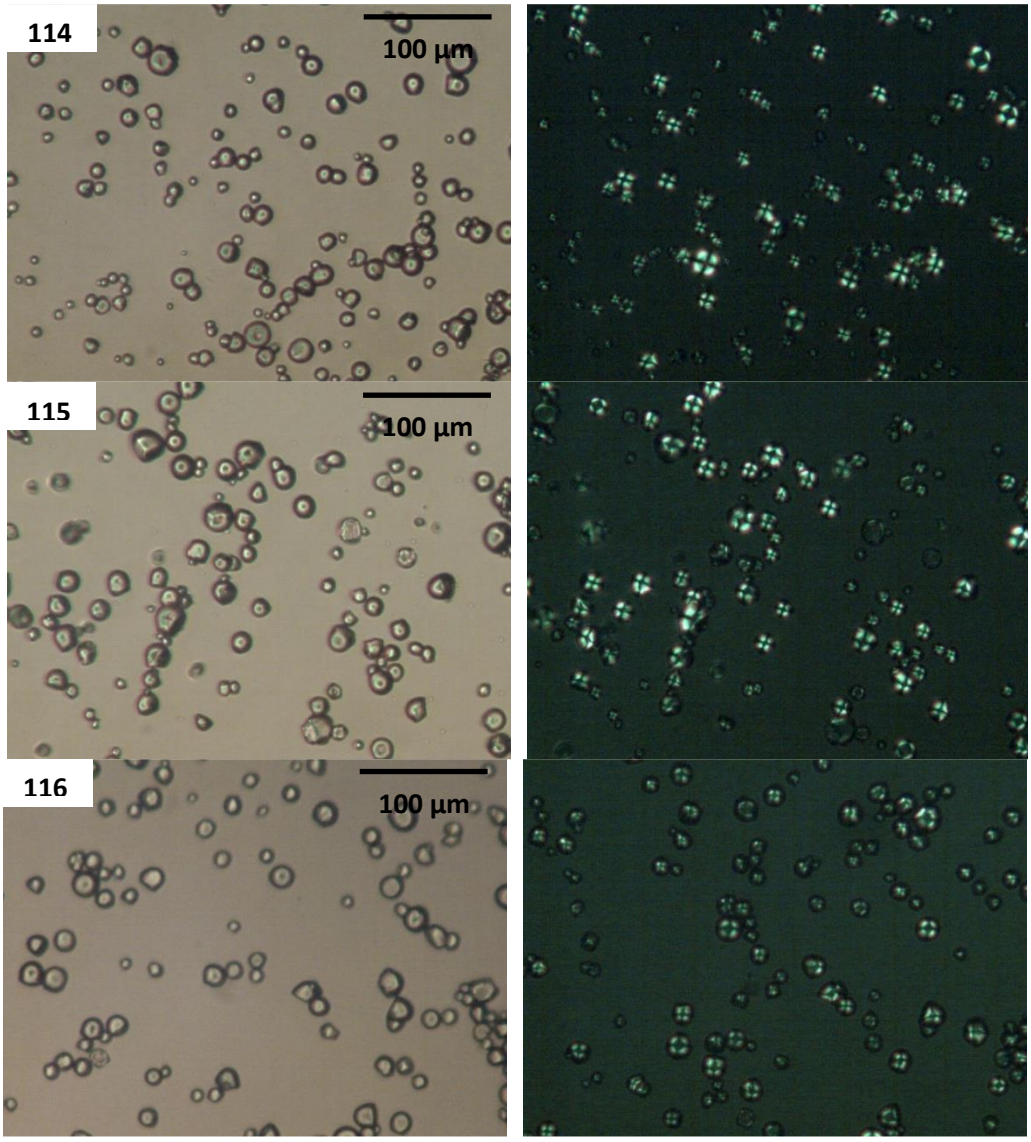


Figure 3.14: Simple light microscopy images for samples 114-116. Right hand side shows the polarised pictures indicating the Maltese crosses.

3.2.6 Total starch Assay

The 15 modified starches (see Table 2.3) were assessed for the total starch levels using the Megazyme total starch assay according to the methods described in section 2.2.2.1 and 2.2.2.2. Figure 3.15 presents the total starch as determined by enzyme degradation to D-glucose. Six of the modified starches (106, 108, 111-112 and 115-116) showed very low total starch contents between 48.3-62.4%. Starch sample 108 was the highly converted pea dextrin. Logically the dextrins will be more easily accessible for the enzymatic reaction that will convert the dextrins into

glucose and therefore could result in a higher total starch value, if this was based on glucose levels. However, the starch may be so highly converted that the starch granular structure was completely destroyed so that the dextrin formed agglomerates upon addition of DMSO creating a physical barrier making the dextrin less accessible for enzyme attack. This explanation was supported by the high particle size distribution (see Table 3.1). The other low content starch samples were all tapioca starches stabilised with propylene oxide and samples 106, 111 and 115-116 had been crosslinked with phosphorous oxychloride as well. Sample 112, with the lowest starch content (48.3%), was only stabilised with propylene oxide. Starch sample 102 had a slightly higher total starch content of 70.6% and this high amylose maize sample had also been stabilised, but with acetic anhydride.

The observed trend was that the stabilised starches had lower total starch contents, as assessed by the Megazyme method (see section 2.2.2.2), than the other modified starches. The starches, which were only crosslinked (101, 103-104, 110 and 114), acid-treated (109, 113) or low converted dextrin (107) had relatively high total starch contents ranging from 81.6-93.1%. Although the amount of total starch appeared to be different, it needed to be considered that these results most likely were an artefact of the Megazyme assessment, since the level of starch in these samples was likely to be very similar. It is interesting and worthy of note that there were limitations in the Megazyme assay that gave rise to the incorrect total starch contents once the starch was physically or chemically modified. It could be suggested that the total amount of starch determined by the Megazyme assay indicated the amount of accessible starch or reflected changes to the glucose units.

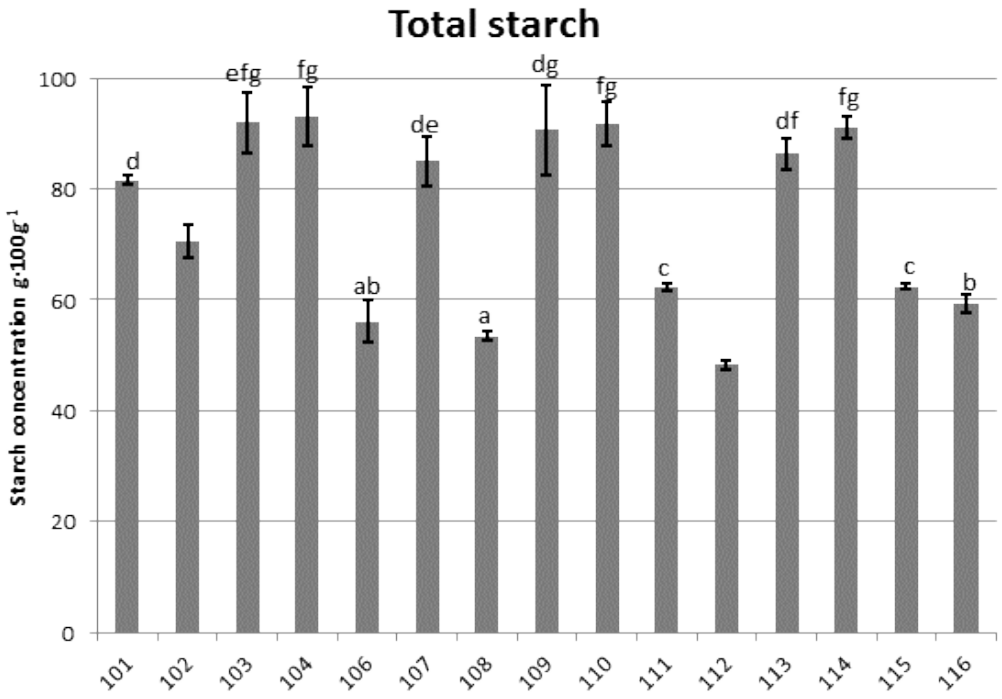


Figure 3.15: The average total starch of sample 101-104 and 106-116. The bars represent the mean value of 4 replicates (measured 2x2) and the error bar denote the standard deviation of the mean value. The column marked with the same letter showed no significant difference (P>0.05) in t-test.

The total starch values and the swelling power values did not correlate (see Figure 3.16) although the amount of accessible starch in the sample would have had an effect on the swelling power. These results showed that the amount of accessible total starch in the sample (determined by the Megazyme assay) had a limited effect and lower impact than the immediate modification on the obtained swelling power values. Hence the modification of the starches may have had a higher impact on the starch properties compared to the amount of accessible starch (determined by the Megazyme assay) and hence it appeared that the total amount of accessible starch was not the major factor in the regulation of the properties of the starches.

To obtain a measure of the internal structuring features of the granule, e.g. the crystalline structure and helical order, the technique of differential scanning calorimetry (DSC) can be used.

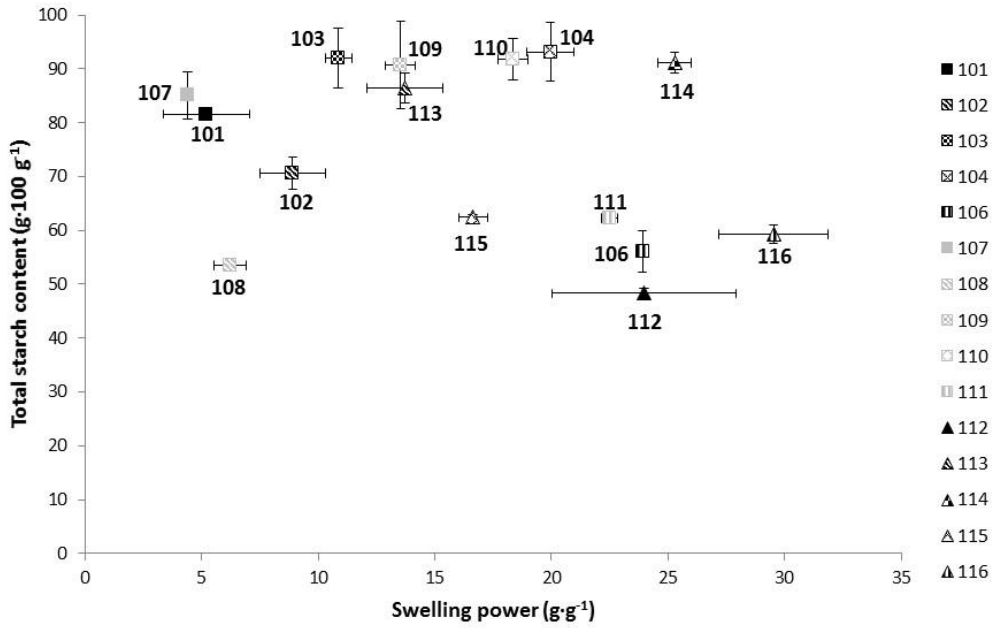


Figure 3.16: The swelling power versus the total starch content (as assessed by the Megazyme assay) of samples 101-104 + 106-116. Replicates are n = 4 for total starch content and n=3 for swelling power. Error bars denote the standard deviation of the mean.

3.2.7 Differential scanning calorimetry

DSC is a technique of many capabilities, but the focus of the work described in this section was to look at the enthalpies (energy required to alter the status of the materials, e.g. melt helices, solubilise materials) and the peak temperature of crystalline melting (method is given in section 2.4.5). Figure 3.17 presents the enthalpies recorded on the first heating of the samples in water (starch: water 1:2) and the enthalpies after 1 week storage at 4°C (retrogradation enthalpies) of the modified starches. The range of initial enthalpies were 9.0 - 20.0 $J \cdot g^{-1}$ of dry starch (wb, corrected for moisture), except for the high converted pea dextrin (108) with a small enthalpy of 0.7 $J \cdot g^{-1}$ of dry starch (wb). The lower values of 9.0 $J \cdot g^{-1}$ and 10.5 $J \cdot g^{-1}$ of dry starch (wb) were observed for the stabilised and medium crosslinked tapioca starch (111) and the acid treated maize (109) respectively (see Figure 3.18). However, after storage the acid treated maize (109) showed two different peaks in the thermogram both with lower enthalpies than the initial value for the sample (see Figure 3.18).

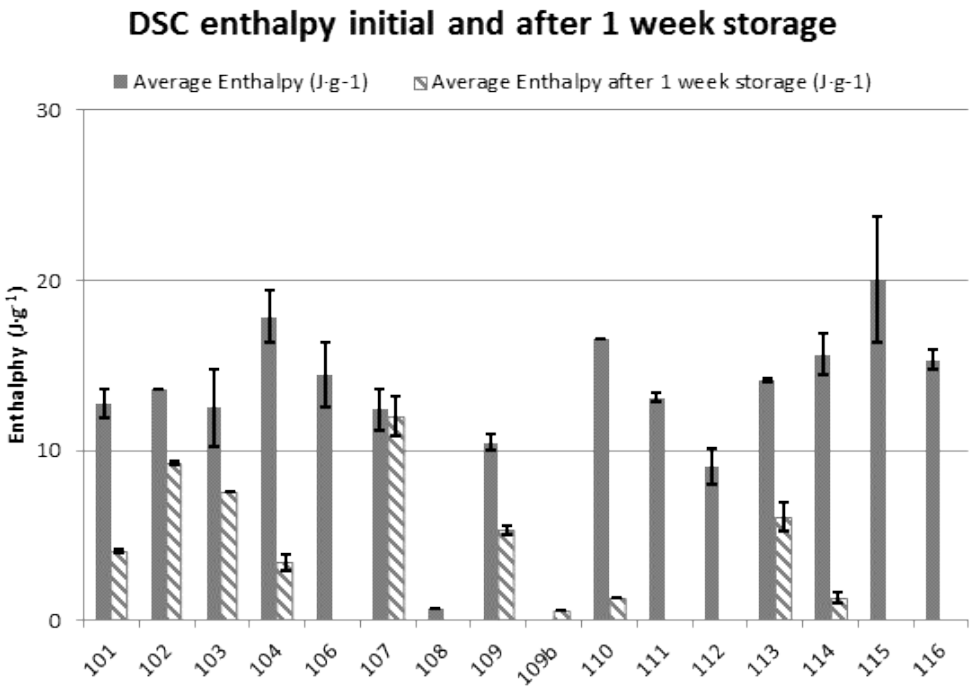


Figure 3.17: The average enthalpy for samples 101-104 and 106-116 initial and after 1 week storage (retrogradation). Sample 109 had two peaks after storage 109 and 109b. The mean of duplicate values (n = 2) are shown and the error bars denote the standard deviations. Experiments were done in duplicate (n = 2), starch: water ratio 1:2, with a heating rate of 10°C /min and in the interval 15-130 °C (see section 2.4.5).

The retrogradation enthalpies gave an indication of whether the samples were prone to amylopectin retrogradation upon storage i.e. re-crystallisation after gelatinisation. The results showed that the stabilised tapioca starches (106, 110-112 and 115-116) did not retrograde whereas the tapioca starch, which was only crosslinked (110 and 114), retrograded upon storage. This was relevant for processed products containing starches. If the starch was processed at high temperatures, e.g. frying the starch would gelatinise and upon cooling and storage at the relevant moisture contents, it may or may not recrystallise and form the crystalline regions. The ordering of the starch can be crucial for network formation and film forming capabilities (Liu and Han, 2005).

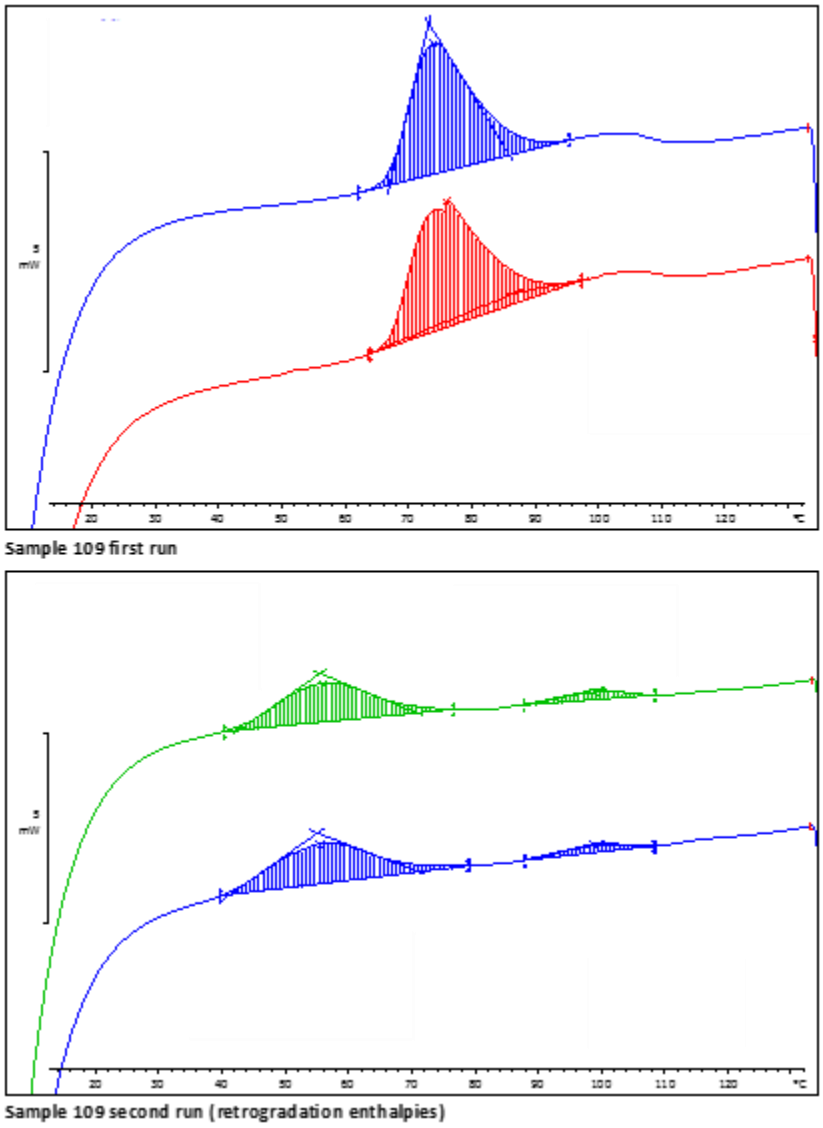


Figure 3.18: DSC thermograms of sample 109. The first run is showing the initial melting and the second run is showing the retrogradation profile. Experiments were done in duplicate (n = 2), starch: water ratio 1:2, with a heating rate of 10°C /min and in the interval 15-130 °C (see section 2.4.5).

The peak temperatures (Figure 3.19) indicated the temperature necessary to melt the helices within the granules and could therefore be affected by the amylose/amylopectin levels and modifications due to possible increased rigidity of the granules. The initial peak temperatures for the modified starches spanned from 62.0 °C - 78.6 °C. Lower peak temperatures (62.0 °C - 69.2 °C) were observed for samples 103, 106-108, 111-112 and 115-116, where five of these starches formed the tapioca group of stabilised and increasing crosslinking in the order of 112 (only stabilised), 116, 106, 111 and 115. This rank order of increasing crosslinking for the

tapioca group did not correlate with either decrease or increase in peak temperatures. The higher peak temperatures in the temperature interval of 70.3 °C - 78.6 °C were observed for the modified starch samples 101, 104, 109-110 and 113-114 which were starches of different origin, but either acid treated (109, 113) or crosslinked (101, 104, 110, 114). The highest melting temperature at 84.1 °C was observed for sample 102 (stabilised high amylose maize) which was similar to previous findings for high amylose maize starches (Shi et al., 1998).

The values for the peak temperature after retrogradation were similar for all retrograded samples except for the stabilised high amylose maize (102) and the second peak of the acid treated maize starch (109b). Sample 102 reached a peak temperature of 89.8 °C, which may indicate that high amounts of amylose had co-crystallised into double helices, which then required high temperatures and energies to disorder these (Shi et al., 1998). The act of retrogradation typically involves amylopectin for long term changes during storage and amylose for short term development of crystallinity (Fredriksson et al., 1998).

It is commonly found (Orford et al., 1987, Sandhu and Singh, 2007, Shi and Seib, 1992, Yuan et al., 1993) that the melting peak for retrograded starch is observed at lower temperature than the gelatinisation peak. The higher peak temperature for retrogradation of sample 102 was however obtained from a very broad temperature range of 48.7-106.3°C. The observed peak could therefore be a mix of the typical melting of retrograded amylopectin (about 60 °C) and another peak around 95 °C which may account for the typical melting of amylose-lipid complexes present in maize starches (Shi et al., 1998). For the acid treated maize starch (109) two peaks were observed for the melting of the retrograded sample (see Figure 3.18). This indicated the separation of the melting of the retrograded amylopectin (peak

temperature 55.8 °C) and the melting of the amylose-lipid complexes (peak temperature 99.0 °C, see Figure 3.18) which is discussed above.

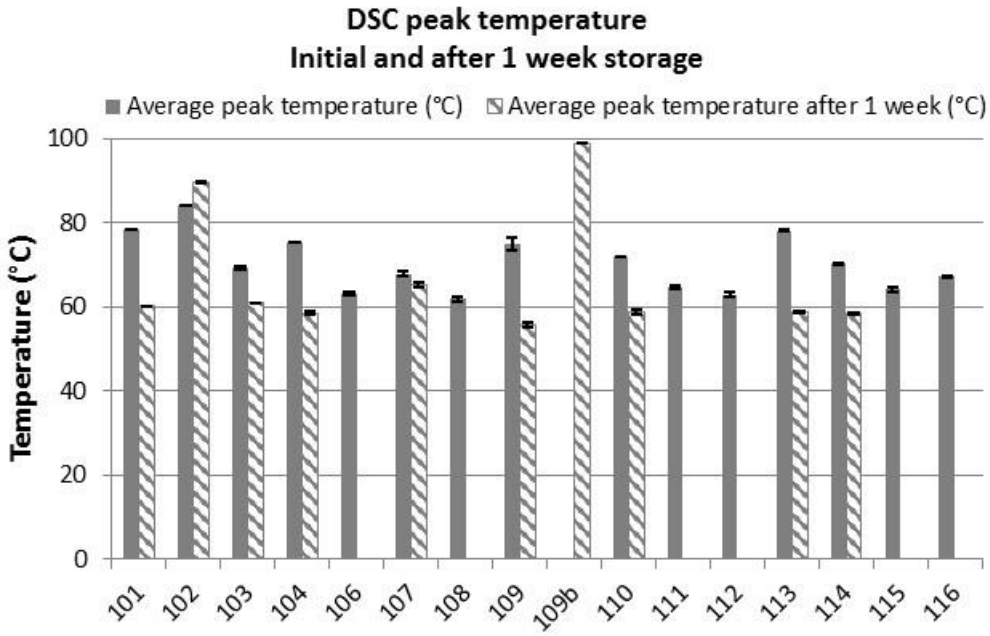


Figure 3.19: The peak temperature for samples 101-104 and 106-116 initial and after 1 week storage (retrogradation). Sample 109 had two peaks after storage 109 and 109b. Experiments were done in duplicate (n = 2), starch: water ratio 1:2, with a heating rate of 10°C /min and in the interval 15-130 °C (see section 2.4.5).

3.2.8 Measurement of Amylose levels

A factor in the crystalline forms within the starches and their behaviour on heating were the relevant levels of amylose and amylopectin. Measurements of the relative amylose/amylopectin contents were attempted using the Megazyme amylose/amylopectin assay procedure (see section 2.2.2.4). The Megazyme assessment was very difficult to conduct due to the complex structure of the starch granule after modification. An example was that very high values for amylose levels were obtained for waxy maize starches (sample 101 and 104 obtained levels of 56.8% and 53.0 % respectively) with high variation in duplicates. Further a nonviable amylose level of more than 100 % was obtained for sample 103 (crosslinked pea starch). The assay was therefore concluded not to be valid for use with modified starch samples. The traditional method to obtain amylose levels is to investigate the

starch's ability to form a complex with iodine and therefore the iodine staining method adopted from Knutson (Knutson, 1986) was used to measure the apparent amylose levels.

3.2.8.1 Iodine staining (section 2.4.3)

Figure 3.20B shows the amylose levels determined for the native starches to confirm that these levels were according to literature and the method was appropriate to measure the amylose content of native samples. Two control samples with known amylose contents were used to determine the equation for the calculation of the unknown amylose levels in the native starch samples.

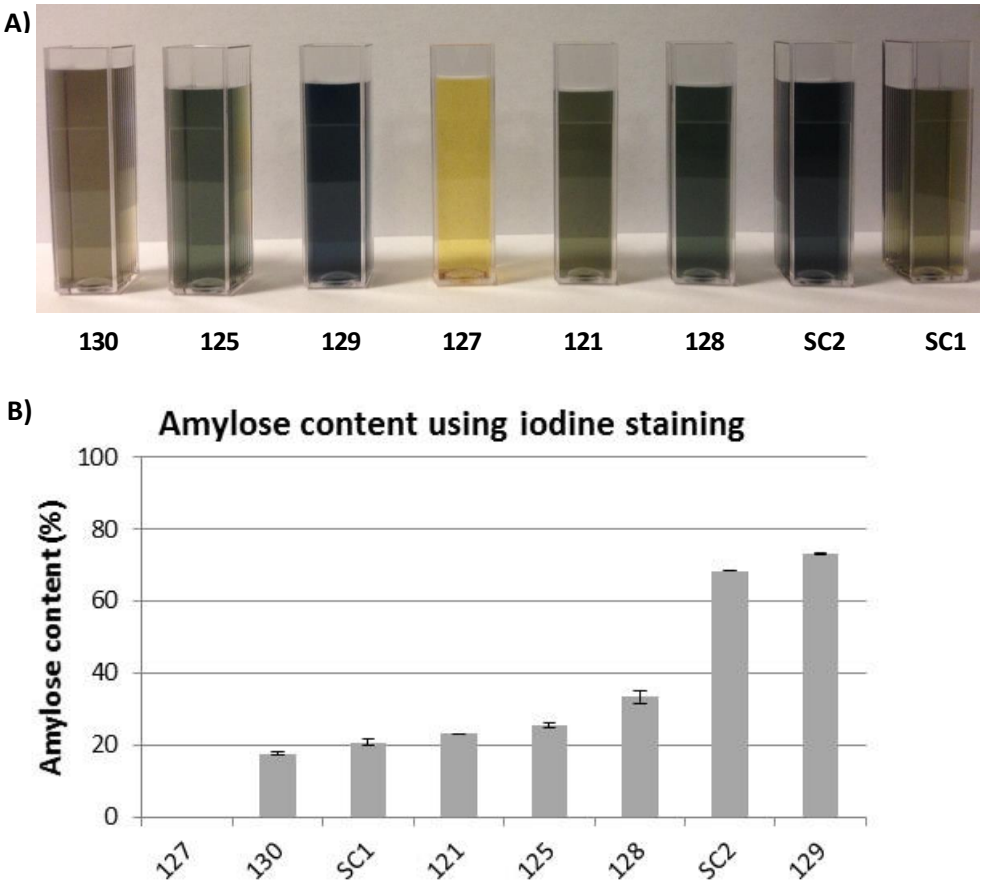


Figure 3.20: A) The colouration when staining with iodine indicated the amount of amylose in the native starch samples (121-131, few selected). B) The graph shows the increasing amount of amylose in the samples determined from SC1 and SC2 (127 was negative but has been zeroed). For method see section 2.4.3.

The native sample 127 was a waxy maize sample which should contain no or very little amylose, which was also visualised by the yellow colour of the sample (see Figure 3.20A). All native starches i.e. tapioca starch (130), maize starch (121), sago starch (125) and pea starch (128) gave amylose levels similar to those reported in the literature (Huang et al., 2007, Singh Sandhu et al., 2007, Zhu, 2015) with values of 17.7%, 23.1%, 25.5% and 33.4% respectively. The high amylose content for sample 129 (73.1%) was expected due to it being high amylose maize starch. The method therefore was generally thought to be acceptable for an estimate of amylose levels. The results shown in Figure 3.20 indicated that for the native starches results were as anticipated.

The group of stabilised and crosslinked tapioca starches were analysed to determine the amylose levels with the above method. However it was very difficult to obtain correct absorbance values due to sedimentation of an aggregate binding the iodine in a complex as seen in Figure 3.21. This result demonstrated that the difficulties in the determination of amylose levels were linked to the modification of the starches. These findings therefore confirmed the difficulties observed for the determination of amylose levels using the Megazyme amylose/amylopectin assay procedure as addressed above.

The observation of an interaction between iodine and modified starches to form a precipitating complex could be indicative of important interactions occurring with the modified starches that could impact on their physicochemical properties. It would appear that the crosslinked starches are especially prone in forming this complex and it is worth speculating that it may be formed under other conditions for example with lipids (see section 4.4.2). It must be noted that the precipitate was not analysed and it could be due to an iodine-amylose complex or even that the

amylopectin played an important role in the formation of the complex. It was not possible to quantify the amount of sediment and therefore it was not concluded whether the iodine-starch complex correlated with the increasing crosslinking of the modified tapioca group (112 (no crosslinking) < 116< 106< 111< 115), but it did appear to follow the suggested levels of crosslinking.

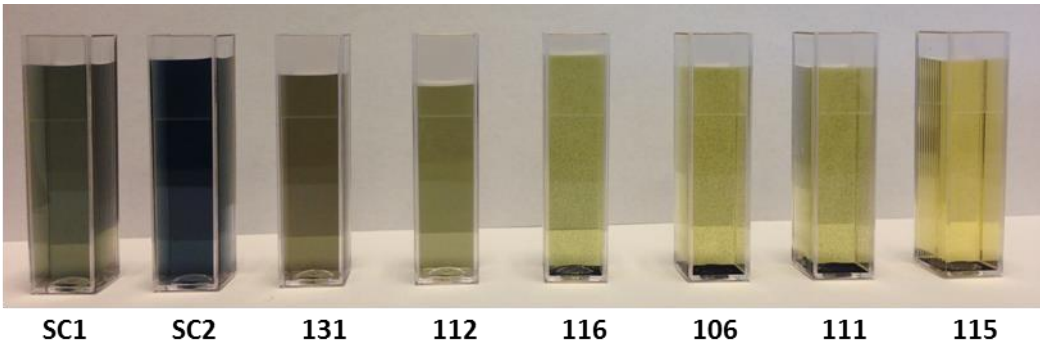


Figure 3.21: The colouration when staining with iodine used as an indicator for the amount of amylose. Samples are the tapioca group (131, 112, 116, 106, 111, 116) of starches and two standard controls (SC1 and SC2).

3.3 Discussion

The physicochemical parameters measured for starches as presented in this chapter are all considered important representations of their properties when used in a food application. The desirable properties for film forming starches vary and depend on the targeted application, but factors such as the starches' chemical compositions, the relative amounts and sizes of the amylose and amylopectin molecules, the hydration and stability characteristics are all likely to play a role. A feature of the samples used in this study was that commercial starches from different manufacturers were used and data on these samples were limited. Statutory information, such as botanical source and type of chemical modification was given, but precise levels of modification were not available. The major reason for the work on characterisation was to establish the differences between the group of starches, but this exercise has also demonstrated that many of the methods, typically used for characterisation of starches, are not valid if the starches are chemically modified.

For example one feature of starches that is considered important for film formation is the amount of amylose they contain (Liu and Han, 2005). However, with modification of the starches the amylose may be either trapped or bound within the granule and therefore might not able to form the required networks. It was therefore relevant to know the amount of amylose in the starches with the intention to further examine how the amylose would be released from the granule. However, the two methods used to estimate amylose contents gave misleading results. Once the starches were crosslinked, the action with iodine caused the complex to precipitate. This is interesting in terms of whether other compounds capable of forming complexes, for example monoglycerides, would cause macro structure differences with modified starches.

Investigations were undertaken to see if the chemical modification of native starches caused changes in the gross granule structure. The impact of modifications on the shape and surface of the granules, as visualised by SEM images, could be expected to be relevant to the behaviour, due to different packing order and polymer loss from the packed granules. Although the starches showed differences due to their botanical source, physical modifications or major depolymerisation, the chemical modifications did not seem to impact their surface morphology or their overall size and shape. Granule shape and crystallinity were also investigated by taking microscopic images using simple light and polarised light (Figure 3.11 to Figure 3.14). The polarised images revealed that only one of the modified starches had lost crystallinity (108), but more samples had slightly altered Maltese crosses. This indicated that the crystalline regions in the granule had been affected by the modification.

Estimates of particle size as measured by LLS did vary, but this was thought to be due to aggregation of the starch granules. This apparent size difference could influence parameters based on hydrodynamic volume, e.g. RVA pasting curves and swelling power of the starches. While the RVA represented the viscosity of the samples when subjected to shear, the swelling volumes showed how much the granules could swell with very limited breakdown.

To understand the viscosity data it was necessary to understand the amount of starch in the system, but again the starch assays used gave results which may well reflect flaws in the assay procedure when used for chemically modified samples. There has been some discussion with the manufacturer (McCleary, 2015) of the kit for starch assessments and it was agreed that its use for chemically modified starches was not advisable. Dextrins were not assessed as starch in the total starch

method, which is reasonable, but stabilisation also apparently reduced the measured starch levels by as much as 52% (see section 3.2.6). Although the stabilisation substitutions will add to the weight of the glucose units, the low levels of starch assessed by the assay suggested some flaws in the rationale of the assay procedure. Another factor that needs to be highlighted is that the data was often based on starch amounts. If data on starch amounts were uncertain, then results for swelling power, DSC endotherms etc. were also questionable. The results for the swelling power and the pasting properties did show some similarities but did not correlate with the total starch content as assessed.

The two pea dextrins (107-108) had very low pasting viscosities and also a very low swelling power due to the conversion of the starch. Samples 101-103 also had low swelling power values, however they did increase with increasing end viscosity for the pasting curves. For the tapioca group the peak viscosities from the pasting curves were decreasing in the order of 111> 106> 116> 115> 112> 130 and the end viscosities in the order of 115> 106= 111> 116> 130> 112 for 13.4% starch. None of these ranking orders correlated with the order of the swelling power for that group which was 116> 106> 112> 111> 115. It was observed that the tapioca group was not showing any trends in the expected order of increasing crosslinking 115> 111> 106> 116> 112> 130 and this will be further explored and addressed in chapter 4.

The calculated enthalpies and peak temperatures retrieved from the DSC thermograms indicated that the modification changed the heat required to melt the helices in the crystalline regions, but no trends were observed for the tapioca group. Only the stabilised tapioca (112) had markedly lower values for both enthalpy and peak temperature compared to the stabilised and crosslinked tapioca starches. The enthalpy values after storage indicated that stabilisation and crosslinking of the

tapioca starches led to no retrogradation of the starches, which can be an important asset for their film forming capabilities.

The knowledge of the physicochemical properties addressed in this chapter can be necessary to guide the selection of suitably modified starches in food applications. It was important to understand the properties which led to the behaviour of the starches in order to select the desirable properties for a specific product. The tapioca group of stabilised and crosslinked starches showed some interesting results for their pasting properties and their binding of iodine and formation of an iodine-binding complex. In the following chapter (chapter 4) this group of modified starches is addressed in more detail, to establish if other methods are available to predict the functionalities of the starches at moisture contents used in film forming applications.

CHAPTER 4 DISSOLUTION OF STARCHES IN LOW WATER ENVIRONMENTS AND DIFFERENTIATING THE CHEMICALLY MODIFIED STARCHES

In the previous chapter data on the starches supplied for this project were shown. The assessments reflected many methods that would be typical for the characterisation of native starches in excess water. However, it was clear from the data sets that these analyses did little to show the behaviour of starches in the environments that could be experienced when batters are subjected to high temperatures and subsequently rapid dehydration during a process such as frying. Also the first sets of analyses did not satisfactory detail the differences between the starches that had been chemically modified. A set of tapioca starches had been provided that were listed as having different levels of crosslinking and with stabilisation levels said to be similar (see Table 2.3). The aims of the work presented in this current chapter were therefore:

1. To investigate the behaviour of starches when heated in limited water, but fluid systems.

2. To use the tapioca family of chemically modified starches to investigate novel testing regimes that could relate their behaviour to the expected levels of modification.

4.1 Starches in low water environments

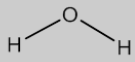
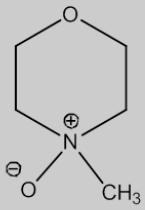
Starches in coating batters for food often need to function at low moisture levels. The necessary attributes to create films may not be readily predicted from the types of high moisture testing that is typically used for starch characterisation. Behaviour in low water content systems, such as N-methyl morpholine N-oxide (NMMO), may

provide information that can predict the starches' properties as film formers. NMMO, in the presence of some water, dissolves rather than swells granular starch (see section 1.2.1.4.1) and therefore the interpretation of the behaviour of the starch dissolution properties may be probed from that perspective. NMMO in this study was used in a mix of 78% NMMO - 22% water, since some water had to be present in order for the granule to dissolve (Koganti et al., 2011, Koganti et al., 2015). As the modified starches presented in chapter 3 were also potential film formers, these samples were used to investigate if the modification levels of the tapioca group can be better understood using the model system of NMMO.

4.1.1 NMMO-water as a solvent for starch dissolution

NMMO is an organic solvent derived from morpholine and it is classified as a heterocyclic amine oxide (see Table 4.1). NMMO is generally known for its dissolution properties in the Lyocell process where cellulose is dissolved in NMMO-water to create cellulose fibres (Fink et al., 2001). Previous studies have used NMMO-water as a solvent to explore starches granular structures (Koganti et al., 2011, Koganti et al., 2015). The NMMO-water system has been probed at different water levels by Koganti and co-workers (Koganti et al., 2011) and it was concluded that the 78% NMMO - 22% water solvent system was best suited for the examination of the granule morphology of starches.

Table 4.1: Solvent characteristics of water and NMMO

Water	N-methylmorpholine N-oxide
Standard plasticiser	Organic solvent
Normal media used for granule structure investigations	Allows investigation of polymers without granule morphology
Interact with the granule by penetration of the granule leading to swelling and hence solubilisation of the linear polymers. With shearing there is loss of swollen granule integrity	Interaction with the granule leading to partly dissolving the granule layer by layer
Deionised water	Low water content system (78% NMMO - 22% water)
	

The possibilities to use the NMMO-water system in the food industry are limited due to its chemical composition. Therefore the NMMO-water system is only used as an analytical tool to better understand the behaviour of starches in limited water environments, which is not currently readily or easily achievable. Various techniques can be used to explore the granule morphology utilising the NMMO-water system. Focus in this study was on the pasting properties (RVA and rheology) and thermal analysis (DSC and hot stage microscopy).

4.2 Pasting characteristics in NMMO-water

The pasting of starches in excess water is a well-known technique for fingerprinting starches and provides information about the swelling capabilities and therefore the morphology of the starch granule. Figure 4.1 shows a schematic presentation of swelling and concomitant breakdown of the starch granule, including the standard parameters derived from a standard pasting curve.

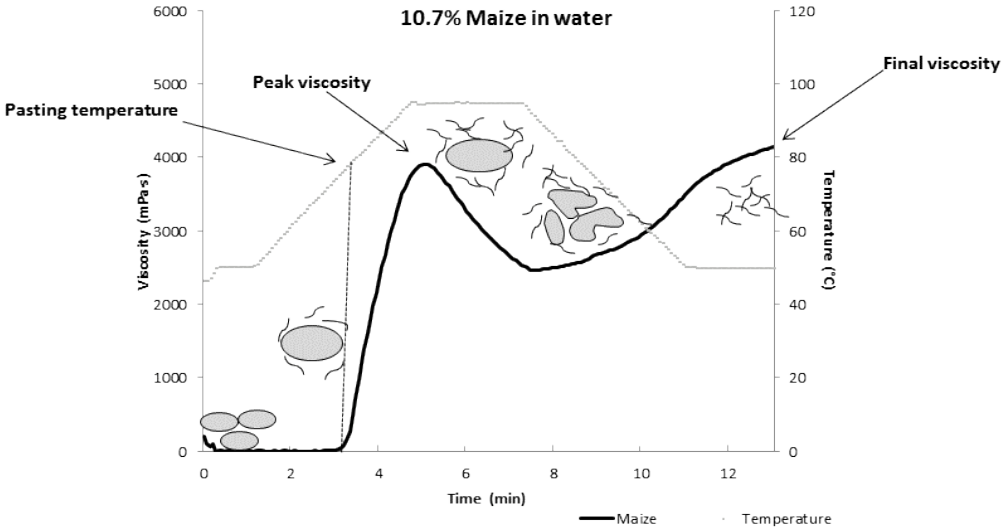


Figure 4.1: A schematic presentation of the theoretical behaviour and associated viscosity when pasting native starch granules in water. 10.7% normal native maize (121) is used as an example.

From the pasting profile of normal maize starch (121) values of pasting temperature, peak viscosity and end viscosity can be determined and trough and setback can be calculated. These characteristics are important for starch properties in excess water and they are normally those used in quality control for commercial starch samples. However, if the starches are used in a low water content system, their behaviour may not correlate with high water performance. When the starches only have limited access to water, their hydrodynamic volume can change accordingly. Using the group of tapioca starches (Table 2.3) with similar stabilisation levels and increasing crosslinking levels (112< 116< 106< 111< 115) in an excess water environment, it was shown in the previous chapter (Figure 3.4-3.5) that the rank order of peak viscosities changed going from high starch concentration (14.3% starch) to a lower concentration of 7.1%. The order of the peak viscosities changed as the concentration was decreased, but without an obvious trend. This was further supported by the data at 10.7% starch concentration (data not shown).

