Pectic Substances in the Plant Cell Wall and their Role in Potato Processing.

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Dedication

To my mother and father I offer this as scant appeasement for all the worries that I have caused them and for the substantial grief that will inevitably follow.

Acknowledgements

Firstly I am forever indebted to my mentor Dr Mike Jarvis who's laughter at my apparent misfortune never ceased and finally taught me that negative results are not necessarily a bad thing.

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My friends and family who never really understood why I wanted to study potatoes but offered their support anyway I will be eternally grateful.

Summary

The objective of this thesis was to characterise the chemistry of the pectin in the primary cell wall of two potato varieties, cv. Record and cv. Saturna, used in the crisp industry. The varieties chosen produce crisps with different textures. The original aim was to relate chemical characteristics of the pectin in the raw cell wall to textural differences in the crisp.

A new procedure for the isolation of the cell wall, free from starch was developed. Methodologies to measure the pectic galacturonan, total ester and the extent of β-elimination in the isolated cell wall were developed.

Cell walls were isolated from raw, cold wash, hot wash, cold wash crisp and hot wash crisp samples from the 1995 and 1996 harvest. No significant differences in the pectin content or structure were found between the cell walls isolated from cv. Record and cv. Saturna. Nevertheless, the cell walls isolated following the crisping process had a reduced pectic galacturonan and methyl ester content. The neutral sugar content of the cell wall remained unchanged; however, the galactan and arabinan side chains had a greater mobility. As no significant differences between the varieties were found, the objectives were changed to monitor the pectin changes during processing.

For comparison with crisping, slices of cv. Maris Piper were boiled for 2.5 minutes. Boiling resulted in pectin solubilisation (8%) with no reduction in the percentage galacturonan methyl esterification. Saponification followed by boiling caused 44% pectic galacturonan solubilisation. The pectin solubilised during cooking following saponification had a greater proportion of neutral sugars to galacturonan and had a smaller molecular-size distribution than the fraction solubilised by boiling alone.

The maximum extent of β-elimination in isolated cell walls of boiled cv. Saturna was estimated at 1%. Non-methyl esters were found at values of 14-15% in cv. Marfona and cv. Fianna. Chelation of divalent cations from the middle lamella resulted in the preferential solubilisation of a predominantly linear, non-methylated fraction of pectin. Saponification resulted in cleavage of ester cross-links between pectic chains. Pectic chains whose anchorage in the cell wall relied on ester links were now held in the cell wall by divalent bridges. Boiling of the saponified tissue would result in the cleavage and loss of the cations into the cooking water, thus resulting in pectic solubilisation. Acid hydrolysis may have been an additional mechanism of cleavage of the pectic chain.

Slices of the 1996 cv. Saturna harvest were crisped after saponification. The effect of saponification on the quantities of pectin solubilised prior to the cooking process was reversed from boiling. Saponification prior to frying reduced the quantities of pectin solubilised due to the crisping process, 8% being solubilised from the saponified crisps and 40 % solubilised from the non-saponified crisps. Both saponification experiments resulted in solubilisation of the rhamnogalacturonan I fraction suggesting that the non-methyl esters occur within or close to the rhamnogalacturonan I fraction. The galacturonan and rhamnose units within the rhamnogalacturonan I do not provide sufficient sites for non-methyl esterification. Ester linkages between the ester group of the homogalacturonan and the galactan side chains may provide alternative sites for ester linkages.

A taste panel experiment was run on crisps following different pre-treatments. The pre-treatments that reduced the softening upon heating also conferred strength to the crisp.

The crisping process involves cleavage of the methylated pectic chain due to β-elimination in the cell wall resulting in the preferential solubilisation of a methylated homogalacturonan fraction. This mechanism of cleavage is prevented in the saponified crisp due to removal of the methyl ester groups. Anchorage of

pectic chains whose ester cross links have been broken by saponification is maintained by divalent bridging. The saponified galacturonan has a greater potential for the formation of bridging due to the removal of the ester groups.

There is a clear role for pectins in controlling the texture of the crisp; however, varietal differences can not yet be explained by differences in the raw cell wall.

