PROCESS FOR RECOVERY OF SMOOTH FIBRE INGREDIENT FROM POMACE

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Abstract

This research aimed to develop a process to convert apple pomace into a food ingredient which can provide functional properties such as water-binding in baked goods, stability in aqueous suspension and smooth mouthfeel. This was achieved by modifying the apple pomace through three main steps: heating, shearing and enzymatic hydrolysis.

Firstly, the effect of sample preparation (addition of water to fresh pomace, temperature and shearing apple pomace) on the solubility of pectin was investigated. Secondly, kinetics of main reactions involved in pomace while heating at temperatures between 90-140 °C (10 °C intervals) and incubation times between 0-360 min, were studied at bench scale. These reactions were: solubilisation and depolymerisation reactions of pectin, degradation of sugars and production of secondary products such as organic acids and 5-hydroxymethylfurfural (5-HMF). After that, the kinetics of these reactions were modelled for determining the rate constants and activation energies. The kinetic models of pectin solubilisation and 5-HMF formation were then used for scaling up the hydrothermal process in a more complex heat transfer situation, using a pilot scale retort. Finally, the effect of particle size distribution and molecular weight of solubilised components (mainly pectin) on physicochemical and sensorial properties of pomace material was investigated.

Solubilisation of pectin at room temperature was independent of addition of water and shearing treatment of pomace. However, heating at temperatures > 100 °C, combined with increasing the amounts of water added to pomace (from 0 to 8 mL water/ g pomace) resulted in increasing the pectin solubility up to a pomace-water ratio of 1:2. The maximum amount of solubilised pectin (~ 605 μmol galacturonic acid/ g dry pomace) was determined when heating pomace at 130 and 140 °C for 15 and 7 min, respectively. Hydrothermal depolymerisation of pectin through acid hydrolysis and β-elimination reactions also showed temperature-dependent behaviour. Depolymerisation reactions resulted in degradation of pectin polymers into ethanol-soluble forms (galacturonic acid). Depolymerisation seemed more likely to happen from non-esterified sites of pectin polymers, as suggested by the high degree of esterification of the remaining insoluble pectin.

Increasing amounts of glucose and fructose were observed in the serum phase of pomace to about five times their initial values when pomace was heated at temperatures >120 °C. This was

accompanied by a reduction in sucrose content, suggesting hydrothermal hydrolysis of sucrose to its subunits. A complete conversion of sucrose was recorded at temperatures > 120 °C and times > ~30 min.

Glucose, fructose and galacturonic acid underwent further transformation at temperatures > 100 °C forming secondary products of organic acids, (such as acetic acid, formic acid and lactic acid), furfural and 5-HMF. Modelling the kinetics of pectin solubilisation and 5-HMF production resulted in activation energies of 81 and 105 kJ/mol, respectively. The effects of hydrothermal treatment were modelled using COMSOL for heating a slab of pomace in a pilot scale retort with a maximum steam temperature of 125 °C. In this model, heat transfer through the pomace and chemical reactions of pectin solubilisation and 5-HMF production were predicted. The aim for this model was to identify conditions permitting solubilisation enough to double the amount of pectin from the amount initially present at room temperature while limiting the production of 5-HMF to the range permitted in food standards (bulk averaged). The validity of the model was confirmed in the pilot plant condition.

Another objective of this research study was to investigate the effects of particle size distributions and molecular weight of pectin on sensory properties and physical stability of pomace samples. A controlled modification of particle size and molecular weight of solubilised pectin was achieved by fractionation of heat-treated pomace into insoluble solid and serum parts.

Shearing insoluble particles for 5 min showed significant particle size reduction from 496 μm (initial shearing for 2 min) to 165 μm. Further shearing did not affect the average size of the particles.

Microscopy revealed the effect of shearing in separating cell aggregations, resulting in individual cells. Shearing also fractured some individual cells, the oval shape of most cells was still visible.

The pectin in the serum phase of pomace before and after heat treatment was analysed for molecular weight distribution. Results confirmed the effectiveness of heat treatment on solubilising high molecular weight pectins into the serum phase. Two ranges of high (between 4000-73 kDa) and low (< 73 kDa) pectin molecular weights were analysed in heat-treated pomace. These two ranges were separated from each other by ultrafiltration. High molecular weight of fraction (with the average size of 380 kDa) was enzymatically hydrolysed into two pectin components with average molecular weights of 30 and 150 kDa.

Finally, six pomace ingredients were produced from the pomace fractions with two insoluble particle size distributions (with the average size ranges of 500 and 160 μm) and three ranges of molecular weights (380, 150 and 30 kDa) being blended. Particle size reduction had a significant

effect on the physical stability of pomace suspensions. Samples containing particles >496 μm showed phase separation during storage (5 days at 4 °C), while samples with smaller particle size did not show any phase separation.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

5-HMF	: 5-Hydroxymethyl furfural
BPHMw	: Big particles high molecular weight
BPMMw	: Big particles medium molecular weight
BPLMw	: Big particles low molecular weight
CCD	: Central composite designs
DE	: Degree of esterification
DP	: Degree of polymerisation
DLVO theory	: Derjaguin, Landau, Vervey, and Overbeek Theory
EDTA	: Ethylenediaminetetraacetic acid
EtOH	: Ethanol
FDA	: Food and drug administration
G	: Grainy
GNS	: Grainy not sure
GNVS	: Grainy not very sure
GalUA	: D-galacturonic acid- as part of a pectic polysaccharide
GRAS	: Generally recognised as safe
HG	: Homogalacturonan
HPLC	: High performance liquid chromatography
HPAEC-PAD	: High-performance anion-exchange chromatography with pulsed amperometric detection
HMw	: High molecular weight
HRGs	: Hexuronic acid reducing end groups
IFFJP	: International federation of fruit juice processors
LMw	: Low molecular weight
LS	: Light scattering
MALLS	: Multi-angle laser light scattering detector
MMw	: Medium molecular weight
NaOAc	: Sodium acetate
NaCl	: Sodium chloride
PAE	: Pectin acetyl esterase
PG	: Polygalacturonase
PME	: Pectin methyl esterase
RG-I	: Rhamnogalaturonan I
RG-II	: Rhamnogalaturonan II
RT	: Room temperature
RI	: Refractive index
S	: Smooth
SEC	: Size exclusion columns
SEM	: Scanning electron microscopy
SDF	: Soluble dietary fibre
SNS	: Smooth not sure
SNVS	: Smooth not very sure
SP	: Small particles
SPHMw	: Small particles high molecular weight
SPMMw	: Small particles medium molecular weight
SPLMw	: Small particles low molecular weight
UnUs	: Unsaturated uronides
UV	: Ultraviolet

WHC : Water holding capacity
WSP : Water soluble pectin
XG : Xyloglucan
XGA : Xylogalacturonan

CHAPTER 1 **Research Objectives**

1.1 Introduction

There are some opportunities to make valuable functional ingredient from fruit processing waste. Pomace is the left-over solid residue remaining after pressing fruit and vegetables for juice production. The solid part of apple pomace mainly consists of cell wall components such as cellulose, xyloglucan and pectin (Bhushan et al., 2008; Sharma et al., 2014).

Disposal of pomace is a huge problem for the juicing industries. Direct disposal is an economical loss as well as posing serious environmental problems (Bhushan et al., 2008; Sharma et al., 2014). Although much pomace is utilized as a feedstock for animals, pomace in general are poor feed supplements because of the high amount of carbohydrate and low protein content present. Despite many efforts to extract useful material from pomace (e.g., pectin, polyphenols, organic acids and single cell protein) large amounts of apple pomace still remain as waste (Bhushan et al., 2008; Mandhatri et al., 2014; Sharma et al., 2014).

Apple pomace is rich in plant cell wall polysaccharide that cannot be digested by the human system (although some may be fermented by gut bacteria). The definition of dietary fibre proposed by the AACC Dietary Fibre Definition Committee in 2001 is "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances." (AACC, 2001). In recent years, great attention has been taken towards characterising dietary fibre (soluble and insoluble), understanding their properties, functionality and their applications in food products (Alba et al., 2018; Bhushan et al., 2008; Chang et al., 1993; Chau et al., 2007; Masoodi et al., 2001; Miceli-Garcia, 2014; Sudha, 2011; Sudha et al., 2007; Zhu et al., 2015).

Apple pomace is a good source of dietary fibre containing a well-balanced ratio of soluble and insoluble fibre (1:2) as well as polyphenols, carotenes and flavonoids (Bhushan et al., 2008; Sudha, 2011). For these reasons, apple fibre is considered to have better nutritional quality in comparison to cereal fibres (Sudha, 2011). Higher amounts of soluble fibre of pomace can

positively affect the water holding capacity (WHC) of the food product. The WHC of material is the ability to hold its own and added water. This property helps to enhance the viscosity and stability of food, as well as decrease the rate of nutrient absorption in the intestine. This makes pomace suitable for inclusion in healthy low-calorie foods.

Devising a process that can convert pomace to a high fibre food ingredient product with desirable functional purposes (such as texturising agents that can provide creamy mouthfeel) can deliver a profitable material out of processing waste. Production of food ingredients from fruit and vegetable by-products are usually connected with developing a process containing thermal and mechanical steps (Espinosa-Muñoz et al., 2013; Hemar et al., 2011; Lopez-Sanchez et al., 2012; Lopez-Sanchez et al., 2011). Enzymatic treatment may also be used to make a particular functional property in the plant product. Thermal processing of fruit and vegetable results in softening tissue, separating cells across the middle lamella by dissolving pectin into the serum phase. Mechanical and enzymatic processes can also affect the functional properties of plant-based ingredients such as thickening, stabilising and texturising properties. These treatments can change the size and the shape of particles, structure of the soluble polymers (such as pectin), WHC and physical stability of final products (Kunzek et al., 2002; Taherian et al., 2009).

Early discussions with ingredient companies selling fibre ingredients into nutritional and bakery products revealed a need for a fibre product with creamy fat-mimetic properties for use in drinks and smoothies plus a product with good water-binding properties for use in baked systems. Existing fibre ingredients, such as those from cane bagasse or brans, are primarily cellulosic and offered little physical functionality to a food system. Hence the goal arose to design a simple, robust process which could be executed with compact equipment easily located on an existing juice processing site. The complete process must render fresh pomace into dried ingredient which exhibited the sensory and water binding properties desired.

This thesis reports my work to discover what size insoluble fibre must be reduced to, and what molecular weight pectic oligomers must be maintained at, to achieve the sensory properties desired in the target ingredient. I report development of a system to guide the selection of time, temperature and shear required of the full-scale process when acting on apple pomace.

1.2 Objectives

I intended to develop reaction kinetic model supporting a process to solubilise pectin in oligosaccharide form while physically fragmenting the insoluble residue (containing cellulose and hemicellulose polysaccharides). It was expected that both size of insoluble particles and the

molecular weight distribution of pectic oligosaccharides will influence mouthfeel and stability of the system.

I obtained apple pomace from a commercial juicing plant and used it to:

1. Investigate the likely process steps (e.g., hydrothermal, mechanical and enzymatic) to solubilise pectin (with high molecular weight), fragment the residual material (composed of cellulose and hemicellulose polysaccharides) by applying mechanical shear and reducing the molecular weight of solubilised pectin into lower molecular weights;
2. Describe the effect of time, addition of water to pomace and temperature parameters during the hydrothermal and shearing processing steps on quality attributes (e.g., pectin solubilisation and hydrolysis, decomposition of sugars, production of unwanted components such as organic acids, furfural and 5-hydroxymethyl furfural etc and to characterise the kinetics of these reactions;
3. Apply the kinetics of the main reactions to models for scaling up the process in a more complex situation (pilot plant scale) and preparing high quantity of hydrothermally treated pomace for the next step of the study;
4. Fractionate and recombine hydrothermally-treated pomace in order to prepare samples with different characteristics of particle sizes and pectin molecular weights for sensory evaluation;
5. Understand the influence of insoluble particle size distribution and pectic oligomer molecular weight distribution on mouthfeel properties of pomace ingredients;
6. Understand how an industrial pectinase enzyme may modify the molecular weight distribution of pectin and what is their effect on physical stability and sensorial quality of the pomace ingredients;

Ideally, the process developed would employ a small number of standard equipment items and achieve near 100% yield on incoming pomace solids. Preferably, it would be a compact process, with employing little added water.

CHAPTER 2 **Literature Review**

2.1 Apple Production

Apple (*Malus domestica*) is a popular fruit that is produced in temperate regions around the world (Hui, 2007). China and the USA together produce more than 50% of apples in the world (Bhushan et al., 2008). Worldwide production of apples in 2014 was 85 million tonnes, with China accounting for 48% of the total (www.fao.org, 2014).

New Zealand grows a substantial number of apples with a total production in 2014 of 430,000 tonnes (www.fao.org, 2014). The total area planted in apples increased between the years of 2013 and 2016 from 8,630 to 8,809 hectares, with an average yield of 60.7 tonnes per hectare (2013-2015). In 2016 apples were one of the few fruit and vegetables that were not imported into New Zealand. New Zealand apples are exported to other parts of the world (as fresh or processed products) with the value of $692 M. In 2016, a million more new apple trees have been planted across New Zealand (Anon, 2016). In New Zealand, fresh apples and apple products obtained the second place –after bananas- of "consumer spending on fruit" with a value of more than $100 M in 2016 (Anon, 2016).

Apples may be processed into products such as juice and cider, juice concentrate, dehydrated or frozen apple, and canned apple slices and cubes, baby foods, applesauce and vinegar (Hui, 2007; Sudha, 2011). Some of the commercial apple varieties which are grown around the world are Braeburn, Fuji, Gala, Golden Delicious and Granny Smith.

2.2 Apple Composition

Apple composition is affected by growing location, environment, varieties and harvest maturity. An apple contains approximately 85% water, 12-14% carbohydrate, 0.3% protein and 2.0 % fibre. Malic acid is the primary organic acid in apple fruits (0.3-1%). Minerals and vitamins present in

apples include calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, vitamin A and C. The average concentration of total phenolic content in flesh and skin of apple are 0.4 and 1.3 mg/g fresh weight, respectively (Hui, 2007). The soluble sugar content in 100 gram edible portion of apple is about 10 g/100 (5.4 g/100 fructose, 1.8 g/100 glucose, 2.6 g/100 sucrose) (Hui, 2007).

The basic anatomy of apple fruit is shown in Figure 2.1. Apple skin is comprised of layers of cuticle (a non-cellular waxy coating), epidermis and hypodermis. Inside the fruit there are two parts, the hypanthium and pericarp. The hypanthium (fruit flesh) is made up of parenchyma cells with large vacuoles, each surrounded by a thin non-lignified primary cell wall. The pericarp (apple core) extends from the centre of the fruit to the core line and contains the ovary and seeds (Drazeta, 2002; Harris et al., 2006).

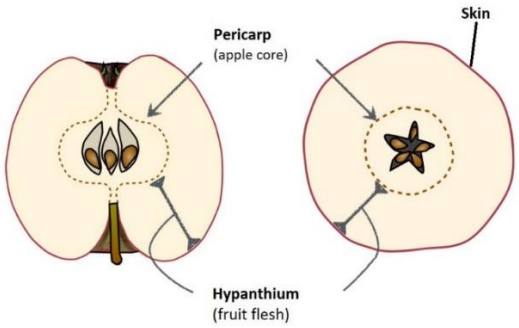


Figure 2.1 The basic anatomy of apple fruit (adapted from https://botanistinthekitchen.blog/2014/11/24/apples-the-ultimate-everyday-accessory/).

Figure 2.2 shows the arrangement of parenchyma cells of the flesh and the structure of the cell. The vacuole occupies most of the cell space and contains the juice. Primary cell wall mainly contains cellulose, hemicellulose (primarily xyloglucan) and pectin with a very small amount of protein (Harris et al., 2006; Redgwell et al., 2008b).

Figure 2.2 General arrangement of parenchyma cells and structure of the cell. Adapted from Redgwell et al. (2008b).

2.3 Apple Cell Wall

2.3.1 General Layout of Fruit Cell Wall

Plant cell wall composition and physical properties may vary enormously from cell type and plant species. Nevertheless, all plant cell walls, have similar basic constructions of crystalline cellulose microfibrils and a matrix of non-cellulosic polysaccharides such as xyloglucan (XG), pectin, structural protein and enzyme and lignin. Interactions between these polymers in cell wall networks are unresolved (Harris et al., 2006; Panouille et al., 2006). Electron micrographs of unripe, mature and over-ripe fruits show some difference in cell wall arrangements which usually starts from the pectin-rich layer between adjacent cells (middle lamella) (Figure 2.3 A and B) (Benarie et al., 1979; Brummell, 2006). These changes include pectin solubilisation and decreased amount of neutral sugar side chains that have been reported in apples during maturation and ripening to decrease the firmness (Brummell, 2006; Ng et al., 2015). The following sections describe the main polysaccharides in plant cell walls.

Figure 2.3 Ultrastructure of cell wall of apple flesh, at different stages of ripening. A: mature hard apple, cell walls (cw) of adjacent cells tightly packed and darkly stained, and middle lamella (m). B: over-ripe apple started to dissolve from outer parts while the inner part still contains tight fibrillar arrangement. Cell wall (cw), middle lamella (m). bar =0.5 μm. Adapted from Benarie et al., (1979).

2.3.2 Cellulose

Among the different polymers and interactions in plant and fruit cell walls cellulose is the only polysaccharide with well-known composition. It consists entirely of β-(1→4) linked glucose residue long, linear rigid and inextensible chains (Figure 2.4) (Agoda-Tandjawa et al., 2010; Brummell, 2006; Scheller et al., 2010). These parallel linear chains are tightly bound together through hydrogen bonds and form microfibrils (Brummell, 2006; Jarvis, 2009; Zykwinska et al., 2005). The interior region of cellulose microfibrils is highly crystalline (ordered domains), while the outer part contains some discorded (amorphous) regions (Agoda-Tandjawa et al., 2010; Brummell, 2006).

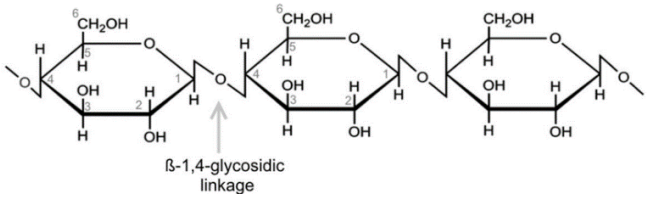


Figure 2.4 Cellulose structure. Aadapted from Gurunathan et al. (2015).

Pure commercial cellulose is obtained by treating plant processing waste with alkali solutions and alkaline hydrogen peroxide. This sort of powdered cellulose is used in food products in order to increase fibre content of the daily diet. Unmodified, it can provide poor mouthfeel due to detectable particles. In order to overcome this issue powdered cellulose can be further treated with other physical and chemical processing steps. The resultant modified cellulose products, such as carboxymethyl cellulose, methyl cellulose and methyl ethyl cellulose are widely used as texturising, thickening and bulking agents, as well as emulsifiers, and stabiliser (Harris et al., 2006; Imeson et al., 2010).

2.3.3 Hemicellulose

Hemicellulose is a general term to describe the group of cell wall polysaccharides that are not cellulose or pectin (Scheller et al., 2010). Xyloglucan (XG) is the most abundant hemicellulose in the dicotyledon primary cell wall (20-25% w/w). Xylan is the major hemicellulose in secondary cell wall, which plays an important role in cell wall expansion (Scheller & Ulvskov, 2010; Watt et al., 1999; Bhushan et al., 2008). XG is composed of a backbone of β-(1→4) glucose units (cellulose-like main chain) regularly substituted at the O-6 position with xylose and with sub-branches of galactose, fucose and arabinose (Jarvis, 2009; Watt et al., 1999). There are variations of XG branching pattern dependent on the plant variety and plant tissue types (Scheller & Ulvskov, 2010). In apple, XGs are the major hemicellulose (up to 18% of the cell wall polysaccharides) and are mostly present as fucogalactoxyloglucans (Figure 2.5), containing a repeating subunit of four glucosyl residues with the first three bearing a single xylose residue. About half of the other subunits have fucose-galactose side chains attached to xylose residues (Bhushan et al., 2008; Harris et al., 2006; Ng et al., 2014; Renard et al., 1995; Scheller et al., 2010).

Figure 2.5 The structure of xyloglucan oligosaccharides; XLFG, XXFG and XXXG. Adapted from Watt et al., (1999).

2.3.4 Pectin

Pectin is part of the cell wall matrix and is particularly present in the intercellular spaces of dicotyledons, (middle lamella region). It acts as cementing material, with several interactions (covalent and non-covalent) with cellulose and XG (Berjenholt, 2010; Bonnin et al., 2014; Cucheval, 2009; Zykwinska et al., 2005). It is an extremely complex cell wall polymer (Bonnin et al., 2014).

The structural properties of pectin vary among the different plant species and can be affected by environmental conditions and plant development stage. The native pectin in plants mainly consists of three polysaccharide structures: homogalacturonan (HG), rhamnogalaturonan I (RG-I) and rhamnogalaturonan II (RG-II) (Berjenholt, 2010; Zykwinska et al., 2005).

Homogalacturonan is a linear polymer of α-(1→4)-D-galacturonic acid (GalUA). The carboxylic acid group on C-6 can be methylated. Acetylation of O-2 or O-3 is possible but rare. Degree of esterification (DE) is defined as the percentage of GalUA units which have been methyl-esterified (Berjenholt, 2010; Bonnin et al., 2014; Zykwinska et al., 2005). These ester groups can be removed during plant or organ development, and the polysaccharide can then become highly negatively charged. Chains of unesterified HG can associate through divalent ionic bridges with Ca^{2+}, forming junction zones for chain association (Bonnin et al., 2014). Voregan et al., (2009) estimated the average length for a HG backbone in citrus, sugar beet and apple is between 72-100 GalUA residues. They also reported that about 36% of pectins in apple are present as HG.

RG-I is a highly branched structural group and consists of up to 100 or more repeating units of the disaccharide α-(1→2)-L-rhamnose-α-(1→4) D-galacturonic acid (Berjenholt, 2010). Depending on the plant source, about 20-80% of rhamnose residues on RG-I backbone can be substituted with arabinose or galactose at the C-3 or C-4 positions (Bonnin et al., 2014; Zykwinska

et al., 2005). Two chains of RG-I can be linked to each other by oxidative coupling of ferulic acid units (Berjenholt, 2010).

RG-II is minor substructure of pectin and is highly a branched molecule. It consists of a short HG backbone (around 9 galacturonan units) linked to several sugars (GalUA, rhamnose, galactose and some rare sugar such as apiose, methyl-fucose and methyl-xylose).

RG-I and RG-II domains are referred as "hairy region" whereas HG sometimes referred to as smooth (Figure 2.6) (Berjenholt, 2010; Zykwinska et al., 2005).

Figure 2.6 Three major pectic polysaccharides domains present in cell wall, HG= homogalacturonan, RG-I= Rhamnogalascturonan I, RG-II= Rhamnogalacturonan II. Adapted from Berjenholt et al., (2010).

A further substructure of pectin, xylogalacturonan (XGA), has been also reported in some plant cell walls (such as apple) but it is not present in large amounts compared to the other substructures. It consists of a HG backbone with single xylose side chains. XGA might be attached to O-3 of pectin backbone as a side chain of RG-I (Bonnin et al., 2014; Brummell, 2006; Zykwinska et al., 2005).

Apple RG-I is rich in arabinan and arabinogalactan side chains and some XGA structures (Redgwell et al., 2008a; Voragen et al., 2009). The structural properties of pectin polymers, such as molar mass, composition and frequency of side branches, DE and distribution of their ester groups highly affect its properties, stability and utilisation in the food industry (Bonnin et al., 2014; Sila et al., 2009).

The optimal pH for pectin polymer stability is at its pKa value (3.5-4.1) (Berjenholt, 2010; Sila et al., 2009). It is known to be unstable above pH 5 especially when exposed to high temperatures (Dominiak et al., 2014). Pectin may be affected by chemical de-esterification and de-polymerisation as well as being susceptible to pectinolytic enzymes. Highly methylesterified pectin can degrade at temperatures higher than 80 °C at alkaline or acidic pH by depolymerisation reactions (β-elimination and acid hydrolysis, respectively). During these reactions, glycosidic bonds at C-4 position of galacturonic acids in the backbone are broken and the molecular weight declines (Berjenholt, 2010). This explained more fully in Section 2.6.1.1.

2.3.4.1 Relevant Cell Wall Interactions that may Affect Pomace Modification

A number of models have been suggested to describe interactions in the plant cell wall. One early model was based on the view that cellulose microfibrils were completely coated by XG. In this model XGs cross-linked adjacent microfibrils as well as other cell wall components (such as pectin polysaccharides) (Cosgrove, 2001). This model has since been rejected by several researchers due to the reports on insufficient XG in cell wall to cover whole surface of microfibrils, between adjacent cells and within the cellulose fibres (Bootten et al., 2004; Dick-Perez et al., 2012; Dick-Perez et al., 2011; Peaucelle et al., 2012; Zykwinska et al., 2007; Zykwinska et al., 2005). A new model for primary cell wall, "the tethering network model", has been proposed by Dick-Perez et al., (2011) and is given in Figure 2.7 below. This model is important in outlining the way pectin and XG may interact with other polysaccharide in the cell wall and these interactions are described in more details below (Dick-Perez et al., 2011; Park et al., 2012; Peaucelle et al., 2012; Scheller et al., 2010; Zykwinska et al., 2007; Zykwinska et al., 2008a).

Figure 2.7A model of plant cell wall. Pectin (green curve lines) represent numerous interactions with the surface of cellulose microfibers (red tubes) and xyloglucan (blue curves). Limited number of xyloglucan intercalates (dashed blue lines) into cellulose microfibers. All the three types of cell wall polysaccharides interact in a single network. Adapted from Dick-Perez et al., (2011).

Pectin interactions

Pectins play a part in the structural firmness of plant tissue as a part of the primary cell wall and as the main component of the middle lamella (Zykwinska et al., 2008a). One of the models which has been suggested for the three main pectin substances (HG, RG-I and RG-II) is that HG constituents are depicted as side chains of RG-I and other pectic molecules can also interact with RG-I or HG polymers (such as galactan, arabinogalactan, arabinan and RG-II). Interactions of these subunits with each other in the cell wall is likely through the covalent and ester linkages which combine them into a macromolecular structure (Bonnin et al., 2014; Vincken et al., 2003; Zykwinska et al., 2005). HG chains can be linked to RG-I through hydrogen bonds, hydrophobic interactions and ionic links which greatly depend on methylation of pectin and the methyl group

distribution (Zykwinska et al., 2005). RG-II can cross-link two HG molecules by ester bonds. Galactan, arabinogalactan and arabinan can also be linked to RG-I through the glycosidic linkages. It has been suggested that ionic and ester bonds of pectin can affect the porosity of primary cell wall (Peaucelle et al., 2012; Vincken et al., 2003). Moreover, two unesterified HG polymers might interact via an ionic bridge with calcium as described by the "egg box model" and resultant Ca^{+2}-pectate gels (Vincken et al., 2003). Another feature of the tethering model is the possibility of covalent linkages between RG-I side chains and XG. Pectin can also be associated with some parts of cellulose fibres through the weak bonds of RG-I's side chains or by tangling effects. The amount of pectin which is associated with cellulose is quite small (Cumming et al., 2005; Dick-Perez et al., 2011; Jarvis, 2009; Oechslin et al., 2003; Popper et al., 2008; Voxeur et al., 2016; Zykwinska et al., 2005).

Work using *in vitro* systems has been shown that pectin arrangements in the wall may be influenced by the amount of XG present. In the presence of high concentrations of XG, pectin may fill the gap between XG and cellulose, enabling XG to interact more easily and tightly to cellulose and maintain the cell wall architecture. In conditions of low XG content, pectin may bind to cellulose fibres directly via ester-linkages to phenolic compounds) (Oechslin et al., 2003; Zykwinska et al., 2005). It is as yet unproven that these interactions occur in the native wall.

XG interaction

In the tethering network model, XG can bind to the adjacent microfibrils and play an important role in dicotyledon cell wall expansion (Bhushan et al., 2008; Zykwinska et al., 2005). XGs are reported to be available in cell wall as free XG chains and XG-pectin complexes. It is suggested that XG-pectin complexes can provide stronger links to cellulose microfibrils than free XGs (Popper et al., 2008). XGs are able to bind the cellulose fibres by two main mechanisms: by tangling between fibres during the cell wall growth and by hydrogen bonds (Fu et al., 2006; Scheller et al., 2010; Zykwinska et al., 2005).

2.4 Apple Juice and Pomace Production

2.4.1 Processing Steps for Apple Juice Processing

The main steps of apple juicing are given in Figure 2.8. The initial process steps are sorting, washing and crushing/milling and pressing. Pressing can be achieved by belt and/or hydraulic presses. With the belt press, apple pulp is trapped and squeezed between belts by the rollers, and

with the hydraulic press, milled apples inside the body of the equipment are compressed by the hydraulic pressure that can be applied from the top or sides.

Juicing may/or may not include addition of enzymes. This depends on the customer requirement for clear or cloudy juice. Mixtures of pectin-acting enzymes (pectinases) are the usual industrial enzyme preparations used to increase the yield of juice. Pectic enzymes are mainly obtained from fungi such as *Aspergillus* spp. These enzymes can successfully operate in the acidic condition during the fruit juice porduction (pH~3-4) (Hui, 2007; Kashyap et al., 2001).

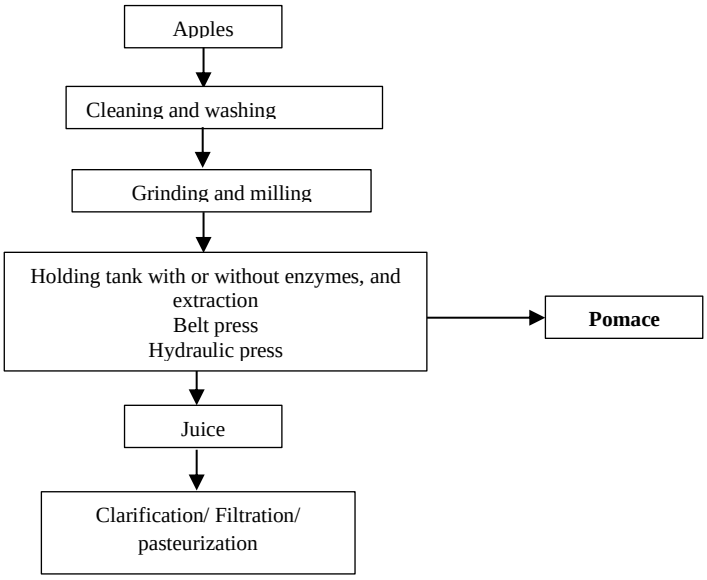


Figure 2.8 Basic steps of juicing apples adapted from Sinha (2007).

2.4.2 Apple Pomace

Pomace is the solid residue remains after pressing apples for juice (Hui, 2007; Sudha, 2011). About 70% of the apples produced worldwide are consumed as fresh fruit, while near to 30% are processed to other products such as apple juice (65%), cider, apple purees, jams and dried apple products (Bhushan et al., 2008). New Zealand exports most of its apple crop as fresh fruit. Figures are not available for the proportion of crop that is used for juicing. It appears though that this change from season to season, depending on the quality of the fresh crop and the relative market demand for juice and whole fruit.

Apple pomace is the major by-product of the apple juice industry, representing 30% of the original fruit weight (Sudha, 2011; Bhushan et al., 2008; Tiwari, Brunton, & Brennan, 2013). It mainly contains flesh/skin (95%) seeds (2%-4%) and stalk (1%) (Bhushan et al., 2008; Tiwari et al., 2013).

It has high moisture content (70-80%) although the composition of pomace can vary by batch, processing procedure and processing site (especially effectiveness of the pressing step). The moisture makes the pomace bulky and susceptible to microbial growth (Bhushan et al., 2008; Sharma et al., 2014). The approximate pH of the fresh apple pomace is around 4 which might be decreased to around 3 by storing at 8℃ (Sharma et al., 2014; Sudha, 2011). The percentage of pectin by fresh weight in milled apples before pressing is about 0.5-1.6% and after pressing is 1.5-2.5% (Prasanna et al., 2007).

According to Bhushan et al., (2008) who summarized the composition of apple pomace from 13 research papers, the total carbohydrate and pectin content in pomace (dry weight basis) is 48-62% and 3.5-14.3%, respectively. The alcohol-soluble fraction of carbohydrate was reported as sucrose (3.8-5.8%), glucose (19.5-19.7%) and fructose (48.3%).

The approximate composition of apple pomace is given in Figure 2.9 below:

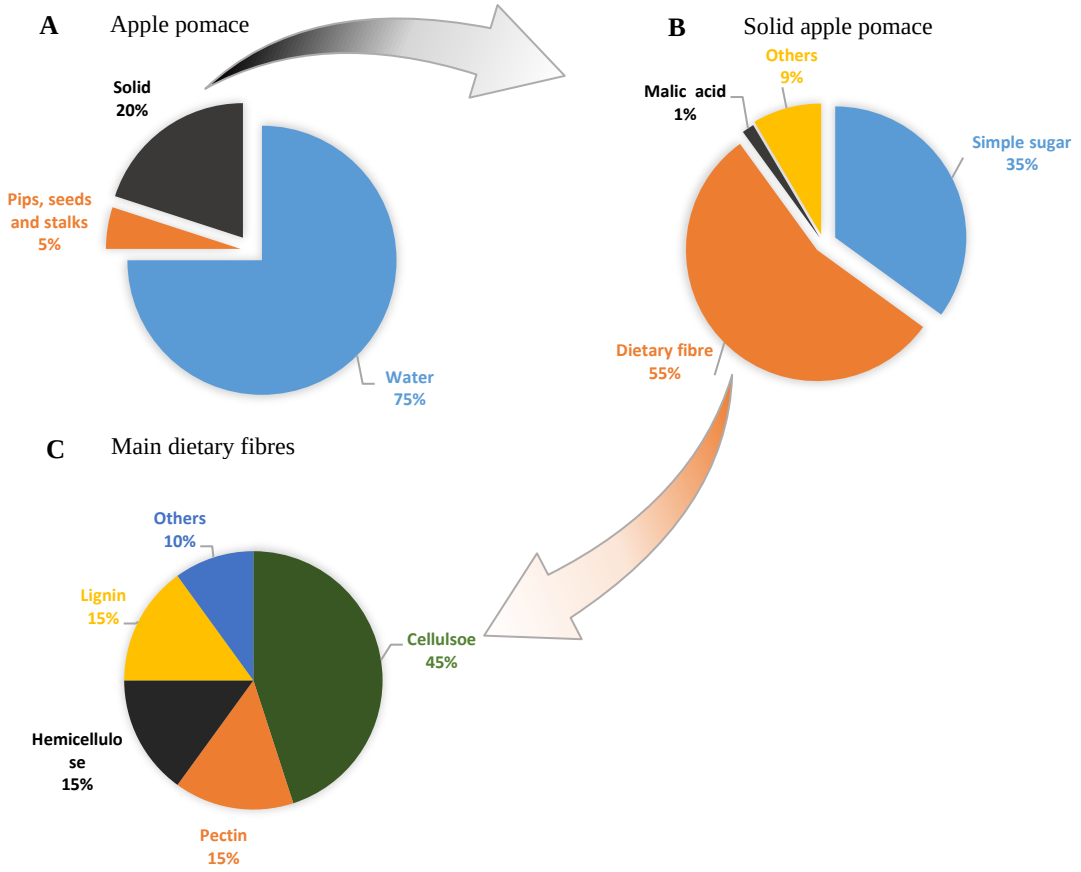


Figure 2.9 Approximate composition of apple pomace. A: Whole apple pomace (wet basis). B: solid part of apple pomace (dry basis). C: Main dietary fibres (dry basis). Adapted and modified from Bhushan et al., (2008); Sudha (2011) and Sharma et al., (2014).

2.4.3 Current Uses for Pomace

Direct disposal of pomace close to the processing plant or transportation to a landfill site for disposal (which is still being done by many factories) represents a huge loss of biomass, creates additional costs and can cause serious environmental problems (Bhushan et al., 2008; Sharma et al., 2014; Van Dyk et al., 2013). It has been reported that about $US10M is spent annually on disposal of apple pomace in the USA (Van Dyk et al., 2013).

Many efforts have been made to utilise apple pomace in edible products such as extraction of pectin, polyphenols and antioxidants, pigments and whole fibre. These are described briefly below.

2.4.3.1 Pectin Extraction

Among all approaches for the utilisation of pomace, pectin production is the most common option on a large scale (Miceli-Garcia, 2014; Sharma et al., 2014). Pectin is a safe food additive. The US Food and Drug Administration (FAD) accords pectin "generally recognised as safe" (GRAS) status (Berjenholt, 2010). It is usually extracted for various applications in food, pharmaceutical and cosmetics, such as gelling agent, emulsifier, film coating, encapsulation, binding agent, texturiser, stabiliser and thickener (Miceli-Garcia, 2014; Sharma et al., 2014; Sila et al., 2009).

Pectin is classified as a dietary fibre because it is not broken by the enzymes of the human gut. However, pectin may be fermented by intestinal bacteria. In these cases, pectin can provide substantial health benefits (see Section 2.4.3.4 for further information on dietary fibre). Non-toxicity, non-digestibility and biocompatibility of pectin also support its application as a drug carrier, for example for colon drug delivery. Food industries commonly use pectin in jam, jellies, bakery products, confectionery, acidified milk drinks, beverages and frozen foods (Bhushan et al., 2008). Annual consumption of pectin around the world is estimated to be 45,000 tonnes (Willats et al., 2006).

Although pectin is naturally present in large proportions in the primary cell wall of all dicotyledons, citrus peel and apple pomace are the two major commercial sources of extracted pectin (Harris et al., 2006; Willats et al., 2006). There are two main reasons for this: firstly, the large concentrated quantity of the raw material available annually and secondly, the high quality of pectin present (for example. size and DE (>50%)). For these reasons other potential sources of pectin (such as beet pulp, banana, mango, peach, pineapple, strawberry, tomato fruit, sunflower head and tamarind), even though they may contain reasonable amounts of pectin have not been commercialised (Harris et al., 2006; Thakur et al., 1997; Willats et al., 2006). Pectin is usually

extracted by dilute mineral acids or organic acids at high temperature followed by alcohol precipitation (Bhushan et al., 2008). The structure of commercial pectin is less complex than native pectin because of the structural changes taking place during the pectin extraction (Stephen et al., 2006).

The pectin in fresh apple pomace is very susceptible to degradation by pectinolytic enzymes in apple pomace (endogenous enzymes in apple fruit or added during juicing) and microorganisms (yeast and fungi) which can also produce pectolytic enzymes (Berjenholt, 2010; Thakur et al., 1997). Therefore, it is necessary to extract pectin from raw material immediately or dry the pomace as soon as possible before it degrades (Bhushan et al., 2008; Thakur et al., 1997). Pectin is heat sensitive, therefore drying the pomace at high temperatures can cause a substantial loss in the yield and functionality of the pectin extracted (Sharma et al., 2014; Sudha, 2011). Moreover, apple juice production is usually coupled with enzymatic treatment of pulp and this damages the pectin considerably (Thakur et al., 1997). Although apple pectin is considered to have superior gelling properties than citrus pectins, its application in the food industry is sometimes limited due to its brown hue which can make it difficult to incorporate into light-colour foods (Djilas et al., 2009).

2.4.3.2 Polyphenol and Pigment Molecule

Apple skin is a good source of polyphenols and anthocyanins that are not extracted during the juicing process (Sudha, 2011; Tiwari et al., 2013; Wikiera et al., 2016). Major bioactive phenolic components extracted from apple pomace are quercetin glycosides, followed by phloridzin and its oxidative products, chlorogenic acid, catechin and procyanidin (Bhushan et al., 2008; Miceli-Garcia, 2014). These polyphenols, especially epicatechin and quercetin glycosides, have shown good free radical scavenging properties, with two times higher activity in comparison to vitamins C and E (Bhushan et al., 2008). Convectional extraction by methanol or ethanol solvents, subcritical water, subcritical CO_2/ethanol extraction or enzyme treatment techniques are used to extract polyphenols from the pomace (Bhushan et al., 2008). Extracting polyphenols and pigments from pomace is expensive, and large amounts of waste still remain after the extraction of these components (Miceli-Garcia, 2014).

2.4.3.3 Stock Feed

Pomace has traditionally been used as cattle feed. It has very low nutritional value for stock, however, due to high sugar content and low protein and vitamins (Djilas et al., 2009; Vendruscolo et al., 2008).

2.4.3.4 Direct Incorporation into Food

Apple pomace is a rich source of dietary fibre (non-starch polysaccharide). The definition of dietary fibre proposed by AACC Dietary Fibre Definition Committee in 2001 is "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances." Codex Alimentarius further defines dietary fibre as "dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans." (Codex, 2008). Increasing the intake of dietary fibre helps to decrease the risk of colorectal cancer, constipation, diverticulosis and cardiovascular disease (Harris et al., 2006). The ability of fibres to bind water helps enhance the viscosity of added food, as well as decrease the rate of nutrient absorption in the intestine, makes pomace suitable for inclusion in healthy low low-calorie foods (Sudha, 2011).

Dietary fibre content in whole apples (with peel) is about 2.4% (fresh weight basis) (Hui, 2007) and total dietary fibre in apple pomace is 35-60% (dry weight basis) (Bhushan et al., 2008). Main constituents of dry apple pomace dietary fibre are cellulose (7%-44%), lignin (15%-23%), hemicellulose (4%-24%) and pectin (18-19%) (Bhushan et al., 2008; Sharma et al., 2014).

Dietary fibre of apple pomace contains a well-balanced ratio of soluble and insoluble fibre, as well as polyphenols, carotenes and flavonoids (Bhushan et al., 2008; Sudha, 2011). For these reasons, apple fibre is considered to have better nutritional quality in comparison to cereal fibres (Sudha, 2011).

Dried apple pomace powder has been used directly in bakery products such as cake, bread, cookies and muffins replacing a portion of wheat flour (Miceli-Garcia, 2014; Sudha, 2011). This substitution can have positive or negative influences on the final product. Replacing part of the wheat flour with dried milled apple pomace in cakes, cookies and muffins resulted in products with fewer calories than controls (Bhushan et al., 2008; Chang et al., 1993) and slightly better colour (Sudha et al., 2007). Addition of apple pomace at 15% helped to increase water absorption in bread dough from 60.1% to 70.6% (Sudha et al., 2007). Moreover, those breads which have been produced by replacing a portion of the wheat flour with apple pomace were softer, with higher weight in comparison with the ones without pomace (Sudha, 2011). It has been also reported that dough stability of the samples with 15% pomace added was decreased during the mixing (Sudha et al., 2007). Replacing flour with apple fibre (the dried milled apple pomace) up to 11%, resulted in dough development time (the hydration rate and development of gluten) increased from 1.9 min to 3.9 min (Masoodi et al., 2001). To overcome this problem, using

hydrated apple fibre (hydration ratio 1:7 for 12 h) rather than dry apple fibre has been suggested. This decreased the deleterious impacts of adding fibre on the bread making quality and was thought to be because pre-hydrated apple fibre competed less for water than wheat flour and other dough components (Chen et al., 1988).

2.5 Opportunities and Requirements

Previously some uses of pomace were explained. Here we introduce some other opportunities for pomace as an ingredient in food industry as well as the requirement of them (Table 2.1).

Table 2.1 Opportunities for pomace in food industries.

Opportunities	Requirements	
	Pectin	**Solids**
Low calorie texturing agent	➢ Provide high hydration properties and suitable viscosity and texture in product.	➢ Do not detect in mouth as individual particles. ➢ May be individual cell particles of 100-200 μm with smooth surface.
Bakery products	➢ Have good water holding capacity to postpone the staling. ➢ Provide good porosity and protect bubbles walls in dough and final product.	➢ Do not have adverse effect on mouth feel. ➢ In cookies, support the shape ability of cookies dough. ➢ Do not make the gluten network too tight or too loose (depends on hydration properties of solid particles). ➢ Do not increase the hardness of bread, provide good colour, without off smells.
Spray drier carrier (e.g. for drying of fruit juices)	➢ High molecular weight of pectin (~DP16 or dextrose equivalent of 6).	➢ Solid particles < 50 μm with high temperature of glass transition (higher than the temperature on outlet of spray dryer "~≥90°C").
Dry blending (Dry mixed vegetable soups, Breader and Batter)	➢ Have high hydration properties and provide suitable viscosity in mixture.	➢ Distribution of solid particles depends on the final product (particles with sharp edges might be detectable in the mouth). ➢ Rate of water uptake of solid particles (good hydration properties) and increase the viscosity.

DP= degree of polymerisation
dextrose equivalent is the amount of reducing sugars present in a product, expressed as percentage on a dry basis relative to glucose.

2.6 Pomace Processing Steps

Many strategies to reprocess fruit juice waste streams into useful materials start with drying, in order to stabilise material (by reducing the water and microbial content), make it less bulky and easier to handle. Drying can be achieved by using high or low air temperature (105°C or 60°C) and by infrared techniques. In some countries sun drying is possible. However, these methods make the pomace darker (by Maillard or enzymatic browning), reduce the nutritional value, lower the pectin yield and reduce the gelling properties of pectin (Bhushan et al., 2008; Hwang et al., 1998; Redgwell et al., 2011).

The following sections will focus on processing operations commonly used on wet pomace or on whole fruit and vegetables where the treatments have affected cell wall constituents similar to those found in apple pomace. Physical (hydrothermal and shearing) and enzymatic treatments will be reviewed.

2.6.1 Physical Processing

2.6.1.1 Hydrothermal Process

A process in the presence of water at high temperatures and pressures is called a hydrothermal process. An effective heat treatment will denature the pectinolytic and browning enzymes in the pomace (e.g. polygalacturonase (PG), pectin lyase, cellulase, polyphenoloxidase and peroxidase) as well as reducing its microbial content (Kunzek et al., 2002). Hydrothermal processes can also depolymerise and solubilise pectin and separate cells across the middle lamella thereby reducing the size of tissue clumps (Lopez-Sanchez et al., 2012).

Impact on Cell Wall Components

Mild treatment at 70°C for 10 min was not sufficient to reduce the cell adhesion along the middle lamella of carrot tissue (Lopez-Sanchez et al., 2011). Day et al., (2010) found that blanching carrots at 80°C for 10 min partially solubilised pectin from middle lamella which then resulted in partial detachment between adjacent cells (Figure 2.10 b). A more severe treatment at 100°C for 30 min separated the adjacent cells (Figure 2.10 c).

Figure 2.10 Confocal laser microscopy images of raw carrot tissue (A), blanched tissue at 80°C for 10 min (B), heat treatment at 100°C for 30 min (C). Adapted from Day et al. (2010a).

Sila et al., (2006a) showed that heat treatment could increase water soluble pectin (WSP) content extracted from plant tissue and soften the tissue, through different depolymerisation reactions such as β-elimination.

Hydrothermal treatments can solubilise pectin in plant tissue by depolymerisation reactions through acid hydrolysis and β-elimination (Sila et al., 2006a). Figure 2.11 shows HG pectin de-polymerisations through the enzymatic and non-enzymatic reactions (Sila et al., 2009). Hydrothermal treatment of apple pomace at 130, 150 and 170°C for 5 min showed that maximum pectin solubilisation was obtained at 130°C while the highest yield of alcohol precipitated insoluble solid pectin was gained at 150 °C. This is possible because of the extraction and precipitation of other polysaccharides (eg. XG) associated with pectin at higher temperature. The amount of neutral sugar associated with the pectin polysaccharides (e.g. xylose, mannose, glucose, galactose) increased at high temperature suggesting hydrolysis of side chains of pectin (Wang et al., 2014).

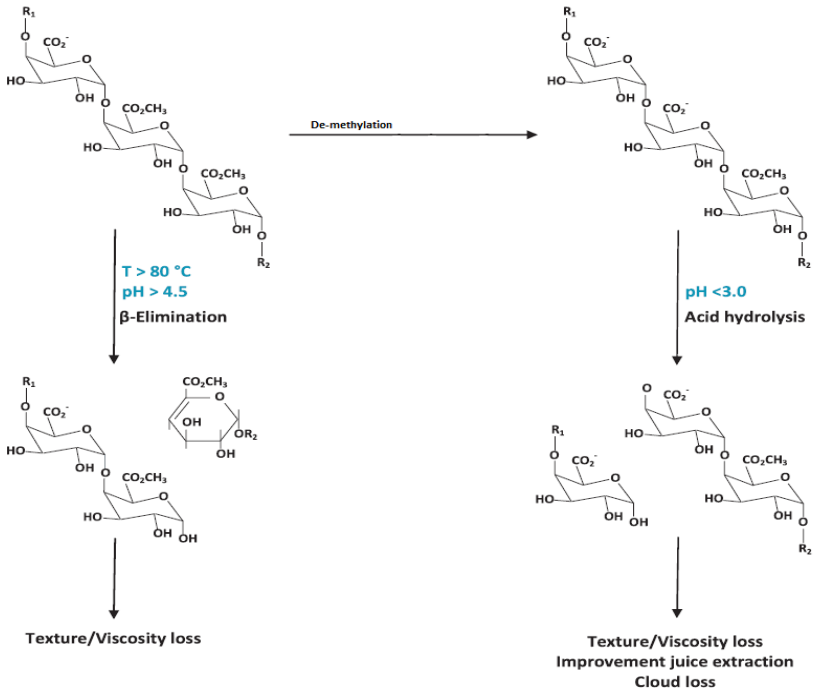


Figure 2.11 Possible pectin (only homogalacturonan) conversion reactions. Adapted from Sila et al. (2009).

β-Elimination Reactions and Acid Hydrolysis

The specific nature of pectin depolymerisation reactions (β-elimination and acid hydrolysis) is not very clear. Here we tend to introduce some prevalent conditions related to each of these reactions. β-elimination is one of the non-enzymatic mechanisms which can degrade pectin to lower molecular weight, and if happening in a plant tissue containing pectin might result in increased pectin content in solution (Diaz et al., 2007; Kravtchenko, Arnould, et al., 1992; Kuhnel et al., 2011; Sila et al., 2006b). This reaction only proceeds on GalUAs with a glycosidic bond on C-4 in the β-position of a neutral carboxyl group at C-5 (Figure 2.11). The reaction will take place by removing a hydrogen atom on C-5 of GalUA. The unstable anion intermediate will be stabilised by losing C-O linkage in the β-position. Consequently, a double bond will be formed between C-4 and C-5 at the non-reducing end which absorbs at 235 nm.

The rate of the β-elimination reaction is accelerated by DE > 60% (the main criterion), temperatures higher than 80°C and pH higher than 4.5. The extent of the reaction is also affected by molecular weight distribution of pectin, length of side chains and polymer-solvent interactions (Diaz et al., 2007; Muhidinov et al., 2010; Shpigelman et al., 2014). The β-elimination reaction competes with de-methylation reaction therefore, the β-elimination reaction never goes to completion even in optimal conditions (Kravtchenko et al., 1992). The β-elimination reaction results in pectin depolymerisation especially from middle lamella and therefore increases cell

separation (leading to softening plant tissue) as well as decreasing the molecular weight of pectin polymer and intrinsic viscosity (de Roeck et al., 2010; Sila et al., 2006a; Sila et al., 2009). Thermal pre-treatment of carrot discs at high temperature short time (90°C for 4min) resulted in higher WSP content and retained higher DE (~ 59%) in comparison to a low temperature long time pre-treatment (60°C for 40 min) with lower WSP content and DE (~ 53%) (Sila et al., 2006a). This has been explained that the optimal temperature for the most plant pectin methyl esterase (PME) is between 50 to 80°C. Therefore, at pre-treatment condition of 60°C the resulted pectin contained lower DE in comparison to the raw carrot (~60%). A low temperature pre-treatment before cooking resulted in lower DE and lower WSP as a consequence of unfavourable β-elimination reaction (Sila et al., 2006a; Sila et al., 2005).

According to de Roeck et al., (2010), high pressure processing (600 MPa) of carrot pieces enhanced contact between pectin and PME and resulted in lower DE pectin (~31%). Subsequent thermal processing resulted in less pectin depolymerisation again presumably due to unfavourable β-elimination conditions.

The second thermal degradation mechanism on pectin chain is acid hydrolysis, which occurs at pH ≤ 3.0. This reaction takes place at high temperature (> 100 °C) and hydrolyses the glycosidic linkage between GalUA residues in pectin chain (Figure 2.11). It has been shown that at temperatures less than 80°C, acid hydrolysis was limited (Diaz et al., 2007; Fraeye et al., 2007). Acid hydrolysis is only partially reliant on the DE and can occur in de-methylated pectin (Krall et al., 1998). It has been reported by Fraeye et al., (2007), that a decrease in DE from 78% to 59% doubled the rate of acid hydrolysis, but further reduction in DE to 19% resulted in only a slight increase in reaction rate.

Secondary Products from Sugar and Pectin Degradation

5-hydroxymethyl furfural production and degradation products

Figure 2.12 shows the possible degradation products generated during hydrothermal treatment. It is well known that thermal degradation of sucrose solutions can happen during heating. Acid hydrolysis of sucrose produces equal amounts of glucose and fructose (Dawber et al., 1966; Edye, 2001). Degradation of sucrose will start with production of fructose and an intermediate which will quickly convert to glucose. In acidic conditions, the rate of decomposition of sucrose to its monomers is faster than the rate of degradation of its subunits to other products such as 5-HMF (Edye, 2001).