It was therefore important to obtain a better understanding of the hydrodynamic behaviour of starches used in low water environments, such as batters, to establish

their response at very low water contents. The schematic dissolution and breakdown of the starch granule in a NMMO-water system is presented in Figure 4.2. The dissolution of the granule in NMMO-water has been suggested to generally dissolve from the surface (Koganti et al., 2015) with layer by layer of the concentric growth rings solubilising. This means that the viscosity of the dissolved starch does not reflect the granule morphology and will only reflect the polymer-polymer or polymer-solvent interactions. These interactions will indicate how the starch could behave in limited water systems. The NMMO-water system will therefore be used as an indicator to predict the behaviour of dissolved starch in limited water environments.

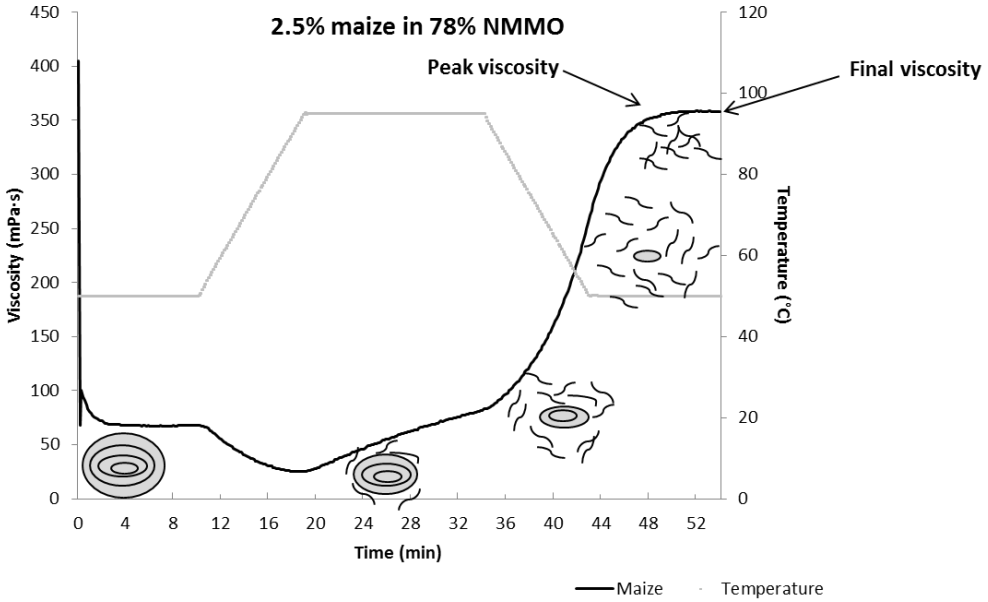


Figure 4.2: A schematic presentation of the theoretic dissolution of the starch granule in 78% NMMO. 2.5% normal native maize (121) is used as an example.

4.2.1 The behaviour of the tapioca group in limited water systems

A few of the standard techniques presented and discussed in chapter 3 have been used to explore the starches in the NMMO-water system.

4.2.1.1 Rapid visco analysis

The tapioca group of stabilised and crosslinked starches were expected to have different characteristics due to their levels of modifications, even if they had been

studied in excess water where no discernible trend was observed (see chapter 3). The differences occurring due to changing the solvent from excess water to NMMO-water is visualised in Figure 4.3, which shows the pasting curves for the stabilised tapioca starch (112, 7.1%) in water and NMMO-water. The two pasting curves comply with the theory presented in Figure 4.1 and Figure 4.2 for both solvent systems. The viscosities for the starch in NMMO-water were higher than those achieved in water and therefore the typical concentrations of starch used when being pasted in NMMO-water were low (5% was routinely used for these experiments, see section 2.4.4). The length of the pasting programme was also much extended with the NMMO-water solvent compared to water alone. These factors, plus the higher viscosity of the NMMO-water solvent compared with water should be taken into account when comparing NMMO pasting data with that for water alone.

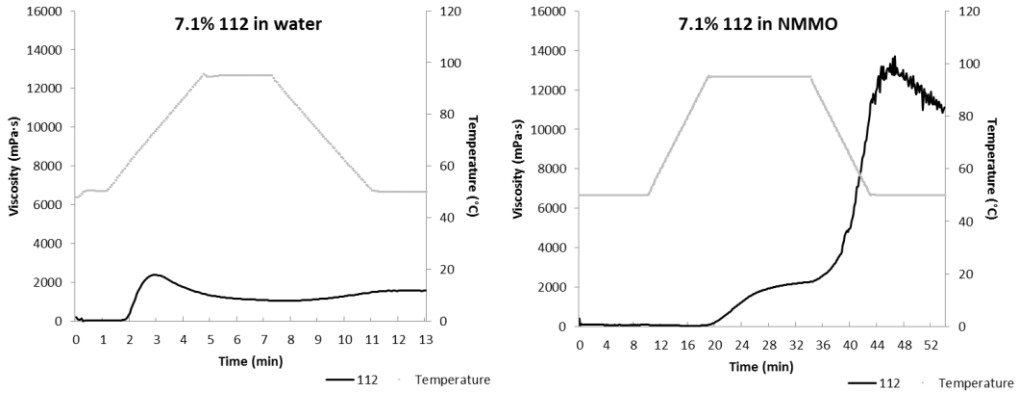


Figure 4.3: The RVA pasting curve for the stabilised tapioca starch (112) in water (left) and 78% NMMO - 22% water (right).

To establish if NMMO-water pasting could assist in understanding the behaviour of modified starches, the tapioca family of starches was investigated. The pasting curves for the tapioca group (2.5%) in 78% NMMO - 22% water is given in Figure 4.4. The order of the end viscosities (112>106>116>130>111>115) was not consistent with any of the orders for the peak viscosities in water, indicating that the solvent system expressed differences in hydrodynamic volume of the starches.

(Wattanachant et al., 2003) have shown that increasing level of stabilisation (keeping the crosslinking level the same) led to a surprising change in pasting viscosities using water as the solvent. They found that with increasing level of stabilisation the viscosity increased. However, the granules became more restricted or rigid with higher levels of stabilisation (10% propylene oxide) and therefore viscosity decreased with higher stabilisation (10 and 12%). It was proposed that the higher the level of stabilisation, the easier crosslinking would be. This would be due to hydroxypropylation which had weakened the bonding between starch molecules thus facilitated more crosslinking within the starch polymers (Wattanachant et al., 2003). Therefore the proposal put forward implies that crosslinking levels are very dependent on stabilisation, and less dependent on the levels of chemicals used for crosslinking.

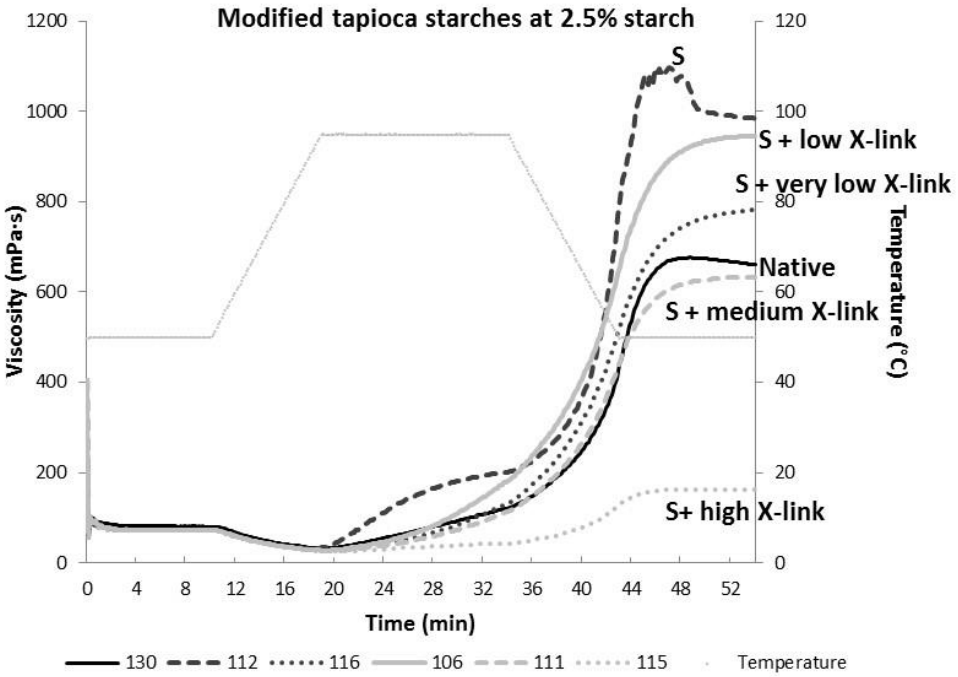


Figure 4.4: The RVA pasting curve for the group of stabilised (S) and crosslinked (X-link) tapioca starch (130, 112, 116, 106, 111,115, see table 2.3) in 78% NMMO – 22% water.

The information obtained from the commercial suppliers about the samples used in this study was that the group of tapioca samples had been treated to achieve the

same level of stabilisation, but different levels of crosslinking were created by variation in the amount of added chemicals. Figure 4.4 shows that the end viscosities followed a trend related to the level of crosslinking suggested by the supplier with the exception of very low and low crosslinked samples (116 and 106 respectively). It can be suggested that a similar trend as for increasing stabilisation in water, (Wattanachant et al., 2003) is possible for increasing level of crosslinking in NMMO-water. Although the NMMO-water solvent provides information on polymer-polymer and polymer-solvent interactions rather than granule morphology, i.e. gelatinisation and swelling, the results may indicate a similar trend for the dissolution pattern of stabilised and crosslinked starches. The results obtained show that the end viscosities increased in the order 106>116 (low crosslinking > very low crosslinking). However, when the crosslinking increased to the medium level, the polymer-polymer and polymer-solvent interactions were so restricted/rigid in the limited water environment that the viscosities dropped below the native tapioca starch (130). Viscosities dropped further with higher crosslinking 130> 111>115 (native tapioca > medium crosslinked > highly crosslinked).

For the crosslinking it could be expected that the NMMO-water solvent would not break the chemical link and therefore the polymer would be greater in size and therefore resulted in greater viscosity (for sample 106 and 116). However, too high a level of crosslinking (sample 111 and 115, see Figure 4.4) could mean that the polymer could not open up to its full dynamic volume and therefore viscosities were lower.

The orders of the peak viscosities in water were different at several concentrations (see section 3.2.1) and also different compared to the order of end viscosities in NMMO-water. The peak viscosity in water should reflect the stability and potential

swelling of the granule, while the end viscosity in the NMMO-water should show the viscosities associated with the starch macromolecules (polymer-polymer and polymer-solvent interactions) when fully loosed from the granule structure. For 14.3% starch in water the peak viscosities increased in the order medium crosslinking (111) > low crosslinking (106) > very low crosslinking (116), but with the highly crosslinked sample (115) having the lowest peak viscosity. As the concentration was decreased to 7.1% starch in water the order of the peak viscosities of crosslinked starches changed to very low (116) > low (106) > medium (111) > high (115) with the highest crosslinked tapioca starch (115) only developing very small viscosity values (Figure 3.5).

It could be considered that at low concentrations the swollen granules did not impinge much upon each other allowing for highly swollen granules, which were not broken by contact with the other swollen granules. At the higher concentrations the breakage of the granules dominated over the advantages of higher swelling and moderate crosslinking gave higher peak viscosity values. Very high crosslinking (115, Figure 3.4) stopped the granule swelling. The effect of different levels of crosslinking has also been studied by (Wongsagonsup et al., 2014). They found that increasing the level of crosslinking (in this case sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP)) decreased the viscosity of the resulting pasting curves (in water), which supported the findings for the concentration of 7.1% in water, but highlighted the relevance of the method of pasting in such conclusions. The dynamic volumes of the macromolecules at low water environments and the pasting and packing together of the swollen granules that resulted in the viscosity that occurred in high water environments could not be directly compared. It was shown that in excess water the concentrations were crucial for the behaviour of the modified

starches. The pasting behaviour in limited water environment changed the dissolution of the granule and emphasised the importance of the presence of water upon starch dissolution.

4.2.1.2 Differential scanning calorimetry

Figure 4.5 shows examples of starch DSC thermograms in water and in 78% NMMO-22% water. The energy requirements to disrupt the structures in the starch were measured by the change in enthalpy. The overall enthalpy was positive (energy being required to disrupt the order) when heating starches using water as a solvent and therefore the thermogram showed an endothermic peak. Whereas for the NMMO-water solvent the overall heat capacity was negative resulting in an exothermic peak. It is well known that the melting of helices, resulting in disruption of the crystalline regions of the amylopectin packing within native starches (gelatinisation) is an endothermic event hence energy is absorbed during the order-disorder transition. The intensity and temperature of the transitions depend on the type of starch (botanical source) and the plasticiser level (normally water). Other factors, such as whether the starch contains lipids can also be relevant.

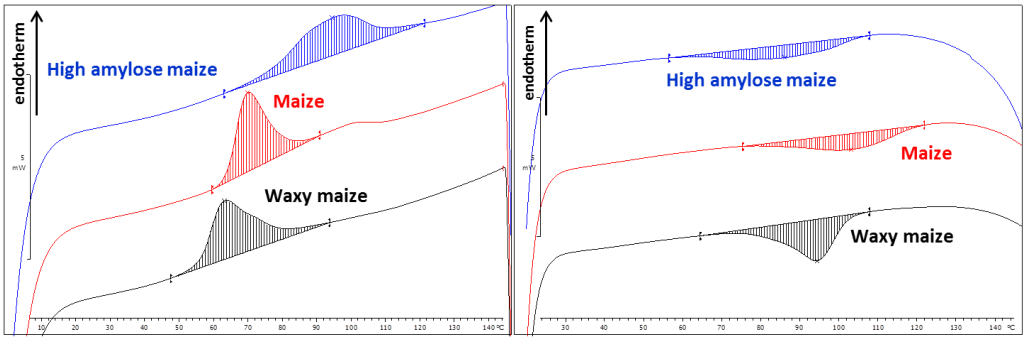


Figure 4.5: DSC thermograms for high amylose maize starch (129), maize (121) and waxy maize starch (127) in water (left) and 78% NMMO - 22% water (right). Experiments were done in triplicates (n = 3), starch: water ratio 1:2 or starch: NMMO - water ratio 1:9, with a heating rate of 10°C /min for water and a heating rate of 5°C /min for water-NMMO, both in the interval 0-150 °C (see section 2.4.5).

The NMMO-water solvent system clearly influenced the order-disorder transition resulting in an overall exothermic transition for the starches in the limited water environment. (Koganti et al., 2011) proposed that the exothermic event was seen

due to the higher negative energy transition of mixing the solvent-starch system. They suggested that the balance between the heat of melting (endotherm) and the heat of mixing (exotherm) changed to a net negative exotherm, indicating that the power of the solvent caused major polymer-solvent interactions. These interactions may be occurring without a significant contribution of heat of melting due to changes in the crystalline morphology. There may be a hidden endothermic peak representing melting, but it was cancelled out by the strong transition exotherm. The enthalpic values and the peak temperatures for the tapioca group are given in Figure 4.6 and Table 4.2.

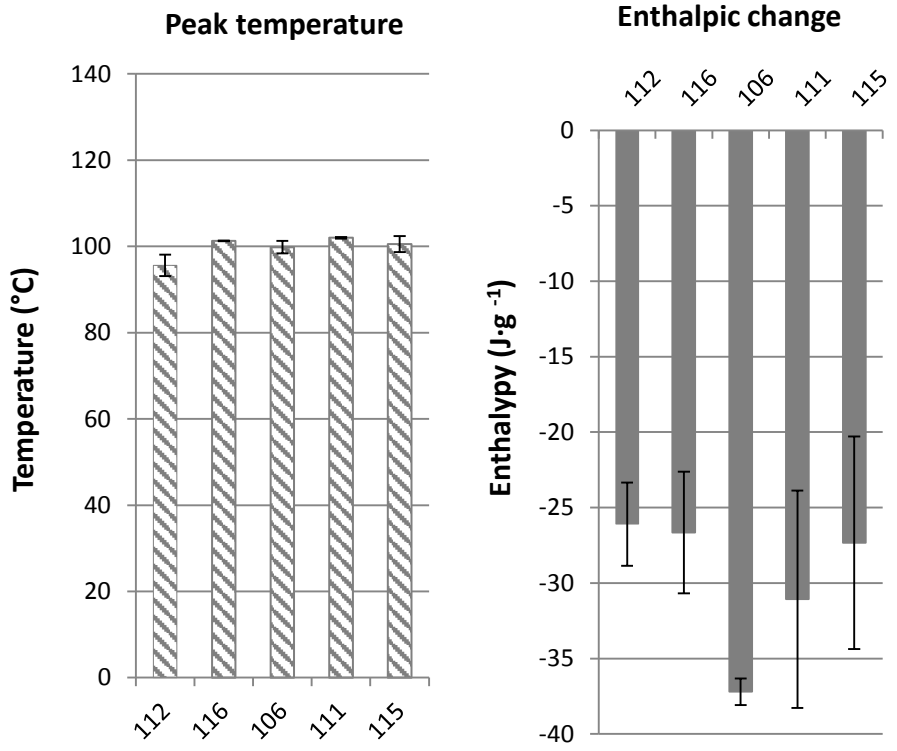


Figure 4.6: The change in enthalpy and peak temperature for the tapioca group (130, 112, 116, 106, 111, 115) from the DSC thermograms. Starch: NMMO - water ratio 1:9, with a heating rate of 5°C /min and in the interval 20-150 °C (see section 2.4.5). The bars show mean values and error bars denote the standard deviation of duplicate samples.

The peak temperatures recorded for the exotherms for the stabilised and crosslinked tapioca starches (106, 116, 111, 115) were not different (100.3 ± 2.0 °C), whereas the stabilised tapioca starch had a slightly lower peak temperature of 95.6

± 2.5 °C. The transition enthalpies showed high levels of variance, but it was possible that sample 106 (the stabilised and low crosslinked starch) was different to 112, 116 and 115, but not to 111. It would seem that the chemical modifications that occurred to the starches, although having a major impact on their behaviour in excess water, did not alter the solvation properties of the samples to any great extent as measured by DSC when the solvent used was NMMO-water. Stabilisation without crosslinking may reduce the temperature at which the material solvated into the mixed solvent, but the energies released for the solvation of the starches was not impacted much by the chemical modifications. This was not expected as the NMMO-water system did seem to be very sensitive to detecting some changes in the starch. This was exemplified when investigating a series of control tapioca starches that were suitable for comparison with the chemically modified starches.

Table 4.2: The calculated change in enthalpy and peak temperature for the stabilised tapioca group with increasing crosslinking in the order 112, 116, 106, 111, 115 from the DSC thermograms. Starch: NMMO - water ratio 1:9, with a heating rate of 5°C /min, and in the interval 20-150 °C (see section 2.4.5). n = 2 and all values are shown.

Sample	Crosslinking	Enthalpic change ($J \cdot g^{-1}$)	Peak temperature (°C)
112	None	-24.14, -28.04	93.83, 97.38
116	Very low	-23.80, -29.50	101.21, 101.35
106	Low	-36.58, -37.82	98.79, 100.84
111	Medium	-25.98, -36.16	101.89, 102.15
115	High	-22.34, -32.30	99.24, 101.83

4.3 Three native tapioca starches differentiated using the NMMO-water solvent as a primary tool

In the course of the studies discussed in this thesis it was difficult to determine which controls were appropriate. For example, many of the starches were from

commercial sources and there was no indication of the starting starches for the chemical modifications. A series of modifications were available for the tapioca starches, so this set of samples was considered important and hence more than one tapioca starch was tested as a control to represent the samples before the modification.

Three native tapioca starches (130-132), from different commercial sources, were tested in some detail including the use of the two different solvents, water and 78% NMMO-22% water. The standard pasting curves at 12.5% starch in water for the three tapioca starches are shown in Figure 4.7 i). The pasting curves in water were very similar and only sample 132 seems to have slightly lower viscosities. The pasting curves for the same three tapioca starches (130-132) in NMMO-water are shown at two concentrations 2.5% and 5% in Figure 4.7 ii) and iii) respectively. It was evident from the pasting curves that the three starches behaved differently in the low water system. The three starches could be differentiated using the NMMO-water solvent system, which the pasting curves in water did not predict.

Properties of the three tapioca starches are given in Table 4.3. Both the crystallinity and the amylose levels were similar for the three starches and also in accordance with previously reported results (Zhu, 2015). The particle size of the three tapioca starches (130-132) was significantly different. However, these were all in the range of 17-22 μm, which is in accordance with previous results (Zhu, 2015).

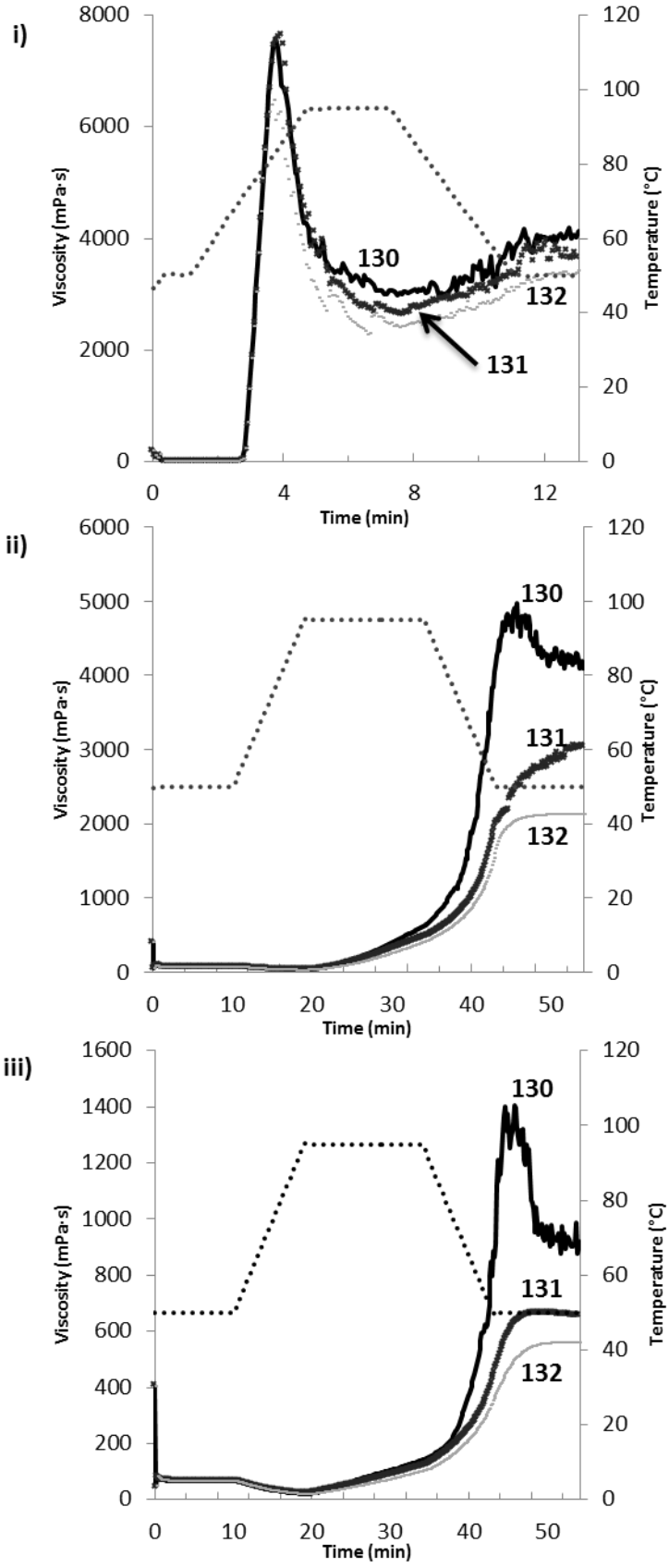


Figure 4.7: Three native tapioca starches (130-32) i) 12.5% in water, ii) 5% in 78% NMMO- 22% water, iii) 2.5% in 78% NMMO- 22% water. All samples were run in triplicates, but not shown.

Table 4.3: Properties of the three tapioca starches 130-132. Data shows mean ± SD and different superscripts indicate P<0.05. n is the number of replicates and [i] n = 5 and [ii] n = 6. Table modified from Brøgger et al. (2015)

Sample	130	131	132
Amylose content (n=2, %)	15.9, 15.5	11.6, 19.4	18.8, 17.4
Crystallinity (n=1, %)	28	26	28
Particle size (n=3, µm)	$17.71^{a} \pm 0.12$	$18.01^{b} \pm 0.05$	$21.63^{c} \pm 0.06$
T_o (°C) onset temperature (H_2O)[a]	$62.3^{a} \pm 0.4$	$62.3^{a} \pm 0.2$	$61.0^{b} \pm 0.2$
T_p (°C) peak temperature (H_2O)[i]	$68.5^{a} \pm 0.5$	$68.8^{a} \pm 0.2$	$67.5^{b} \pm 0.4$
T_e (°C) end temperature (H_2O)[i]	$78.1^{a} \pm 0.4$	$76.8^{b} \pm 0.6$	$76.2^{b} \pm 0.6$
ΔH ($J \cdot g^{-1}$) enthalpic change (H_2O)[i]	$11^{a} \pm 1.4$	$12^{a} \pm 1.4$	$14^{a} \pm 2.1$
T_o (°C) onset temperature (NMMO)[ii]	$80.4^{a} \pm 5.2$	$83.3^{a} \pm 2.9$	$85.1^{a} \pm 0.7$
T_p (°C) peak temperature (NMMO)[ii]	$93.0^{a} \pm 3.7$	$95.5^{a,b} \pm 3.5$	$99.0^{b} \pm 2.5$
T_e (°C) end temperature (NMMO)[ii]	$105.8^{a} \pm 4.8$	$108.9^{a,b} \pm 4.2$	$112.7^{b} \pm 3.7$
ΔH ($J \cdot g^{-1}$) enthalpic change (NMMO)[ii]	$-25.9^{a} \pm 5.4$	$-30.6^{a,b} \pm 5.7$	$-36.4^{b} \pm 4.7$

When using the technique of DSC to measure the order-disorder transition for the starches in water, the enthalpy and peak temperature values were similar (Table 4.3). Changing the solvent to NMMO-water resulted in the expected negative enthalpic changes, but also differentiated the three starches with significantly different values for both enthalpic changes and peak temperature.

The NMMO-water solvent system was able to differentiate the starches using RVA pasting techniques and DSC thermal analysis. Rheological measurements were conducted to explore whether the results from the RVA pasting could be explained by the molecular size of the released polysaccharides. The dependence of molecular size would result in differences in complex viscosity following dissolution of the starches leading to differences in the viscoelastic properties. Figure 4.8 shows the angular frequency versus the complex viscosity of the three tapioca starches (130-132) after an RVA experiment including two weeks storage and then after being heated for additional 30 minutes at 95 °C (see section 2.4.12).

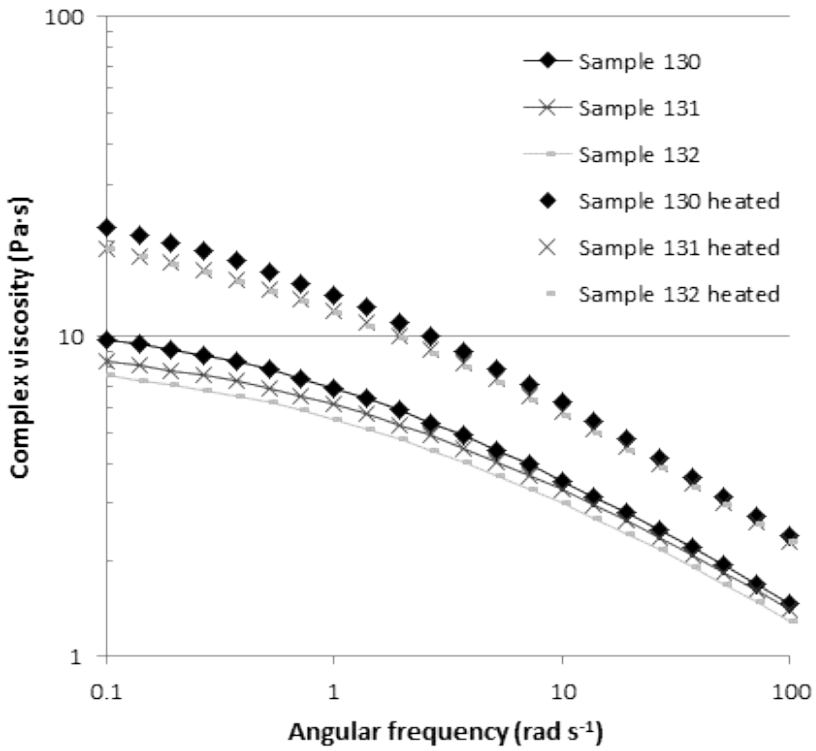


Figure 4.8: Rheological measurement showing the angular frequency versus the complex viscosity for the three tapioca starches (130-132, 5% in 78% NMMO- 22% water, see section 2.4.12) after 2 weeks storage at 20-30 °C and then after additional 30 minutes heat treatment at 95 °C. Data show one out of two similar replicates.

The results obtained show that the rheological data ranked in the same way as for the RVA pasting measurements both after RVA experiment and two weeks storage as well as after further heat treatment. However, the viscosity of all three samples increased after heating indicating that the samples were not completely dissolved in the NMMO-water system. The tapioca starches were also measured immediately after RVA to verify that the storage of the samples did not influence the dissolution leading to change of viscosity in the rheological measurements.

The dissolution of the starches in the NMMO-water solvent system was explored using hot stage microscopy. Polarised light microscopy was used to observe the dissolution of the granules during heating (no shear). Figure 4.9 show the three starches (130-132) after RVA treatment with NMMO-water as the solvent (40 °C), heated to 94 °C and then held at 95 °C for various time intervals depending on the

starch and the amount of intact granules left. All three tapioca starches showed intact granules in the solvent after RVA treatment (40 °C) which indicated that the dissolution in NMMO-water was not complete. During heating to 94 °C some of the granules dissolved, however only after reaching 94 °C and holding for 10 minutes was it observed that the granules of tapioca starch 130 dissolved completely. The tapioca starch 131 also dissolved gradually on being heated to 95 °C and with an additional 15 minutes at 95 °C most granules were dissolved. For sample 132 the granules were much more robust and after being held for 30 minutes at 95 °C there was still some intact granules remaining. The differences in dissolution properties of the starches in NMMO-water confirmed that the three native tapioca starches (130-132) possessed different properties when investigated at low water content.

The RVA method of pasting the starches in the NMMO-water system did not seem to provide sufficient time or temperature to cause the solvation of the whole granules for the native tapioca and therefore the viscosity data was a snapshot of the situation as the granules dissolved. It may be that the difference in the size of the granules was relevant to their subsequent dissolution, but the mechanism in the NMMO-water system may be more subtle than this with the water acting as the plasticiser and the solvent decoupling the hydrogen bonding to allow solubilisation. It is possible that the dominating factors for each of these mechanisms were slightly different in starches even if they were from the same botanical source. Based on these findings it was thought appropriate to measure the complex viscosity for the tapioca group of stabilised and crosslinked starches. This may indicate changes to the dissolution of the granules in the NMMO-water system and therefore possibly differentiate various levels of modification for the group of tapioca starches.

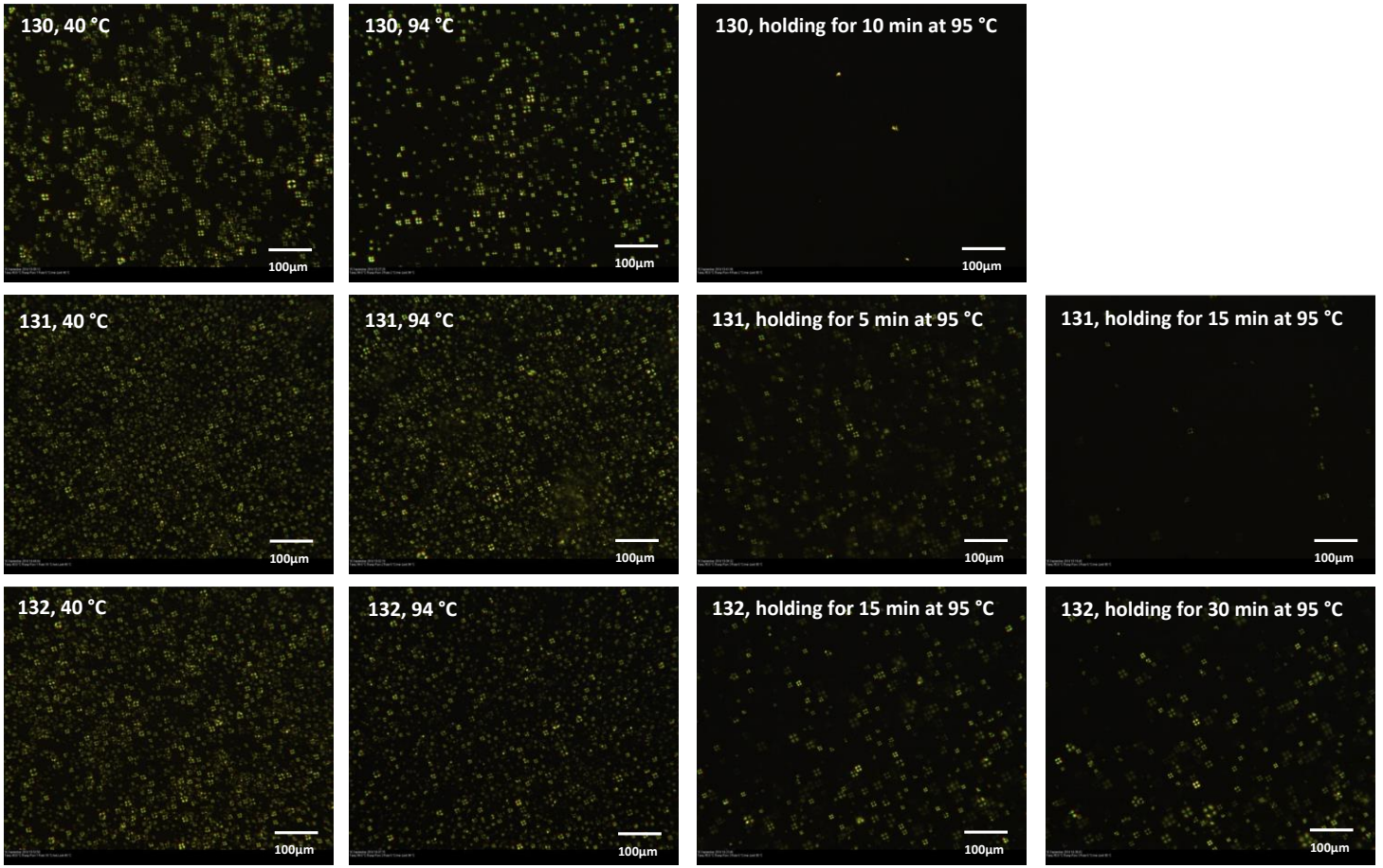


Figure 4.9: Hot stage microscopy of the tapioca starches 130 -132 (section 2.4.8.2). Polarized images are obtained to visualise dissolution of the granules in NMMO – water.

4.4 Further analysis of the tapioca group

4.4.1 Complex viscosity in NMMO-water

The complex viscosity in the NMMO-water system measured immediately after the RVA measurement (as explained in section 2.4.12.1) is shown for the tapioca group in Figure 4.10. The data for angular frequency versus the complex viscosity showed marked differences between the starches in the tapioca group and especially the crosslinked starches showed an increase in complex viscosity compared to the native tapioca starch (130) and the stabilised tapioca starch (112). At low angular frequencies the order of increasing complex viscosity for the crosslinked starches was 111> 115> 106> 116 with the increasing crosslinking order being 115> 111> 106> 116. However at higher angular frequencies (e.g. 10) the order changed and the resulting order of complex viscosity was 111> 106> 115> 116.

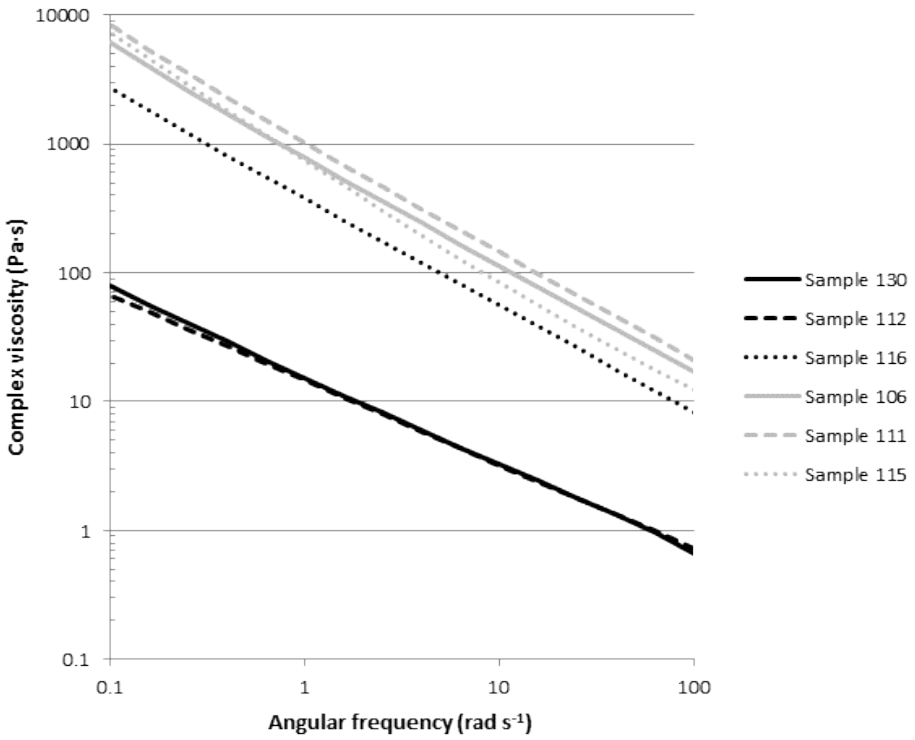


Figure 4.10: Rheological measurement showing the angular frequency versus the complex viscosity for the tapioca group of stabilised and crosslinked starches (130, 112, 106, 116, 111, 115) right after RVA measurement. Data show one out of two similar replicates.

These rheological data showed differences that previously had not been observed in the pasting viscosities at high shear in the NMMO-system (Figure 4.4) and had not been shown in the pasting viscosities for water either (Figure 3.4 and 3.5). The pasting in the RVA followed by complex viscosity measurements showed clear differences between the crosslinked and non-crosslinked samples, but the level of crosslinking did not seem to be reflected in the assessments. This could have been masked by the fact that the samples were also stabilised which added complexity to the interpretation of the data.

4.4.2 Iodine staining combined with pasting properties

An observation that was reported in chapter 3 was that the modified starches interacted with iodine. Another possible approach to differentiate between the modified starches could be to follow the addition of either iodine or lipids to the starch solutions that could then perhaps result in the formation of the amylose-iodine (and maybe amylopectin-iodine) or amylose-lipid complexes. These tests could indicate if any amylose was available for these complexes. If the complexes were formed, their behaviour in terms of solubility of the polymers or impact on granular integrity could be examined.

Samples from the group of tapioca starches (see Table 2.3) were pasted using the method given in section 2.4.4 and adapted to examine the pasting profiles when iodine was added. The amount of iodine relevant for these tests was determined during the preliminary experiments with sample 112. Figure 4.11 shows how the pasting properties of the stabilised tapioca starch (112) were affected by the increasing amount of iodine added to the mixture. The stepwise increase in iodine concentration showed a clear trend of increasing viscosities for the chemically

stabilised starch. The concentration of starch remained the same for all experiments and only the amount of iodine changed. The addition of the different concentrations of iodine had a clear effect on the colour of the samples as well. With increasing concentrations of iodine the blueness (purpleness) of the sample increased markedly. Also the colour intensity increased upon storage of the sample (observed for sample 111-112). The blueness of the sample was dependent on the amount of iodine added thus at very low concentrations the samples were light pink (data not shown).

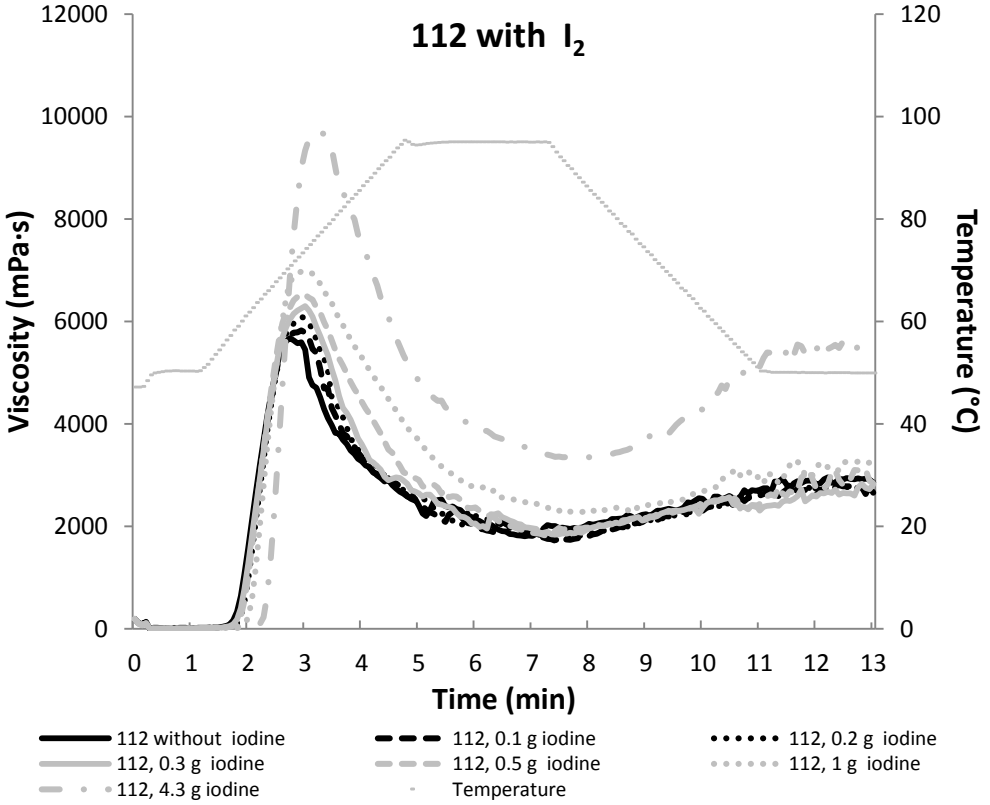


Figure 4.11: Different levels of iodine (0.05M) were added to the stabilised tapioca starch (112) and pasted resulting in the RVA pasting profiles displayed.