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List of Abbreviations

CDTA	cyclohexandiamine tetra-acetic acid
conc	concentration
CP	cross polarisation
cv.	cultivar
DMSO	dimethyl sulphoxide
EELS	electron energy loss spectroscopy
FW	formula weight
GAL	galactose
Gal UA	galacturonic acid
K_{av}	relative retention volume
LSD	least significant difference
MAS	magic angle spinning
MHDP	m-hydroxy diphenyl
NMR	nuclear magnetic resonance
NS	neutral sugar
PME	pectin methylesterase
PMT	pectin methyltransferase
RG I	rhamnogalacturonan I
RG II	rhamnogalacturonan II
rpm	revolutions per minute
s.d.	standard deviation
SEM	scanning electron microscopy
SIMS	secondary ion mass spectroscopy
$T_{1\rho}$	rotating frame spin-lattice relaxation time
T_1	spin-lattice relaxation time
TBA	thiobarbituric acid
UV	ultra violet

Chapter 1

Introduction

Potato Crisps

Potato crisps –also known as potato chips - are obtained by slicing potato tubers to a range of thickness from 0.7mm to 1.8mm (Lisinska & Leszczynski, 1989). The raw slices can be given various pre-frying treatments to influence the colour or texture of the resultant crisp. The slices are then fried in oil at a temperature in the range 175°C to 190°C to obtain a moisture content of ~2% (Lisinska & Leszczynski, 1989), with an average fat content of between 36 and 42% using a conventional industrial line frier (Ufheil & Escher, 1996). If the moisture content is higher, then the product will not feel crisp when eaten (Gamble & Rice, 1988). The low moisture content has an additional advantage in making them resistant to microbiological spoilage (Mottur, 1989). Following frying the crisps are flavoured and seasoned. This also extends the shelf life of the crisp due to the antioxidant properties of the seasoning (Lisinska & Leszczynski, 1989).

The ideal tuber for crisp processing should possess: shallow eyes; resistance to bruising; specific gravity greater than 1.080 and an insignificant accumulation of reducing sugars (Lisinska & Leszczynski, 1989; Gould, 1984). The crisps should have less than 15% blisters and a maximum of 10% minor defects; however, ideally both these should be zero. Many existing cultivars do not meet these requirements.

History

Potato crisps are thought to have originated from a chef in Saratoga Springs, New York in 1853 (Mottur, 1989; Willard, 1993). A party of guests complained that his fried potatoes were too thickly cut; consequently, he sliced them wafer thin and fried them. The fried slices became a Saratoga speciality (Mottur, 1989).

It was not until the 1920's that crisps became commercially available in the shops; firstly wrapped in brown paper bags (Mottur, 1989) followed in 1926 by bags made from ironing wax paper (Willard, 1993). Cellophane and glassine wrappings followed shortly. The first continuous fryer was used in 1929 (Willard, 1993).

Cultivation

Yield, specific gravity, glucose content and chip colour are all influenced by the variety and vary from harvest to harvest, dependent on the conditions experienced during the growing season (Sinha et al., 1992). These conditions influence crisping quality as follows:

- Drainage/irrigation; excess water at the end of the growing season reduces the specific gravity.
- Nutrient status; high nitrogen content prolongs the growing season, consequently they are immature at harvest, resulting in low specific gravity and high reducing sugars.
- Killing of potato vines causes an abrupt break in growth, preventing translocation of nutrients to the tuber, resulting in lower specific gravity (Lisinska & Leszczynski, 1989).
- The soil type also influences yield; sandy soils produce tubers with higher specific gravity and yield than those grown on clay soils.
- The yield will also be dependent on the harvest date (Sinha et al., 1992). The same variety, grown under different conditions, results in tubers with different composition.

Sugar produced by the leaves is translocated to the tuber and stored as starch (Swokinos, 1978). As the tuber matures, the sugar content decreases and the starch content increases. In the mature tuber the major constituent of the dry matter is starch, with levels between 65 and 75% (Burton, 1989), which exist as starch grains with sizes varying from 5 to 100 microns (Mottur, 1989).

Freshly harvested, fully mature tubers produce crisps of the highest quality. They are also less susceptible to damage during shipping, storage and reconditioning (Lisinska & Leszczynski, 1989); however, their quality will diminish on storage. The storage quality is affected by the level of maturity at harvest (Sowokinos, 1978). Sprouting during storage can be controlled chemically or by a drop in storage temperature to 2-4°C (Burton, 1989). The process of curing, whereby the tubers are held at 21°C for 7-10 days, thickens the peridem and protects the tuber against dehydration and bruising (Mottur, 1989). However, extended storage will increase the levels of reducing sugars (Burton, 1989). During frying these sugars will react with amino acids in the potato cell, resulting in brown/ black crisps (Sowokinos, 1978). These darkened crisps are unacceptable to the consumer. Senescent sweetening is an irreversible process whereby starch molecules are broken down, possibly by a combination of phosphorolysis and hydrolysis (Burton, 1989). Phosphorolysis is cleavage of the non-reducing end of the glucose chain at the end of the amylose molecule or an amylopectin chain, to form a glucose-1-phosphate. Starch hydrolysis is catalysed by amylases; α-amylases attacks within the amylopectin branch or along the amylose chain; while β-amylase attacks the end of the glucan chains. Storage of tubers at low temperature increases the reducing sugar problem, due to the phosphorolysis mechanisms (Burton, 1989). Reconditioning of the tubers stored at low temperature is required prior to frying. Reconditioning occurs between 15-20°C, the higher the temperature the quicker the process (Lisinska & Leszczynski, 1989). Conditioning minimises further starch conversion, as well as reversing the low-temperature induced increase in reducing sugars (Sowokinos, 1978; Burton, 1989). The length of conditioning time required is dependant on the levels of reducing sugars, mature tubers require shorter periods (Lisinska & Leszczynski, 1989).