In general, 5-HMF formation is linked to hexose sugar degradation. Fructose is known as one of the favourable substrates for this conversion. Heating fructose in acidic condition results in an

enolisation reaction which produces 1,2-enediol intermediates. This can be followed by a dehydration reaction and produce 5-HMF. Conversion of glucose through enolisation and dehydration to 5-HMF can happen directly, however it is more likely that first glucose converts to fructose through an isomerisation reaction then the fructose degradation happens as described (Hayes et al., 2008; Vandam et al., 1986).

Continued heating can result in further degradation of 5-HMF into equal amount of formic acid and levulinic acid as well as polymerisation of 5-HMF into dark colour substances (Dawber et al., 1966; Enomoto et al., 2018; Rackemann et al., 2013; Vandam et al., 1986). Continuous heating in an aqueous system results in oxidation of levulinic acid into acetic acid (Zhang et al., 2018).

According to Rackemann et al., (2013) the decomposition of a glucose-xylose mixture into formic acid and levulinic acid at ≥160°C for at least 15 min had the average ratio of 1.18: 1 (formic acid to levulinic acid). Higher production of formic acid can be related to other decomposition pathways for glucose and xylose to formic acid.

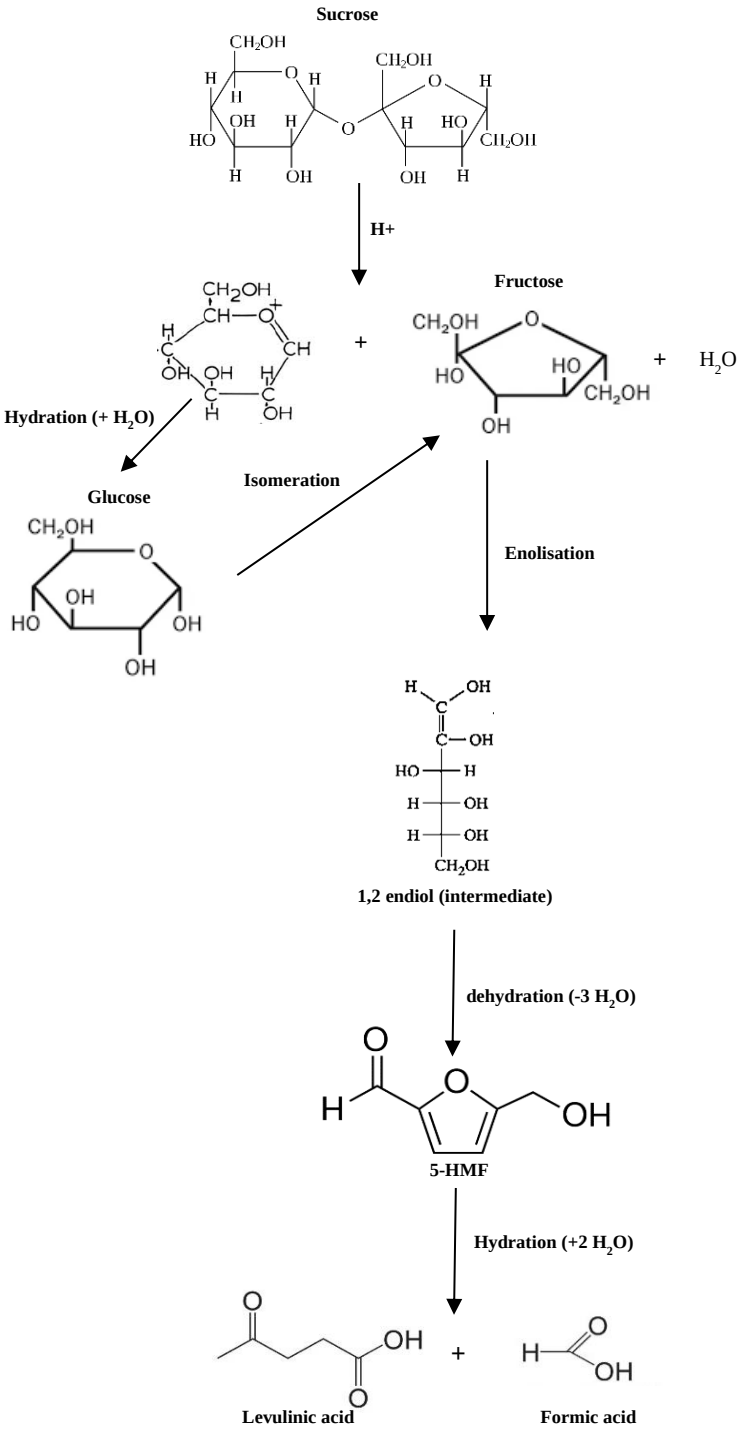


Figure 2.12 Decomposition pathway of sugars into 5-hydroxymethyl furfural and its secondary products.

Furfural production and degradation products

Furfural formation is linked to decarboxylation of pentoses such as xylose, ribose and arabinose and hexuronic acids such as glucuronic acid and galacturonic acid at high temperatures >130 °C. The first step in production of furfural from pectin is hydrolysis of de-esterified pectin to galacturonic acid. Next step is conversion of galacturonic acid to furfural, which depends on the

presence of hydrogen ions in the system. Formation of furfural increases at elevated temperatures and at higher concentration of hydrogen ions (Dounlop, 1948; Usuki et al., 2008).

There are also some side reactions which can contribute to the degradation furfural into other products such as formic acid, acetic acid and dark-coloured polymers (Dounlop, 1948; Zeitsch, 2000). Furfural decomposition can occur through three reactions (Figure 2.13). 1-Auto-oxidation: the presence and concentration of oxygen can accelerate the rate of the reaction and results in increasing the acidity and developing dark colour polymers (Dounlop, 1948). The dark coloured components are produced through resinification reaction. In this reaction, furfural may interact with itself or with the intermediates before producing furfural (Lamminpää et al., 2016). 2- Acid-hydrolysis: In this reaction, the rate of furfural decomposition is related to the concentration of hydrogen ions. Formic acid and acetic acid are the main carboxylic acids produced through this pathway (Dounlop, 1948; Zeitsch, 2000). 3- Thermal hydrolysis: this reaction can occur when furfural is exposed to high temperature (> 180°C) for a long time (> 8 h, depends on the temperature) (Dounlop, 1948; Zeitsch, 2000).

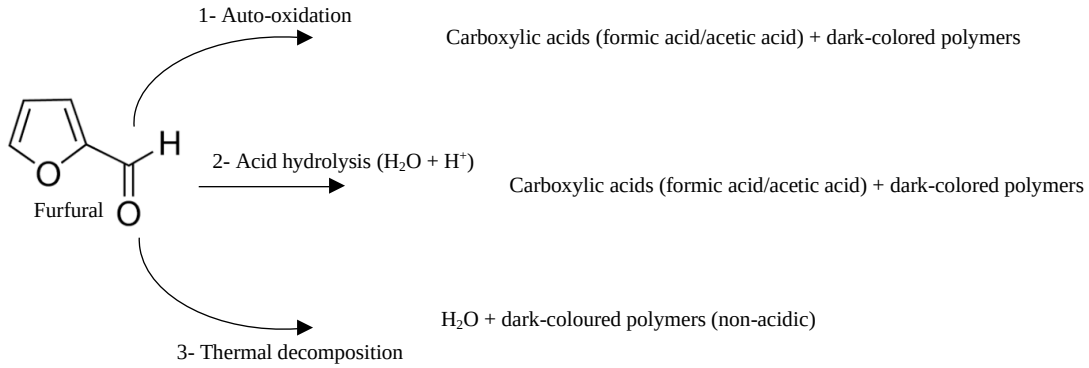


Figure 2.13 Decomposition pathways of furfural into organic acids and dark-coloured polymers.

Water that maintains its liquid state under pressure at temperatures higher than its natural boiling point (100°C) is called subcritical water. Critical temperature is considered as any temperature above 100°C- 374°C (Usuki et al., 2008). In this condition, ionic product (level of H^+ and OH^-) is higher than normal water, which means that water can act as acid or base catalyst to accelerate reactions (e.g dehydration of carbohydrates) without addition of further additives (Toor et al., 2011; Usuki et al., 2008).

Formic acid has also been reported to be produced by further degradation of 5-HMF at temperatures ≥140°C (Dashtban et al., 2012; Kuhnel et al., 2011). Acetic and lactic acids have also been reported in sugar beet pulp < 140°C, (Kuhnel et al., 2011), although this has also been attributed to contamination of the vegetable and fruit materials with microorganisms (Kuhnel et al., 2011).

Lamminpää et al. (2012, 2016) showed that formic acid can increase both the rate furfural production as well as the rate of furfural decomposition due to higher hydrogen ions in the system which can proceed the dehydration of substrate.
Galacturonic acid is more susceptible to degrade into furfural and its degradation products during hydrothermal treatment than the pentose sugars such as xylose (Usuki et al., 2008).

2.6.1.2 Shearing Processes

A shearing process involves applying mechanical energy to reduce particle size in materials and aiding particle dispersion. Redgwell et al (2008a) showed that shearing apple tissue using a laboratory Ultra-Turrax homogeniser at 24000 rpm for 1 min on apple tissue resulted in particles containing several contiguous cells. As shown in Figure 2.14 high shear treatment helped disperse the dense structure and aggregates of apple cell wall. The actual fabric of cell wall however remained intact (Redgwell et al., 2008a, 2008b). Breaking the particles into smaller units provides a larger surface area which can increase the viscosity of suspensions (Redgwell et al., 2008a).

Figure 2.14 Apple cell wall material suspension (3%) optical micrographs, before shearing (left) after shearing (right). Adapted from Redgwell et al. (2008a).

Espinosa-Muñoz et al (2013), found a bimodal particle size distribution (1000 µm and 200 µm) in the puree prepared by sieving (1.2mm sieve) and cooking at 98°C for 4 min. Shearing treatments on this puree could decrease the particle size distribution (cell clusters) to a major peak of 1000 µm in medium ground (at condition of 5000 rpm for 15 seconds) to one sharp peak at 200 µm in highly ground (at condition of 10000 rpm for 10 min). The highly ground sample also represents some individual cells (Figure 2.15).

Figure 2.15 Confocal laser scanning images (A), particle size distribution (B) of cooked apples at 98°C for 4 min to produce apple puree; From left to right: NP= natural puree seived through 1.2 mm, MG= medium grinded puree from shearing at 5000 rpm for 15 s, HG= highly grinded puree from grinding at 10000 rpm for 10 min. Adapted from Espinosa-Munoz et al. (2013).

Applying shear before thermal treatment was not as effective as applying it after heating (90°C for 40 min) to generate small particles with smooth edges (Lopez-Sanchez et al., 2011). This suggests that depolymerisation of pectin in the middle lamella (possibly through β-elimination at temperatures > 90 °C) reduces the cell adhesion and helps the shearing treatment to separate tissue cluster fragments. Lopez-Sanchez et al. (2012) were able to generate particle sizes of 68-319 μm with smooth edges by shearing cooked carrot using a kitchen blender. Particle size analysis indicates that the particles contained (on average) 1-5 cells.

The impact of shear is sensitive to temperature. Day et al. (2010a) found that heating carrots at temperatures < 90 °C and then shearing using a kitchen blender resulted in cell clusters (Figure 2.16a). These results suggested that heating temperature was not sufficient to separate cells through their middle lamella and therefore shearing broke up cell wall structure to random cell clusters (particle size 5-600 μm). However, applying the same shearing condition after heating at 100 °C resulted in cell separation and produced single cells with smooth surface and intact cell wall (Figure 2.16b). Doubling the shear time on these single cells caused rupture of intact cells to irregular small cell fragments without particular cell structure (Figure 2.16c).

Figure 2.16 Confocal laser scanning microscopy images of sheared carrot; A: cluster cells from sheared tissue after heating (80°C for 10 min), B: single cells from sheared tissue after heating (100°C for 30 min), C: cell fragments from double sheared single cell tissue. Adapted from Day et al. (2010a).

High shear treatment of apple cell wall could not change the alignment of the glucose chains in cellulose microfibrils (Redgwell et al., 2008b). Moreover, Agoda-Tandjawa et al. (2010) have reported that homogenisation treatment did not change the cellulose crystallinity of sugar beet cellulosic residue.

2.7 Enzymatic Modification

2.7.1 Kinetics of Enzymatic Reactions

Enzymatic reactions typically follow Michaelis-Menten kinetics. Reactions will start by mixing enzyme and substrate followed by incubation at a specific temperature and pH (Figure 2.17). At the beginning of this process, reactions followed zero-order kinetics due to the excess substrate for the enzyme. Measuring rate of the reaction at initial regime will be equal to the maximum rate (Figure 2.17). However, as the reaction progresses, substrate becomes the limiting factor in the mixture; reaction speed starts to decrease and reach a plateau, resulting in first order kinetics. The relevant equation 2.1 for a typical enzymatic reaction is given below.

$$\frac{dS}{dt} = -\frac{Vmax \times S}{Km + S}$$

Equation 2.1 Michaelis-Menten equation.

According to Michaelis-Menten equation at high concentration of substrate (K_m<< S) rate of reaction will be equal to the V_{max}. However, at the end of the reaction when concentration of substrate is very low (K_m>> S) rate of reaction will depend on V_{max} and K_m (Figure 2.17).

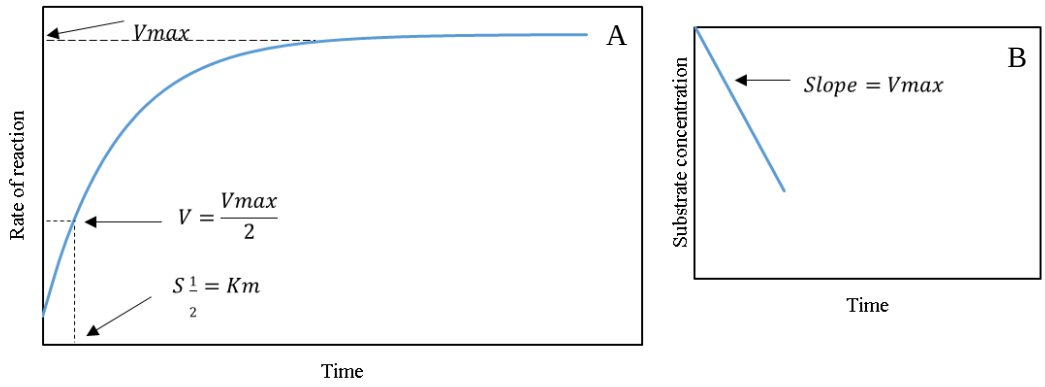


Figure 2.17 Schematic of the kinetics of a typical enzymatic reaction according to Michaelis-Menten. A) rate of reaction vs. time and B) substrate concentration vs. time.

Although Michaelis-Menten kinetic is a good fundamental equation in enzymology, it is not the only way to predict the enzyme's reaction. The response surface methodology with a central

composite design is an easy empirical way to model a reaction contains variables. In the central composite design, α is known to be the distance from the centre of the design to each axial point. The equation for calculating α is given below (equation 2.3). Two variable factors in central composite design will be measured α to be 1.41.

$$\alpha = \left(2^k\right)^{\frac{1}{4}}$$

Equation 2.2 Determining α, in central composite design. k=number of variables.

The 2 factorial design will contain 5 central points, 4-star points and 4 cube points as shown in Figure 2.18.

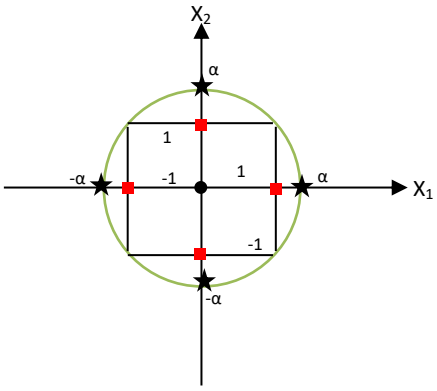


Figure 2.18 Diagram of central composite design generated for two factor variables. Central point (black circle), star points (black stars) and cube points (red squares).

For this modelling, 13 number of experiments were calculated using the equation 2.4.

$$N = 2^k + 2k + C_p$$

Equation 2.3 Determining number of experiments runs using response surface regression, where k is number of variables and C_p is the number of central points.

2.7.1.1 General Description of the Enzymes Attack Cell Wall

Pectinase and cellulase are the generic names for groups of enzymes that act on pectin and cellulose and other glycans. Industrial enzymes are usually of fungal origin. Plant also has many (but not all) of these cell wall modifying enzymes too.

Pectin Breakdown

The main pectin degrading enzymes (based on their action site) are given in Figure 2.19A.

Pectinmethylesterase (PME) removes the methyl groups on esterified galacturonic acid backbone and produces de-methylated pectin and methanol. De-methylated pectin will be sensitive to Ca^{+2} and may produce Ca^{+2} pectate gels (according to egg box model (Jarvis et al., 1995). PME is active in the range of pH 4-7.5. Plant PME de-methylated pectins in a more blockwise fashion (a processive mode of action), while fungal PME act more randomly and generate a random distribution of de-esterified GalUA. Pectin acetyl esterase (PAE), removes acetyl groups from pectin.

Endo/Exo polygalacturonase (endo-PG and exo-PG) can only hydrolyse pectin from un-esterified HG. This type of pectin is mainly present in middle lamella and hydrolysis can therefore reduce cell-to-cell adhesion (Figure 2.19B). Endo-PG hydrolyses the glycosidic bond at α-(1→4) of HG randomly, while exo-PG can attack the pectin from the non-reducing end only to produce GalUA monomers. PG enzymes require de-esterified pectin to act on and the activity of both enzymes increases the number of reducing end groups. A small amount of endo-PG activity can profoundly affect pectin viscosity.

Pectin lyases are able to break the glycosidic bonds of the pectin chain by a β-elimination reaction, producing a double bond (refer to 2.6.1.1). The activity of this enzyme is decreased as the DE decreases (Babbar et al., 2016; Bonnin et al., 2014; Sharma et al., 2017).

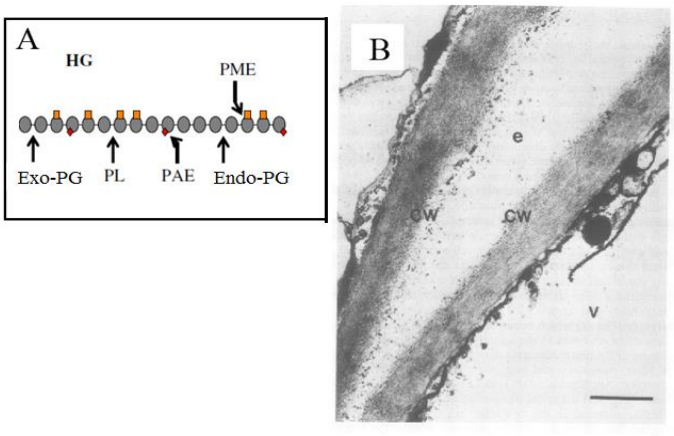


Figure 2.19 Main pectin degrading enzymes and effect of PG on apple cell wall: A) Endo-PG, Exo-PG, PL, pectin lyase; PME, pectin methyl esterase; PAE, pectin acetyl esterase. B) Endo and exo-PG (obtained from tomato) on apple tissue resulted in dissolving pectin in middle lamella (m) and partially separate adjacent cells, while fibrillar structure of cell wall (cw) remained unchanged. Adapted from Benarie et al. (1979) and Bonnin et al. (2014) with some modifications.

Cellulose and Hemicellulose Breakdown

Cellulases (such as endo-glucanase, exo-glucanase and β-glucosidase as a complex) are a group of enzymes that can degrade cellulose polymer chains into oligomers or even monomers if left to go to completion (Figure 2.20A). Endo-xylanase and β-xylosidase degrade xylans by attacking internal linkages or from the non-reducing end, respectively (Figure 2.20B). Some cellulolytic enzymes such as endo-glucanase can also hydrolyse XG as the backbone is similar to cellulose (Bonnin et al., 2014; Sharma et al., 2017). It has been suggested however, that in XG with a high amount of α-L-arabinose (1→2), this arabinose can block the endo-glucanase action and make the XG resistant to cellulase (Figure 2.20C) (Vincken et al., 1996; Watt et al., 1999).

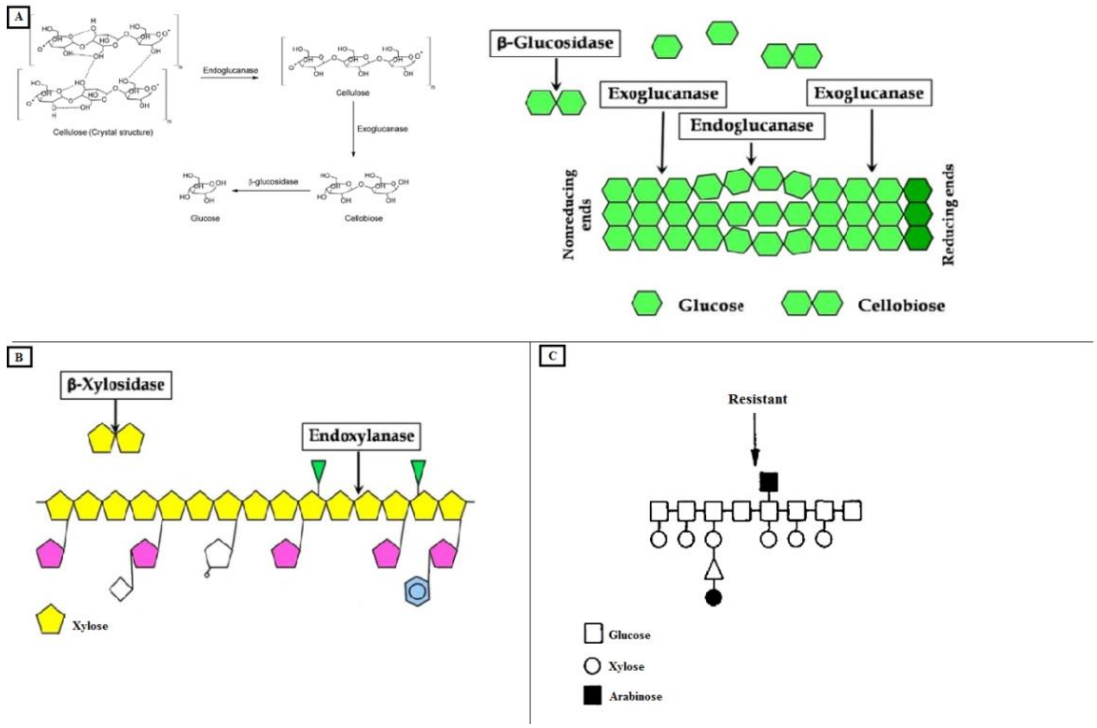


Figure 2.20 Cellulolytic and hemicellulolytic enzymes. A, cellulase enzymes and their site of action t; B, hemicellulase enzymes and their site of action; C, resistant XG fragment from degradation by endo-glucanase. Adapted from Vincken et al.(1996) and Ratanakhanokchai et al. (2013).

2.7.1.2 Use of Enzymes

Juicing

Enzyme preparations are used in the juice industry for increasing the juice yield and for clarifying or stabilising the extracted juice.

Increasing the juice yield can be achieved by pectinases and/or cellulases, the effectiveness of which depends on the type of raw material. Some of the commercially available cell wall-degrading enzyme preparations are described in Table 2.2.

Enzymatic maceration

Enzymatic maceration uses commercial pectinase enzymes on crushed fruit to extract juice, releasing aroma/colour and facilitating the compression step. These enzymes usually contain high amounts of PG, PME, PL and RG-I to solubilise pectin, especially from the middle lamella to break down cell-to-cell adhesion. Commercial pectinase enzymes are commonly isolated from *Aspergillus niger* and other *Aspergillus* species (Table 2.2).

Enzymatic liquefaction

Degrading and liquefying fruit cell walls with a wide spectrum of cellulases, hemicellulases and some pectinases is called enzymatic liquefaction. High amounts of cell wall-degrading enzymes as well as a synergistic effect between pectinase and cellulase enzymes leads to extreme degradation of the mash cell wall and release of the cellular contents. There are also some problems involved in the liquefaction process with high enzyme doses in these preparations including solubilising high amount of polyphenol (which affect colour of the juice).

A range of pectinase enzymes are also used on extracted juice in order to clarify the juice (removing turbidity by solubilizing pectin in juice), to improve the sedimentation speed of small particles and to stabilise pectin in cloudy juices (by using PME) (Bonnin et al., 2014; Demir, 2001; Kashyap et al., 2001; Will et al., 2000)

Table 2.2 Commercial plant cell wall enzymes

Cell wall degrading enzymes	**Enzymes preparations**	**Supplier**	**Type of saccharolytic activities**						**References**
			Cellulase	Hemicellulase (includes xylosidase)	PG	Rhamnogalacturonase	Arabinan and galactan hydrolase	Protease	
Pectinases	Pectinex	Novozymes & Sigma Aldrich	Low	Low	High	High	High	~0	(Combo et al., 2012; Panouille et al., 2006)
	Rapidase	DSM	Medium	High	High	High	Medium	Minor	(Zykwinska et al., 2008a)
	Viscozyme L	Sigma Aldrich	nd	nd	High	nd	nd	nd	(Combo et al., 2012)
	Macer8	Biocatalysts	nd	nd	High	nd	nd	nd	(Combo et al., 2012)
Cellulases	Cellulyve	Lyven	High	Medium	Low	Minor	Minor	~0	(Panouille et al., 2006; Zykwinska et al., 2008b)
	Celluclast	Novozymes & Sigma Aldrich	High	High	Low	Medium	~0	~0	(Panouille et al., 2006; Wikiera, et al., 2015a; 2015b)
	Maxazyme	DSM	Medium	Medium	~0	~0	~0	~0	(Panouille et al., 2006)
	Laminex	DuPont Industrial Biosciences	High	nd	Minor	Minor	nd	nd	(Dominiak et al., 2014)
	Validase	DSM	High	nd	Minor	Minor	nd	nd	(Dominiak et al., 2014)
	Multifect	DuPont Industrial Biosciences	High	nd	Minor	Minor	nd	nd	(Dominiak et al., 2014)
	Viscoferm	Novozymes	Medium	High	Low	~0	Medium	nd	(Wikiera et al., 2015a)
Proteinases	Neutrase	Novozymes	~0	~0	~0	Minor	Low	Medium	(Panouille et al., 2006; Zykwinska et al., 2008b)
	Papain	DSM	Minor	~0	Minor	Minor	~0	Low	(Panouille et al., 2006)
	Promod	Biocatalysts	~0	~0	Minor	Minor	Low	High	(Zykwinska et al., 2008a)

nd= not determined

Extracting Polysaccharides

Commercial juicing-aid enzymes (pectinases and cellulases) have been increasingly used on fruit pomace in order to extract pectin (so-called "green pectin") and other cell wall polysaccharides. Use of enzymes can reduce or eliminate the hazardous chemical substances normally used for pectin extraction. Effective use of cell wall degrading enzymes for pectin extraction is based on the assumption that degrading (or loosening) the whole cell wall by a range of enzymes in combination helps to facilitate pectin release from the cell wall network (Zykwinska et al., 2008a). Moreover, enzymes are usually applied at lower temperature and weaker acid conditions than conventional extraction methods. This can also help to decrease the corrosion of equipment as well as the amount of wastewater (Adetunji et al., 2017). The efficiency of enzyme-extracted pectin production strongly depends on the selection and dosage of the enzymes and the time, temperature and pH (Adetunji et al., 2017; Babbar et al., 2016). The effect of enzymatic preparations also related to the tissue type and their cell wall composition (Zykwinska et al., 2008a).

Soluble cell wall polysaccharides (soluble dietary fibre, SDF) are widely used in food industry for providing better texture in low-calorie foods and have valuable human health benefits. Cellulolytic or pectinolytic enzymes can be used in extracting these soluble polysaccharides by disrupting the linkages in cell wall and releasing soluble polysaccharides in the water medium. It has been suggested by Li et al (2014) that cellulase enzymes can extract higher soluble polysaccharides than chemical methods. The maximum amount of SDF (18.7% basis on dry pomace weight) obtained from dried apple pomace powder was achieved by cellulase treatment at 50°C for 1 h (pH 4.5) while the acid treatment (sulfuric acid at pH 2) for 4 h at 80°C resulted in 10.3% of SDF. These observations are in a good agreement with other reports (Naghshineh et al., 2013; Panouille et al., 2006; Zykwinska et al., 2008a). Pectinolytic enzymes have also been used to extract pectin from plant material. These enzymes release more pectin as oligomers than polymers in comparison to cellulase enzymes and conventional extraction. For example, Min et al. (2011) showed that extracting pectin from apple pomace (previously heat-treated at 121°C for 10 min) by commercial pectinase at 40°C for 1 h resulted in slightly lower amounts of GalUA than when using oxalic acid/ammonium oxalate at pH 4.6.

Commercial pectinases are a mixture of fungal pectinases with low cellulase activity (usually detectable in the mixture). The synergistic effect between pectinase and cellulase has been reported and results in solubilising the neutral sugar side chains of pectin during extraction. Min et al. (2011) and Panouille et al. (2006) have both reported that the pectin extracted by commercial pectinase had retained higher amount of sugar side chains of pectin (such as arabinose, galactose,

rhamnose and xylose) than acid extraction. Moreover, this synergistic effect of cellulase and pectinase can greatly affect the amount of glucose and solubilise pectin as small oligo-and monomers (Panouille et al., 2006).

The type of enzymatic extraction process can strongly influence the quality of pectin produced. These parameters include the combination of enzymes, dosage of the enzyme preparations, pH, process time, temperature and solvent:biomass ratio (Adetunji et al., 2017; Babbar et al., 2016; Wikiera et al., 2015). High concentrations of pectinolytic enzymes, with their ability to hydrolyse pectin side chains result in decreased pectin yield, producing more pectin oligomers and monomers than polymers as well as contaminating the pectin with sugars from hydrolysed pectin side chains (Babbar et al., 2016; Li et al., 2014). Low concentration of enzymes also can result in low yield of pectin extraction (Wikiera et al., 2015). Therefore, it is important to optimise the process parameters to produce the pectin with expected characteristics.

Optimisation of the parameters for enzymatic process has been investigated. According to the intended final pectin product, different conditions need to be chosen. For example, Zykwinska et al., (2008a) examined different combinations of commercial cell wall degrading enzymes to extract long-chain pectin. They reported that a combination of protease and purified cellulase on chicory root at 50°C for 4 h could solubilise the maximum amount of pectin (42% of original dry cell wall material) and arabinose (43% of dry cell wall material) in water. The majority of the GalUA extracted was present as polymers while only a minor part (1-5%) was present as oligomers and monomers. The analysis of the total arabinose content in solution showed near to half of total extracted arabinose was released in polymer form. Moreover, a 10-fold dilution of the enzyme mixture had only a minor effect on the amount of pectin and arabinan extracted. Similar results were reported by Panoullie et al. (2006) who extracted pectin using a combination of cellulase and protease (Cellulyve and Papain) on chicory root for 16 h at 40 °C. This combination of commercial enzymes solubilised more than 70% of the pectin present and much of this was released as polysaccharide.

Production of pectin oligosaccharides by enzymatic or fermentation treatments is gaining attention due to the specific biological activities of pectic oligomers such as reducing blood glucose level, their prebiotic potential to increase the number of beneficial bacteria in human gastrointestinal tract, prevention of lipid accumulation in the liver, decreasing the incidence of colon cancer and protecting the immune system (Gullon et al., 2013). According to Combo et al. (2012) who used different commercial pectinase (Endo-PG-M2, Viscozyme L and Pectinex) for producing oligosaccharides from LM citrus pectin (~6.7%), efficiency of the oligosaccharide production strongly depended on enzyme preparation and time of the reaction. Endo-PG-M2 could release the highest amount of oligo-GalUA as dimers and trimers (at 40°C and pH 5.5) even

after 2 h reaction time (~76% of total oligo-GalUA solubilised) than Viscosyme L and Pectinex. Production of more oligo-GalUA might be related to the random cleavage mechanism of endo-PG (in Endo-PG-M2), while exo-PG would produce solely mono-GalUA.

Prolonged pectinase treatment and increasing the monosaccharide production from plant material has also been reported by Leijdekkers et al. (2013). Using commercial pectinases at 45°C from sugar beet pulp showed a slight increase in monosaccharide level by increasing the time of reaction from 6 to 48 h. During this time the amount of soluble oligomeric and polymeric GalUA slightly increased, while the amount of arabinan released decreased. This is likely to be due to further degradation of cell wall components to monomers.

The pH is another process parameter which is usually optimised in order to extract the maximum pectin content with particular properties. Dominiak et al. (2014) trialled four different commercial cellulase preparations on lime peel at four different pH conditions (pH 3-4.8) and showed that at pH 4.8 all enzyme preparations were very effective to extract high pectin yield (~24-32% basis on dry matter) with the DE > 78%. The viscosity-average molecular weight of extracted pectins solutions at pH 4.8 was lower than other pH conditions.

Other parameters of the process such as size of the plant particles at the start and solid: liquid ratio are important for optimising pectin extraction from plant material. For example, Wikiera et al. (2015b) found that enzymatic treatment of larger particles (4.76mm) resulted in higher pectin release than fine particles (0.42mm). Increasing the amount of water in the reaction (up to 15 ml/g dry pomace) also improved the pectin release by 10%.

Enzyme effects on Particle Properties

Particle size appears to be affected by enzyme treatment. Pickardt et al. (2004) reported that the average particle sizes of fresh homogenised carrot tissue after commercial pectinase and commercial cellulase treatments were 121 and 24 μm, respectively. After drying, materials treated with pectinases were slightly deformed and aggregated. Particles in the samples treated with cellulases however, were highly aggregated and these aggregates could not be dispersed when water was added. These findings were in agreement with Vetter et al. (2002) who also showed that particles in fresh cellulase added materials were smaller than pectinase added materials (50-250um and 5-50 μm, respectively). Small cell fragments in material treated with cellulase aggregated more during drying than those treated with pectinase (>500 μm and 80-500 μm, respectively). This means that the type and mixture of enzymes used to break down the plant materials can affect not only the amount and the size of pectin solubilised but also the size of the insoluble particles remaining.

Pectin and cellulose degradation of plant material can result in different polysaccharide breakdown products that might be used as functional food ingredients. When treating fruit tissues with commercial pectinases whole cells were achieved (Kabbert et al., 1997, Forster et al., 2002 and Vetter & Kunzek, 2002), but digestion of cellulose with a mixture of commercial cellulases and pectinases produced cell wall fragments (Forster et al., 2002; Pickardt et al., 2004; Vetter et al., 2002). The amount of GalUA in samples treated with commercial pectinase was more than four times higher than with commercial cellulase. However, the treated sample with commercial cellulase resulted in 1.5 times more glucose content than sample treated by commercial pectinase (Forster et al., 2002; Vetter et al., 2002). The DE of pectin in cell wall material after enzymatic treatment by commercial cellulase was 8.7% while in material after commercial pectinase action was 43% (Pickardt et al., 2004).

2.8 Physicochemical Properties of the Enzymatic and Non-enzymatic Treatments on Pomace

Physicochemical properties of material include WHC, swelling, gelling, viscosity properties and stability of dispersions from phase separation. These qualities which are also related to the hydration properties of materials and can prevent syneresis, improve texture and shelf life of food products (Li et al., 2014). Enzymatic and non-enzymatic treatments have already been reported on plant tissues (refer to Sections 2.4.1.1, 2.4.1.2 and 2.4.2). These treatments can greatly affect the hydration properties of plant material.

Water holding capacity is the ability of material to take up water (through capillary suction) and swell. This is one of the most influential properties of modified pomace type material that are dried and then rehydrated. Cell walls extracted from fruit and vegetables are known to have good WHC and more acceptable flavour and taste in comparison to cell wall material extracted from cereal brans and some plant roots (Redgwell et al., 2005). The WHC of isolated plant cell wall material depends on its composition (quantity and type of polysaccharides present), microstructure of particles (pore size and surface area), conditions applied during rehydration (temperature and mechanical stress) and the prior treatments (non-enzymatic or enzymatic treatments) that plant tissue has received (Redgwell et al., 2008b; Reiter, 2002).

<u>After non-enzymatic treatments:</u>

Some research has been published on the effect of chemical modification of plant cell wall components (such as de-esterification with monovalent and divalent cations) on rehydration properties of plant material. Vetter et al. (2003a; 2003b) have investigated these modifications

on hydration properties of apple cell wall. They have shown that the cell wall containing low methoxyl pectin that has been treated with potassium ions before solvent drying (from ethanol) resulted in increased WHC and swelling properties. It has been suggested that charging the de-methylated pectin with potassium ions resulted in forming the potassium carboxylate structures (salification process). The carboxylate ions later prevent the hydrogen binding between pectin chains (by generating higher repulsive forces on surface of cell walls) and help them to stabilise and reduce their shrinkage during drying. High hydrophilic properties of potassium carboxylate on cell wall material resulted in highest hydration properties during the rehydration process (~80% ml water/g dry matter) (Vetter et al., 2003a; 2003b). These findings were in good agreement with Godeck et al. (2001) and Kunzek et al. (2002) who charged the apple low methoxyl pectin cell wall materials with sodium ions prior to drying, which showed the maximum hydration properties (during rehydration process). The WHC of low methoxyl pectin material that was charged with Na^{+} was higher than 70% (ml water /g dry matter) in both papers. Treating cell wall material with Ca^{+2} before drying leads to an irreversible rehydration state. This might be due to free generating ionic calcium pectate bonds between carboxyl groups in low methoxyl pectin and Ca^{+2} (Kunzek et al., 2002).

Solvent drying of plant material (such as with ethanol) prior to air drying has a pronounced effect on rehydration (Godeck et al., 2001). According to Vetter et al. (2003a) water: ethanol exchange of apple cell wall before air drying helped to increase the porosity of cell wall materials and protect the integrity of cell wall (Figure 2.21b) during drying. The rate of absorbing water and re-forming original size upon rehydration increased (Figure 2.21c).

A **B** **C**

Figure 2.21 Light microscopy pictures of the apple cell wall materials. Fresh (before drying) (A), dried (B), rehydrated with water (C). Adapted from Vetter et al. (2003a).

Mechanical size reduction of dried cell wall particles also can affect their hydration properties and their ability to form a strong gel network. For example, milling of dried pomegranate peel to particle size <0.5 mm showed a stronger gel network upon rehydration with added calcium than the gel structure of the materials which contained cell wall particles >0.5 mm (Abid et al., 2017).

This was suggested to be due to higher hydration properties of fine particles with increased surface area.

In order to produce the cell wall material with high hydration properties, the condition of rehydration process is as important as the condition of plant treatments and the drying step. Rehydration of apple cell wall material in 0.1M potassium acetate solution resulted in better water uptake than rehydration in 0.1M malic acid solution (Vetter et al., 2003a). Redgwell et al. (2008a) showed that applying moderate shear (Ultra-Turrax 9000 rpm, 5s) during the rehydration of plant cell wall materials (apple, kiwifruit and tomato) produced stable, high viscosity suspensions of particles which did not settle. This might be due to increasing the chance of particles to absorb water. In addition, the effects of NaCl solutions at different concentrations (0 to 10%) on these colloidal suspensions were also examined. Increasing the concentration of NaCl resulted in a decrease in the viscosity and an increase in the sedimentation of particles in dispersion.

The hydration properties and producing a stable dispersion of plant cell wall can be explained with Derjaguin, Landau, Vervey, and Overbeek (DLVO) theory. DLVO developed a theory about stability of the colloidal systems and their aggregation. According to this theory, interaction forces can be well approximated by van der Waals forces (assume to be always present in solution/medium) and double layer interactions. Van der Waals forces resulting from rotating and fluctuating of dipoles in atoms and molecules in solution. Double layer interaction related to the surface charge of the substrates presents in suspension (Figure 2.22). Two particles which started to get close to each other (maybe through the van der Waals forces between the solution and particles) will eventually repel each other by the double electrical layer on surface of particles. The strength of this repulsion depends on the zeta potential. Zeta potential (or thickness of double electrical layer) is known as the potential difference between the charges of the particles and amount of opposite charges which are available in the solution. At high zeta potential (lower amount of charge in medium and high surface charge of particles) a suspension stays stable and particles will not aggregate, while by increasing the charge of solution (for example higher concentration of salt in solution or more opposite charges in comparison to charge of particles) the particles will aggregate and settle (Redgwell et al., 2008a; Trefalt et al., 2014).

Figure 2.22 Electrical double layer effect on viscosity of cell wall materials and stability of dispersions. Adapted from Redgwell at al. (2008a).

After enzymatic treatments:

Alterations to the level of inter- and intramolecular interactions after any non-enzymatic or enzymatic treatments can affect the hydration properties and viscosity of the resulting material. Moreover, enzymatic treatment which can cause severe degradation of cell wall can result in increased interactions between particles and consequent loss of molecular mobility (Kotcharian et al., 2004). Rehydration of plant material which has been treated with commercial pectinase (macerated material) showed higher WHC than ones affected by commercial cellulase (liquefied material) due to higher amount of single cells in macerated materials and, more cell collapse in liquefied plant material which has negative effect on its water uptake properties (Kotcharian et al., 2004). Micrographs, particle size distribution and porosity analysis also show that the surface of macerated plant material particles was smoother, with more porosity and similar particle size distribution between fresh and rehydrated samples. Liquefied material however, contained much lower GalUA and great amount of cell fragment aggregation which make liquefied material unfavourable material to absorb water and swell (Kotcharian et al., 2004; Vetter et al., 2001).

The type of the plant cell wall as well as treatment condition can affect rehydration properties of dried cell wall material. For example, applying Celluclast on apple tissue reduced WHC and

subsequently the apparent viscosity of the suspension due to high reduction of cellulose content in the plant material (Redgwell et al., 2008b). On the other hand, WHC of sugar beet pulp after high temperature steam treatment (resulting in a great reduction of pectin content) was still about 50% greater than the untreated sugar beet pulp (Kuhnel et al., 2011; Reiter, 2002).

All in all, the shape, size and interactions of the particles in fresh and rehydrated dispersions as well as concentration of soluble cell wall polysaccharides influences the WHC, final viscosity of the dispersion and sedimentation rate of the particles (Appelqvist et al., 2015; Redgwell et al., 2011).

2.9 Sensory Properties of Treated Plant Cell Wall Suspensions

Sensory evaluation in the food industry is a combination of the fields of psychology, statistics, and human biology and is used to determine the acceptability, similarity or differences between sets of samples from people's point of view. Any changes in food products can be a highly risky venture for the industry. Sensory evaluation can provide valuable information on a product with lower cost and without changing the current process line. The food industry usually uses sensory tests for product development, improving or reformulating a current sample, quality assurance of a product, as well as investigating the effects of changing production equipment or ingredients in the current process (O'Mahony & Rousseaub, 2003). These tests determine if the new idea is developed enough to launch on an industrial scale. In food production different attributes such as colour, taste, texture, flavour and overall acceptance of a product can be evaluated. Texture analysis can be investigated using panellists or measuring instruments such as texture analyser and rheometer which measure physical properties related to texture perception. However, the instrumental techniques do not give a clear idea of how a food product is actually sensed in the mouth with human receptors (Chen et al., 2013).

Sensory testing in the food area can generally be divided into three main categories; affective, descriptive and discrimination tests. The affective test is mostly about the acceptance or preference by the consumers for a product. The descriptive test measures the intensity differences of one or more attributes in the selected products. This test usually needs trained panellists to be able to indicate the presence of the target attribute in the product as well as measuring its intensity. The discrimination test determines whether there are considerable differences between samples or not on an attribute. Selecting an appropriate evaluation protocol depends on the aim of the project and is very important to extract any meaningful and applicable results (McClure, 2008).

The discrimination test is mostly conducted when the difference between samples is not easy to detect by the judges (O'Mahony & Rousseaub, 2003). A data analysis method which is normally used for this test is Thurstonian Modelling. This statistical analysis is already accepted in sensory science and provides a value called δ or d′ as a measure of the sensory differences between two samples. This model is based on two assumptions: 1- Perception of a target component in the food product has a normal distribution as it is more likely to have different homogeneity within the sample, and 2- Judges are dedicated to execute the decision rule of the sensory test (O'Mahony & Rousseaub, 2003).

With this analysis two products have obviously different sensory magnitudes when their normal distributions do not have any overlapping area (Figure 2.23A), while two similar samples will have an overlap area (Figure 2.23B). The distance between the average of normal distributions of two products is d′. This value provides information on the discriminability of consumers to understand the differences between products (Bi et al., 2010). If d′ is small (d′< 0.8), it means that samples were more similar and if d' is large (d′> 0.8) two products are easy to distinguish as different samples (Lee et al., 2004; McClure, 2008; Morten et al., 2007; O'Mahony & Rousseau, 2003).

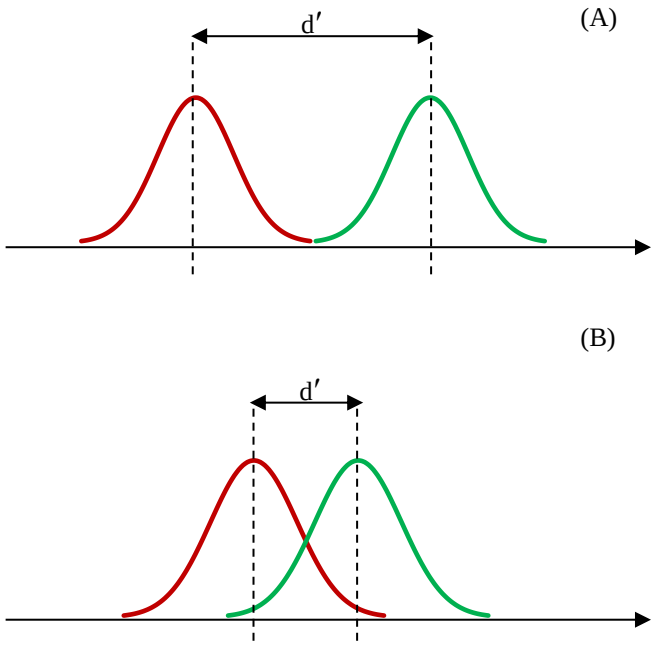


Figure 2.23 Thurstonian analysis for a food product with one stimulus (eg. Sweet and sweeter). A) Easy distinguishable samples and B) confusable samples to distinguish, where degree of differences between samples is shown as d′.

The human sensory system for perceiving mouthfeel of a food product depends on the mechanoreceptors in the mouth which includes the hard and soft palate, tongue, gums, teeth (and

the area surrounded by teeth) and the system of muscles/tendons for chewing food. Mouthfeel or texturising properties of food products (solid, semi-solid and liquid) refer to all senses that are perceived from the time that a food is placed in the mouth until it is swallowed (Guinard et al., 1996). Mouthfeel properties of semi-solid food products, such as thickness, graininess, stickiness, and creaminess are known as textural mouthfeel. The residual mouthfeel after swallowing (afterfeel) such as mouthcoating and throat-catching can also be included in sensory tests of semi-solid food products. Sensory testing of a semi-solid food product can be investigated by test panels (trained or native) (Appelqvist et al., 2015; Guinard et al., 1996). The definition of some sensory attributes is given in Table 2.3.

Table 2.3 Definition of some sensory attributes used for the evaluation of texture and mouthfeel of fruit dispersions. Adapted from Colin-Henrion et al., (2009); Espinosa-Munoz et al., (2012) and Appelqvist et al., (2015).

Attribute	Definition
Texture	
Thickness	Perception of the sample's thickness in mouth. Evaluated by pressing the sample between tongue and the palate.
Stickiness	Adhesion of the product to palate. Evaluated by unsticking the tongue from palate several times.
Grainy	Perception the amount of solid particles in mouth. Evaluated by pressing the sample between palate and the tongue and sweeping the tongue to the palate.
Creaminess	Perception of velvety and smooth consistency of the sample. Evaluated by moving the tongue parallel to the palate in circles.
Cohesiveness	The degree that a mass of sample holds together. Evaluated by pressing the sample between palate and tongue. Low cohesive sample is the one which two distinct phases (particles and liquid) are felt in mouth.
Afterfeel	
Mouthcoating	Smooth and creamy perception on surface of the mouth and throat. Evaluated after swallowing.
Throat-catching	Feeling of throat-catching because of the remained particles in mouth and throat. Evaluated after swallowing.

Nowadays, consumer demands for high fibre food products are increasing and these products are not attractive if they cannot provide acceptable mouthfeel and consistency (Bengtsson et al., 2011). There are also some limitations in plant fibre applications in food products due to sensorial and technological constraints (Redgwell et al., 2005). In semi-liquid or semi-solid foods chewing is not required but a force is required to initiate the flow associated with swallowing. This force is generated by contact of the food between palate and tongue followed by squeezing to making the material ready to swallow. Sensory properties of semi-solid foods depend on concentration, shape and size of dispersed particles, their interactions with themselves, and matrix (before

putting a sample in mouth), viscosity of matrix as well as the particles and matrix interactions with mechanoreceptors (especially palate and tongue) and saliva in mouth (Appelqvist et al., 2015; Kunzek et al., 2002).

Mouthfeel properties of plant food suspensions (purees) have been investigated in some publications (Colin-Henrion et al., 2009; Bengtsson et al., 2011; Espinosa-Muñoz et al., 2012: Appelqvist et al., 2015). Graininess and smooth perception are highly related to the particle size distribution. Espinosa-Munoz et al. (2012) reported on sensory evaluations done using 14 trained panellists (4 men and 10 women). A descriptive sensory method was used to rate the intensity of visual appearance and mouthfeel properties of 12 apple puree products with different particle sizes, serum phase viscosity and total solids contents. They decreased the particle size of apple puree with a lab blender (tip speed of ~31 m/s for 15 s and 62 m/s for 3 min) from 1000 μm to 500 μm and 230 μm. These smaller particle sizes contained 90% of the particles in the puree and helped to decrease the perception of graininess of the sample. These results were in general agreement with Appelqvist et al. (2015) who decreased the average particle size of carrot puree from 225 μm to 92 μm and 56 μm by different homogenisation conditions (using a kitchen blender at 7000 rpm for 8 min or 3 passes through a high-pressure homogeniser at 60 MPa). In this study, 9 trained judges assessed 10 carrot dispersions by determining the intensity of 6 textural attributes and 2 afterfeel attributes on an unstructured line scale labelled from low to high. The carrot dispersions with average particle size of 92 μm had the least graininess and greatest creaminess among test panellists.

Creaminess perception seems to be a universal sensation and does not change by training the test panels or using untrained consumers. Creaminess (also known as multimodal perception) is a combination of texture (smooth and velvety) and taste/flavour (De Wijk et al., 2006). It is correlated with particle size distribution, surface properties (particularly the smooth surface of particles) and viscosity of the dispersal medium. Regarding the shape and surface of particles on mouthfeel, Appelqvist et al. (2015) showed that producing smoother carrot suspensions (through the presence of more single cells) than very small sub-cellular fragments could help in not feeling the separate particles in the mouth and provide a better and smoother mouthfeel than small cell fragments. These results were in good agreement with Bengtsson et al. (2011) who decreased the size of insoluble particles of different fruit and vegetable suspensions by homogenising them in a kitchen blender or in a high-pressure homogeniser. This sensory test was also carried out on 9 trained panellists using a descriptive method.

It has been shown that in apple suspensions created by high pressure homogenisation, the average particle size distribution was ~1.5 times smaller than suspension made in a kitchen blender. This greatly decreased the graininess perception of the apple suspension. In contrast, high pressure

homogenised suspensions of carrot were still perceived to be grainier than apple suspensions. In addition, micrographs of apple and carrot suspensions show that high pressure homogenised apple suspensions contains more single swollen cells, whereas carrot suspensions (even after high pressure homogenisation) consisted of large cell clusters (Bengtsson & Tornberg, 2011).

Viscosity of the surrounding matrix can also affect the sensory properties of homogenised plant materials. For example, Espinosa-Munoz et al. (2012) and Appelqvist et al. (2015) showed that by increasing the viscosity of the surrounding medium through the addition of pectin or xanthan solutions created a perception of less particles and more consistency and smoothness (creaminess) in plant dispersions even when the particles in these dispersions were large. This is likely to be due to the lubricant role of the soluble polymers that help to cover the rough edges and surfaces of the particles. Increasing the viscosity of the surrounding medium could also help to provide better afterfeel perception of the dispersions by slipping the particles from the mouth without sticking to throat surfaces (Espinosa-Muñoz et al., 2012; Appelqvist et al., 2015).

2.10 Conclusions

Over the years many researchers have applied different processing methods to recover and make valuable products out of fruit and vegetable wastes. However, most of these proposed methods were focused on extracting one component such as pectin, polyphenols, pigments or bio-ethanol from these by-products. These approaches consequently left behind most of the cell wall components representing a huge loss of biomass. Other processes which have been developed to incorporate them in food products (such as drying and blending into food) do not usually meet the acceptability criteria by consumers or have only been used in small quantities compared to the huge amounts of plant wastes. In addition, fruit and vegetable wastes have been considered to be grainy or sharp in the mouth when consumed. Until now little attention has been given to developing a process to convert these waste streams directly into a profitable food product or ingredient with acceptable mouthfeel properties.

The proposed process steps focused on in this thesis were heating, shearing and enzymatic treatments to modify the cell wall structure, producing apple pomace-based ingredients. Heating fruit and vegetable pomace promoted several reactions which took place simultaneously. Kinetics of pectin solubilisation in fruit pomace during heating have been studied before, however they have been a limited focus on other chemical reactions which are taking place at the same time such as depolymerisation reactions or degradation of sugars. Moreover, kinetics of reactions such as degradation of pectin (through acid hydrolysis or β-elimination) and sugars (such as sucrose, glucose and fructose) were mostly studied on the pure components.