There could be several possible mechanisms resulting in the observed increase in viscosities:

- The iodine dissolved in the water and the resulting iodide ions may have increased the solvent quality causing greater swelling of the granules and higher hydrodynamic volumes of the starch polymers in solution.

- Upon formation of the iodine-amylose complexes these robust and rigid helices may have stabilised the swollen granule and reduced the rate at which it was broken down. This may have resulted in high viscosities.
- As the amylose was released from the granule the iodine-amylose complexes were formed and increased the viscosity of the system. These may have occurred as single helices, but also as major complexes perhaps with the iodine inside and between the chains.

It should be remembered that the sample 112 was a stabilised starch and would have had propylene groups associated with the amylose. These substitutions would have reduced the amyloses solubility so it is possible that increasing solvent quality was an important factor. Despite the starches being chemically substituted dark blue colouration was observed at high iodine concentrations, so it did appear that iodine complexes were formed and they could have played a stabilising role either inside or outside the granule.

The relevance of the stabilisation of the starch and the iodine was evident when comparing the results for sample 112 with the native tapioca starch (130). For the native sample the addition of 1 g of iodine led to an increase in peak and end viscosities whereas adding 4.3 g iodine resulted in no viscosity development (see Table 4.4). This suggested that at these high levels of iodine addition, the polymers aggregated and precipitated. These results also suggested that there was a level where the iodine-amylose complexes remained in solution and added to the perceived viscosity. The increase in iodine aggregation caused a net decrease in hydrodynamic volume.

Table 4.4: Peak and end viscosities for the stabilised and crosslinked tapioca group (increasing native (130), 112, 116, 106, 111 and 115) with increasing addition of iodine (RVA pasting see section 2.4.4)

Sample	Iodine (g)	Peak viscosity (mPa·s)	End viscosity (mPa·s)
130 : Native tapioca	0.0	5700	3693
130: Native tapioca	1.0	6249	6175
130: Native tapioca	4.3	36	92
112: Stabilised tapioca	0.0	5677	2855
112: Stabilised tapioca	0.006	5927	2944
112: Stabilised tapioca	0.1	5826	2658
112: Stabilised tapioca	0.2	6095	2691
112: Stabilised tapioca	0.3	6300	2820
112: Stabilised tapioca	0.4	6399	2846
112: Stabilised tapioca	0.5	6538	2842
112: Stabilised tapioca	1.0	6984	3216
112: Stabilised tapioca	4.3	9720	5503
116: Stabilised very low x-link tapioca	0.0	9763	8831
116: Stabilised very low x-link tapioca	1.0	8998	10730
116: Stabilised very low x-link tapioca	4.3	6755	9714
106: Stabilised low x-link tapioca	0.0	12195	13810
106: Stabilised low x-link tapioca	1.0	11033	13741
106: Stabilised low x-link tapioca	4.3	8596	14000
111: Stabilised medium x-link tapioca	0.0	12497	15387
111: Stabilised medium x-link tapioca	0.1	12330	15130
111: Stabilised medium x-link tapioca	0.2	11737	15020
111: Stabilised medium x-link tapioca	0.3	11427	15242
111: Stabilised medium x-link tapioca	0.5	11015	15037
111: Stabilised medium x-link tapioca	1.0	9970	14395
111: Stabilised medium x-link tapioca	4.3	5767	9223
115: Stabilised high x-link tapioca	0.0	855	2382
115: Stabilised high x-link tapioca	1.0	1001	3062
115: Stabilised high x-link tapioca	4.3	395	1174

For the stabilised and medium crosslinked tapioca starch (111) increasing levels of iodine resulted in decreasing viscosities (see Figure 4.12). A similar trend was observed for the other stabilised and crosslinked tapioca starches (see Table 4.4). The consistent decrease in viscosity for all the stabilised and crosslinked tapioca starches suggested that the crosslinking is critical. If much of the amylose was held covalently together in a pre-existing crosslink then only the free flexible portions of the chain would be able to interact with the iodine. It appeared that the net impact was to reduce the overall volume occupancy of the joined macromolecules. Even at the highest levels the iodine did not cause total loss of viscosity. Therefore the chain lengths available for the interaction was likely reduced due to their covalent linkages to other amylose chains which may have inhibited mass aggregation.

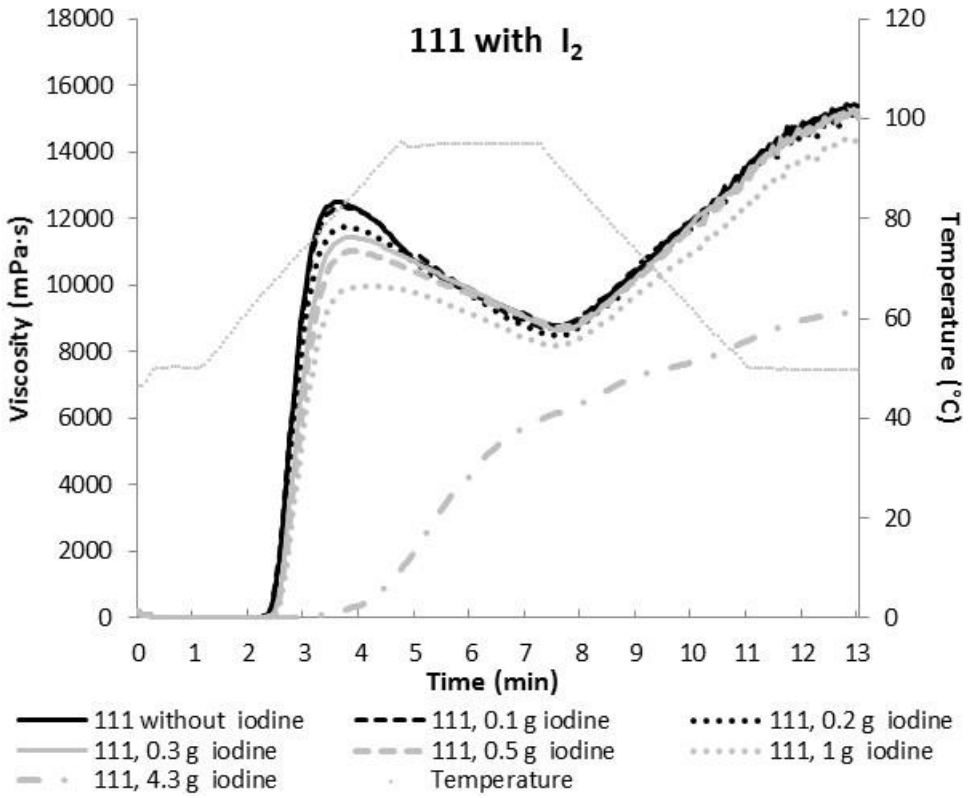


Figure 4.12: Different levels of iodine was added to the stabilised and medium crosslinked tapioca starch (111) and pasted resulting in the RVA pasting profiles displayed (for method see section 2.4.4).

By adding the iodine, which would bind to the already crosslinked amylose/amylopectin, granule rigidity would be expected to increase while there

would be a decrease in the capability of granular swelling and leaching of amylose. The results for the iodine staining, combined with the pasting properties, imply that some free amylose/amylopectin were available in the modified starches and that their interaction with ligands was different from unmodified starches. The study of modified starches with iodine may aid in understanding the behaviour of the starches, and such functionality in batters may be relevant. Iodine is not the only material known to form complexes with the amylose. Amylose is also known to form amylose-lipid complexes and their creation may have a profound impact on the starches functionality. The concept of whether amylose-lipid complexes are different for the modified starches is discussed in the next section.

4.4.3 Glycerol monostearate combined with pasting properties

The addition of glycerol monostearate (GMS) to a solution of starch will be thought to interact with the available amylose upon gelatinisation of the starch. The addition of GMS was a modification of the standard method for pasting properties (see section 2.4.4). The solid GMS particles were dry mixed with the starch before adding the water leading to a homogeneous mixture. Several concentrations were tested and the pasting curves are presented in Figure 4.13. It was observed that the addition of GMS to the stabilised tapioca starch (112) only slightly changed the pasting curve, increasing the peak viscosity with the addition of 1 g GMS. The iodine induced changes reported in the previous section therefore could have been due to the solvent quality enhancement.

The stabilised and crosslinked tapioca starches appeared to react differently to GMS addition. Sample 116, 106 and 111 with increasing crosslinking were all influenced by the addition of the high amount (1 g) of GMS to the solutions resulting in decrease in pasting viscosities. The stabilised and highly crosslinked tapioca starch (115) changed its pasting properties, but contrary to the other modified starches,

the pasting viscosity increased with increasing addition of GMS. Higher viscosities were also observed for the non-modified tapioca starch (130).

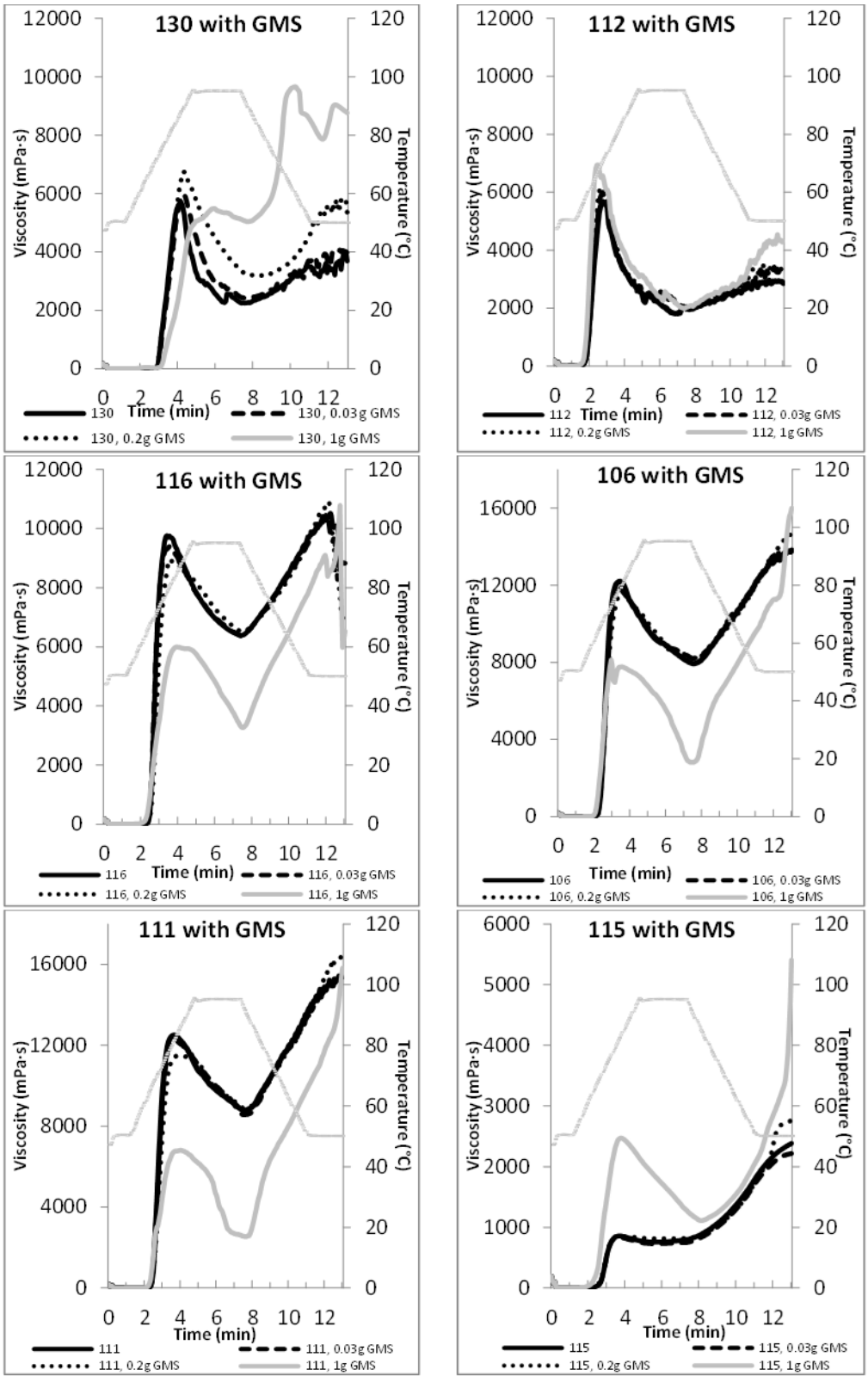


Figure 4.13: Different levels of GMS was added to the group of stabilised and crosslinked tapioca starches (130, 112, 116, 106, 111, 115) and pasted resulting in the RVA pasting profiles displayed.

The mechanisms for the interplay between materials and the starches would appear to be complex and only tentative suggestions for these could be explored. For the materials where the substitution of the amylose did not occur or was not relevant, as the amylose was so heavily tied into the structures due to substantial crosslinking, the addition of 1g GMS resulted in an increase in viscosity. This could be due to micellar formation of the lipids dominating the viscosity profiles. For the stabilised sample alone or with samples with lower crosslinking, perhaps there was sufficient interactions of the amylose with the lipids to stop the micelles being a major issue in the observed viscosities. For the lower levels of crosslinked samples with stabilisation at high lipid concentrations the viscosities were lower and the reasoning could be the same as put forward for the iodine.

The behaviour of the tapioca group of modified starches was divergent and difficult to interpret. The stabilised and highly crosslinked starch (115) seemed to stand out from the other stabilised and crosslinked starches, especially in its pasting properties (in water, water-NMMO, with addition of iodine or GMS). The information obtained from the supplier of the starches was that the starches had all been stabilised to the same level, but with different levels of crosslinking. However, there was no indication of the exact levels of substitution. The processing variables that enabled the chemical modification of the starch were also not detailed, but these could possibly impact on the behaviour of the starch.

Prior to modification the starches are usually pre-treated with a basic solution (NaOH) and sodium sulphate solution (Na_2SO_4) to open up the structure of the starch granules and facilitate modification. Work reported in this section did indicate that solvent quality and the influence of an ionic environment may be relevant to the starches performance. In order to understand whether the pre-treatment could

influence the final properties of the starches an experiment containing a pre-treatment procedure was conducted.

4.4.4 Pre-treatment of native tapioca starch

In the chemical modification of native starches, the starches undergo quite harsh conditions before addition of the actual modifying agent. Four pre-treatments were chosen to mimic the most relevant pre-treatments for starches. The method for pre-treatment was adopted and modified from (Wattanachant et al., 2003). It was set-up to test the condition of pre-treatment prior to modification of starches. The method was developed to understand the difference between the pre-treatments and the cause and effect properties.

4.4.4.1 Pre-treatment method

The four samples prepared were as follows

I. native tapioca (130) + water (reference sample)

II. native tapioca (130) + 10% Na_2SO_4

III. native tapioca (130) + 5% NaOH solution

IV. native tapioca (130) + 10% Na_2SO_4 + 5% NaOH

Pre-weighed portions of 50 g starch (130) were transferred to 250 ml Duran flasks. Then 100 g 10% Na_2SO_4 solution, 100 g 5% NaOH solution and 50 g 20% Na_2SO_4 + 50 g 10% NaOH solutions were added to each respective flask. Lumping was avoided by shaking. When the solutions were properly mixed, they were left overnight to hydrate. Five washes with water were done by letting the starch sediment and removal of the supernatant by pouring gently. Centrifugation at 2400 g for 3 minutes was required for sample III and IV to sediment. For each washing step 100 g water was added to the blend and the dispersions were shaken severely. The fourth washing step was left mixing overnight on a bench-top roller mixer. The pH value of

the fifth waste water was noted. The samples were dried in an oven overnight at 40 °C, ground with a mortar and pestle and sieved to a final particle size of < 425µm.

The pH values of the fifth waste water samples were similar for samples I and II and were pH 8.2, whereas the pH of samples III and IV were similar with a value of pH 12.3. The samples I - IV were all off white powders and their pasting viscosities in water was tested to explore the effect of the pre-treatments.

4.4.4.2 Pasting properties after pre-treatment

Figure 4.14 presents the pasting curves for the samples I - IV in water including the reference sample 130 without any pre-treatment. It is observed that samples I and II develop a higher peak viscosity compared to the reference sample (130).

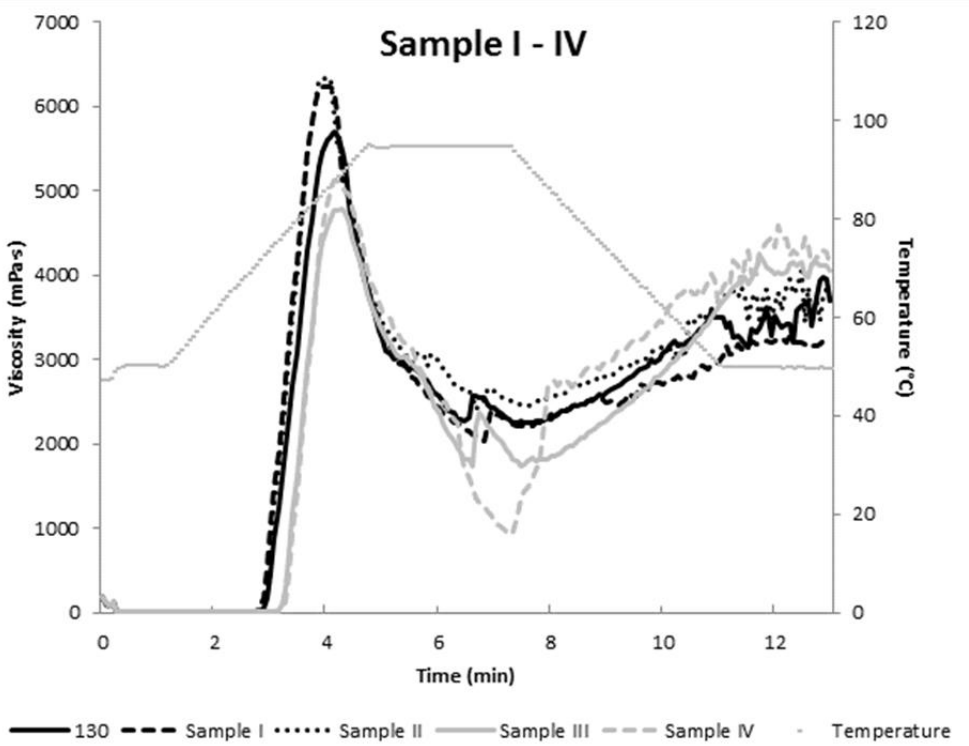


Figure 4.14: RVA pasting profiles (10.7%) for sample 130 and the pre-treated sample I – IV (see section 2.4.4)

The pasting curves for samples I and II were very similar indicating that washing with water (sample I) did influence the properties of the starch compared to the non-treated sample 130. The sample treated with 10% Na_2SO_4 had a similar effect as

washing with water (II). However, the two samples pre-treated with 5% NaOH solution (III) and 10% Na_2SO_4 + 5% NaOH solution (IV) showed a decrease in pasting viscosities. Due to the impact of the harsh pre-treatment it was interesting to explore the immediate effect of changing the solvent from water to a sodium sulphate solution.

4.4.4.3 Pasting in 10% sodium sulphate solution

The group of stabilised and crosslinked tapioca starches remained interesting due to their divergent properties and therefore their pasting in 10% Na_2SO_4 was carried out. The resulting pasting curves are presented in Figure 4.15. A consistent trend was that all the pasting temperatures increased with the change of solvent from water to 10% Na_2SO_4. Sample 130 attained a similar peak viscosity and the stabilised tapioca starch only obtained a slightly lower peak viscosity. The stabilised and crosslinked starches 116, 106, 111 and 115 all showed a decrease in their peak viscosities when the 10% Na_2SO_4 solution was used as the solvent.

Comparing the pre-treated samples (Figure 4.14) with these results it only showed a shift in pasting temperature for the samples treated with 5% NaOH (III) and 10% Na_2SO_4 + 5% NaOH (IV). The sample treated with 10% Na_2SO_4 solution (II) remained at the same pasting temperature as the references. The samples were washed after pre-treatment which might suggest that the pre-treatment did not have an effect at room temperature and the salts were washed out prior to the RVA pasting measurement. The impact of pre-treatments, as mimicked by the addition of salts in this work, was insufficient to explain the differences in behaviour of the modified starches.

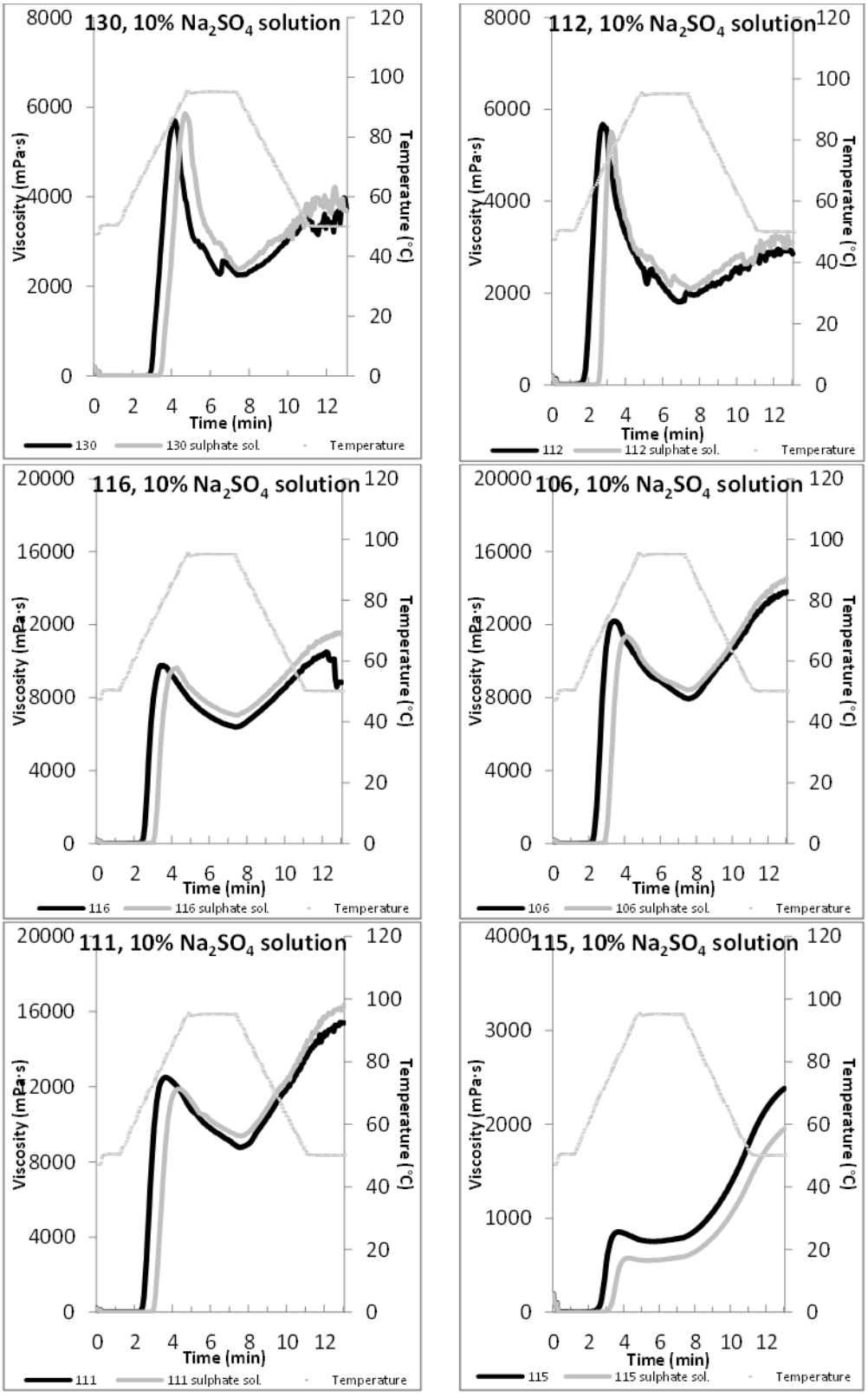


Figure 4.15: RVA pasting profiles (10.7%) with 10% Na_2SO_4 solution for reference sample 130 and the tapioca group (112, 116, 106, 111, 115)

The modifications of stabilisation and crosslinking have proved to be a very complex system even though information was given by the commercial supplier on the

relative levels of stabilisation and crosslinking. However, it is important to note that these given values were based on the level of chemicals added and not necessarily on the actual level of stabilisation and crosslinking (measured by degree of substitution or molar substitution).

A numerical value for the levels of modification would be much better for correlation of the functionality. The empirical measures that were tried to achieve more understanding of the materials were difficult to interpret in terms of the suspected levels of substitution and therefore more direct assessment of the chemical entities was attempted. The results are given in the following section.

4.4.4.4 The use of nuclear magnetic resonance in the understanding of stabilisation and crosslinking of starches

Nuclear magnetic resonance (NMR) is a powerful technique to visualise chemical bonding and it was therefore used to understand the modifications of starches from the tapioca group. ^{13}C-NMR and ^{31}P-NMR techniques were recorded with the support from the Centre for Biomedical Sciences, University of Nottingham, University Park and in collaboration with Huw Williams and Dr. Bill MacNaughtan who kindly recorded the required data. The data recorded using solid state NMR was analysed with the focus of detecting the stabilisation and crosslinking levels of the starches. The complex structure of the starch polymers (amylose and amylopectin) resulted in a very complicated NMR spectrum (Sperger and Munson, 2011, Thérien-Aubin et al., 2007) and these signals were acknowledged, but not assigned in the recorded spectra. Figure 4.16A shows the ^{13}C-NMR spectrum recorded for some selected starches. It is seen that the spectrum was very complex and therefore areas appointed to modification have been enlarged (B and C).

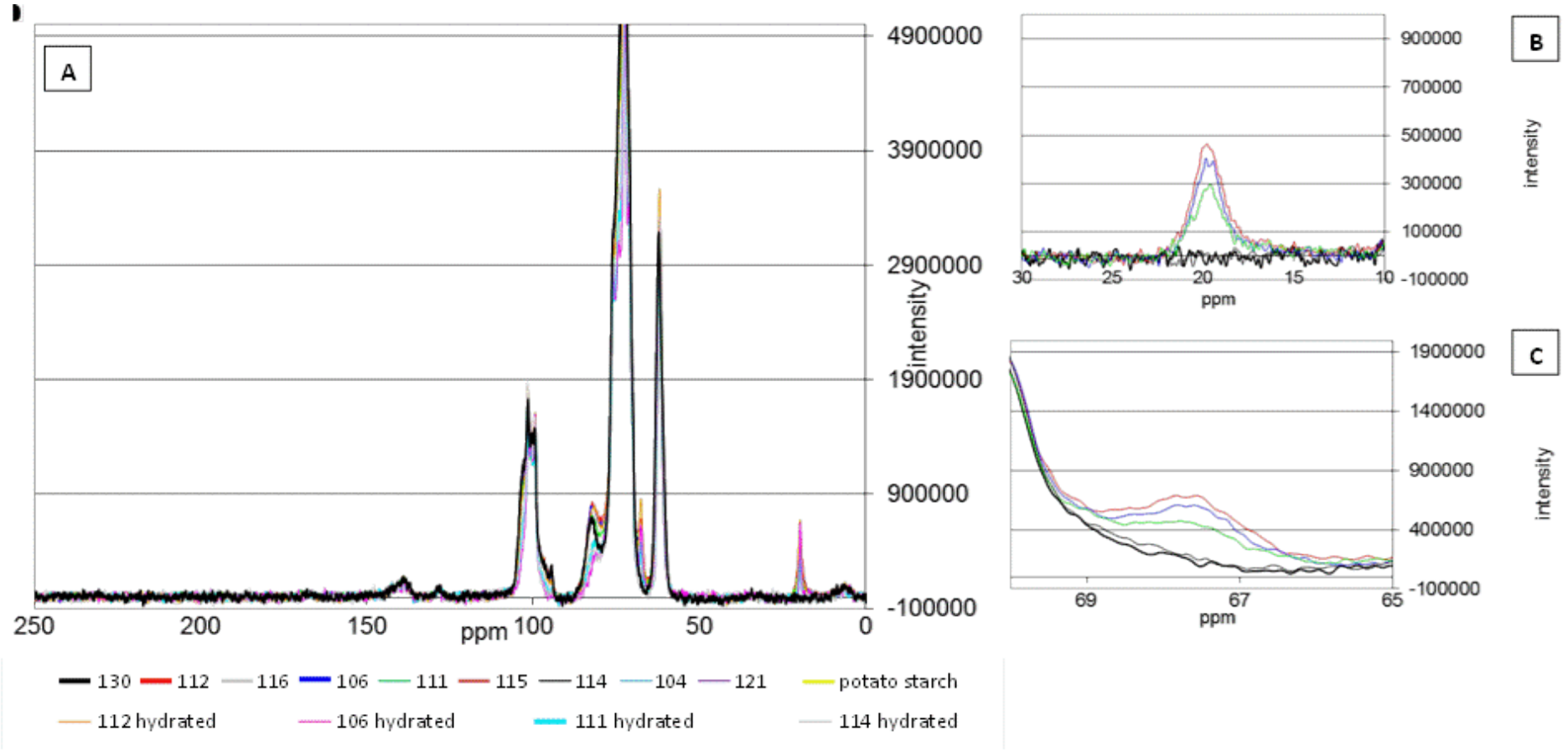


Figure 4.16: solid state ^{13}C-NMR of selected samples to explore the modification of the starches. Some samples were hydrated in order to increase the signal intensity.

Signals are enlarged for the area of 65-70 ppm (C) and 10-30 ppm (B). These areas of the spectrum showed a difference in intensity of the signal, which was indicating the change in modification. Both signals at around 67.5 ppm and 20 ppm showed the similar order of intensity. These signals were predicted to be connected and refered to the stabilisation of starches (Dragunski and Pawlicka, 2001).

In spectra B and C only the native tapioca starch (130), the stabilised tapioca starch (112), the stabilised and low crosslinked tapioca starch (106), the stabilised and medium crosslinked tapioca starch (111)) and the low crosslinked maize starch (114) are displayed. An increase in intensity of these signals (Figure 4.16 B and C) was only observed for the stabilised samples (112, 106 and 111). Neither the native starch (130) nor the low crosslinked maize starch (114) showed any increase in intensity. The signals were therefore presumably connected to the stabilisation. It was unclear whether it was the level of stabilisation or maybe a combined factor with the crosslinking that caused the differences in ^{13}C-NMR signal intensities.

The intensity order of the signals were 112> 106> 111 which complied with the increased crosslinking (the level of stabilisation was thought to be similar, but this remained untested). Further ^{13}C-NMR experiments were conducted (data not shown,) including the rest of the tapioca group, which resulted in the intensity order 112> 106> 116> 111= 115 and therefore it did not totally correlate with the suggested level of crosslinking. The crosslinking agent for these starches was phosphorous oxychloride and hence phosphorous was incorporated upon crosslinking. This led to believe that the amount of phosphorous might be detectable using ^{31}P-NMR. Figure 4.17 shows the ^{31}P-NMR spectrum of the stabilised and highly crosslinked tapioca starch (115) and potato starch, which naturally contained phosphorous, as a reference sample. As seen in Figure 4.17 the signal

from the crosslinking was not detectable and a signal was only observed for the native potato starch. The use of nuclear magnetic resonance to determine stabilisation and crosslinking might be possible if a controlled experiment with known (and high) amounts of stabilisation and crosslinking was obtained and the values of the levels of modification could be verified.

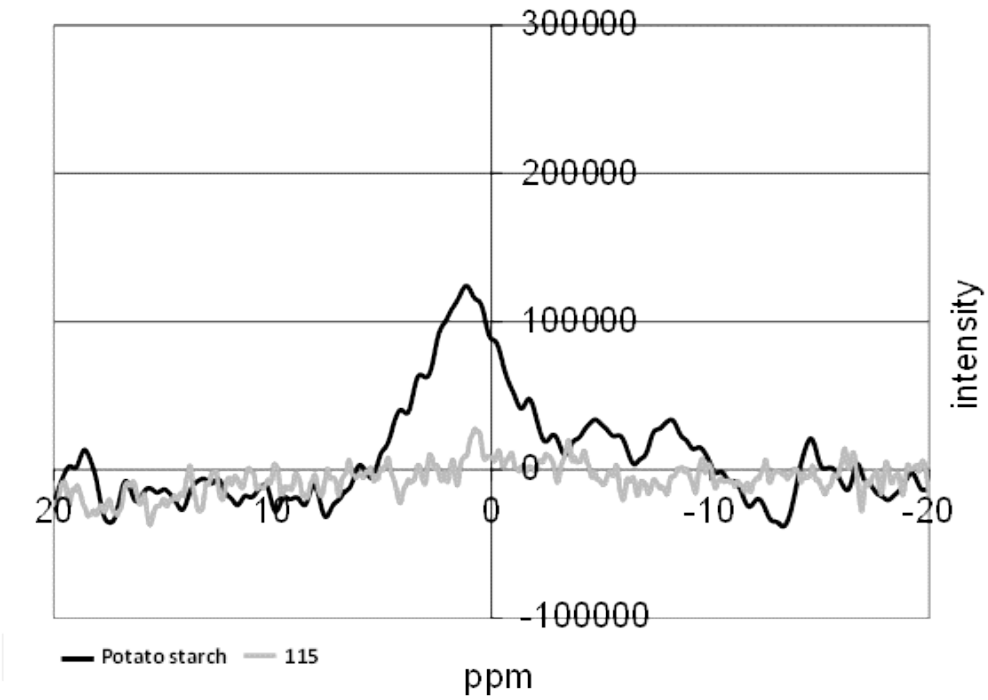


Figure 4.17: Solid state ^{31}P-NMR for potato starch and the stabilised and highly crosslinked tapioca starch (115). The analysis was done in cooperation with Huw Williams and Bill MacNaughtan (for method see section 2.4.11).

4.5 Discussion

A feature of the work carried out and presented in this thesis was that commercial samples were to be used. Only little information was provided by the commercial suppliers regarding the actual levels of stabilisation and crosslinking which made the understanding of the characteristics of the modified starches difficult. The tapioca group should have formed a family of materials where the changes in the chemical modification should have dominated their differences and therefore this set of samples was examined in some detail. Several studies have investigated different levels of modification, either stabilisation and/or crosslinking, but only little is known about the combination of stabilised and crosslinked starches with increasing levels of crosslinking (see chapter 1).

The group of modified tapioca starches (112, 116, 106, 111 and 115) had unique pasting properties in both water and NMMO-water, which resulted in different orders of peak and end viscosities dependent on concentration and solvent. These results indicated that pasting properties were dependent on the complexity of the starch granule. They confirmed the relevance of the original granule structure, its robustness on swelling and the importance of other materials, inside, outside and at the surface of the granule. The complexity of these factors seemed to have increased due to modification and therefore the predicted ranked orders were not achieved. The differences in the measured viscosity whilst heating and shearing in the RVA in excess water and limited water (NMMO-water) could to some extent be interpreted in terms of starch granule swelling and the relevance of the liberation of free polymer from the granule.

The ultimate aim of this work was to predict starch behaviour when processed in a recipe for a batter. For these potential film formers it was relevant to measure the

amount of available amylose/amylopectin since especially amylose is known to play an important role in film formation related to fried products (Altunakar et al., 2004, Mohamed et al., 1998). Hence the availability of amylose in the stabilised and crosslinked starches could indicate their strength as film formers. Even the measurement of the amylose levels proved difficult for these starches. One of the reasons suggested in the previous chapter was that if there was modification of the starches, precipitation may have occurred in the presence of iodine. This experimental work led to a modification of the normal RVA standard measurement through addition of iodine or glycerol monostearate. This modification was made to reveal the amylose/amylopectin available for complex binding if the polymers were forming helical structures.

The iodine staining RVA measurement suggested that the iodine did bind to some available amylose/amylopectin resulting in different pasting properties for the modified tapioca group. It was suggested that the iodine amplified stability, perhaps by increasing the solvent quality of the water for the more hydrophobic stabilised chains by the presence of the iodine. For the stabilised tapioca starch (112) this resulted in increased pasting viscosities with increasing iodine concentration. For the stabilised and crosslinked tapioca starches (116, 106, 111 and 115) the amylose forming interactions with the iodine will be limited as a result of the crosslinking of the chains. However, the iodine-amylose helices formed would reduce the swelling of the granule.

Glycerol monostearate did also bind amylose in the amylose-lipid complex upon gelatinisation. Interestingly, the addition of GMS (1 g) to the stabilised and highly crosslinked tapioca starch (115) resulted in an increase in pasting viscosities, whereas the other stabilised and crosslinked samples (116, 106 and 111) showed a

decrease in pasting viscosities (Figure 4.13). The high viscosities may have been an artefact of the high concentrations where the lipids may have formed micelles. The lower viscosities may have indicated that where the lipid can associate with the amylose, it reduces swelling of the granule. The stabilised and highly crosslinked tapioca starch (115) had a markedly different behaviour compared to the other stabilised and crosslinked starches. This was supported by the pronounced differences in pasting characteristics in different solvents (see Figure 3.4 and Figure 4.4).

The work in the presence of iodine and GMS demonstrated the dominant role of the granular structure in the typical measurements of starch functionality. The use of NMMO with only low levels of water present was designed to target the macromolecular structures rather than the swollen granule. Interestingly, the levels of water previously mixed with the NMMO and the time temperature regimes used to ensure full solubilisation of the granules did not seem to fully affect the solubilisation with these starches but did highlight how different the starches were in this limited water environment.

Prior to any chemical modification step when processing starches, the starches are pre-treated to facilitate the modification. Having shown the importance of the granular structures some minor work was undertaken to see the impact of this pre-treatment. The pre-treatment of the native tapioca starch (130) was visualised by pasting properties and confirmed that the pre-treatment with 5 % NaOH solution (III) and 10% Na_2SO_4 solution + 5% NaOH solution (IV) caused a decrease in pasting viscosities and increase in pasting temperature hence changing the properties of the starch considerably. Sample soaked in water or sodium sulphate did alter the pasting properties a little, but no major effects were observed. The pasting of

starches directly in a 10% Na_2SO_4 solution caused a change to the pasting properties of the starches and therefore it was explored, as the iodine effects could have been due to ionic composition, whether pasting in 10% Na_2SO_4 solution could reveal any new information on the group of tapioca starches. However results showed that all the stabilised and crosslinked starches behaved in a similar manner with a slight decrease in viscosity and shift to a higher pasting temperature.

The knowledge of the actual amounts of crosslinking and stabilisation levels would have been helpful throughout this work. To try and source this information the use of the solid state ^{13}C-NMR technique was tested and resulted in detection of the signals present due to stabilisation of the starches, but it remained unclear whether the stabilisation and crosslinking were connected to the intensity of the signals. The signal for crosslinking (incorporation of phosphorous) was too weak to be detected by solid state ^{31}P-NMR.

The use of the 78% NMMO - 22% water solvent system as a limited water environment differentiated between stabilised and crosslinked tapioca starches although the ranking order was not consistent with the information provided. However it did highlight the important difference in properties of a low moisture system compared to that in excess water, which is most commonly used in starch analysis. The NMMO-water system proved to be very powerful in differentiating three native tapioca starches revealing different pasting characteristics in NMMO-water, but similar pasting properties in excess water. Also the DSC measurement in NMMO-water confirmed the differences of the starches which were not obvious when using water as a solvent. Hence NMMO-water can be used as solvent to differentiate between native starches of same origin which is not easily revealed by other methods.

CHAPTER 5 CHARACTERISATION AND BEHAVIOUR OF BATTERS AND HOW THEY BEHAVE AT VARIOUS WATER LEVELS

The previous chapters looked at analyses of some of the individual starches that are used in the manufacture of batters. In practice starches and other materials are used in combination to create the properties required in the finished food product. The work reported in this chapter was targeted at understanding the behaviour of batter formulations that contained a combination of starches.

5.1 Batters and their natural environments

Batters in the food industry are most often used to provide functionality at very low moisture levels in the final product. The batters are prepared in excess water before being applied to a base. This is then followed by processing which is usually either frying or cooking depending on the desired product. The change in moisture as the batter is cooked is likely to be a critical factor for the phase transitions occurring in the starches. The hypothesis therefore was that the creation of the desired characteristics of the batters is related to the hydration levels of the carbohydrates, that individual starch components handled moisture differently and that this difference impacted on the desired properties of the batter.

The moisture loss or gain within the batter after frying/cooking are crucial for the final quality attributes of the product. Changes in moisture are dependent on the chemical nature of the ingredients and on the physical format of the batter. The importance of the functionality of the individual ingredients in the batter formulations has been addressed in chapter 3-4 and this chapter will focus on the composition of the batters and their behaviour at various water levels.

To understand the interactions of the batter ingredients with water at different stages of the manufacturing and upon storage of the battered product, the process needed to be considered. The stages used in batter processing are given below.

1. Initially the batter is hydrated at a concentration of 40% solids. This suspension is used to coat the base material. (This is presented in section 5.2 and 5.4.1 for a model system for coating and frying batters).
2. The batter coating is subjected to heat (this may or may not result in dehydration). Depending on the moisture levels, the starches in the batter may gelatinise, resulting in thick paste coverings of the base material. (Data on the pasting properties of the batters in excess water is given in section 5.3).
3. Storage of the preheated batters may allow reorganisation of the starchy components leading to changes in texture. (Data on storage of hydrated batter materials is given in section 5.3).
4. Critical to the final texture of the product are the final moisture contents of the batters. How the ingredients interact with low moisture or rapidly changing levels of moisture is important. Several different factors will be relevant:
    a. Diffusion of moisture from base to batter and final moisture contents (this is looked at in some detail in section 5.4.3).
    b. Macro structuring of the batter layer during rapid moisture loss (some details given in section 5.2 and 5.4.1 within this chapter when frying of model systems was investigated).
    c. Crispiness of batter layer (details of texture assessment are given in 5.4.4 and more understanding of this is presented in the following chapter).

Batters are made from blends of different materials where the dominant components are starch based. Provided to this project were 10 batters that were coded 2001-2010 (Table 2.5). These batters had been selected by the commercial partners to show differences in behaviour due to their various different starch components.

5.2 Frying of batters

A major stage in the creation of a battered product is the frying steps. The high temperatures (frying is typically carried out at 180-185 °C) and thin coating of the batter allows for a rapid dehydration of the batter coat that ideally leaves behind a layer that reduces fat uptake into the product and produces a crispy outside layer, while retaining a soft core.

When starches within batters undergo frying as part of an industrial process they may undergo structural changes. This change may involve the starches' non-processed form undergoing loss of molecular order upon frying resulting in film formation surrounding the base. The focus of this section was to explore the different batter properties with regard to behaviour upon frying, the wet batter pickup and the oil uptake of the batters. As batters are used on products, it would be expected that the moisture content of the materials forming the base matrix onto which the batter is placed will also influence the batter's performance. To avoid this complication, a model base was used for this stage of the study. Filter papers were used as the base matrix onto which a batter can be added with the expectation that this will mimic the industrial process of fried products sufficiently to allow elucidation of the relative importance of the different components in the batters.

5.2.1 The behaviour of batters 2001-2010 upon frying

The method for testing the batter properties upon frying is given in section 2.5.1.3. Due to the different non-carbohydrate ingredients such as spices and aromas added to the batters, the colour of the batters were very different, which therefore also resulted in different colours upon frying. Figure 5.1 shows examples of the batters 2001-2010 after frying and cooling to room temperature. The fried samples were visually assessed immediately after frying and it was noted that samples 2005, 2007 and 2009 all had bubbles in the created films (some of which had burst so the filter paper was visible). The other samples (2001-2004, 2006, 2008 and 2010) were similar in film forming properties when visually assessed.

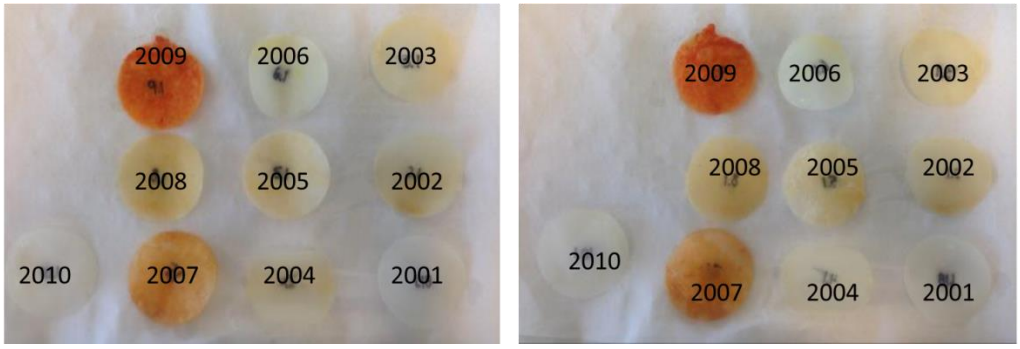


Figure 5.1: Batter 2001-2010 applied to filter paper and fried. The samples were visualised from the top (left) and the bottom (right) since bubbles mainly appeared on the bottom of the samples.

The wet batter pickup values and the oil uptake values (for methods see section 2.5.1.3 Table 2.9) were measured as these parameters were important for the performance of the batters and the attributes of the final product (see Figure 5.2). The wet batter pickup value denoted how much batter mixture stayed on the base after applying the batter. This was relevant for the amount of moisture required to flash off during frying and the thickness of the final film. The oil uptake was measured as the weight gain after frying (subtraction of the weight of the filter paper (base) and the batter solid uptake from the weight of the fried filter paper, see equation 2 Table 2.9) and reflected the film's porosity or capability to create a barrier for the oil not to access the filter paper, but also the batter's ability to take

up oil itself. Figure 5.2 shows the correlation of wet batter pickup versus oil uptake and it is clear that the batters can be differentiated into three groups.

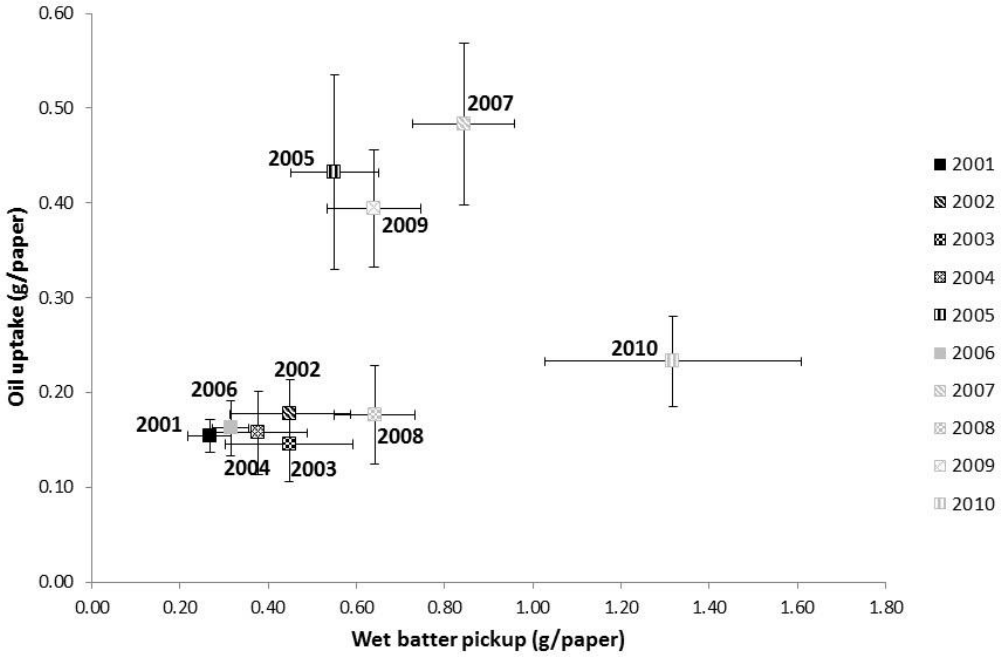


Figure 5.2: The wet batter pickup versus the oil uptake for the batters 2001-2010 for a 36% solids mixture. n = 10 and error bars are showing the standard deviation.

Batter mixtures 2001-2004, 2006 and 2008 were in the same group with low wet batter pickup values and low oil uptake values. Another group included the batter mixtures 2005, 2007 and 2009 which had clearly higher oil uptake values, whilst similar wet batter uptake values as the other group of 2001-2004, 2006 and 2008 were observed. The third group was sample 2010 which was remarkably different from the other batters with a very high wet batter pickup, but a relatively low oil uptake value. It was noted at the time of the frying experiment that batter 2010 had a very high cold viscosity compared to the other batters and hence the change in solids must have altered the properties of the mixture. This is discussed further below.

Table 2.5 shows the major components in the batter blends. From this knowledge some linkage of the frying performance to the ingredients can be postulated. The

notable ingredient for sample 2010 is xanthan gum, which is not contained in any of the other batters. The addition of gum to the batter mixture is most likely directly linked to the high viscosity of the batter mixture before it was applied to the base. Thus this high viscosity of the suspension resulted in a very high wet batter pickup value.

It can be seen from Table 2.5 that the batter samples 2005, 2007 and 2009 contained wheat flour, which was not contained in any of the other batter formulations. Previous studies have shown that wheat flour is known for creating bubbles in the film formed by batters containing this material (Coates, 2014). The reasons why the flours give rise to such bubbling and hence high oil uptakes has not yet been fully understood. However, it is reasonable to link the burst bubbles, as observed for these samples, with higher oil uptake values due to the base (filter paper) being accessible for the oil. The other indicator that some of the batters caused an aerated and non- flat surface for the films was from the stacking height data.

The measurement of the stacking heights for the model fried system is given in section 2.5.1.3.1. The stacking data of the batters is given in Figure 5.3. The two batters with the greatest stacking height (2005 and 2007) contained wheat flour, but the third sample, 2009, that also had wheat flour as an ingredient and showed high oil uptake, did not have a high stacking height (see Figure 5.4). Although the stacking heights of two of the wheat samples were high, they were not significantly different from many of the other batter samples (see Figure 5.3).

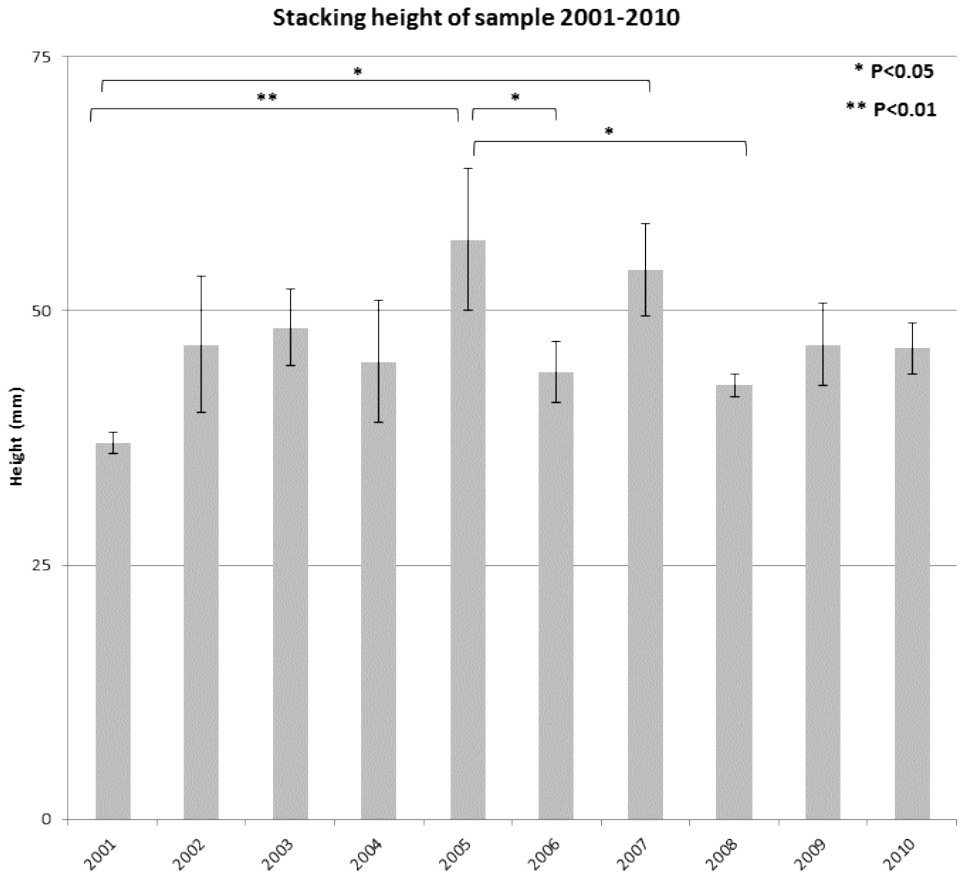


Figure 5.3: The stacking height of fried batters 2001-2010 (36% solids mixture). The average value of 10 replicates (n = 10) has been given and error bars mark the standard deviation. The one-way analysis of variance (ANOVA) test has been used for statistical analysis.

The stacking height for batter sample 2001 appeared to be rather different from the other batters. However, the stack height data for sample 2001 was only significantly different from wheat flour containing batter samples 2005 and 2007 ($p<0.01$ and $p<0.05$ respectively) and not statistically different from the other samples. The individual ingredients for the batter sample 2001 were not markedly different from the other samples. Sample 2010 had a very high wet batter pickup (see Figure 5.2) that was not reflected in the stack height data. This may be due to that batter not forming aerated bubbles and as a result the batter film was closely covering the filter paper.

The results shown in Figure 5.3 emphasise that especially samples 2005 and 2007 form a different film (creating bubbles) compared to the other batters, which led to

the higher stacking height. The other batter sample forming bubbles (2009) had a stacking height similar to the other batter mixtures and the formation of bubbles in that batter was not confirmed by the stacking height value. This was likely due to the bubbles created being fewer or smaller.

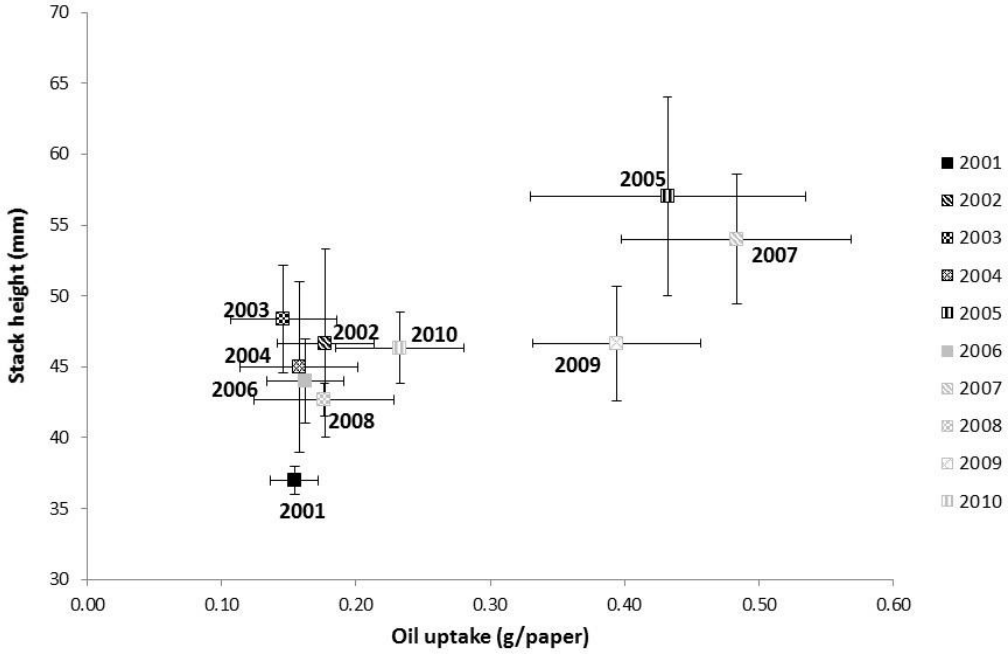


Figure 5.4: The wet batter pickup versus the oil uptake for the batters 2001-2010 for a 36% solids mixture. n = 10 and error bars are showing the standard deviation.

The attributes of the batter mixtures 2001-2010 vary and it was clear that some ingredients play an important role in the perception of the final product. The results obtained showed that the wheat flour and the stabiliser (xanthan gum) had a big impact on some properties of the batters, namely bubble formation and initial viscosity of the batter mixture respectively.

For the preparation of the batter solution (see section 2.5.1.3.1) the mixing was done at room temperature and therefore the gelatinisation temperature of the starches would not be reached. Hence cold soluble stabilisers (like xanthan gum) would be the main viscosity developer together with the solids content. If pregelatinised starches were present, these would increase viscosity as well. Due to

different batter components (see Table 2.5) the initial viscosity of the batter solutions would be slightly different which may have led to different wet batter pickup. The wet batter pickup could be adjusted during the processing e.g. by altering time and speed of batter application or (blow) drying before frying. The pasting behaviour of the batters in excess water may indicate the development of viscosity and the initial gelatinisation behaviour prior to a possible melting of the starches at lower water levels and film formation during the frying process (see section 1.1.1.1).

5.3 Batters and their properties at high water levels

The 10 batters 2001-2010 were investigated to demonstrate if there were significant differences in their performance at moisture levels in excess of those typically used in batters and at levels not directly comparable to the different stages of batter functionality.

The initial testing was to paste the batters 2001-2010 in excess water using the standard pasting method (see the method in section 2.4.4). Although the batter concentration was lower (12.5%) compared to a standard batter solution (solids:water = 40:60), the properties in excess water could indicate some of the batter characteristics, e.g. the viscosity development under controlled heating and shear. Figure 5.5 shows the pasting curves for the batters and indicates that they were different in terms of the peak and end viscosities, the breakdown (difference between peak and lowest viscosity) and the temperatures at which the viscosities first started to be recordable. Batter 2006 developed a markedly higher viscosity compared to the other samples. Most of the batter mixes had peak viscosities between 1200-3200 mPa·s (2001, 2003-2005 and 2007-2009). Only samples 2002

and 2010 had abnormally shaped curves (no peak viscosities) and lower viscosity development.

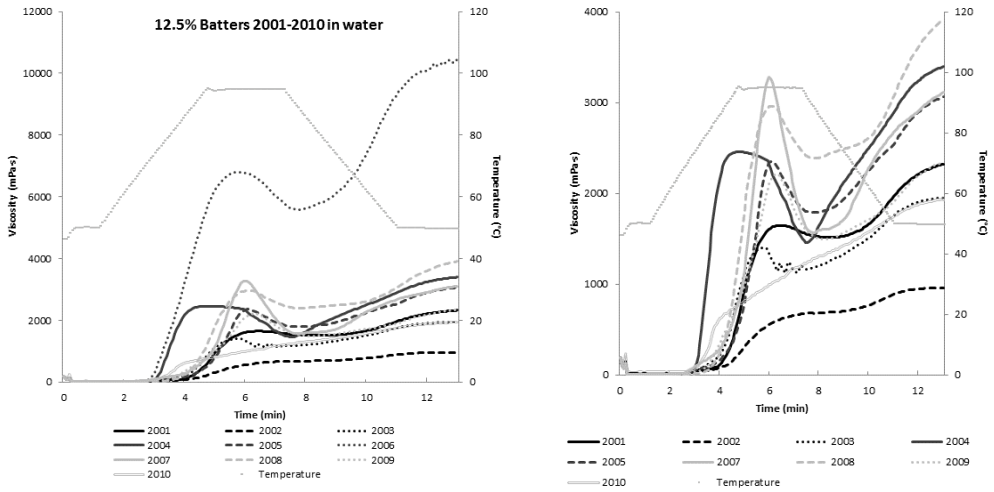


Figure 5.5: The standard RVA curves for 12.5% 2001-2010 in water. The right graph is an enlargement of the left graph without showing the RVA pasting curve for 2006.

On completion of the RVA pasting the samples were stored, in the canister in which they were pasted, at 4 °C for 24 hours and their gel strengths were assessed according to the methods given in section 2.4.7. The gel strengths of the batter samples after RVA measurements and cooling for 24 hours are shown in Figure 5.6.

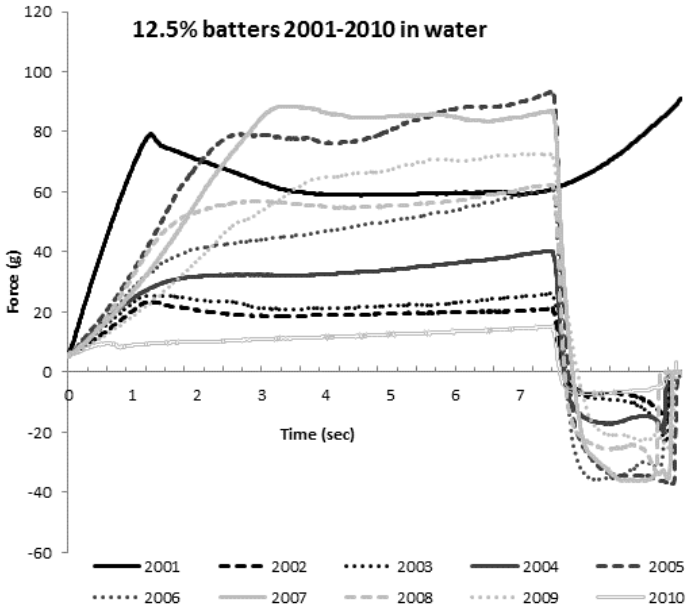


Figure 5.6: The breaking force measured by texture analysis of the gels prepared from RVA measurements of samples 2001-2010.

The gel strength of the samples roughly followed the order of 2007> 2001> 2005> 2009> 2008> 2006> 2004> 2003> 2002> 2010 however some of the maximum peaks were very difficult to determine due to the flat profile (see Figure 5.6). The samples 2002 and 2010 which developed low viscosity in the RVA pasting measurement also showed very low gel strength after cooling. However, sample 2006 with the highest pasting viscosities showed a rather weak gel strength and was not distinguishable in terms of gel strength from some other batters. The gels formed from samples 2007, 2001 and 2005 appeared strong despite the pasting properties being very diverse. There was no obvious correlation between the peak viscosities and gel strengths for these batters.

Figure 5.7 shows the gel samples after the gel strength measurements and it was clear that the samples were very different in hardness and flow capabilities. For example, samples 2001 and 2010 (compared lower right) show marked differences, with sample 2001 being a rigid gel and sample 2010 resulting in a thin flowing paste.

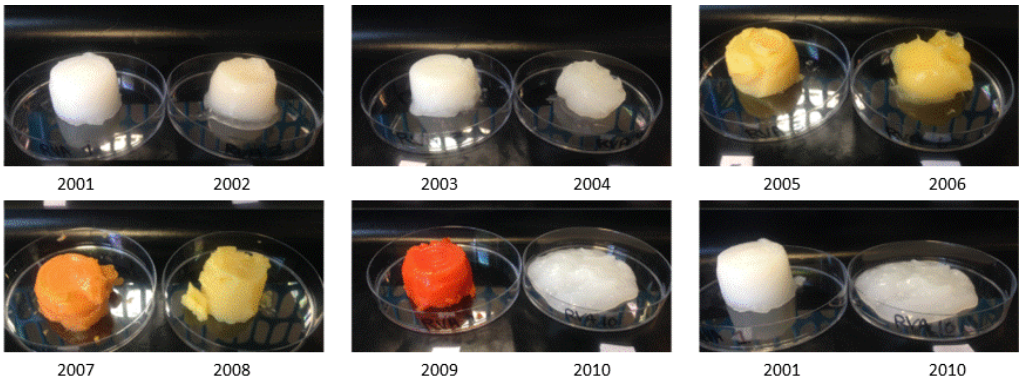


Figure 5.7: The visual characteristics of the batters 2001-2010 after gelatinisation by RVA and cooling for 24 hours at 4°C before measuring the gel strength. Images taken prior to drying of the processed batters.

The characteristics in excess water indicate that these batters (2001-2010) could be expected to have markedly different properties in the early steps of the industrial processing. The variation in the behaviour of the batters and batter composition will

be discussed further in section 5.4 when other functionalities and composition information have been detailed.

In addition to using the RVA pasting method to achieve a measurement of the behaviour of the batters, this method can be used as a precise process to create samples prepared under controlled cooking with constant shear and controlled temperature. The RVA treatment was therefore used in this study to create processed (cooked) batters. The controlled cooking of the batters was targeted to mimic an industrial process where the batters reach the starch gelatinisation temperature upon processing. The resulting behaviour of the batter would therefore be relevant in understanding the film forming properties of the batters.

5.3.1 Controlled cooking of batter solutions and their behaviour

The methodology used to create the cooked samples was kept as simple as possible in order to minimize variations in the process. The samples were pasted using the standard RVA procedure (section 2.4.4.1), cooled for 24 hours at 4 °C and tested for gel strength. These samples were then transferred to Petri dishes and dried in a vacuum oven at 40 °C for six days. The samples were ground to a particle size < 335 µm. To achieve a moisture content relevant to a midpoint of processing i.e. somewhere between the starting moisture of 60% and a final moisture of about 1.5%, the dry powder was moderately hydrated. The powder batter samples were equilibrated over saturated sodium chloride for at least seven days in order to achieve samples of similar moisture content (at the same water activity level) and these samples would then be ready for further analyses. Concurrent with other thermal analyses (see section 6.1.3), the moisture content of the samples were determined by standard oven drying (after DSC measurement) and TGA. The results obtained are shown in Figure 5.8. It was consistent that the TGA moisture values

were lower than the moisture values determined by oven drying except for sample 2009. This difference could be due to the different methodology of the measurement where the TGA measurement was much faster with a set heating rate and also reaching a much higher temperature (see section 2.4.6).

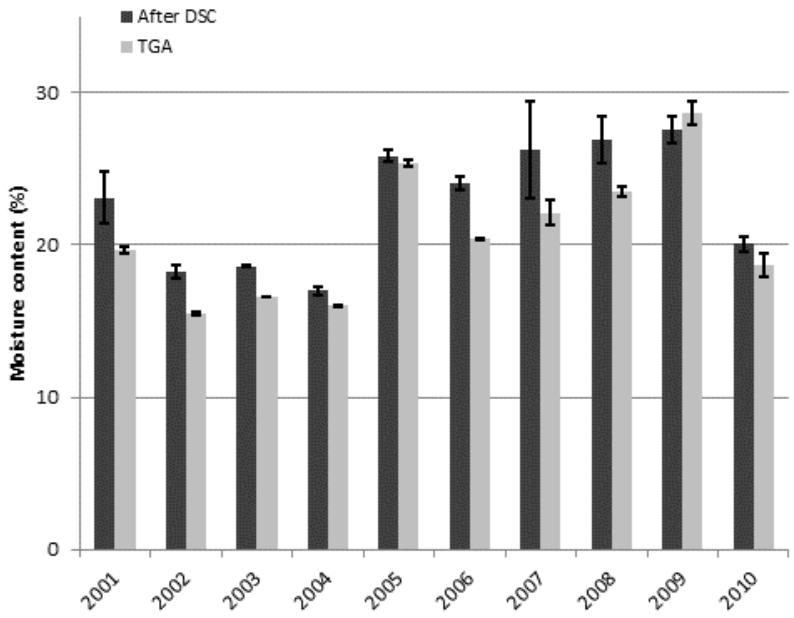


Figure 5.8: The moisture content of treated sample 2001-2010 measured by standard oven drying after DSC measurement and determination of moisture contents using TGA. n = 2 and error bars indicate the standard deviations.

It is clearly seen that the moisture contents of the processed batter samples 2001-2010 are different although they were stored at the same water activity level. These results were very surprising since previous studies have shown that the moisture content of various starch samples were similar at the same water activity level (Sair and Fetzer, 1944). The moisture contents for samples 2001-2010 were between 15.5-28.7% for both drying methods and indicated that the moisture content of these processed batters were not similar at the same water activity level. The batter mixtures 2001-2010 were very complex due to their many different starch components (see Table 2.4) and therefore three other batters (the 3000 series) were selected to clarify and understand the surprising results obtained upon storage at the same water activity level.

Data from the 2000 series of samples indicated marked differences in performance. However, some of these differences seemed to be linked with the non-starch components of the batter formulations, e.g. wheat flour was deemed to be the key ingredient in bubble formation in the batters 2005, 2007 and 2009 and the gum in batter 2010 dominated the wet batter pickup. The impact of the different starches could have been masked by the other ingredients thus three batter samples 3001-3003 (created by the sponsoring companies and thought by them to show differences in performance) were selected to explore some of the other starches or other carbohydrate components and their attributes in the batter mixture.

5.4 Specific selected batters 3001-3003

5.4.1 The behaviour of batter sample 3001-3003 upon frying

Batter samples 3001-3003 contained 16 different carbohydrate materials (301-316) divided between the three different batters (see Table 2.6). Some of the ingredients were the same type of carbohydrate material (e.g. 301, 308 and 312 were all modified potato starches), but from different commercial sources and might also be modified differently. The individual materials in these batter blends all contributed with their individual properties to the final attributes of the batter.

To explore the importance of solid contents in the batter, two frying experiments were conducted with 36% solids and 50% solids. Figure 5.9 shows the battered filter papers after frying with 36% and 50% solids respectively. The battered samples were visually evaluated and it was seen that the batter samples 3001-3003 at 36% solids were similar in texture and batter uptake. For the batter samples at 50% solids the battered filter papers appeared to be very different. The battered filter paper with batter mixture 3001 (50% solids) resulted in a thin film covering the filter paper

whereas the battered filter papers with batter mixtures 3002 and 3003 both obtained a very thick film (see Figure 5.9). The texture of the thick batters (3002-3003) was slightly different with batter mixture 3002 appearing more airy and porous than sample 3003.

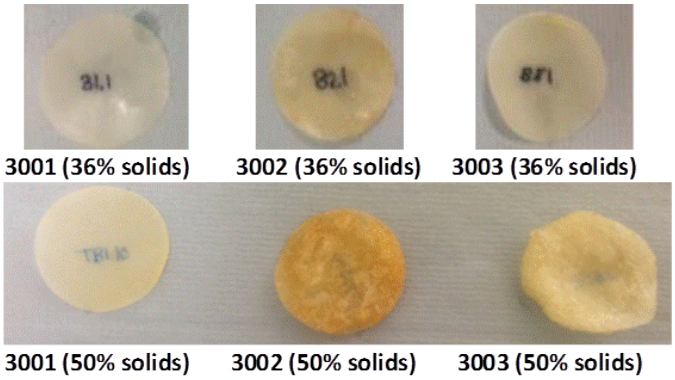


Figure 5.9: Fried battered filter paper for samples 3001-3003

The wet batter pickup versus the oil uptake shown in Figure 5.10 confirms the visual assessment that the wet batter pickup was similar for all the batters at 36% solids and also the oil uptake was similar (see Figure 5.10 enlarged insertion). For the batter mixtures at 50% solids the wet batter pickup for the battered filter paper with sample 3001 was slightly higher than the wet batter pickup for the 36% solids samples whereas the oil uptake was similar. However, the battered filter paper with batter mixture 3002 and batter mixture 3003 (50% solids) had increased wet batter pickup values of 2.8 g/paper and 4.3 g/paper respectively. The increase in wet batter pickup confirmed the visual assessment of the batter mixtures at 50% solids. The oil uptake also increased for the battered filter papers with batter mixtures 3002 and 3003 (50% solids), but with high and overlapping standard deviations possibly due to variance in wet batter pickup. The experiment clearly showed that the solids content of batter mixtures were crucial both for the attributes of the final product, but also for the properties of the batter mixtures and their capabilities to form films with the required properties. The difference in texture (influenced by wet batter pickup) and

change in oil uptake were important factors to consider when assessing a batter mixture.

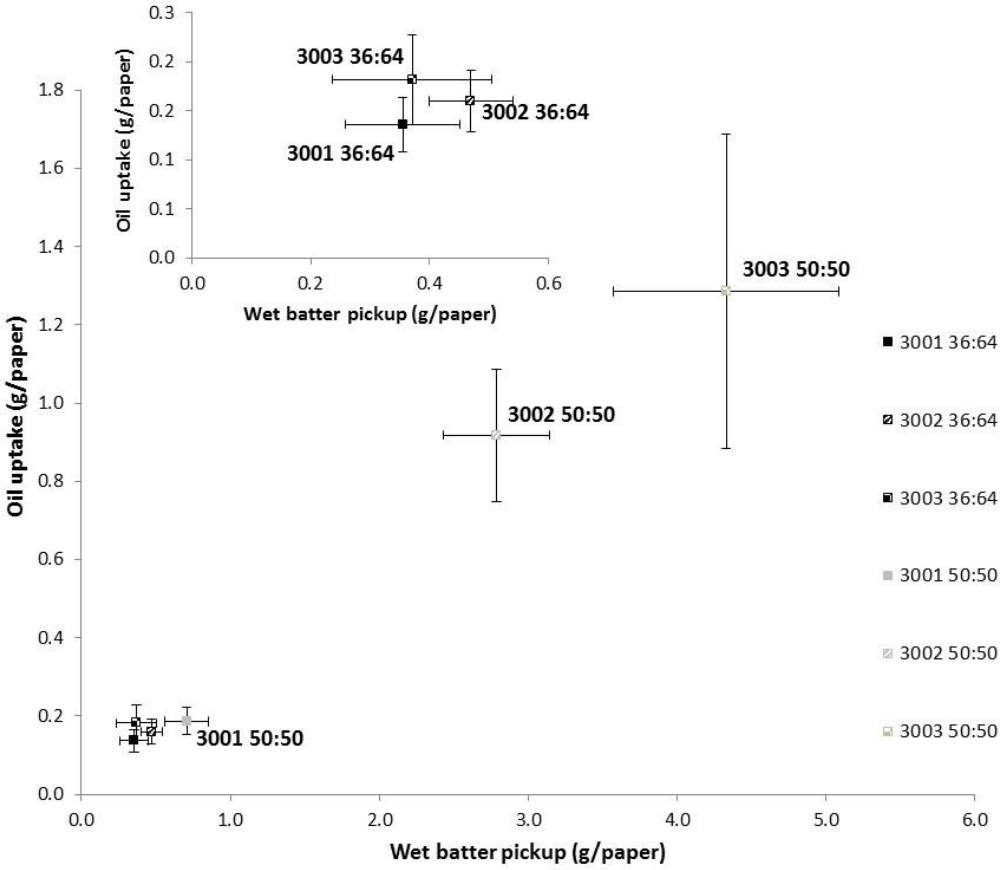


Figure 5.10: The wet batter pickup versus the oil uptake for the batters 3001-3003 for a 36% solids mixture and a 50% solids mixture.

The variations in batter compositions clearly resulted in different properties of the batter mixtures at high solids (50%). However, the standard procedure for battered potato chips uses about 36% solids in the batter mixture (which is adjusted to the individual batter) and hence the differences at high solid contents are less relevant for industrial use. It was not clear from these preliminary frying experiments which individual materials contributed to the difference in behaviour (at 50% solids) of the three batters. It was attempted to distinguish the batter properties (36% solids) by measuring the crispiness of the battered filter paper (Analysis on a Microsystem Texture Analyser), but the data showed high standard deviations and it was

considered that the major contribution of the filter paper base may dominate over the properties of the batter (data not shown).

The properties of the fried batter mixtures at standard solids (40%) were further investigated without the impact of the filter papers. The method was an alteration of the standard method (section 2.5.1.3.1). Wooden sticks were used instead of filter paper to support the batter film. This allowed the fried batter to be scraped off the sticks after frying and cooling to room temperature. These cooked batters were stored for further analysis.

5.4.2 Moisture contents of samples 3001-3003

The batter samples 3001-3003 were carefully selected to show the diversity of the batter mixtures and the impact of the individual starch component in one specific batter. The characteristics of the batters in excess water showed marked differences between the three samples both for pasting viscosities (Figure 5.11, for method see section 2.4.4) and gel strengths (Figure 5.12, for method see section 2.4.7).

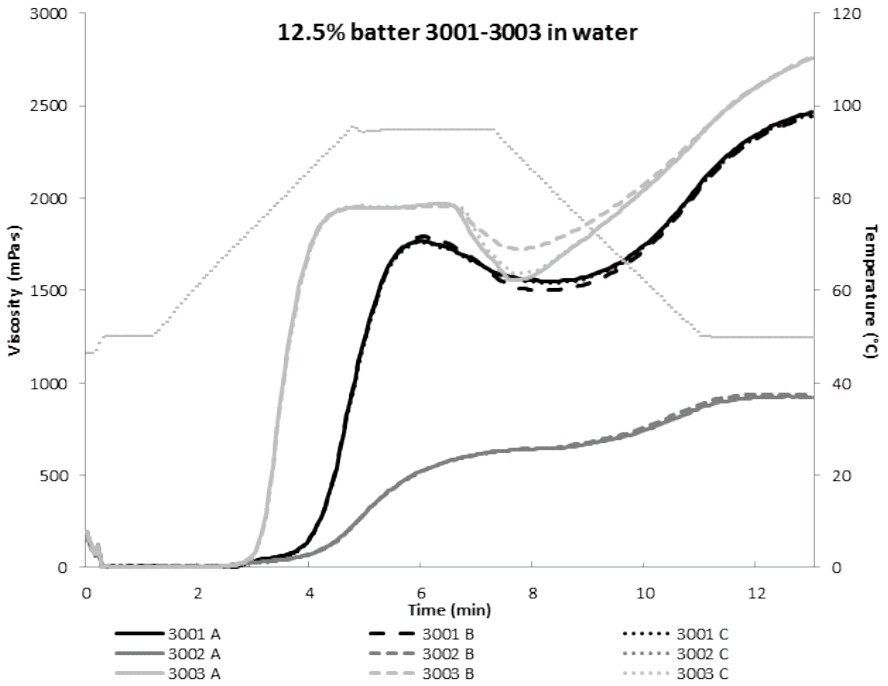


Figure 5.11: The pasting curves (RVA) for 12.5% samples 3001-3003 in water.

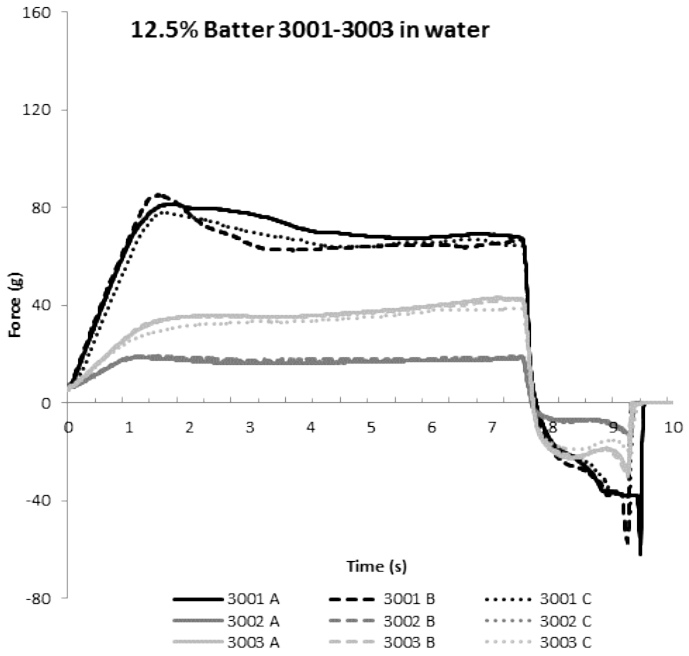


Figure 5.12: The breaking force for samples 3001-3003 measured by texture analysis (after RVA processing).

After being processed (section 5.3.1) the batter samples were equilibrated over saturated salt (NaCl) and their moisture contents were measured by the standard oven drying method and by TGA. Figure 5.13 (left) presents the results for the treated batters, which clearly indicates that the moisture contents for these three treated batters were different with especially treated sample 3001 having a higher moisture content of about 20%.

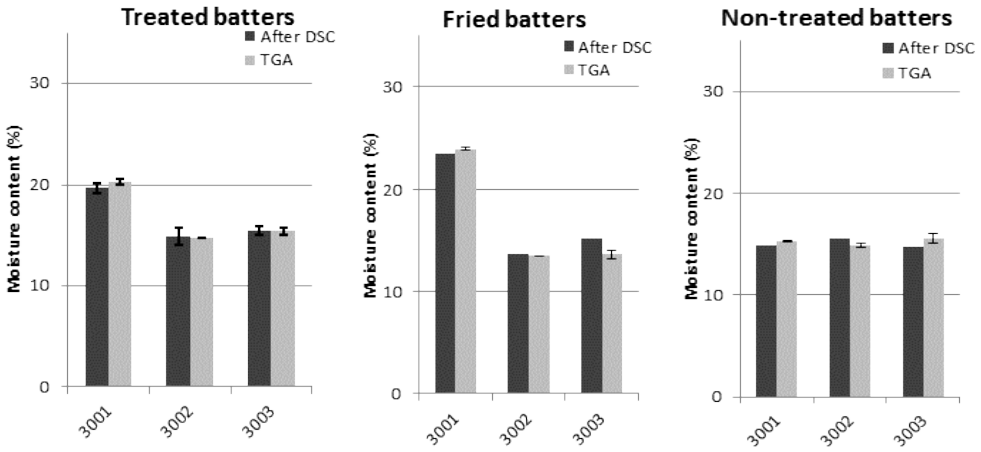


Figure 5.13: The moisture contents of RVA-treated, fried or non-treated sample 3001-3003 measured by standard oven drying after DSC measurement and determination of moisture content using TGA.

Figure 5.13 also showed the total moisture contents of the equilibrated samples when they were not cooked (non-treated, right) and when they were fried (middle) (see section 2.5.1.3.1) and then equilibrated over saturated sodium chloride. For the non-treated samples the moisture contents appeared to be similar for the three batters (14.7% - 15.6%) whereas the fried samples (13.5% - 23.9%) showed a similar trend as to the treated (RVA processed) samples. The individual starches 301-317 within the three batters 3001-3003 were tested using the same conditions and the resulting moisture levels are given in Figure 5.14. None of the non-treated samples 301-317 obtained moisture contents higher than 18.2% and the moisture content was very similar for all samples (13.4-15.4%), except for the modified potato starch samples (301, 308 and 312) that had slightly higher moisture levels (18.2%, 17.6% and 18.0% respectively).