The use of pectinase and cellulase on fruit and vegetable pomace in literature has mainly been focused on extracting cell wall components such as pectin, hemicellulose or studying cellulose in the plant cell wall. The enzymatic hydrolysis of these polysaccharides in a more complex matrix of soluble cell wall components has also been studied but, accurately predicting the enzymatic hydrolysis kinetics in these systems is challenging and not often reported.

Although the effect of particle size distribution and the addition of pure pectin on sensorial properties have been studied, to the best of our knowledge, no study has been done on modifying the soluble and insoluble parts of whole apple pomace sample and using sensory evaluation to prove their effects. In addition, using a discrimination test in combined with Thurstonian Modelling has rarely been used for conducting and analysing the sensory results when textural properties of a food product are investigated.

Considering the gaps in each part explained above, this opportunity arises to study the kinetics of the main reactions involved in pomace while heating. Modelling the kinetics of main reactions to predict the expected amounts of each reaction under more complex heating situation for example pilot scale retort. This can govern and prove the predictability of hydrothermal treatment for industrial purposes. Controlled modification of particle size distribution and modelling the enzymatic hydrolysis of solubilised pectin is also useful in proving the effects of each modification on sensorial properties of pomace ingredient from graininess and smoothness point of view.

Developing a simple reliable process with the proved scale-up, and delivering acceptable mouthfeel properties for consumers, can provide a way for recovering fruit and vegetable pomace into profitable food ingredients. This can also help managing plant wastes to protect our environment.

CHAPTER 3 **Effects of Sample Preparation**

3.1 Introduction

Pomace mostly contains cell wall components (skin and flesh) of apple fruit (~95%) (Bhushan et al., 2008; Tiwari et al., 2013). Fruit and vegetable pomace contain different polymeric components such as pectin, cellulose and hemicellulose which interact with each other in a cell network called "tethering network" (Zykwinska et al., 2007; Zykwinska et al., 2008a; Scheller et al., 2010; Dick-Perez et al., 2011; Park et al., 2012; Peaucelle et al., 2012). Any physical, chemical and hydrothermal treatments may affect the arrangement of this network resulting in opening the structure, changing the porosity, size and shape of cell wall materials and dissolving soluble polymers such as pectin (Kunzek et al., 2002).

Therefore, a set of preliminary experiments were undertaken in this chapter in order to understand the effects of sample preparation on pectin solubilisation. In order to understand the effect of water addition to fresh pomace in combination with temperature on the amount of solubilised pectin, a set of experiments was also carried out. Moreover, the effect of particle size on solubilisation of pectin from cell walls was also investigated.

3.1.1 Material and Methods

3.1.2 Plant Materials

Apple pomace was obtained from Turners & Growers Apple Juice Concentrate Plant (Hasting, New Zealand) for this trial. This pomace collected was from one variety of apple (Pink Lady) and did not contain added exogenous pectinolytic enzymes which are sometimes added during juicing to enhance juice yield. Pomace which came off the production line was collected into 100 g bags and kept frozen at -18 °C until next use. Pomace was thawed overnight at 1°C before use.

3.1.3 Shearing

A Kenwood kitchen hand blender (model HB714, Tokyo, Japan) with a blade diameter of 0.05 m operating at 15200 rpm was used to homogenise the pomace sample. The tip speed of the homogeniser blade was calculated as 39.8 m/s using the related equation 3.1 from (Valentas et al., 1990):

$$tip\ speed = \pi DN$$

Equation 3.1 Tip speed equation

where D= diameter of the blade and N=rotational speed.

3.1.4 pH

The pH of samples was measured before and after heat treatment. Cooled pomace (20 g) was homogenised with water using a kitchen blender (Section 3.1.3) at room temperature of 20 °C (RT) for 1 min and pH was recorded using an electrode attached to a pH meter (CyberScan pH 510, Thermo Scientific Eutech, USA).

3.1.5 Dry Matter

Dry matter of wet insoluble pomace materials was determined by drying at 70 °C using a Contherm BIOCELL 1000 incubator (Scientific Ltd, Auckland, New Zealand) at atmospheric pressure until a constant dry weight was achieved (~ 4 days).

3.1.6 Soluble Solids

Soluble solids were estimated using a hand-held refractometer (ATAGO, Saitama, Japan). Samples were measured at RT (20 °C). Samples were centrifuged at 20 °C and 14170 g prior to measurement. Duplicate readings were taken, and the results were recorded as °Brix. °Brix is a measure of the low molecular weight solute content of an aqueous solution (standardised on sucrose content).

3.1.7 Pectin Analysis

In this study, different measurements were done on different portions of dried or heat-treated pomace. Figure 3.1 below gives a description of each portion. A) pellet which contains insoluble polymeric components with some occluded supernatant, B) Supernatant (SN) is the

serum phase remaining after centrifugation. C) Ethanol (EtOH) -insoluble pectin are the components which can precipitate in 80% EtOH and D) EtOH-soluble galacturonic acid (GalUA) is the soluble components which remain soluble in 80% EtOH. All measurements were represented per g of dry pomace.

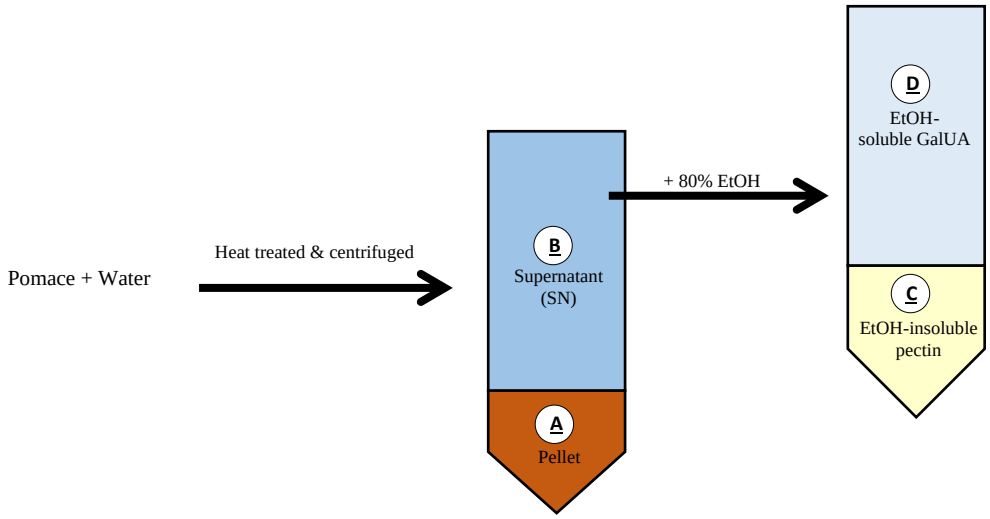


Figure 3.1 Schematic overview of apple pomace portions separated for different analysis measurements.

Sugars may interfere with GalUA determination through developing a dark colour background. Supernatant was precipitated with four volumes of ethanol (99%), then two more washes were done on the recovered EtOH-insoluble pectin using 80% ethanol. EtOH-insoluble pectin was separated from the EtOH-soluble portion by centrifugation using an Eppendorf bench top centrifuge (5430R, Eppendorf, Hamburg, Germany). The EtOH-insoluble pectin was re-dissolved in the original volume of water. In some circumstances, the EtOH-soluble GalUA (Fig 3.1, D), was used for further analysis (e.g., Chapter 4, Section 4.2.4.1).

When assaying dry pomace/pellet, small quantities (~ 100 mg) were washed with 1 mL EtOH (80%). The EtOH-washed pellet was washed eight times with 80% EtOH, then air dried. The number of times the samples were washed was determined by testing the soluble sugars remaining in the EtOH, until no sugar was detected. For this, the total carbohydrate method of Dubois et al. (1956) was used with some modifications to fit a microplate (O'Donoghue et al., 2017).

3.1.7.1 Total Pectin Hydrolysis

Total pectin in dry samples was hydrolysed using the method of Ahmed & Labavitch (1978), scaled down to fit a microplate. In this method, 1 mg sample was placed in a microfuge tube, then

cold concentrated sulphuric acid was added slowly, mixed thoroughly on a vortex mixer, and kept for 1 h in an ice bath. The samples were vortexed every 10 min during this period. Ice-cold water was then added gradually to reach a total volume of 1.475 mL. The sample was centrifuged before GalUA content was assayed using the method of Blumenkrantz & Asboe-Hansen (1973) (see below).

3.1.7.2 Galacturonic Acid Content

The GalUA content was determined by a colorimetric method according to Blumenkrantz & Asboe-Hansen (1973) modified to fit a microplate (O'Donoghue et al., 2017). In this method, 40 µL of sample (or GalUA standard) and 200 µL of di-sodium tetraborate in concentrated sulphuric acid (with the solution final concentration of 12.5 mM) were pipetted in the microplate well. The microplate was covered and heated in a 90 °C oven for 1 h. The plate was pre-read at 520 nm directly after removal from the oven using a microplate reader (SpectraMax Plus 384, Molecular Devices, San Jose, USA, with Softmax Pro software version 5.4.5, Molecular Devices). After 30 min cooling the plate down on ice, colour was developed by the addition of 10 µL of 2.9 mM 3-phenylphenol in 125 mM sodium hydroxide). The absorbance was read at 520 nm after 5 min of colour development. Standards of GalUA (0-1.11 µmol/mL were used to quantify the amount of GalUA in the sample via a calibration curve. Results were converted to µmol GalUA/ g dry pomace.

3.2 Experimental Design

The purpose of this set of experiments was to identify 1) does the solubility of pectin in apple pomace change as a function of temperature? 2) does the solubility of pectin in apple pomace depend on the amount of water present? and 3) does shearing before heat treatment affect the pectin solubility in apple pomace?

A set of experiments was undertaken to quantify the amount of pectin solubilised into the supernatant in response to different temperatures, added water to fresh pomace and homogenisation of pomace.

First: The effect of water addition on solubilised pectin from sheared and non-sheared pomace was tested. In sheared samples (+shear) one batch of pomace was sheared with a stick blender for ~1 min, while the other pomace batch was not sheared (-shear). All samples taken from these batches were mixed with water in the following pomace:water ratios (w/v) of 1:0, 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:6 and 1:8. In these experiments pomace: water ratio of 1:0 means that no additional

water was added to pomace or pomace was treated with its water content as it had originally. The mixtures remained at RT or were placed in an autoclave (121°C) for 10 min. The autoclaved samples were cooled down to RT by placing them in the water bath. The pH of the samples before and after autoclaving was measured. All samples were then centrifuged at 10000 rpm for 15 min at RT using Sorvall RC6 plus centrifuge (rotor model F21-8x50y, Thermo Fisher Scientific, USA). Supernatants and dry pellets (oven-dried at 70°C) were assayed for GalUA content and the °Brix. All the methods involved in measuring the related parameters are given in section 3.1.1. This trial was run twice providing replicate samples as shown in Figure 3.2. each replicate was taken from individual batch and the laboratory tests taken on each set of experiment was done triplicate.

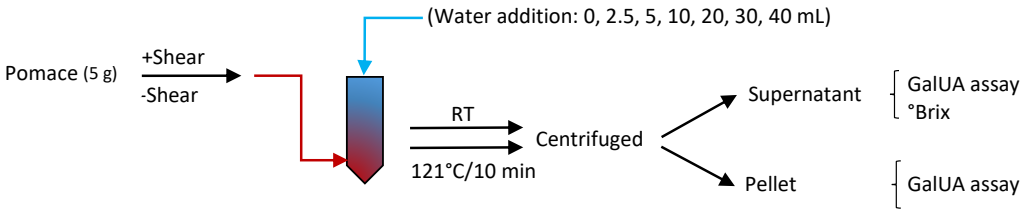


Figure 3.2 Outline of trials for pectin solubility in different amounts of water present at RT and after treatment at 121°C for 10 min.

Second: The effect of order of water addition (before or after heat treatment) on pectin solubilisation was examined (Figure 3.3). Two sets of samples were prepared. In one set pomace samples were autoclaved (121°C) for 10 min, cooled to RT and then water added to reflect pomace:water ratios of 1:0, 1:0.5, 1:1, 1:2 and 1:4. In another batch, samples with the same pomace:water ratios of 1:0, 1:0.5, 1:1, 1:2 and 1:4 were autoclaved (121°C) for 10 min. All samples were centrifuged, then the supernatants of all samples were assayed for GalUA. This experiment was done in two replicates. Each replicate was taken from an independent batch. Laboratory analysis which taken place on each treated pomace was done triplicate.

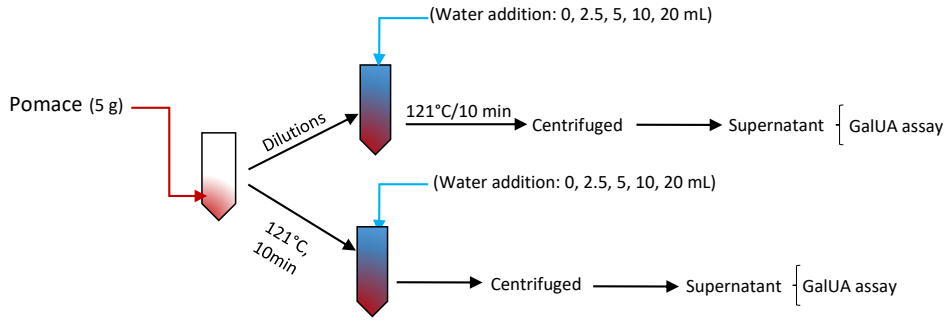


Figure 3.3 Trials for understanding the effect of the order of water addition (before or after autoclaving) on pectin solubilisation.

Third: The effect of temperature on pectin solubilisation was tested according to the method designed in Figure 3.4. In this experiment a pomace:water ratio of 1:1 was chosen and samples were prepared and heated at temperatures of RT, 30, 50, 70, 80, 90, 100 and 121°C. After heat treatment samples were cooled to RT by placing them in a water bath for 10 min followed by centrifugation at RT for 15 min at 10000 rpm. The supernatants were assayed for GalUA. The experiment was repeated three times. Each replicate as taken from an individual batch. Laboratory analysis of each treated pomace was done three times.

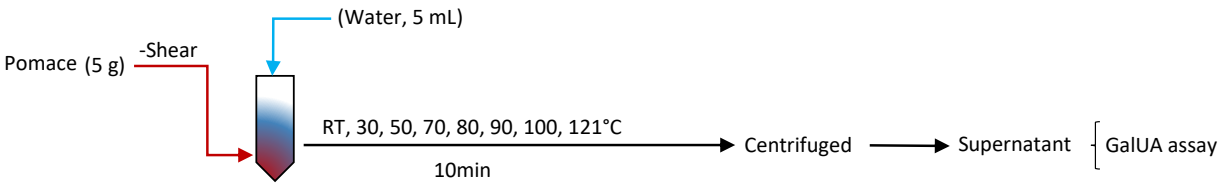


Figure 3.4 Trials for pectin solubility as a function of temperature.

3.2.1 Results and Discussion

3.2.1.1 Effect of Water Addition on Solubilised Pectin from Sheared and Non-sheared Pomace

The effects of added water and temperature (RT and 121 °C) on the soluble solids content of supernatants as measured by °Brix are shown in Figure 3.5A. Increasing the amounts of water added to pomace resulted in decreasing the soluble solids of supernatants, the effect being

predominantly in accord with the level of dilution. Autoclaving resulted in higher °Brix of supernatants than RT samples. This was expected due to increased solubilisation of sugars from cell walls with heating. It has been previously demonstrated that apple, carrot, sugar beet and onion contain high amounts of neutral sugar side-chains associated with pectin (such as arabinose, xylose, glucose and mannose), heat treatment might help to release these sugars from the pectin side chains (Zykwinska et al., 2005; Redgwell et al., 2008a; Voragen et al., 2009; Wang et al., 2014).

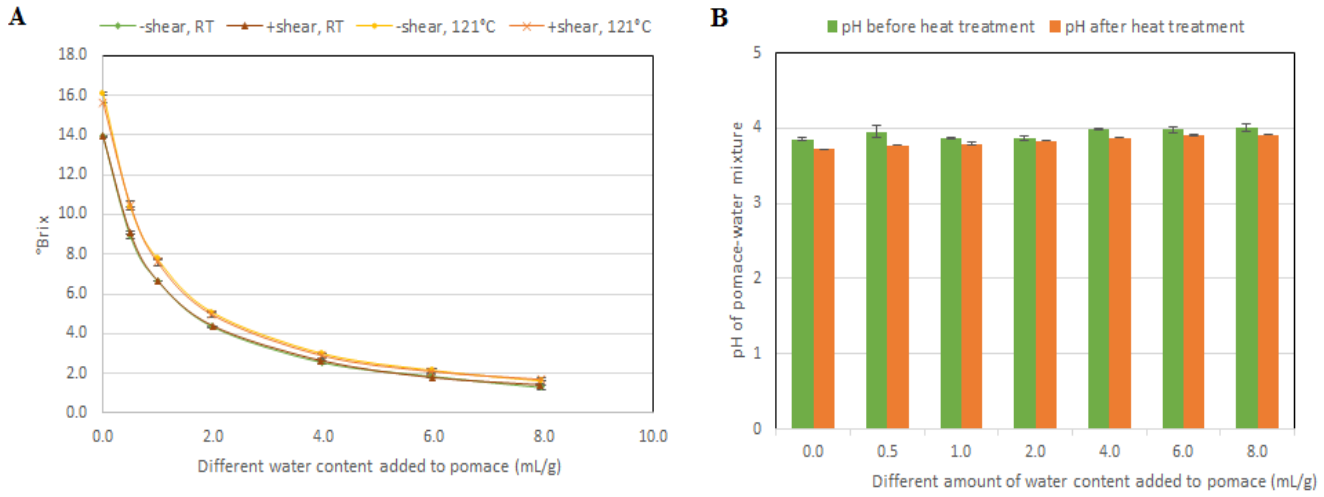


Figure 3.5 A) °Brix values in the soluble phase at RT and 121 °C of ±sheared samples in increasing amounts of water /g fresh pomace. B) pH of the pomace-water mixture before heating at 121 °C and after heating treatment. The error bars represent the maximum and minimum value of two replicate samples.

Shearing can affect the cell wall arrangements by disintegrating the particles and cell walls. As a result of this physical changes, higher amounts of soluble components may become soluble. Results in Figure 3.5A showed that shearing did not affect the amounts of soluble solids in supernatants in comparison to non-sheared supernatants. These results may show that the shearing techniques, duration or severity of this shearing were not enough to disintegrate the pomace cells and release and release measurable quantities of soluble material.

Heating at 121 °C can degrade the sugars present in the mixture to acidic components such as acetic acid, lactic acid and formic acid (Kuhnel et al., 2011). From this point of view pH of the samples before and after autoclaving were examined (Figure 3.5B). The results showed a small reduction in sample pH. Increasing organic acid content during the heating at high temperature may be linked to de-acetylation of pectin and to products of the Maillard reaction (Kuhnel et al., 2011). The pH change may indicate such a degradation process has occurred. Brands et al. (2001)

showed that heating glucose or fructose solutions separately at 120 °C for 0-40 min resulted in the production of acetic acid and formic acid, even after only 10 min heating. They found production of these deleterious components was higher in fructose solution than glucose. The presence of acidic components in the current samples needed to be determined further to confirm their production.

The total content of GalUA in fresh pomace was 913 ± 50 µmol GalUA/g dry pomace. Figure 3.6 shows the amount of GalUA that was solubilised into the supernatant by the treatments. This shows that the amount of pectin solubilised in water at RT was independent of amount of water added. This suggests that at RT such pectin as was available to be solubilised was already dissolved in the soluble phase of the fresh pomace or not limited by solubility. The average amount of soluble GalUA was about 169 µmol GalUA/g dry pomace. In 1:0 pomace: added water ratio the amounts of solubilised pectin were not affected by the treatment temperature (RT and 121 °C). These results imply that the original water content of pomace was already saturated soluble pectin and did not have capacity to solubilise more when pomace was heated at 121 °C. For heated samples, increasing the amount of water to 2 mL/g fresh pomace increased the pectin solubilisation to about 563 ± 9 µmol GalUA/g dry pomace, then it reached a plateau. Shearing did not have an effect on pectin solubilisation.

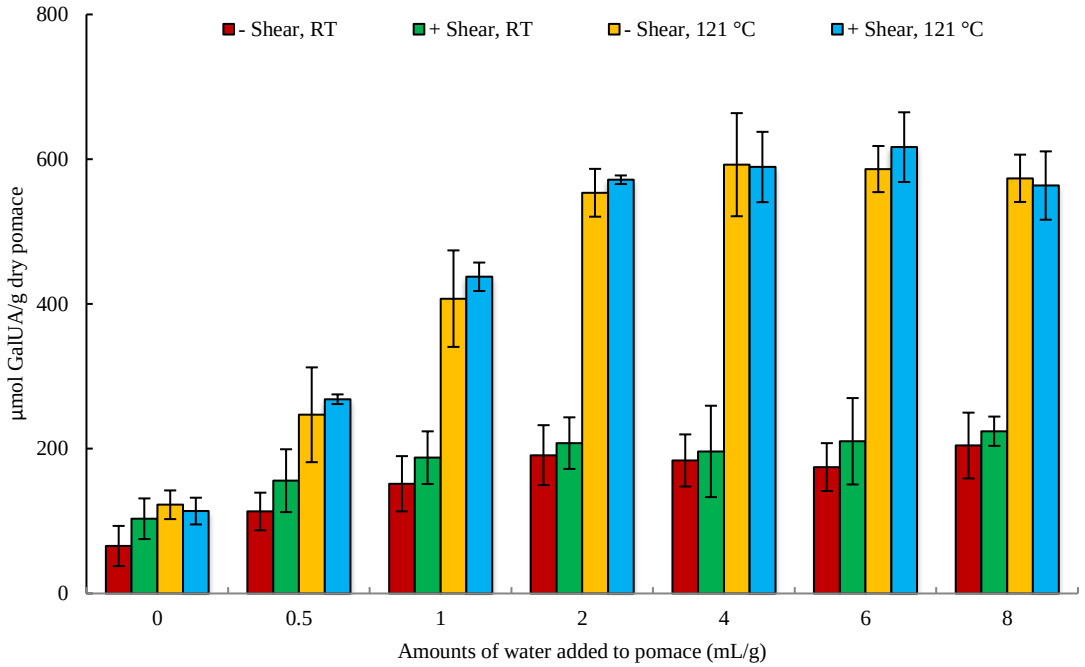


Figure 3.6 Amount of GalUA solubilised (µmol GalUA/ g dry pomace) in aqueous phase at RT and 121°C of ±sheared samples in increasing amounts of water/g fresh pomace. The error bars represent the maximum and minimum value of two replicate samples.

Pectin polysaccharides in plants can be inter-linked by divalent metal ions especially Ca^{+2} (Jarvis et al., 1995), and can also be linked to other cell wall components and other pectin polymers through interactions such as weak Van der Waals forces, covalent and hydrogen bonds. Weak van der Waals forces linking pectin with other cell wall components (due to the tangling effect) might be influenced by cold water and some amount of pectin may become solubilised in water. This pectin is also known as unbound pectin (Chang et al., 1993; Thakur et al., 1997; Zykwinska et al., 2005). Some pectin chains might be linked to other pectin polymers (such as RG-I) and other cell wall components by heat-labile bonds (e.g. hydrogen bonds) which could be disrupted during heating at high temperature releasing pectin from the insoluble particles of pomace (Chang et al., 1993; Zykwinska et al., 2005).

3.2.1.2 Effect of Water Addition Before or After Heat Treatment

From the previous experiment, it was established that increasing the amounts of water added to pomace could help to solubilise more pectin during heating at 121 °C. However, the effect of order of water addition (before or after heat treatment) was still unknown. Figure 3.7 shows that the amount of GalUA solubilised in water was independent of the time when water was added. High temperature had more influence on releasing pectin from pomace than the amount of water available within pomace during the heating. This experiment was directly related to processing pomace in an industrial scale where minimum added water is desirable.

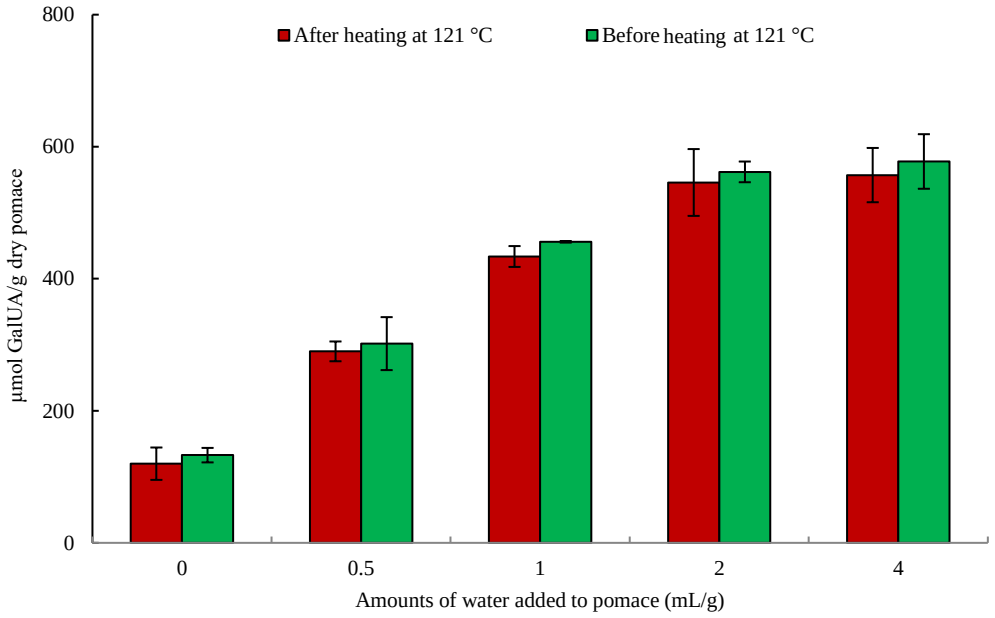


Figure 3.7 Amount of solubilised GalUA expressed as µmol GalUA/g dry pomace. Pomace was mixed with increasing amounts of water before or after heating at 121 °C for 10 min. The error bars represent the maximum and minimum value of two replicate samples.

3.2.1.3 Effect of Temperature on Solubilising Pectin from Pomace

The previous experiment identified that the solubility of pectin from pomace did not increase beyond a pomace:added water ratio ≥ 1:2 (w/v) when heated at 121 °C for 10 min. Figure 3.8 shows the effect of temperature on pectin solubilisation from the pomace. Increasing the temperature from RT to 70 °C slightly increased the amount of solubilised GalUA by softening the tissue and swelling the pomace cell wall materials which increases the surface exposed to water. In addition, pectin reactions such as acid hydrolysis and β-elimination are temperature dependent. Therefore, increasing temperature of plant material will increase the rate of these reactions and can help to solubilise and de-polymerise pectin into serum phase (Berjenholt, 2010). Solubilisation and de-polymerisation of pectin through these reactions may influence the pore size of the cell wall follows by increasing the possibility of more pectin solubilisation from cell wall into the serum phase.

Heating at 80 °C increased the pectin solubilised to 203 µmol GalUA/g dry pomace. Pectin solubilisation did not change between 80 to 100 °C. The stable amount of solubilised pectin between 80-100 °C may show that the pomace cell wall has reached to maximum expansion as well as the rate of pectin chemical reactions (acid hydrolysis and β-elimination) might have not changed significantly between 80-100 °C. Autoclaving at 121 °C had the most effect on releasing pectin from apple pomace (550 µmol GalUA/g dry pomace).

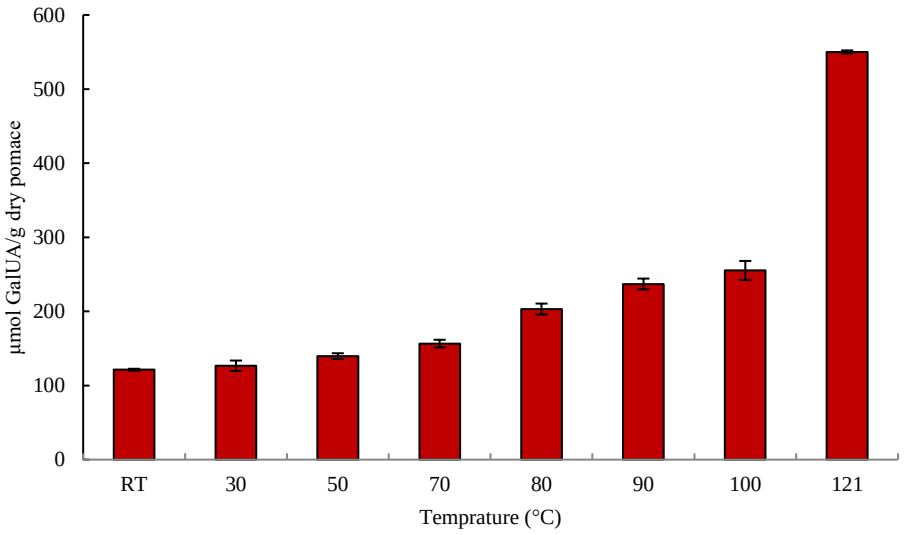


Figure 3.8 Amount of solubilised GalUA (µmol GalUA/ g dry pomace) in aqueous phase of total available GalUA in pomace after incubation for 10 min at the temperatures shown, ±standard error. Experimental replications n=3.

It has been reported that rigidity of whole apple cell wall decreases at thermal treatments of ~100 °C, due to de-polymerisation and solubilisation of pectin from the middle lamella (Espinosa-Muñoz et al., 2012; Lopez-Sanchez et al., 2012). Higher temperatures for shorter periods were also effective, as shown by Wang et al. (2014) who found that maximum pectin extraction from dried apple pomace could be achieved at 130 °C for 5 min. They also found that temperatures beyond 140 °C resulted in solubilisation of non-pectic cell wall carbohydrates such as xyloglucan. Current results fit this pattern, with maximum solubilisation at the highest temperature tested (121 °C). In order to understand the kinetics of pectin solubilisation from apple pomace different heating intervals needed to be investigated at different temperatures.

3.2.2 Conclusion

Apple pomace contains significant amounts of soluble and insoluble fibre. Among all the fibres, pectin can have a great effect on textural properties of the product. Therefore, trials in this chapter included a set of preliminary experiments to concentrate on how different pomace preparation conditions such as water addition to fresh pomace, shearing and temperature could affect the quantity of solubilised pectin. The results revealed that shearing of pomace before heat treatment had little effect on accessibility of pectin. This means that the shearing process was not sufficient to disintegrate the cell wall and increase the soluble solids in the serum phase. Increasing the amounts of water added to pomace could effectively increase solubilisation of pectin up to a pomace-water ratio of 1:2. Water addition before or after heat treatment showed similar levels of pectin solubilisation. Addition of two parts of water to one of pomace has a significant impact on the size of equipment and energy required at full industrial scale. Later optimisation work may be required to reduce the added water level with least impact on pectin solubilisation.

Understanding the effects of sample preparation on amounts of solubilised pectin can guide the study of the kinetics of main reactions such as pectin solubilisation, depolymerisation and degradation reactions of cell wall components when heating pomace at different temperatures and durations.

CHAPTER 4 Kinetics of Reactions in Apple Pomace During Hydrothermal Treatment

Processing of fruit and vegetable waste streams usually contains a heating step to solubilise cell wall polymers and increase shelf life of the product by reducing microbial content of the product and inactivating enzymes. Heating strategies including drying, irradiation, or hydrothermal treatment (direct or indirect) can be used for this purpose.

In this study, water was added as solvent to solubilise more pectin into the serum phase of apple pomace. A number of reactions can take place in fruit and vegetable residue which directly affect the quantity and quality of the pectin as well as safety of the heat-treated product. Heating temperature and incubation time are two main factors influencing these possible reactions. This chapter focuses on kinetics of the reactions involved in pomace when temperature and time are varied.

4.1 Introduction

Heat treatment of many food products is a necessary step to provide a safe product with a longer shelf life. This process might be applied at the beginning or the end of a production line (de Roeck et al., 2010). Heating is usually used for pasteurisation (mostly for very acidic products such as fruits, temperatures < 100°C) or sterilisation which has a higher intensity of heat (mostly for low acid food products such as vegetables, temperatures >120°C) (Colin-Henrion et al., 2009; Li et al., 2016; Ranganathan et al., 2016). In addition, several physico-chemical reactions can take place while heating which can change the physico-chemical properties of the final product. These reactions can affect texture, chemistry, colour and nutrients of processed food (de Roeck et al., 2010).

Fruit and vegetables contain complex components such as lignin, cellulose, hemicellulose, xyloglucan and pectin (de Moura et al., 2017). These components can undergo several temperature-sensitive reactions during hydrothermal treatment (Colin-Henrion et al., 2009; de Oliveira et al., 2015; de Roeck et al., 2010; Osorio et al., 2008). Pectin is a part of the cell wall

matrix and is the main component of the middle lamella (the intercellular spaces of dicotyledons) (Zykwinska et al., 2005). It acts as a cementing material, interacting with both cellulose and XG (Zykwinska et al., 2005; Cucheval, 2009; Berjenholt, 2010; Bonnin et al., 2014). Pectin is known to be heat sensitive and to be an extremely complex cell wall polymer which undergoes different chemical reactions during thermal processing (Bonnin et al., 2014; Ranganathan et al., 2016). Any changes in its properties can directly affect the final properties of the plant product. Therefore, understanding the kinetics of the reactions involving pectin such as pectin solubilisation and its fragmentation provides important information in developing any process for processing fruit and vegetable residues.

Optimal stability pH of pectin polymer in apple pomace and many fruit residues are in the ranges of 3.5-4.1 depending on the fruit pH (Sila et al., 2009; Berjenholt, 2010). Highly methylated pectin can degrade at temperatures higher than 80 °C at alkaline or acidic pH by depolymerisation reactions of β-elimination and acid hydrolysis, respectively. During these reactions, the glycosidic bond at the C-4 position of galacturonic acid (GalUA) in the backbone is broken and the molecular weight declines (Berjenholt, 2010). Solubilisation of pectin can also expose it to degradation reactions. In addition to solubilisation of pectin and its fragmentation into smaller pectin oligomers during heating, other chemical reactions occur including the degradation of sugars into secondary products such as 5-hydroxymethyl furfural (5-HMF), furfural and organic acids, which are not desirable in hydrothermally treated food products (Dounlop, 1948; Kus et al., 2005; Gaspar et al., 2009; Kuhnel et al., 2011; Wang et al., 2019).

The purpose of this chapter is to understand the kinetics of the main reactions involved in apple pomace during heating at different temperatures and times, using the sample preparation condition selected from Chapter 3.

4.2 Material and Methods

4.2.1 Plant Materials

Apple pomace was obtained from Turners & Growers Apple Juice Concentrate Plant (Hasting, New Zealand). This pomace was a mixture of apple varieties include Pacific Rose, Gala, Granny Smith and Jazz which contained added pectinolytic enzymes (Pectinex Smash XXL, Novozyme, Denmark). Pomace was sub-sampled in 50 g and 1 kg bags, vacuum packed and kept frozen at -30 °C until further use. Pomace was thawed overnight at 1°C before use.

4.2.2 Shearing

Shearing of apple pomace was done using a rotor-stator high shear mixer (Silverson L4RT, blade diameter of 0.03 m, Chesham, UK) at a tip speed of 12.56 m/s. Tip speed can be used in scaling up the process to apply the same tip speed using another mixer with different blade size and revolution.

4.2.3 Dry Matter

Dry matter of pomace materials was determined according to the procedure given in Chapter 3 Section 3.1.5.

4.2.4 Pectin Analysis

Total pectin content and solubilised pectin from apple pomace were measured according to the procedure given in Chapter 3, Section 3.1.7.

4.2.4.1 Mono/di-galacturonic Acid

The collected ethanol (EtOH) supernatant (EtOH- soluble GalUA) which was collected from the original EtOH precipitation in Chapter 3, Section 3.1.7, Figure 3.1D, was air-dried. Water was added back to the original volume and the sample was dissolved completely. The mono- and di-GalUA in this sample were analysed on a Dionex HPLC system (Dionex Summit, Dionex, California, USA) with an autosampler (ASI-100), pump (P680) and column oven (TCC-100). A 50 μL of sample was injected onto a strong cation-exchange Sugar Pack 1 column (Waters Corp, Massachusetts, USA) at 65 °C, with a mobile phase of 50 mg/L ethylenediaminetetraacetic acid (EDTA) at a flow rate of 0.5 mL/min. Components were detected with a refractive index (RI) detector Shodex RI-101, Tokyo, Japan. Standards of mono- and di-GalUA were injected in the range of 0.05-2 mg/mL.

The HPLC system was unable to separate these two components from each other because their retention times and response factors (ratio of peak area: concentration) were similar. This meant that although GalUA could be detected, mono- and di-GalUA could not be distinguished one from the other. Figure 4.1 shows an example of separate injection of mono- and di-GalUA with similar concentration of 1mg/mL using HPLC. Therefore, in this study, the combined peak is assumed to be all mono-GalUA and reported as mono/di-GalUA with the unit of GalUA μmol/g dry residue, using the mono-GalUA formula weight (194.2 g/mol).

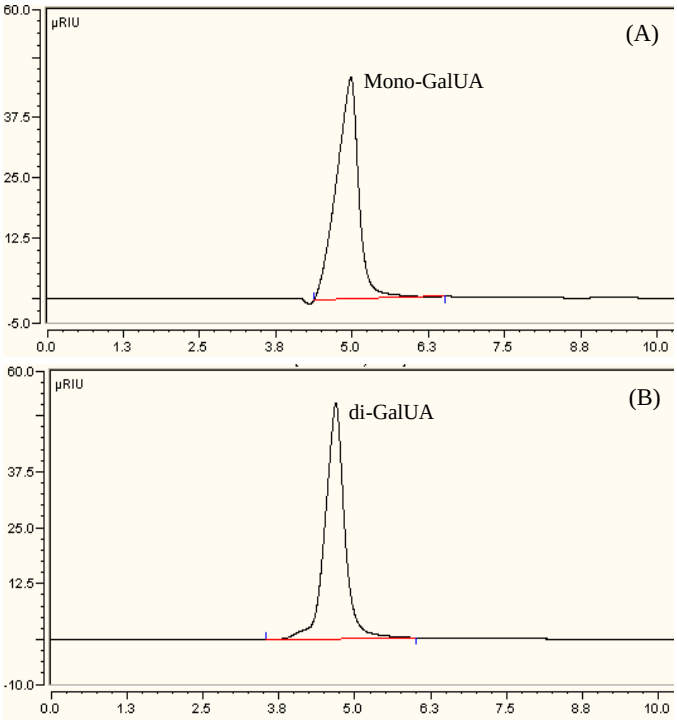


Figure 4.1 HPLC chromatograms of the standard solutions of mono-GalUA (A) and di-GalUA (B), when using cation-exchange column of sugar peak 1 at 65 °C with the EDTA as mobile phase with the flow rate of 0.5 mL/min and refractive index detection.

4.2.5 Determination of Hexuronic Acid Reducing End Groups

The presence of hexuronic acid reducing end groups (HRG) was assayed using the method of Milner & Avigad (1967). For this method, two reagents were prepared. A copper solution was prepared by dissolving 280 g anhydrous Na_2SO_4 and 40 g NaCl in 720 mL water with continuous heating and stirring, then 100 mL of 2 M acetate buffer (pH 5.1) and 65 mL of 0.32 M $CuSO_4$ were added to the mixture. The pH of the final copper solution was adjusted to 4.8 with the total volume made up to 1 L.

An arsenomolybdate reagent was prepared by first dissolving 50 g of $(NH_4)_6Mo_7O_{24}.H_2O$ in 900 mL water followed by addition of 42 mL concentrated H_2SO_4. Sodium arsenate (6 g $Na_2HAsO_4.{}_7H_2O$ in 50 mL water) was added. This solution was stored in the dark.

Hexuronic acid reducing end groups were assayed by addition of 300 µL copper solution to the EtOH washed sample (100 µL), followed by mixing and boiling for 10 min in a water bath. Samples were cooled in an ice bath, then 200 µL of arsenomolybdate reagent was added and the sample was mixed. A volume of 200 µL aliquot was pipetted into microplate wells and absorbance was read at 600 nm using a plate reader (Molecular Devices, San Jose, USA). Standards of GalUA (0-1.11 µmol/mL) were used to quantify the HRG.

4.2.6 Determination of Unsaturated Uronides

The β-elimination reaction breaks pectin down resulting in unsaturated uronides (UnU). Samples (200 µL) were pipetted into a UV transparent microplate and the unsaturated bonds in the polygalacturonides (UnU) resulting from β-elimination were detected by absorbance at 235 nm using a SpectraMax Plus 384 plate reader. Absorbances were converted to a 1 cm path length using the microplate's Softmax pro software. The concentration of UnU was calculated using Beer's law. This equation 4.1 is given below:

$$\text{Absorbance} = \varepsilon l c$$

Equation 4.1 Beer's law equation, where ε = extinction coefficient, l = the path length and c = concentration of UnU. An ε value of 5200 $M^{-1}cm^{-1}$ was used for UnU according to Diaz et al. (2007).

The production of UnU in pure citrus pectin (DE >94%) after heating at 110°C for ≥ 2h at pH 8.5 was tested before to examine the maximum absorbance that could be recorded by spectrophotometry when high amounts of UnU are available. This test also confirmed the operational region in which the response was still linear.

4.2.7 Sugar Analysis and Degradation Products

The main simple sugars (glucose, fructose and sucrose), as well as their degradation products 5-HMF, furfural and organic acids were determined using an UltiMate RS 3000 HPLC system coupled with an autosampler and a pump (Thermo Scientific, Dreieich, Germany). Samples (20 µL) were injected onto an Aminex HPX-87H column (Bio-Rad, California, USA) at 60 °C operating in 5 mM H_2SO_4 at a flow rate of 0.5 mL/min. Sugars were detected using a RI detector, while organic acids were detected by absorbance at 210 nm. Sugar (sucrose, glucose and fructose) and organic acid (acetic acid, lactic acid, formic acid) individual standard solutions were used to determine their retention time at the relevant wavelength. The maximum absorbance for 5-HMF and furfural were determined by scanning their individual standard solutions in the wavelength range of 200-600 nm. Results showed that 5-HMF and furfural had maximum absorption at 284 and 277 nm, respectively.

4.2.8 Degree of Esterification

Degree of esterification (DE) of the dry cell wall and solubilised pectin were measured according to McFeeters & Armstrong (1984) with some modifications. In this method, esterified methyl groups were measured by gas chromatography detection of methanol after release by NaOH saponification. In detail, 2 mg dry EtOH washed pomace or pellet was mixed with 25 µL of 50

mM citric acid, containing 1 M NaCl at pH 5 and 225 µL of water was added. For supernatant, a 100 µL aliquot containing 2.5 µmol GalUA, was mixed with 20 µL of 50 mM citric acid, containing 1 M NaCl at pH 5, and 125 µL water was added. A volume of 20 µL of 1 M NaOH was then added to all samples (pomace and supernatant) to induce saponification. Samples were kept at 1 °C overnight. The next day, 82.5 mM citric acid (30 µL) and 25 mM *n*-propanol as internal standard (33.3 µL) were added. The samples were centrifuged at 14,170 g for 10 min at RT. A blank sample for supernatant was also prepared in the same way explained above except for the addition of NaOH. This procedure was used to account for methanol that may already be present in the supernatant. A 25 mM methanol solution (33.3 µL) and water (191.7 µL) replaced the sample volume in a standard for quantifying the amount of methanol. For detection of methanol, 1 µL aliquot was injected onto a gas chromatograph (Shimadzu GC17A, Kyoto, Japan) equipped with a BP-20 column (15 m x 0.53 mm, 1 mm film thickness, SGE) at 80 °C with He as a carrier gas. A flame ionization detector was used for measuring the amount of methanol released from the saponification. The percent of DE, either in dry cell wall fraction or supernatant was determined by calculating the ratio of the moles of methanol to the moles of GalUA present in the sample.

4.3 Experimental Design

In order to understand the kinetics of reactions in pomace (mainly pectin), a heating process was applied in a controlled system at laboratory scale. This approach is discussed below. AISI 316 stainless steel tube with outside and inside diameters of 12 and 9 mm, respectively and matching fittings including tube fitting caps, nut and ferrule set were bought from Swagelok (New Zealand branch). Similar reactors of 205 mm length were built in the Massey University workshop. Figure 4.2 shows the dimensions of these reactors.

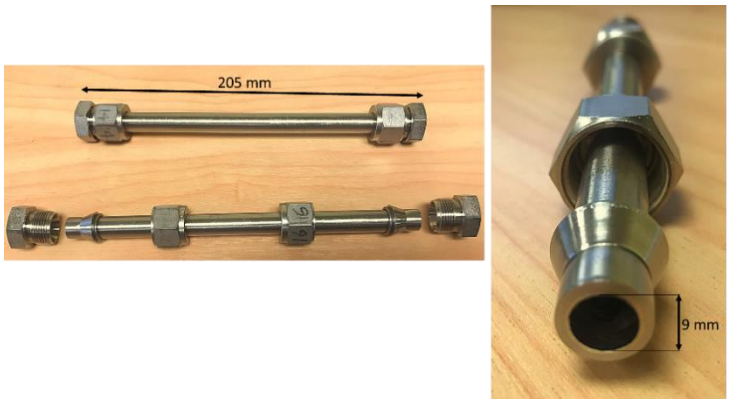


Figure 4.2 Stainless steel reactors used for heating pomace at laboratory scale using oil bath.

The hydrothermal treatment steps and related measurements are given in Figure 4.3. Temperature and time are two main factors affecting any temperature dependency reactions. In this study, the hydrothermal treatment was applied by immersing the stainless steel reactors in a silicon oil bath at temperatures between 90-140 °C (at 10 °C intervals) for durations between 0-360 min. Table 4.1 shows the experimental matrix of temperatures and times used in this study.

Table 4.1 Treatment layout.

Temperature (°C)	Time (min)	Temperature (C)	Time (min)
90	0*	120	0
90	10**	120	5
90	20	120	10
90	30	120	15
90	60	120	20
90	90	120	30
90	120	120	40
90	150	120	50
90	180	120	60
90	210	120	70
90	240	120	90
90	270	120	120
90	300	120	150
90	330	120	180
90	360	130	0
100	0	130	2
100	10	130	5
100	20	130	7
100	30	130	10
100	60	130	15
100	90	130	20
100	120	130	25
100	150	130	30
100	180	130	40
100	210	130	60
100	240	130	90
100	270	130	120
100	300	130	150
110	0	140	0
110	5	140	2
110	10	140	5
110	20	140	7
110	30	140	10
110	40	140	15
110	50	140	20
110	70	140	25
110	90	140	30
110	120	140	40
110	150	140	50
110	180	140	60
110	210	140	90
		140	120

* Time= o min, means reactors were immersed in silicon oil for 4.5 min. ** All incubation time were started to record after 4.5 min immersion in silicon oil at relevant temperature.

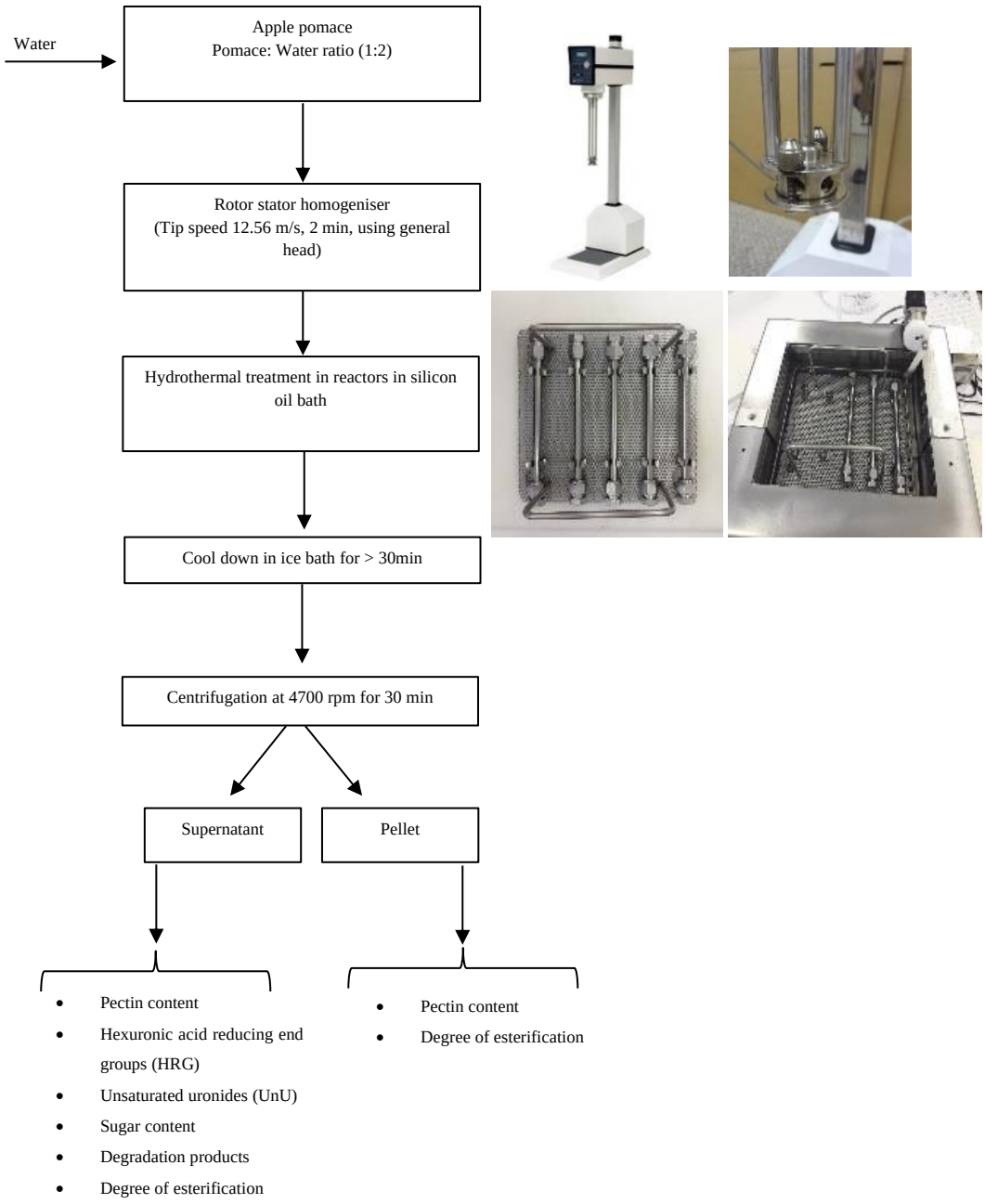


Figure 4.3 Hydrothermal treatment pathway of apple pomace and relevant analysis.

Heat transfer is the flow of thermal energy, which can transit due to the temperature differences between two materials. This transfer can happen through convection, conduction or radiation. Convection heat transfer can happen between a solid (or surface) and liquid materials with different temperatures. In this study convection heat transfer from silicone oil to surface of reactor was ignored due to high heat transfer coefficient of the silicon oil. Moreover, it has been assumed that the steel of the reactors was at the oil temperature at the beginning of the heat transfer due to the small thickness of the reactor walls and high thermal conductivity of stainless steel.

Heat transfer from reactor to pomace was assumed to be limited by conduction heat transfer within the pomace mass.

Zero time should be when the bulk of the pomace has reached a temperature where reaction rates are becoming significant. At the point, pomace near the centre will be cooler and reacting slowly while pomace near the walls will be near the oil temperature and reacting rapidly. Since selection of zero time has only a relatively minor influence on estimation of kinetic parameters, the estimate was not highly refined. The condition judged reasonable was the point when average mass of the pomace mass had reached to the target temperature change from 20 C to oil bath temperature. The time taken to meet this criterion was calculated at the two extremes used for oil bath temperature, 90 and 140 C and averaged. For these estimates, unsteady state conduction heat transfer calculations were conducted assuming an infinite cylinder etc of diameter 9 mm using the third kind of boundary condition (Equation 4.2).

Equation 4.2 Conduction heat transfer through infinite cylinder of diameter 2R using third kind of boundary condition

$$Y_{av} = \sum_{m=1}^{\infty} \frac{4\,Bi^2}{{\beta_m}^2({\beta_m}^2 + Bi^2)}\, exp\left[-{\beta_m}^2\, Fo\right]$$

Where Y_{av} is average mass of pomace in reactor, Bi is biot number, β_m are the roots of equation when m=1, 2, 3, … and Fo is Fourier number.

The eqautions for Biot number (Bi), biot numebrs roots (B_m) and fourier number (Fo) are also given in Equations 4.3, 4.4 and 4.5.

Equation 4.3 Biot number

$$Bi = h_c R/\lambda$$

Where h_c is heat transfer coefficient of silicon oil, R is inside radius of reactor, and λ is thermal conductivity of the pomace.

Equation 4.4 Roots of the equation of conduction heat transfer equation

$$\beta\, J_1(\beta) - Bi\, J_0(\beta) = 0$$

Where J_1 and J_0 are Bessel's function.

Equation 4.5 Fourier number

$$Fo = \lambda t/\rho C_p R^2$$

Where λ is thermal conductivity of pomace, t is incubation time, ρ is the density of pomace, Cp is specific heat capacity of pomace and R is inside radius of reactor.