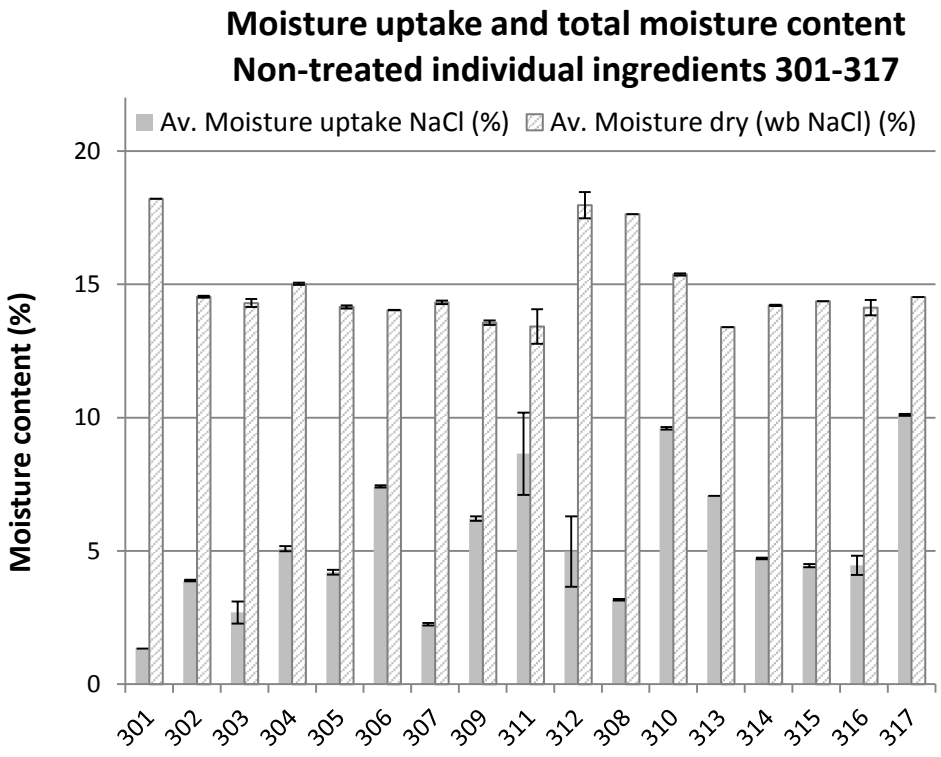


Figure 5.14: The moisture content of non-treated samples 301-318 equilibrated over saturated salt and measured by standard oven drying after DSC measurement.

This experimental work demonstrated an expected finding for the non-processed samples. It is generally accepted that equilibrium moisture content approximates to the weight average of the components in the mixture (Enrione et al., 2007, Iglesias et al., 1980). The results for the raw individual ingredients could suggest that raw mixtures of these would possibly comply with this general rule as the moisture contents of the non-treated batters 3001-3003 fell within the range of the values for the raw individual ingredients. To achieve these average moisture contents for the non-processed batters 3001-3003, there would have to be significant levels of potato starch present and some interactions to elevate the equilibrium moisture content at 75.6% RH.

However, the moisture content for batter 3001 when it had been processed, either cooked or fried, was much higher than any of the raw ingredients. The feature that seemed to be relevant for the moisture content of sample 3001 was whether it was processed or not. The equilibrium moisture contents of starches, at these low moisture contents, are generally not considered different between the crystalline and amorphous starches. However, the water holding capacities would be markedly different in high moisture systems. It was also noted that the marked increase of moisture content for sample 3001 was not evident for the other two batter samples, as the moisture contents of these were similar whether processed or not. A moisture content difference of 5% between the processed batters at 75.6% RH needed further examination. The technique of dynamic vapour sorption (DVS) (see section 2.5.2.4 and 1.3.2.2) can provide information about the sorption and desorption of materials and therefore it was used to further analyse the processed batters.

5.4.3 Use of dynamic vapour sorption to understand the differences in moisture contents of batter 3001-3003

The sorption and desorption isotherms measured by DVS for the processed (treated) and non-treated batters 3001-3003 are given in Figure 5.15. The methodology used is given in section 2.5.2.4. The sorption and desorption were measured in duplicate. For the treated batters it was seen that the sorption and desorption isotherms were clearly different at higher relative humidity (RH) values. Above a RH value of about 65% the treated batter samples could be differentiated as sample 3001 increased markedly for the change in dry mass with increasing RH values.

These DVS isotherms confirmed the results obtained for measuring the total moisture contents of the treated and non-treated batters (3001-3003) when stored over saturated sodium chloride (Figure 5.13). The isotherms for the non-treated batter samples 3001-3003 (Figure 5.15) also showed a slight difference between the non-treated samples with the same trend as for treated samples i.e. the non-treated batter 3001 had the greater change in mass at high relative humidity followed by non-treated batters 3003 and 3002. The differences for the non-treated samples were not as marked as for the treated samples, which could indicate a difference in properties of the batters upon processing. The sorption properties and hence the moisture uptake or loss for the batter samples were important factors when assessing their film forming properties towards their final attributes. This will be discussed in more detail in the next section.

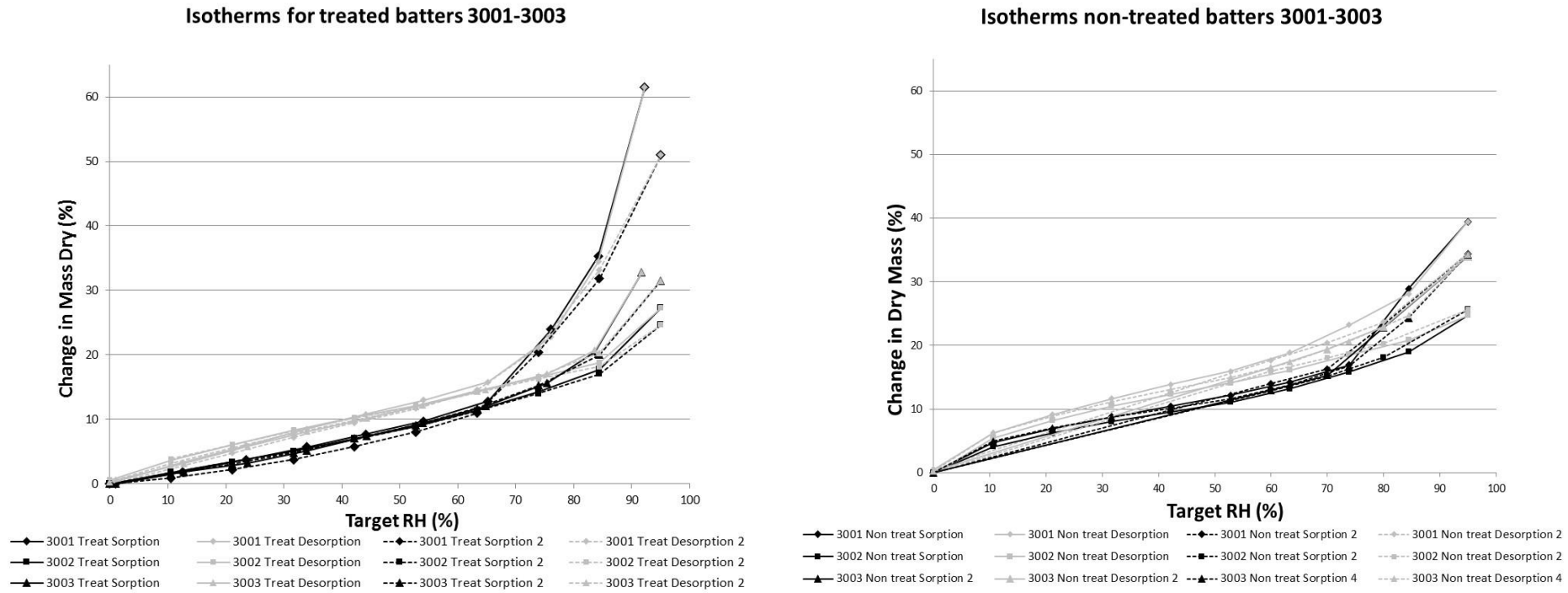


Figure 5.15: The dynamic vapour sorption of RVA-treated or non-treated samples 3001-3003 (in duplicate).

5.4.4 The relation between moisture uptake, isotherms and crispiness

The importance of the moisture uptake and moisture loss of the batters is crucial when determining the properties of the film. The quality of the final product is often determined by the crispiness of the batter layer, the texture and moisture content of the base and the capabilities to maintain the product in a state of crispiness, e.g. under a heat lamp. If the moisture uptake of the batter is high and water movement into the batter is pronounced, then the film formed by the batter will go soggy and lose its crispiness (Nicholls et al., 1995, Roudaut et al., 1998). However, if the moisture loss through the batter film is excessive it could result in a dry film but also a drying of the base layer resulting in an overall dry product. The balance between moisture uptake and loss is very delicate and these properties may also vary depending on the base layer.

The crispiness of a fried food product is often associated with the desirable attributes of the product. It is a major challenge for the food industry to preserve crispiness over time, especially when the crispy layer is not in equilibrium with its neighbouring environments. The batter film could be expected to be influenced by the moisture of the atmosphere and the potato base. Holding the sample under heat lamps for periods of time will provide additional drying and heating, but perhaps even more crucially it allows time for moisture diffusion.

Crispiness of products is a well-recognised sensory attribute, but its measurement using analytical techniques is difficult. Crispiness is mostly assessed by sensory evaluation, but the data collected can be supported by texture and accompanying acoustic measures (Seymour and Hamann, 1988). Studies on crispiness of starch based products have shown that both crispiness and crunchiness decrease with increasing water activity levels (Katz and Labuza, 1981, Seymour and Hamann,

1988). Also the hardness of a product inversely correlates to crispiness and crunchiness values and increases with increasing water activity levels (Seymour and Hamann, 1988). Although a_w is often used to vary the sample to examine different behaviour, the change in the textural parameters is generally thought to relate to the plasticising effects of the increasing moisture levels at the higher a_w values.

The analysis of the moisture content of the batter samples 3001-3003 (using different methods) and the individual ingredients (301-317) at the same water activity level (a_w = 0.756) indicated that the batter samples had different properties than seen from the individual ingredients. The DVS data confirmed the first observations that at high relative humidity, achieved over saturated sodium chloride (RH 75.6%), the treated batter sample 3001 would obtain higher moisture uptake (seen in Figure 5.13 (left) consistent with the shown high change in mass at the high relative humidity (Figure 5.15 (left)). These differences in the sorption isotherms, although they occur at high relative humidity where perhaps the sample is already in the rubbery state, could lead to differences in the batter's ability to create and then retain a film with a crispy texture. The crispy texture created by processed batter sample 3001 (whether fried or cooked) seemed particularly vulnerable to a change in humidity.

There were some factors that needed to be considered:

- The batter samples that showed differences were processed. It is therefore tempting to think that the difference in properties between processed and non-processed batters might be explained by the difference in crystallinity of the samples. Non-processed batter samples will still have intact crystalline regions due to the starches being in their natural state with alternating amorphous and crystalline regions. However, the processed batters have been gelatinised and

hence lost their crystallinity. Therefore they mostly consist of amorphous material (some retrogradation might have occurred). At high moisture levels the amorphous materials absorb more water than crystalline materials (Al-Muhtaseb et al., 2004). The change in polymer order can therefore explain the difference between the isotherms for the treated and non-treated batters.

- The marked differences were only apparent for one of the batter samples and therefore this difference between samples was indicative that the samples consist of various starches with different absorptive properties. For example could the amylose/amylopectin levels, different types of crystallinity or different modifications be responsible for the responses. These factors could have been explored, however the ratios of the starches in the batters remained confidential thus it was difficult to predict responses using the commercial recipes.
- In addition to the chemical composition there were differences in physical parameters following the creation of the treated starches. The particle sizes and shapes of the batter components were different due to the treatment and concomitant milling of the treated samples. Hence the packing of the powder (granules or the matrix) in the treated batter samples might be different thus changing diffusion paths into the powders. Upon moisture uptake, the structures may also change and impact on the moisture uptake rates. It should be remembered that moisture levels were measured using different methods and the hydration time should have reached equilibrium. The moisture measures, from after DSC, TGA and DVS could all be imprecise because of the small samples sizes, so the samples measured may not reflect the total sample. This error should have been negated by the fact that several different samples and methods were used, it would seem unlikely that the same error occurred

on each occasion. The fact that in the processed batter samples the components were in more intimate contact compared to the powder blends of the non-processed samples also needs to be remembered.

These results were puzzling and difficult to explain and therefore major discussions with the supplier of the samples were undertaken. It was finally revealed that the batter samples 3001-3003 that were thought to be totally starch based had in fact been formulated to contain different salt levels (including raising agents) and also small amounts of stabilisers (hydrocolloids). The rank order for salt addition was 3002<3003<3001. This information led to an understanding why the moisture levels may be so high. The moisture content over 75.6% RH would rise considerably if salt was present due so sodium chlorides' distinctive sorption isotherm shown in Figure 5.16. However, why there were such differences between the samples was not so obvious. The focus was now to determine whether the sorption behaviour for batters, with and without gum and salt, were different and if the treatment of the batters were crucial for the sorption behaviour.

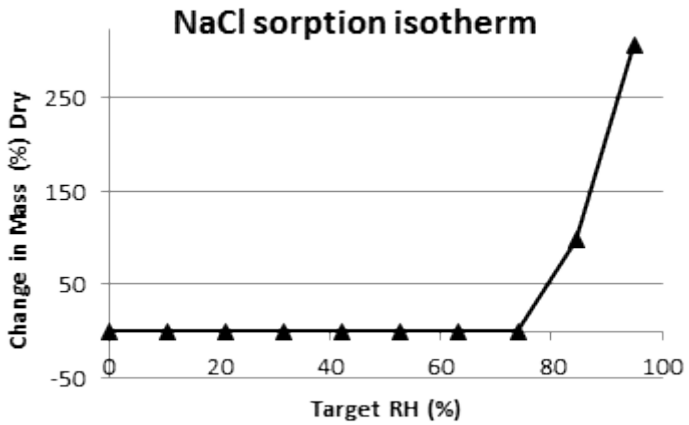


Figure 5.16: Dynamic vapour sorption analysis of sodium chloride. Only the sorption isotherm is shown.

The new batches of batters, not containing salts and stabilisers, were provided and the sorption and desorption isotherms for these batters, treated and non-treated, were determined. The results are given in Figure 5.17. The isotherms for the non-

treated batters without salt and gum were very similar for all batters 3001-3003 and only reached a change in mass of about 26% at high RH values. For the treated batters the shape of the isotherms had changed a little as there was a rather steep increase in the change in mass when increasing the RH values above 85%. The change in mass at the highest relative humidity (95%) was only slightly higher than for the non-treated samples. Hence the sorption behaviour of the batters without salt and gum appeared to be largely similar whether they were processed or not.

In order to test the salt influence on the sorption and desorption isotherms, the batters without gum and salt were dry mixed with 5% NaCl before the RVA processing and then tested along with the dry mixed blend without processing (non-treated batters without gum and salt + 5% NaCl). The isotherms in Figure 5.18 showed that the addition of salt to the batter mixtures and subsequent cooking led to very high change in mass at high RH. The change was similar for all three treated batters 3001-3003 without gum and salt + 5% NaCl. The isotherms for the non-treated batters 3001-3003 without gum and salt + 5% NaCl (Figure 5.18) showed that the three batters did show an increase in moisture compared to the samples without salt so the starchy formulations did not appear to have an effect on the moisture levels. The samples used in the DVS were small and there would be critical issues of mixing and possible imprecision in the accurate sampling of mixtures, especially for the non-treated samples. However, the differences in the isotherms on processing were confirmed. The addition of salt did seem to accentuate the differences between processed and non-processed samples (see values in Table 5.5). Although trying to fit the isotherms to models was not originally the purpose of this work, this was thought to be a worthwhile exercise as it may throw more light on why the processing impacts on the sorption behaviour.

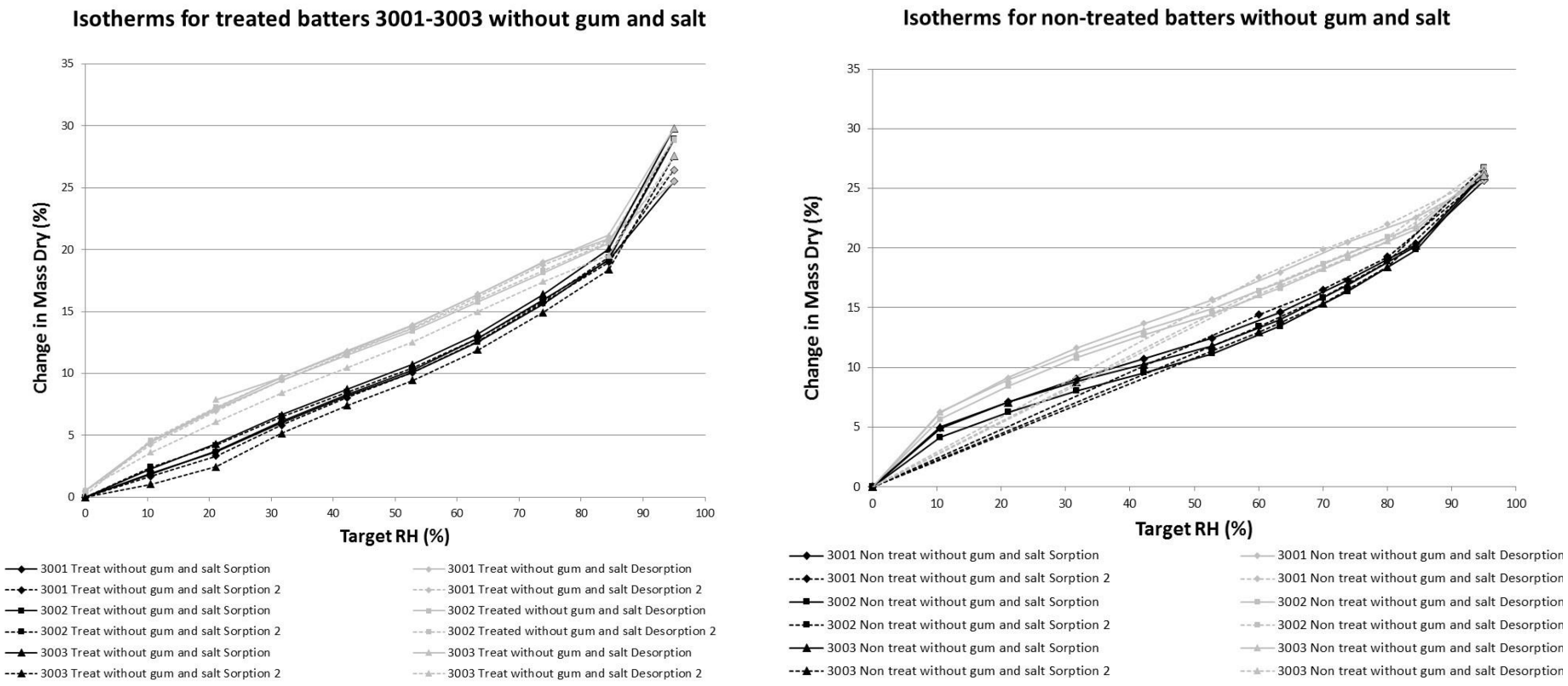


Figure 5.17: Dynamic vapour sorption analysis of RVA-treated or non-treated samples 3001-3003 without gum and salt (in duplicate).

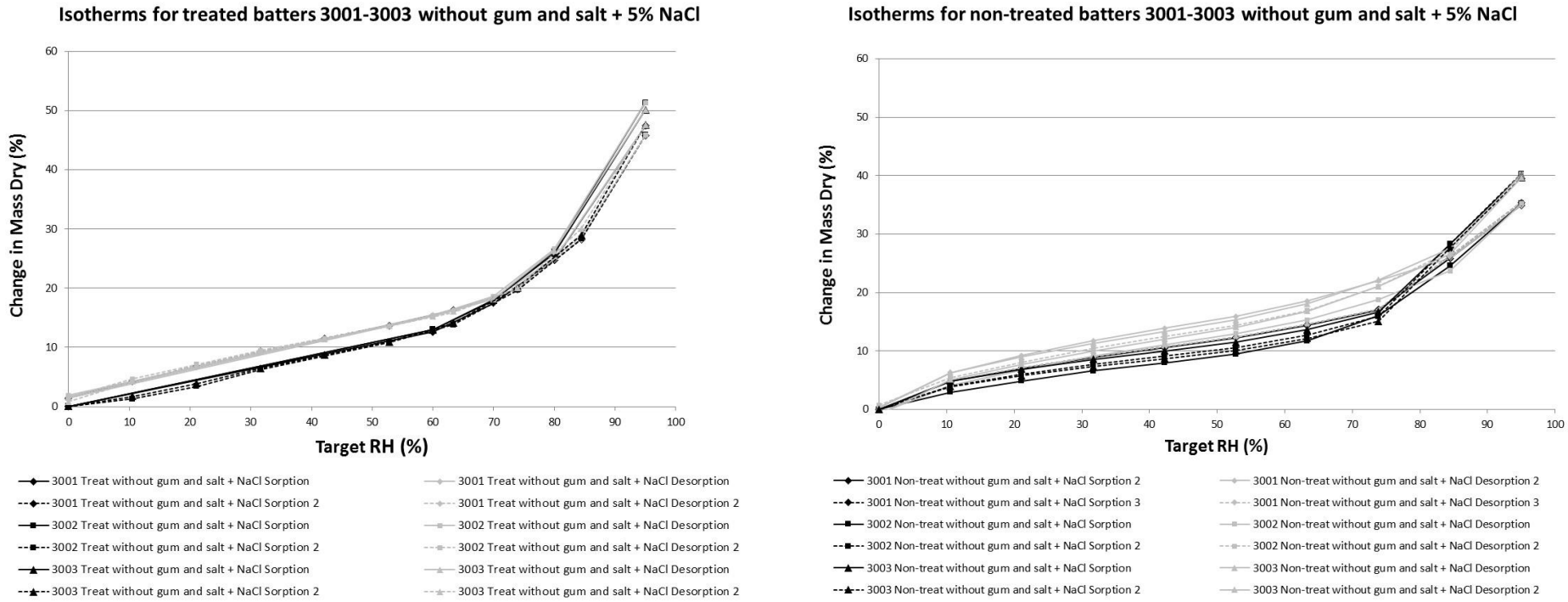


Figure 5.18: The dynamic vapour sorption of RVA-treated or non-treated samples 3001-3003 without gum and salt + 5% NaCl (in duplicate).

Table 5.5: DVS data from batter samples 3001-3003 without gum and salt but added 5% NaCl, treated or non-treated (see section 5.3.1)

Non-treated without gum and salt + 5% NaCl (Sorption)							Treated without gum and salt + 5% NaCl (Sorption)							
RH (%)	3001	3001	3002	3002	3003	3003	**RH (%)**	3001	3002	3003	**RH (%)**	3001	3002	3003
0.00	0.01	0.00	0.03	0.73	0.38	-0.80	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.82
10.56	4.80	4.84	2.97	5.47	6.29	5.09	**60.00**	12.61	13.03	12.95	**10.56**	1.23	1.30	4.22
21.11	7.00	7.06	4.89	8.13	9.01	7.75	**70.00**	17.44	17.94	18.04	**21.11**	3.13	3.41	6.81
31.67	8.92	8.98	6.58	10.45	11.31	10.03	**80.00**	24.74	26.18	25.89	**31.67**	5.99	6.21	9.24
42.22	10.57	10.65	8.00	12.45	13.33	12.05	**95.00**	47.39	51.32	50.15	**42.22**	8.42	8.57	11.39
52.78	12.25	12.34	9.50	14.37	15.35	14.05					**52.78**	10.67	10.83	13.61
63.33	14.37	14.46	11.74	16.96	18.06	16.84					**63.33**	13.68	13.81	16.09
73.89	17.05	17.14	16.12	21.05	22.19	21.06					**73.89**	20.08	19.63	19.97
84.44	25.86	26.18	24.60	27.02	27.58	26.61					**84.44**	28.07	28.29	30.06
95.00	34.90	35.37	35.23	40.31	40.08	39.63					**95.00**	45.78	45.86	47.57

5.4.5 Batter sorption data and their relation to moisture distribution (GAB equation)

There are many equations available to represent sorption data and relate the water activity (a_w) and the change in moisture content of a sample (Boki and Ohno, 1991). The Guggenheim-Anderson-de Boer (GAB) equation was selected to interpret the sorption data produced for the batters because of the larger range of applicability (covering the whole a_w range) compared to the BET model (Branauer-Emmett-Teller) that only covers the lower a_w range (Timmermann, 2003). This was particular important because of the salt affecting the high a_w range of the isotherm. The GAB equation can be utilised to determine the characteristic monolayer value (M_0) as well as the energy constants C and K which describe the differences in free enthalpy between pure liquid and the monolayer and between the pure liquid and the second sorption layer respectively (Timmermann, 2003).

The theory leading to the GAB equation has evolved since the early 1950's when William James Scott first described the term water activity and related it to the microbial growth in foods (Chirife and Fontana, 2008). The thermodynamic concept activity was first described by the ratio of fugacity of a substance f (the escaping tendency of a substance) to the fugacity of the substance at a standard state f_0 at the same temperature (indicated by the subscript T).

Equation 5

$$\boldsymbol{a} = \left(\frac{\boldsymbol{f}}{\boldsymbol{f^0}}\right)_{\boldsymbol{T}}$$

For a system at equilibrium state the water activity can then be defined as the ratio of fugacity of water f_w to the fugacity of the pure liquid water f^0_w

Equation 6

$$a_w = \left(\frac{f_w}{f_w^0}\right)_T$$

Due to the equilibrium state at which the water activity is defined and the definition of fugacity that represents an effective pressure (with non-ideality of the gas phase taken into account), then fugacity can be replaced by the vapour pressure of the system and the vapour pressure of pure liquid water and hence linking the water activity to the pressure (Reid, 2007).

Equation 7

$$a_w = \left(\frac{p_w}{p_w^0}\right)_T$$

An important factor to bear in mind is that water activity relations are strictly dependent on the system being in an equilibrium state. The relative humidity (measured by DVS) is related to water activity as a_w = RH/100 and data obtained from the DVS analysis can therefore be modelled using the GAB equation utilising the water activity values to predict water distribution in the system (Raschip et al., 2008).

Equation 8: The GAB equation

$$M = \frac{M_0 C K a_w}{(1 - K a_w)(1 - K a_w) + C K a_w}$$

With M denoting the equilibrium water content, M_0 is the monolayer capacity, a_w is water activity and the constants C and K are defined as

$$C = c' exp\ \frac{(h_m - h_n)}{RT} \qquad and \qquad K = k' exp \frac{(h_l - h_m)}{RT}$$

Where c' and k' denote the equation constants, h_m, h_n and h_l is the total heat sorption of the first layer, multilayers and the heat of condensation of pure water respectively, R is the universal gas constant and T is the absolute temperature. The monolayer and multilayers can be defined as

$$M_0 = M\,(1 - Ka_w) \qquad and \qquad M_{multilayer} = MKa_w$$

The parameters determined by utilising the GAB equation can indicate the water distribution in the samples provided that the samples are at equilibrium (see section 5.5).

From the data the sorption values were retrieved and the monolayer capacity (M_0) and the energy constants C and K were determined using the solver function in Excel. The data are given in Table 5.6 for the treated and non-treated batters with and without gum and salt. As stated, when the experimental work was first started, there was not the intension to fit this to models and for some sorption isotherms only a few points within the sorption curve were measured. It is noticeable that some of the replicates did give different values. The values for the monolayer capacity appeared to be similar for all the batters with values ranging between 5-12%, except for two very high exceptions were the duplicate values were widely different.

The dimensionless energy constant C, which expressed the measure of the difference of free enthalpy between the pure liquid and the monolayer, showed a large range (0-20). Despite these values being widely spread and some large deviations between the duplicates, paired T-testing between the non-treated and the treated samples showed a significant difference between the treatments ($P<0.01$). The treated batter had lower C values than the non-treated batters thus indicating that the water binding to the treated batters had lower energy constants than for the non-treated samples. The decrease in C-value indicated that water is less strongly bound in the monolayer in the treated batter formulations.

Table 5.6: Data retrieved from modelling to the GAB equation

NON-TREATED BATTERS														
Parameters	**3001**	**3001**	**Av. 3001**	**STDEV**	**3002**	**3002**	**Av. 3002**	**STDEV**	**3003**	**3003**	**3003**	**3003**	**Av. 3003**	**STDEV**
M_0	7.15	11.55	9.35	3.11	7.73	7.38	7.55	0.25	6.66	7.38	8.68	6.79	7.37	0.92
C	15.24	2.24	8.74	9.19	11.78	27.48	19.63	11.10	14.11	27.48	4.85	23.26	17.43	10.07
K	0.87	0.75	0.81	0.08	0.73	0.75	0.74	0.01	0.91	0.75	0.77	0.85	0.82	0.07
NON-TREATED BATTERS WITHOUT GUM AND SALT														
Parameters	**3001**	**3001**	**Av. 3001**	**STDEV**	**3002**	**3002**	**Av. 3002**	**STDEV**	**3003**	**3003**	**Av. 3003**	**STDEV**		
M_0	8.89	8.30	8.59	0.42	7.67	7.74	7.70	0.05	7.89	7.76	7.83	0.09		
C	12.95	79.99	46.47	47.40	11.72	14.72	13.22	2.12	16.91	22.92	19.92	4.25		
K	0.70	0.72	0.71	0.01	0.75	0.75	0.75	0.00	0.74	0.75	0.75	0.01		
NON-TREATED BATTERS WITHOUT GUM AND SALT + 5% NaCl														
Parameters	**3001**	**3001**	**Av. 3001**	**STDEV**	**3002**	**3002**	**Av. 3002**	**STDEV**	**3003**	**3003**	**Av. 3003**	**STDEV**		
M_0	7.33	7.30	7.31	0.02	6.53	6.45	6.49	0.06	6.66	6.11	6.39	0.39		
C	17.33	17.12	17.22	0.15	6.08	11.24	8.66	3.65	19.73	11.71	15.72	5.68		
K	0.84	0.84	0.84	0.00	0.87	0.89	0.88	0.02	0.88	0.90	0.89	0.01		

TREATED BATTERS												
Parameters	**3001**	**3001**	**Av. 3001**	**STDEV**	**3002**	**3002**	**Av. 3002**	**STDEV**	**3003**	**3003**	**Av. 3003**	**STDEV**
M0	7.58	43.85	25.72	25.64	6.34	7.03	6.68	0.48	5.32	6.76	6.04	1.02
C	1.67	0.20	0.93	1.04	4.42	3.82	4.12	0.42	4.64	3.28	3.96	0.96
K	0.96	0.75	0.86	0.15	0.82	0.78	0.80	0.03	0.92	0.84	0.88	0.05
TREATED BATTERS WITHOUT GUM AND SALT												
Parameters	**3001**	**3001**	**Av. 3001**	**STDEV**	**3002**	**3002**	**Av. 3002**	**STDEV**	**3003**	**3003**	**Av. 3003**	**STDEV**
M_0	10.29	81.31	45.80	50.22	7.28	7.15	7.21	0.09	7.51	7.98	7.74	0.33
C	2.99	0.40	1.70	1.83	4.53	5.80	5.17	0.90	5.42	2.77	4.09	1.87
K	0.69	0.39	0.54	0.21	0.80	0.80	0.80	0.00	0.80	0.78	0.79	0.01

TREATED BATTERS WITHOUT GUM AND SALT + 5% NaCl												
Parameters	**3001**	**3001**	**Av. 3001**	**STDEV**	**3002**	**3002**	**Av. 3002**	**STDEV**	**3003**	**3003**	**Av. 3003**	**STDEV**
M_0	8.77	10.62	9.70	1.31	8.45	11.59	10.02	2.22	8.20	11.61	9.90	2.41
C	2.26	1.32	1.79	0.66	2.55	1.13	1.84	1.00	2.93	1.15	2.04	1.26
K	0.87	0.85	0.86	0.01	0.87	0.86	0.86	0.01	0.88	0.85	0.87	0.02

For the energy constant K, which expressed the measure of difference of free enthalpy between the pure liquid and the second sorption layer (multilayer), the values were all less than one, which was a criterion of the GAB equation. The K-values suggested a difference between the batters containing added salt (treated and non-treated) (paired T-test, $P<0.001$) with lower K-values being calculated for the batters without gum and salt. This indicated a difference in binding energy of the multilayer when salt was present. The K-values were not affected by the treatment of the starches ($P=0.77$). The absorption of a multilayer was believed to be at the higher end of relative humidity and therefore it supports the sorption isotherms (Figure 5.18) suggesting that the addition of salt changed the moisture uptake drastically at high relative humidity. However the data set was not adequate to determine whether there was a difference in the multilayer binding energy between the different batters.

The determination of the GAB parameters suggested that both the processing (C-values varying for the treated or non-treated batters) and the addition of salt (K-values varying) influenced the moisture uptake. The GAB parameters indicated that the differences were seen in the monolayer with regard to the processing and the multilayer sorption upon salt addition. The obtained data set was known to be of limited quality and that the analyses may lack some rigour. This will be further discussed below.

5.4.5.1 The deviations associated with the GAB equation

The sorption isotherms were measured in duplicate for most batter samples. However, one of the duplicates was only analysed in the relative humidity intervals 0-60%, 60-70%, 70-80% and 80-95% (short analysis) instead of the long analysis with intervals every 10% humidity change (see section 2.5.2.4). The short analysis data

therefore did not contain any data point for the interval 0-60% (only for 0% and 60%) and then further three more data point. This may not have given sufficient data to model an equation like the GAB equation and be able to derive appropriate GAB parameters. The value of the monolayer (M_0) and the energy constant C are both related to the monolayer sorption which is believed to take place in the lower or middle region of relative humidity. Thus not having collected data points in this region allowed a somewhat inappropriate fitting of the data and the M_0 and C values might change substantially although having used the same solving function. The change of the energy constant C will be reflected in the shape of the curve at low relative humidity and hence the sigmoidal curve might not be observed.

In order to test the data set and obtained GAB parameters, the literature was consulted. It was found that for starches or carbohydrate materials, the obtained C-values were in the range of 0.25-26.7 (Al-Muhtaseb et al., 2004, Doporto et al., 2012, Raschip et al., 2008, Timmermann, 2003), but could also be experimentally determined to 120 for gas/solid systems (Timmermann, 2003). To verify the GAB parameters, the constant C was changed towards the limits of the starch material and set to 0.1 and 30 and then solved with regard to all three parameters (see Table 5.7). The resulting GAB values (only calculated for the non-treated batters and the non-treated batters without gum and salt) were very different from the earlier obtained values, but not necessarily incorrect. The difference in sum of squares value (ΔSS) is determined to obtain the most appropriate values calculated for the GAB parameters by choosing the lowest value for the best fit of data.

Table 5.7: Parameters calculated using the GAB equation probing the values for C = 0.1 and C = 30. ΔSS is the calculated square minimum and ΔSS Org. is the calculated square minimum of the original samples (for comparison).

NON-TREATED BATTERS C = 0.1														
Parameters	3001	3001	Av. 3001	STDEV	3002	3002	Av. 3002	STDEV	3003	3003	3003	3003	Av. 3003	STDEV
M0	175.32	49.07	112.20	89.27	86.02	48.04	67.03	26.86	6.66	92.87	44.57	6.79	37.72	40.87
C	0.15	0.43	0.29	0.20	1.21	0.70	0.95	0.36	14.11	0.20	0.51	23.25	9.52	11.22
K	0.49	0.56	0.53	0.05	0.20	0.42	0.31	0.16	0.91	0.54	0.53	0.85	0.71	0.20
ΔSS	6.50	0.44			1.58	0.23			4.30	0.52	0.23	0.20		
ΔSS Org.	1.43	0.44			0.019	0.0017			4.30	0.52	0.049	0.20		
NON-TREATED BATTERS C = 30														
Parameters	3001	3001	Av. 3001	STDEV	3002	3002	Av. 3002	STDEV	3003	3003	3003	3003	Av. 3003	STDEV
M0	7.15	13.39	10.27	4.42	127.37	7.34	67.35	84.87	6.66	43.12	7.79	6.79	16.09	18.03
C	15.23	1.69	8.46	9.58	3.74	31.26	17.50	19.46	14.12	0.41	8.61	23.26	11.60	9.60
K	0.87	0.73	0.80	0.10	0.05	0.75	0.40	0.50	0.91	0.59	0.79	0.85	0.79	0.14
ΔSS	1.43	0.42			1.93	0.0017			4.30	0.52	0.062	0.20		
ΔSS Org.	1.43	0.44			0.019	0.0017			4.30	0.52	0.049	0.20		
NON-TREATED BATTERS WITHOUT GUM AND SALT C = 0.1														
Parameters	3001	3001	Av. 3001	STDEV	3002	3002	Av. 3002	STDEV	3003	3003	Av. 3003	STDEV		
M0	146.05	65.59	105.82	56.90	72.22	50.47	61.35	15.38	94.13	50.55	72.34	30.82		
C	1.98	0.85	1.41	0.80	1.02	0.65	0.84	0.26	1.38	0.69	1.03	0.49		
K	0.09	0.32	0.20	0.17	0.26	0.42	0.34	0.11	0.17	0.42	0.30	0.17		
ΔSS	1.73	0.26			1.79	0.20			2.38	0.25				
ΔSS Org.	0.014	0.0021			0.013	0.0002			0.019	0.0022				
NON-TREATED BATTERS WITHOUT GUM AND SALT C = 30														
Parameters	3001	3001	Av. 3001	STDEV	3002	3002	Av. 3002	STDEV	3003	3003	Av. 3003	STDEV		
M0	125.09	8.85	66.97	82.20	125.63	9.48	67.56	82.13	130.54	9.51	70.02	85.58		
C	8.53	18.12	13.33	6.78	1.21	4.63	2.92	2.42	3.94	5.20	4.57	0.89		
K	0.03	0.70	0.36	0.48	0.14	0.70	0.42	0.40	0.05	0.71	0.38	0.46		
ΔSS	1.84	0.01			1.99	0.03			2.65	0.04				
ΔSS Org.	0.014	0.0021			0.013	0.0002			0.019	0.0022				

The new calculated values (Table 5.7) reveal that more than one solution might be correct mathematically, hence demonstrating that there can exist more local minima or saddle points, which results in variable GAB parameters. It should be noted that inserting a new value for C and solving the equation will determine new values for M_0, C and K, but it was noted that these values were not robust solutions. Values were different if the same parameters were used and the equation solved again, hence resulting in various values for the same data set. A reasonable explanation for lack of robustness for the GAB parameters could be due to insufficient data points at low relative humidity which will influence i) C, which reflected the initial slope, ii) the overall curve shape, iii) no plateau region hence M_0 not well defined.

The exercise of enforcing different levels for the C energy constant in the GAB equation demonstrated the care needed to interpret the data collected for the different batters, salt additions and starch treatments. However, the DVS data set has been analysed with the restricted data sets available and the sorption data confirms the expected results for salts having an impact on the behaviour of the batters in term of moisture uptake.

The processing of the batters also seemed to influence moisture behaviours. What also seemed to be shown and could be important if confirmed was that salts interacted with the treated starch to induce a greater change than with the non-treated starchy materials. Although sorption and desorption data were collected and the samples showed some hysteresis, the differences in the levels did not seem to be indicative of changes in the formulations. In these current studies the GAB parameters were not sufficiently robust to give anything more than an indication of trends within the batters and more experiments need to be conducted to verify this data.

5.5 Discussion

Industrial batters tend to be formulated with multiple components and the attributes of the different starches may be needed at different stages within the manufacturing and eating of the battered product. The key characteristics of the starches therefore may need to be manifested at specific water and temperature environments. The functionality of the batter formulations at different water levels was therefore addressed and discussed in this chapter using different approaches.

A logical approach to explore the batter behaviour during processing and observing the immediate result was to fry samples. As it was the batter that was to be investigated rather than the underlying matrix, a model system using battered filter paper was developed. Measures of the wet batter pickup and the oil uptake were undertaken as these are important attributes for the final product. Batter samples 2001-2010 represented a wide spectrum of attributes towards the final product. Batter samples 2005, 2007 and 2009 demonstrated the ability to form bubbles and sample 2010 indicated the importance of viscosity of the starting batter solution. Interestingly, the major differences were not driven by the starches in the batter, but by other materials. Flours encouraged aeration and oil uptake, while gums increased viscosity and batter pickup. The results for batters 3001-3003 confirmed a strong dependence of the viscosity on the pickup volumes and the associated film thickness. Results for these also showed the non-linearity between the pickup volumes and the concentration of the batter suspension.

To prepare processed samples for other assessments (see chapter 6) and to allow examination of the batter behaviour during and after gelatinisation of the starches a controlled cooking of the batter solutions was undertaken. The experiments in

excess water (see section 5.3.1) indicated that the batter samples 2001-2010 adapted different behaviours due to their individual components. Although both analyses were conducted at the same solids content, the viscosity of the batter samples during heating and shearing (RVA measurement) did not correlate with the gel properties (TA measurement) of the pastes. Again, the most striking difference in the gelling properties for the samples did not seem to be due to the differences in the starch, but because of the xanthan present in the system. It could be imagined that the viscosity reached as the batter suspensions were heated and/or the properties of the gelled films could be very relevant for the films behaviour. The measures undertaken were not directly applicable to the batter coatings as the moisture contents were too great. It was therefore a major objective to examine the batter formulations at moderate or low water contents.

To achieve moderate water levels, the processed commercial batter samples 2001-2010 from the RVA were dried and milled. In order to create a controlled moisture level, the samples were equilibrated at the same water activity level by using saturated sodium chloride. It was found that the moisture contents of the batter samples prepared in this way were very different. This was confirmed by several moisture determination methods. Batter samples 3001-3003 were formulated for this programme of work to explore the dependence of moisture content in the batters made from combination of starches. One of these batter samples was clearly different in behaviour and the degree of difference was dependent on whether the sample had been processed or not (see Figure 5.13).

The sorption isotherms confirmed the differences seen for the batters. As it was very difficult to explain these differences in terms of the starch behaviours, other possible factors relevant for batter mixtures were considered. Other non-

carbohydrate based ingredients contained in the batters were distinct possibilities, e.g. stabilisers, leavening agents and salt. It was agreed with the suppliers of the original three batter samples that these samples may contain other materials in addition to the starches. Batter samples 3001-3003 were therefore reformulated and this time it was assured that no gum or salt would be present in the batters. For these new samples the sorption isotherms were very similar for all three batters, indicating that the variation in the starches did not influence the outcome of the sorption. It should be noted that each batter contained a mixture of starches and that the isotherms would be a generalisation of the effects of the individual components. Addition of 5% salt (wet weight to starch) caused a significant shift in the sorption isotherms, which was most noticeable at the higher moisture contents.

Although the data collected for the sorption isotherms were not ideal for use to model the water uptake such a task was attempted. The GAB equation was utilised to determine the monolayer capacity M_0 and the energy constants C and K. Although the data must be treated with some caution, statistical differences could be ascertained for the dimensionless energy of absorption functions with K values varying depending on the salt addition and C values varying for the treated and non-treated batter formulations.

It seems surprising that there is not more in the literature regarding the sorption performance of processed and non-processed starches. There are some references regarding the changes in the isotherms due to loss of water or crystallinity in hydrothermally treated starches (Enrione et al., 2007, Kaminaski and Al-Bezweni, 1994) and some general statements about the processed starches (Iglesias et al., 1980). However, little systematic work can be found in the public domain that includes the different starches and modified starches. An intriguing observation has

been the relevance of processing of starch in the presence of sodium chloride. The equilibrium moisture contents seemed considerably higher for the treated samples than for the non-treated samples with the same level of salt. These results may be due to poor mixing of the salt with the starch, or a change in matrix (particle size and shape) for the processed samples. It therefore may be a consequence of the physical attributes of the samples rather than a basic change in their interactions with water at a molecular level. This area is certainly worthy of further investigation as rates and levels of moisture are critical for the batters' processing and eating qualities.

The batter formulations supplied had a complex composition and the understanding of their behaviour was difficult. Minor ingredients did seem to dominate the performance to the extent that it was difficult to observe the contributions due to the starchy components. Moisture will play an important role not only towards crispiness, but also towards the interaction between individual ingredients including non-carbohydrate materials as films are formed. The batters' ability to form films and function at low moistures would seem to be the key area for a successful batter formulation and therefore required further examination. This is discussed in the following chapter.

CHAPTER 6 BEHAVIOUR, IN TERMS OF FILM FORMING PROPERTIES, OF STARCHES CREATED IN LIMITED SHEAR REGIMES

Chapter 3-5 have addressed the modification of starches and their behaviour in limited water content systems. This research concluded that the modification of starches with focus on the stabilised and crosslinked tapioca series is a complex matter. It requires more detailed knowledge into the actual levels of modification and position of modification in order to correlate these with the behaviour of the starches. The assessment in a limited water system (NMMO-water, chapter 4) revealed the change in functionalities under said conditions and also concluded that this solvent system is capable of differentiating starches with the same origin and similar behaviour in water. These results led to investigation of the starch blends (batters) under their usual conditions i.e. frying (chapter 5), which concluded that specific ingredients such as wheat flour and xanthan gum had a great impact on film forming capabilities. It was also found that the solids content was important for the final film attributes, such as wet batter pickup and oil uptake. Chapter 5 also discussed the relevance of moisture sorption of the processed and non-processed formulations and concluded that minor ingredients (e.g. salt) may cause major changes to the water sorption reflecting the final attributes of the film i.e. the crispiness of the film. The frying experiment showed the importance of the film formation at controlled conditions. Therefore the objectives of the work conducted and detailed in chapter 6 in relation to previous results were to:

1. Investigate two film forming methods under controlled cooking and limited shear regimes.

2. Assess film forming capabilities in relation to batter formulations and their individual ingredients.
3. Assess the relevance of moisture within the batter formulation including the impact on the film forming properties.

6.1 Film formation in relation to coating with batters

The formation of starch films is applied in many food products due to the functionality of the film, which can include maintaining crispiness over time perhaps under heat lamp conditions and the control of water movement (moisture loss/gain). The many methodologies used to create films are variable and are often chosen to accord the desired final functionality. The application of heat and pressure at low moisture content will allow melting of the starches to form films in a way that relates directly to the creation of the batter film on potato chips. The techniques used in this study were the use of a “popping” or hot press equipment that will allow the film forming procedure to be carried out reproducibly. The films formed from the procedures needed to be created at controlled moisture contents as this was proven to be important in chapter 4 and 5. The relevance of moisture in the batter formulations was addressed according to properties such as moisture sorption of different starch blends (chapter 5) and pasting characteristics in limited water environment (NMMO-water, chapter 4). Due to the starch functionalities being highly dependent on moisture, it is an important factor to keep constant (or known).

The samples created by pressing or popping can be used as powders or directly as films for DMA and thus provide information on the rheological properties and the glass transition properties of the formed films. The rheological behaviour of a starch film plays an important role in the functionality of the film as it indicates the capability of the film to form a porous or strong film material. The batter film

covering a moist base such as a potato chunk will require different functionalities, e.g. to act as a barrier for moisture uptake and loss, to help maintain crispiness or to control oil uptake. These functionalities can be obtained by different blends of starches. Therefore it is important to understand the film forming abilities at different moisture contents, and also to explore the individual starches' film properties. This was partly accomplished in chapter 5 when conducting the frying experiments. However, the film forming capabilities may be more clearly accessed if the film itself was evaluated.

6.1.1 The formation of popped samples

The technique of treating the starches at high temperature at controlled moisture levels and pressures and the subsequent rapid release of the pressure, thereby allowing moisture to evaporate, is referred to in this work as popping. The sample created is a popped sample (see section 2.5.1.1). Available to this project was a "popping" machine (Figure 6.1) that was a specially designed instrument that could control the parameters of heating, pressure and time. It was originally designed for the study of popped rice samples. The advantages of the popping machine were the easiness of manually controlling the temperature and the duration of the experiment.

6.1.1.1 Method development

The popping machine was a bespoke built piece of equipment for a different project and consisted of a heated cup and a heated piston (see Figure 6.1) which was automatically temperature controlled, but could be adjusted. However, it must be noted that it was not possible to control either the heating rate or the cooling rate. The pressure could be adjusted as well but it was kept constant (see section 2.5.1.1.1).

The sample preparation was developed into the standard procedure described in section 2.5.1.1.1. Initially, samples of pre-weighed starches were evenly dispersed in the cup and water was added with a pipette. This created samples with uneven distribution of water and also the sample was difficult to remove from the heated cup upon formation of the popped sample. Another approach was to hydrate the starches by water addition and leave them shaking overnight to evenly distribute the water. However, due to the very low amount of water added, the water was not dispersed evenly and created lumps within the starch sample. Inspired by the frying experiment (chapter 5) and using the filter paper as a base, the filter paper was adapted as a base material transferring the moisture into the starch sample. This method was concluded to be the most appropriate to transfer the moisture evenly into the sample.

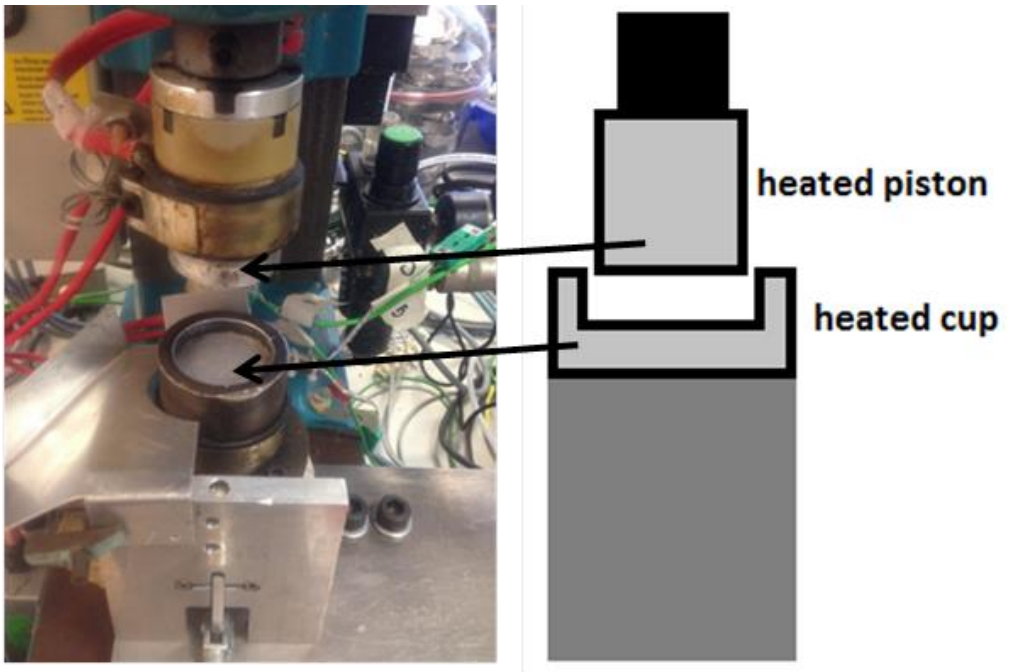


Figure 6.1: An enlarged picture of the popping machine showing the heated cup and piston including a schematic drawing (right). The machine was able to press samples at about 400 kPa and at various temperatures (changeable for upper and lower plate). The machine was manually operated and pressing time could be adjusted (section 2.5.1.1).

However, the sample recovery from the cup was challenging and different materials were used to ease this recovery. A pre-shaped foil cup (prepared by pressing the cup

on its own) was used as the holder of the sample and various materials were tried to create a sandwich type structure to recover the sample avoiding breakage and loss of sample. Filter paper, silicon paper, foil and baking paper were all used to optimise the conditions of recovery. The final set-up used a pre-shaped foil cup, followed by a filter paper (to transfer moisture), the starch sample and then silicon paper on top of the sample. This set-up minimised the sample loss (flowing of the sample, denoted as creeping on the edges of the cup) by using the silicon paper as a lid and also to minimise the sample adhesiveness to the piston. Further the samples were easily recovered by using the edges of the pre-shaped foil cup.

The method development was crucial to create reproducible samples for assessment of the film forming properties. However, it was also a necessity since a method had not previously been developed for starches or blends of starches using the novel technique of the popping machine. The popping machine was limited in only creating one popped sample for each experiment and the experiment duration was approximately 10 seconds. The popped samples were recovered immediately after popping.

6.1.1.2 Screening of popped batter samples 2001-2002

The standard procedure (section 2.5.1.1) was used to test batter samples 2001-2002 at different moisture levels. Prior to screening of all the samples in the 2000-series, the two batters (2001-2002) were popped to test the ability of the method to distinguish between samples. Figure 6.2 shows the samples next to each other in order to visualise the difference between the popped samples. The samples were evaluated on visual parameters such as film forming capabilities (i.e. formation of a popped film with a strong network), moisture distribution, flowing of the sample, expansion power and porosity. A scheme was later used to evaluate these

parameters (see Table 6.1 p. 213). The samples were scored right after popping and weight and measures were noted (the two latter parameters proved to be difficult to determine due to the flowing/creeping of the samples). The batter sample 2001 showed good film forming capabilities with even moisture distribution, little flowing and a hard film (little porosity). However, batter sample 2002 showed poor film forming capabilities with little expansion power (flat sample), flowing of the sample (leading to loss of sample due to overflow) and high crumbliness.

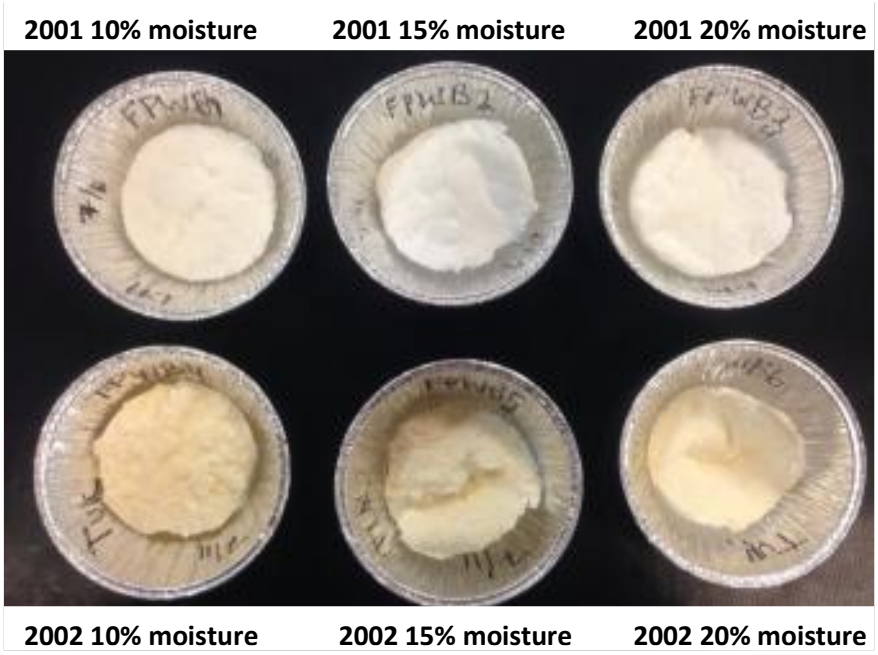


Figure 6.2: The popped batters 2001-2002 at various moisture contents (10, 15 and 20%) and popped at 185°C.

The visual evaluation indicated differences between the two batter samples, although the different moisture levels (10, 15 and 20%) did not seem to have a pronounced effect on the popped samples. However, the difference in moisture content was reflected to some extent for sample 2002 due to higher flow ability thus more loss of sample. The somewhat limited dependence of moisture content was surprising due to other functionalities such as pasting properties (chapter 3) being highly dependent on water (and concentration) and also considering the importance of moisture to plasticise the sample. Due to the successful screening of sample

2001-2002 to differentiate between batters, the rest of the batter blends 2003-2010 were screened.

6.1.1.3 Popped batter samples 2003-2010

Since it was concluded that the difference in moisture content did not clearly change the properties of the film formed, it was decided to screen the batter samples 2003-2010 at a moisture content of 15%. Surprisingly, the popping of batter samples 2003-2010 showed no or only minor differences between the samples (Figure 6.3) and it was not possible to clearly distinguish between the popped batter samples 2003-2010. They all showed good film forming capabilities with high expansion and low levels of crumbliness. A possible explanation for the similarities of the sample could be due to only partial melting of the starches, so the differences in the starch composition of the blends forming the batters was not expressed. The texture of the film could be dominated by the partly melted starches and then the other starches in the blend could act as simple fillers. In order to probe this theory, DSC measurement could be conducted to determine the (remaining) crystallinity.

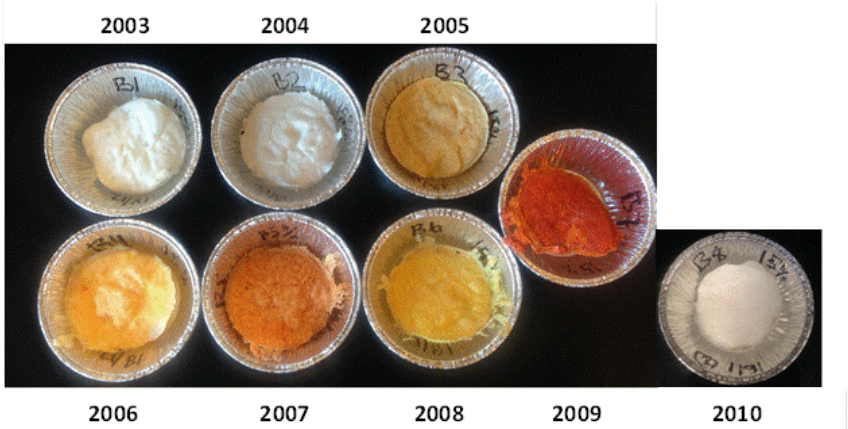


Figure 6.3: The popped batters 2003-2010 at 15% moisture content and 185°C.

However, the DSC measurements (standard method starch:water = 1:2, see section 2.4.5) of the popped batter samples showed that all samples except one sample (2010) revealed no endothermic event indicating that the order within all the starch

granules was lost. This result indicated that melting of the crystallites had occurred (see Figure 6.4). The loss of order and crystallinity may indicate that the popped batter samples 2001-2009 have been completely melted as they were popped and therefore the melting materials of the individual starches would contribute to the overall film network.

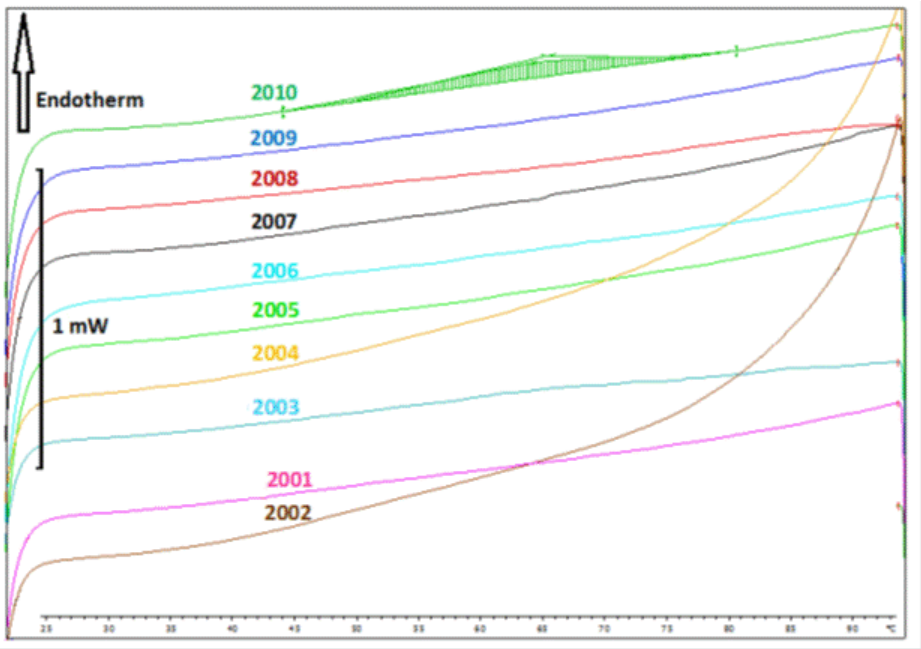


Figure 6.4: Differential scanning calorimetry traces of popped batter samples 2001-2010. n = 1, for sample 2010 n = 2, starch:water ratio 1:2, with a heating rate of 10°C /min and in the interval 20-95 °C (see section 2.4.5).

The samples were also inspected using polarized light microscopy, which clearly confirmed for batter sample 2010 that some intact granules remained on the edges of the popped sample (visualised by Maltese crosses, see Figure 6.5). Some of the other samples also had a few intact granules left at the edges of the popped samples (data not shown), which suggested that the criteria to achieve melting was better achieved at the centre of the device then at the edge. At the edge there could be some flow and there would be lower pressures. Although the moisture content at the edge may be less, as water vapour may escape, water content variation seemed not to be a major feature for this work over the ranges investigated (see Figure 6.2).

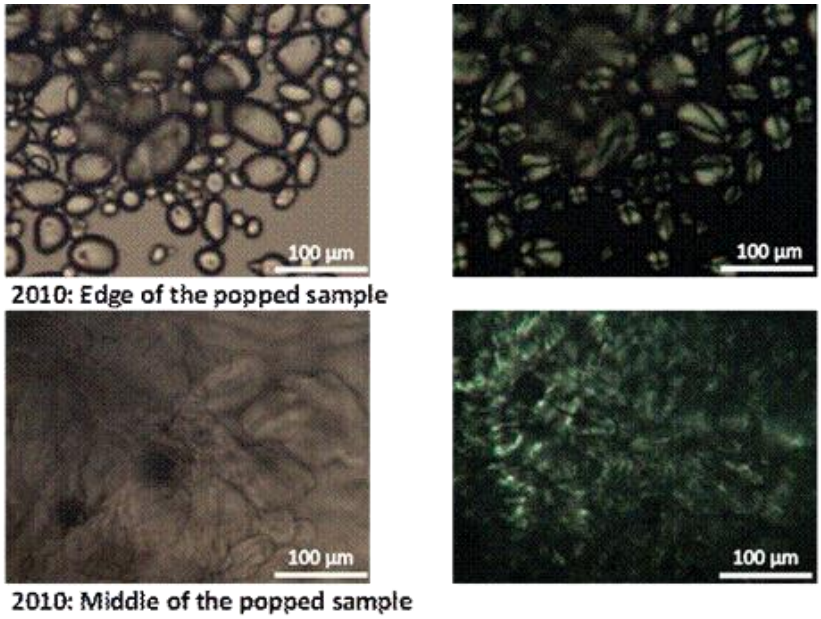


Figure 6.5: Microscopic images of popped batter sample 2010 under simple light (left) and polarised light (right).

Since the batter samples 2001-2010 showed only minor differences in the popping experiment, it was interesting to investigate the individual ingredients for the batters (201-220). This was to confirm if any of the individual ingredients gave rise to different film forming abilities. The individual components of each batter were assessed using the popping machine (see section 2.5.1.1) and the results were found to be surprisingly similar (see Figure 6.6).

The popped samples were placed into relevant groups and it was seen that all the flour type ingredients (201, 202, 203, 205 and 213) had a tendency to possess high flow ability (less for sample 213). However, they all formed a firm popped sample.

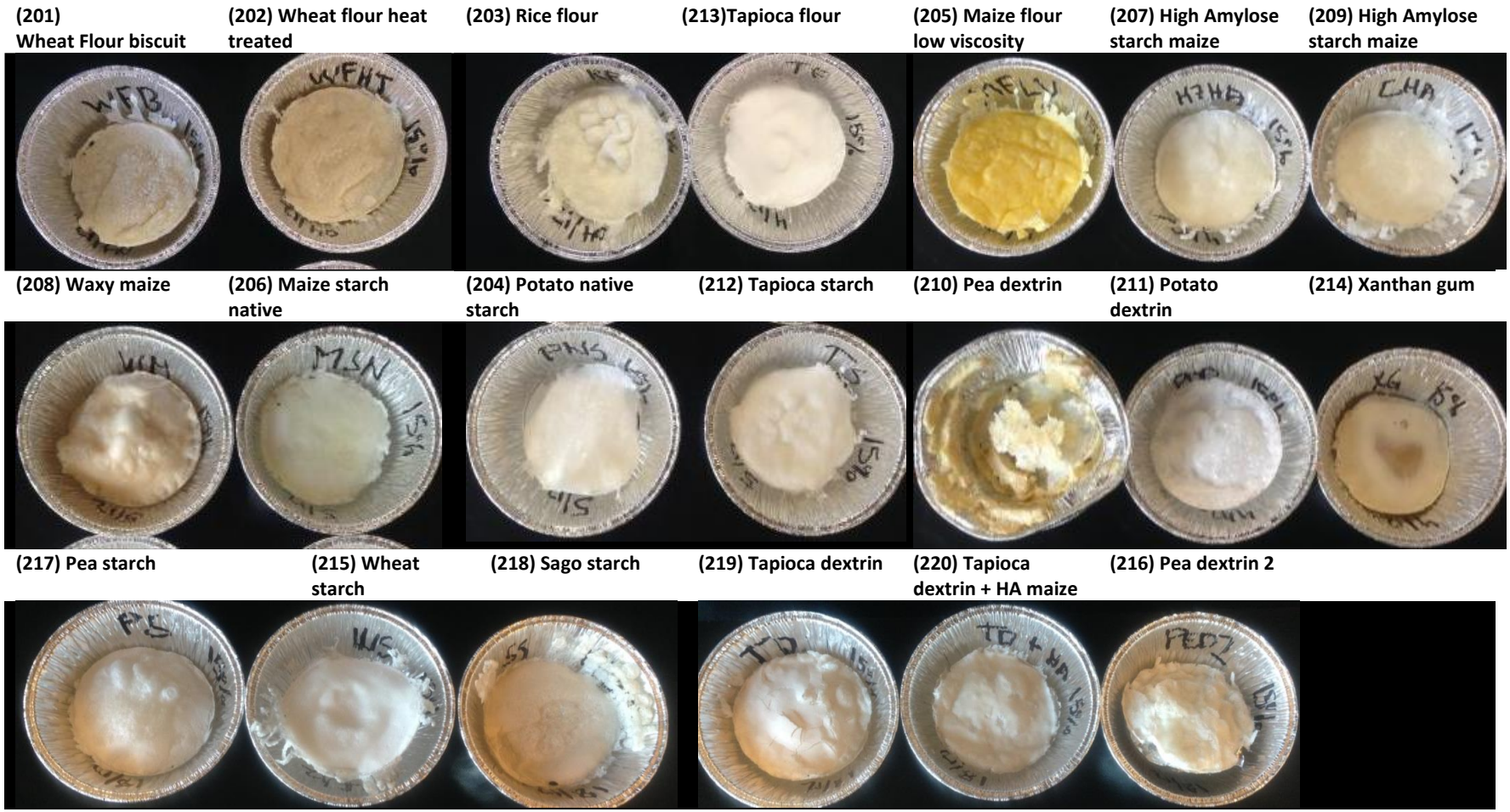


Figure 6.6: The popped individual ingredients 201-220. The blends were popped according to the method in section 2.5.1.1 with addition of 15% moisture (calculated on starch "as-is").

Samples containing maize starches (205-209 and 220) formed a popped film as well and there were no differences observed between the high amylose maize starches (207, 209), the normal maize starch (206) and the waxy maize starch (208) (see Figure 6.6). Due to the marked different functionalities related to their amylose levels, these samples were expected to be different, since melting of the starches (and gelatinisation temperature if in excess water) would be increased at higher amylose levels. Thus the high amylose samples (207, 209) were predicted to not form film, but maintain the powder structure.

A range of native starches were present in the batter formulations (2001-2010) and those samples showed good film forming abilities as well (204, 206, 212, 215 and 217-218) as seen by the firmness of the popped samples. Xanthan gum was the only stabiliser in the batter formulations and showed a markedly different behaviour due to lack of hydration, however still forming a film. Other samples that showed marked differences were the dextrins (210-211, 216 and 220), which will be discussed in the below section.

6.1.1.4 Popped dextrin samples

Where most individual ingredients formed films (popped samples), the four dextrin samples (210-211, 216 and 219) behaved very differently. Figure 6.7 shows the four dextrin samples next to each other and Table 6.1 shows the observed visual parameters and their marking. The three samples 211, 216 and 219 (potato, pea and tapioca dextrin respectively) appear to form an even film (see Table 6.1), but upon collection of the sample it was discovered that the filter paper (carrying the moisture and functioning as a base) had disappeared.

Figure 6.7: The popped individual ingredients 210-211, 216 and 219 (dextrin samples). The blends were popped according to the method in section 2.5.1.1 with addition of 15% moisture (calculated on starch "as-is").

For sample 210 (pea dextrin) the disappearance of the filter paper was clear due to the high flow of this sample and no traces of the filter paper were found (reproduced at least five times). This very peculiar result was not shown for any of the other starches. This observation may explain why the batter containing pea dextrin 210 was distinguishable from the other samples (see section 6.1.1.2).

Table 6.1: The scheme for marking visual parameters used in the assessment of the popped samples. This scheme shows the evaluation of sample 210, 211, 216 and 219. The samples were scored from 1-5 in the order 1 --> 5 i.e. if there was no loss of sample creep up --> stay will be marked 5 since all the sample stayed in the cup. The parameters were assessed organoleptically (no tasting) thus no instrumental values for noise and thickness were obtained.

Samples with 15 % moisture	Powder --> film[a]	Porous --> firm[b]	Creep up --> stay[c]	Low noise --> high[d]	Thin --> thick[e]	Notes
(210) Pea dextrin	?	1	1	3	1	No film, no filter paper, pieces
(211) Potato dextrin	4	3	3	3	5	Lot of air in film, high
(216) Pea dextrin	4	3	2*	4	4	Uneven pop, no filter paper
(219) Tapioca dextrin	4	4	4	2	4	Uneven pop, powder cracked, no filter paper

[a] Assessment of film network after recovery from the cup. Score 1 was powder and score 5 was a cohesive film.

[b] Porous assesed as crumbliness (1) and firm as a strong film network (5) by handling the sample right after popping.

[c] Creep up assessed while popping and immediately after pressure release as how much sample overflow.

[d]Noise level was assessed while popping (t = 4.6 s) and was assessed as the fizzy noise (water evaporation) coming from the sample.

[e] Thickness was visually assessed after the sample had been recovered from the cup.

[*] Significant amount of sample was attached to the piston.

Dextrins would usually be produced by a roasting process (heat and shear, see section 1.2.1.5.2) under acidic conditions. It was possible that there were some residual chemicals left in the dextrin samples from production of the sample hence causing the destruction of the filter paper. In order to examine this theory, the pH values of the dextrin samples were tested. Acidic pH values were measured for all the samples (pH 2.2-4.9). However, testing the acidic conditions on a filter paper (0.01 M HCl solution without starchy material, pH 1.9) did not result in the destruction of the filter paper (data not shown). The cause of the destruction of the filter paper was not clear from initial experiments and further analysis was not within the scope of this thesis. Nonetheless, it may be important that these remarkable properties were observed for the dextrins.

Popped samples were expected to clearly show differences in the film forming abilities of the batters (as seen for 2001-2002) and the findings were anticipated to be supported by the popping behaviour of the individual ingredients. However the batters and the major individual ingredients did not show any marked differences upon film formation. These results raise some very important issues.

Of concern for understanding the frying of batters is the moisture content that may be critical during the frying process. Although the water content starts at about 60% wet weight basis, the content in the final fried batter is about 1.5%. Water contents in excess of 50% allow starches to gelatinise at 60-80 °C, but at moisture contents of 15% the melting temperatures are likely to be well in excess of 120 °C (Steeneken and Woortman, 2009) and movement of the amylose from the granule to form a cohesive network would seem unlikely. Results from the popping work for the starches seemed to demonstrate that the temperature used (185 °C for 4.6 sec, see

section 2.5.1.1.1) was sufficient to melt all the ordered components and allowed sufficient connectivity of the macromolecular structure to form a cohesion film.

The actual level of moisture, from 10 to 20%, seemed to make little difference to the performance, so perhaps at these temperatures and pressures the moisture levels are not the critical factor for film forming. It is possible that the change to the starch morphology may have also been influenced by the rapid expansion of the sample when the top plate was lifted rather than when the sample was being heated. The batter sample where there was still crystalline and helical order present (see Figure 6.5) contained xanthan and this may have influenced the amount of water available for the starch. The batters that created bubbles upon frying (see section 5.2.1) were not notably different in the popping experiments and this may indicate different expansion mechanisms.

Further it needs to be reemphasised that the difference in amylose levels (see section 6.1.1.3) did not impact the ability to form a popped sample, as even the high amylose maize samples (207 and 209) formed films. It is often suggested that high amylose starches are good film formers, but this presupposes that the amylose is able to leach from the granule and form a network. This molecular movement was questionable in a system of such limited water and with the melting points in native high amylose starches always being considered to be very high (Richardson et al., 2000). A feature of the popped samples was that the formed films were uneven and showed some variation across the sample (microscopic images, Figure 6.5). When this work was originally conceived it was hoped that films formed by this mechanism could then be used for further analysis such as DMA analysis (see section 2.5.2.1). The films could be cooled in the popping machine, without the rapid release of water vapour and the popping, but the samples would need to be left for several

hours to cool down *in-situ* to achieve this. An alternative method was therefore sought.

6.1.2 Film formation utilising a controlled heating and cooling press

Film formation in food products is often applied by coating with a batter solution about 40% solids. Upon processing (frying or baking) the moisture evaporates concomitant with starch melting which creates a low moisture film (see section 1.1.1.1). This film formation could be studied using a hot press, especially if this had controlled heating and cooling. Such equipment needs to maintain pressure so that the starch and moisture is evenly distributed and a film of melted starch is formed.

The heating press used for this work (see Figure 6.8) was based at the Engineering facility at University Park Campus (University Of Nottingham) which required external support and training from Davide De Focatiis and his group. The method for film formation was adopted from the technique for making plastic biopolymers (De Focatiis and Buckley, 2008) hence the moulds needed to be redesigned to meet the demands of the starch film formation. The main requirement for the moulds was to have a sealed system where the moisture was unable to escape under high temperatures and high pressure (185 °C and 50 bars, for method see section 2.5.1.2.1). Further the sample size was important due to thickness of the film and dimensions to obtain suitable films for analysis. The initial testing of the hot press equipment was done with existing moulds (for plastic polymers) and an initial set up with use of a filter paper to carry moisture and a PTFE paper to ensure release of the film. Figure 6.9 shows the initial set-up with addition of 15% water to the filter paper, application of sample and film formation.

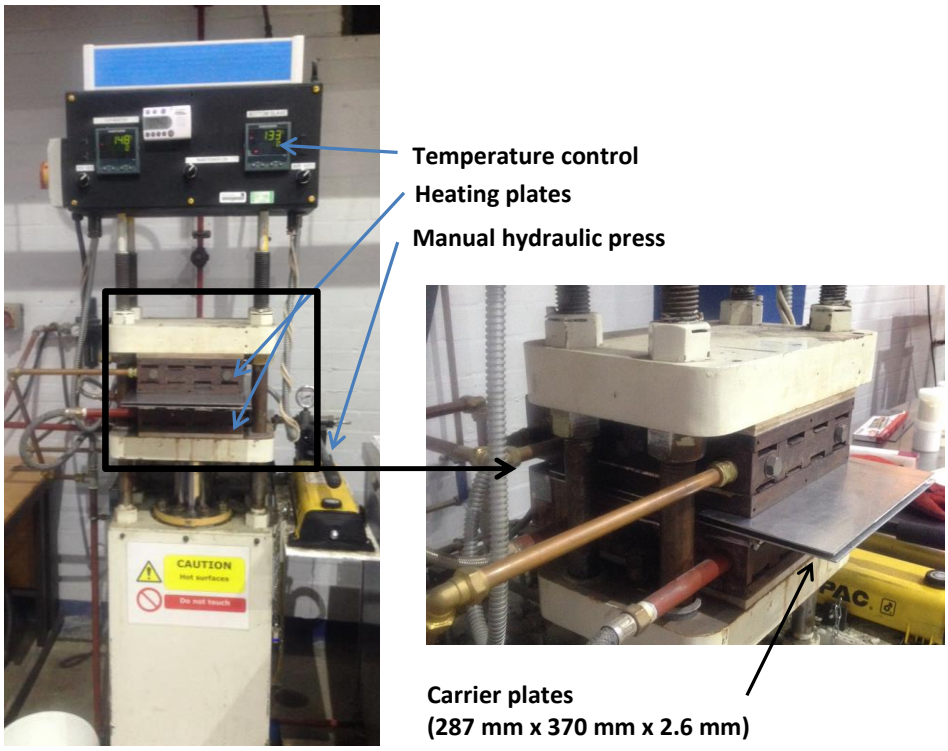


Figure 6.8: The heating press at University Park Campus. Used with support from Davide De Focatiis (Faculty of Engineering). The heating press was equipped with a temperature control for upper and lower plates (connected to water for cooling) and a hydraulic press to apply pressure.

It is seen from Figure 6.9 that the addition of 15% moisture (based on wet batter weight) was not sufficient to hydrate the sample and create an even film (batter 2002, high amylose maize (207) and waxy maize (208) showed similar results, not shown). The addition of moisture was again a challenge in the film formation although it did not appear to be important for the previous investigated popped samples (see Figure 6.2).

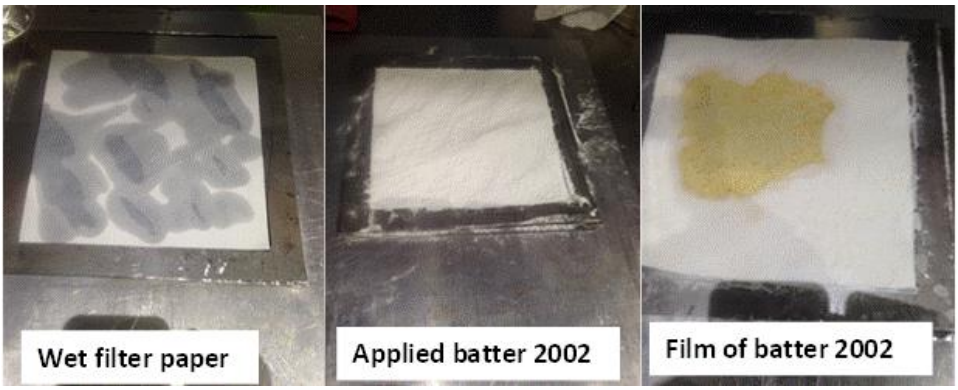


Figure 6.9: The initial testing of the method used for the heating press. Filter paper was wetted, batter applied and film formed.

The big size of the mould (160 mm x 160 mm x 0.7 mm) may had been a limitation for the water to hydrate evenly so special designed moulds were made to decrease sample size and make it possible to form replicates within the same batch. Figure 6.10 shows the developed sandwich type set-up including a schematic drawing of the moulds. The set-up was developed with the experience from the popping experiments. The carrier plates were necessary to handle the mould and insert it within the two hot plates (see Figure 6.8).

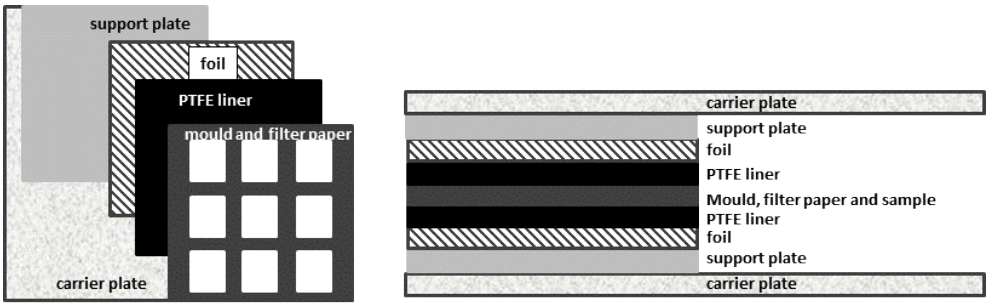


Figure 6.10: A schematic diagram of the "sandwich set-up" used for the hot press experiments. (The dimensions shown are not in accordance with the real plates etc., see section 2.5.1.2.1)

Using the new moulds, the addition of moisture still utilised filter paper as a carrier, however it was soon observed that the method was not adding sufficient moisture to the batter systems. The screening was done on batters 2001-2002 and the individual ingredients with the varying amylose levels (206-208). Different moisture levels were probed and it is seen in Figure 6.11 that the addition of 15-34% moisture content was not sufficient to create a film with evenly distributed moisture. The filter paper was not able to absorb more moisture and therefore another method of water addition was required. Suspensions of the materials were prepared prior to the film formation in order to make it possible to create films with initial 40-60% moisture. Mixing of the suspensions and final texture of the suspensions were very different between samples as seen in Figure 6.12 (before and after images of some selected results). It was clear that a higher amount of moisture was needed to create films with evenly distributed moisture.

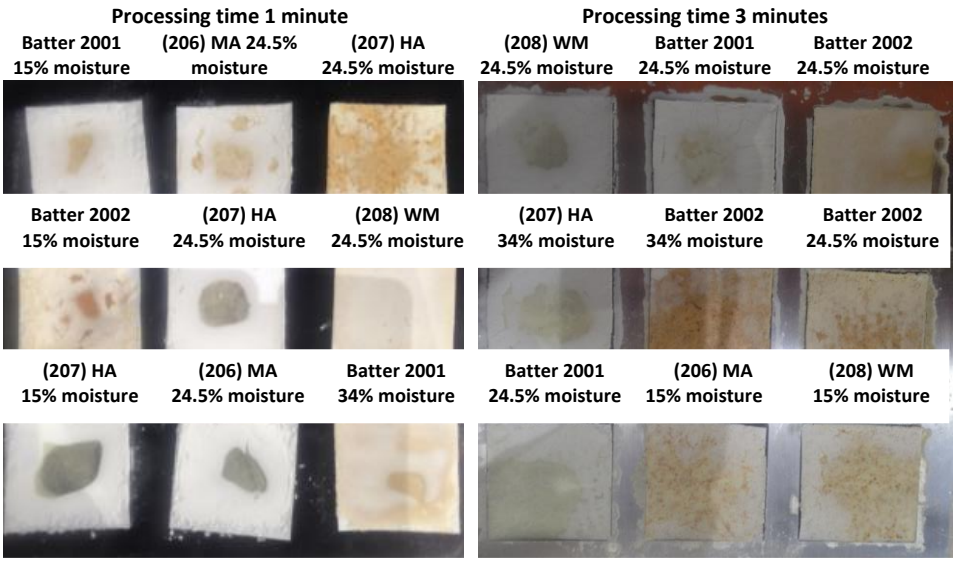


Figure 6.11: Screening with different moisture contents 15-34 % added to the filter paper. MA maize starch, HA high amylose maize starch and WM waxy maize starch. The change in processing time (from 1 to 3 minutes) was a part of the screening process but it did not appear to make a difference in film formation.

The amount of suspension required did not appear to make a difference in the thickness of the film or whether the films were formed evenly. Another variable was the processing time, which was changed to 1, 2 or 3 minutes (Figure 6.11, not all data shown), and did not appear to have a pronounced effect on the film formation.