According to this measurement after 4.5 min the average mass of pomace inside the reactor was reached to temperature of silicon oil. Therefore, this time was selected as zero time for heating all experimental conditions given in Table 4.1.

Table 4.2 Physical and thermal properties of stainless steel, silicon oil and apple pomace, and the variables used in equations 4.3 and 4.5.

Stainless steel 316	**Unit**	**Amount**	**Variable**
Outside diameter	m	0.012	OD
Inside diameter	m	0.009	ID
length of tube	m	0.2	L
Density	kg/m^3	7990	$\rho_{reactor}$
Specific heat capacity	J/kgK	500	$Cp_{reactor}$
Oil	**Unit**	**Amount**	
Heat transfer coefficient	W/m^2K	300	$h_{silicon\ oil}$
Apple pomace	Unit	Amount	
Density of pomace*	kg/m^3	1000	$\rho_{apple\ pomace}$
Specific heat capacity*	J/kgK	4185.5	$Cp_{apple\ pomace}$
Thermal conductivity*	W/mK	0.67	λ

*Assume to be the same as water.

4.3.1 Process of Hydrothermal Treatment Runs

The steps for the hydrothermal treatment are shown in Figure 4.3. Apple pomace was thawed overnight at 1°C. On the next day seeds, stickers and stalks were removed manually. Preparing pomace sample prior to heat treatment was done by mixing one part of pomace with two parts of water using the rotor-stator high shear mixer (Section 4.2.2) at a tip speed of 12.6 m/s for 2 min using a general screen. This mixing produced a uniform sample for filling the reactors. Reactors were filled using a plastic syringe with nozzle diameter similar to the reactor diameter and left for 1 hour at RT to equilibrate. Other filled reactors were immersed in the silicon oil at the temperatures and times listed in Table 4.1. Actual heating times were started after the calculated zero time of 4.5 min.

Reactors containing the heat-treated samples were immediately transferred to an ice bath for 20-30 min, then the treated pomace was ejected from the reactor into 50 mL tubes using a long stainless steel rod with a similar size to the inside diameter of the reactors as a plunger. Samples were centrifuged using a Heraeus multifuge 1S-R centrifuge (Thermo Scientific Massachusetts, USA) with a Sorvall Heraeus 75002002 G rotor at 4618 g for 30 min at 4 °C. Supernatant was

transferred into separate tubes and frozen. The wet pellet was dried in an oven at 70 °C until it reached a constant weight. Each supernatant and insoluble pellet was analysed separately (see Figure 4.3) using methodology given in Material and Methods (Section 4.2).

Each hydrothermal treatment time/temperature combination was carried out in duplicate. These are called Run 1 (R1) and Run 2 (R2).

Prior to measuring the GalUA content in supernatant and pellet, soluble sugars were removed because they can severely interfere with the detection of GalUA. Sugars were removed by EtOH precipitation of supernatant or extensive EtOH washing of the pellet according to the method in Chapter 3, Section 3.1.7.

The dried insoluble pellet (Figure 3.1A) was ground using a customised metal pestle, then 100 mg of ground sample was mixed with 1 mL of 80% EtOH, vortexed and centrifuged. The resulting supernatant contained soluble sugars and was discarded. This was repeated eight times then the pellet was air-dried.

The supernatant of hydrothermally treated pomace (Figure 3.1B) was precipitated with EtOH and followed by two more washes of EtOH-insoluble pectin (Figure 3.1C). The EtOH-insoluble pectin was separated from the EtOH-soluble portion (Figure 3.1D) using centrifuge. The EtOH-insoluble pectin was re-dissolved in the original volume of water.

All prepared samples (EtOH-insoluble pectin, EtOH-soluble GalUA and pellet) were kept frozen until further analysis.

4.4 Results and Discussion

4.4.1 Kinetics of Pectin Solubilisation During Hydrothermal Treatment

In the current study, the pH of the apple pomace as received was 3.5 with a moisture content of 83.4%. Apple pomace was used in this chapter was contained pectinolytic enzymes which were added during juicing process. To minimise artefacts created by enzyme activity, pomace was collected freshly off the process line in small bags and packed in ice. It was stored frozen, thawed rapidly and thermal treatment experiments were performed without delay. The total GalUA content of the original apple pomace (including soluble and insoluble polysaccharides) was 1078 µmol/ g dry pomace, which was ~ 21% of the pomace solid components. The degree of esterification (DE) of the pomace pectin was 54%. Sucrose, fructose and glucose made up ~ 20% of the pomace solid content. The main composition of apple pomace is given in Table 4.3. Microbial content, a complete list of pomace composition as well as the range of chemical elements present in pomace are given in Appendix 1.

Dietary fibre of the apple pomace used for this trial was measured by the Nutrition Laboratory, Massey University (refer to Appendix 1). The insoluble, soluble and total dietary fibre of pomace was 48, 8 and 57 % of dry pomace, respectively. These results were in good agreement with the determination of dietary fire in commercial apple fibre with the amounts of 45, 15 and 60% for insoluble, soluble and total dietary fibre, respectively (Rosell et al., 2009).

Table 4.3 Composition of the original/unheated apple pomace

Fraction	Amount (µmol/ g dry pomace)
Sucrose	66
Fructose	736
Glucose	274
Galacturonic acid (from polymeric form)	885
Mono/di-galacturonic acid	194

Figure 4.5 shows the distribution of GalUA content between fractions of each heat treatment (time-temperature conditions are given in Table 4.1. The three fractions are insoluble solid, EtOH-insoluble pectin and EtOH-soluble GalUA. The results are presented based on µmol GalUA/g dry pomace. For this purpose, total GalUA content in each fraction of pomace was divided by the total GalUA recovered from heat-treated pomace. This ratio was then multiplied by the total GalUA content per g dry pomace to make the final unit of GalUA/g dry pomace. This normalisation in results helped to better identify any trend in final GalUA content from each fraction by avoiding the losses in material while emptying the reactors.

The % of GalUA losses in each run was determined by dividing the total GalUA of dry pomace recovered in each reactor from the total GalUA content initially present in reactor (Figure 4.4).

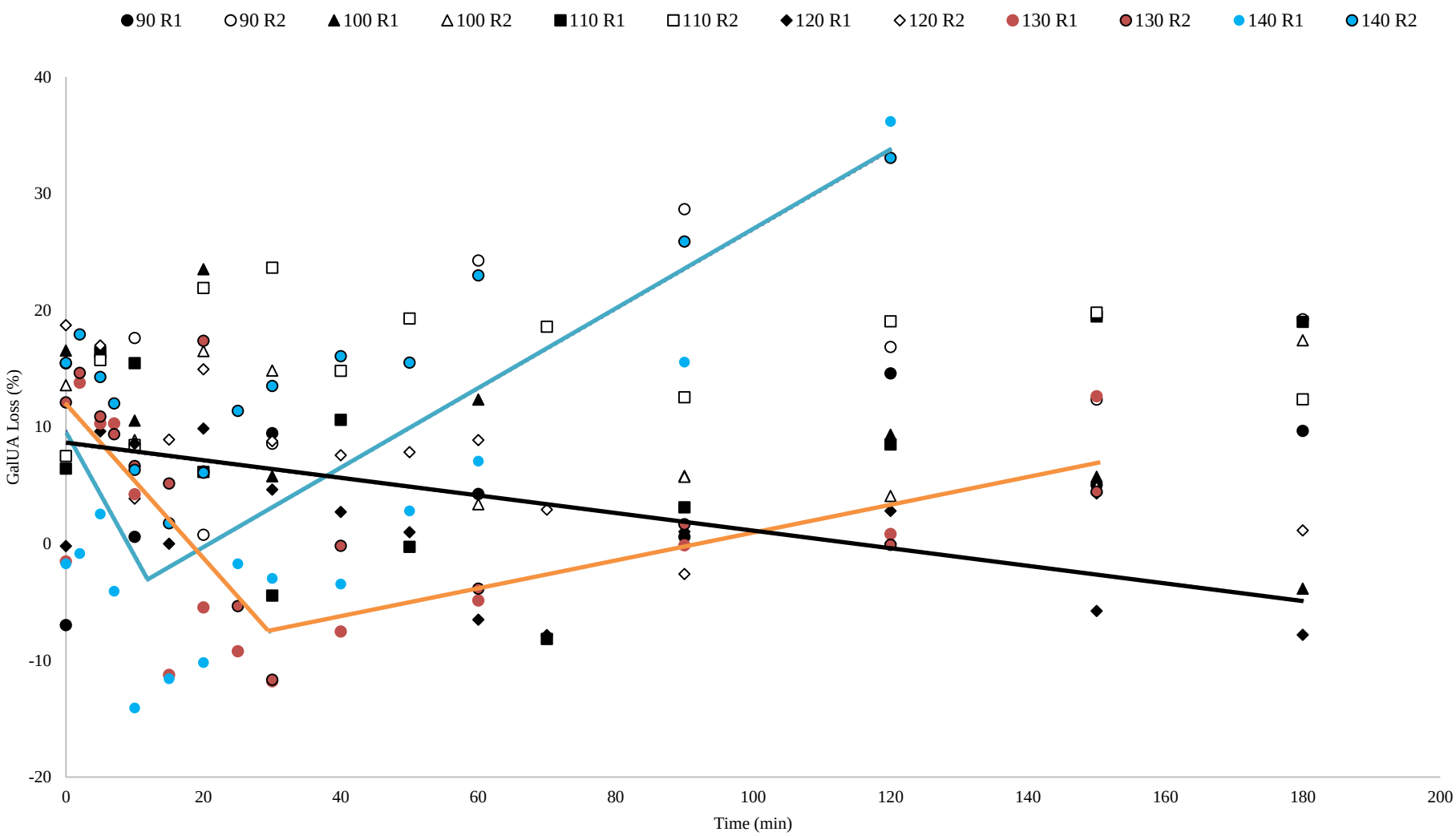


Figure 4.4 Percent of the losses in GalUA content of heat treated pomace using reactors at temperatures between 90-140 °C and treatment times between 0-300 min for experimental replications (R1 and R2). Trend lines of average % of GalUA loss for 120-140 °C were plotted.

According to Figure 4.4, losses are mainly below 20%. These losses could be due to incomplete removal of the material from reactors. Some negative percentage in loss was also observed. Heat treated pomace using reactors was always assumed to have a uniform pomace:water ratio of 1:2 throughout inside the rectors. This may not have always been the case and the negative losses might be due to changes in this proportion (pomace-water). Heating at temperatures <120 °C showed a stable % of GalUA losses in the range of 0 to 15%, while at 120, 130 and 140 °C a trend of increasing loss developed. In this trend, the % of loss first decreased then gradually increased towards the end of heating time resulted in a "checkmark shape" in the % of losses (especially at 140 °C). The downstream part of in % of GalUA loss can be related to how pectin was involved in cell wall complex structure. If more intact pectin was in the cell wall, sulphuric acid digestion analysis would be less complete. Therefore, less amounts of GalUA would be accounted for total GalUA in pamace. The reason for upstream trend at temperatures > 120 °C might be related to degradation of GalUA into other degraded products such as furfural and formic acid. These degraded components will be discussed further in Section 4.4.4. Separate trend lines were plotted for these temperatures before and after the minimum GalUA loss for the relevant times.

Figure 4.5 illustrates the competition between the reactions solubilising cell wall pectin, mobilising it into the serum phase as ethanol-insoluble oligomers, with further depolymerising reactions converting oligomers to smaller ethanol-soluble species represented as mono-GalUA. According to these results, prior to heating, about 60% of GalUA content remained insoluble in the pellet. Increasing the incubation time as well as temperature decreased this to about 10 and 8%, when pomace was heated at 130 and 140 °C for 60 and 50 min, respectively. Heating at temperatures between 90-100 °C slightly increased the amount of solubilised pectin to about 1.2 times more than the initial amount of solubilised pectin at RT. However, heating between 110-120 °C solubilised pectin to about 2.5 times more than its initial content (at RT). Heating at temperatures > 120 °C, had the greatest effect on the amounts of mono-GalUA by increasing the rate of pectin degradation in the serum phase. The amounts of mono-GalUA increased from about 11% in pomace without heat treatment to about 47% after heating at 140 °C for 90 min.

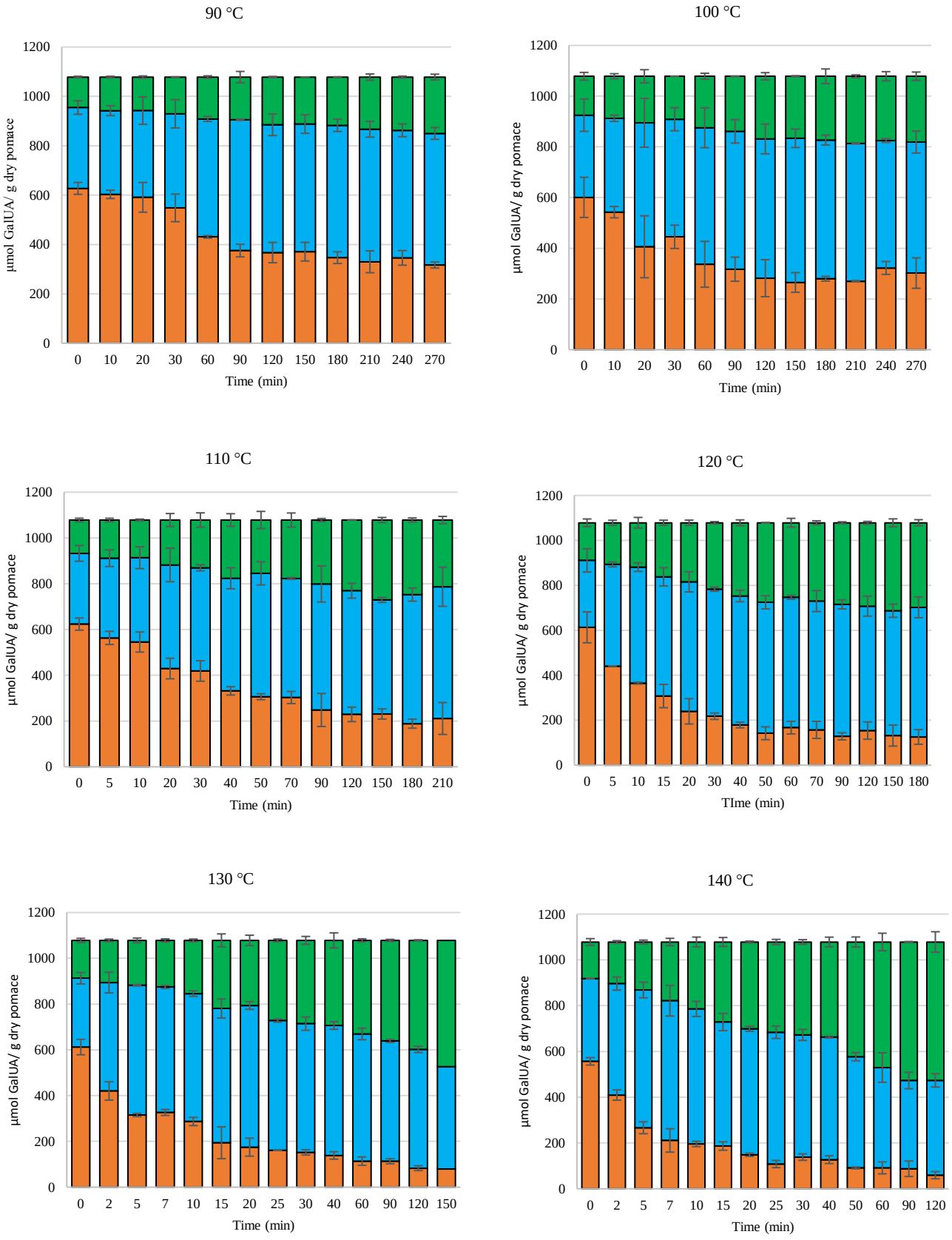


Figure 4.5 The GalUA distributions of the pectin in the pellet (orange), EtOH insoluble pectin (blue) and EtOH-soluble GalUA (green) of heat-treated pomace at different temperatures of 90-140 °C and duration of time 0-210 min. The error bars represent the maximum and minimum value of two replicate samples.

At RT 290 ±11 GalUA (μmol/g dry pomace) is solubilised in supernatant (as EtOH-insoluble pectin). Kinetics of pectin solubilisation and its degradation to GalUA subunits are given in Figure 4.6A & B. The amount of GalUA remaining as insoluble pectin in the pellet is given in Figure 4.6C. Error bars on the graph show the standard error of two experimental replications.

Figure 4.6A shows that increasing temperature from 90 to 140 °C increased pectin solubilisation from cell walls in the pomace. Heating at 90 °C, which was selected as minimum temperature, showed a slight increase in the solubilised pectin from 329 to 484 μmol GalUA/ g dry pomace, for heating times between 0 to 90 min, respectively. After that solubilised pectin content reached a plateau regardless of time at this temperature. Heating at temperatures > 100 °C showed faster and greater solubilisation in comparison to heating at ≤100 °C. For example, heating at 100 and 110 °C resulted in reaching similar solubilisation of GalUA (~540 μmol GalUA/ g dry pomace), but this ~ 90 min heating at 100 °C and 50 min at 110 °C.

Heating of plant materials in water seems to result in increasing the pore size of the pomace tissue and opening its structure. Subsequently, more water diffusion into the loose structure can positively affect solubilisation of pectin (Taherian et al., 2009; Contreras-Esquivel et al., 2014). Sila et al. (2006a) showed that both heating temperature and time could increase the amount of water-soluble pectin extracted from carrot tissue and could soften the material. In Figure 4.6A, heating at 130 °C for 15 min and 140 °C for 7 min resulted in 56 % of solubilisation of pectin, (i.e., ~ 605 μmol GalUA/ g dry pomace). This amount was also considered as the maximum solubilised GalUA in this study. Solubilisation of GalUA at higher temperatures have been reported. For example, Martínez et al. (2009) showed that the percent of pectin solubilisation from sugar cane bagasse was increased from 24 to 58% when heating condition changed from 140 to 167 °C.

Heating for longer than 25 min at temperatures above 120 °C resulted in lower amounts of solubilised polymeric pectin. It is likely that the same amount of pectin is solubilised, but more of this pectin is degraded to its GalUA units and is soluble in EtOH rather than precipitated by it. Lim et al. (2015) reported that hydrolysis of pectin into its individual GalUA subunits can take place when pectin solutions are heated at 90 °C. The pectin analysis technique used in this study Blumenkrantz & Asboe-Hansen (1973) cannot quantify the amount of pectin subunits soluble in EtOH due to high concentration of co-extracted sugars, also present in the supernatant. These sugars contributed to a dark background in the assay masking the colour developed by pectin. Therefore, in order to

capture the extent of this breakdown to mono/di-GalUA, EtOH-soluble fractions were assayed by HPLC.

Figure 4.6B shows the production of mono/di-GalUA during heat treatment. Production of mono-/di-GalUA slightly increased during heating of pomace at 90 and 100 °C, while at the severe conditions of 120, 130 and 140 °C, production of mono-/di-GalUA was accelerated. Miyazawa et al. (2004) showed that the degradation of unesterified pectin into its subunits is much easier than for highly esterified pectin. In the current study, apple pomace pectin had a DE of 54% before thermal treatment. Therefore, some of this pectin might have been more sensitive to decomposition from the non-methylated sites.

In line with the other results, the amount of pectin remaining insoluble in the pellet decreased with increasing temperature and time (Figure 4.6C).

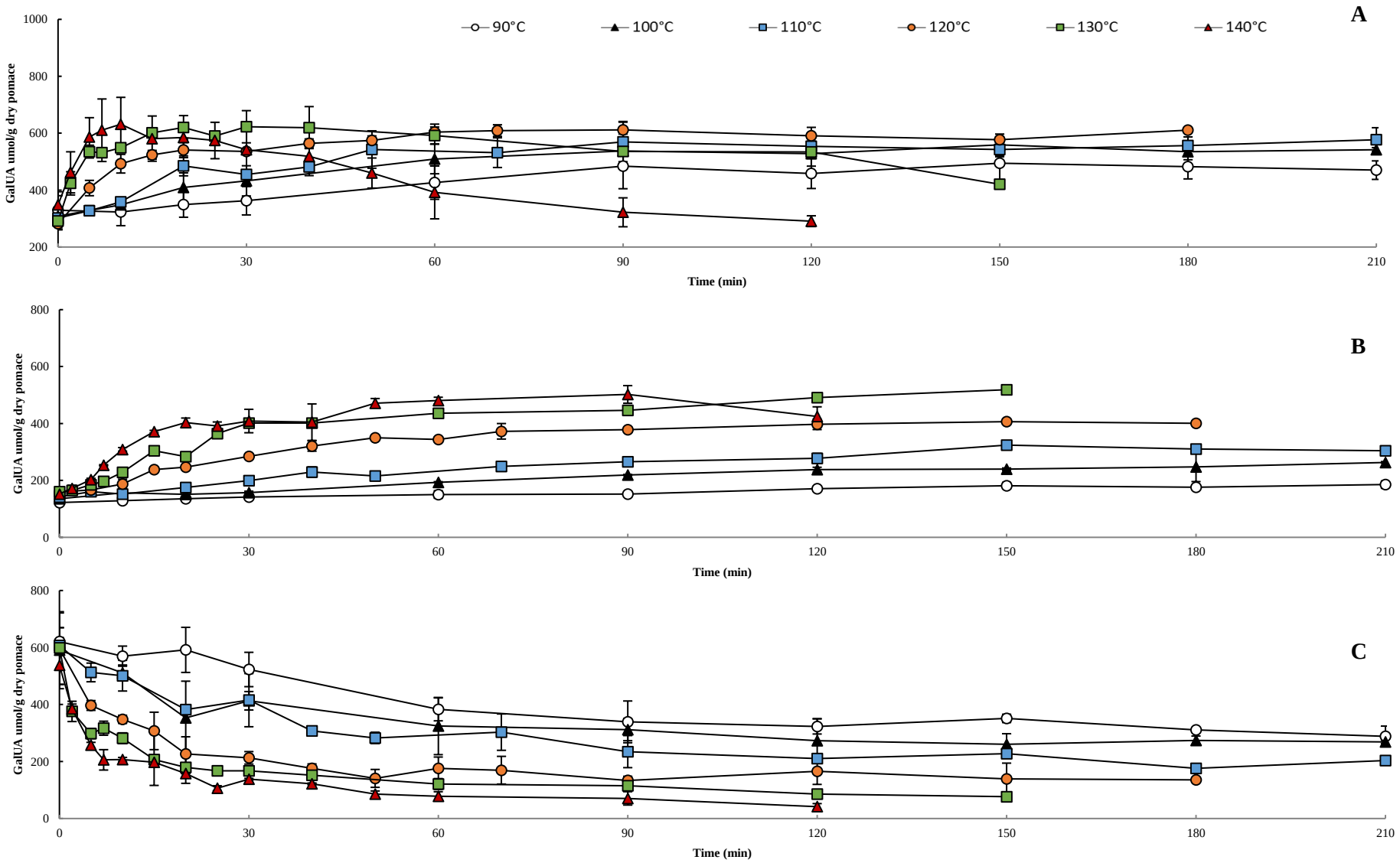


Figure 4.6 Kinetic of solubilised pectin in supernatant (A), mono-galacturonic acid production (B) and in-solubilised pectin remained in pomace (C) during hydrothermal treatment of apple pomace at various temperatures of 90-140 °C and time between 0-210 min. The error bars represent the maximum and minimum value of two replicate samples.

Kinetic prediction of pectin solubilisation

The differential rate equation for a first order reaction, which reached an equilibrium concentration, is given below:

Equation 4.2 First order differential rate equation

$$Rate = \frac{dC_p}{dt} = -K(C_p - C_{eq})$$

Where C_p is concentration of pectin at a time, C_{eq} is concentration of pectin at equilibrium point. K is the rate constant of the pectin solubilisation reaction.

$$\int_{C0}^{C_p} \frac{1}{(C_p - C_{eq})} dC_p = -K \int_0^t dt$$

Where C_0 is the concentration of solubilised pectin at time (t) of 0 min.

$$Ln \frac{C_p - C_{eq}}{C_0 - C_{eq}} = -Kt$$

If X will be the extent of the reaction, then:

$$X = 1 - \frac{C_p - C_{eq}}{C_0 - C_{eq}}$$

Adding the extent of reaction into the equation above will give:

$$1 - X = \exp(-Kt)$$

K is given by Arrhenius equation as:

$$K = K_{ref} * \exp(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{T}{T_{ref}}\right))$$

Where K_{ref} is the reference rate constant at reference temperature (T_{ref}).

This will lead to the differential rate equation to:

$$t = -\frac{Ln\,(1 - X)}{K_{ref} * \exp(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{T}{T_{ref}}\right))}$$

Solubilised pectin at RT (18.4 µmol GalUA/mL) was considered as the concentration of pectin at t= 0 min. In this study 100 °C was chosen as the reference temperature (T_{ref}) and rate constant (K_{ref}). Concentration of solubilised pectin at 100 °C reached a plateau around 33 µmol GalUA/mL

when heating for 90 min. This value was also taken as the equilibrium concentration of solubilised pectin (C_{eq}) at this temperature. Therefore, from the above equation rate constant and activation energy for pectin solubilisation at 100 °C will be 0.030 (1/min) and 72 (kJ/mol), respectively. Figure 4.7, shows the actual measurement and predicted values of pectin solubilisation at temperature between 90-140 °C and incubation times between 0-360 min.

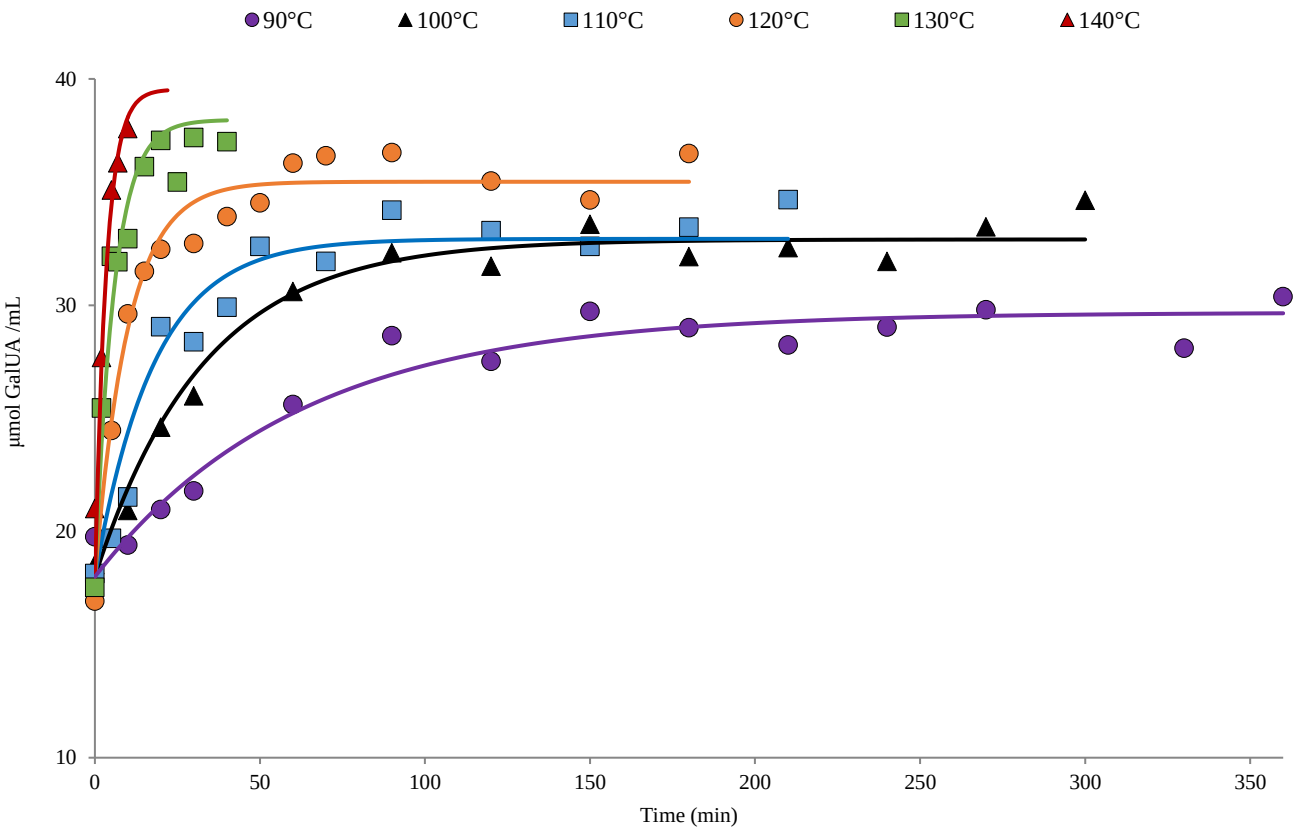


Figure 4.7 Concentration of solubilised pectin in supernatant while heating apple pomace at 90-140 °C for 0-360 min. Actual measurements of pectin (symbols) and predicted model (solid lines) using the equation 4.7.

The kinetic model fitted for solubilised pectin showed a good agreement between predicted and measured results with an R^2 of 0.95 at the reference temperature of 100 °C. This information can be used for predicting the concentration of solubilised pectin in other heating conditions.

4.4.2 Kinetics of the Pectin Fragmentation During Hydrothermal Treatment

Pectin may undergo depolymerisation reactions such as acid hydrolysis and β-elimination when heated. Although the specific nature of these reactions is not very clear, it is known that they are temperature dependent (Sila et al., 2009; Berjenholt, 2010). It is known that the acid hydrolysis reaction takes place mostly at temperatures > 80 °C and the glycosidic linkage between GalUA

residues in the pectin chain is broken (Diaz et al., 2007; Fraeye et al., 2007). This reaction forms a reducing end group on the broken site of the pectin chain. The second thermal degradation reaction on pectin is β-elimination. When this reaction is taking place the hydrogen atom at the C-5 position of GalUA will be removed, resulted in breaking the pectin polymer. This reaction forms a reducing end group on one side and a double bond (at non-reducing end) of the broken chain. The number of reducing end groups (HRGs) formed from these reactions, can be measured according to the method explained in Section 4.2.5 and 4.2.6. The formation of double bonds (UnUs) can be identified by measuring absorbance at 235 nm. Kinetics of these reactions were monitored in the EtOH-insoluble fraction of supernatant in this study. Figure 4.8 shows the formation of HRGs (A) and UnUs (B) during the hydrothermal treatment of the pomace at various temperatures and times.

Concentration of HRGs in the EtOH-insoluble supernatant fraction increased only slightly during heating from 0-210 min to 1.2 and 3.3 μmol GalUA/g dry pomace when pomace was heated at 90 and 100 °C (Figure 4.8A). Heating at temperatures >110 °C accelerated production of HRG with the maximum production of 9.8 μmol GalUA/g dry pomace at 140 °C after 20 min. Further heating at higher temperatures resulted in decreasing in the amount of HRG suspected to be due to further degradation of pectin into GalUA sub-units (mono-/di-GalUA) which are then degraded to other products.

The UnUs in the EtOH-insoluble supernatant fraction increased slightly over temperature and time (Figure 4.8B). At temperatures ≥120 °C, amounts of UnUs reached ~ 3.7 μmol GalUA/g dry pomace and stayed constant for the rest of the heating time. That UnUs reached a plateau suggests that the conditions for the reaction occurring were not being met (i.e., no esterification condition). This suggests that β-elimination is a self-limiting reaction in heated apple pomace.

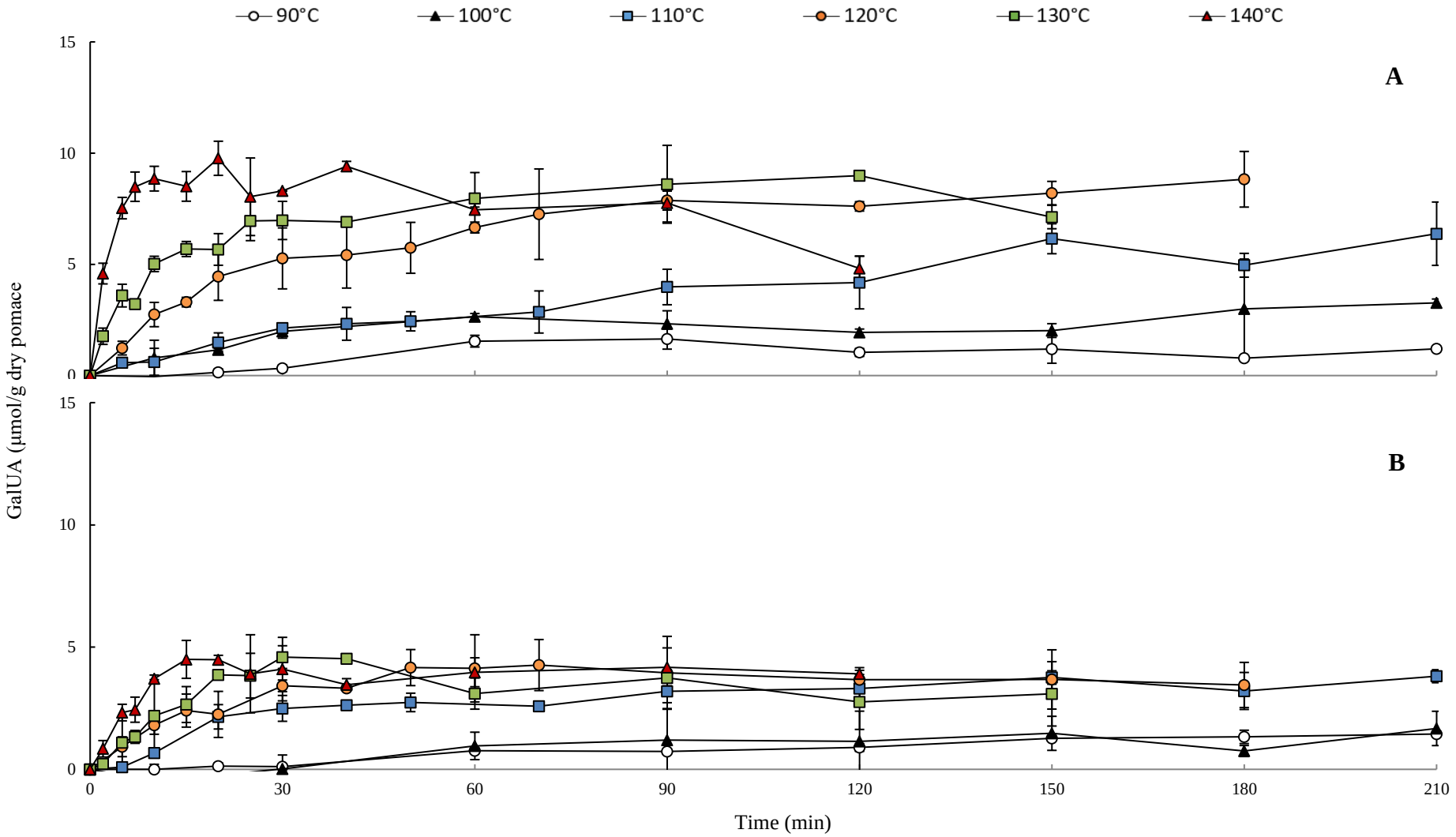


Figure 4.8 De-polymerisation of the apple pomace solubilised pectin through acid hydrolysis (A) and β-elimination (B) while heat treatments at 90-140 °C for duration of 0-210 min. The error bars represent the maximum and minimum value of two replicate samples.

De-polymerisation reactions can directly affect the molecular weight, viscosity and the gelling properties of solubilised pectin. Heating fruit and vegetable with naturally high pH such as carrots (pH~ 6.2) usually undergo β-elimination as the dominant depolymerisation reaction (Greve et al., 1994; Schultz et al., 2014). However, Krall et al. (1998) suggested that softening in acidic fruit tissues during heating is more related to the acid hydrolysis reaction. Degradation of passionfruit pectin through acid hydrolysis at temperatures between 80-160 °C and duration times between 0-25 min was highly dependent on temperature (Klinchongkon et al., 2017). Klinchongkon et al. (2017) also reported that the amount of HRG increased from 96 to 259 GalUA μmol /g pectin when heating for 5 min at 140 °C. Acid hydrolysis reactions can also help to solubilise more pectin into the aqueous phase as well as increase the sugar content, especially the sugars that may be side branches of pectin (RG-I). In the current study, the concentration of HRGs and UnUs increased at temperatures (between 90 to 140 °C) and incubation times, however these amounts were much lower than the amount of solubilised pectin (Figure 4.6A).

Pectin is known to be resistant to hydrolysis in its optimal pH (natural pH of the origin of pectin) but becomes more sensitive to hydrolysis at pH above or below its optimal pH when exposed to high temperatures (Sila et al., 2009; Berjenholt, 2010; Dominiak et al., 2014). Krall et al. (1998) reported that regardless of the degree of esterification citrus pectin at pH 3.5 formed minimum amounts of HRGs when heated at 100 °C. In the current study, pH of apple pomace was not adjusted before heat treatment. Acid hydrolysis can only influence the amounts of HRGs but β-elimination produces both UnUs and HRGs. With this in mind, the amount of HRGs produced in this study was about twice that of UnUs. This means that both reactions were taking place to a limited extent while heating pomace.

4.4.3 Effects of Heating on Degree of Esterification of Pectin

The DE of pectin is conventionally used for classifying pectin into two very broad categories of low methyl-esterified pectin (DE <50%) and high methyl-esterified pectin (DE >50%) (Alba et al., 2017; Chan et al., 2017). In the current study, DE of the fresh apple pomace (in total) was in the mid range of 54%. This is an average measure so is likely to be made up of a number of populations of pectins with different DEs.

Figure 4.9 shows DE of the pectin in supernatant and pellet of heated apple pomace. The DE of solubilised pectin at RT (without heating) was less than 30%. Previously, pectin solubilisation showed an increase in the amounts of pectin at higher temperatures. Higher DE values measured in supernatant could be due to greater amounts of solubilised pectin which carried more ester groups than the pectin already solubilised at RT. Figure 4.6 showed that the amounts of solubilised pectin started to decrease after 10and 40 min of heating at 130 and 140 °C, respectively due to

degradation of pectin into its subunits (mono-GalUA). The DE did not show a decrease however, it is possible that depolymerisation may happen faster at the unesterified sites on this pectin. Therefore, the esterified groups remaining on the smaller amounts of remaining pectin would have even higher values than before. The increasing trend in DE of solubilised pectin was observed mostly at temperatures > 120 °C. These results of DE in solubilised pectin were in good agreement with Wang et al. (2014) who extracted pectin with a DE of 90% from apple pomace and citrus peel using subcritical water at 170 °C.

In the current study, the DE of insoluble pectin remaining in the pellet (Figure 4.9B) after heating at temperatures between 90 and 120 °C (for >180 min) were mostly in the range of 50-80%. However, increasing temperature to >120 °C and heating for more than 40 min resulted in higher DE measurements (between 60-100%). It is clear that the pectin polymers remaining insoluble at this stage were mostly esterified. It is probable, that the highly esterified pectin is more resistant to hydrolysis by heat.

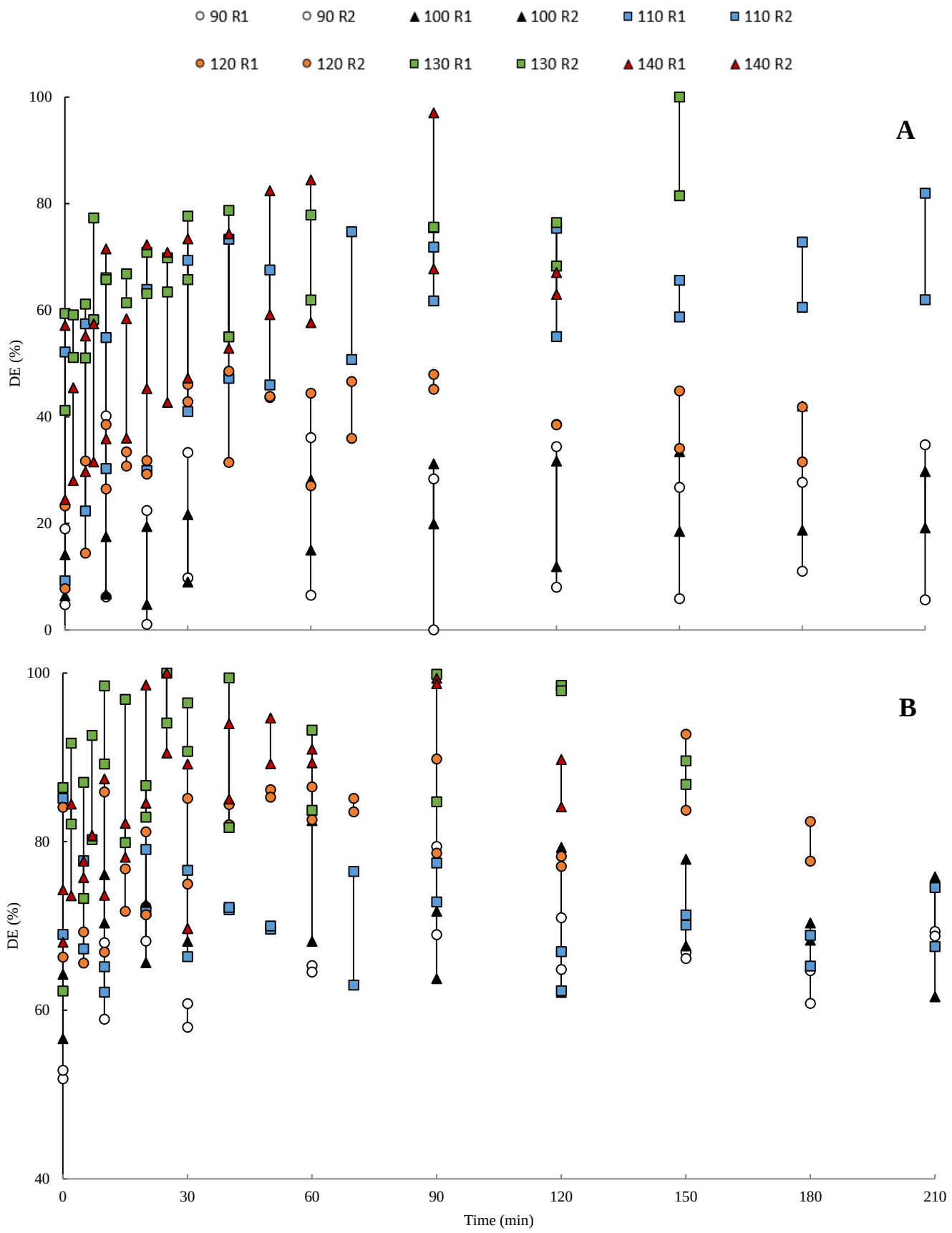


Figure 4.9 Degree of esterification of pectin in supernatant (A) and pellet (B) after heating apple pomace at temperatures between 90-140 °C and time durations between 0-210 min. Each point two similar symbols shows two experimental replications of R1 and R2 for each temperature.

4.4.4 Degradation of Sugars and Production of Unwanted Components During Hydrothermal Treatment

4.4.4.1 Sugar Degradation

In the current study sucrose, glucose and fructose made up ~20% of the dry weight of fresh apple pomace as received (Table 4.3). Sucrose is a disaccharide with high sensitivity to temperature (Oomori et al., 2004; Haghighat Khajavi et al., 2005). Hydrolysing the glycosidic bonds of sucrose in aqueous solution can happen thermally or enzymatically. Both reactions produce glucose and fructose. Heat-related degradation of sucrose depends on the operating temperature and time (Haghighat Khajavi et al., 2005). Heating apple pomace at temperatures higher than 110 °C resulted in loss of sucrose (Figure 4.10A) with increased amounts of glucose and fructose (Figure 4.10B & C). Heating at 130 and 140 °C for longer than 40 and 25 min showed complete loss of sucrose (Figure 4.10A). This was accompanied by increased glucose and fructose concentrations to their maximum values of ~4 and 5 μmol/mL, respectively. Decomposition of sucrose is usually accompanied by an increase in hydrogen ion concentration (Haghighat Khajavi et al., 2005). Edye (2001) contends that hydrogen ion provides a catalyst for more sucrose degradation. It has been reported by Haghighat Khajavi et al. (2005) that heat associated decomposition of sucrose does not obey first order kinetics due to the decreasing pH of the solution and recycling catalyst for its faster degradation. In this study pH of the supernatants were measured after each heat treatment but no reduction in the pH was observed from the original untreated pomace value of pH 3.5.

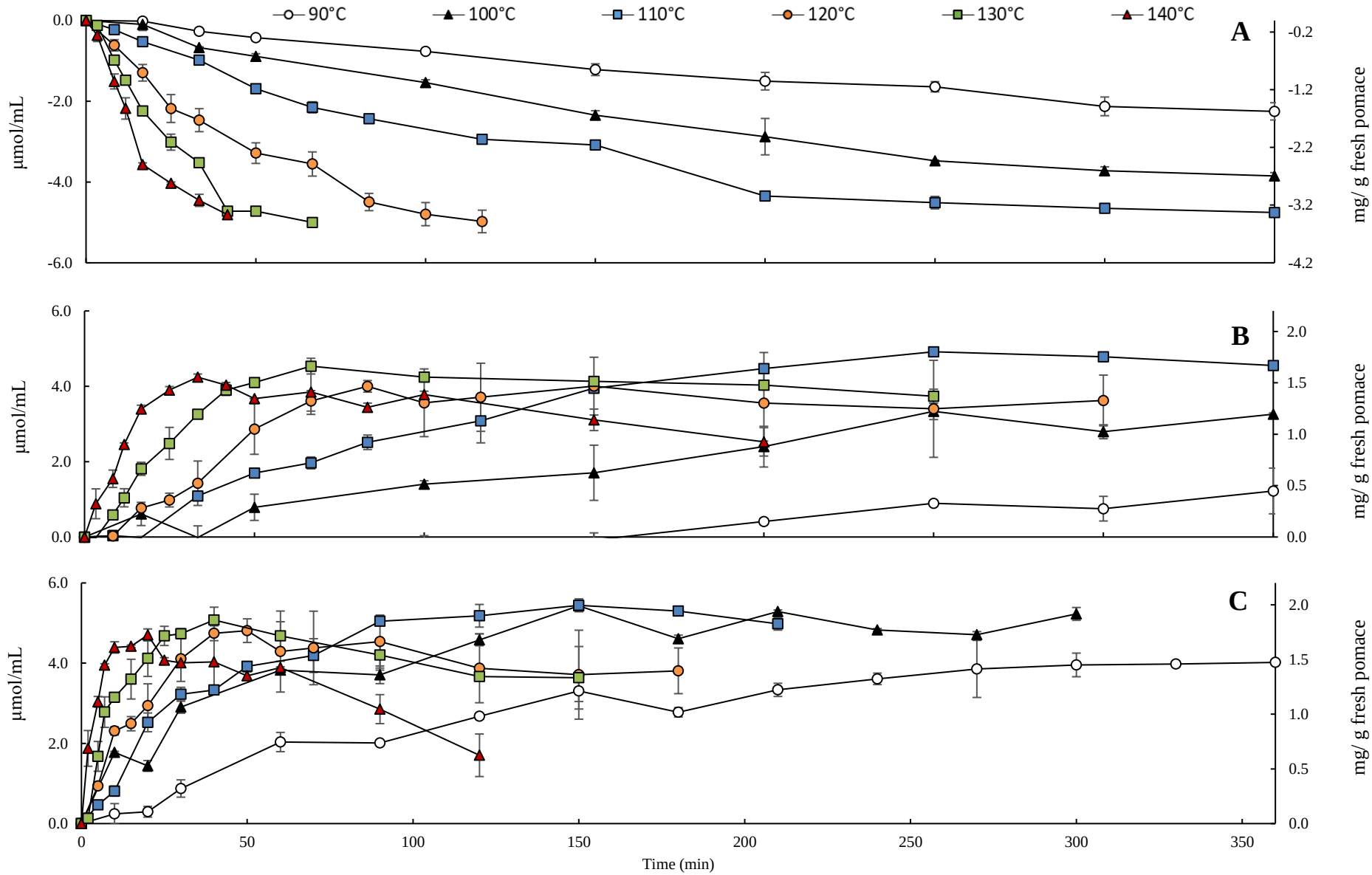


Figure 4.10 Changes in concentration of sugars of sucrose (A) glucose (B) and fructose (C) in apple pomace while heating at 90-140 °C for 0-360 min. The error bars represent the maximum and minimum value of two replicate samples.

Loss of the sucrose and maybe other cell wall saccharides (hemicellulose and xyloglucan branches) from the apple pomace resulted in increasing the concentration of the simple sugars (e.g. glucose and fructose) in the supernatant. Prolonged heating at severe conditions (≥120 °C) resulted in decreasing amounts of glucose and fructose in supernatant. This is likely to be due to degradation of these sugars into other secondary products such as 5-HMF and organic acids (e.g. formic acid and lactic acid) (Choudhary et al., 2013; Gomes et al., 2015; Haghighat Khajavi et al., 2005; Kuhnel et al., 2011; Yin et al., 2011; Zhang et al., 2017). This is addressed in the following sections.

4.4.4.2 5-HMF and Furfural Production

Furfural and 5-HMF are known to be secondary degradation products from heating of pentose and hexose sugars (Selen Burdurlu et al., 2003; Zhao et al., 2007). They are usually monitored in processed food products due to potential health risks when consumed. Production of furfural is related to decarboxylation followed by de-hydration of GalUA under severe heating conditions (>130 °C) (Kuhnel et al., 2011; Martins et al., 2000; Voigt et al., 2010). The formation of 5-HMF however, is linked to enolisation of the hexose sugars (Steinbach et al., 2018). Fructose and glucose (most likely to be vulnerable to enolisation) reached their maximum concentration while heating at 130 and 140 °C.

Production of these components during apple pomace hydrothermal treatment is given in Figure 4.11. Production of furfural and 5-HMF at 90, 100 and 110 °C was negligible, but increasing temperatures to ≥120 °C resulted in significant and time-dependent increase in production of these components. According to Figure 4.11, heating for 20 min at 130 and 140 °C resulted in a slight increase in the concentration of 5-HMF. However, continued heating resulted in reduction in concentration of fructose and glucose and an accelerated production of 5-HMF in supernatant.

The results of this study were in good agreement with the study conducted by Kuhnel et al. (2011), who examined the production of 5-HMF and furfural from sugar beet pulp in presence of water at 120-170 °C. They also found a temperature-time dependency for 5-HMF and furfural production. Temperature was found to be the key element as longer incubation time at lower temperature was not very effective to form these components. Steinbach et al. (2018) showed that conversion of fructose to 5-HMF is generally faster than from glucose because glucose conversion needs an extra step of glucose isomerisation to fructose. They also showed that the heating 1% w/w solutions of glucose or fructose in 5 mmol/L sulphuric acid for 10 min at 220 °C showed conversion of 25% of glucose and 96% of fructose to 5-HMF. Haghighat Khajavi et al. (2005) showed that sucrose degradation resulted in production of 5-HMF.

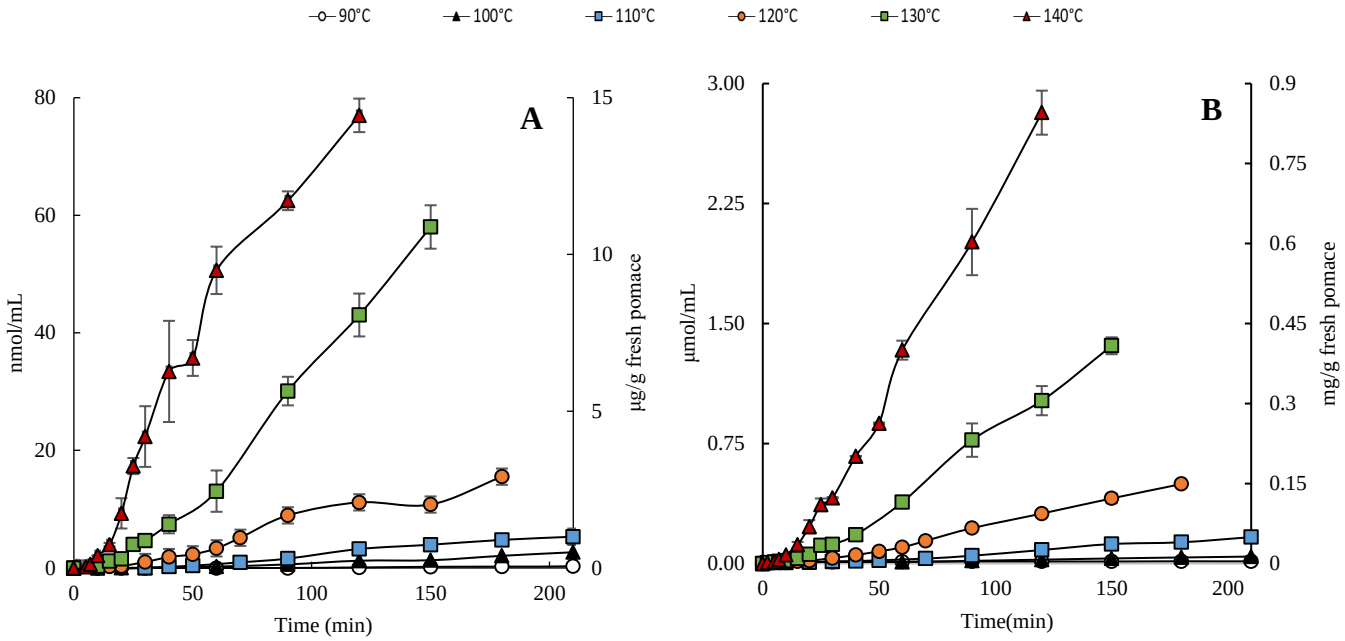


Figure 4.11 Production of furfural (A) and 5-HMF (B) in supernatant during heating apple pomace at temperatures of 90-140 °C for 0-210 min. The error bars represent the maximum and minimum value of two replicate samples.