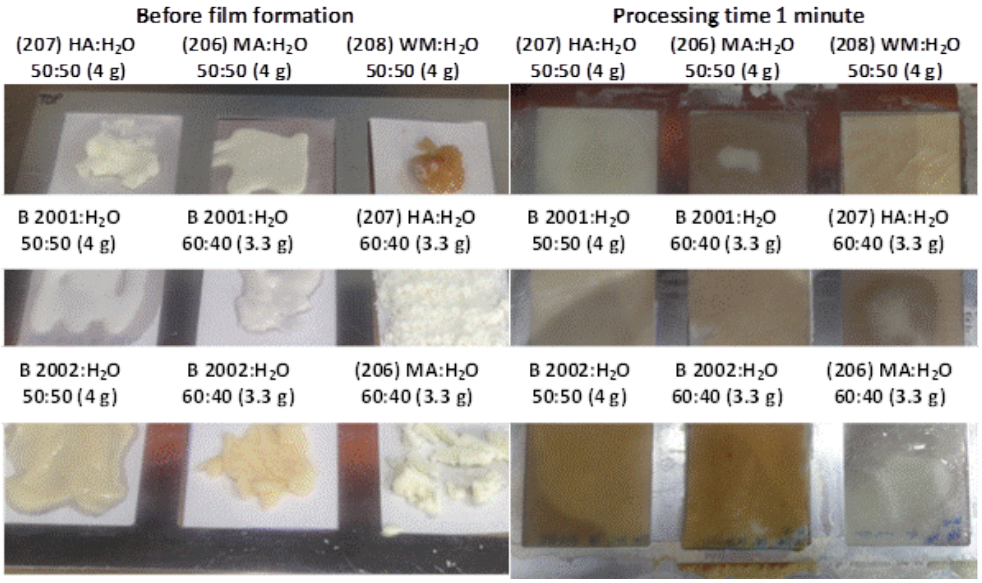


Figure 6.12: Screening with different moisture contents (40-60 %) added as a suspension of the starch material, before and after film formation. One screening variable was the amount of suspension added i.e. 3.3 g or 4 g. MA maize starch, HA high amylose maize starch, WM waxy maize starch and B batter.

The method development was probed by screening some selected materials and it was concluded that the sandwich type set-up presented in Figure 6.10 was the most appropriate set-up to avoid moisture and material escape. A suspension of 50:50 starch material/water (4 g) was the moisture content which created the right texture of the film (not too wet and yet hydrated) with one minute processing time.

The moulds were packed into the sandwich type system with the moulds protected by carrier plates and both PTFE liner and thick aluminium foil to seal the mould in order to prevent evaporation of moisture and escape of sample (see Figure 6.10 and section 2.5.1.2.1). However, the high moisture content of the 50:50 batter/water suspensions, which was required to ensure enough moisture in the sample for even film formation, resulted in the easy flow of the suspension. Therefore some material did flow out of the mould system (Figure 6.13). This flaw could not be easily solved. Less moisture would result in dry areas on the film hence limited gelatinisation or melting of the starches creating the film. Keeping in mind that flow of sample and moisture from the mould system were unavoidable, this method was adopted as the standard method (section 2.5.1.2.1) and the three batters 3001-3003 were assessed.

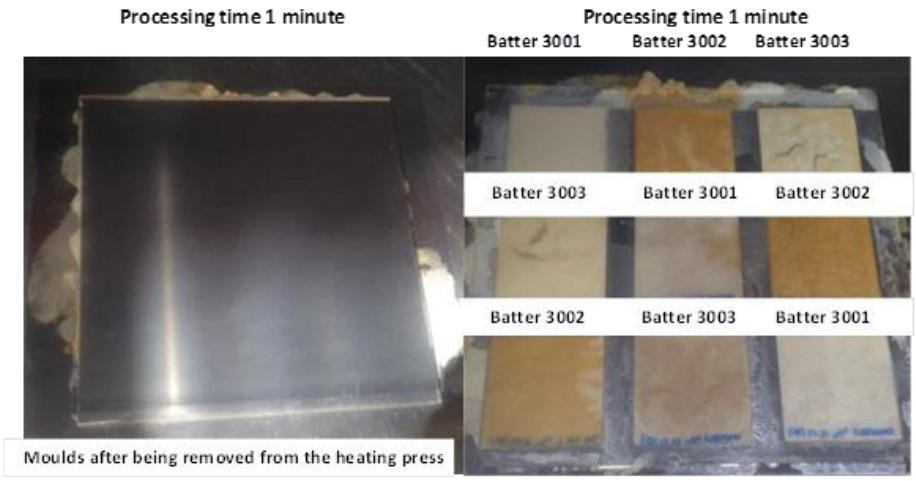


Figure 6.13: Moulds after being removed from the heating press. Left picture demonstrate the escape of batter suspension and moisture from the mould system. Right picture shows triplicate samples (n = 3) of the batter samples 3001-3003 randomly placed (batter:starch 1:1, 4 g).

Figure 6.13 shows the films of the three batters 3001-3003 in triplicates. This picture demonstrated the escape of batter solution and moisture from the mould, resulting in different levels of thickness of the three replicates and most likely differences in moisture contents across the samples. The method developed for the formation of a film using the heating press was not successful since it was anticipated that it was possible to maintain the moisture and sample material (see introduction to chapter 6). This proved very difficult to obtain. A major goal had been to use films formed by pressing for further analyses, especially to establish the viscosity of the films and their glass transition temperatures. Since the moisture content and thickness of the films could not be sufficiently controlled, the films were not adequate for subsequent comparative analysis and examination of the film forming properties.

6.1.3 Dynamic mechanical analysis (DMA)

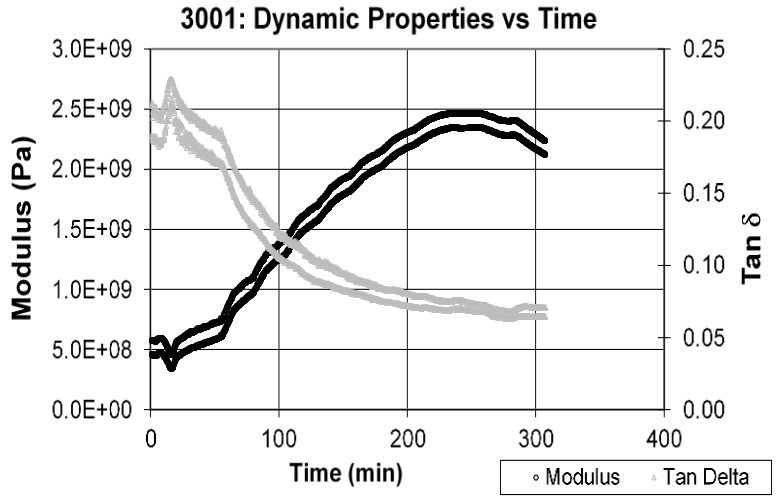
A useful technique to analyse film forming properties is dynamic mechanical analysis (DMA, see section 2.5.2.1) in which modulus (flexibility) and transition temperatures can be determined. The DMA method is normally used to obtain material stiffness as a function of temperature.

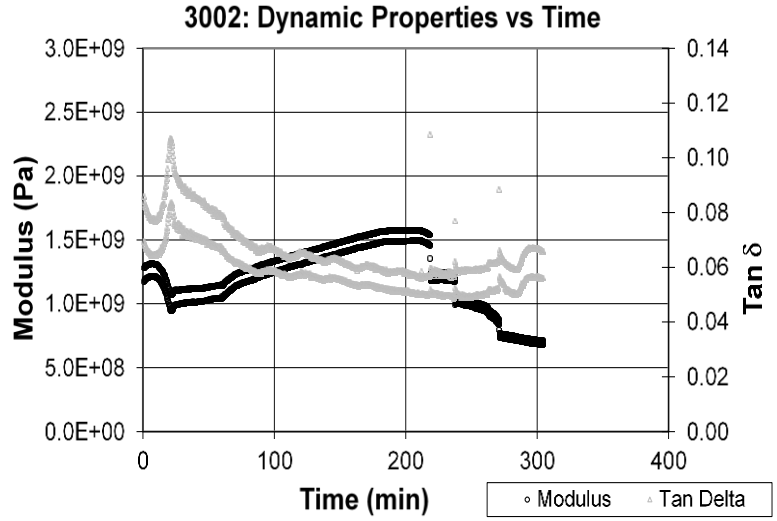
Although the three batter films (3001-3003, Figure 6.13) did vary in moisture content and thickness, they were tested using the DMA technique (for method see section 2.5.2.1). The standard method was slightly altered to use a pre-cut piece of film and not the powder pocket technique. The films were prepared using the heating press (see 2.5.1.2) and it was observed that the moisture content may vary between samples. In order to interpret the obtained data from the DMA it was important to know the moisture contents of the samples or as a minimum adjust the

samples to similar moisture contents since the retrieved data would be moisture dependent.

The DMA equipment is restricted to either control the relative humidity of the sample using the humidity control system, or to control the temperature using a standard oven set-up equipped with a liquid nitrogen supply. Hence the film could not be adjusted to similar relative humidity (to ensure “expected” similar moisture content and avoid fracturing) and then analysed for glass transition (temperature scan). Because of this limitation of the DMA, the films were used “as-is” after 1 week storage in a closed container at room temperature.

Figure 6.14 shows the storage modulus at two frequencies (1Hz and 10Hz) and the respective tan δ values of the films formed from batters 3001 to 3003. The modulus will indicate cracking or pores in the film and can therefore conclude whether the film will be suitable for analysis. It is shown that only the film formed from batter 3001 could be analysed using the DMA technique. The two other films formed from batters 3002 and 3003 broke during the analysis which was indicated by the big drop in modulus after approximately 220 minutes and 240 minutes for film 3002 and film 3003 respectively. The sudden drop in modulus (representing the stiffness in the film) indicated that the samples had suddenly gained a lot of flexibility due to the cracking of the film. This was confirmed upon removal of the film samples 3002 and 3003 from the cantilever clamps where it was seen that the films had fractured.





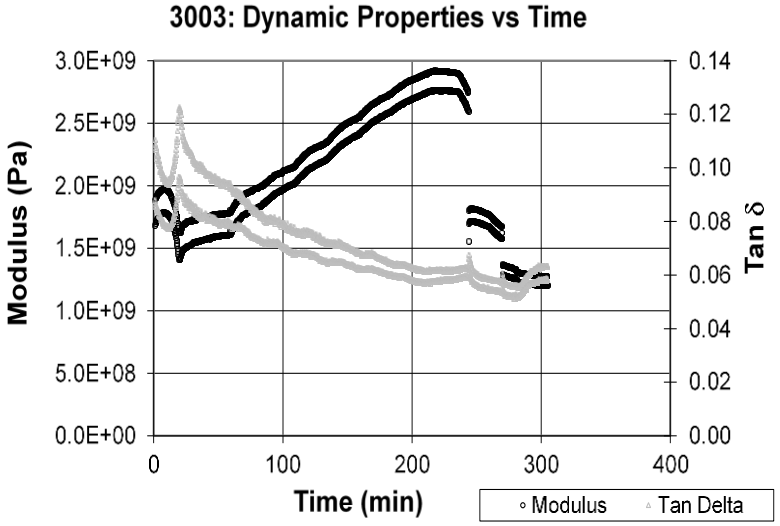


Figure 6.14: The dynamic mechanical analysis of the films formed from batters 3001-3003 by utilising the heating press procedure (see section 2.5.1.2.1). The modulus for two frequencies (1Hz and 10Hz) was plotted against time and the respective calculated tan δ are shown. The temperature was kept constant (30 °C ± 2 °C) during the experiment.

The difficulties in producing films with known and similar moisture contents using the controlled heating and cooling press and the issues experienced with the DMA analysis of the films resulted in another approach to investigate processed batters using the DMA technique. The popped work (see section 6.1.1) showed that most of the granular structures had been lost on the sample formation. Although pasting with high water and shear may not replicate the batter film formation exactly, it may not be such a bad representation as originally anticipated. Therefore batters were pasted in the RVA (see section 2.4.4) and the samples dried in order to create a processed batter (see also section 5.3.1). The DMA powder pockets technique (section 2.5.2.1) was used to characterise these processed batters.

6.1.3.1 DMA on processed batters

The batter samples 2001-2010 had been processed in excess water under controlled heating and shearing (RVA measurement) and the target was to pre-gelatinise or melt the starches within the batters so they were in the same state as they would be upon film formation during a frying process. However it may be that some of the batters containing high amylose starches (samples 207 and 209) had only been partly gelatinised. The processed batters were dried and milled (as described in section 5.3.1) and then stored over saturated sodium chloride for one week in order to obtain a similar moisture content (e.g. at one water activity level, see section 2.5.2.3) in the processed batter samples. Prior to the DMA experiment, it was expected that the moisture content of the samples would be similar at one water activity. Measures of moisture content of the samples were only conducted after the DMA measurements (see section 5.3.1). Increasing the moisture content had two advantages, the glass transition temperature would be lowered and the sample

would mimic a midpoint in the frying process (between the starting and final moisture contents of 60% and about 1.5% respectively).

The DMA set-up (equipped with the oven for temperature scan) is shown in Figure 6.15. After the experiment and upon removal from the pocket, the powder samples 2001-2010 (Figure 6.15, lower left corner) were visually assessed and showed differences between the samples. Samples 2001-2004 remained powders whereas samples 2005-2010 had formed thin cohesive films within the pocket.

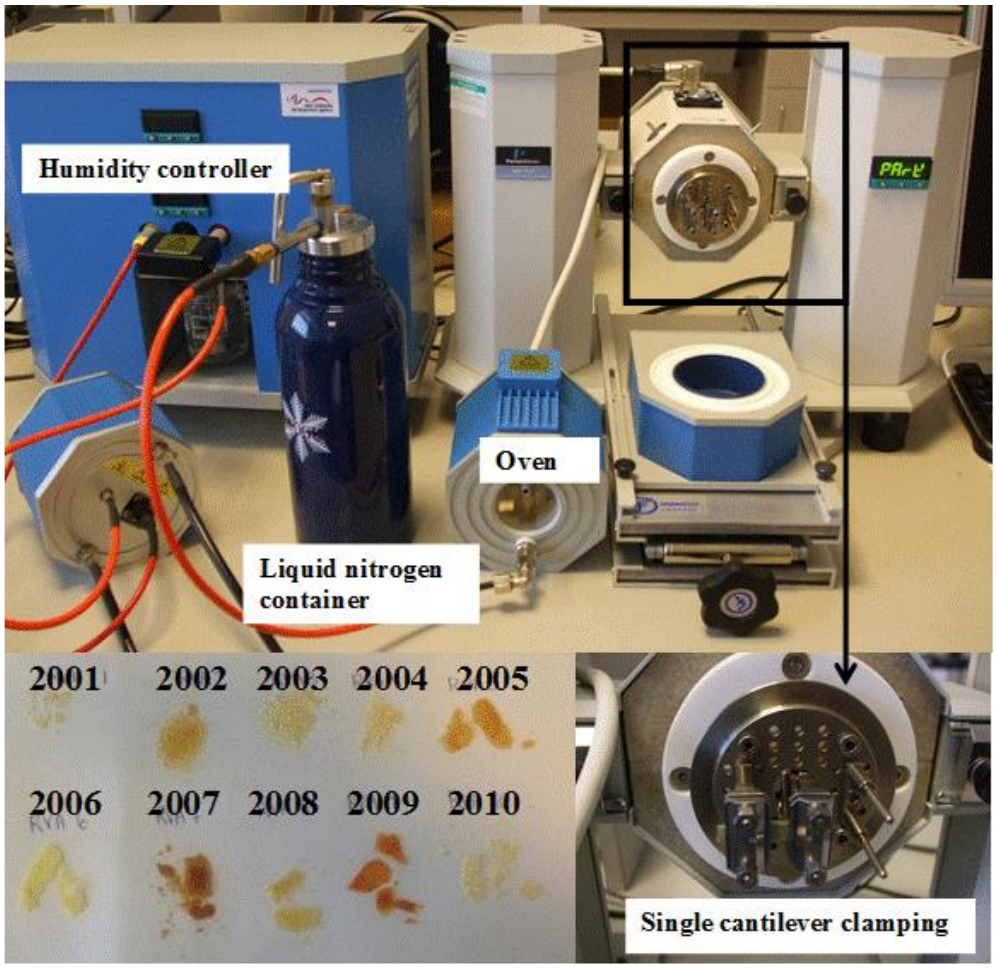


Figure 6.15: Dynamic mechanical analysis equipment (for method see section 2.5.2.1). The single cantilever clamping mode is enlarged (lower right corner). Lower left corner displays the resulting powders after analysing the processed batter samples 2001-2010 using the powder pocket technique.

Figure 6.16 displays the analysis of batter samples 2003 and 2006 presenting tan δ as a function of temperature. The graph shows tan δ at four different frequencies

(0.1 Hz, 1 Hz, 10 Hz and 30 Hz) in order to measure the mechanical properties of the processed batters at different shears. The modulus (both storage and loss) are highly dependent on the frequency as well as the measuring conditions and the history of the material. The mechanical properties at different frequencies (shear) indicate the flexibility of the materials, which is important for their use as potential film formers. Furthermore, glass transition is frequency dependent and thus can be observed by applying different frequencies.

It is seen that for sample 2003, there was no clear glass transition indicated by similar peak temperatures at different frequencies. However, for batter sample 2006 there was a clear glass transition with the peak temperature increasing with higher frequencies (Blanshard and Lillford, 1993). If there was more than one transition shown e.g. sample 2006, the glass transition was assigned to the peak exhibiting frequency dependence. However, for samples with less clear glass transitions (sample 2003) the shown transition above 0 °C was assigned as the glass transition peak. The glass transition temperatures for the batter samples were determined from the average of peak temperatures for all frequencies.

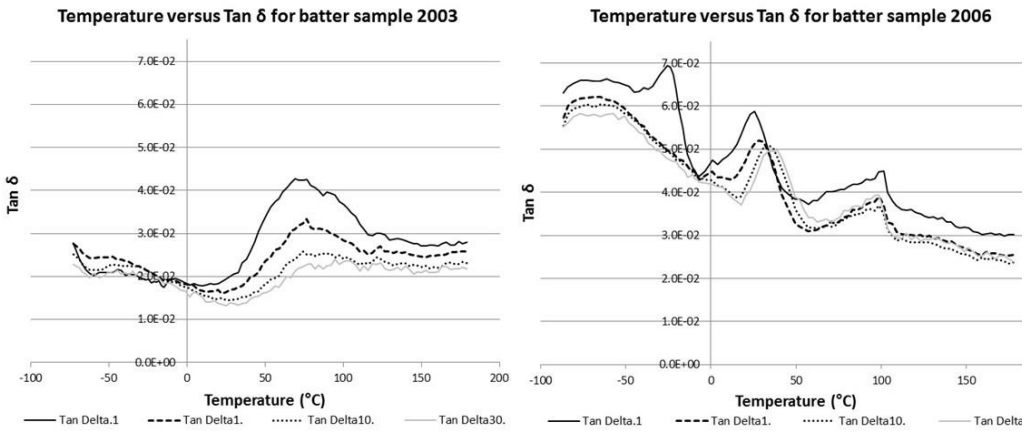


Figure 6.16: Dynamic mechanical analysis of the processed batter samples 2003 and 2006 using the powder pocket technique.

The activation energies for the batters were calculated using the Arrhenius equation given as $A = A_0 \exp(-E_a/RT)$ where A is the rate constant, A_0 is the pre exponential

factor, E_a is the activation energy, R is the universal gas constant (8.3136 J mol^{-1} K^{-1}) and T is the temperature (K). The activation energies were determined by the equation retrieved from plotting log (frequency) as a function of 1/T. The activation energy values appeared different (Figure 6.17), but due to the high standard deviation, no clear trend was observed. Although the activation energies showed differences, most of them were similar to previously reported values for glass transition in biopolymers found in the range of 200-400 kJ·mol^{-1} (Blanshard and Lillford, 1993, Mizuno et al., 1998).

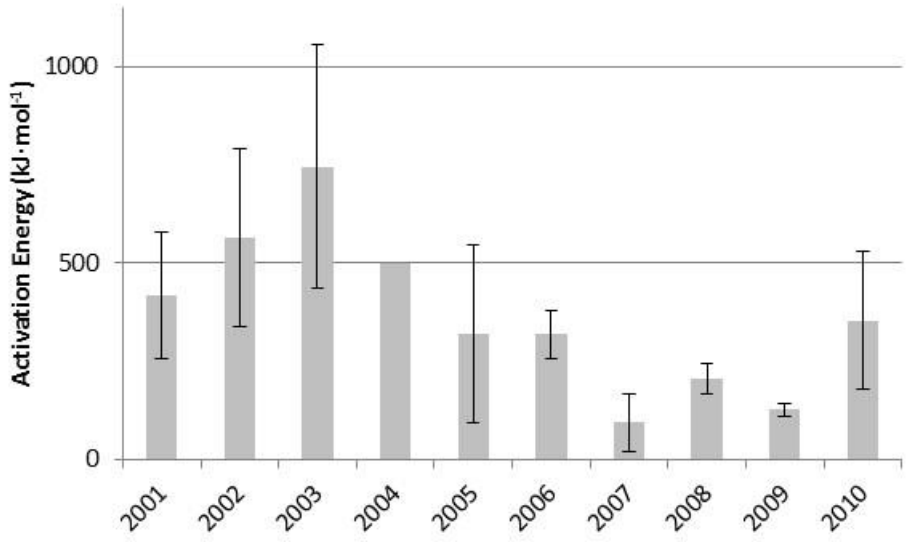


Figure 6.17: The activation energy for batter samples 2001-2010 determined using the Arrhenius equation and the data from dynamic mechanical analysis.

The processed batter samples were also assessed by other thermal analysis techniques such as TGA (see section 5.3.1) and DSC to gain additional information about the samples. Further the moisture contents of the samples were determined (section 5.3.1) which proved to be very relevant for the interpretation of the data.

6.1.3.2 DSC analysis

DSC analysis was used to determine the glass transition temperatures of samples 2001-2010 (to compare to values determined by DMA). However, no clear transition

could be seen for the second measurement (see Figure 6.18) and therefore the glass transition temperature could not be determined by DSC for these samples. A possible explanation for the lack of visible transitions could be due to the complexity of the batters or the processing conditions (see 5.3.1). It was observed that some of the processed batters (2002, 2005, 2007-2008 and 2010) showed a transition upon the first measurement (see Figure 6.18).

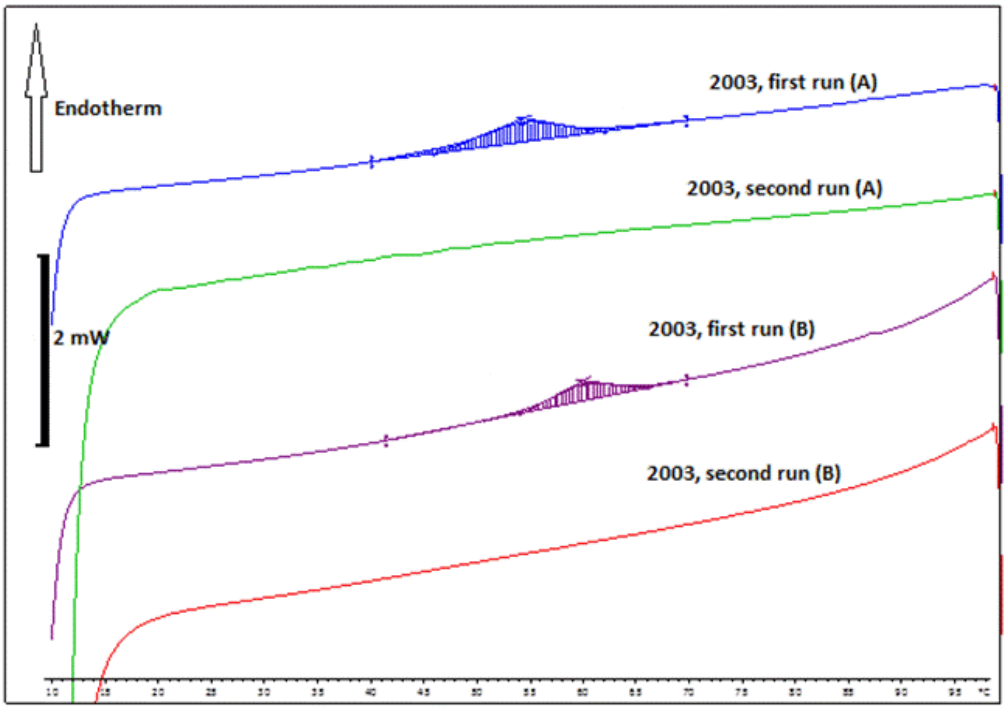


Figure 6.18: DSC thermogram for processed batter sample 2003. Experiment done in replicates (n = 2) with rerun to determine glass transition temperature. Experiments were done on hydrated batter samples (75.6 RH), with a heating rate of 10°C /min and in the interval 5-100 °C (see section 2.4.5).

The DSC analysis of the processed batter samples indicated that the samples were different with regards to composition and crystallinity (possibly retrogradation behaviour) and thus suggested that the samples behaved differently upon storage. The re-ordering of the starches could have been expected to have an impact on the rheological performance of the materials. These results confirmed that the diversity of ingredients within the batter formulations would alter the behaviour of the batters at the macromolecular level.

The DSC analysis suggested that the processing conditions of the batters were very important in terms of recrystallization behaviour after treatment. It also indicated that the DSC technique had some limitations when analysing complex materials such as the batter formulations. It may be that due to the amount of transitions within the batter formulations, that each is so small that they are indistinguishable from the base line.

6.1.3.3 Glass transition temperature

Concurrent with the thermal analysis of the processed 2000 series samples, the moisture content of the samples were tested (after DSC and TGA, see Figure 5.8 section 5.3.1). The results were surprisingly very different, with moisture contents spread in the interval of 15.5-28.7%, hence despite the samples being stored at the same water activity, the moisture contents of the batter samples were different (see section 5.3.1).

Figure 6.19 presents the graph of the moisture contents of the batter samples versus their glass transition temperature (determined by DMA, see section 6.1.3.1). A clear correlation was observed between the moisture content and the glass transition temperature. The higher the moisture content, the lower the glass transition temperature. This correlation has previously been reported by Slade and Levine (Levine and Slade, 1991) to be one of the essential theories in the understanding of the glass transition and glass behaviour. It was shown that the correlation was true for multi component blends of starches, but also that the components of the starch batters seemed to be less relevant for the glass transition than the moisture dependence.

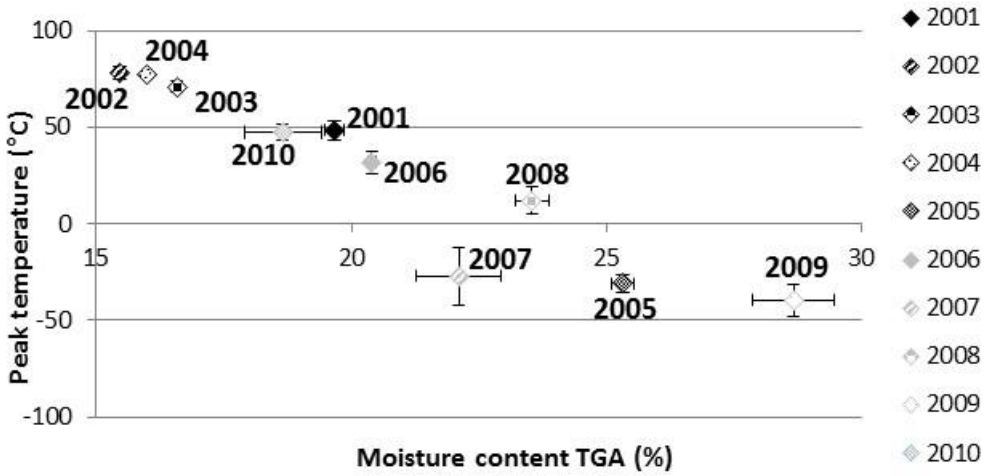


Figure 6.19: The peak temperature determined by DMA versus the moisture content determined by TGA for the processed batter samples 2001-2010.

It was presumed that the glass transition temperature would be able to distinguish the batter samples thus differentiating the batter formulations according to glass transition and thermal behaviour. The aim was to create an insight into the batter performance based on the glass transition temperature. However, since the glass transition temperature could not be confirmed by DSC (see 6.1.3.2) and the moisture content could not easily be adjusted to the same level, the true glass transition for the batter formulations could not be related to the batter properties in the coating process. An important finding was that the moisture contents of the processed batter samples appeared to be very different although stored at the same water activity level. This interesting finding was covered in more detail in chapter 5.

6.2 Discussion

The work described in this thesis was to establish the roles of different starches in the film formation that is thought to be an essential feature of the functionality of batters. The thickness of the batter film on French fries is in the region of 0.7-0.9 mm (Coates, 2014) and therefore to use the film to establish its rheological properties is difficult, especially since the fried film may be aerated and contain oil. Forming starch films is known to be challenging (Rindlav-Westling et al., 1998) and they are typically cast from low concentrations or made via thermomechanical extrusion with associated high shear. Neither of these is similar to the processing for a batter which includes moderate levels of solids (40%) and rapid flash off of water due to immersion in oil at 185 °C. One unknown for prediction of batter performance is the temperature-moisture content relationship as the water flashes off during frying. With the strong relationship between the process of starch melting and the level of plasticiser, the loss of water may be critical for the formation of a starch film. To understand film formation, it is essential to be able to form films from batters in a controlled way, with variables of temperature and moisture contents. This chapter has focused on the development of new techniques to form films utilising starch formulations. The formation of films was achieved by applying pressure and heat to starches with limited moisture contents, hence creating a film network as a result of the melting of the starches.

The hot press and the popping machine were operated at 185 °C. It was interesting that reasonable films could be formed using the popping machine, but not the hot press at the lower (< 40%) moisture contents. The critical feature seemed to be the even spread of moisture in the samples. Even at moisture levels down to 15% the popping machine still produced samples where there was little ordered structure

left. It is possible that the destruction of the order in the starches was not so much due to the heating stage, but occurred during the rapid release of pressure. The pressures on heating the samples may have aided in forcing water into the granules and then the rapid drop in pressure disrupts the granules. This is not a mechanism one can imagine in the frying of batters. Whatever the mechanism, sufficient polymer-polymer interaction must occur to allow a solid film to be created for all the starches examined. Despite major differences in these samples in terms of their amylose/amylopectin ratios, physical and chemical modification, their properties were not widely different.

The hot press should have enabled the same type of film formation, but despite its better heating and cooling facilities, it was very difficult to obtain homogenous films using this equipment. In an effort to make films suitable for rheological determinations, they were made at moderate water contents (50%).

The starch films made by pressing were in the region of 0.7 mm in thickness. Their brittleness was evident in the way they cracked when being examined by DMA. Without adequate films, the alternative of using processed powders and the pocket technique was tried. The loss of order in the popped films suggested that the use of a processed batter sample created by pasting may be valid. In an effort to manipulate the T_g to a reasonable value for measurement, the amorphous processed batter samples were all hydrated at 75.6% RH. Later DSC measurements showed that some retrogradation of these samples had occurred, again highlighting that the different starches in the batters could be acting differently. Although the DMA traces were very complex, the expected change in T_g with frequency was often observed and the activation energies were in line with expected values for these transitions. The glass transition determination using the DSC technique was not

possible due to the complexity of the batter formulations and/or the processing conditions and confirmed that these small transitions are easier to see by rheological techniques.

The DMA data could have been helpful in understanding more about the starch mixtures, but there was the serious complication that the samples had different moisture levels. The correlation of the measured T_g with moisture showed that this was a much more dominant factor than the change in batter formulations. Reasons why the batters came to different equilibrium moisture contents have been dealt with in Chapter 5 where it was concluded that salts had been added to the starchy mixtures.

Another ingredient that seemed to have a major impact was one of the dextrins. Assessment of the individual ingredients (201-219) led to surprisingly small differences between the popped samples. Only the dextrin samples stood out with remarkably different textures and destruction of the filter paper that was used as a base. The most distinct dextrin (210) was included in the batter 2002, which had showed marked differences in the popping experiments compared to the other batters.

CHAPTER 7 DISCUSSION AND CONCLUSION

7.1 Batters leading to crispy films

The key attribute of a batter is that it will improve the eating quality of the product, in this work potato, onto which the batter is added. The batter needs to form a film and that film is required to control oil uptake and to provide a crispy layer. The batter-film transition occurs during processing where the key characteristics are that heat is applied and that water content is reduced from about 60% in the batter mixture down to very low levels, perhaps in the region of 1%. However, only little moisture should be lost from the potato base layer to maintain a soft and moist interior of the final product. Loss of moisture from the batter occurs within seconds during a frying process. After frying the film is required to maintain crispiness for minutes, whilst acting as the interface between the potato base and the often humid ambient air.

The sponsors of this work have substantial experience in the manufacture and application of batter formulations that fulfil the basic requirements for coating potatoes. According to the manufacturer, to achieve the desired functionality, a batter requires:

- Blends of starches
- Modified starches within the blends

A schematic representation that described the key factors in a batter and film formation was drawn up (Figure 7.1). The theories put forward, resemble the process where on heating and moisture loss some of the starches retained either some or all of their granule structure while others formed a matrix of starchy materials holding the granules in place. The theory is presented in Figure 7.1a and it

is based on the principle of multiple granules present with different functionalities. Some granules will act as bricks and retain the granular shape whereas other granules will swell and yet others are broken down leaching amylose (or less branched amylopectin) allowing polymers to solubilise in water to act as glue between bricks (intact granules) and thereby create a film by connecting the different materials. This mechanism will be completed upon the final frying and thus the process requires a fast temperature increase to flash off of moisture to be completed.

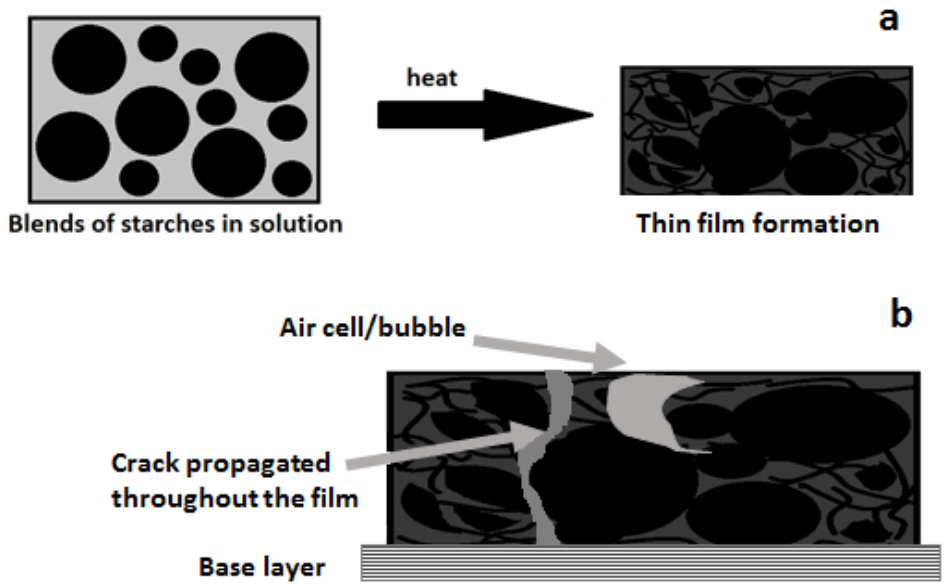


Figure 7.1: Schematic of the "bricks and glue" theory. a) Some starch granules are still intact, some are swollen while others are broken down and leaching amylose (or less branches amylopectin) acting as a glue between bricks, densification of the system as water is lost. b) Other factors for the film are the relationship between the film and the base layer and the cracking and air pocket formation within the film.

The rapid transition from high moisture to low moisture upon heating would be presumed to be the dominant factor for the film formation and may therefore affect the final attributes of the film. Figure 7.1b captures some of these factors and indicates the importance of the interaction between the film and the base layer material. Of relevance to this work the base layer would be treated potato. Figure 7.b also indicates the importance of spaces within the film, either cracks or bubbles.

These are relevant in terms of oil getting into the batter and base layer (Llorca et al., 2003, Sahin and Sumnu, 2008). The cracks and bubbles will also change the moisture sorption behaviour of the film.

To validate and extend the model put forward and to comprehend the empirical findings of the sponsoring companies, several objectives would need to be met. These included understanding the role of modified starches in batter formations, how and when the batter results in a film and the quality of that film. Underlying factors that needed to be considered throughout the work were moisture levels and their impact on the starches and the films. This essential factor was made more difficult by the fact that during processing water loss and temperature of the materials were interdependent and very difficult to measure as changes may occur very rapidly and may never be in equilibrium.

7.2 Commercial samples

A feature of the work discussed in this thesis was that all the materials were commercial samples and information about their origin and modification were supplied by Newly Weds Foods Ltd. and McCain Foods Ltd. as part of their sponsorship towards this PhD study. In total the companies supplied 77 individual ingredients and combinations to form batters that became the backbone samples used for the studies. Although the use of these samples ensured that the work undertaken was of practical relevance, there were some difficulties encountered due to the restricted information available for the materials. In some cases the chemical nature of the samples was unknown or the proportions within the mixtures were withheld.

7.2.1 Starches with explicit levels of modification

One of the major objectives of the work was to examine chemically modified starches as their altered functionalities were believed to have a considerable impact on the properties of batters. Examination of the modified starches (chapter 3 and 4), concluded that various characteristics and physicochemical properties were confirmed in literature to be similar to the ones reported in this study. Of relevance to the batters could be the etherification of the starches. The introduction of the propylene group, for example, has been shown to lower the gelatinisation point of the starch (White et al., 1989). This could be relevant in these low moisture systems where opening of the granule structure to allow polymer interaction could be important for the film formation. However, contrary to this idea is that the more hydrophobic chains introduced, the more the starch chains are prevented from associating. The prevention of retrogradation is the key use of stabilised starches where their structure prevents formation of amylose-amylose helices and this "stabilisation" reduces syneresis. Although the cooked batters are held and frozen, it would seem unlikely at the moisture contents of the batters that the key function of the modified starches is to prevent water loss from the system.

In addition to etherification, most of the modified starches are also crosslinked. The crosslinking can be inter- or intramolecular and is generally considered to limit the swelling of the granules, which are then much more robust, so that they can retain their structure even on heating and shearing. The number of crosslinks that impacts on the granule behaviour can be low, perhaps one crosslink per 3000 anhydroglucose units can be sufficient to cause the changes (Williams, 2011). The validity of the new models for amylopectin structure, based on the backbone model ((Bertoft, 2013) and see chapter 1), may well be tested by ascertaining where the crosslinking and the stabilising substitution occurs within the structures. However,

accurate assessments of the levels and position of the chemical changes would need to be known.

A set of modified starches, stated to have the same level of stabilisation and different levels of crosslinking, were examined in some detail as it was thought that they represented a series and could be comparable. The levels of modification as given by the suppliers were not specific and relied on the levels of added chemicals and not on measured levels of chemical substitution. The information on the addition of chemical reagent used for the modification was not disclosed. When 25% propylene oxide is used, it is thought that about 40 ether linkages are added per 100 glucopyranose units and if only 5% propylene oxide is used this falls to 4-6 groups per 100 glucopyranose units (Organization and Assembly, 1972). These numbers will be dependent on other factors such as the pre-treatment of the starch, its botanical origin etc. It is also known that the addition of the stabilisation impacts on the levels of crosslinking achieved with the same level of chemical (Wattanachant et al., 2003).

The family of samples investigated in some depth were based on tapioca and as these starches have little or no lipid in the starch granules and have a typical ratio of amylose (≈20%), this source of starch appeared to be appropriate to investigate for chemical modification. For many of the assessments in excess water, the results did not follow the expected behaviour based on the suggested crosslinking of the samples. Sample 115, was suggested to be highly crosslinked and this material did seem to develop little viscosity when pasted.

For several of the assessments it would be normal to base the values on the levels of starch, or amylose present in the system. During these studies, it was noted that the normal assay procedures failed. Iodine interacted with the crosslinked starches to

form precipitates. Whether this was relevant in situations where lipid inclusions were formed in the dense networks occurring in the batters could be considered.

The functionalities of the group of stabilised and crosslinked tapioca starches were difficult to interpret in relation to the modifications levels and suggested that the information provided was inadequate. Attempts were made to get quantifiable data for the modification levels, but it was felt that there were too many unknowns for these samples to warrant the effort that seemed to be required to get estimations.

7.3 Replication of the thin film on a potato chip (French fries)

Application of batter formulations onto a base material, such as a potato chunk, is a complex process and was described in chapter 1. As the starting materials are processed, the starches within the batter formulation undergo a range of transitions due to changes in the primarily moisture content and temperature. One objective of this research was to replicate the film formation of the batter formulation in a test system in order to interpret and understand the film forming abilities and properties of the starch mixtures.

Batter formulations were added to a base of hydrated filter paper and the batter/filter base system was fried. Although this did give some insight into batter to film transitions, the dynamics of moisture loss and the concomitant changes to the starches were difficult to follow. Upon heating changes to the starches occur and the levels of water and temperature have crucial roles. Figure 7.2 indicates the generally accepted view of the state diagrams that describes the zones for melting (T_m) and the glass transitions (T_g) for starches (wheat starch in this case) at different moisture levels and temperatures. It shows the trend that as moisture content decreases, higher temperatures are required for the starch to melt (disruption of the granular structure). Further the evaporation of water becomes significant at

temperatures above 100 °C and this rapid flash off of moisture is very relevant at water contents below 60%. At these moisture contents (<60%) the temperature required to melt the starches increases. At very low water content (< 10%), the temperature needs to be above 200 °C to achieve loss of starch order. The dynamics of moisture loss as the samples are heated must be very relevant for the formation of the film.

Starches used in the batter will absorb water differently and it is possible that the flashing off of the water may occur before loss of order for the starches. For example, the melting temperatures of the high amylose starches are known to be high. From DSC measurements the peak temperature was quoted as 88 °C and completion not occurring until > 100 °C, and therefore it is unlikely to occur in the batters as the water is lost (Kibar et al., 2010).