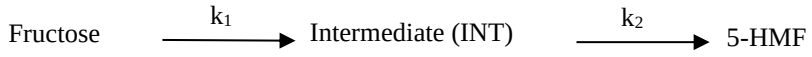
Furfural formation is linked to decarboxylation of pentoses (such as xylose, ribose and arabinose) and hexuronic acid (such as glucuronic acid and galacturonic acid). Formation of furfural increases at elevated temperatures (especially >130 °C) and with higher concentration of hydrogen ions (Dounlop, 1948; Usuki et al., 2008). In the current study, the concentration of substrate for furfural formation (mono-GalUA) started to increase at temperatures >120 °C, which is in good agreement with higher formation of furfural at 130 and 140 °C. It is important to mention that furfural can also be formed from glucose and fructose by conversion of these sugars into pentoses (e.g. xylose) (Steinbach et al., 2018). Comparison between the formation of furfural and 5-HMF in Figure 4.11 reveals that under the conditions of the study production of 5-HMF was more dominant than furfural. This can be related to the higher concentration of available substrate (fructose) for 5-HMF formation than furfural. Degradation of these components to other products such as organic acids have different rates as well.

Kinetic Prediction of 5-HMF Production

5-Hydroxymethyl furfural can be found in processed food products such as apple and citrus juices, dried fruits and coffee. Formation of 5-HMF directly depends on the processing temperature and storage duration. This component may have negative effects on human health (Shapla et al., 2018). Therefore, a limited concentration of 5-HMF 20 mg 5-HMF/ kg juice in fruit juices has been specified by European Economic Community Association of the Industry of Juices and

Nectars (Tamer et al., 2019) and a limit of 25 mg 5-HMF /kg concentrates has been stipulated by the International Federation of Fruit Juice Processors (IFFJP) (IFFJP, 1974).

In the current study, understanding the kinetics of 5-HMF formation can be used to avoid the extensive processing conditions that may introduce an unwanted, non-nutritive and possible detrimental component. It has been explained before that 5-HMF can be produced by further degradation of hexose sugars especially fructose. A summarized pathway of 5-HMF production is given below:



Kinetics of 5-HMF formation during heating of apple pomace have been determined in cooperation with Professor John Bronlund. Production of 5-HMF from fructose has two steps of firstly formation of an intermediate from fructose and secondly- conversion of the intermediate into 5-HMF (Enomoto et al., 2018; Wang et al., 2011). The first step is obeying a first order reaction which is dependent on the rate constant of fructose conversion (k_1) and accumulation of the intermediate. The second step, converting intermediate to 5-HMF, also obeys the first order reaction kinetics: rate depends on the concentration of intermediate. As the second step rate constant (k_2) is small, the pool of intermediate is likely to be small. Once the pool has built, the second reaction step can be expected to be fast enough to allow the first reaction's kinetics to dominate. Therefore, the overall reaction to produce 5-HMF is obeying the first order kinetics. Production of 5-HMF at 90 °C has been ignored as the concentration of 5-HMF at this temperature was very low.

Rate of formation of intermediate is given in equation 4.8 below:

Equation 4.3 Rate equation of intermediate formation

$$\frac{dC_{INT}}{dt} = k_1 C_f - k_2 C_{INT}$$

Where C_{INT} is concentration of intermediate, C_f is concentration of fructose, k_1 is the rate constant to convert fructose to intermediate and k_2 is the rate constant to convert intermediate to 5-HMF.

$$\int_0^{C_{INT}} \frac{dC_{INT}}{k_1 C_f - k_2 C_{INT}} = \int_0^t dt$$

$$\frac{-1}{k_2} \ln \frac{k_1 C_f - k_2 C_{INT}}{k_1 C_f} = \mathrm{t}$$

$$\ln \frac{k_1 C_f - k_2 C_{INT}}{k_1 C_f} = -k_2 t$$

$$k_2 C_{INT} = k_1 C_f - k_1 C_f \exp(-k_2 t)$$

$$k_2 C_{INT} = k_1 C_f (1 - \exp(-k_2 t))$$

Rate of the 5-HMF formation is given in equation 4.9 below:

Equation 4.4 Rate of 5-HMF formation

$$\frac{dC_{HMF}}{dt} = k_2 C_{INT} = k_1 C_f (1 - exp(-k_2 t))$$

$$dC_{HMF} = k_1 C_f \int_0^t (1 - \exp(-k_2 t))\, dt$$

$$C_{HMF} = k_1 C_f (\mathrm{t} + \frac{\exp(-k_2 t)}{k_2} - \exp\frac{0}{k_2})$$

$$C_{HMF} = k_1 C_f (\mathrm{t} - \frac{1 - \exp(-k_2 t)}{k_2})$$

$$C_{HMF} = \frac{k_1 C_f}{k_2} (k_2 \mathrm{t} - (1 - \exp(-k_2 t))$$

According to the equation given above, three assumptions were taken for 5-HMF production:

1- k_2 is much smaller than k_1. This means that the intermediate converts to 5-HMF very quickly. Therefore, rate of the equation highly depends on k_1.
2- Fructose concentration (C_f), assumed to be a constant value. This amount was determined from the average of fructose concentration in all treatments and times, except 90 °C treatment. In the current study, the effect of other sugars such as glucose to 5-HMF has been ignored due to faster degradation of fructose.
3- From assumptions 1 and 2, it can then be assumed that $\frac{C_f}{k_2}$ is a constant and small value which can be ignored in the last equation.

Re-arrangement of the last equation gives:

$$C_{HMF} = k_1 (k_2 \mathrm{t} - (1 - \exp(-k_2 t))$$

This leads to:

$$k_1 = k_{1ref}\, exp(\frac{E_1}{R}(\frac{1}{T_{ref}} - \frac{1}{T}))$$

$$k_2 = k_{2ref}\, exp(\frac{E_2}{R}(\frac{1}{T_{ref}} - \frac{1}{T}))$$

Therefore, the following values were obtained:

Parameter	Unit	1	2
k_{ref}	1/min	0.307781	0.002506
E	J/mol	113971.8	105249.8
T_{ref}	K	373.15	373.15

The constant k_1 and k_2 were calculated at each temperature as well as the predicted concentration of 5-HMF production. Figure 4.12 shows the actual measurement and predicted values of 5-HMF production using k_1 and k_2.

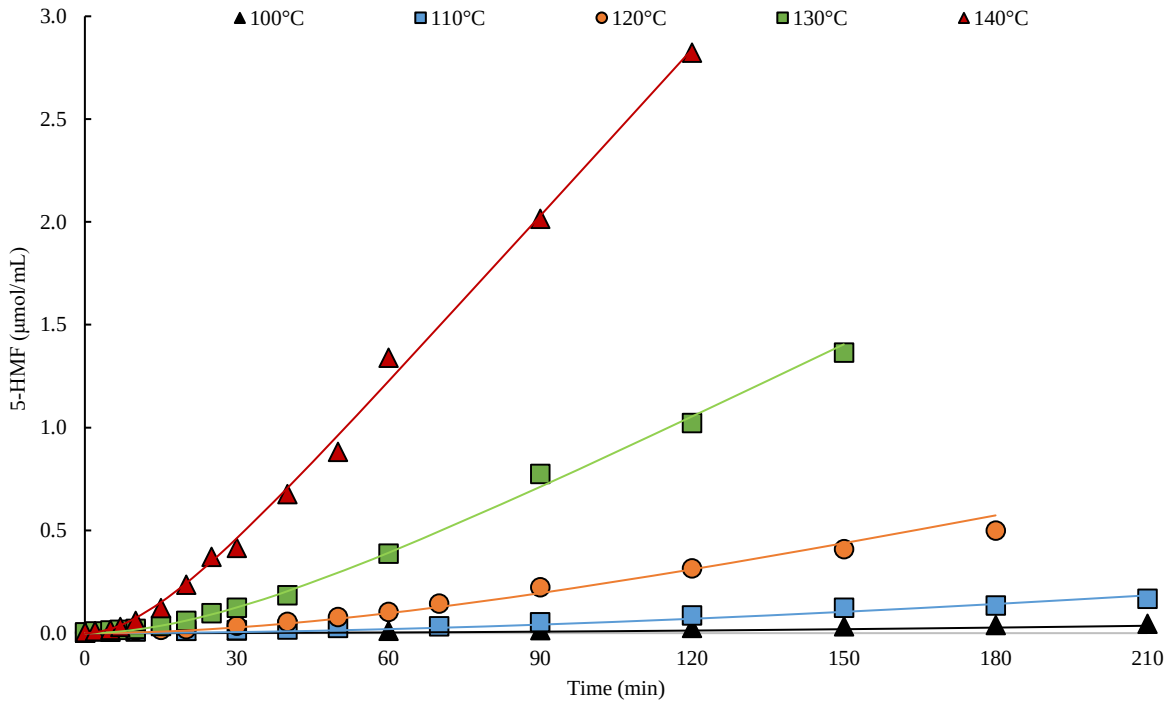


Figure 4.12 Concentration of 5-HMF produced while heating apple pomace at 90-140 °C for 0-210 min. Actual measurements of 5-HMF production (symbols) and predicted model (solid lines).

The kinetic model fitted for 5-HMF production showed an excellent prediction of formation of this component. The R^2 for this kinetic model at reference temperature was 0.99. This information can be used for predicting the concentration of 5-HMF in other heating conditions and especially when applying the process at greater scale.

4.4.4.3 Organic Acid Production

Organic acids were also produced during heat treatments. Formic acid and acetic acid are usually produced as the secondary products of 5-HMF and furfural degradation. The rate of furfural decomposition depends on the concentration of the accumulated furfural, as well as the

concentration of hydrogen ions present. At temperatures ≥100 °C, water can act like acid as the source of hydrogen ions and can be considered as a reactant. Furfural degradation into organic acids (such as acetic acid, formic acid and lactic acid) is also known to be autocatalytic, but only when its contribution to the initial hydrogen ions in the aqueous solution becomes significant. The possibility of this reaction has been reported by Dounlop (1948).

Figure 4.13 shows the great increase in acetic acid, formic acid and lactic acid production during heating at 90-140 °C. In the current experimental design, heat treatments above 110 °C increased the rate of formic acid production and accelerated the rate of furfural decomposition (Figure 4.13A). Degradation of furfural into the acid components can also accelerate furfural production from its precursors and at the same time as increasing the rate of furfural degradation into acidic components.

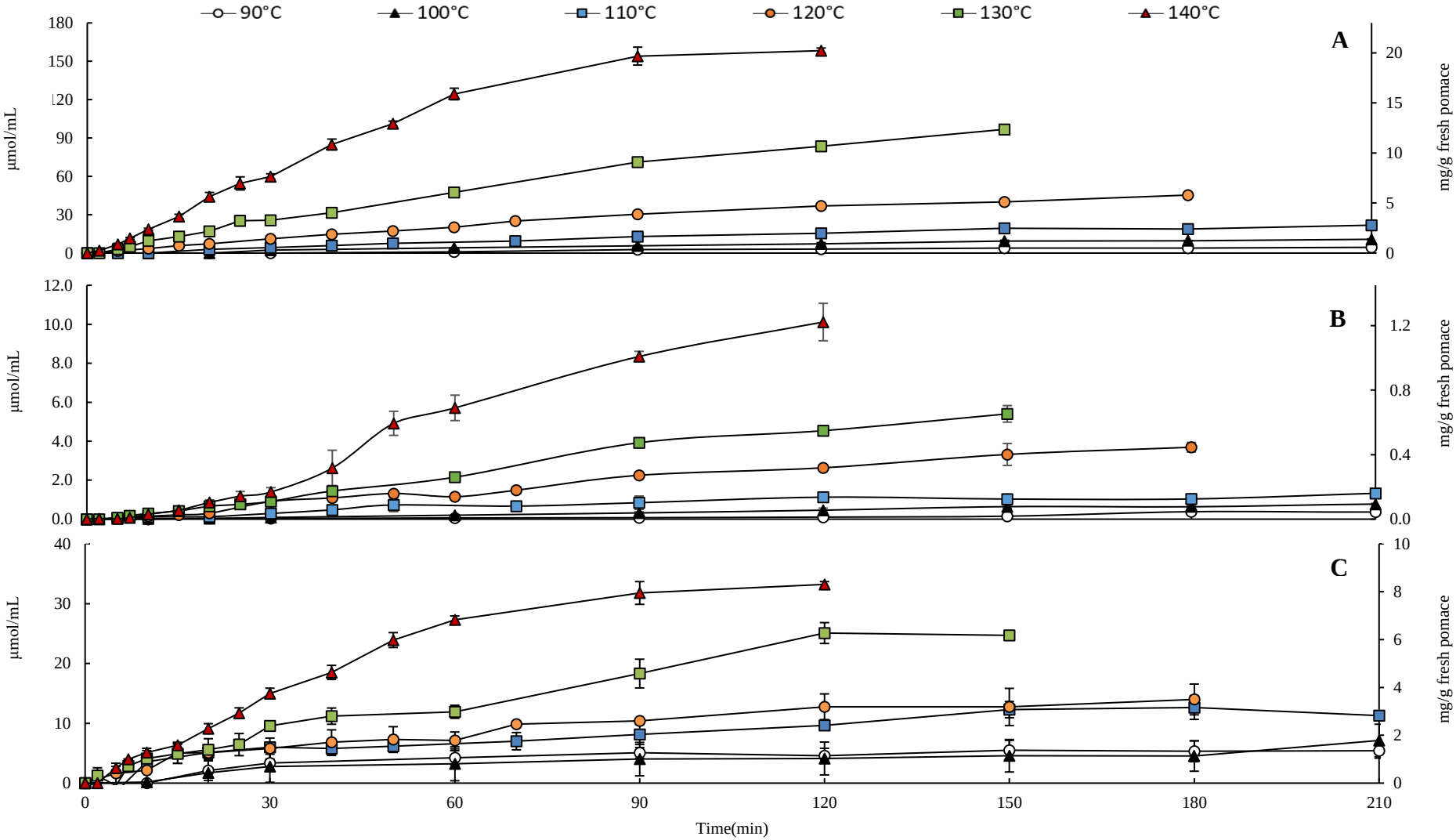


Figure 4.13 Production of degradation components of formic acid (A), acetic acid (B) and lactic acid (C) in supernatant during hydrothermal treatments at 90-140 °C between 0-210 min. The error bars represent the maximum and minimum value of two replicate samples.

Results of this study were in good agreement with Lamminpää et al. (2016) who heated xylose solutions at temperatures ≥130 °C. They reported that that production of formic acid can increase the rate of furfural production as well as speed up decomposition of furfural. This was thought to be due to higher concentration of hydrogen ions in the system. Usuki et al. (2008) reported that GalUA is more susceptible to degrade into furfural and its degradation products (e.g., organic acids) during heating than other pentoses. In addition to furfural, 5-HMF can undergo degradation reactions at high temperatures (≥130 °C) resulting in formation of carboxylic acids e.g., formic acid, acetic acids and lactic acid. The main product of 5-HMF degradation was shown to be formic acid (Kuhnel et al., 2011; Dashtban et al., 2012). These results were in good agreement with higher amounts of formic acid produced in the current study (Figure 4.13A).
It is also possible that continuous heating of the pomace promoted the Maillard reaction. Acetic acid and lactic acid may also increase as secondary products of this reaction (Martins et al., 2000; Voigt et al., 2010; Kuhnel et al., 2011).

4.5 Conclusions

Apple pomace is a good source of soluble and insoluble fibre and sugars. These components can be heat-sensitive, undergoing different reactions such as de-polymerisation, de-esterification, degradation and browning reactions under certain conditions of temperature and time. These reactions can change the structural and functional properties of the cell wall components and directly affect the technical application of apple pomace. The work in this chapter was conducted to understand the kinetics of the main reactions involved in the pomace especially those of pectin when pomace undergoes hydrothermal treatments at different temperatures and times.

Results of this study revealed that increasing temperature and time on homogenised apple pomace, up to a certain point, can increase the amounts of soluble pectin. However, prolonged heating can drive de-polymerisation reactions such as acid hydrolysis and β-elimination as indicated by increased HRGs and UnUs. As these reactions proceed, these are increased amounts of pectin subunits (mono- and di- GalUA) and formation of unwanted products such as 5-HMF, furfural and organic acids. The remaining polymeric pectin was highly esterified, suggesting that less esterified pectin was more sensitive to hydrothermal treatment. These results suggested that pectin solubilisation are mostly happening from the unesterified sites of pectin molecules.

The kinetics of pectin solubilisation and 5-HMF formation reactions were modelled successfully. The order of reaction, rate constant (at reference temperature) and activation energy of these reactions are given in the table below:

Reaction	Order of reaction	T_{ref}	K_{ref}	E
Pectin solubilisation	1st	100 °C	0.021 min^{-1}	81 kJ mol^{-1}
5-HMF formation	1st	100 °C	0.0025 min^{-1}	105 kJ mol^{-1}

Understanding the kinetics of the main reactions involved in apple pomace during hydrothermal treatment can be used for predicting the extent of any selected reaction, and for finding the best operating condition to achieve the expected properties in the heat-treated apple pomace. Studying the kinetics of pomace reactions is a useful approach for scaling up the heating process to achieve a safe product with expected properties.

CHAPTER 5 Scaling Up the Hydrothermal Process to Pilot Plant

In Chapter 4, the kinetics of the reactions involved in the hydrothermal processing of pomace were studied at temperatures between 90-140 °C. This information can be used for modelling and scaling up the heat treatment as well as finding the best heating condition (temperature and time) to achieve specific extents of reactions. Chapter 5 outlines the challenges of modelling and scaling up the heat treatment step to the pilot plant.

5.1 Introduction

The transition of a process from the laboratory to the pilot plant and finally to the industrial scale is not only about increasing the quantity of the materials about 10-100 times but also using different processing equipment (William, 2013). For example, hydrothermal treatment of pomace in this study at laboratory scale was done in small tubular reactors submerged in a silicon oil bath. This system heated quickly and was easy to control and provided close to uniform heat treatment. In contrast, heating at pilot plant scale can be applied using a steam-jacketed pan, extruder, heat exchanger, direct steam injection or using a retort. The selection of the equipment will directly affect the processing time and temperature to achieve a desired characteristic in a sample.

Each of these equipment items has advantages and disadvantages. For instance, operating a steam jacket pan is easy, but can affect the moisture content of the product as the pan is open to the environment. Direct steam injection will increase the water content of the final product after heat treatment. Indirect heat exchangers are usually in the shape of plate or tube, through which (in the case of this study) pumping the semi-solid pomace material into the system would be difficult. In this part of the study, a pilot scale retort was used as the heating equipment for hydrothermal processing with pomace sealed inside a flat pouch in a shape of a slab. With this system heat transfer can be relatively slow, and temperature variations could occur with the position in the product. The surface will heat faster than the centre.

The objective of Chapter 5 was to find an operating condition (time and temperature) for the heat treatment step which can solubilise pectin near two times more than the amount which was already dissolved at room temperature (RT) or near the maximum amounts of solubilised pectin. This goal is to be met on average across the bulk of the pomace pouch. It was favourable for this study that the solubilised pectin will be mostly in the form of the polymer because small oligomers and monomeric forms of pectin may result in the production of other unwanted secondary products in heat-treated pomace (refer to Chapter 4).

Hydroxymethylfurfural (5-HMF) is produced as a secondary product while heating fruit and vegetables and is known to be a toxic component when is excess (Selen Burdurlu et al., 2003; Zhao et al., 2007). The international agency of research on cancer introduced it as "possibly carcinogenic to humans" (Tamer et al., 2019). It was explained in Chapter 4 that this component is produced during the heating of solutions containing sugars (especially fructose). Therefore, another objective of this study was that production of 5-HMF (on average across the bulk of the pack) should remain in the safe range recommended for apple products. The limit for this component in fruit nectars and concentrates is specified by European Economic Community of Association of the Industry of Juices and Nectars to be 20 mg/kg juice (Tamer et al., 2019). The International Federation of Fruit Juice Processors (IFFJP) reported the maximum allowable concentration of 5-HMF to be 5 mg/L juice or 25 mg/ kg concentrate (IFFJP, 1974).

The prediction of pectin solubilisation and 5-HMF production during heating in the retort was carried out by coupling heat-transfer modelling with the kinetics of two the reactions determined in Chapter 4.

5.2 Material and Methods

5.2.1 Plant Material

Apple pomace was collected from Turners and Growers Apple Juice Concentrate Plant (Hasting, New Zealand). This pomace was drawn from the same batch used in Chapter 4 Section 4.2.1.

5.2.2 Aluminium Laminated Bag

Plain foil pouches (A3 sized) with the internal dimensions of 0.29 m × 0.17 m (length × width) and thickness of 99 μm (Counter Sales Packing System, Tauranga, New Zealand) were used for heat-treating the pomace in the retort.

5.2.3 Pilot Plant Retort

A 900 mm diameter rotary static multi microflow pilot plant retort (Steriflow Thermal Processing, Roanne, France) was used for the heat treatment of the pomace. The heating process was applied with steam at 125 °C and without agitating the retort basket. The cooling down step was achieved with a shower of water and again without agitating the retort basket.

5.2.4 Pectin Analysis

The measurement of the galacturonic acid (GalUA) content as an estimation of pectic content was carried out using the method given in Chapter 3, Section 3.1.7.

5.2.5 5-Hydroxymethyl Furfural Measurements

The determination of the 5-HMF formation was carried out using the method given in Chapter 4, Section 4.2.7.

5.3 Design of Retorting Conditions

Heating samples using a retort is known to be slow. The rate of heating depends on temperature and the position within the sample inside the retort. For example, the centre of the sample will usually heat up or cool down more slowly than the sample close to the surface or edges of the retort pouch. Moreover, due to the uneven heat treatment, throughout the bulk material, reactions such as pectin solubilisation and 5-HMF production will proceed to different extents during the heating cycle.

It was necessary to find a way to predict the temperature as a function of time and position (temperature = f (time, position)), which allows prediction of the extent of the reaction versus time and position using the kinetics characterised in Chapter 4. It was also important to estimate the average levels of products formed from each reaction across the whole volume of the sample. To achieve this level, a mathematical model for heat transfer and chemical reactions in the retort pouches was developed.

5.3.1 The Physical System to be Modelled

Apple pomace was added to the aluminium bags, which were then heat-sealed and located in the retort basket for heat treatment. It is already known that heat transfer into a thin layer product is

faster and more uniform than for a thicker layer. Therefore, the foil pouch was filled to a thickness of 15 mm. In order to keep a solid rectangular shape to the filled bags, aluminium structures were built from expanded metal sheet and channel-section by the Massey University workshop. These structures could fit inside the retort basket and control the spacing between pouches. Figure 5.1 shows the bag, loaded retort basket and retort.

Figure 5.1 A) Aluminium laminated bags filled with pomace and heat-sealed. B and C) metal structure to hold bags in a thin layer inside the retort basket. D) retort.

5.3.2 Heat Treatment of the Pomace in The Retort

Temperature profiles of the retort to reach a set temperature (125 °C) and cool down to 60 °C were recorded continuously through each run using one thermocouple which was located in the middle of the retort basket measuring the condition experienced by the outside of the pomace bags. Both heating and cooling were achieved using a water curtain, in a steam atmosphere. This style of retort is expected to ensure all pomace bags had an identical heating and cooling profile. These heating profiles are given in Figure 5.2. It took about 15 min to heat the retort up to the target temperature (125 °C) and 12 min to cool it down to about 60 °C.

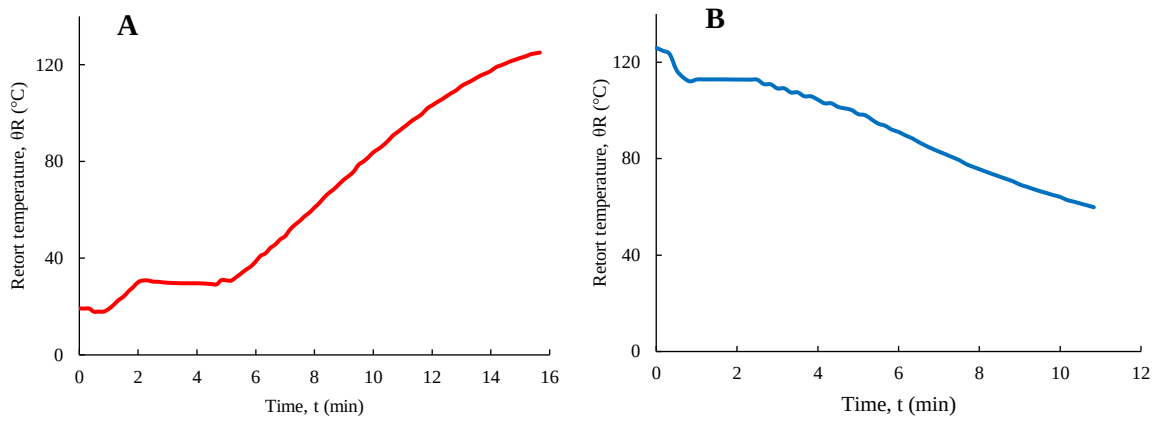


Figure 5.2 Temperature profile of retort during: A) heating up to 125 °C and B) cooling down to 60 °C.

At the end of the retort cycle, the cooling down step was achieved using a combination of compressed air (to remove residual steam and maintain the pressure) and a shower of cold water. Compressed air helped to prevent bags bursting during the cooling cycle of the retort. When the pressure inside the retort reached 0.2 bar (~ 60°C), the door of the retort was opened, and samples could be removed. The bags were taken out of the retort basket and cooled further in an ice bath to stop the reactions. Heat treatment of pomace using retort was done in three separate runs on three different days.

5.3.3 Conceptual Model of the Heat Treatment Process

In this study, the aluminium bag, described in section 5.2.2 was modelled in a 3D situation. The modelled bag had free accessibility to steam and cooling water on all its surfaces. The aim of this model was to predict the temperature-time profile of the pomace and extent of the pectin solubilisation and 5-HMF formation based on the recorded retort temperatures applied to the external surfaces of the bag.

To do this, several assumptions were made:

1. Filled bags with the prepared sample inside the structure have a rectangular block shape without losing or deforming its shape, with the dimensions of 0.29 × 0.17 × 0.015 m, for length, width and height of the bag, respectively.
2. Heat transfer is even from all sides of the rectangular bag.
3. Temperature inside the retort remains constant during the holding time once it reaches 125 °C until cooling.
4. Thermal properties of the pomace material are constant during the heating.
5. Production of 5-HMF obeys zero-order kinetics. According to the kinetics of 5-HMF production in Chapter 4 Section 4.4.4.2, it is known that the reaction rate is dependent on

the conversion rate of the fructose to the intermediate because the conversion rate of the intermediate to 5-HMF can be ignored (very small and fast conversion reaction). Therefore, 5-HMF production is assumed to obey the zero-order kinetic (Appendix 2).

6. Solubilisation of pectin from pomace into the serum phase obeys first order kinetics (refer to Chapter 4, Section 4.4.1).
7. Heat transfer inside the bags can occur from all sides (3D heat flow), and heat transfer from the retort into the samples is a conduction heat transfer until reaching a constant value (near to the maximum retort temperature of 125 °C).

5.3.4 Model Formulation

Due to the assumption explained above that all surfaces are exposed to retort temperature condition, axial symmetry could be applied in each dimension, as shown in Figure 5.3. The solution space in this smaller region can be mirrored in each direction to define the whole geometry.

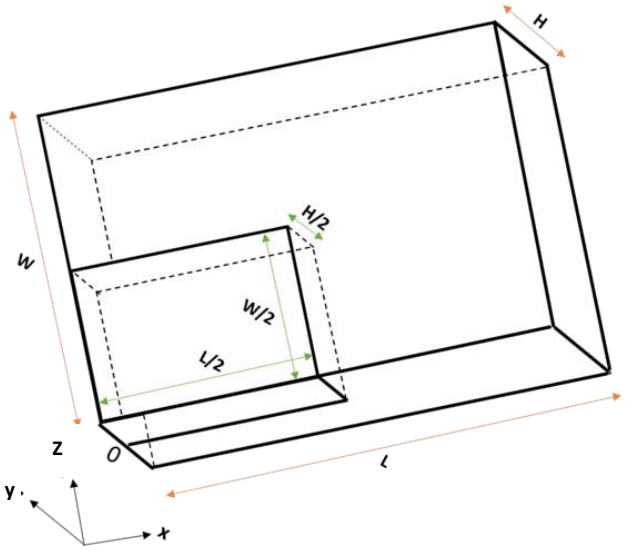


Figure 5.3 Selection of a rectangular piece of filled bag with dimensions of L/2 × W/2 × H/2 from a whole bag with dimensions of L × W × H, using axial symmetry boundary conditions.

According to the Fourier's law conduction heat transfer equation 5.1:

Equation 5.1 Fourier's conduction heat transfer equation.

$$Q = \lambda A \frac{d\theta}{dx}$$

Where here λ is thermal conductivity of the material, A is the surface heat is transferring through and dx is the distance heat is transferring.

And general unsteady state heat transfer equation 5.2:

Equation 5.2 Un-steady state heat transfer equation.

$$Q = \rho\, v\, C_p \frac{\delta\theta}{\delta t}$$

Where ρ is mass of the material, v is the volume of the sample (as dz × dy × dx), C_p is specific heat capacity of the material and dθ/dt is rate of the temperature changes inside a material.

Therefore, for the selected piece of the bag, the heat transfer will be:

$$\rho C_p \frac{d\theta}{dt} = \lambda \frac{d^2\theta}{dx^2} + \lambda \frac{d^2\theta}{dy^2} + \lambda \frac{d^2\theta}{dz^2}$$

When $0 < x < \frac{L}{2}$, $0 < y < \frac{W}{2}$ and $0 < z < \frac{H}{2}$, at $t > 0$

5.3.5 Boundary Conditions

In order to represent the symmetrical theory on the selected piece of the whole bag we will have:

Heat transfer from the right side of the selected piece will be: $\lambda \frac{d\theta}{dx} = 0$

When $x = \frac{L}{2}$, $0 \le y \le \frac{W}{2}$ *and* $0 \le z \le \frac{H}{2}$, at $t > 0$

Heat transfer from the back of the selected piece will be: $\lambda \frac{d\theta}{dy} = 0$

When $0 \le x \le \frac{L}{2}$, $y = w/2$ and $0 \le z \le \frac{H}{2}$, at $t > 0$

Heat transfer from the bottom of the selected piece will be: $\lambda \frac{d\theta}{dz} = 0$

When $0 \le x \le \frac{L}{2}$, $0 \le y \le \frac{W}{2}$ and $z = 0$, at $t > 0$

Some resistance to heat transfer on the exposed surfaces will occur, so convention boundary condition was applied on these locations.

According to Newton's law heat transfer equation:

$$Q = h\, A\, d\theta$$

Where h is heat transfer coefficient, A is the surface of exposed sample to heat and dθ is the temperature differences.

Heat transfer from the left side of the selected piece will be: $-\lambda \frac{d\theta}{dz} = h(\theta_R - \theta)$

When $x = 0$, $0 \leq y \leq \frac{W}{2}$, $0 \leq z \leq \frac{H}{2}$, at $t > 0$

Heat transfer from the front side of the selected piece will be: $-\lambda \frac{d\theta}{dz} = h(\theta_R - \theta)$

When $0 \leq x \leq \frac{L}{2}$, $y = 0$ *and* $0 \leq z \leq \frac{H}{2}$, at $t > 0$

Heat transfer from the top of the selected piece will be: $\lambda \frac{d\theta}{dy} = h(\theta_R - \theta)$

When $0 \leq x \leq \frac{L}{2}$, $0 \leq y \leq \frac{W}{2}$ and $z = \frac{H}{2}$, at $t > 0$

The initial temperature at time= 0 will be equal to θ_i for all locations within the geometry.

5.3.6 Reactions

A general mathematical expression on the rate of reaction, when concentration of a component is changing by time is given below:

$$\frac{dC}{dt} = k$$

Where dC is the concentration of target component changed by reaction time (dt) and k is a constant rate value of the reaction.

From the zero-order reaction equation given above, rate of reaction only depends on the constant value (k). The kinetic model was used for 5-HMF production (refer to Appendix 2) which will be:

$$\frac{dC_{HMF}}{dt} = k_{HMF}$$

$C_{HMF} = 0$ $(\mu mol/ml)$ *which is the initial concentration of* $5-HMF$ *at* $t = 0$ for all x, y and z positions, k_{pec} is the rate constant of 5-HMF at reference temperature (110 °C).

If rate of change in target concentration ($\frac{dC}{dt}$) is dependent on the quantity of that component, then reaction will be a first order. This kinetics was used for pectin solubilisation reaction (refer to Chapter 4, Section 4.4.1) and will be:

$$\frac{dC_{pec}}{dt} = k_{pec}(C_{equ} - C_{pec\,i})$$

$C_{pec\,i} = 18.4$ (µmol/ml) which is the initial concentration of pectin at t = 0 for all x, y and z positions, k_{pec} is the rate constant of pectin at reference temperature (110 °C) and C_{equ} is the maximum concentration of solubilised pectin in serum phase.

5.3.7 Solution

Heat treatment using a retort contains three steps of heating-up, holding and cooling-down (Figure 5.4). Heating up is the time taken to increase the retort steam temperature to the maximum temperature selected (125 °C). Holding time is the duration for which temperature is kept at maximum temperature. Cooling down is the time when the temperature of the retort in decreasing to a target temperature (60 °C) before the door of the retort is opened.

The retort was observed to achieve linear ramps on both heating up and cooling down to within 2 °C of target temperature at any time point. The time for heating up was selected after several modelling runs to be achievably fast without the penalty of the maximally heat-treated outermost pomace producing dramatically more 5-HMF than the average. The cooling down time was selected similarly. The holding time was chosen to give enough pectin solubilisation without unacceptably high 5-HMF as explained further below.

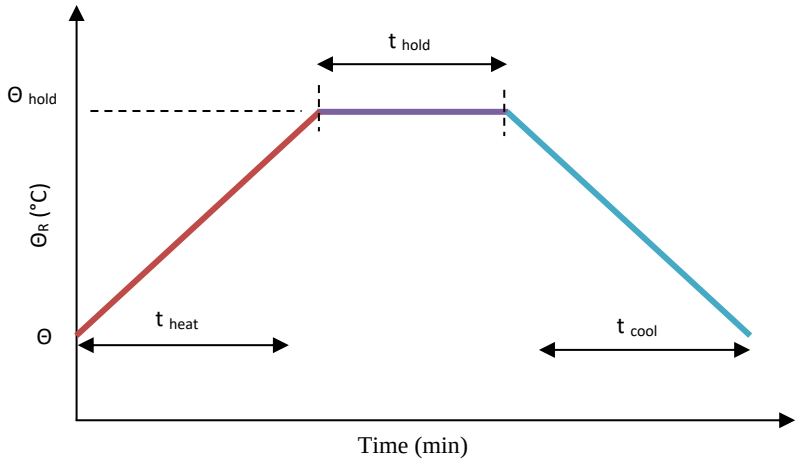


Figure 5.4 Schematic temperature-time profile of retort in three steps of heating up to Θ_{hold}, holding at Θ_{hold} and cooling down to Θ.

The model of heat transfer through apple pomace in combination with chemical reactions of pectin solubilisation and 5-HMF formation was solved using COMSOL Multiphysics 5.3a using the finite element method. The selected parameters for solving the problem with COMSOL are given in Table 5.1 below.

Table 5.1 Parameters defined for prediction of the solubilised pectin and the 5-HMF production using COMSOL.

Name	Value	Description
H	0.015 m	Height
L	0.29 m	Length
W	0.17 m	Width
Θ_R	125 °C	Retort temperature at holding time
Θi	20 °C	Initial temperature
h_retort	500 W/(m^2.K)	Heat transfer coefficient
Ea_pec	71.89 kJ/mol	Activation energy for pectin solubilisation reaction
kref_pec	0.056 1/min	Reference rate constant for kinetic of pectin solubilisation
Θref_pec	110 °C	Reference temperature for kinetic of pectin solubilisation
C_pec i	18.4 µmol/mL	Initial pectin concentration
C-equ	36.64 µmol/mL	Pectin concentration at equilibrium point
R_gas	8.314 J/(mol·K)	Ideal gas constant
t_heat	1000 s	Time for heating up the retort to 125°C
t_hold	500 s	Holding time
θ_hold	125 °C	Temperature at holding time
t_cool	940 s	Time for cooling down retort to 55°C
θref_HMF	110 °C	Reference temperature for kinetic of 5-HMF production
kref_HMF	0.0008 µmol/(mL·min)	Reference rate constant for kinetic of 5-HMF production
Ea_HMF	150.2 kJ/mol	Activation energy for 5-HMF reaction

Figure 5.5 shows the temperature profile penetration of the heat into selected piece of the bag at the end of heat treatment of pomace in retort.

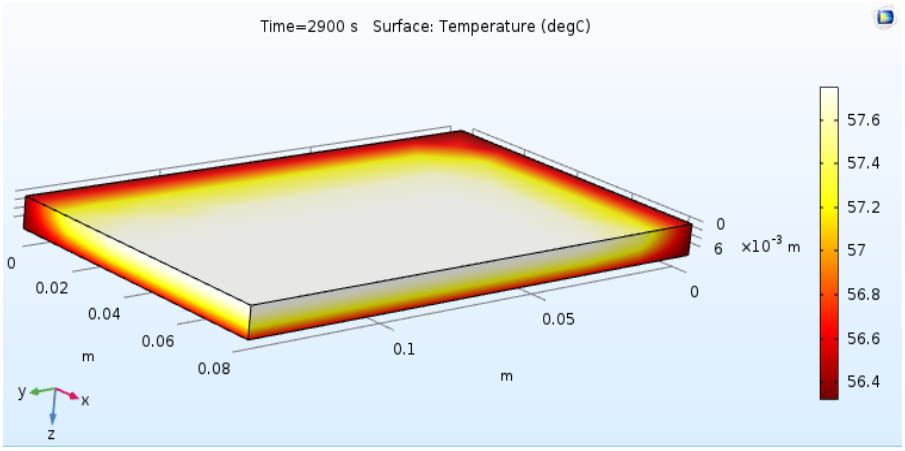


Figure 5.5 Profile of temperature at the end of the cooling step of the retorted pomace. Coloured scale bar temperature calibration by colour with the range of 56.4 -57.6°C.

Figure 5.6 shows the prediction of average temperature profile of the pomace and solubilised pectin during hydrothermal treatment in the retort.

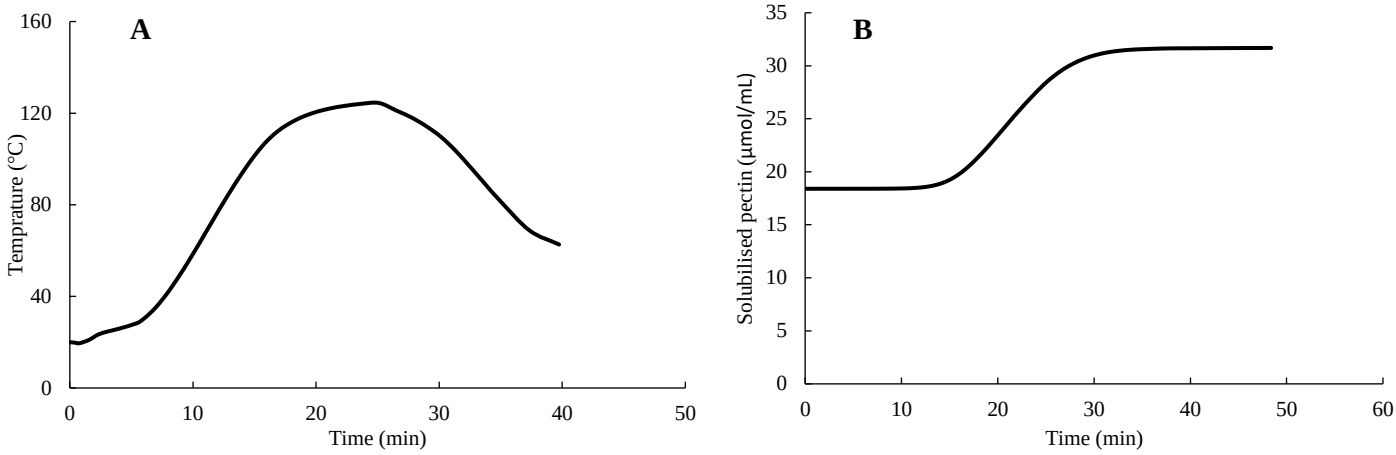


Figure 5.6 A) Prediction of average temperature profile inside the bag during the heat treatment of pomace in the retort, and B) prediction of average solubilised pectin from retorted pomace.

During the hydrothermal treatment, the edges and the surface of the bag are first parts which reach to retort temperature and will remain hot for a longer time than the materials in middle of the bag. Therefore, two prediction of 5-HMF concentration were made: average across the whole sample and maximum concentration of 5-HMF on the outer edges of the bag. Figure 5.7 shows the average and maximum production of 5-HMF while retorting.

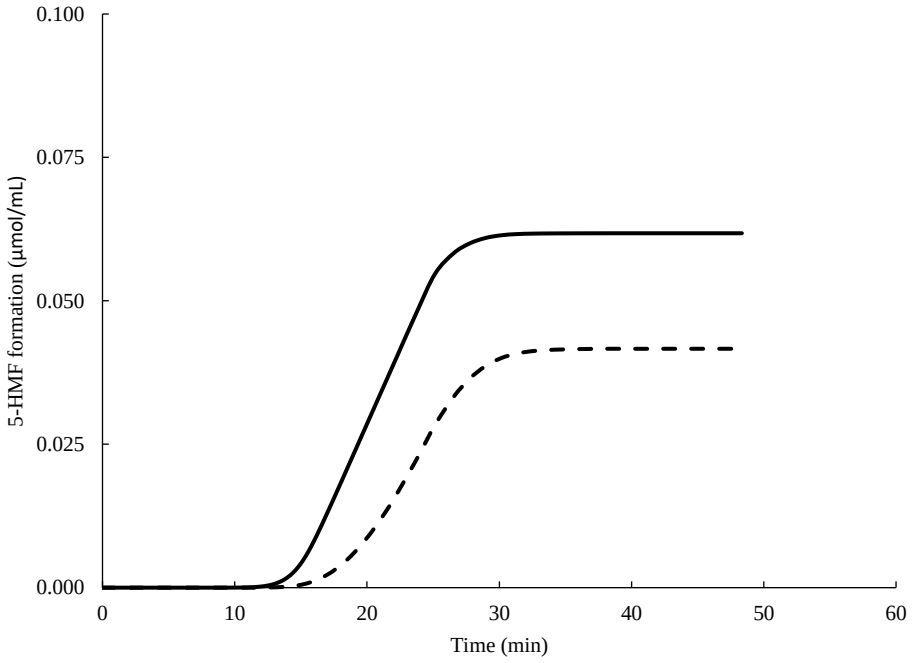


Figure 5.7 Prediction of the average and the maximum production of 5-HMF while heating in the retort. Dashed line (average 5-HMF production) and solid line (maximum 5-HMF production).

According to the prediction results from COMSOL and the temperature profile of the retort operation, an overall operation time of 40 min was needed to achieve 31 µmol/mL solubilised pectin and 5-HMF of 0.0387 µmol/mL, when holding time is 8.3 min. Figure 5.8 shows the prediction amounts of solubilised pectin and formation of 5-HMF when holding time (at 125 °C) is changing from 0 to 33 min.

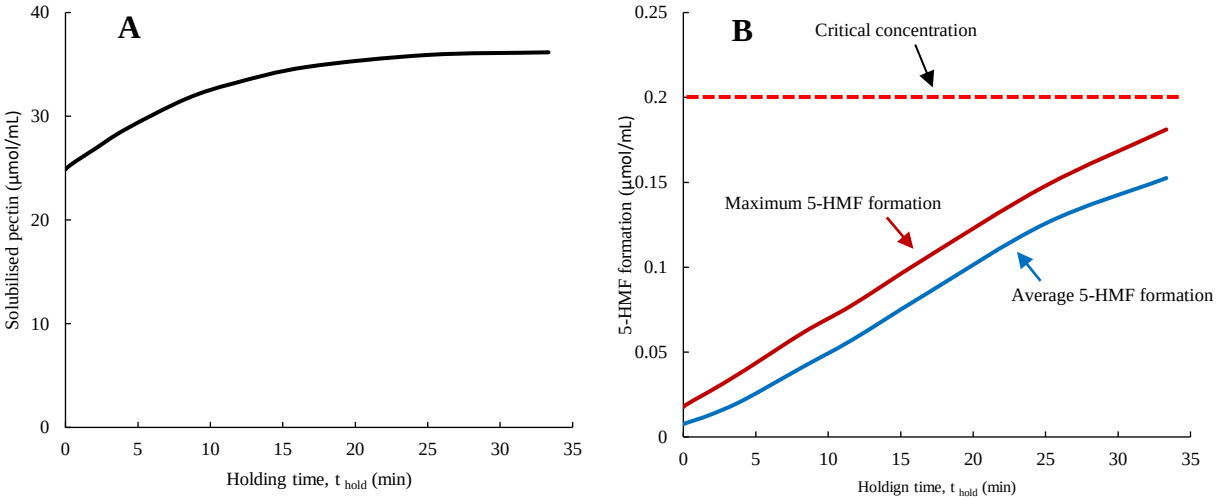


Figure 5.8 A) Prediction of average solubilised pectin, and B) formation of 5-HMF, during holding time of 0-33 min and at holding temperature of 125 °C.

According to the prediction results given above, increasing the holding time from 0 to 33 min resulted in increasing the amounts of solubilised pectin to ~36 µmol/mL (maximum amounts of pectin solubilised from pomace), then it reached a plateau. Production of 5-HMF was also increased at higher holding times. Concentration of 5-HMF did not reach to the target limit of 0.2 µmol/mL (IFFJP, 1974) even at the highest holding time of 35 min. In this study, the incubation time for pomace to heat up from RT (20 °C) to 125 °C then cool down to 70 °C was calculated using COMSOL to be 40 min. Of this, the time taken for the retort to heat up from RT to 125 °C was 15.5 min (Figure 5.2). An 8.3 min holding time for a retort at 125 °C would let all the pomace reach a temperature near that of the retort. The cooling step should reduce the retort temperature from 125 °C in a safe way to below 60 °C. The COMSOL solution provided a cooling down time of 16.2 min. In the retort operational settings, this time was separated into three cooling phases (cooling 1, 2 and forced cooling) to prevent any explosion of pouches due to the drop in report pressure. This combined profile gave an average thermal experience for each voxel of pomace closest to the target treatment.

In this study, a holding time of 8.3 min was selected for testing the validity of the model in pilot plant condition. Table 5.2 shows the final set-up conditions for hydrothermal treatment of the pomace in the retort.

Table 5.2 The operational conditions of temperature, pressure and time inside the retort.

Phase	Retort temperature (°C)	Pressure (bar. g)	Time (min)
Heating up	125.0	2.0	15.5
Holding at temperature	125.0	2.0	8.3
Cooling 1	100.0	2.0	5.0
Cooling 2	60.0	0.4	10.0
Forced cooling	0.0	0.2	2

5.4 Model Validation

Pomace was prepared by mixing two parts water with one-part apple pomace using a pilot plant rotor-stator high shear mixer (Silverson AX5, blade diameter of 0.05 m, Chesham, UK). Previously in Chapter 4, a benchtop high shear rotor-stator mixer (Silverson L4RT, blade diameter of 0.03 m, Chesham, UK) with the tip speed of 12.56 m/s was used to mix pomace and water before the heat treatment. Scaling up of this step in a pilot plant scale was done according to the blade size of the pilot plant mixer (0.05 m) and its rotational speed. The tip speed for pilot plant high shear mixer was calculated in the same way (Chapter 3, section 3.1.3). Therefore, a speed of 4,800 rpm was applied to provide 12.57 (m/s) tip speed. Mixing was done for 2 min.

A specific amount of mixed sample (1 kg) was filled into the aluminium bags so that the thickness of the bag did not exceed 0.15 m. Before heat sealing the bags, air was taken out as much as possible by pressing the bag by hand. This also helped to ensure the thickness of the bags. Heat treatment in the retort was applied according to the settings given in Table 5.2.

The heat-treated pomace was separated into serum phase and sediment solids using a Pilot plant solid bowl clarifier (Model CTC 3-03-107, GEA Westfalia Separator, Oelde, Germany) with the speed of 10,000 rpm at RT. The cloudiness of the supernatant was removed using a benchtop centrifuge (Model CR 22GII, Hitachi, Tokyo, Japan) equipped with a R20A2 rotor at 12,880 g for 10 min at RT. This centrifugation helped to separate small particles in serum and provide clear supernatant after clarification. The remaining pellet was added to the sedimented solids.

Solubilised pectin and 5-HMF production were measured in the supernatant of retorted pomace. Table 5.3 shows a comparison between the actual measurements on heat-treated pomace and the predicted amount. The amount of solubilised pectin was about 5% over-predicted, while the

average concentration of 5-HMF was about 37% over-predicted by COMSOl. The amount of 5-HMF produced was still lower than the limit of 25 mg 5-HMF/L of concentrate.

Table 5.3 The predicted amount and actual measurements of solubilised pectin and 5-HMF in heat treated pomace.

Sample	Solubilised pectin		5-HMF	
	Prediction	Actual amount	Prediction	Actual amount
	GalUA (µmol/mL)	GalUA (µmol/mL)	5-HMF (µmol/mL)	5-HMF (µmol/mL)
Retort supernatant	31.6	29.9 ±0.2	0.04	0.024 ±0.001

5.5 Conclusion

Scaling up the heat treatment process is not only about increasing the volume of the sample, but also it needs knowledge about the kinetics of the main reactions involved in the heat process. Modelling the scale-up is one way to predict and examine of scaling up the process. In this study, modelling was conducted by COMSOL, to calculate the temperature-time profile for each element of pomace in a pouch during heating in a pilot plant retort, and also to predict the extent of reactions (pectin solubilisation and 5-HMF production) taking place as well. Results revealed that the heat transfer model through apple pomace was able to predict the temperature changes over time. In addition, the kinetic models of pectin solubilisation and formation of 5-HMF corresponding to pomace temperature were partially validated. Scaling up the hydrothermal process from bench scale to a larger scale (pilot plant) was validated. Moreover, production of heat-treated pomace in larger scale also gave an opportunity to apply other processing steps (such as shearing and enzymatic treatments) on the same batch of heat-treated pomace.

CHAPTER 6 Controlled Modification of Soluble and Insoluble Fractions

Production of a large quantity of heat-treated pomace and its separation into supernatant and sedimented solids in Chapter 5 enabled each part of pomace to be modified separately in a controlled environment. This chapter focuses on these modifications (physically and enzymatically) on solubilised portion and sedimented solid parts and how to recombine the treated fractions of pomace to obtain an apple pomace with similar composition to unmodified heat-treated pomace. The prepared reconstituted pomace samples will be later (Chapter 7) used to investigate their physical stability and sensorial properties.

6.1 Introduction

Production of food ingredients from fruit and vegetable by-products are often connected with developing a process with thermal and mechanical steps (Hemar et al., 2011; Lopez-Sanchez et al., 2011; Lopez-Sanchez et al., 2012; Espinosa-Muñoz et al., 2013). Enzymatic treatment may also be used to make particular functional properties. Thermal process and the main reactions involved in heated pomace were studied in Chapter 3 and 4. Mechanical and enzymatic processes can also be introduced to change the technological functional properties of plant-based ingredients such as thickening, stabilising and texturising properties. These treatments can change the size and the shape of the particles, the structure of soluble polymers (such as pectin), the water holding capacity and physical stability of the final product (Kunzek et al., 2002; Taherian et al., 2009).

The main objective of the current chapter was producing, characterising and modifying the structural and physicochemical properties of insoluble particles and soluble polymers (especially pectin) of apple pomace in a controlled system, which can be later recombined to obtain apple pomace again. Physical and chemical properties of the insoluble solids and soluble phase can directly affect the functional properties of the reconstituted samples. Therefore, in the next part of the study (Chapter 7) the sensory evaluation of recombined pomace samples with different physical (particle size distributions) and chemical (molecular weights of pectin) properties will be tested using consumer panellists.

6.2 Material and Methods

6.2.1 Plant Material

Apple pomace was collected from Turners and Growers Apple Juice Concentrate Plant (Hasting, New Zealand). This pomace was taken from the same batch used in Chapter 4 Section 4.2.1.

6.2.2 Ultra-Filtration

Supernatant of heat-treated apple pomace was collected as described previously (Chapter 5), then was fractioned into retentate and permeate using ultra-filtration equipment built on-site by Massey University. This separation was done using a membrane of spiral HFM-180 (Koch Membrane System, USA) with a nominal pore size of 100 kDa. The applied transmembrane pressure while filtering was 2-3 bar.

6.2.3 Particle Size Analysis

Particle size analysis of sheared pomace was carried out using static light scattering with a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). Shearing had to be done in wet conditions, therefore sedimented solid (Figure 6.3, A) was mixed with permeate (resulting from ultra-filtration, Figure 6.3, D) as the isotonic diluent. This action also helped to maintain the osmotic pressure and ionic strength of apple pomace.