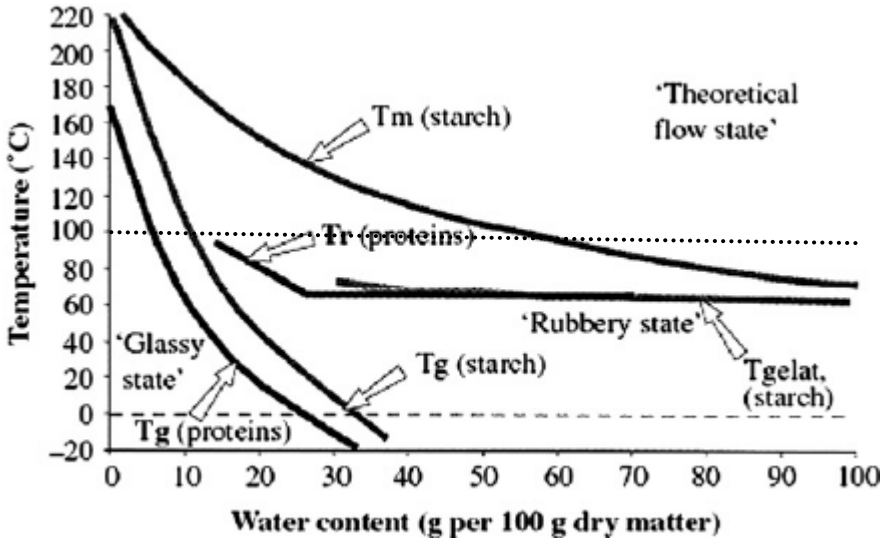


Figure 7.2: A schematic representation of the temperature-water content state diagram of wheat starch and gluten protein (main components in wheat flour). T_g denotes the glass transition temperature, T_m denotes the melting temperature, T_{gelat} denotes the starch gelatinisation temperature and T_r denotes the minimum temperature of protein thermosetting. The figure is adapted and reprinted from (Cuq et al., 2003).

If another component such as wheat flour is present in the starch formulation, then the protein transitions need to be considered as well as the starch transitions (see Figure 7.2). The change of state for the starch material can be used to predict the

processing behaviour of food materials containing starch (Cuq et al., 2003), however attention toward the specific system is important and the state diagram may change slightly depending on type of starch, modification levels etc.

For film formation these transitions needed to be considered and therefore the replication of thin films used for potato chips was a challenging process. With frying the water leaving the sample would be hard to control and therefore two techniques were developed to look at thin film formation from the starches used to create batters. Interestingly, these methods (popping and hot press) gave different insights into the film formation.

The popping equipment used is discussed in chapter 6 and although this was designed for a different purpose, with development it did allow insight into the potential film properties of the starches. One of the technical challenges was to create even moisture distribution at low levels of water addition to the starch. This was done as it was considered to be interesting to monitor starch behaviour at low moisture levels. The assumption was that water being removed from the batter on frying would keep the batter at approximately 100 °C due to the latent heat of evaporation. Therefore the starches retained their ordered state until a high temperature and low moisture regime persisted. For the popped samples, a low moisture content of 15% was enough to create a firm, cohesive, popped (somewhat expanded) film for all samples (excluding the dextrins, which will be discussed in section 7.5).

These films were not very different from those created at 10 or 20% moisture. These results were a major finding and led to a question of whether the critical factor was i) that heating the starch at 185 °C for 4.6 seconds was sufficient, at the moisture

contents used, to disrupt order and allow sufficient starch polymers from the granule to cause the “glue” to hold the starch together as a film (see Figure 7.1) or ii) that it was the rapid release of moisture vapour upon pressure release that lead to the disruptions of the starch granules so effectively that they were able to form an expanded film. Upon expansion of the starch materials and loss of the water, there would be a sudden transition from rubbery state to the glass state enforced by the very low moisture content and rapid drop in temperature.

According to the state diagram (see Figure 7.2), the temperature required for melting of starches at 15% moisture was about 180 °C which was about the temperature set for the popping machine (185 °C). Thus for normal starches a film could be expected to form in the popping machine, however high amylose starches would be thought to behave differently due to their different melting profiles (Liu et al., 2006). Since the popping of starches is a novel technique, no literature reports have been found with similar findings. However, the popping of long grain brown rice was reported to show a similar effect (Hsieh et al., 1989). Hsieh and co-workers found that 14% moisture added to the rice resulted in increased puffing (compared to addition of 16%, 18% and 20% moisture) and suggested that this unexpected result was an effect of reduced elasticity at lower moisture content which prevented the puffed porous structure from shrinking upon cooling (Hsieh et al., 1989).

All the starches and batters formed slightly expanded films and there was no correlation between the samples that showed expansion of frying and those that were expanded in the popping device. The starches lost their structural organisation through the popping procedure and only starches at the very edge of the film or where high levels of gums were known to be present, showed starch helical order.

To avoid the added complication of expansion, it is possible to keep the starches in the popping machine as it cooled before opening it up. This was carried out for some samples and these are shown in Figure 7.3.

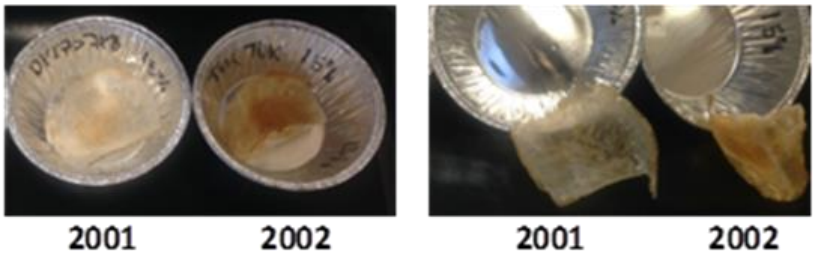


Figure 7.3: Samples 2001 and 2002 cooled in the popping machine.

These samples seem to show uneven moisture distributions, but the starches have lost their structure order. It took over 45 minutes for the samples to cool in the machine so this was not a practical way of creating films for further analyses.

The hot press should have allowed films to be made in the same way as the popping machine, but the temperature control should have been far superior and cooling of the plates should have allowed multiple film samples to be formed reproducibly. It was not possible to create films at the low moisture contents achieved in the popping machine. Films at higher moisture contents of 50% tended to flow when being heated, however sufficient samples were made for some comparisons. It was thought that the variation between samples of the same type was no greater than that observed between the different samples. Films of about 0.7 mm formed from pressing could be used for measurement of T_g using dynamic mechanical analyses, but the films were very brittle. To try and create films close to their T_g for mechanical measurement, processed batter samples were stored at an a_w of approximately 75%. This lead to the appreciation that the formulations used in the processed samples were not just based on carbohydrates and the dominant factor was the amount of salt contained in the batters.

7.4 Examination of starches at low moisture

If the structures of the batters were to be understood, an alternative way of monitoring starch behaviour at low moisture levels was necessary. In the proposed model of the film formation (Figure 7.1), the concept that starch needed to be considered in both its particulate and solubilised form when creating a batter film is crucial. Normally water acts as the plasticiser and solvent in high water systems, but it is generally considered that water is a poor solvent for starch. Thus to interpret a normal high water pasting curve, one has to consider the free polymer and the granule remnants. In chapter 4 the functionalities of starches in low moisture systems were examined by utilising the 78% NMMO-22% water solvent system. It was expected that this system would cause the starches to solubilise and that their structure could therefore be evaluated when not confined to the granule.

A group of stabilised and crosslinked tapioca starches (increasing in level of crosslinking) was tested in this low moisture system and it was noted that there was a very distinct change in behaviour for pasting. Other studies by (Koganti et al., 2011, Koganti et al., 2015) have shown that the 78% NMMO- 22% water system was suitable to investigate the dissolution of starches thus avoiding the impact of the granular morphology. In the current work, the dissolution of some starches in NMMO-water was incomplete since polarised microscopy showed that some granules were still intact after RVA-pasting (confirmed by hot stage microscopy). An explanation for the incomplete dissolution of the tapioca starches could be that they do not have any pores within their granules. Close investigation of the work carried out by (Koganti et al., 2011, Koganti et al., 2015) would confirm this concept. They showed that maize starch (pores) dissolved much faster in 78 % NMMO-22% water

than did pea and potato starch (known to not contain pores). This observation may indicate that the pores may be important for dissolution in low water environments.

Furthermore the type and level of modification may be of greater relevance in this solvent system. The hydrophobic groups added during stabilisation may cause steric hindrance within the granular structure. Together with crosslinking, this could create a rigid molecule with a complex structure thus resulting in a complex dissolution pattern. These hypotheses may be supported by the iodine staining experiments (see section 3.2.7) that suggests that stabilisation and crosslinking may change the manner in which the solubilisation occurs. Another observation that highlights that the understanding of the starch solubilisation is incomplete came from the differences observed for three native tapioca starches that seemed very similar when assessed by the typical measures used to benchmark starch behaviour, but were clearly different in the NMMO-water solvent.

7.5 Impact of minor ingredients

One of the main discoveries in this research was the relevance of minor ingredients in a batter multi component system. The major focus of the work was to understand the starchy materials, but although these often composed the bulk of the ingredients, the minor ingredients made such a large impact that it was difficult to ascertain the roles played by the different starches. Examples of the actions of the minor ingredients included:

- Wheat flours seemed to be the primary cause of the aeration and therefore oil uptake in fried batters. The flour's propensity to expand was not noticeable in the popped samples.

- Gums influenced the amounts of batter pickup. Inclusion of gum in a batter formulation reduced the loss of order in the starches for the popping experiment.
- Some pea dextrins destroyed the cellulose based filter paper layer onto which the batter was placed for the popping experiments. Although this effect was not observed when the dextrin was incorporated with the rest of the ingredients into a batter, the relevance of the base matrix interactions with the film was highlighted by the dramatic impact of the pea dextrin on the amorphous cellulose base.
- Inclusions of salts will impact on equilibrium moisture contents on the samples sufficiently to dominate T_g measures etc.

Three batter formulations and their ingredients were supplied to this project for some in depth analyses. It was supposed that these samples only contained starches. Upon examination of batter samples 3001-3003, some very interesting findings were revealed for the sorption data of these mixtures. A clear difference was seen between the batters' sorption isotherms at high relative humidity. This variation in moisture dominated the T_g measures for the starches.

Communication with McCain Foods Ltd. and Newly Weds Foods Ltd. revealed that the supplied ingredient lists of these batter formulations were not complete. Minor ingredients such as salt, leavening agents and stabilisers were present in the batter mixtures, but not reported on the supplied ingredient lists. Experimental work using starch blends alone and with addition of sodium chloride revealed that the marked differences in sorption data were due to the salt addition. Differences due to the starchy materials in the batters were not observable. This conclusion emphasised the importance of the minor ingredients in a batter mixture and the significant impact it may have on batter properties.

An additional observation that may need more work to confirm the findings was that the influence of salt in the batters' moisture pickup at high water activity seemed greater after they had been processed and dried, rather than just a dry mix of starch with salt crystals. This may be just due to the particle effects such as size and porosity, but there is other evidence that the interactions between starch and salt impact on the starches behaviour (Moreau et al., 2011). Figure 7.4 shows the rate at which the two samples (sample 3001 but one with and one without salt, both processed) are coming to equilibrium in the DVS at high RH values. These data have not been fully evaluated, but could establish if rates of moisture uptake differ in these samples as well as provide information on the equilibrium moisture contents that the samples achieve. Rates of moisture migration could be a relevant issue for the film function and the porosity of the film may be very relevant for its final performance (see Figure 7.1).

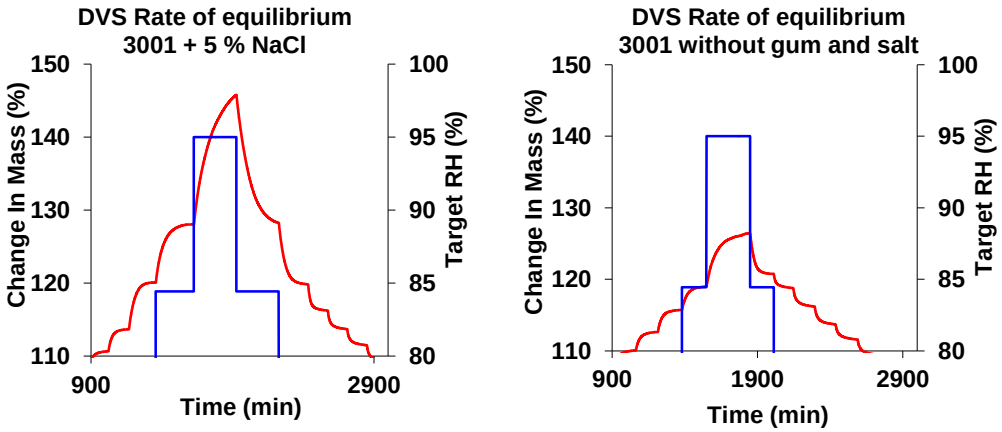


Figure 7.4: DVS Rate of equilibrium for sample 3001 + 5% NaCl and sample 3001 without salt/gum. The moisture content is expressed on a dry weight basis with 100% corresponding to a completely dry solids material. 110% corresponds to 10g of water added to 100g of solid.

7.6 Further work

7.6.1 Interesting observations

Research reported in this thesis has led to several interesting observations that did not form part of the main work, but are worthy of further examination.

Observations worthy of additional study:

- The NMMO-water system was able to differentiate between starches of same origin (tapioca). What needs to be addressed is if these starches have different functionalities, particularly at low water content and therefore if the NMMO method could help predict starches performance in low water environments.
- The moisture isotherms showed marked differences at high relative humidity as salt was added. Differences were also observed for processed versus non-processed materials and seemed to be extenuated in the presence of sodium chloride. Further work is required to establish if processing of starches has a major impact on the moisture sorption and the role of salt in this mechanism. Rates of moisture pickup and loss may need to be studied as well as the equilibrium state. If there is a salt processing interaction, it could be important to establish if this is relevant for the testing of barrier properties of films.
- The interactions of the chemically modified starches with ligands (iodine or lipids), if explored more fully and with samples where the modifications are better quantified, may lead to a better understanding of how the modification impacts on the different levels of the starch structure.
- The popping of samples using the popping machine showed that most of the starches behaved in a similar manner. However, the dextrin samples showed a peculiar behaviour. Destruction of the cellulose-based filter paper was the result of the popping of dextrins and no explanation for this phenomenon was readily available. Further studies on the role of the batter when interacting with the base materials assists in providing practical

knowledge about pre-treatment of the potato base and the necessary qualities of the batter to create the optimal film.

7.6.2 Further work to test the main suppositions

The formation of films and their properties were focal points of the research reported in this thesis and the understanding of the mechanisms of the film forming has been addressed using different techniques such as popping and film formation retaining moisture. Batter formulations are designed to form films creating a barrier between a high moisture base material (potato) and the ambient air thereby avoiding moisture loss and/or uptake, thus maintaining soft and moist interior texture and retaining the crispiness of the film (crust). The relevance of this and the theory of "bricks and glue" (Figure 7.1) was examined to account for the mechanism of film formation using a blend of starches, but more work is required before there is an understanding of how the starches impact on the barrier properties.

7.6.2.1 Film formation and evaluation

To examine the films they have to be created and this proved very difficult. The popping of the samples suggested that at low moisture contents and the temperatures used, films could be formed. Thus enough “glue” could be generated from all starches to hold the system together. A recommendation could be to continue and improve the studies on popped starch films. The question whether the film forming is generated by the expansion theory with destruction of granules or whether another mechanism is causing the range of materials to form the films remains unsolved. More work is required to establish if the compression and the amount of water present in the popping experiment are the critical factors. Further examination using this technique may reveal unique properties of the materials, which have not previously been elucidated and reported in the literature.

Modification of the equipment to allow fast cooling could make it reasonable to create films that had not expanded and could be used for other investigations of the films performance (moduli, T_g, porosity, textural parameters).

7.6.2.2 Choice of starches

Although the use of industrially relevant formulations and sources of starches has helped to keep the work commercially focused, it has made certain areas difficult for fundamental interpretation. Fewer samples chosen for their expected functionality that then could be tested would aid the understanding of the complex systems. To create tagged samples that would allow the assessment of which starches formed inclusions and which the holding matrix in the films would be a good focus of further work. Surface microscopies and atomic force microscopy (AFM) for surface rheology may also help establish the format of the film.

7.6.2.3 Dynamics and modelling of processing

The theory presented in Figure 7.1 is based on the principle of multiple granules present with different functionalities. The concept is that granules act as the fillers, while the leached starch polymers act as the continuous matrix. The change in state of the starches will be dependent on moisture, temperature and the other ingredients present. Key changes are thought to occur during the dynamic processing. Rapid change in moisture and the impact of this on the starch films produced will be critical to the product performance. To ascertain the key steps in this process through analytical assessments has proved very difficult. One of the critical problems is that the crucial temperatures and moisture contents of the systems are unknown. As there is significant knowledge on some of the parameters needed to measure diffusion of moisture and temperature in the starch systems and to measure beginning and end values, it is possible that the dynamics of the system

could be modelled. This model may aid in ascertaining where the critical time points may be in terms of moisture and temperature and the status of the batter as it moves from starch slurry to amorphous glassy film.

BIBLIOGRAPHY

AHMAD, F. B. & WILLIAMS, P. A. 1999. Effect of salts on the gelatinization and rheological properties of sago starch. *Journal of agricultural and food chemistry,* 47**,** 3359-3366.

AL-MUHTASEB, A. H., MCMINN, W. A. M. & MAGEE, T. R. A. 2004. Water sorption isotherms of starch powders: Part 1: mathematical description of experimental data. *Journal of Food Engineering,* 61**,** 297-307.

ALTUNAKAR, B., SAHIN, S. & SUMNU, G. 2004. Functionality of batters containing different starch types for deep-fat frying of chicken nuggets. *European Food Research and Technology,* 218**,** 318-322.

ANNABLE, P., FITTON, M. G., HARRIS, B., PHILLIPS, G. O. & WILLIAMS, P. A. 1994. Phase behaviour and rheology of mixed polymer systems containing starch. *Food Hydrocolloids,* 8**,** 351-359.

ASHOGBON, A. O. & AKINTAYO, E. T. 2014. Recent trend in the physical and chemical modification of starches from different botanical sources: A review. *Starch - Stärke,* 66**,** 41-57.

BARBOSA-CÃNOVAS, G. V., FONTANA JR, A. J., SCHMIDT, S. J. & LABUZA, T. P. 2008. *Water activity in foods: fundamentals and applications*, John Wiley & Sons.

BERTOFT, E. 1991. Investigation of the fine structure of alpha-dextrins derived from amylopectin and their relation to the structure of waxy-maize starch. *Carbohydrate Research,* 212**,** 229-244.

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

BERTOFT, E. 2013. On the Building Block and Backbone Concepts of Amylopectin Structure. *Cereal Chemistry Journal,* 90**,** 294-311.

BERTOFT, E. & KOCH, K. 2000. Composition of chains in waxy-rice starch and its structural units. *Carbohydrate Polymers,* 41**,** 121-132.

BERTOFT, E., PIYACHOMKWAN, K., CHATAKANONDA, P. & SRIROTH, K. 2008. Internal unit chain composition in amylopectins. *Carbohydrate Polymers,* 74**,** 527-543.

BIKIARIS, D., PRINOS, J., BOTEV, M., BETCHEV, C. & PANAYIOTOU, C. 2004. Blends of polymers with similar glass transition temperatures: A DMTA and DSC study. *Journal of Applied Polymer Science,* 93**,** 726-735.

BLANSHARD, J. M. V. & LILLFORD, P. 1993. *The Glassy State in Foods*, Nottingham University Press.

BOKI, K. & OHNO, S. 1991. Equilibrium isotherm equations to represent moisture sorption on starch. *Journal of Food Science,* 56**,** 1106-1107.

BORBÉLY, É. 2008. Lyocell, The New Generation of Regenerated Cellulose. *Acta Polytechnica Hungarica* 5**,** 11-18.

BRØGGER, A. F., MITCHELL, J., KOGANTI, N., SWALI, A. & HILL, S. 2015. Can pasting in N-methyl morpholine N-oxide (NMMO) differentiate between tapioca starches? *Starch - Stärke,* 67**,** 985-988.

CARMONA-GARCIA, R., SANCHEZ-RIVERA, M. M., MÉNDEZ-MONTEALVO, G., GARZA-MONTOYA, B. & BELLO-PÉREZ, L. A. 2009. Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (Musa paradisiaca). *Carbohydrate Polymers,* 76**,** 117-122.

CHIRIFE, J. & FONTANA, A. J. 2008. Introduction: Historical highlights of water activity research. *Water Activity in Foods: Fundamentals and Applications***,** 3-13.

CLOSS, C., CONDE-PETIT, B., ROBERTS, I., TOLSTOGUZOV, V. & ESCHER, F. 1999. Phase separation and rheology of aqueous starch/galactomannan systems. *Carbohydrate Polymers,* 39**,** 67-77.

COATES, J. 2014. *Batter ingredients and their effect on batter coating integrity.* Master thesis, University of Nottingham.

CROSBIE, G. 1991. The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. *Journal of cereal science,* 13**,** 145-150.

CUQ, B., ABECASSIS, J. & GUILBERT, S. 2003. State diagrams to help describe wheat bread processing. *International Journal of Food Science & Technology,* 38**,** 759-766.

DE FOCATIIS, D. S. A. & BUCKLEY, C. P. 2008. Determination of craze initiation stress in very small polymer specimens. *Polymer Testing,* 27**,** 136-145.

DOPORTO, M. C., DINI, C., MUGRIDGE, A., VIÑA, S. Z. & GARCÍA, M. A. 2012. Physicochemical, thermal and sorption properties of nutritionally differentiated flours and starches. *Journal of Food Engineering,* 113**,** 569-576.

DRAGUNSKI, D. C. & PAWLICKA, A. 2001. Preparation and characterization of starch grafted with toluene poly (propylene oxide) diisocyanate. *Materials Research,* 4**,** 77-81.

ENRIONE, J. I., HILL, S. E. & MITCHELL, J. R. 2007. Sorption behavior of mixtures of glycerol and starch. *Journal of agricultural and food chemistry,* 55**,** 2956-2963.

FERRY, J. D. 1980. *Viscoelastic properties of polymers,* New York, Wiley.

FINK, H. P., WEIGEL, P., PURZ, H. J. & GANSTER, J. 2001. Structure formation of regenerated cellulose materials from NMMO-solutions. *Progress in Polymer Science,* 26**,** 1473-1524.

FOA/WHO 2011. Compendium of food additive specifications. *FAO JECFA Monographs,* 11**,** 57-75.

FREDRIKSSON, H., SILVERIO, J., ANDERSSON, R., ELIASSON, A. C. & ÅMAN, P. 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydrate Polymers,* 35**,** 119-134.

FRENCH, D. 1972. Fine structure of starch and its relationship to the organization of starch granules. *Journal of the Japanese Society of Starch Science,* 19**,** 8-25.

GALLANT, D. J., BOUCHET, B., BULEON, A. & PEREZ, S. 1992. Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition* 46 Suppl 2**,** S3-16.

HERMANS, P. H. & WEIDINGER, A. 1948. Quantitative X-Ray Investigations on the Crystallinity of Cellulose Fibers. A Background Analysis. *Journal of Applied Physics,* 19**,** 491-506.

HSIEH, F., HUFF, H. E., PENG, I. C. & MAREK, S. W. 1989. Puffing of Rice Cakes as Influenced by Tempering and Heating Conditions. *Journal of Food Science,* 54**,** 1310-1312.

HUANG, J., SCHOLS, H. A., VAN SOEST, J. J. G., JIN, Z., SULMANN, E. & VORAGEN, A. G. J. 2007. Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. *Food Chemistry,* 101**,** 1338-1345.

HUME, P. & ROSE, M. 2013. *RE: McCain Foods Ltd & Newly Weds Foods Ltd: Potato chip processing.*

IGLESIAS, H. A., CHIRIFE, J. & BOQUET, R. 1980. Prediction of sorption isotherms of food models from knowledge of components sorption behavior. *Journal of Food Science,* 45**,** 450-452.

JANE, J. L. 1993. Mechanism of starch gelatinization in neutral salt solutions. *Starch-Stärke,* 45**,** 161-166.

JENKINS, P., COMERSON, R., DONALD, A., BRAS, W., DERBYSHIRE, G., MANT, G. & RYAN, A. 1994. In situ simultaneous small and wide angle x-ray scattering: A new technique to study starch gelatinization. *Journal of Polymer Science Part B: Polymer Physics,* 32**,** 1579-1583.

JENKINS, P. J. & DONALD, A. M. 1995. The influence of amylose on starch granule structure. *International Journal of Biological Macromolecules,* 17**,** 315-321.

JYOTHI, A. N., MOORTHY, S. N. & RAJASEKHARAN, K. N. 2006. Effect of Cross-linking with Epichlorohydrin on the Properties of Cassava (Manihot esculenta Crantz) Starch. *Starch - Stärke,* 58**,** 292-299.

KAMINASKI, W. & AL-BEZWENI, M. 1994. Calculation of water sorption isotherms for multicomponent protein-containing mixtures. *International Journal of Food Science & Technology,* 29**,** 129-136.

KATZ, E. E. & LABUZA, T. P. 1981. Effect of Water Activity on the Sensory Crispness and Mechanical Deformation of Snack Food Products. *Journal of Food Science,* 46**,** 403-409.

KAUR, M., OBEROI, D. P. S., SOGI, D. S. & GILL, B. S. 2011. Physicochemical, morphological and pasting properties of acid treated starches from different botanical sources. *Journal of food science and technology,* 48**,** 460-465.

KIBAR, E. A. A., GÖNENÇ, İ. & US, F. 2010. Gelatinization of waxy, normal and high amylose corn starches. *The Journal of Food,* 35**,** 237-244.

KNUTSON, C. A. 1986. A simplified Colorimetric Procedure for Determination of Amylose in Maize Starches. *Cereal Chemistry Journal,* 63**,** 89-92.

KOGANTI, N., MITCHELL, J., MACNAUGHTAN, W., HILL, S. & FOSTER, T. 2015. Effect of granule organisation on the behaviour of starches in the NMMO (N-methyl morpholine N-oxide) solvent system. *Carbohydrate Polymers,* 116**,** 103-110.

KOGANTI, N., MITCHELL, J. R., IBBETT, R. N. & FOSTER, T. J. 2011. Solvent Effects on Starch Dissolution and Gelatinization. *Biomacromolecules,* 12**,** 2888-2893.

KOO, S. H., LEE, K. Y. & LEE, H. G. 2010. Effect of cross-linking on the physicochemical and physiological properties of corn starch. *Food Hydrocolloids,* 24**,** 619-625.

LEACH, H. W., MCCOWEN, L. & SCHOCH, T. J. 1959. Structure of the starch granule. I. Swelling and solubility patterns of various starches. *Cereal Chemistry,* 36**,** 534-544.

LESLIE, R., CARILLO, P., CHUNG, T., GILBERT, S., HAYAKAWA, K., MAROUSIS, S., SARAVACOS, G. & SOLBERG, M. 1991. Water diffusivity in starch-based systems. *Water relationships in foods.* Springer.

LEVINE, H. & SLADE, L. 1991. *Water relationships in foods : advances in the 1980s and trends for the 1990s,* New York, Plenum Press.

LIGHT, J. M. 1990. Modified food starches: why, what, where, and how. *Cereal foods world,* 35**,** 1081-1092.

LII, C. & LEE, B. 1993. Heating A-, B-, and C-type starches in aqueous sodium chloride: effects of sodium chloride concentration and moisture content on differential scanning calorimetry thermograms. *Cereal chemistry,* 70**,** 188-192.

LILLFORD, P. J. 1988. 6 - The polymer/water relationship - its importance for food structure. *In:* BLANSHARD, J. M. V. & MITCHELL, J. R. (eds.) *Food Structure - its creation and evaluation.* Woodhead Publishing.

LIU, H., RAMSDEN, L. & CORKE, H. 1999. Physical properties of cross-linked and acetylated normal and waxy rice starch. *Starch/Staerke,* 51**,** 249-252.

LIU, H., YU, L., XIE, F. & CHEN, L. 2006. Gelatinization of cornstarch with different amylose/amylopectin content. *Carbohydrate Polymers,* 65**,** 357-363.

LIU, Z. & HAN, J. H. 2005. Film-forming Characteristics of Starches. *Journal of Food Science,* 70**,** E31-E36.

LLORCA, E., HERNANDO, I., PÉREZ-MUNUERA, I., QUILES, A., FISZMAN, S. M. & LLUCH, M. Á. 2003. Effect of batter formulation on lipid uptake during frying and lipid fraction of frozen battered squid. *European Food Research and Technology,* 216**,** 297-302.

LÓPEZ, O. V., GARCÍA, M. A. & ZARITZKY, N. E. 2008. Film forming capacity of chemically modified corn starches. *Carbohydrate Polymers,* 73**,** 573-581.

LÓPEZ, O. V., ZARITZKY, N. E. & GARCÍA, M. A. 2010. Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity. *Journal of Food Engineering,* 100**,** 160-168.

MAHLIN, D., WOOD, J., HAWKINS, N., MAHEY, J. & ROYALL, P. G. 2009. A novel powder sample holder for the determination of glass transition temperatures by DMA. *International Journal of Pharmaceutics,* 371**,** 120-125.

MARLIDA, Y., SAARI, N., KARIM, R., MOHAMED, S. & KARIM, A. 2007. Improvement of glucose production by raw starch degrading enzyme utilizing acid-treated sago starch as substrate. *ASEAN Food Journal,* 14**,** 83-90.

MCCLEARY, B. 2015. *RE: Megazyme Assay Procedure.* Type to HILL, S.

MCCLEARY, B. V., GIBSON, T. S. AND MUGFORD, D. C. 1997. Measurement of Total Starch in Cereal Products by Amyloglucosidase-α-Amylase Method: Collaborative Study. J. AOAC International. *Journal of AOAC International,* 80**,** 571-579.

MCCORMICK, K., PANOZZO, J. & HONG, S. 1991. A swelling power test for selecting potential noodle quality wheats. *Crop and Pasture Science,* 42**,** 317-323.

MITRUS, M. 2005. Glass transition temperature of thermoplastic starches. *International Agrophysics*.

MIZUNO, A., MITSUIKI, M. & MOTOKI, M. 1998. Effect of Crystallinity on the Glass Transition Temperature of Starch. *Journal of Agricultural and Food Chemistry,* 46**,** 98-103.

MOHAMED, S., HAMID, N. A. & HAMID, M. A. 1998. Food components affecting the oil absorption and crispness of fried batter. *Journal of the Science of Food and Agriculture,* 78**,** 39-45.

MOREAU, L., BINDZUS, W. & HILL, S. 2011. Influence of salts on starch degradation: Part II–Salt classification and caramelisation. *Starch-Stärke,* 63**,** 676-682.

MURPHY, P., PHILLIPS, G. & WILLIAMS, P. 2000. Starch. *Handbook of hydrocolloids***,** 41-65.

NIAZI, M. B. K. 2013. *Production of plasticized thermoplastic starch by spray drying.* Doctoral dissertation, University of Groningen.

NICHOLLS, R., APPELQVIST, I., DAVIES, A., INGMAN, S. & LILLFORD, P. 1995. Glass transitions and the fracture behaviour of gluten and starches within the glassy state. *Journal of Cereal Science,* 21**,** 25-36.

ORFORD, P. D., RING, S. G., CARROLL, V., MILES, M. J. & MORRIS, V. J. 1987. The effect of concentration and botanical source on the gelation and retrogradation of starch. *Journal of the Science of Food and Agriculture,* 39**,** 169-177.

ORGANIZATION, W. H. & ASSEMBLY, W. H. 1972. *WHO food additives series*, World Health Organization.

PAES, S. S., SUN, S., MACNAUGHTAN W., IBBETT, R., GANSTER, J., FOSTER, T.J., MITCHELL, J.R. 2010. The glass transition and crystallization of ball milled cellulose. *Cellulose,* 17**,** 693-709.

PAL, J., SINGHAL, R. S. & KULKARNI, P. R. 2002. Physicochemical properties of hydroxypropyl derivative from corn and amaranth starch. *Carbohydrate Polymers,* 48**,** 49-53.

PÉREZ, S. & BERTOFT, E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch - Stärke,* 62**,** 389-420.

PERRY, P. & DONALD, A. 2000. The role of plasticization in starch granule assembly. *Biomacromolecules,* 1**,** 424-432.

PERUMAL, R. 2013. *Mechanistic understanding of starch components used in potato chip batters.* Doctor of Philosopy, Universiy of Nottingham.

RASCHIP, I. E., YAKIMETS, I., MARTIN, C. P., PAES, S. S., VASILE, C. & MITCHELL, J. R. 2008. Effect of water content on thermal and dynamic mechanical properties of xanthan powder: A comparison between standard and novel techniques. *Powder Technology,* 182**,** 436-443.

RATNAYAKE, W. S., HOOVER, R. & WARKENTIN, T. 2002. Pea Starch: Composition, Structure and Properties — A Review. *Starch - Stärke,* 54**,** 217-234.

REID, D. S. 2007. Water activity: fundamentals and relationships. *Water activity in foods. Fundamentals and applications***,** 15-28.

RICHARDSON, P. H., JEFFCOAT, R. & SHI, Y.-C. 2000. High-amylose starches: From biosynthesis to their use as food ingredients. *MRS Bulletin,* 25**,** 20-24.

RINDLAV-WESTLING, A. S., STADING, M., HERMANSSON, A.-M. & GATENHOLM, P. 1998. Structure, mechanical and barrier properties of amylose and amylopectin films. *Carbohydrate Polymers,* 36**,** 217-224.

ROCHA, T. D. S., CARNEIRO, A. P. D. A. & FRANCO, C. M. L. 2010. Effect of enzymatic hydrolysis on some physicochemical properties of root and tuber granular starches. *Food Science and Technology (Campinas),* 30**,** 544-551.

ROUDAUT, G., DACREMONT, C. & MESTE, M. L. 1998. Influence of water on the crispness of cereal-based foods: acoustic, mechanical, and sensory studies *Journal of Texture Studies,* 29**,** 199-213.

ROUDAUT, G., DACREMONT, C., VALLÈS PÀMIES, B., COLAS, B. & LE MESTE, M. 2002. Crispness: a critical review on sensory and material science approaches. *Trends in Food Science & Technology,* 13**,** 217-227.

ROUDAUT, G. & WALLECAN, J. 2015. New insights on the thermal analysis of low moisture composite foods. *Carbohydrate polymers,* 115**,** 10-15.

ROYALL, P. G., HUANG, C.-Y., TANG, S.-W. J., DUNCAN, J., VAN-DE-VELDE, G. & BROWN, M. B. 2005. The development of DMA for the detection of amorphous content in pharmaceutical powdered materials. *International Journal of Pharmaceutics,* 301**,** 181-191.

SAHIN, S. & SUMNU, S. G. 2008. *Advances in deep-fat frying of foods*, CRC Press.

SAIR, L. & FETZER, W. 1944. Water sorption by starches. *Industrial & Engineering Chemistry,* 36**,** 205-208.

SANDHU, K. S. & SINGH, N. 2007. Some properties of corn starches II: Physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chemistry,* 101**,** 1499-1507.

SANGWICHIEN, C., ARANOVICH, G. L. & DONOHUE, M. D. 2002. Density functional theory predictions of adsorption isotherms with hysteresis loops. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 206**,** 313-320.

SEYMOUR, S. K. & HAMANN, D. D. 1988. CRISPNESS AND CRUNCHINESS OF SELECTED LOW MOISTURE FOODS1. *Journal of Texture Studies,* 19**,** 79-95.

SHAH, B. N. & SCHALL, C. A. 2006. Measurement and modeling of the glass transition temperatures of multi-component solutions. *Thermochimica Acta,* 443**,** 78-86.

SHI, Y.-C., CAPITANI, T., TRZASKO, P. & JEFFCOAT, R. 1998. Molecular Structure of a Low-Amylopectin Starch and Other High-Amylose Maize Starches. *Journal of Cereal Science,* 27**,** 289-299.

SHI, Y.-C. & SEIB, P. A. 1992. The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydrate Research,* 227**,** 131-145.

SING, K. S. 1985. Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity (Recommendations 1984). *Pure and applied chemistry,* 57**,** 603-619.

SINGH, J., KAUR, L. & MCCARTHY, O. J. 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review. *Food Hydrocolloids,* 21**,** 1-22.

SINGH SANDHU, K., SINGH, N. & LIM, S.-T. 2007. A comparison of native and acid thinned normal and waxy corn starches: Physicochemical, thermal, morphological and pasting properties. *LWT - Food Science and Technology,* 40**,** 1527-1536.

SLADE, L. & LEVINE, H. 1993. The glassy state phenomenon in food molecules. *The glassy state in foods***,** 35-102.

SLAVUTSKY, A. M., BERTUZZI, M. A. & ARMADA, M. 2012. Water barrier properties of starch-clay nanocomposite films. *Brazilian Journal of Food Technology,* 15**,** 208-218.

SPERGER, D. M. & MUNSON, E. J. 2011. Analysis of structural variability in pharmaceutical excipients using solid-state NMR spectroscopy. *AAPS PharmSciTech,* 12**,** 821-833.

STEENEKEN, P. A. M. & WOORTMAN, A. J. J. 2009. Identification of the thermal transitions in potato starch at a low water content as studied by preparative DSC. *Carbohydrate Polymers,* 77**,** 288-292.

TESTER, R. F., KARKALAS, J. & QI, X. 2004. Starch—composition, fine structure and architecture. *Journal of Cereal Science,* 39**,** 151-165.

THANATUKSORN, P., KAJIWARA, K. & SUZUKI, T. 2010. Characteristics and oil absorption in deep-fat fried batter prepared from ball-milled wheat flour. *Journal of the Science of Food and Agriculture,* 90**,** 13-20.

THÉRIEN-AUBIN, H., JANVIER, F., BAILLE, W. E., ZHU, X. X. & MARCHESSAULT, R. H. 2007. Study of hydration of cross-linked high amylose starch by solid state 13C NMR spectroscopy. *Carbohydrate Research,* 342**,** 1525-1529.

TIMMERMANN, E. O. 2003. Multilayer sorption parameters: BET or GAB values? *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 220**,** 235-260.

UTHUMPORN, U., WAHIDAH, N. & KARIM, A. A. 2014. Physicochemical Properties Of Starch From Sago (Metroxylon Sagu) Palm Grown In Mineral Soil At Different

Growth Stages. *IOP Conference Series: Materials Science and Engineering,* 62**,** 012026.

VAN DEN BERG, C. 1985. Development of BET-like models for sorption of water on foods, theory and relevance. *Properties of water in foods.* Springer.

VARELA, P. & FISZMAN, S. M. 2011. Hydrocolloids in fried foods. A review. *Food Hydrocolloids,* 25**,** 1801-1812.

WANG, S. S., CHIANG, W.-C., YEH, A.-I., ZHAO, B. & KIM, I.-H. 1989. Kinetics of Phase Transition of Waxy Corn Starch at Extrusion Temperatures and Moisture Contents. *Journal of Food Science,* 54**,** 1298-1301.

WANG, T. L., BOGRACHEVA, T. Y. & HEDLEY, C. L. 1998. Starch: as simple as A, B, C? *Journal of Experimental Botany,* 49**,** 481-502.

WATTANACHANT, S., MUHAMMAD, K., HASHIM, D. M. & RAHMAN, R. A. 2003. Effect of crosslinking reagents and hydroxypropylation levels on dual-modified sago starch properties. *Food Chemistry,* 80**,** 463-471.

WEXLER, A. H., S. 1954. Relative humidity-temperature relationships of some saturated salt solutions in the temperature range 0 degree to 50 degrees C. *Journal of Research of the National Bureau of Standards,* 53**,** 19-26.

WHITE, P., ABBAS, I. & JOHNSON, L. 1989. Freeze-Thaw Stability and Refrigerated-Storage Retrogradation of Starches. *Starch-Stärke,* 41**,** 176-180.

WILLIAMS, P. A. 2011. *Renewable Resources for Functional Polymers and Biomaterials: Polysaccharides, Proteins and Polyesters*, Royal Society of Chemistry.

WOLFF, I. A., DAVIS, H. A., CLUSKEY, J. E., GUNDRUM, L. J. & RIST, C. E. 1951. Preparation of Films from Amylose. *Industrial & Engineering Chemistry,* 43**,** 915-919.

WONGSAGONSUP, R., PUJCHAKARN, T., JITRAKBUMRUNG, S., CHAIWAT, W., FUONGFUCHAT, A., VARAVINIT, S., DANGTIP, S. & SUPHANTHARIKA, M. 2014. Effect of cross-linking on physicochemical properties of tapioca starch and its application in soup product. *Carbohydrate Polymers,* 101**,** 656-665.

XUE, J. & NGADI, M. 2006. Rheological properties of batter systems formulated using different flour combinations. *Journal of Food Engineering,* 77**,** 334-341.

YEH, A. L. & LI, J. Y. 1996. Kinetics of Phase Transition of Native, Cross-linked, and Hydroxypropylated Rice Starches. *Starch-Stärke,* 48**,** 17-21.

YIU, P. H., LOH, S., RAJAN, A., WONG, S. C. & BONG, C. F. J. 2008. Physicochemical properties of sago starch modified by acid treatment in alcohol. *American J. of Appl. Sci,* 5**,** 307-311.

YUAN, R., THOMPSON, D. & BOYER, C. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wx-containing genotypes in two inbred lines. *Cereal Chemistry,* 70**,** 81-81.

YUN, S.-H. & MATHESON, N. K. 1990. Estimation of Amylose Content of Starches after Precipitation of Amylopectin by Concanavalin-A. *Starch - Stärke,* 42**,** 302-305.

ZHU, F. 2015. Composition, structure, physicochemical properties, and modifications of cassava starch. *Carbohydrate Polymers,* 122**,** 456-480.

ZOBEL, H. F. 1988. Molecules to Granules: A Comprehensive Starch Review. *Starch - Stärke,* 40**,** 44-50.