A refractive index of 1.5 and absorption of 0.001 were used by the instrument to calculate the particle size distribution of samples. The mean particle size was calculated from both values of the D [4, 3] and D [3, 2]. D [4, 3] is the average of the volume mean diameter and D [3, 2] is the average surface mean diameter of particles.

$$D\,[4,3] = \sum_{1}^{n} \frac{D^4{}_i\, n_i}{D^3{}_i\, n_i} \qquad D\,[3,2] = \sum_{1}^{n} \frac{D^3{}_i\, n_i}{D^2{}_i\, n_i}$$

Where n_i is the number of particles of diameter D_i. The D [4, 3] value is more sensitive to big particles and aggregations while, D [3, 2] is more sensitive to small particles. The measurement of diameter was carried out in triplicate and results were reported as average of the replicates.

6.2.4 Scanning Electron Microscopy

Scanning electron microscopy was used to study the effect of shearing on the size and shape of particles. Samples of pomace were clamped between two membrane filters and fixed by immersing into Karnovsky's fixative (glutaraldehyde + formaldehyde). The fixed sample was washed three times in 0.1 M phosphate buffer. After that samples were washed and dehydrated with graded series of aqueous ethanol (EtOH) solutions (25%, 50%, 75%, 95%, and 100%) and finally with liquid CO_2. Prepared samples were mounted onto aluminium stubs and sputter-coated with approximately 200 nm of gold, using a BAL-TEC SCD 005 sputter coater. Samples were viewed using a FEI Quanta 200 scanning electron microscope (Thermo Fisher Scientific, Massachusetts, USA) at an accelerating voltage of 20 kV.

6.2.5 Light Microscopy

Light microscopy images were taken by an Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan) equipped with a model XC50 video camera. Reconstituted pomace samples were diluted 20 times using deionised water then stained with 0.5% safranin solution to make cells more detectable. Samples were pipetted onto a glass slide, covered with a cover slip and observed with a ×10 dry objective lens. Two replications were prepared for each pomace samples and seven images were taken from each replication with cellSens Dimensions Software.

6.2.6 Determination of Hexuronic Acid Reducing End Groups

Hexuronic acid reducing end groups (HRGs) were assayed using the method of Milner & Avigad (1967) explained in Chapter 4 Section 4.2.5.

6.2.7 Pectinase Enzyme

Molecular weight of pectin was changed using the food grade enzyme of Kleerase 100XL (ZYMUS International Limited, New Zealand). According to the manufacturer notes, this preparation was extracted from non-genetically modified strains of *Aspergillus niger*. Kleerase 100XL includes enzymes such as pectin esterase, polygalacturonase (PG) depolymerase, arabinase, cellulases and hemicellulases. According to the manufacturer's notes, the activity of Kleerase 100XL was reported to be >30000 units/mL with an effective range of pH 2.5-5.0 (optimum pH of 3.5) and an effective temperature range between 15 °C- 60 °C (optimum 45 °C). This enzyme would be inactivated immediately above 70 °C (pasteurisation temperature).

6.2.8 Determination of Molar Mass Distribution

Molecular weight distribution of pomace samples' supernatant was determined using size exclusion chromatography, either by low-pressure liquid chromatography or by HPLC with multi-angle laser light scattering (MALLS) detection. These techniques are explained below.

6.2.8.1 Low-Pressure Liquid Chromatography

Molecular weight distribution of pectin was monitored by low-pressure liquid chromatography (Waters). A Superose 6HR column (300 mm × 10 mm, GE Healthcare, Chicago, USA) was used for separating polyuronides according to their molecular weights. The elution buffer contained 30 mM sodium acetate (pH 6.5), 20 mM NaCl and 10 mM EDTA with flow rate of 0.5 mL/min was used. A volume of 1 mL sample containing 1 mg galacturonic acid (GalUA) was injected and 36 fractions of 0.5 mL/min were collected. Fractions were assayed for total carbohydrate and GalUA content (see below). Size markers of dextran solutions with molecular weights of ≥5000, 500, 73, 40 and 9.3 kDa, and glucose with the concentration of 1 mg/mL were injected onto the column to identify their positions of elution (Figure 6.1). All samples and standard solutions were injected at least in two replications.

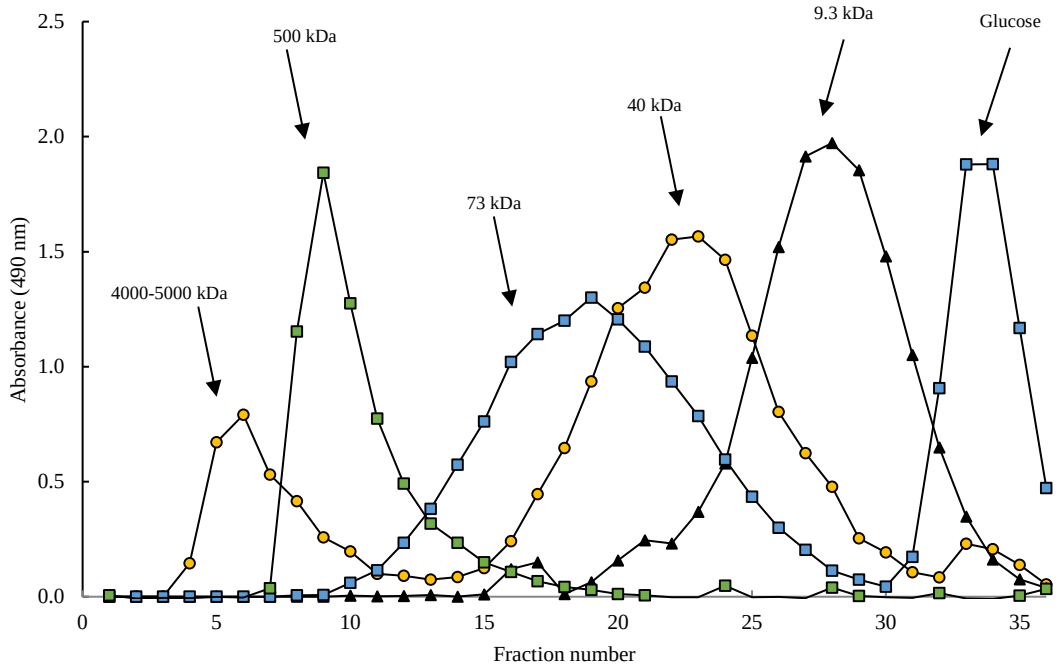


Figure 6.1 Size distribution profiles of dextrans with molecular weights of ≥5000, 500, 73, 40 and 9.3 kDa, and glucose (0.18 kDa) separated on Superose 6HR at a flow rate of 0.5 mL/min. Thirty-six fractions of 0.5 mL were collected and assayed for total carbohydrate as described in Section 6.2.8.1.

In addition, a Superdex Peptide column (300 mm× 10 mm, GE Healthcare, Chicago, USA) was used for separating low molecular weight polyuronides. Pooled fractions from Superose 6HR column (see Section 6.4.5.1) were injected on to the column. The elution buffer contained 30 mM NaOAc (pH 6.5), 20 mM NaCl and 10 mM EDTA with a flow rate of 0.5 mL/min. Fractions (0.5 mL) were collected and assayed GalUA content. In this column size markers of 9.3 kDa dextran, stachyose, raffinose, sucrose and glucose, tri-GalUA, di-GalUA and mono-GalUA with the concentration of 0.5 mg/mL were injected onto the column to identify their positions of elution (Figure 6.2).

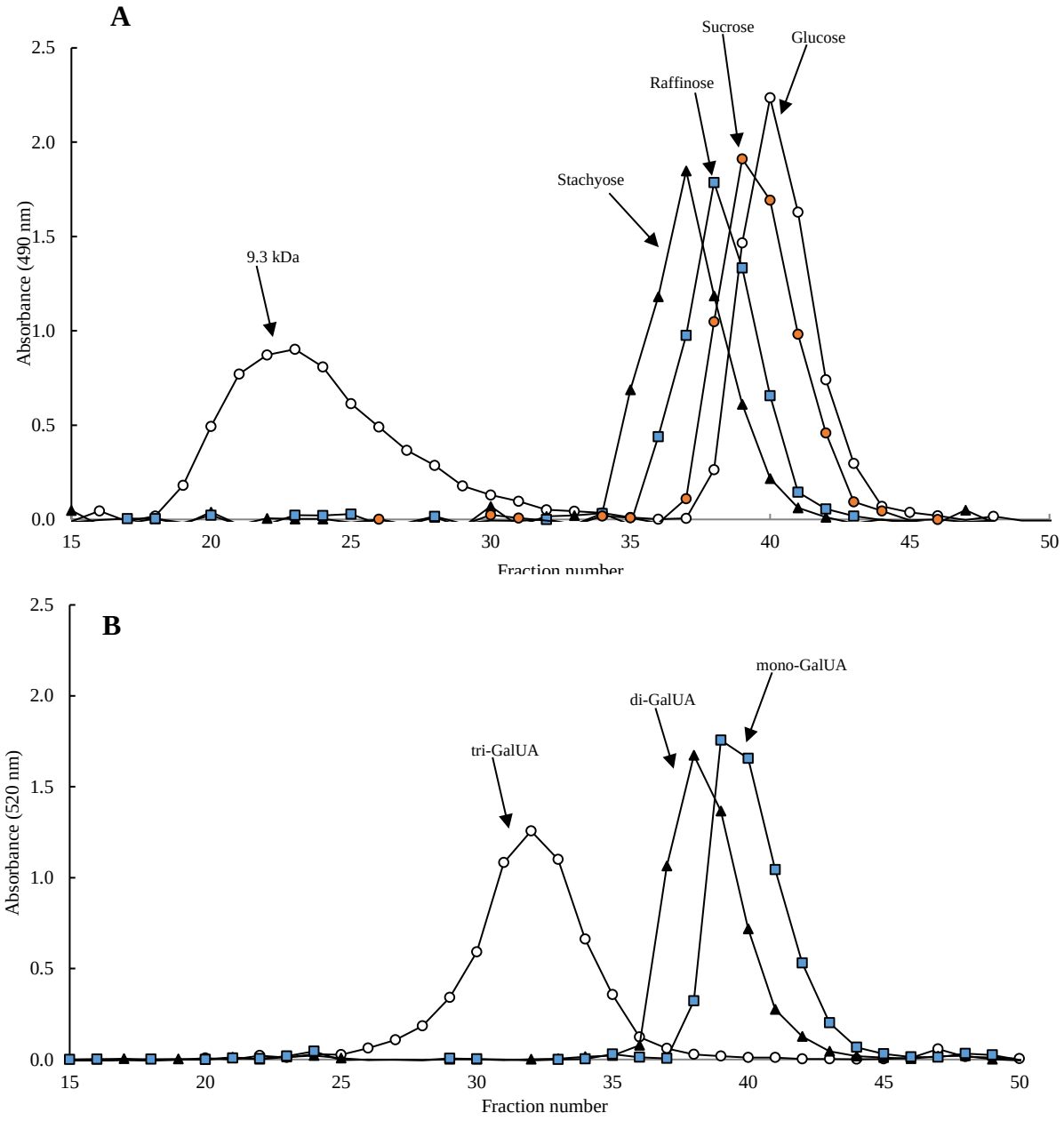


Figure 6.2 Size distribution profile of A) dextran (9.3 kDa), Stachyose (0.66 kDa), Raffinose (0.50 kDa), Sucrose (0.34 kDa), Glucose (0.18 kDa) and B) tri-GalUA (0.54 kDa), di-GalUA (0.37 kDa) and mono-GalUA (0.19 kDa) separated on Superdex Peptide at a flow rate of 0.5 mL/min. Thirty-six fractions of 0.5 mL were collected and assayed for total carbohydrate as described in Section 6.2.8.1.

Total carbohydrate and GalUA assays were done on the collected fractions. These methods are explained below:

Determination of Total Carbohydrate Content

Total carbohydrate content was measured according to the Dubois et al. (1956) method with modifications to fit a microplate (O'Donoghue et al., 2017). In this method, 50 µL of the standard solution or sample fractions collected from low-pressure chromatography was transferred into a microplate well and 25 µL of 5% phenol solution and 150 µL of concentrated sulphuric acid was added. Colour was developed by this addition. The 96-well plate was shaken carefully, and absorbance was recorded at a wavelength of 490 nm.

Determination of Galacturonic Acid Content

Galacturonic acid content was determined by a colorimetric 3-phenyl-phenol method according to Blumenkrantz & Asboe-Hansen (1973) modified to fit a microplate (O'Donoghue et al., 2017). The methodology for this measurement is given in Chapter 3, Section 3.1.7.

6.2.8.2 Size Exclusion Chromatography Coupled with MALLS Detector

A more accurate molar mass distribution of serum samples was provided by using HPLC size exclusion chromatography with multi angle laser light scattering detection (SEC-MALLS) with three columns respectively (SBG 805, 803 and 802.5) connected in series. These columns had exclusion limits of 100-1000, 1-100 and 0.5-10 kDa (Shodex, Shanghai, China). Columns were kept at 35 °C while in operation. A mobile phase of 100 mM acetic acid (pH 4.4) with 100 mM NaCl that was filtered through 0.025 µm overnight was used in this study. An HPLC system (Model SIL-20A, Shimadzu Prominence System 20, Japan) coupled with HPLC pump (Model LC 1150), degasser (LC-20AC) and autosampler (SIL-20A) was used. A sample volume of 100 µL (filtered through 0.2 µm filter) was injected. The eluent was passed through a refractive index (RI) detector (Model RID-20A, Shimadzu) then ultraviolet (UV) detector (Model LC 1200, Shimadzu), followed by an 8+ multi angle laser photometer (Wyatt Technology, USA). A flow rate of 0.5 mL/min of eluent buffer and refractive index increment (dn/dc) of 0.146 were used in this study. The molecular weight calculation was done by using ASTRA software 6.1.1.17 (Wyatt Technology, USA) with the Debye fitting model. The standard solutions of 20 mg/mL bovine serum albumin, 10 mg/mL dextran with molecular weight of 526 and 9.3 kDa, 2 mg/mL tri-GalUA and di-GalUA solutions were also used for calibration in this method. All samples and standard solutions were injected at least in three replications. Standard solutions of sucrose,

glucose and fructose were also injected to find out their retention time. Results showed that these sugars were eluted ~ 60 min. The intensity of the light scattering (LS) signal was very low after 60 min in all samples and due to unknown value of dn/dc for sugars, the cut off time in chromatograms was decided to be until 60 min.

6.2.9 Dry Matter

The dry matter of the samples was measured according to the method described in Chapter 4, Section 4.2.3.

6.3 Experimental Design

Heat treated pomace was separated into high molecular weight pectin, permeate and sedimented solids using the system designed in Chapter 5. This enabled better control when applying different treatments on each fraction separately. The schematic overview of this preparation, fractionation and the relevant treatments on solid particles and retentate is given in Figure 6.3. Mechanical destruction is about decreasing the size of the insoluble particles into a uniform dispersion, while enzymatic treatment mostly affects the size of the soluble polymers.

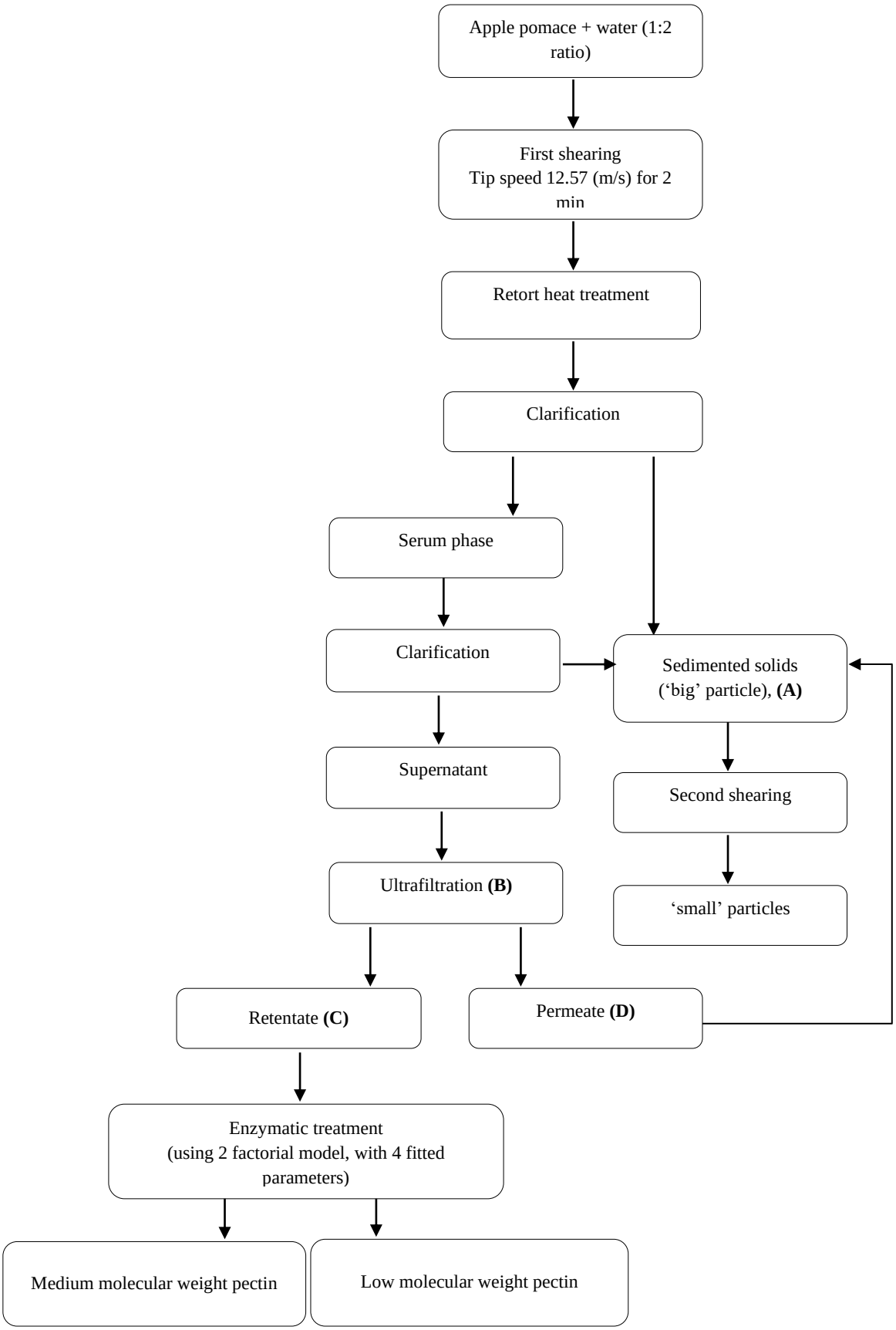


Figure 6.3 An overview of process steps for fractionating and modifying pomace in controlled environment. A, B, C, and D letters are further explained on Figure 6.6.

Homogenisation of apple pomace samples were done at two different steps called ‘first shearing’ and ‘second shearing’. First shearing: This step was done on apple pomace before heat treatment. In this step, one part of pomace was mixed with two parts of water and sheared for 2 min at tip speed of 12.56 m/s and at room temperature (RT) using a rotor-stator high shear mixer (Silverson L4RT, blade diameter of 30 mm, Silverson Machines Ltd, Chesham, UK) with the general screen (containing 1 hole of 10 mm per 100 mm^2) (Figure 6.4A). Sheared pomace-water underwent a heat treatment in retort at 125 °C as explained in Chapter 5.

The second shearing was done after heat treatment on a mixture of solid particles and permeate (as isotonic diluent) for 2, 5 and 10 min at tip speed of 15.7 m/s and at room temperature (RT) using the same rotor-stator high shear mixer used in Chapter 4, Section 4.2.2. In this shearing, a medium screen (containing 20 holes with the diameter of 1 mm in 100 mm^2) was used (Figure 6.4B). Size and shape of first and second sheared particles were analysed by light scattering method and microscopy techniques (light microscopy and scanning electron microscopy).

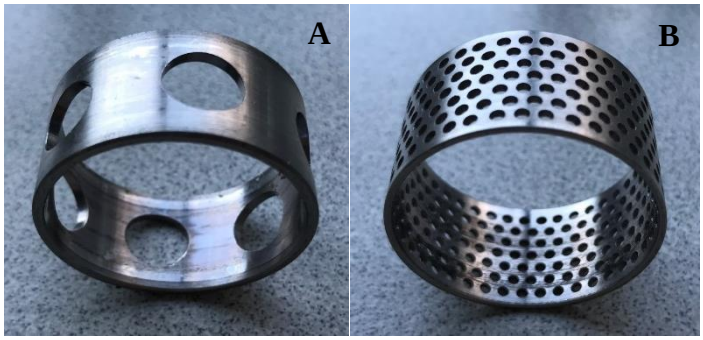


Figure 6.4 Homogenising apple pomace before and after heat treatment using two screen size of general (A) and medium (B) for 2 min with general screen at tip speed of 12.56 m/s and 2, 5 and 10 min) at tip speed of 15.7 m/s with medium screen at RT.

Retentate was subjected to enzymatic hydrolysis using commercial pectinase (Kleerase 100XL) to produce two distributions of pectin molecular weights. Selecting a suitable condition in enzymatic treatment is an important step to achieve different molecular weight ranges. For this reason, a response surface regression was developed using Minitab with two variables of enzyme concentration and time. Section 6.3.1 explains how this model was designed and selected from a set of 4, 3 and 2 factorial designs.

Molecular weight distribution of pomace supernatant (before and after heat-treatment), permeate, and retentate (before and after enzymatic treatment) were determined using low-pressure size exclusion chromatography and high-pressure SEC-MALLS. For low-pressure chromatography sample (1mL) containing 1mg GalUA/mL were injected in low-pressure size exclusion chromatography. For SEC-MALLS, sample (100 µL) containing 2 mg GalUA/mL was injected.

Standard solutions of sucrose, glucose, fructose, tri-, di and mono GalUA were also injected on the HPLC columns finding the retention time of these components when coming off the columns. A solution of inactivated enzyme was also injected into the system as well.

6.3.1 Experiments Suitable Enzyme Condition

Pectinases are able to break the pectin chain resulting in smaller pectin fragments with lower molecular weight. In the current study, the food grade pectinase enzyme Kleerase 100XL was incubated with retentate which was expected to contain high molecular weight pectins. The effect of this enzyme preparation on substrate was monitored by measuring the concentration of hexuronic acid reducing end groups (HRGs) released as an indicator of molecular weight change.

To generate pectic oligomer mixtures to order it was first necessary to determine the enzyme concentration and reaction time gave what degree of hydrolysis. Two approaches were possible: mechanistic via measuring Michaelis-Menton enzyme kinetics or empirical by factorial experiment. The former applies only to single enzyme activities whereas Kleerase is a complex mixture of several cell degradation enzymes such as pectinases and cellulases sold to aid fruit maceration. Therefore, in this study, the empirical route was chosen. A factorial experiment of central composite design was created for the initial stage of the enzymatic hydrolysis (using a short time incubation) when the substrate was still in excess within the reaction. In this study, the combined effect of incubation time, enzyme concentrations, pH and temperature on depolymerisation of pectin polymers was studied using response surface methodology with a central composite design. The central composite design was done using Minitab 18.1 (Minitab Inc. USA, 2017) to predict the amount of HRGs produced during enzymatic treatment. Production of HRGs were used as measure of degree of hydrolysis and thus an indicator for reduction in molecular weight of solubilised pectin. Increasing in concentration of HRGs resulted in decreasing the viscosity and physical stability of plant suspensions.

The production of HRGs from Kleerase digestion was modelled with the three separate experiments, using 4, 3 and 2 factorial designs to find the best fit model for this formation. These are presented below.

The model adequacy of each set was tested by calculating the coefficient of determination (R^2), Mallows' Cp, and Lack-of-Fit of the model. The R^2 value shows the general capability of the model to predict formation of the HRGs. Lack-of-Fit is another statistical analysis. This value is the average differences between experimental results and the predicted values from the model. An accurate model should have an insignificant effect from Lack-of-Fit when p-value >0.05 (Yolmeh et al., 2017). Mallow's Cp is a test statistic that compares the accuracy of the model with

the subset of fitted parameters in the model. This value should be small and close to the number of variables fitted to the model plus a constant number of 1(Rawlings et al., 1998).

4-Factorial design:

The rate of an enzymatic reaction depends on factors such as substrate concentration, pH, temperature, concentration of enzyme and incubation time. In this study, the volume ratio of the enzyme to substrate was constant for all samples. The 4-factorial design was developed for pH (2.5-6.5), temperature (20-70 °C), enzyme concentration (0.09-8.59 unit/ µg GalUA) and time (0-35 min). A set of thirty-one experiments was designed with Minitab. The pH conditions derived from Minitab were 3.5, 4.5, 5.5, and 6.5 (using 100mM sodium citrate buffer) and temperatures of 20, 30, 45, 55 and 65 °C which were obtained using a water bath. The conditions were applied, and model was tested in the laboratory.

The experimental results were fitted for the linear, linear-interactions, linear-squares and full quadratic models (Table 6.1). The R^2 (prediction) results for linear-square and full quadratic were zero. The linear and linear-interaction model showed insignificant differences (p-value >0.05) for pH, temperature and time factors and any of their interaction with temperature, time and enzyme concentration (except the time-enzyme concentration interaction). At the conclusion of this experiment, pH of the prepared retentate with buffers (according to the designed) were separately tested. Results revealed that the presence of buffers could not change the pH of the samples sufficiently due to high buffering capacity of natural pomace pectin (pH~3.5), meaning that the variation of pH intended by experiment was absent. The R^2 values, Mallow's Cp and Lack-of-Fit for this design is given in Table 6.1- column A.

Table 6.1 Statistics of the linear and linear-interaction models designed with response surface methodology (central composite design) using Minitab.

		A	**B**	**C**
		4-factorial design (pH, temperature, enzyme concentration, time)	**3-factorial design (temperature, enzyme concentration, time)**	**2-factorial design (enzyme concentration, time)**
Model	**Statistics**	**Model accuracy**	**Model accuracy**	**Model accuracy**
Linear	R^2	77%	70%	79%
	R^2 adjusted	73%	65%	75%
	R^2 predicted	56%	45%	52%
	Mallow's CP	5.0	4.0	3.0
	Lack-of-Fit p=	<.001	0.010	0.001
Linear-interaction	R^2	88%	84%	94%
	R^2 adjusted	82%	76%	92%
	R^2 predicted	54%	56%	86%
	Mallow's CP	11.0	7.0	4.0
	Lack-of-Fit p=	<.001	0.022	0.011
Linear square	R^2	84%	73%	84%
	R^2 adjusted	78%	60%	76%
	R^2 predicted	0%	0%	41%
	Mallow's CP	9.0	7.0	5.0
	Lack-of-Fit p=	<.001	0.007	0.001
Full quadratic	R^2	95%	87%	99%
	R^2 adjusted	91%	74%	98%
	R^2 predicted	0%	0%	93%
	Mallow's CP	15.0	10.0	6.0
	Lack-of-Fit p=	<.001	0.014	0.145

3-Factorial design

The previous experiment showed an insignificant effect of pH to influence the system. Therefore, in the next experiment, the effect of three variables of temperature, enzyme concentration and time at a fixed pH of 3.5 was tested with the similar ranges given above. The results of a 3-factorial model was fitted for linear-squares and full quadratic models, and the R^2 (prediction) was zero (Table 6.1- column B). Therefore, linear and linear-interaction models were used to fit the results (Table 6.1- column B). The analysis of variance showed insignificant effects of temperature and its interaction with time and enzyme concentration (p-value > 0.05). This result suggests there was a much higher influence of enzyme concentration and time on pectin HRGs than the incubation temperature.

2-Factorial design

A new prediction model for HRGs formation was developed using two variables of time and enzyme concentration since the temperature effect was not significant. The incubation temperature was selected to be 45 °C as recommended by enzyme supplier to be the optimum temperature for the enzyme.

Two variables of time (between 0-30 min) and enzyme concentration (between 0-5.15 unit/ μg GalUA) at fixed pH of 3.5 and reaction temperature of 45 °C were used to model the formation of HRGs. Table 6.2 shows the experimental plan for these parameters designed by Minitab for incubation time (between 0-30 min) and enzyme concentration (between 0-5.15 unit/ μg GalUA) and their effect on HRG formation. The experimental results were fitted to linear, linear-interaction, linear-square and full quadratic model in Table 6.1. The statistical results for these fitted models are given in Table 6.2- column C. The statistical results revealed that the full quadratic model was able to predict the HRG values better than other fitted models.

Table 6.2 Central composite design using Minitab when incubation time (0-30 min) and enzyme concentration (0-5.15 unit/µg GalUA) are two variable factors. The amount of HRG was measured as a response for this model. The predicted values using the full quadratic model were determined and compared with experimental values.

RunOrder	PtType	Blocks	Time (min)	Enzyme (unit/µg GalUA)	HRG (µmol/mL)	
					Predicted value	Experimental value
1	0	1	15	2.58	12	12.96
2	0	1	0	0.43	4	3.43
3	1	1	25	4.30	22	23.01
4	-1	1	25	0.43	5	4.66
5	-1	1	30	2.58	15	15.20
6	1	1	15	2.58	13	12.96
7	-1	1	15	5.15	20	20.04
8	-1	1	15	2.58	12	12.96
9	0	1	15	0	4	4.82
10	0	1	15	2.58	13	12.96
11	1	1	0	2.58	4	4.46
12	0	1	15	2.58	14	12.96
13	1	1	0	4.30	5	4.77

The statistical results of the variables in this model are given in Table 6.3. According to this, time, enzyme concentration and their interactions showed significant contribution to the model. Lack-of-Fit of the model had a p-value > 0.05 which means a suitable fitting for the predicted model.

Table 6.3 Analysis of variance for the full quadratic model when two variables of time and enzyme concentration were used.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	479.593	95.919	138.47	0
Linear	2	344.324	172.162	248.53	0
Time	1	161.656	161.656	233.36	<0.001
Enzyme concentration	1	179.263	179.263	258.78	<0.001
Square	2	22.686	11.343	16.37	0.002
Time × Time	1	22.208	22.208	32.06	0.001
Enzyme concentration × Enzyme concentration	1	0.521	0.521	0.75	0.414
2-Way Interaction	1	73.982	73.982	106.8	0
Time × Enzyme concentration	1	73.982	73.982	106.8	<0.001
Error	7	4.849	0.693		
Lack-of-Fit	3	3.426	1.142	3.21	0.145
Pure Error	4	1.423	0.356		
Total	12	484.442			

The interaction of enzyme concentration × enzyme concentration was eliminated from the model due to its insignificant effect (p-value of 0.414, Table 6.3). A new 2-factorial model equation was therefore developed in Minitab using a quadratic model with the 4-fitted parameters of time, enzyme concentration, time × enzyme concentration and time × time. The analysis variance for this model is given in Table 6.4. Analysis of variance showed the significant effect of all 4 fitted parameters on predictive ability of the model. The Lack-of-Fit value for this model was 0.173, which revealed an insignificant effect of this error on HRG formation prediction. The R^2, R^2 (adjusted) and R^2 (predicted) are also given in Table 6.4 with the values of 99, 98 and 96%, respectively, representing a good predicted model. In this model, the Mallow's Cp also decreased to 4.8 with the ideal value being 5, providing more accurate model.

Table 6.4 Analysis of variance of a 2-factorial model of time and enzyme concentration using a modified quadratic model with the 2-factorual 4-fitted parameters of time, enzyme concentration, time × time and time × enzyme concentration.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	479.071	119.768	178.41	0
Linear	2	343.842	171.921	256.1	0
Time	1	163.014	163.014	242.83	<0.001
Enzyme concentration	1	178.94	178.94	266.55	<0.001
Square	1	22.165	22.165	33.02	0
Time × Time	1	22.165	22.165	33.02	<0.001
2-Way Interaction	1	73.801	73.801	109.94	0
Time × Enzyme concentration	1	73.801	73.801	109.94	<0.001
Error	8	5.37	0.671		
Lack-of-Fit	4	3.948	0.987	2.77	0.173
Pure Error	4	1.423	0.356		
Total	12	484.442			
R^2= 99%	R^2 (adjusted)= 98%	R^2 (predicted)= 96%	Mallow's Cp= 4.8		

Another way to judge the accuracy of a model when factorial variables are fixed is by measuring the mean absolute deviation (MAD). This value shows the average distance between each data point and the mean or the average distance between experimental points and the fitted model. A model with lower MAD is known to have better fitting for predicting experimental results (Ghorbannezhad et al., 2016; Yolmeh et al., 2017). The MAD values for models with a similar number of variables are comparable. The MAD value is calculated according to the equation given in (Ghorbannezhad et al., 2016; Yolmeh et al., 2017).

In the current study, the MAD value for 2-factorial model of time and enzyme concentration was calculated when 2-factorial (full quadratic) model (Table 6.3) and 2-factorial 4-fitted parameters model (Table 6.4) were selected. For the full quadratic model, the MAD was calculated to be 8.1 while for 2-factorial 4-fitted parameters model MAD measured to be 7.9. This result also supports all previous statistics on the superiority of 2-factorial 4-fitted model.

In conclusion, in this study, 2-factorial 4-fitted parameters model of time, enzyme concentration, time× time and time× enzyme concentration, was selected to be the best fitted model, for determining HRG formation. The regression equation for HRG formation with this model is given in equation 6.1 below.

$$HRG = 3.41 + 0.32\ Time + 0.03\ Enzyme\ concentration - 0.014\ Time \times Time + 0.015\ Time \times Enzyme\ concentration$$

Equation 6.1 HRG formation of 2-factorial 4-fitted parameter model.

Figure 6.5 shows the response surface and counter plot of the 2-factorial 4-fitted parameters model. The response surface plot (Figure 6.5A) shows the relationship of dependent and independent variables on the output of the model in a three-dimensional graph, while the counter plot (Figure 6.5B) is the two-dimensional representation of the surface plot (Yolmeh et al., 2017). In the iso-respond counter plot (Figure 6.5B), the curves inside the plot show the amount of HRGs produced in different combinations of time and enzyme concentration. For example, the curve labelled "15" means that any combinations of enzyme and time which will meet on this curve will result in formation of 15 HRGs from retentate.

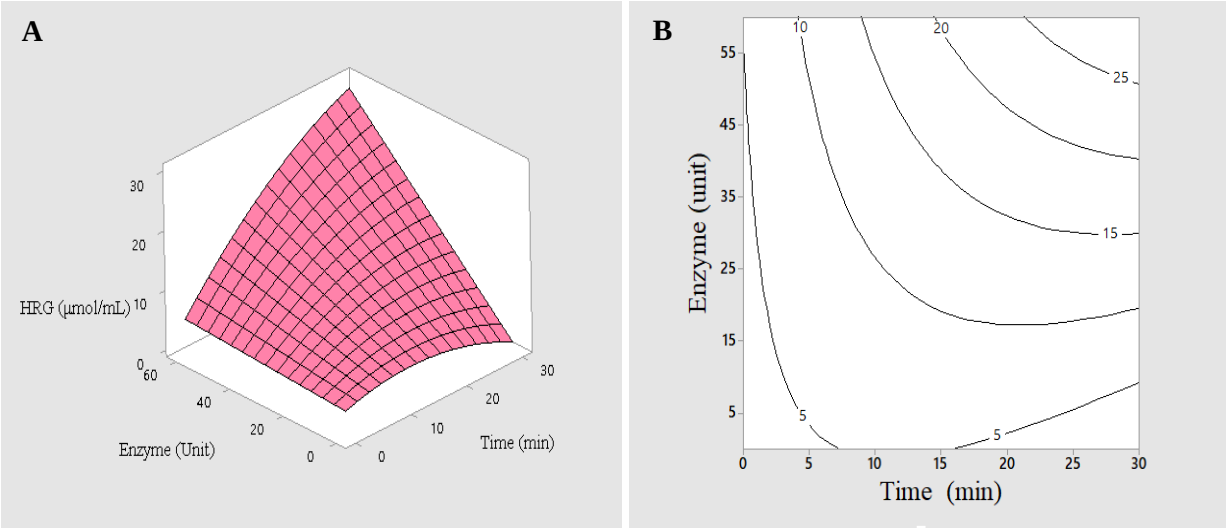


Figure 6.5 A) Three-dimensional surface plots and B) iso-respond counter plots of reducing end groups versus enzyme concentration and time.

The final equation and iso-respond counter plot are useful tools in finding the best operation condition of time and enzyme unit for producing specific amounts of HRGs in retentate. From this work and by developing this model, it is possible to design a condition to achieve pectin break-down in a controlled way.

6.4 Results and Discussion

6.4.1 Chemical Properties of Pomace Fractions

Heat-treated pomace was fractionated into solid particles, retentate and permeate using ultra-filtration (Figure 6.6). Retentate and permeate were further separated into ethanol (EtOH)- soluble and -insoluble fractions

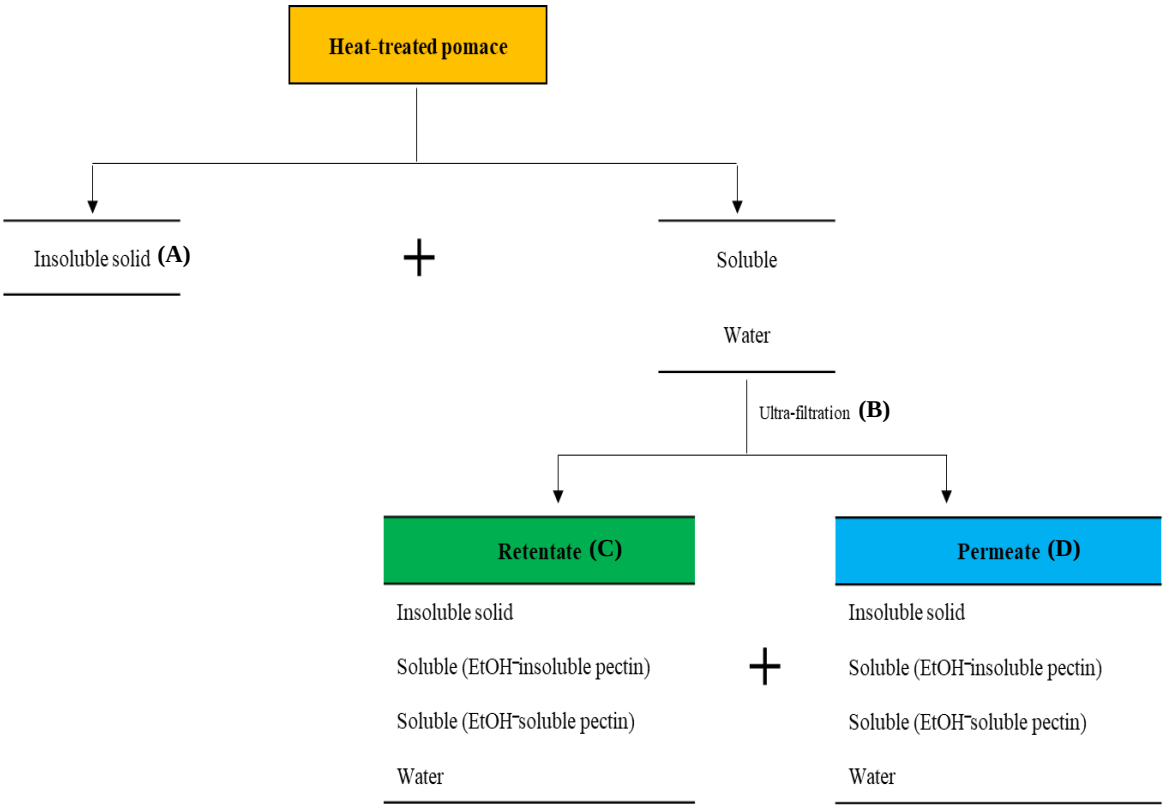


Figure 6.6 Fractionation of heat-treated pomace into retentate and permeate using ultra-filtration and their sub-fractions. See figure 6.3 for the process origins of samples A= Insoluble solids, B= Ultra-filtration, C= Retentate and D= Permeate and their positions during processing are shown in the overall scheme in Figure 6.3.

The total amount of solubilised pectin in supernatant (EtOH-insoluble and EtOH-soluble) was about 60% of total GalUA content in the original apple pomace (Table 6.5). The concentration of EtOH-insoluble pectin in retentate and permeate were ~ 100 and 11 µmol GalUA /mL, respectively. However, according to Table 6.5, the mass of this component in retentate and permeate were close as much more volume of permeate than retentate arose in the filtration step. EtOH-soluble GalUA mostly contained low molecular weight components such as monomers.

The amounts of mono-/di- GalUA in permeate was about 12 times more than in retentate (Table 6.5).

Table 6.5 The total GalUA content (µmol/g dry pomace) of insoluble solid, retort supernatant (EtOH-insoluble pectin and EtOH-soluble GalUA) in heat-treated pomace and after retort supernatant was fractionated by ultrafiltration into retentate and permeate .

Component		GalUA (µmol/g dry pomace)
Sedimented solid (insoluble solid)		433.7 ±9.58
Retort supernatant	EtOH-insoluble pectin	463.9 ±3.41
	EtOH-soluble GalUA	180.8 ±0.42
Retentate	EtOH-insoluble pectin	168.8 ±0.96
	EtOH-soluble GalUA	12.9 ±0.05
Permeate	EtOH-insoluble pectin	151.7 ±0.50
	EtOH-soluble GalUA	149.9 ±0.56

The degree of esterification in heat-treated pomace was 91%, which was higher than raw pomace (54%). The higher DE measurement in heat-treated pomace might be related to several reasons such as accurate determination of GalUA content in heat-treated pomace. It was already explained that heating resulted in increasing the mono-GalUAs, these monomers won't precipitate by EtOH. Therefore, less amounts of GalUA in heat-treated pomace was determined for more ester groups. The degree of esterification in EtOH-insoluble pectin of supernatant, retentate and permeate was determined to be 49, 37 and 23%, respectively. This is suggesting some loss of esterification on pomace treatments.

6.4.2 Reconstituting Pomace From its Fractions (Calculating the Recombination Ratio)

Fractionation of pomace into retentate, permeate and insoluble solids was a step towards preparation of samples with different physicochemical properties for sensory analysis. Before modifying the separated components, it was necessary to calculate the recombination ratio to be able to reconstitute the apple pomace back from its fractions, so that it had similar insoluble solid content and solubilised pectin content to the heat-treated pomace. Table 6.6 gives a description and composition of the fractions prepared from heat-treated pomace.

Table 6.6 Component descriptions

Name	Description	Amount
Solid (A)	Weight of fresh pellet (g)	

Retentate (B)	Amount of retentate (mL)	
Permeate (D)	Amount of permeate (mL)	
CSn	Concentration of GalUA in supernatant after heat treatment (µmol GalUA/mL supernatant)	29.9
CB	Concentration of GalUA in retentate (µmol GalUA/mL retentate)	100.1
CD	Concentration of GalUA in permeate (µmol GalUA/mL permeate)	11.0
TS	Total dry matter of heat-treated pomace (g solids/g heat treated pomace)	0.06
SA	Dry matter of insoluble solid (g solids/g insoluble solids)	0.13
SB	Dry matter of retentate (g solids in Retentate/mL Retentate)	0.06
SD	Dry matter of permeate (g solids in permeate/mL permeate)	0.02

Three equations below were devised to achieve the recombination ratio.

Equation 6.2 Total balance

$$A + B + D = 1$$

Equation 6.3 GalUA balance

$$(CB \times B) + (CD \times D) = CSn \times (B + D)$$

Equation 6.4 Total dry matter balance

$$(SA \times A) + (SB \times B) + (SD \times D) = TS \times (A + B + D)$$

The ratio of components was solved for the equations and are given in Table 6.7.

Table 6.7 Recombination ratio of solid, retentate and permeate to produce heat-treated pomace with similar amounts of solubilised pectin and dry matter content.

Component	**Volume ratio**
Solid (A)	0.38
Retentate (B)	0.13
Permeate (D)	0.49

Calculation of reconstituted pomace composition, and actual analysed composition are given in Table 6.8 below:

Table 6.8 Comparison dry matter content and solubilised pectin between heat-treated pomace and reconstituted pomace.

Heat treated pomace	Expected amount	Reconstituted pomace	Measured amount
Dry matter apple pomace (g dry pomace/g fresh pomace)	0.065±0.00	Dry matter reconstituted apple pomace (g dry pomace/g fresh pomace)	0.064±0.00
Solubilised GalUA (µmol/mL)	29.93±0.22	Solubilised GalUA (µmol/mL)	29.96±1.67

Results showed a great match in the dry matter content and solubilised GalUA of heat-treated and reconstituted pomace.

6.4.3 Microstructural Properties

A shearing process involves applying mechanical energy to reduce particle size in materials and aid particle dispersion. The analysis of particle size distribution of pomace after first shearing (shearing whole pomace before retorting) and second shearing (shearing sedimented solid with permeate after clarification) (see Figure 6.3) are given in Figure 6.7. First shearing resulted in a broad distribution of particles, with majority at ~ 1000 µm and, a group at ~ 200 µm. A second shearing decreased the size of the larger particles and resulted in a single particle size distribution. Second shearing for 2 min resulted in decreasing the size of particles to an average range of ~ 200 µm. Shearing for 5 min resulted in decreasing the average size of particles to ~ 165 µm. Shearing for more than 5 min had no further effect on size of particles.

Measuring particle size distribution using light scattering is based on the assumption that particles are spherical. However, shearing of fruit and vegetable tissues produces particles with random shapes, therefore this technique for measuring the particle size should be used with caution (Lopez-Sanchez et al., 2011). Nevertheless, it is clear that shearing reduced the size of particles, and prolonged shearing resulted in less and less additional size reduction.

The results of this study were in good agreement with Espinosa-Muñoz et al. (2013) and Leverrier et al. (2016). They found that pureed apple had similar bimodal particle size distribution (two peaks of 1000 µm and 200 µm) as the pomace described here. They describe the bimodal distribution was a mixture of individual cells and cell clusters. However, further grinding of this puree at 5000 rpm for 15 seconds resulted in a single distribution of particles (cell clusters) of ~ 500 µm. Shearing for 3 min at twice the speed resulted in a tight distribution of particles around 200 µm. Native mango puree also showed a bimodal particle size distribution (first peak of 150 µm and second peak of 650 µm) and homogenisation at 100 MPa resulted in reduction of these particles to sizes about 10 times smaller than the native puree (Kermani et al., 2016). Applying shear before thermal treatment was not as effective as applying it after heating to generate small

particles with smooth edges (Lopez-Sanchez et al., 2011). Lopez-Sanchez et al. (2011) were able to generate particle sizes of 68-319 μm with smooth edges by shearing cooked carrot using a kitchen blender. This suggests that heating which can cause depolymerisation of pectin in the middle lamella reduces the cell adhesion and helps the shearing treatment to separate tissue cluster fragments.

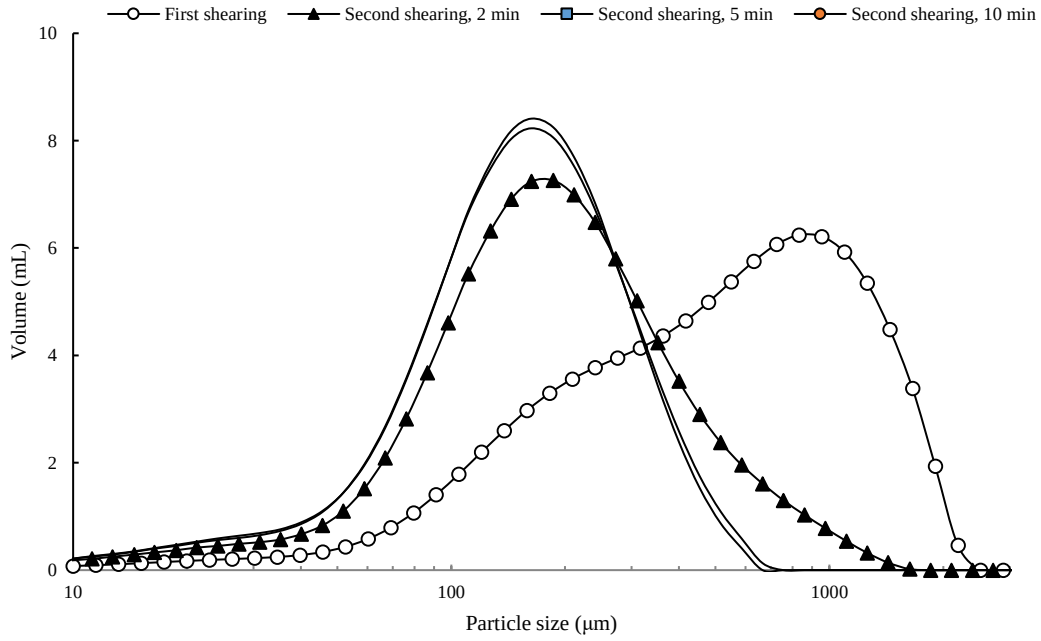


Figure 6.7 Particle size distribution of heat treated pomace after first shearing (pomace-water mixture sheared for 2 min followed by heating in retort) and second shearing (sedimented solid and permeate sheared for 2, 5 or 10 min). The responses were adjusted for the same weight of pomace applied.

The average volume size of particles is called D [4, 3] and the average surface diameter is called D [3, 2]. These values for first and second shearing of apple pomace are given in Table 6.9. First shearing resulted in D [4, 3] and D [3, 2] of 600 and 234 μm, respectively. Shearing of sedimented solid (second shearing) for 2, 5 and 10 min decreased both D [4, 3] and D [3, 2]. As with size distribution, the average volume and surface diameter did not change with increasing shearing time from 5 to 10 min. This showed a limitation of particles that can be generated using rotor stator homogeniser with a medium screen. In Table 6.9, d [0.5] is the midpoint in particle size or 50% of particles size distribution and d [0.9] is the size that 90% of particles can be found below. In the current study, a second shearing for 2 min gave a d [0.5] of 193 μm. Decreasing the particle size in second shearing through increasing the time to 5 and 10 min gave d [0.5] and d [0.9] values of ~164 and 340 μm, respectively.

Table 6.9 Particle size distribution of pomace using two screen sizes after shearing durations of 2, 5 and 10 min at RT. Results are given as volume mean diameter (D [4, 3]), average surface diameter (D [3, 2]), size of 50% of the particles (d [0.5]) and size of 90% of the particles (d [0.9]).

Sample	Screen size	Duration	D [4, 3]	D [3, 2]	d [0.5]	d [0.9]
First shearing	General	2	600	234	496	1264
Second shearing	Medium	2	257	124	193	518
Second shearing	Medium	5	189	108	165	349
Second shearing	Medium	10	185	106	164	339

Heat treatment can solubilise pectin in the middle lamella enabling cells to separate along the middle lamella resulting in individual intact cells while grinding (Espinosa-Muñoz et al., 2013). Appelqvist et al. (2015) showed that increasing the temperature of the heating step from 80 to 100 °C helped to produce more single cells during shearing. In contrast, Moelants et al. (2013) reported that sheared cooked carrots produced average particles of 176 μm which appear to be the result of mechanical disruption (i.e., sharp particles and broken cells) rather than separation of cells through the middle lamella. According to Khan et al. (1993) the size of single cells in apples depends on the variety of the fruit as well as the place inside the fruit. They reported that the size of cells increased from 50 μm (underneath the skin) to about 100-200 μm inside the fruit (~ 5mm depth from skin). Some publications reported the size of single disrupted cell in the apple and carrot to be ~ 200 μm and ~ 90 μm, respectively (Appelqvist et al., 2015; Day et al., 2010b; Espinosa-Muñoz et al., 2013; Leverrier et al., 2016).

According to the particle size parameters of heated sheared apple pomace (Table 6.9), it is likely that the first shearing generated tissue particles containing numerous cells. Smaller cell clusters resulted during the second shearing for 2 min and single cells and cell fragments showed after 5 and 10 min shearing. The results of microscopy images (SEM and light microscopy) confirm the differences in the type of particles resulting from shearing treatments (Figure 6.8).

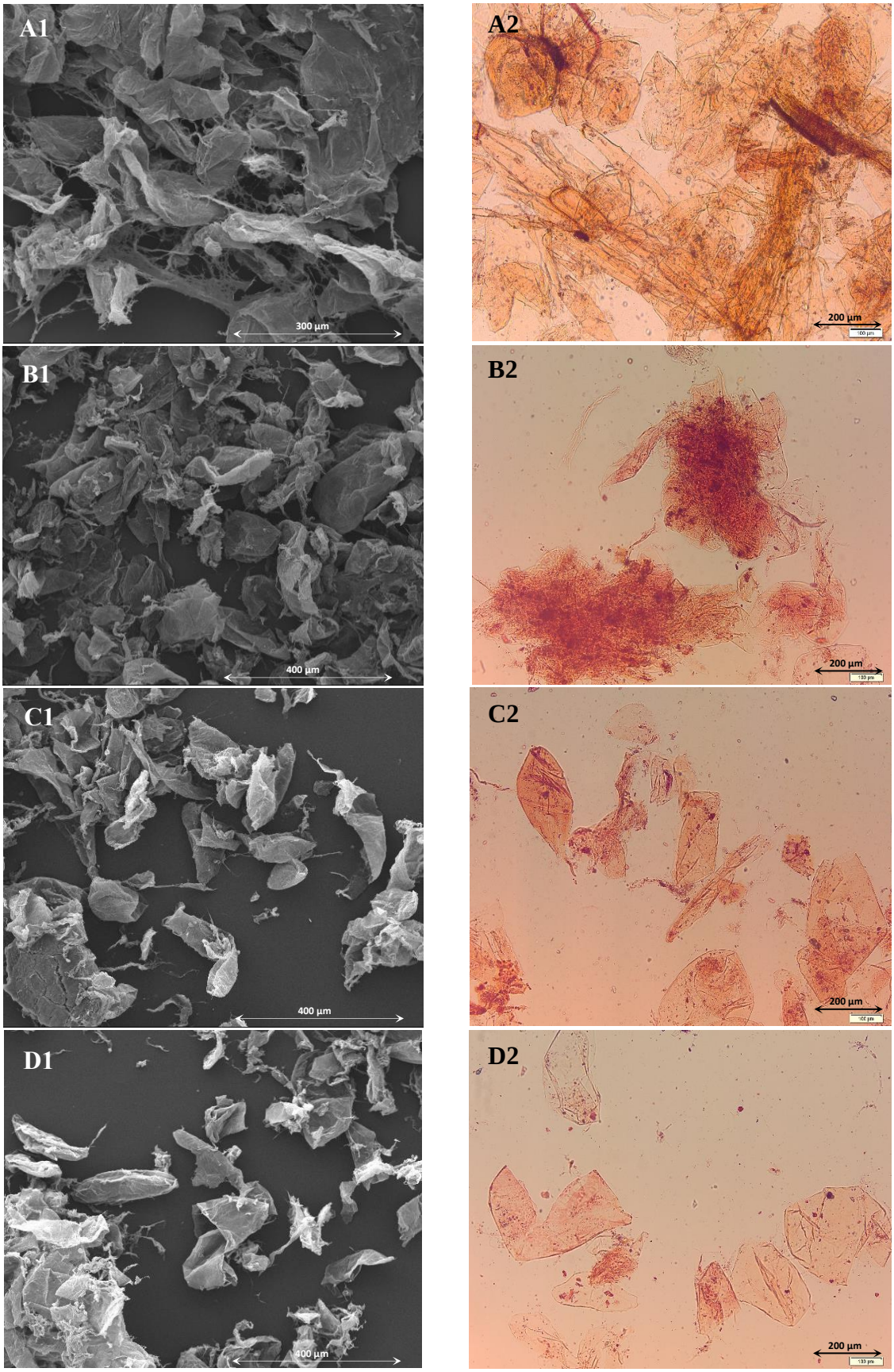


Figure 6.8 SEM (A1-D1) and light microscopy (A2-D2) images of heat treated pomace after first shearing of pomace with water (A1&2), second shearing of solid with permeate for 2 min (B1&2), 5 min (C1&2) and 10 min (D1&2). Size bars at the bottom of each image indicate magnification.

Light microscopy was also used in this study, mostly to investigate and compare the morphology of the particles with SEM images. Using light microscopy to describe the structure of particles should be considered with caution as the samples needed to be diluted (at least 20 times) before microscopy. Figure 6.8A1 & A2 shows that the first shearing was not enough to separate cells from each other. Compact structure and physical connections (matrix) between cells were visible. The size of compact cell structure of this sample was mostly more than 300 μm which is similar to particle size distributions given in Figure 6.7. The second shearing for 2 min (Figure 6.8B1 & B2) helped to break the fibrous matrix and open the structure. The individual cells were more exposed and recognisable, but some cell clusters were still visible. These results were in good agreement with Redgwell et al. (2008b) who sheared apple tissue with a laboratory Ultra-Turrax homogeniser and found that 1 min of shearing at 24000 rpm resulted in particles containing several contiguous cells. The SEM images were in good agreement with the results of particle size distributions which showed average particles size of ~200 μm (Figure 6.7). In contrast, light microscopy was mostly showed the compact group of cells which had a size of ~400 μm.

The purpose of size control in this study was producing pomace sample which mostly contains individual single cells as it has been already studied that individual cells of plant suspensions may help to perceive smooth-mouthfeel (Appelqvist et al., 2015). Five min of shearing appeared to rupture the pomace structure releasing more individual cells but, the shearing force resulted in fracturing them. The broken cells had sharp edges (Figure 6.8C1 & C2). Further shearing for 10 min did not disintegrate the cell structures and the oval shape of individual cell remnants were still recognisable (Figure 6.8D1 & D2). Results of microscopy techniques of 5 and 10 min sheared pomace showed that most of particles were < 200 μm which was in good agreement with particle size distributions given in Figure 6.7. In contrast, Redgwell et al. (2008b) found that longer shearing times dispersed the dense structure and aggregates of apple cell walls while the actual fabric of cell wall remained intact. Aggregation of broken cell remnants was slightly recognisable in this study (Figure 6.8 D1).

Kermani et al. (2016) also found that decreasing the particles of mango puree using high pressure homogenisation mostly occurred due to cell rupture under this severe shearing condition. Consistent with the findings of Moelants et al. (2013) and Appelqvist et al. (2015) using carrot and tomato, heated apple pomace sheared in the current study for >2 min tended to detach cells along the middle lamella, as the oval shape of the cells is recognisable in SEM and light microscopy images. Some of the cells were ruptured from at least one side. Shearing for 5 min resulted in more individual cell remnants with more soft edges in the particles than 10 min shearing.

6.4.4 Enzymatic Hydrolysis

Pectin degradation can occur chemically, thermally (through acid-hydrolysis and β-elimination) or enzymatically and leads to a decrease of the molecular weight of the pectin molecules. One of the aims of this study was to modify pomace pectin size without changing its natural pH so that the impact of solubilised pectin size on mouthfeel of the modified pomace product could be assessed.

Thermal degradation of pectin might need a long incubation time or high temperature to show significant effect (Chapter 4, Section 4.4.2). Enzymic degradation of pectin is a fast and effective way to reduce its size but needs to be controlled to achieve desired outcomes. In this study, degradation of pectin was achieved using Kleerase 100XL a commercial pectinase preparation. Response surface methodology is a statistical technique which uses multivariable quantitative data and solves multivariable equations simultaneously. It was used here to model the enzymatic degradation of pectin in the retentate, considering the parameters which can control the extent of this hydrolysis such as pH, temperature, concentration of enzyme and incubation time. Factorial design of the experiment is the simple way for optimisation of variables with a limited number of experiments (Prakash et al., 2008). Central composite designs (CCD) contain a fractional factorial design with centre points. In the current study, a CCD was used for modelling the enzymatic hydrolysis of pectin in the retentate using two variables of time and enzyme concentration (refer to Section 6.3.1). The selected regression equation for HRGs formation was given in Section 6.3.1. The equation 6.1 of 2-factorial 4-fitted parameter model was:

$$HRG = 3.41 + 0.32\,Time + 0.03\,Enzyme\ concentration - 0.014\,Time \times Time + 0.015\,Time \times Enzyme\ concentration$$

The initial amount of HRG present in retentate was 5.5 ±0.2 μmol/mL. In addition, the GalUA content of retentate was already determined as 100.1 ±0.6 μmol/mL. This value was assumed to be the maximum possible concentration of HRGs, which could be produced from enzymatic treatment to break down retentate into its component GalUA monomers. The regression equation above was used to find the suitable incubation time and enzyme concentration to achieve three HRG concentration of 8, 15 and 25 μmol/mL from retentate. Table 6.10 shows the experimental conditions for time and Kleerase 100XL enzyme concentration chosen for this purpose. The actual measurements of HRG and the predicted values for selected conditions confirmed that the regression equation and model was highly valid (Table 6.10).

Table 6.10 The selected conditions of incubation time and Kleerase 100XL enzyme concentration to achieve 8, 15 and 25 µmol HRG/mL from retentate.

Name of sample	Kleerase 100 XL Enzyme (unit/ µg GalUA)	Incubation time (min)	HRG (µmol/mL)	
			Predicted value	Measured value
Retentate	0	0	-	5.5 ±0.2
HRG 8	4.30	2	8	9 ±0.1
HRG 15	8.59	5	15	16 ±0.1
HRG 25	8.59	12	25	25 ±0.2

6.4.5 Molecular Weight Distribution of Solubilised Polymers

Supernatant of original, unheated pomace at RT and after heat treatment (retort supernatant) as well as the fractions of retentate and permeate from the ultrafiltration of the retort supernatant were analysed for molecular weight distributions using both low-pressure liquid chromatography and HPLC-SEC-MALLS. The molecular weight distribution of samples HRG 8, HRG 15 and HRG 25 were also analysed.

6.4.5.1 Molecular Weight Distribution Profile

In this study, the collected elutions of standard solutions and samples were first analysed to determine their broad molecular weight distribution using low-pressure liquid chromatography with a Superose 6HR column. In size exclusion chromatography, high molecular weight polymers elute early while low molecular weight components have a longer retention time. Molecular weight distribution of unheated pomace (at RT) and retort supernatants, retentate and permeate are given in Figure 6.9. The supernatant of unheated apple pomace at RT mostly contained pectin polymers less than 73 kDa in size. Heating of pomace under retort condition solubilised higher molecular weight pectic polymers. Two main peaks, between 4-16 fractions and 17-36 fractions were present.

Fractionating retort supernatant into retentate and permeate using ultrafiltration was effective in separating high and low molecular weight components. Consequently, high molecular weight peak of retort supernatant was mostly accumulated in retentate fraction, while the low molecular weight part was mostly present in permeate (Figure 6.9A). The retentate had a range of pectin polymers mostly larger than 500 kDa (Figure 6.9A). In this analysis, a small amount of lower molecular weight material was also separated, which might indicate the presence of some di- or mono- GalUA which did not wash through the membrane during ultrafiltration. In contrast to retentate, the ultrafiltration permeate mostly contained components < 40 kDa as expected. A small

amount of high molecular weight pectic material was present in permeate (fractions between 4-12). In theory, permeate should not contain any high molecular weight polymers, however in reality most membrane will have imperfections.

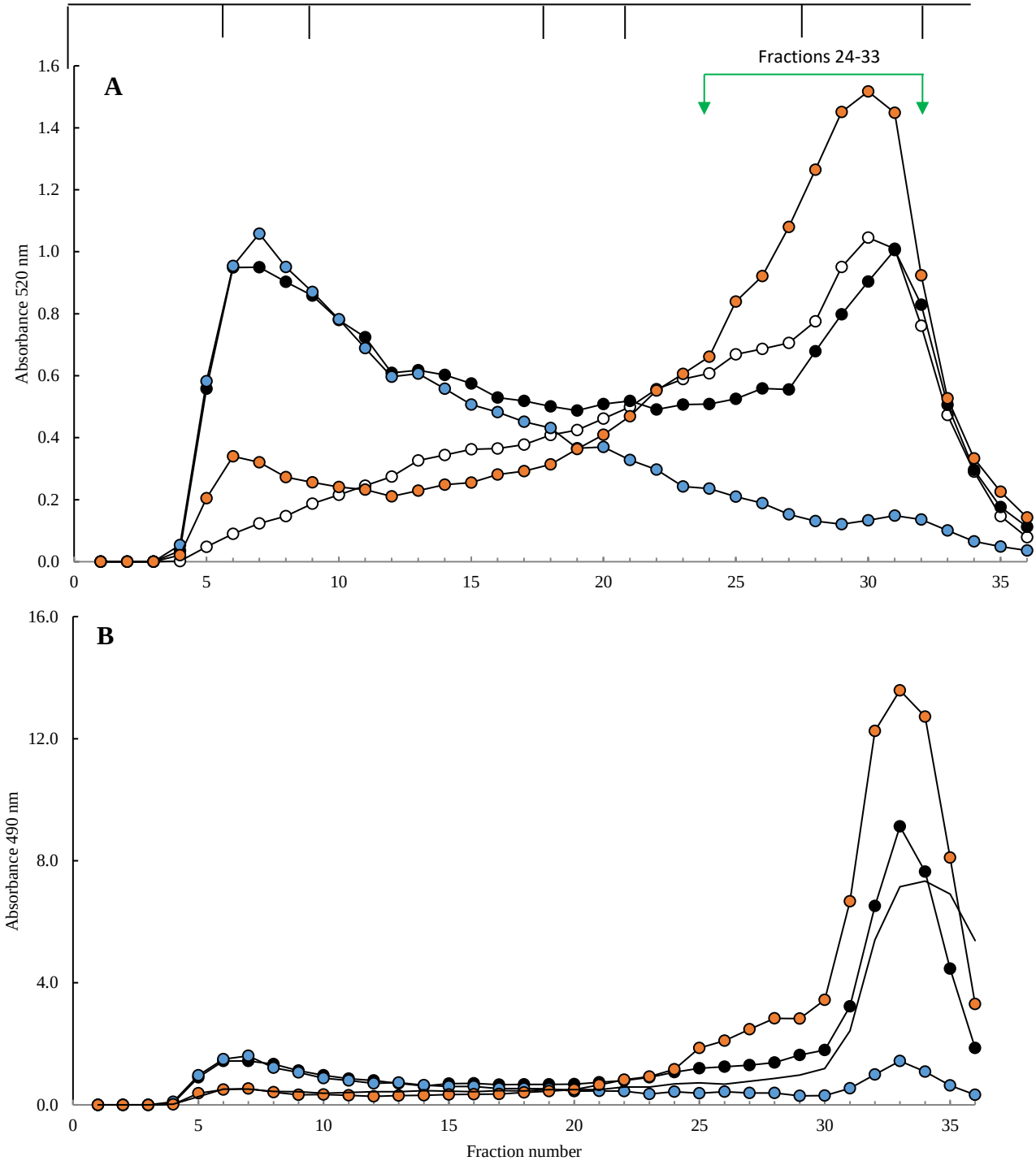


Figure 6.9 Size distribution profile of solubilised polysaccharides unheated pomace supernatant (○), retort supernatant (●), retentate (●) and permeate (●) fractionated on Superose 6HR. A) GalUA content and B) total carbohydrate content. Bars at the top of the distribution profile A show the positions of dextran size markers elution in the order of ≥ 5000, 500, 73, 40, 9.3 kDa and glucose. Profiles are the average of three separate runs.

Total carbohydrate analysis will be positive for pectin and solubilised neutral sugar components such as hemicellulose, xyloglucan and soluble sugars (Fig 6.9B). It is important to mention that the assay response was very high for fraction numbers >24 which was therefore adjusted by dilution. The absorbances given in Figure 6.9 are after considering those dilution factors. These profiles confirmed that the amounts of material in all samples were mostly low molecular weight carbohydrate. This means that in unheated pomace supernatant the soluble carbohydrates which are already dissolved in the aqueous phase of apple pomace are mostly low molecular weight sugars (probably glucose and fructose). Retorting resulted in increasing the amounts of soluble sugars in the aqueous phase of heated pomace. Most of these low molecular weight sugars report to the permeate during ultrafiltration. The results of the low-pressure chromatography analysis were in good agreement with GalUA distributions. Therefore, ultrafiltration was shown to successfully separate high and low molecular weight components from each other.

The three enzymatically-hydrolysed retentate preparations named HRG 8, 15 and 25 were also analysed for molecular weight distribution of GalUA using low-pressure chromatography on Superose 6HR (Figure 6.10). Enzymatic hydrolysis for 2 min using an enzyme concentration of 4.30 unit/μg GalUA (HRG 8) changed the high molecular weight peak of retentate toward the mid-region of the molecular weight distribution. Very few hydrolytic cleavages are needed to change the molecular weight profile significantly. This is similar to the findings of Kravtchenko et al. (1993) who showed that commercial apple pectin mostly contained high molecular weight polymers. Enzymatic hydrolysis (using endo-PG) of their pectin also shifted the main peak of high molecular weight polymers toward the mid-region of molecular weight distribution. The size profiles of HRG 15 and HRG 25 were similar to each other although HRG 25 appeared to have a tighter distribution of low molecular weight materials.

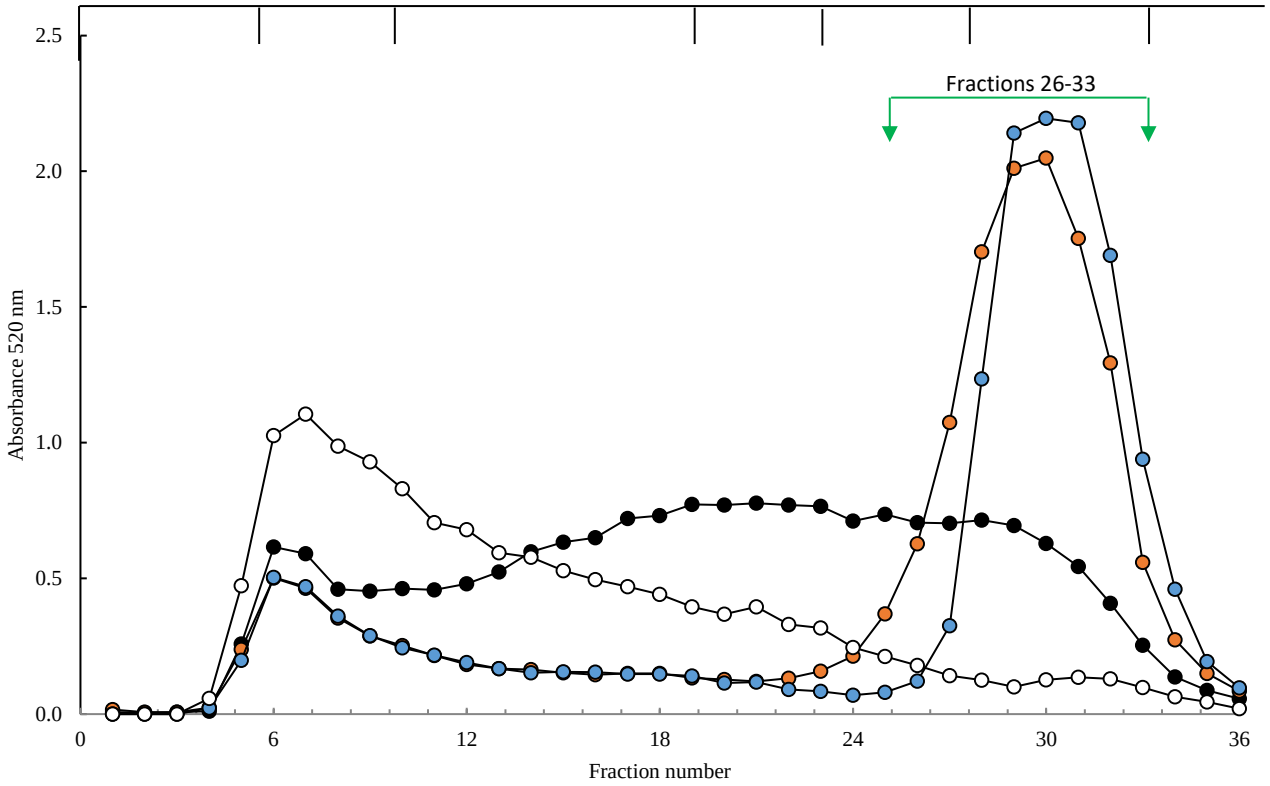


Figure 6.10 Size distribution profile of solubilised pectin of un-treated retentate (○), HRG 8 (●), HRG 15 (●) and HRG 25 (●) fractionated on Superose 6HR, were assayed for GalUA. Bars at the top of the distribution profile show the positions of dextran size markers eluting in the order of ≥5000, 500, 73, 40, 9.3 kDa and glucose. Profiles are the average of three separate runs.

It is important to mention that all enzymatically treated samples retained a small proportion of high molecular weight pectin eluting close to the column exclusion volume (fractions 4-8, Figure 6.10). These findings were in good agreement with de Vries et al. (1982 and 1984) and Kravtchenko et al. (1993) who also identified a group of pectins resistant to degradation by heat (through acid hydrolysis or β-elimination) or added enzymes. They called this region the hairy region which was inaccessible for PG. This part of pectin contains several neutral sugars which are bonded to pectin as side chains.

Pooled fractions of permeate (fractions 24 - 33, Figure 6.9) and HRG 15 (fractions 26-33, Figure 6.10) were analysed for GalUA. The amount of GalUA in mixed permeate and HRG 15 were 0.18 mg/mL and 0.23 mg/mL, respectively. One mL of each mixture was injected onto a Superdex Peptide column to determine the size profile of this small pool (Figure 6.11). Superdex Peptide has a lower separation range than Superose 6HR. Elution position of tri-, di- and mono- GalUA are shown at the top of Figure 6.11.

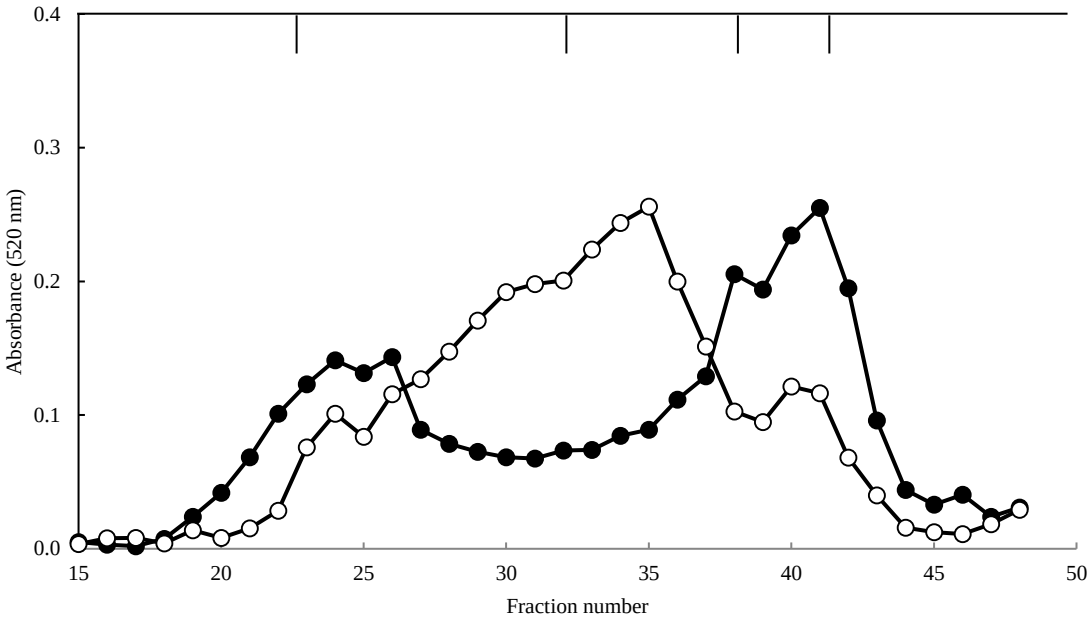


Figure 6.11 Size distribution profile of low molecular weight pooled fractions of permeate (●) and HRG 15 (○) obtained from Superose 6HR separations using a Superdex Peptide column. See Figures 6.9 and 6.10, respectively for the fractions pooled for these samples. Fractions were assayed for GalUA content. Data are the average of two separate runs. Bars at the top of the distribution profile show the positions of size markers elution in the order of dextran 9.3 kDa, tri-GalUA, di-GalUA and mono-GalUA.

Results showed that the permeate mostly contained di-and mono- GalUA, while enzymatically-hydrolysed pectin (HRG 15) mostly produce tri- and mono-GalUA. Kravtchenko et al. (1993) also showed that the enzymatic hydrolysis of pectin using endo-PG was not able to degrade all pectins into mono-GalUA, with significant amounts of di- and tri-GalUA remaining.

6.4.5.2 Estimation of Molar Mass

Molecular weight of complex polymers (such as pectin) is a fundamental characterising measurement and is connected to their functionality in a product (Harding et al., 1991). HPLC-SEC-MALLS is a technique that estimates the molar mass distribution of molecules in solution by measuring the laser light scattering of the sample from multiple angles after separation on an HPLC-size exclusion column. The focus with this method is an estimate of average size rather than obtaining a profile of uronic acid distribution (as was obtained in the low-pressure column chromatography 6.4.5.1).

The refractive index increment of dn/dc describes the change of the refractive index (n) of the solution when the concentration of component (c) is variable. The dn/dc of a component depends on the type of polymer, solvent and temperature condition (Hashim et al., 2014). Knowing the dn/dc value is necessary for measuring the molecular weight distributions of a component in a solution. In general, each polymer has a specific dn/dc value and the presence of other components

in a non-pure solution may affect the calculation of molecular weight of the targeted polymer. According to literature, extracted and purified apple pectins, commercial citrus, dextran, starch and guar gum (contains 3% protein) have been used by many researchers with the calculated dn/dc value of 0.146 mL/g using different eluent buffers (Chapman et al., 1987; Fishman et al., 2001; Yoo, 2002; Shpigelman et al., 2014). The protein content of commercial apple pectin was reported to be about 1.55% without affecting the dn/dc value (Kravtchenko et al., 1992; Shpigelman et al., 2014). These findings confirmed the stability of dn/dc for pectin in the presence of small amounts of protein. It was assumed that the serum phase in the current study was mostly pectin with a dn/dc value of 0.146 mL/g.

In the current study, size distribution of supernatants from unheated pomace (at RT) and after retorting pomace (retort supernatant), retentate and permeate fractions and enzymatically hydrolysed retentate were all monitored by HPLC-SEC-MALLS using light scattering (LS), ultraviolet (UV) and refractive index (RI) detectors. The results of the samples injection are given in Figure 6.12 and 6.13 and 6.14. The LS signal is mostly related to the size of the polymer presented, with large polymer scattering having more intensity. The molar mass of eluted components calculated based on the LS responses is shown as a red line in Figure 6.12, 6.13 and 6.14. Concentration of the polymer can also affect the intensity of this signal. The RI signal is related to the mass concentration of the polymer during elution. UV detection at 280 nm can be used for tracing the presence of other components such as protein and polyphenols.

Average molecular weight of solubilised pectin before and after retorting

The window of interest for scanning sample with LS, UV and RI through the elution time was 30-60 min which covered the fractionation range for all samples. The elution time for monomeric GalUA (as separate injection, data not shown) began at ~56 min, so it is likely elution in this range may also contain some low molecular weight sugars.

Figure 6.12 shows the size exclusion profile and molar mass distributions of solubilised pectin before ('RT supernatant') and after retorting ('retort supernatant'). The LS detector is highly sensitive to high molecular weight components, while RI is more related to their concentration. Therefore, it is possible that very low concentrations of components (eg. pectin-protein or pectin-pectin) are responsible for the initial high LS and UV peaks at the beginning of elution (<48 min) of RT supernatant (Figure 6.12A). The RI signal was detectable after 50 min elution showing the presence of high concentration of components and at the same time, the LS signal voltage decreased showing presence of low molecular weight molecules. Similar results have been reported by Yuliarti et al. (2015) who extracted pectin from kiwifruit and separated it using HPLC-SEC-MALLS. They identified a peak with the estimated molecular weight of 10^7-10^8

g/mol (or 10^4-10^5 kDa) but they suggested that this size of molecule might be too large to consider as pectin (and possibly a product aggregation).

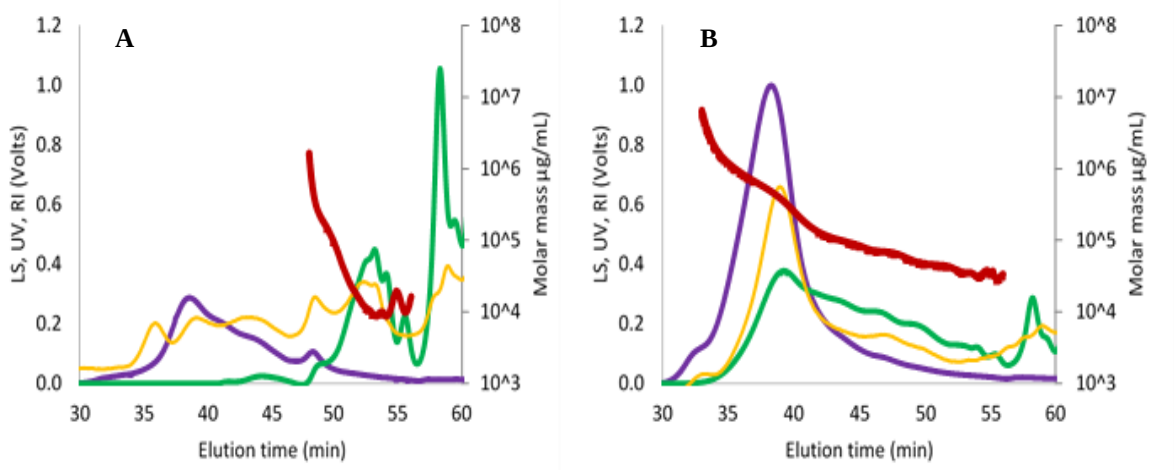


*Figure 6.12 Light scattering (**LS**), ultraviolet (**UV**), Refractive index (**RI**) and calculate log molar mass distribution ( ) of A) RT supernatant, B) retort supernatant. Each sample contained 2 mg/mL GalUA and was prepared in 0.1 M sodium acetate buffer with 0.1 M NaCl. The RI signal was adjusted to allow better visualization (× factor 5). Samples were separated on three columns of SBG 805, 803 and 802.5, connected on series.*

A high intensity peak detectable by both RI and LS at the beginning of retort supernatant's elution revealed the solubilisation of high molecular weight polymers (likely to be mostly pectin) from pomace during heating (Figure 6.12B). Average molecular weight of solubilised components in unheated and retorted pomace was calculated until 56 min (Table 6.11). The average molecular weight of retort supernatant was about eight times higher than RT supernatant.

Table 6.11 Average calculated molecular weight of polymeric components in unheated pomace and retort supernatant.

Sample	Retention time (min)	Molecular weight (kDa)
RT Supernatant	48-56	30 ± 6
Retort supernatant	33-56	232 ± 20

Guo et al. (2014) showed that hot water extraction of pectin from pomelo peels resulted in a wide distribution of molecular weight (~10-800 kDa). The extracted pectin from pomelo peels showed the main RI signal at the beginning of the elution time due to higher amounts of pectin with higher molecular weight solubilised during hot-water extraction. These results were in good agreement with the current study as the heat treatment was applied in hot water conditions and more pectin with higher molecular weight than unheated pomace was obtained.

In the present study, the samples separated on HPLC-SEC-MALLS were not purified, so other soluble components along with pectin were present such as sugars, starch, soluble protein, polyphenols, colour components (from Maillard reaction or enzymatic browning) were also present. Higher intensity of UV signal at the beginning of retort supernatant elution in comparison to RT supernatant (Figure 6.12A and B) may raise some questions about solubilising of other cell wall components (e.g protein and polyphenols) into supernatant. According to the apple pomace composition analysis (refer to Appendix 1), protein and polyphenols are 0.04 and 0.08 g /g dry pomace, respectively. Protein and polyphenol analysis of RT and retort supernatant showed that these components are still present in both samples with the amounts of 0.1 % protein and 0.1% polyphenols in both RT and retort supernatants (Appendix 1).

Presence of protein and polyphenols, as well as coloured components, are expected in pure or natural pectin solutions. Jumel (1994) and Shpigelman et al. (2014) revealed that commercial and purified samples of guar gum or apple pectin still contained 1 and 3% protein, respectively. Total phenolic content of 0.56% (on a dry matter basis) in apple pectin was also published by Kravtchenko et al. (1992).

The intensity of the UV signal was nearly three times higher in retort supernatant (Figure 6.12B) compared to RT supernatant (Figure 6.12A). This may be due to pectin-protein interactions (Kravtchenko et al., 1992; Shpigelman et al., 2014). However, this assumption and the type of protein and pectin interaction (direct covalent or indirect) is still not fully identified (He et al., 1996; Miller, 1997; Oosterveld et al., 2002; Ridley et al., 2001). The quantities of protein and polyphenols did not increase after heat treatment, therefore, the higher intensity of UV signal in the retort supernatant in this study cannot be fully explained.

Average molecular weight characteristics of retentate and permeate

Retort supernatant was fractionated into retentate and permeate using ultrafiltration. The size exclusion profile and molar mass distributions of these fractions examined by HPLC-SEC-MALLS are given in Figure 6.13. Retentate showed high LS, UV and RI signals for the material eluting between ~ 30-50 min (Figure 6.13A). Permeate (Figure 6.13B) mostly contained components which eluted after 45 min. Providing that the ultrafiltration step successfully separated high and low molecular weight components as was also seen by low-pressure chromatography (Figure 6.9).

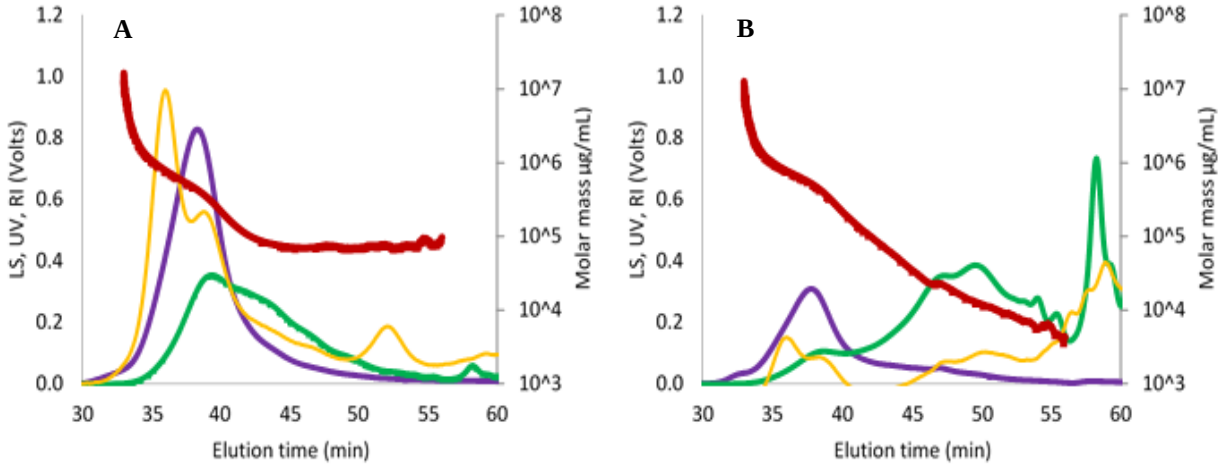


*Figure 6.13 Light scattering (**LS**), ultraviolet (**UV**), Refractive index (**RI**) and calculate log molar mass distribution ( ) of A) retentate and B) permeate. Each sample contained 2 mg/mL GalUA and was prepared in 0.1 M sodium acetate buffer with 0.1 M NaCl. The RI signal was adjusted to allow better visualization (× factor 5). Samples were separated on three columns of SBG 805, 803 and 802.5, connected on series.*

The LS profiles of all samples showed an initial peak between 35-45 min, close to the exclusion limit for the column system used (10^6 Da). It is probable that high molecular weight material may contain very low concentrations of aggregated soluble components. This fraction accounts for 58, 73 and 24% of the mass in retort supernatant (Figure 6.12A), retentate and permeate solids (Figure 6.13A and B), respectively. The degree of pectin esterification in retort supernatant, retentate and permeate were determined to be 49, 37 and 23%, respectively. This initially eluted high molecular weight components may be explained with two possible arguments of; 1- It is possible that the un-esterified pectin in these samples may have interacted with available Ca^{+2} resulting in pectin-pectin aggregations. Unlike the low-pressure chromatography, the HPLC system buffer did not include EDTA which would have disrupted pectin aggregation through Ca^{2+}. 2- It is also possible that during ultra-filtration (depends on the age of the membrane and seals) some high molecular weight components passed through to the permeate as well, resulting in initially high molecular weight region.

The average molecular weight of soluble components in retentate and permeate fractions are given in Table 6.12. for the retention times of 33-56. Comparison between the average molecular weight of soluble polymers in retort supernatant and it fractionation of retentate and permeate, revealed that ultrafiltration could successfully separate high and low molecular weight components from each other.

Table 6.12 Average molecular weight of soluble components in permeate and retentate.

Serum sample	Retention time (min)	Molecular weight (kDa)
Permeate	33-56	91 ± 15
Retentate	33-56	379 ± 80

Average molecular weight of enzymatically hydrolysed retentate

The retentate underwent enzymatic hydrolysis reaction for 2 and 5 min using Kleerase resulting HRG 8 and HRG 15. The chromatograms (LS, RI and UV signals) and molar mass distribution of these samples are given in Figure 6.14A and B. In HRG 8, the intensity of the RI signal between ~35-40 min decreased in comparison with retentate (Figure 6.13A) while the RI signal of HRG 8 at elution times > 50 min (low molecular weight region) increased in comparison to retentate fraction (Figure 6.13A).

The intensity of RI and LS signal at the elution times between 33-45 of the HRG 8 and HRG 15 samples was similar. These results were in a good agreement with low-pressure chromatography results (Figure 6.10), which the fractions of 1 to 21for HRG 8 and HRG 15 had similar absorbance for GalUA assay. The main differences in RI signal of these samples were at elution times between 45-56 min. The RI signal at the beginning of this region (~45-50 min) was higher for HRG 8 than HRG 15, while elution times > 50 min showed higher RI signal in HRG 15 than HRG 8. Based on these considerations, the average molecular weight distribution of HRG 8 and HRG 15 was calculated for the region between 45 to 56 min and appeared in Table 6.13.

Both HRG 8 and HRG 15 showed significant UV and RI peaks at elution times between 56-60 min which was not seen previously in retentate (Figure 6.14A). Inactivated Kleerase 100XL was injected onto the HPLC-SEC (data not shown) which revealed a retention time of 56-60 min using UV and RI detectors. Therefore, obtaining high intensity of RI and UV peaks (at elution time 56-60 min) in HRG 8 and 15 are related to both production of pectin subunits (RI signal) and denatured enzyme (UV signal).

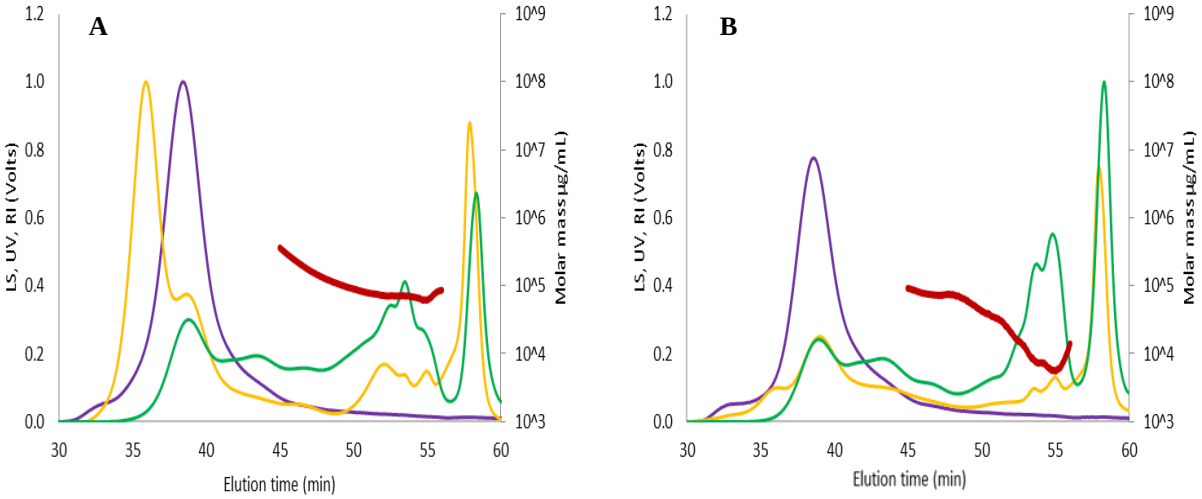


*Figure 6.14 Light scattering (**LS**), ultraviolet (**UV**), refractive index (**RI**) and log molar mass distribution ( ) of enzyme-treated retentate A) HRG 8 and B) HRG 15. Each sample contains 2 mg/mL GalUA and was prepared in 0.1 M sodium acetate buffer with 0.1 M NaCl. Samples were separated on three columns of SBG 805, 803 and 802.5, connected on series.*

The profiles from HPLC-SEC-MALLS for HRG 8 and HRG 15 were in good agreement with the molecular weight profile of these components using low-pressure chromatography (Figure 6.14). The average molecular weight distributions of HRG8 and 15 were also estimated using HPLC-SEC-MALLS for retention times 45-56 (Table 6.13).

Table 6.13 Average molecular weight of soluble components of enzyme treated samples of HRG 8 and HRG 15.

Serum sample	Retention time (min)	Molecular weight (kDa)
HRG 8	45-56	150 ± 57
HRG 15	45-56	27 ± 36

Kermani et al. (2015) showed that the molecular weight of solubilised polymeric components of blanched and homogenised mango puree, halved from 1060 to 435 kDa, when samples were treated with a mixture of PG and pectin methylesterase. In Figure 6.14 at the end of elution time (between 55-56 min) the calculated molar mass (red line) started to increase. Similar trends have been also reported by other authors (Shpigelman et al., 2015). They suggested that this increase might be an error in determination of molecular weight because the LS has very low intensity (~ 0) and RI signal was relatively low at this time.

6.5 Conclusion

Consumer demands for high fibre food products are increasing. These products will not be attractive if they cannot provide acceptable mouthfeel and consistency. It is well known that mouthfeel properties of plant food suspensions (purees) are highly related to insoluble particle sizes and properties of the soluble polymers (e.g pectin) that surrounded them. Therefore, developing a controlled system to change these properties in apple pomace was the main aim of this chapter. This has enabled defined fractions to be produced for subsequent sensory analysis to test the roles of particle size and pectin size on mouthfeel (Chapter 7). Insoluble particles and supernatant of heat-treated apple pomace were separated by centrifugation then the supernatant was fractionated into retentate (~380 kDa) and permeate (~90 kDa) by ultrafiltration. Knowledge of the physical and biochemical properties of these fractions has enabled recombination to be formulated for sensory evaluation (Chapter 7).

A controlled shearing of insoluble solids (shearing on mixture of particles and permeate) helped to disperse and decrease the dense structure and aggregates of cell structures. Particle size was dependent on the equipment used and under the experimental conditions, the Silverson rotor stator homogeniser (with medium screen) resulted in average of particles to be 165 μm when shearing ≥ 5 min. Enzymatic hydrolysis of retentate was also modelled using Minitab. Despite the advantages of using factorial model (e.g lower cost and less time), it also involves some challenges and disadvantages as well. The main disadvantage of using factorial model to predict non-linear reaction (e.g. enzymatic hydrolysis of pectin) is that the model may be less flexible than linear model. In addition, choosing the right number of parameters for non-linear reaction is also very important to predict the enzymatic hydrolysis behaviour in a reasonable way.

According to this model two pectin molecular weights of HRG 8 and HRG 15 with the average size of ~150 and 30 kDa were obtained. All in all, two particle size distribution of insoluble solids and three molecular weight distributions of solubilised pectin would let us to produce a set of pomace ingredients for physical stability and sensory evaluation.

CHAPTER 7 Physical Stability and Sensory Properties of Pomace Ingredients

This chapter reports the physical and sensory testing of pomace-derived ingredients developed in Chapter 6. Sensory tests sought to determine the relative impact of insoluble particle size distribution and soluble pectin molecular weight distribution on products smoothness in the mouth.

7.1 Introduction

Fruit and vegetables are a great source of fibre (soluble and insoluble), polyphenols, vitamins and other nutritional components, which are known to be crucial for a healthy lifestyle. A FAO/WHO report as well as a recent review on food-based dietary guidelines recommended consumption of fruit and vegetable in the daily diet of ~400 g (FAO, 2017; Herforth et al., 2019). However, many people find it hard to take this amount every day. In parallel with this demand from consumers, food industries are getting motivated to produce innovative products which are more natural and healthier such as vegetable soups, fruit desserts, sauces, fruit bars and snacks (Lopez-Sanchez et al., 2011).

Any edible plant suspension, whether it is going to be used as an ingredient in a process line or consumed as by itself (such as puree), needs to meet acceptable stability and sensory properties for consumers (Bengtsson et al., 2011; Hemar et al., 2011; Espinosa-Muñoz et al., 2012). In semi-liquid or semi-solid foods, chewing is not required, but a force is necessary to initiate the flow associated with swallowing. This force is generated by contact of the food between palate and tongue, followed by squeezing to making the material ready to swallow (Appelqvist et al., 2015). Sensory properties of semi-solid foods such as fruits purees (e.g. apple) and jam, depending on concentration, shape and size of dispersed particles, their interactions with themselves, and matrix; before putting a sample in the mouth (Appelqvist et al., 2015; Kunzek et al., 2002).

Sensory testing in the food area can generally be divided into three main categories of affective, descriptive and discrimination tests. The discrimination test is about determining the degree of

differences of an attribute between two products (McClure, 2008). Moreover, the discrimination test is mostly conducted when the difference between samples is not easy to detect by the panellists (O'Mahony & Rousseau, 2003).

One of the quality attributes in fruit and vegetable suspension products (such as cloudy juices, puree and jams) including modified apple products, is their physical stability (Bagheri et al., 2014). The particle size distributions, as well as the stabiliser components which may be naturally present in suspension products (e.g., pectin) or are added to the suspension can affect the stability of product (Corredig et al., 2001; Tatsumi et al., 2000; Sato et al., 2009; Bagheri et al., 2014; Yi et al., 2018). Phase separation of the pomace ingredient mainly affects its shelf stability of the product as well as consumers preference about the product's stability.

The main objective of the current study was to understand the effects of size of insoluble particles and characteristics of the soluble polymers (mostly pectin) on physical stability and sensory properties (especially graininess and smoothness) of the pomace ingredients. Pomace samples were formed from pomace fractions of pellet, retentate and permeate. The recombination ratio was calculated to help to generate these samples with the same relative properties as heat-treated pomace.

7.2 Material and Methods

7.2.1 Plant Material

Apple pomace was collected from Turners and Growers Apple Juice Concentrate Plant (Hasting, New Zealand). This pomace was taken from the same batch used in Chapter 4 Section 4.2.1.

7.2.2 Sedimentation Test

Heat-treated pomace was fractionated then recombined, producing pomace samples for sensory testing, according to the procedure given in Chapter 6, Section 6.4.2.

Phase separation of reconstituted pomace samples was tested by transferring 25 g sample into test tubes. Samples were kept at 4 °C for five days, as microorganisms were started to grow after five days storage. Five days was accepted as an indicator of relative stability of different preparations – no true measure of shelf life can be done except in the final product system. The volume of the sedimented solid was measured each day on the same samples. Results were reported as percent of sedimentation volume change (V_{sc}%), which was calculated as the ratio of the sedimented volume to the total volume of the sample (equation 7.1).

$$V_{sc} = \frac{V_s}{V_{total}} * 100$$

Equation 7.1 Percent of sedimentation volume change (V_{sc}), when V_s = Volume of sedimented solid at a time and V_{total} = total volume of the sample.

7.3 Experimental Design

Recombination of pomace from its fractions was calculated in Chapter 6 Section 6.4.2. However, changing the molecular weight of retentate with the average molecular weight of 380 kDa through enzymatic hydrolysis into two ranges of molecular weights (150 and 30 kDa) resulted in introducing a small dilution factor to the system through the differential addition of liquid enzyme preparation. In order to standardise solids content, equivalent amounts of water were added to the retentate before reconstituting the pomace sample. Therefore, the recombination ratio will be affected by that dilution factor. The new recombination ratio is given in Table 7.1. In this study, retentate is called high molecular weight (HMw) pectin, and two ranges of pectin molecular weight of 150 and 30 kDa are called medium molecular weight (MMw) and low molecular weight (LMw) pectin, respectively.

Table 7.1 Recombination ratio of separated heat-treated pomace fractions to produce reconstituted pomace with similar total solid content and solubilised pectin of original heat-treated pomace.

Component	Relative mass of fraction
Insoluble solid	0.38
Permeate	0.49
Standardise HMw, MMw or LMw pectin	0.17

Reconstituted pomace samples with the average particle size distributions of 500 μm ('big' particles) and 160 μm ('small' particles) (refer to Chapter 6, Section 6.4.3) and three molecular weights of HMw, MMw and LMw pectin were prepared and are shown in Table 7.2. These preparations were devised to help gain a better understanding of the effect of pectin molecular weight on pomace perception in the presence of large or small insoluble particles of cell wall material. One control sample was also designed, which contained only small particles (SP) with no added pectin fraction.

Table 7.2 Apple pomace samples used for sensory evaluation.

Sample	Abbreviation
Big particles+ HMw pectin	BPHMw
Small particles+ HMw pectin	SPHMw
Small particles+ MMw pectin	SPMMw
Small particles+ LMw	SPLMw
Small Particles+ no added pectin	SP

Eighty-four volunteer un-trained panellists participated in this experiment. The panellists replied to a local advertisement. This advertisement was distributed at Massey University and Plant & Food Research, Palmerston North. The ethics approval for the sensory study of apple pomace ingredient is given in Appendix 3.

Before guiding the panellists to the sensory booths, the test procedure and a definition of grainy/smooth were explained to each group of participants in a preparation room. There were 10 panellist per group. These definitions were: "Grainy" is perception the number of solid particles in the mouth, while "Smooth" is a perception of velvety and smooth consistency of sample in the mouth. Sensory evaluation was carried out on samples, which have the potential to be used as food ingredients. The information sheet and consent form which have been signed by panellists are given in Appendix 4.

The SP sample (without attaching its digit code) was presented to panellists in the preparation room. Panellists were able to taste as much as desired until they became familiar with the type of ingredient that they were going to assess in the booths. An anonymous practice sample was presented without identifiers to all panellists for familiarisation during the panellist introduction session prior to evaluations.

For the sensory evaluation, samples of the reconstituted, modified apple pomace (~ 10 g) were presented in 35 mL clear plastic lidded cups. Evaluation was done under white fluorescent light. All five samples were presented on one tray in mixed order and with random three-digit codes provided by the RedJade software. Before evaluation of each sample, the panellists were asked to take the sample from the tray, and match the code presented on their iPad screen. After that, the panellists were asked to remove the lid and take a full plastic teaspoon of sample. Each panellist was asked to answer the discrimination question combined with the certainty about graininess and smoothness. The question was "Is this grainy or smooth? How sure are you?" Each sample was tested and could be swallowed by the panellist. Six responses were permitted: "Grainy (G)", "Grainy not sure (GNS)", "Grainy not very sure (GNVS)", "Smooth not very sure (SNVS)",

"Smooth not sure (SNS)" and "Smooth (S)". Panellists were offered water and a cracker between each sample.

7.3.1 Sensory Evaluation

Recombined pomace samples containing two ranges of solid particles of 'big' and 'small' with three ranges of pectin average molecular weights (HMw, MMw and LMw) were prepared according to the procedure explained in Chapter 6 Section 6.4.2. A discrimination test was designed to determine if subtle differences were perceivable between samples based on their graininess. The test was designed on RedJade consumer and sensory software (RedJade Sensory Solutions LLC, California, USA). All samples were presented to panellists in 50 mL white plastic lidded cups and under fluorescent light. The Thurstonian model was used to analyse the degree of difference between each pair of samples (O'Mahony & Rousseaub, 2003).

This model is mostly used when multiple factors may have changed between samples. For example, in this study, particle size distributions and molecular weight of pectin were changed between pomace samples. In this model, panellists were asked to select a non-numerical answer. Therefore, collected data needs a type of analysis to convert them into an understandable numerical measurement. For this reason, R-index rating analysis was introduced to provide a numerical analysis as probability of differences between two samples. This value gives the probability of whether two samples were correctly discriminated from each other or not. Conversion of this probability to the actual degree of differences between samples is about measuring the d′ value (refer to Chapter 2, Section 2.9). For this analysis, it is assumed that the perception of target factor in the sample has a normal distribution. In this situation, d′ will be the distance between the mean values of two paired samples (Chapter 2, Figure 2.23). Moreover, the relationship between R-indices and d′ are given in tabular form in Appendix 3, originally by Patricia Elliot and reproduced by Swets (1964).

7.4 Results and Discussion

Two performance characteristics of heat-treated apple pomace were evaluated, both interactions between insoluble particles present and pectic residues in solution: physical stability against settling and sensory characteristics. In both cases the presence of large particles was expected to harm performance and presence of enough soluble fibre of sufficient chain length expected to benefit.

7.4.1 Sedimentation of Reconstituted Pomace Samples

A sedimentation test was carried out on recombined pomace samples (according to Table 1.2). The results of combining big particles with HMw, MMw and LMw pectin is given in Figure 7.1 and 7.2.

The effect of storage on phase separation of reconstituted pomace samples after 120 h is given in Figure 7.1, which are representing the photo of one replication until hindered settling phenomena dominate, sedimentation. Results can be explained by Stokes law which is about the movement of spherical particles in a liquid under the force of gravity. In this theory, the size (radius) of particles and viscosity of surrounded matrix directly affect the separation force between particles and serum (Will et al., 2008). In this theory, the particles are assumed not having any effect on each other.

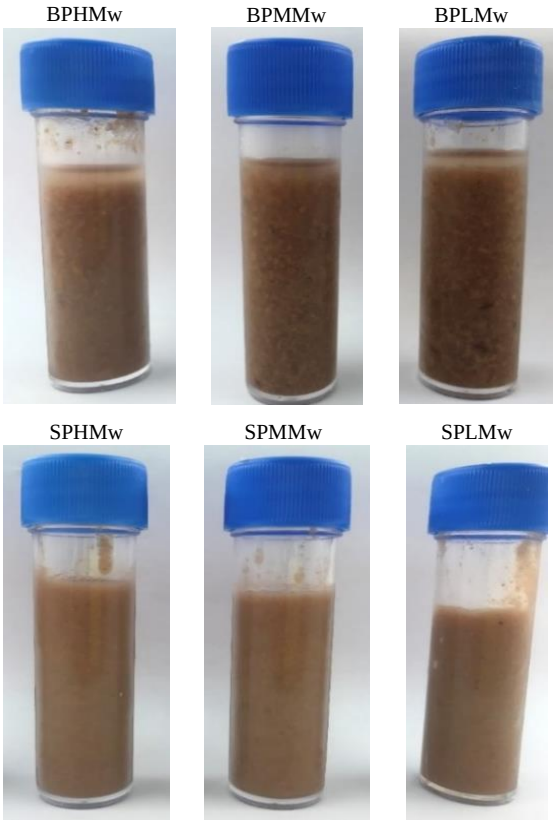


Figure 7.1 Phase separation of reconstituted pomace samples contained two distributions of solid particles ('big' and 'small') and three pectin molecular weight distributions (HMw, MMw and LMw pectins). Samples were kept at 4 °C for 120 h, then volume of each phase separation were measured.

The percent of volume changes of these samples are given in Figure 7.2. According to the results, in pomace samples containing 'big' particles (and regardless of the pectin molecular weight), obvious sedimentation was observed during the first 12 h storage at 4 °C then it reached a plateau with % volume changes of ~8% (changes in the volume of solid particles in the tube) for longer storage time. The reconstituted pomace samples with 'small' particles (regardless of the pectin molecular weight) were stable over time (between 0-120 h) as no phase separation was observed. This test was evaluated in two replications.

The presence of particles in the current study with the average size of ~500 µm ('big') increased the tendency of the samples to sediment. Moreover, it seems that the viscosity provided by high molecular weight pectin (~ 380 kDa) in pomace samples was not enough to prevent this phase separation. It is important to note that the pomace sample in this test still contained at least 2 times water than the original pomace (pomace:water ratio of 1:2).

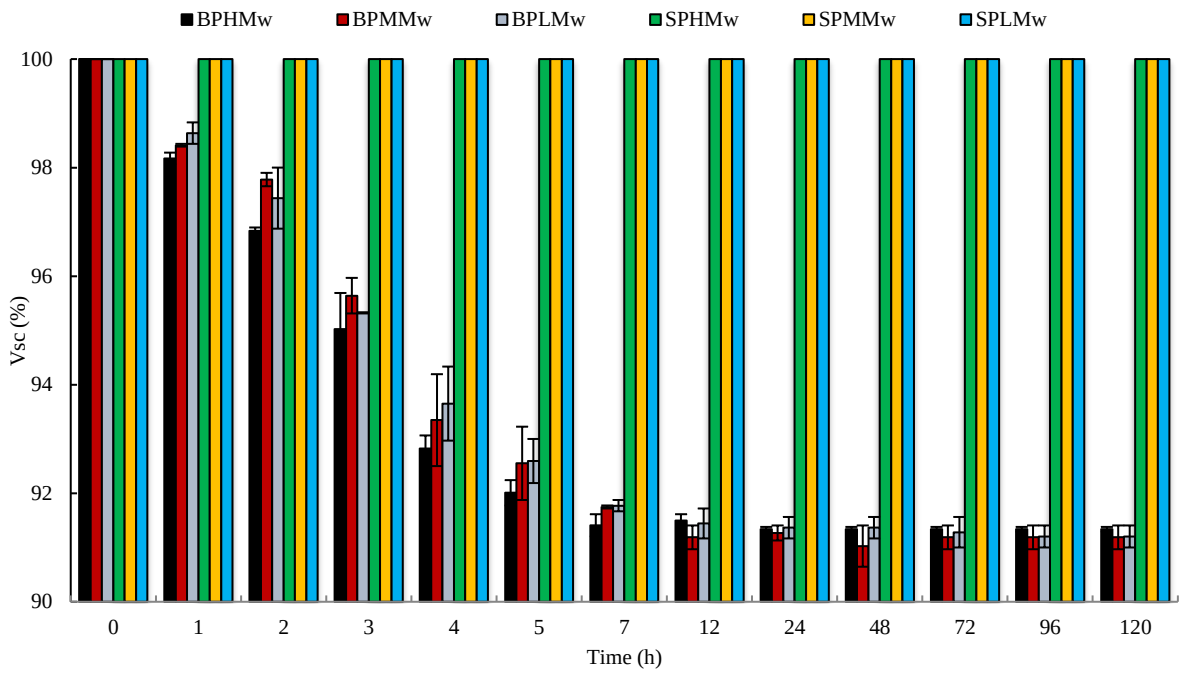


Figure 7.2 Average percent changes in volume of insoluble solids over time when storing samples of BPHMw, BPMMw and BPLMw at 4 °C for 0-120 h. The error bars represent the maximum and minimum value of two replicate samples.

Decreasing the size of solid particles to ~160 µm could effectively prevent phase separation in all pomace samples containing any molecular weight of pectin. Sato et al. (2009) showed that increasing the particle size (> 500 µm) in jaboticaba (a Brazilian fruit) suspension resulted in decreasing the yield stress as one of the rheological parameters. Yield stress shows the tendency of the aqueous phase of suspension to separate by gravity. Bagheri et al. (2014) and Corredig et

al. (2001) showed that the decreasing the size of pulp particles in orange juice to < 150 μm and presence of food stabilisers such as pectin or gelan can increase the stability of orange juice to prevent phase separation. On the other hand, Will et al. (2008) revealed that the addition of apple puree with an average particle size of 200 μm to cloudy apple juice increased the rate of sedimentation.

7.4.2 Sensory Evaluation of Modified Pomace Product

The mouthfeel properties of processed fruit and vegetable products are highly correlated with particle size distribution, surface properties (particularly the smooth surface of particles) and viscosity of dispersal medium (Appelqvist et al., 2015). In this study, a sensory evaluation was designed for testing five pomace samples of BPHMw, SPHMw, SPMMw, SPLMw and SP for graininess/ smoothness characteristics. Two reasons can be given for decreasing the number of pomace samples (i.e., not presenting BPMMw and BPLMw to panellists). Firstly, preventing panellists from getting confused with many pomace samples. Secondly, in the current study, HMw pectin was expected to have better ability to cover sharp edges of particles so that they do not feel grainy in the mouth, than LMw pectins.

Figure 7.3A shows the number of panellists who voted for each sample from being grainy or smooth, based on the choices given, which were G, GNS, GNVS, SNVS, SNS and S. Figure 7.3B shows the raw data of the distribution of the panellists' responses to the sensory question for graininess and smoothness criteria. From this figure, 85% of panellists rate the BPHMw to be 'G' and 12 % rated it as 'GNS'. In contrast, 49 and 31% of the votes were received for SPHMw sample to be 'S' and 'SNS', respectively. The SP sample panellists rarely voted it to be 'G' or 'S'.

A

Responses	Sample name				
	BPHMw	SPHMw	SPMMw	SPLMw	SP
G	71	1	2	1	6
GNS	10	3	4	9	18
GNVS	3	4	3	5	17
SNVS	0	9	8	17	19
SNS	0	26	34	26	18
S	0	41	33	26	6
Sum	84	84	84	84	84

B

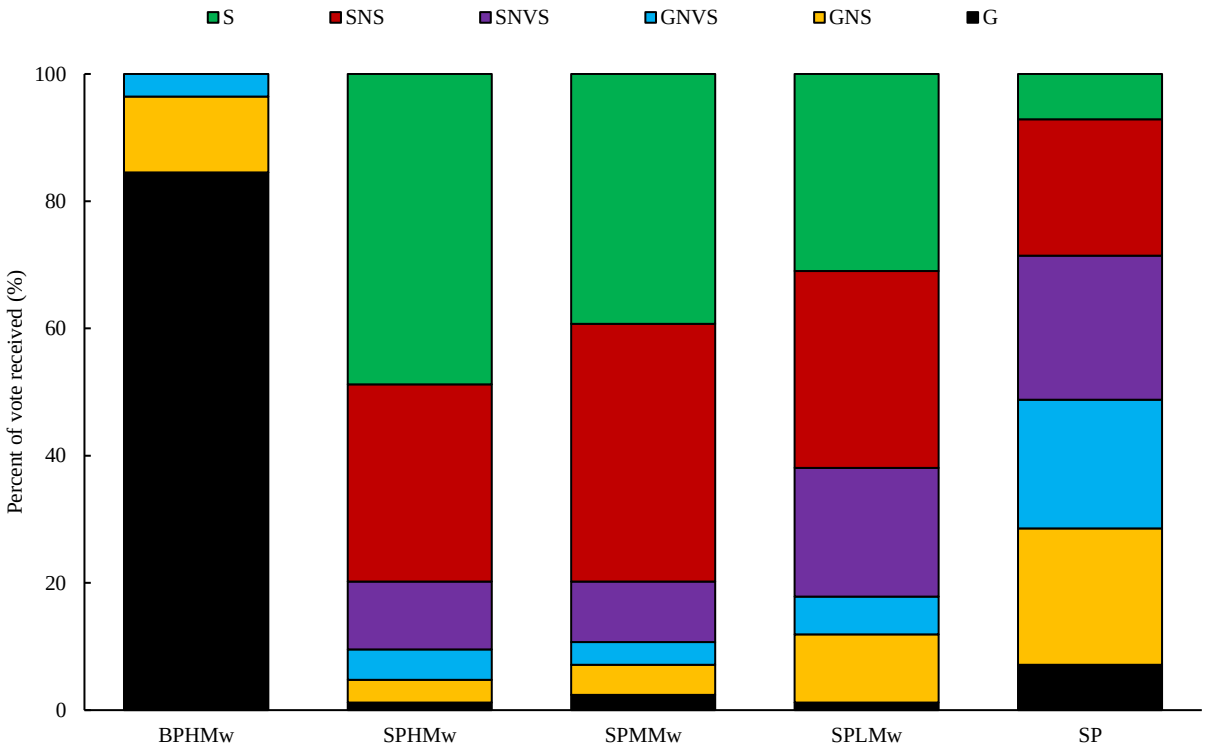


Figure 7.3 A) the number of panellists responded to each sample, and B) the percent of votes received for each pomace sample during sensory evaluation from the graininess and smoothness.

Vote percentage cannot show the discrimination ability of consumers to detect the differences between samples, nor show how significant any differences are. Therefore, Thurstonian modelling was used to analyse the degree of differences between each pair of samples data in this study, when graininess of the samples is considered as the main stimulus. The R-indices of each pair of samples were analysed according to the equation given by O'Mahony (1992). Below one

pair of samples were analysed as an example for calculating R-index value using equation 7.2 below.

Equation 7.2 R-index equation when one pair of samples is analysed statistically.

	G	GNS	GNVS	SNVS	SNS	S
Sample 1	a*	b	c	d	e	f
Sample 2	g	h	i	j	k	l

* The letter shows the number of people selecting the response for each sample.
Note: ∑(a+b+c+d+e+f) = ∑(g+h+i+j+k+l) = 84

$$R-index = \frac{a(h+i+j+k+l)+b(i+j+k+l)+c(j+k+l)+d(k+l)+el+1/2(ag+bh+ci+dj+ek+fl)}{(a+b+c+d+e+f)\,(g+h+i+j+k+l)}$$

Table 7.3 shows the R-index values for each pair of samples.

Table 7.3 Probability of real differences (R-index) between each pair of pomace samples. An R-index of 0.50 means panellists could not discriminate between samples. An R-index > 0.50 indicates panellists were increasingly able to discriminate between samples in a pair presented to them: a perceivable difference is increasingly probable.

Particle size	Pectin size		Small (165 µm)			Big (500 µm)
			MMw (150 kDa)	LMw (30 kDa)	No pectin	HMw (380 kDa)
Small (165 µm)	HMw (380 kDa)	-	0.54*	0.62	0.81	0.99
	MMw (150 kDa)	-	-	0.58	0.79	0.98
	LMw (30 kDa)	-	-	-	0.72	0.98
	No pectin	-	-	-	-	0.93

Note: * numbers in each row show the R-index value when comparing two samples.

The R-index shows the probability of perceivable differences between one pair of samples. In order to measure the degree of differences between samples, the d′ statistic needed to be measured. In order to do this, the relationship between R-indices and d′ given in the table of Patricia Elliot in Swets (1964) was used (refer to Appendix 5). This d′ value shows the degree of differences between each pair of samples and describes the information on discriminability of consumers on understanding the differences between products (Bi et al., 2010). If d′< 0.8, it means that samples

were similar, and if d′> 0.8 two products are easy to distinguish as different samples. The maximum value of d′ will be 3.28 (O'Mahony & Rousseau, 2003; Lee et al., 2004; McClure, 2008; Morten et al., 2007).

Table 7.4 shows the d′ values for analysing each pair of samples. Analysing the results of BPHMw versus any other pomace samples resulted in d′ >2. Therefore, this sample was obviously grainy and easy for panellists to discriminate from others. Analysing results of SPHMw versus the SPMMw and SPLMw showed d′< 0.8. It can be concluded that people could not discriminate between these samples.

In this study, one sample of SP was also tested during the sensory evaluation. This sample contains small particles resulting from sheared pellet and permeate for 5 min using medium screen, with no addition of retentate with pectin of any molecular weight. This design for the sensory test helped to compare the SP sample with the other recombined pomace samples of SPHMw, SPMMw and SPLMw.

Table 7.4 Degree of differences (d') when analysing each pair of sensory samples. A d' value of <0.8 means people could not discriminate. A d'= 0.8 means possible differences between samples and when d'> 0.8 means high confidence of true differences between samples.

Particle size		Small (165 µm)				Big (500 µm)
	Pectin size		MMw (150 kDa)	LMw (30 kDa)	No pectin	HMw (380 kDa)
Small (165 µm)	HMw (380 kDa)	-	0.14*	0.43	1.24	3.28
	MMw (150 kDa)	-	-	0.28	1.14	3.28
	LMw (30 kDa)	-	-	-	0.82	2.90
	No pectin	-	-	-	-	2.08

Note: * numbers in each row show the d' value when comparing two samples.

In the current study, the d′ analysis when pairing SP with SPLMw, SPMMw or SPHMw resulted in d′ values of 0.82, 1.14 and 1.24, respectively. This means that there were significant and distinguishable differences between SP and other recombined pomace samples containing small particles. Moreover, these differences (d′ values) were increased by increasing the molecular weight of pectin. It can be concluded that the smoothness perception was affected by the presence

of pectin and by that pectin's molecular weight. Sensory properties of a food product are mostly evaluated using descriptive test and scale descriptors. However, a scale descriptor test is mostly used when the intensity of one component in the product is changing. In the current study, two attributes were varied at the same time and were expected to have subtle effects on the sensory properties of the final product. In these conditions where the difference between samples might not be easy to detect by the panellists, a discrimination test is often a better technique to use. This type of test is not very common in food industry and may have some downsides such as, not being able to compare all samples with each other at the same time and not being able to make a decision on intensity of each attribute on target sensory properties.

These results of this study were in overall agreement with Bengtsson et al. (2011) who decreased the size of insoluble particles of different fruit and vegetable suspensions by homogenising them in a kitchen blender or high pressure-homogeniser. They showed that in apple suspensions created by high-pressure homogenisation, the average particle size distribution was ~1.5 times smaller than the suspension made in a kitchen blender. This substantially decreased the graininess of the apple suspension.

In addition, Appelqvist et al. (2015) showed that producing smoother solid particles (through the presence of more single cells) with average particle size of 92 μm in carrot dispersions, relative to very small sub-cellular fragments with average particle size of 56 μm (that might also aggregate), could help in not perceiving the separate particles in the mouth. These samples were prepared by homogenising cooked carrots in water. In the current study, shearing was also applied after thermal treatment, for 5 and 10 min using a high shear rotor-stator mixer. The numbers of broken cells with sharp edges increased at higher shearing times (Chapter 6, Fig 5 and 6).

Espinosa-Muñoz et al. (2012) and Appelqvist et al. (2015) revealed that increasing the viscosity of the surrounded medium helps to perceive particles less distinctly and gave more consistency and smoothness (creaminess) in plant dispersions. In contrast to the current study, in both articles given above the preparation of fruit or vegetable suspensions contained the addition of a food thickener (pectin or xanthan solutions). These extra additives might help to cover the particles, resulting in not perceiving them individually. In the current study, the chemical composition of sensory samples was kept similar to the initial heat-treated apple pomace, and no extra source of pectin was introduced into the samples.

Soluble pectin molecular weight can directly affect the viscosity of the serum phase, providing higher viscosity (Sayah et al., 2016). Decreasing the molecular weight of pectin through enzymatic hydrolysis will decrease the viscosity of the serum phase as well (Yamasaki et al., 1967; Pacheco et al., 2019). Unfortunately, in this study, the reconstituted serum was very thin and we were unable to measure the viscosity in the limited range of the rheometer. According to

the reference given above, it is likely that pomace serum phase in the current study may have similar viscosity behaviour.

It is possible that HMw pectin in serum provided higher viscosity and therefore had a better lubricant role in covering the rough edges and surfaces of the insoluble particles. Decreasing the size of pectin polymer decreased this ability, consequently, increasing the perception of particles.

7.5 Conclusion

This chapter reports work to measure physical and sensory performance of the heat-treated pomace material. In both stability against sedimentation and in smoothness of mouthfeel, it was expected that larger particles would make performance worst and long chain pectic oligomers make performance better.

The sensory study was designed to test consumers' ability to detect large or small apple cell wall particles in the presence or absence of soluble pectin, and the impact of pectin molecular weight on any screening effect that pectin might offer. Prepared pomace ingredients with two particle size distributions ('big' and 'small' particles) and three molecular weight of soluble polymers (HMw, MMw and LMw pectin) were tested for physical stability in aqueous suspension and sensorial properties. This comparison showed that increasing the molecular weight of pectin in pomace samples helped samples to feel smoother in the mouth. This is consistent with lubrication and covering residual sharp edges of cell fragments.

CHAPTER 8 Summary and Implications for Industrial Process

Apple pomace is the left-over solid residue remaining after pressing apples. It is rich in plant cell wall polysaccharides that cannot be digested by the human system (although some may be fermented by gut bacteria). Pomace is, therefore, a good source of dietary fibre, and devising a process that can convert pomace to a high fibre food ingredient with desirable functional purposes (such as texturising agents that can provide smooth mouthfeel) can deliver a potentially profitable material out of the processing waste. The goal of this research was devising a process of steps which are simple, robust and which can produce a shelf stable (without phase separation) pomace ingredient which has smooth mouthfeel.

Dried pomace products are not new and flour milled from dried apple pomace has been available on the market for some time. Neither is apple pectin new. For several decades, pectin has been extracted by hot water, dried and sold as a gelling or thickening agent. But here has been no prior report of a product where pectin is extracted from the cell wall then retained as a soluble component of the flour, to modify its sensory and stability properties when used in formulated food systems. The current study was geared to the conditions expected for an industrial process. A process should accommodate the natural pH of the pomace, the hydrothermal step be brief enough to be affected continuously and the shearing step be achieved in the presence of viscosifying pectic oligomers in solution.

The current study developed a systematic approach to select appropriate operational conditions of hydrothermal treatment (time and temperature), and shearing treatments of pomace (using New Zealand's apple pomace as a model) to achieve the sensory and stability targets. The process would employ a small number of standard equipment items and achieve near 100% yield on incoming pomace solids. Figure 8.1 summarises the processing steps employed during the project. These were not anticipating industrial use, but specifically to produce a range of preparations for sensory analysis.

The two most important characteristics of processed pomace are: the effect of solubilised polysaccharides and the size and shape of the remaining insoluble particles. Together these properties can influence mouthfeel, physicochemical properties, product stability as well as handling properties in the processing plant itself. The novelty of this particular study lies in needing to study pectin reactions and extraction within the full complexity of the pomace system. Both shear and time/temperature can influence pectin mobility. Care was taken in setting cell wall: water ratios. Care was taken to ensure the ionic and osmotic conditions of the suspending solution was controlled at all stages, especially when presenting samples for tasting.

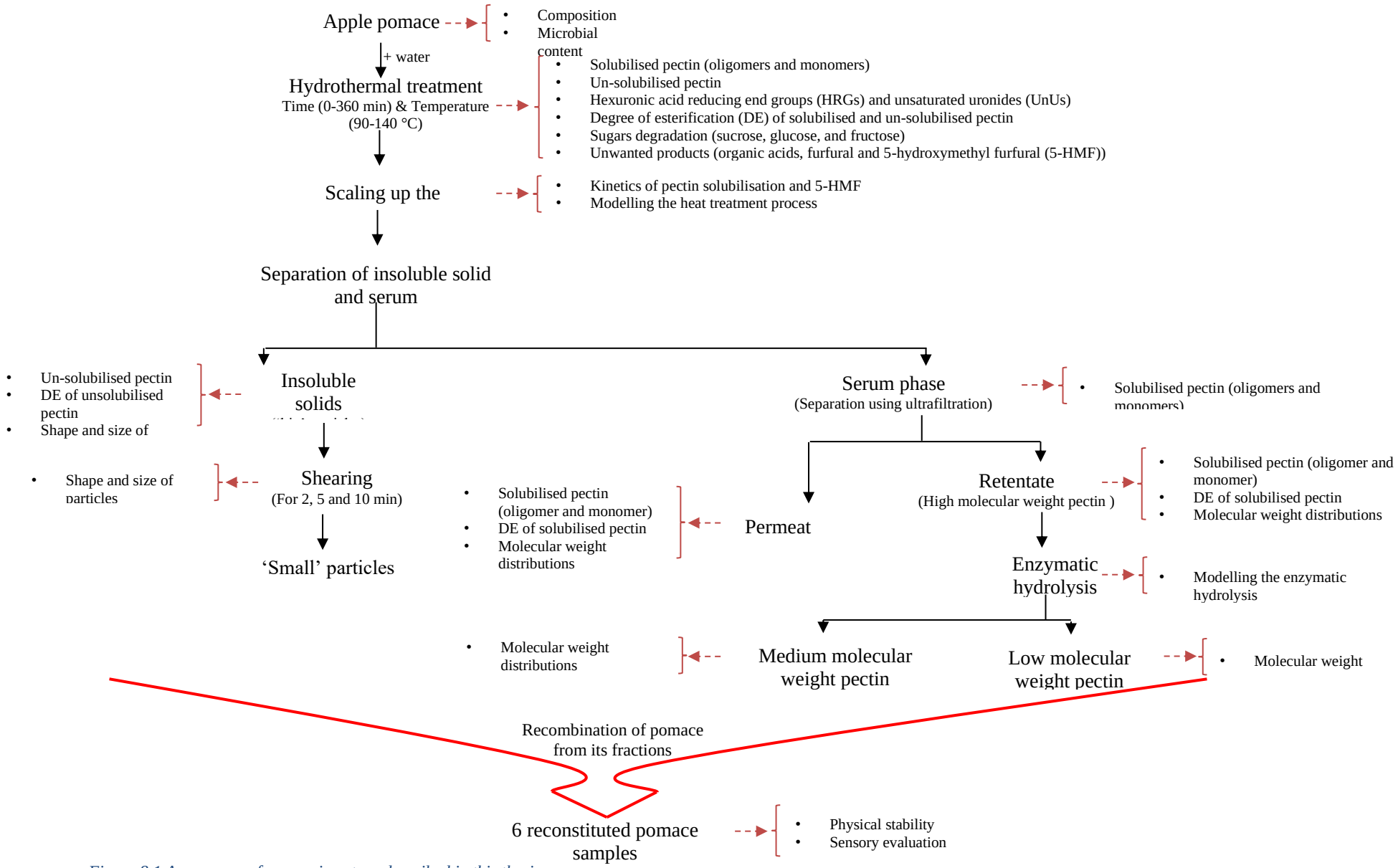


Figure 8.1 A summary of processing steps described in this thesis.

The current study was an applied science project with this purpose to design a simple process converting apple pomace into a functional and valuable food ingredient. This process was aimed to have capability for scaling up to industrial size. The first step in scaling up from laboratory to pilot plant was successfully achieved which confirmed the selection of equipment and kinetic of reactions involved in pomace were correct.

A particular philosophic approach was adopted in the process development: a very small number of simple unit operations to achieve a large array of physico-chemical processes. By combining a hydrothermal and shear process, bacteria and enzymes were to be deactivated, pectin cleaved, rendered soluble and extracted into the serum; cell walls were to be made less rigid on the tongue and easier to tear during shear treatment, cells were to be separated one from another and shorn of sharp projections, cells were to be rounded and made easily deformable during oral processing. All this was to happen without undue production of deleterious components or flavours and without pre-disposing the solutes and dispersed phase to irreversible aggregation upon subsequent drying (not reported here). The simplicity of the process offers industrial adaptability. This process can be applied in an in-line production line directly after releasing the pomace from the juicing belt-press equipment and can be tuned to the needs of different pomaces and products. An international process engineering company has been involved in the latter stages of this study and is currently designing and costing full-scale equipment targeted at a particular application for a first client. The intension is that they will hold a license to sell the process world-wide.

The industrial process anticipated at the beginning of this project is given in Figure 8.2 below. The process aimed to be continuous and contains hydrothermal, physical and enzymatic steps, which deliver a concentrated or dried ingredient product. Evaporation and drying steps in the industrial approach can influence the storage and shelf life properties of the processed pomace by decreasing the water content and slowing down or preventing the microbial growth. In this process, the pips, stalks and labels would first be removed from fresh apple pomace as it came off the juicing production line using a pulper/refiner. Then a coarse shearing may be applied to break big particles in apple pomace and give it the ability to be pumped for the next step (hydrothermal treatment). In this step, pomace would be heated to loosen the compact cell structure of the pomace and to solubilise pectin polymers. In addition, heating would inactivate enzymes and kill microorganisms providing a stable and safe product for human consumption. The next two steps contain the addition of enzymes (commercial pectinase and/or cellulase) to modify the particle size, and solubilise pectin polymers, then inactivating those enzymes by applying heat. The prepared pomace ingredient would then be concentrated into a thick paste-like material, which could be packed as it is or being dried and then packed. The aims of the evaporation and/or drying

steps are increasing stability by decreasing the water content of the product as well as ease of handling of the final product.

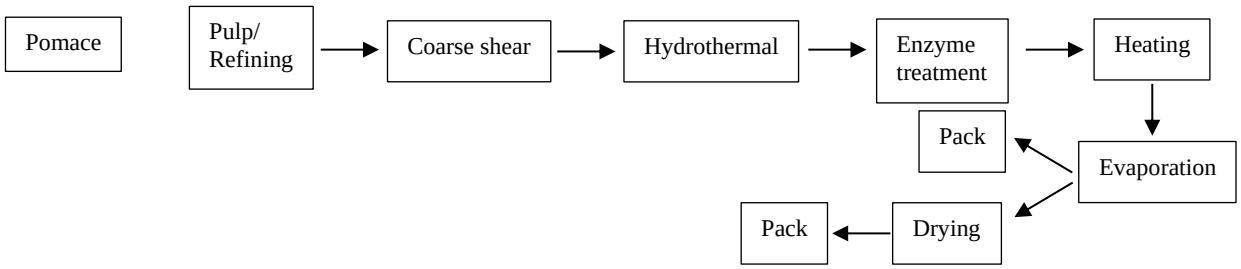


Figure 8.2 An industrial anticipation process for the pomace project.

The results of the sensory evaluation in this research showed that pectin molecules with a higher degree of polymerisation were more effective in covering the solid pomace particles, helping them not to be felt in the mouth. Therefore, enzymatic hydrolysis would not be required for producing a smooth food ingredient. The currently envisaged industrial process omits an enzyme step and for this project is given in Figure 8.3.

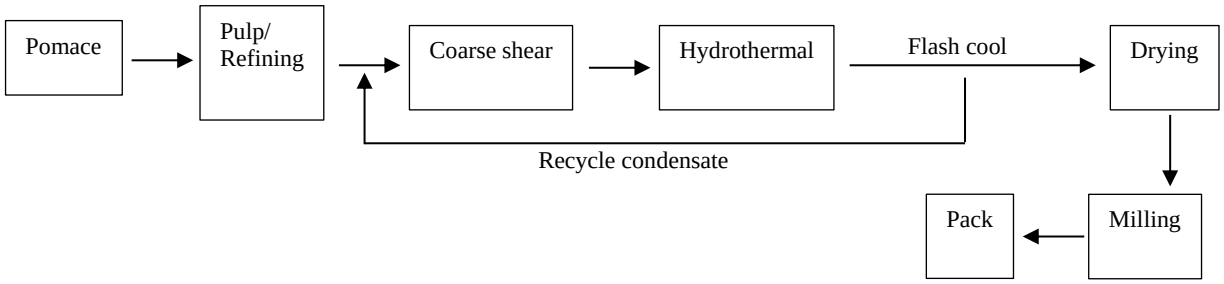


Figure 8.3 Proposed process for conversion of apple pomace into a functional ingredient.

The new proposed process is shorter and easier to operate in industrial scale than Figure 8.2. The final confirmed process for industry scale should meet the requirements of continuous, compact and simple.

In the currently proposed process, hydrothermal treatment of pomace will use UHT direct steam injection for simplicity of heat transfer into pomace. This study showed that addition of water to pomace prior to heat treatment had positive effect on increasing the pectin solubilisation. In the proposed industrial process, some of this water can be supplied by condensed steam and some by condensate recycled from flash cooling of heat-treated pomace. Steam mixing and flashing are expected to contribute some coarse shearing effect. The level of water added prior to heating pomace may well be set by its pumpability through the UHT system. Dried materials would be then milled to smaller particles providing homogeneous dry ingredient according to the demands of each application. This step may also influence the wettability of the final ingredient. Drying

and size reduction and control of properties such as wettability and dispersibility of pomace ingredient demand further study.

8.1 Conclusions

A smooth pomace ingredient was produced through the process steps developed in this study. These steps were heating, shearing. Enzymatic treatment was used for preparing sensory samples and is no longer viewed a necessary to the industrial process. Soluble pectin is a key component for texture. Initial shearing of cold raw pomace did not change the amounts of pectin solubilised, however decreasing the particle size would have a positive effect on the pumpability of pomace at industrial scale. The addition of up to two parts of water to pomace increased pectin solubilised by hydrothermal treatment. The amount of pectin solubilised was similar regardless of whether water was added before or after heat treatment. This finding indicates that heat-treated pomace in a commercial process will release the expected amounts of pectin, but some may remain loosely associate with cell wall, until further water is added. Heat treated pomace could be added to a food production line. In this application, the water required to solubilise the pectin could be supplied by the moisture content of the food. This may result in an increase in the physical and microbial stability of the product.

Heating affects the physical and chemical properties of pomace. Pectin solubilisation showed temperature-time dependent behaviour and obeyed a first-order reaction. The equilibrium amounts of pectin were about two times higher than the amounts of pectin initially solubilised at room temperature (RT). The kinetic model for pectin solubilisation showed a good predicted model with R^2 of 0.94 at reference temperature of 100 °C. The rate constant and activation energy for pectin solubilisation at reference temperature was calculated to be 0.021 min^{-1} and 81 kJ mol^{-1}, respectively.

Solubilised pectin can be followed by subsequent degradation into smaller fragments through acid hydrolysis and β-elimination. These reactions are highly dependent on pH. In this study, the natural pH of pomace remained constant during heating (pH ~ 3.5). Depolymerisation reactions took place to a small extent, revealing that suitable conditions for these reactions were not met in this study. These findings suggested that solubilised pectin is mostly present in polymeric form without a great reduction in its molecular weight. Heating also resulted in breaking down the solubilised pectin into its subunits of mono-galacturonic acids (mono-GalUA). Production of mono-GalUA accelerated at temperatures higher than 110 °C.

An increase in degree of esterification (DE) of solubilised pectin was observed especially at high temperatures > 120 °C. In addition, DE of remained insoluble solids demonstrated an un-expected rising trend during hydrothermal treatment. These finding suggested that low esterified pectin was more sensitive to high temperatures than high esterified pectin. Therefore, depolymerisation had been taken place most likely from un-esterified sites of pectin molecules.
Hydrothermal treatments can also break and rearrange soluble components, producing organic acids (such as acetic acid, lactic acid and formic acid), 5-hydroxymethyl furfural (5-HMF) and furfural. The kinetic model developed for 5-HMF showed a good prediction with an R^2 value of 0.99 at the reference temperature of 120 °C. The rate constant and activation energy of this reaction at reference temperature were 0.0025 min^{-1} and 105 kJ mol^{-1}, respectively.

The kinetic models of pectin solubilisation and 5-HMF production were used to predict the hydrothermal treatment of pomace at pilot scale using a retort. A heat transfer model was used to predict the time-temperature profile throughout the pomace. The validity of pectin solubilisation and 5-HMF formation predictions were confirmed experimentally.

The results of modelling in a more complex heat transfer situation and its relationship with chemical reactions taking place at each time and temperature, give confidence of predictability for the main pomace reactions in other industrial heat treatment equipment.

Scanning electron microscopy of heat-treated pomace suggested that the compact structure of pomace was weakened and the fibrous connections between cells were exposed. Consequently, shearing mostly detached adjacent cell structures along these fibrous connections. This treatment could effectively decrease the particle size distribution to the average of ~ 165 μm. Molecular weight of solubilised pectin in serum phase after heat treatment was about 8 times higher than the dissolved pectin in the raw pomace (before heating). Enzymatic hydrolysis of retentate was modelled and confirmed at laboratory scale, resulting in two ranges of pectin molecular weights from retentate fraction.

Controlled modification of soluble and insoluble elements of heat-treated pomace was done, to generate preparations to examine the sensory properties of treated pomace. Pomace fractions were modified, then recombined in ratios that ensured they were isotonic with respect to freshly treated pomace.

Heat-treated pomace samples containing small particles showed good physical stability during five days storage. Size of particles and molecular weight of pectin both had influence on the smoothness perception. Consequently, the sample contains the smallest particles (~165 μm)

combined with the highest molecular weight of pectin (~ 380 kDa) was recognised as the smoothest when evaluated by the panellists.

In summary, this study provided a guideline for processing the waste streams from fruit juice factories (especially for apple) into a profitable ingredient with functional properties. The proposed steps in the overall process (heating and shearing) can be modified and/or omitted, depending on the final purpose of the pomace ingredient. The final process developed in this study was simple, containing few treatment steps and had been approved its capability for scaling up.

8.2 Recommendations

The recommendations for further research on fruit and vegetable pomace projects are as follows.

- **Shearing technique.** In this study, one shearing equipment type (benchtop Silverson rotor-stator homogeniser) was used. However, optimising other shearing types of equipment such as in-line Silverson homogeniser, colloid mill, high pressure homogeniser may deliver smaller particles or release more single cells from pomace.
- **Insoluble solids and soluble polymers ratio.** To determine the capacity of high molecular weight pectin to cover the solid particles, there needs to be a study on increasing the ratio of solid particles versus pectin content in combination with sensory evaluation.
- **Rheological properties of pomace samples.** Rheological properties of solid-liquid suspensions usually need an in-depth study. The rheology results can be compared with sensory results for better understanding of the effect of viscosity and rheological behaviour of pomace ingredients when its placed in the mouth as well as handling this ingredient in a process at larger scale.
- **Further research on physicochemical and sensorial properties of dried and rehydrated pomace ingredients.** No testing was done on the propensity of small or big cell wall particles to aggregate on drying and if pectin polymers may impede it. Moreover, even if aggregation happening while drying, whether this aggregation is reversible when reconstituting pomace ingredient or not, and what is the effect of pectin polymers on it.
- **Addition to food.** Further research on the application of modified pomace in food products needs to be carried out, particularly in relation to the interaction of pectin and solid particles with other food components such as calcium, protein, and acidity of the food product.
- **Other pomaces.** Apple pomace used in this study is known to be an acidic fruit residue. Expanding the current proposed process to other acidic fruit and vegetable pomaces such

as citrus, pear, kiwifruit and grape pomaces needs to be investigated. In addition, other pomaces such as carrot pomace (with alkaline pH) may need further investigation of kinetics of reaction of heating, and possibly other enzymatic investigations as well.

- **Industrialisation of process.** Applying the current process developed in this study at an industrial scale needs further investigation on the selection of equipment and optimisation the types of equipment for delivering pomace ingredients in a continuous production line. Moreover, the pomace process introduced here contained added water (pomace-water ratio1:2). An industrial scale process may need to be carried out without addition of water.

Appendix 1 Analytical results of apple pomace

The microbial content of apple pomace used in this study was measured in the Microbiology laboratory at Massey University (School of Food and Advanced Technology). Results are shown below:

Appendix Table 1 Microbial content of apple pomace used in the current study.

Sample name	Aerobic plate count	Yeast and moulds	Thermophilic acidophilic bacteria	Method
Apple pomace	$1.1\pm0.1\times10^3$ CFU/g	$5.8\pm0.5\times10^3$ CFU/g	2.0×10^1 CFU/g	Non-selective plate counting; Most probable number method

Composition of apple pomace used in this study (Chapter 4, Section 4.4.1) was done by researcher or Nutrition Laboratory, Massey University, and results are shown below:

Appendix Table 2 Composition of apple pomace used in the current study.

Fraction number	Fraction name	Amount	Unit	Methodology
1	pH *	3.5	-	Chapter 3, Section 3.4.1
2	Moisture content *	83%	-	Chapter 3, Section 3.1.5
3	Degree of esterification (DE) *	56%	-	Chapter 4, Section 4.2.8
4	Galacturonic acid (from the polymeric from) *	884.66	µmol GalUA/g dry pomace	Chapter 3, Section 3.1.7
5	Mono galacturonic acid *	193.69	µmol GalUA/g dry pomace	Chapter 4, Section 4.2.4.1
6	Total galacturonic acid *	1078.35	µmol GalUA/g dry pomace	Sum of fraction numbers 4 and 5
7	Fructose *	735.90	µmol /g dry pomace	Chapter 4, Section 4.2.7
8	Glucose *	237.86	µmol /g dry pomace	Chapter 4, Section 4.2.7
9	Sucrose *	66.15	µmol /g dry pomace	Chapter 4, Section 4.2.7
10	Insoluble dietary fibre **	0.48	g/g dry pomace	Megazyme, AOAC 991.43
11	Soluble dietary fibre **	0.08	g/g dry pomace	Megazyme, AOAC 991.43
12	Total dietary fibre **	0.57	g/g dry pomace	Sum of fractions number 10 and 11
13	Ca **	0.00127	g/g dry pomace	Biological materials digestion. Analysis by ICP-OES
14	Mg **	0.00057	g/g dry pomace	Biological materials digestion. Analysis by ICP-OES
15	Protein **	0.04	g/g dry pomace	AOAC 968.06 (Dumas method). N-P = 6.25
16	Total phenolic **	0.08	g/g dry pomace	Folin-Ciocalteau method, Food chemistry, 120(4):993-1003
17	Ash **	0.01	g/g dry pomace	Furnace 550°C AOAC 942.05 (Feed, meat)
18	Fat **	0.03	g/g dry pomace	(Mojonnier) Acid, AOAC 954.02
19	Sugar **	0.23	g/g dry pomace	(Dubois et al., 1956)
20	Total carbohydrates **	0.90	g/g dry pomace	Subtracted the initial amounts of sample from total amounts of water and fraction numbers 12, 15, 17, and 18.
21	Neutral Detergent Fiber **	0.45	g/g dry pomace	Fibertec, AOAC 2002.04, 973.18
22	Acid Detergent Fiber **	0.37	g/g dry pomace	Fibertec, AOAC 2002.04, 973.18
23	Lignin **	0.19	g/g dry pomace	Fibertec, AOAC 2002.04, 973.18
24	Cellulose **	0.18	g/g dry pomace	Subtracted the fraction number 22 from 23
25	Hemicellulose **	0.07	g/g dry pomace	Subtracted the fraction number 21 from 22

Note: * The measurements were done by the researcher Marzieh Eblaghi. ** The measurements were done by the Nutrition Laboratory, Massey University, Palmerston North, New Zealand.

References: Dubois, M., et al. (1956), 'Colorimetric method for determination of sugars and related substances', Analytical Chemistry, 28 (3), 350-56.

- List of chemical elements were measured in apple pomace (Chapter 4, Section 4.2.1) by Hill Laboratories, Hamilton New Zealand. The final report is given below:

Hill Laboratories
TRIED, TESTED AND TRUSTED
R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand
T 0508 HILL LAB (44 555 22)
T +64 7 858 2000
E mail@hill-labs.co.nz
W www.hill-laboratories.com

Certificate of Analysis

Client:	Marzieh Eblaghi	Lab No:	2074482 SPv1
Contact:	Massey University Institute of Food Science and Technology Private Bag 11 222 Palmerston North 4442	Date Received: Date Reported: Quote No: Order No: Client Reference: Submitted By:	02-Nov-2018 14-Nov-2018 PN428171 Marzieh Eblaghi

Sample Type: Nuts, Fruits and Vegetables and Derived Products

		Sample Name:	Apple Pomace				
		Lab Number:	2074482.1				
Total Nitrogen	g/100g as rcvd		0.11	-	-	-	-
Calcium*	g/100g as rcvd		0.021	-	-	-	-
Magnesium*	g/100g as rcvd		0.0094	-	-	-	-
Potassium*	g/100g as rcvd		0.075	-	-	-	-
Sodium*	g/100g as rcvd		0.0021	-	-	-	-
Phosphorus*	g/100g as rcvd		0.0160	-	-	-	-
Sulphur*	g/100g as rcvd		0.0085	-	-	-	-
Iron*	mg/kg as rcvd		4.7	-	-	-	-
Boron*	mg/kg as rcvd		4.4	-	-	-	-
Copper*	mg/kg as rcvd		1.19	-	-	-	-
Manganese*	mg/kg as rcvd		1.06	-	-	-	-
Zinc*	mg/kg as rcvd		0.8	-	-	-	-

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Nuts, Fruits and Vegetables and Derived Products

Test	Method Description	Default Detection Limit	Sample No
Homogenise*	Mincing, chopping, or blending of sample to form homogenous sample fraction.	-	1
Total Nitrogen	Dumas combustion. AOAC 992.15, 19th edition.	0.02 g/100g as rcvd	1
Biological Materials Digestion*	Nitric and hydrochloric acid micro digestion, filtration.	-	1
Calcium*	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1
Magnesium*	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1
Potassium*	Biological materials digestion. Analysis by ICP-OES.	0.0008 g/100g as rcvd	1
Sodium*	Biological materials digestion. Analysis by ICP-OES.	0.0010 g/100g as rcvd	1
Phosphorus*	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1
Sulphur*	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1
Iron*	Biological materials digestion. Analysis by ICP-OES.	1.0 mg/kg as rcvd	1
Boron*	Biological materials digestion. Analysis by ICP-MS.	0.10 mg/kg as rcvd	1
Copper*	Biological materials digestion. Analysis by ICP-MS.	0.010 mg/kg as rcvd	1
Manganese*	Biological materials digestion. Analysis by ICP-MS.	0.010 mg/kg as rcvd	1
Zinc*	Biological materials digestion. Analysis by ICP-MS.	0.2 mg/kg as rcvd	1

IANZ ACCREDITED LABORATORY

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The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked *, which are not accredited.

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

This certificate of analysis must not be reproduced, except in full, without the written consent of the signatory.

Mark Bryant NZCS (Chemistry)
Senior Technologist - Food & Bioanalytical

Appendix 2 Kinetic models of main reactions in apple pomace

The majority of the model fittings have been reported here was done by Dr. Florencia Michaela Yedro a post-doctoral research engineer on the FIET (Food Industry Enabling Technologies) pomace fracturing project.

Kinetic models for solubilising pectin from insoluble solids of apple as well as acid hydrolysis and β-elimination reactions were developed from the experimental results gained in this study. First order kinetic models were designed for measuring the rate constant and activation energy. The kinetic model for 5-hydroxymethyl furfural (5-HMF) production was also developed to fit the results gained in this study. For simplifying this analysis, a zero-order kinetic was fitted for production of 5-HMF.

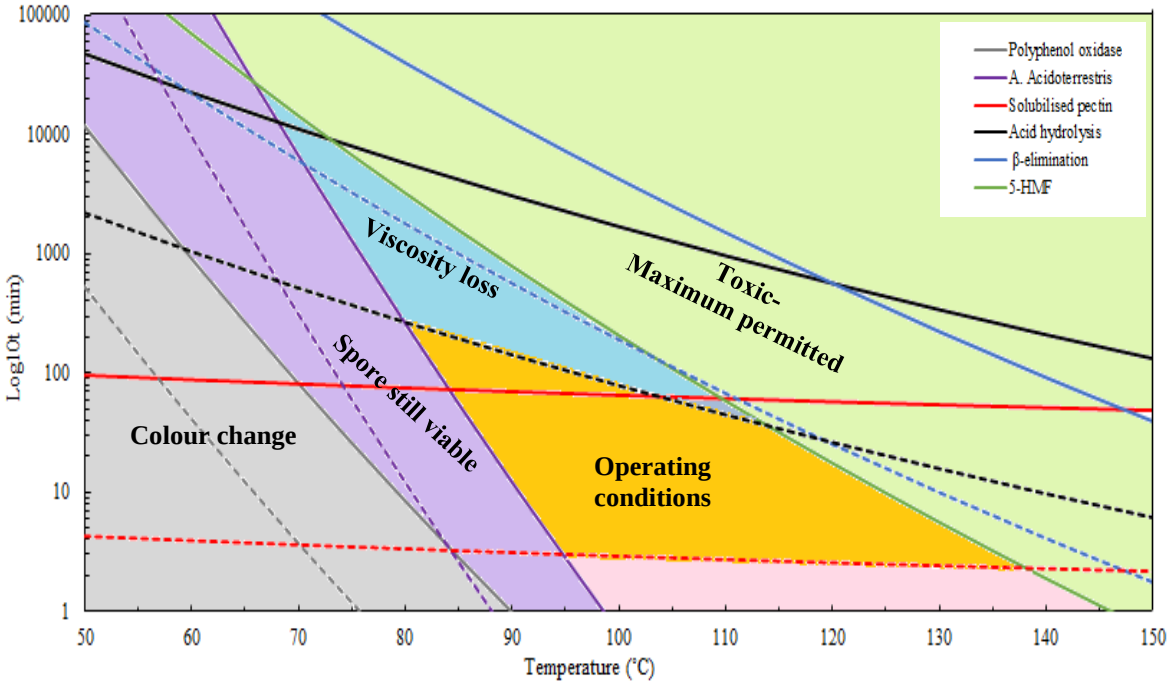
The kinetic model for inactivating the polyphenol oxidase in apple (Yemenicioğlu et al., 1997) and for *Alicyclobacillus acidoterrestris* inactivation (aciduric aerobic spore-forming bacteria found in apple juice (Splittstoesser et al., 1994) were designed according to data extracted from literature. The rate constant (K) and activation energy (E) of above reactions explained above are given in Table 1 below.

Appendix Table 3 Kinetic parameters of main reactions in heat-treated apple pomace.

Reaction name	**Order of reaction**	**T_{ref} (°C)**	**k_{ref} (1/min or µmol/mL min)**	**Ea (J/mol)**
Pectin solubilisation from pomace insoluble solid	1st order	90	0.033	77800
Acid hydrolysis	1st order	90	0.002	61200
β-elimination	1st order	90	0.0008	89100
Polyphenol oxidase inactivation	1st order	68	0.017	229000
Alicyclobacillus acidoterrestris inactivation	1st order	85	0.041	327000
5-HMF	Zero order	110	0.0008	150000

Note= Tref (reference temperature), Kref (reference rate constant) and Ea (activation energy).

According to the kinetic parameters given above, a time-temperature graph was plotted when heating pomace between 50 to 150 °C for 10 or 90% conversion of each reaction given above (Figure 1).



Appendix Figure 1 Inactivation of polyphenol oxidase, microbial content (A. acidoterrestris), pectin solubilisation, non-enzymatic pectin reactions (acid hydrolysis and β-elimination) and degradation products (5-HMF) profile in apple pomace (continues line means 90% conversion, dashed line means 10% conversion).

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Appendix 3 Ethics approval letter for sensory evaluation of apple pomace

In this study, panellists were going to consume food ingredients. It is known that in any study involving human subjects which may put them at any risk requires an ethics approval. Here is given the ethics approval letter for sensory evaluation in this study.

Ethics Notification Number: 4000020821

Title: Sensory evaluations of fruit pomace products

Thank you for your notification which you have assessed as Low Risk.

Your project has been recorded in our system which is reported in the Annual Report of the Massey University Human Ethics Committee.

The low risk notification for this project is valid for a maximum of three years.

Please note that travel undertaken by students must be approved by the supervisor and the relevant Pro Vice-Chancellor and be in accordance with the Policy and Procedures for Course-Related Student Travel Overseas. In addition, the supervisor must advise the University's Insurance Officer.

A reminder to include the following statement on all public documents:

"This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named in this document are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you want to raise with someone other than the researcher(s), please contact Professor Craig Johnson, Director (Research Ethics), email humanethics@massey.ac.nz. "

Please note that if a sponsoring organisation, funding authority or a journal in which you wish to publish require evidence of committee approval (with an approval number), you will have to complete the application form again answering yes to the publication question to provide more information to go before one of the University's Human Ethics Committees. You should also note that such an approval can only be provided prior to the commencement of the research.

You are reminded that staff researchers and supervisors are fully responsible for ensuring that the information in the low risk notification has met the requirements and guidelines for submission of a low risk notification.

Yours sincerely
Professor Craig Johnson
Chair, Human Ethics Chairs' Committee and
Director (Research Ethics)

Appendix 4 Information sheet and consent form for sensory evaluation of apple pomace ingredient

PARTICIPANT INFORMATION SHEET

Study Title: Apple ingredient

Supervisors: Prof Richard Archer (Principle Investigator) – Massey University.

Prof John Bronlund – Massey University.

Dr Erin O'Donoghue –Plant and Food Research.

Researcher: Marzieh Eblaghi – Massey University.

We would like to invite you to take part in our research study. Before you decide if you want to take part, we would like you to understand why the research is being done and what it would involve for you. Please read this information sheet and if you have any questions please email me or ask at the session.

Description of project

This project is aimed at developing a new thickening ingredient from apples.

Participant Identification and Recruitment

- Age group above 18 years old
- Not to be pregnant
- Not on medication which affects their sensory acuity and participants with restricted diets/ food allergies
- Number of participants to be involved are 50 participants which will be organized in 5 group of 10 people based on the given time and suitability between participant and researcher
- Participants will receive a sweet treat at the end of session as a thank you for their time and participation.

Project Procedures

You will be participating in mouthfeel evaluation of apple residue ingredient containing pectinase enzyme and citric acid, which has gone under physical and enzymatic treatments. Apple ingredient will be produced at Massey University food pilot plant/ product development laboratory. There will be 5 individual samples and you will be asked to take a full spoon of each apple ingredients. For each sample, you will be asked to answer the question about graininess and smoothness of sample and how sure you think it is. After choosing your answer and submitting it, you will be asked to take water and a bite of cracker before moving to next sample.

Project procedure

If you are interested in participating in the study, you will be asked to sign a consent form. You will be required to taste some apple ingredient samples and give your feedback on how smooth or grainy they are. You only need to attend one session. The session will last between 25-30 min.

Data management

Details of the participants and the data will be stored in a password protected database, which is only accessible by the research team members. All personal information will be kept confidential by giving number codes to each participant. All date collected will be used only for research purposes and will be used in a thesis as a partial fulfilment of the requirements for the degree of Doctor of Philosophy at Massey University and in other research publications. Confidentiality of identity will be preserved in all such publications. Your data will be kept for 7 years before being destroyed.

Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used unless you give permission to the researcher

Compensation of injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic, and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Project Contacts

If you have any further questions or concerns about the project, either now or in the future please contact the apple ingredient team on specific contacts.

Specific contacts:

Prof Richard Archer (Principle investigator)- R.H.Archer@massey.ac.nz

Marzieh Eblaghi (PhD student)- M.Eblaghi@massey.ac.nz

Human ethic committee Approval Statement

"This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named in this document are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you want to raise with someone other than the researcher(s), please contact Professor Craig Johnson, Director (Research Ethics), email humanethics@massey.ac.nz. "

CONSENT TO TAKE PART IN RESEARCH- INDIVIDUAL

Study Title: Apple ingredient

- I voluntarily agree to participate in this research study.
- I have read the Information Sheet and have had the details of the study explained to me.
- I have had the purpose and nature of the study explained to me in writing and I have had the opportunity to ask questions about the study.
- I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind.
- I understand that all information I provide for this study will be treated confidentially.
- I have no known allergy to the ingredients in the product being tasted.

Signature of research participant

Signature: ……..…… Date:

……

Full Name - printed …….

Signature of researcher

I believe the participant is giving informed consent to participate in this study.

Signature of researcher: ……. Date:

.....

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

Appendix 5 Tables of d′

Relationship between R-indices and d′ are originally developed in tabular forms by Patricia Elliot and reproduced by Swets (1964).

from PATRICIA ELLIOTT Tables of d'
IN: J.A. Swets: Signal Detection and Recognition by Human Observers
John Wiley, New York, 1964, page 683.

P(A)*	d '	P(A)	d '
50%	zero	80%	1.19
51	0.04	81	1.24
52	0.07	82	1.29
53	0.11	83	1.34
54	0.14	84	1.40
55	0.18	85	1.47
56	0.21	86	1.53
57	0.25	87	1.60
58	0.28	88	1.66
59	0.32	89	1.74
60%	0.36	90%	1.81
61	0.40	91	1.90
62	0.43	92	1.98
63	0.47	93	2.08
64	0.51	94	2.19
65	0.54	95	2.32
66	0.60	96	2.48
67	0.62	97	2.66
68	0.66	98	2.90
69	0.71	99	3.28
70	0.74		
71	0.78		
72	0.82		
73	0.86		
74	0.90		
75	0.95		
76	1.00		
77	1.05		
78	1.09		
79	1.14		

Note= * P (A) = R-index

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