Processing

The first step in the crisping process is the washing and peeling of the tuber. The tubers are washed in a drum or a flotation washer, where soil, stones and microorganisms are removed (Lisinska & Leszczynski, 1989). The most

common method of peeling is tumbling them over rotating cylinders coated with carborundum (Mottur, 1989). Various types of slicer are available. The most common are rotary-type (Lisinska & Leszczynski, 1989). The slice thickness will affect the yield. Thin slices have shorter distances for water diffusion, so lose water more rapidly, consequently their frying time is less. Conversely, thicker slices require longer frying times. The quantity of oil uptake is related to surface area. Accordingly, an increase in surface area results in an increase in oil uptake. Hence, thinner slices, having a larger surface area to volume, have a greater yield and fat content (Gamble & Rice, 1988).

The sharpness of the blade determines the smoothness or roughness of the resultant slice. Rough slices will provide a greater surface area for oil absorption and therefore, will result in crisps with a higher fat content (Gamble & Rice, 1988; Lisinska & Leszczynski, 1989).

Washing of slices prior to frying improves the colour and texture of the resultant crisp. Washing the slices in cold water will remove starch grains and other substances from the cut surfaces. If the starch is not removed before frying, then when the starch gelatinises in the frier, the slices stick together and will not crisp properly (Mottur, 1989). Gorun and Potapow found that the cold wash process would remove 42% of the reducing sugars, while the hot wash process would remove 66% of them (Lisinska & Leszczynski, 1989). As a consequence of this reducing sugar leaching, the crisps have an improved, lighter colour. The hot wash process is carried out over a range of temperatures 65-95°C and lasts between 1-5 minutes. Mitchell & Rutledge found that a hot wash process of longer than 5 minutes resulted in grey colours and a loss in taste, with 75°C being the optimum temperature (Lisinska & Leszczynski, 1989). As a consequence of the washing as much as 20% of the dry weight can be lost (Mottur, 1989). The blanching solution is commonly water, although solutions containing sodium bisulfite, phosphoric acid, sodium citrate, citric acid, calcium chloride, and magnesium chloride are also used (Lisinska & Leszczynski, 1989).

Frying

The frying step combines a heat treatment at high moisture with dehydration (Baumann & Escher, 1995). As the internal temperature rises the slices will cook. The water will boil and be released as steam, resulting in dehydration (Selman & Hopkins, 1989). Immersion frying of foods involves two methods of heat transfer; conduction and convection (Singh, 1995). Conduction occurs within the food. Convection occurs between the oil and the food. This interaction is complicated by turbulence in the oil, resulting from the escaping moisture (Singh, 1995). There is also mass transfer; water from the slice in the form of vapour and oil into the slice (Saguy & Pinthus, 1995)
Farkas separated the frying process into four stages (Singh, 1995).

1. Initial heating. The temperature of the submerged slice increases to the temperature of the boiling water. Strock et al. (1966) found that the temperature rose to 102-104°C, but did not rise above this until the bubbling vaporisation ceased. The boiling point of the water within the potato is greater than 100°C due to the dissolved solutes.

2. Surface boiling. The surface and cellular moisture is rapidly lost as steam. A dry crust will concurrently form. (Gamble et al., 1987; Singh, 1995).

3. Falling rate. The middle of the slice has reached boiling point. The crust layer formed by the dehydration of the surface water provides a diffusion barrier. The steam from the middle layer finds paths of weakness, and escapes through a small number of large paths and a larger number of small paths (Gamble et al., 1987; Singh, 1995).

4. Bubble end-point. The rate of bubble loss slows until they are confined to a small number of sites (6-8), until eventually no more bubbles are observed (Gamble et al., 1987; Singh, 1995). The last water to be removed from the slice is associated with starch and requires greater heat energy before removal (Mottur, 1989).

The oil content is dependent on several factors. It is increased by the residence time in oil (Baumann & Escher, 1995; Gamble et al., 1987; Selman & Hopkins, 1989). The oil content is correlated to the square root of the frying time, approximately twice the square root of the frying time in seconds (Gamble et al. 1987). The oil content is also linked to the initial moisture content (Gamble et al., 1987). Baumann & Escher (1995) showed that increased dry matter reduced the required fry time; consequently, slices from high density tubers yielded crisps with lower fat contents than those of lower density (Ufheil & Escher, 1996).

However, there is disagreement concerning when the oil actually enters the slice. Gamble et al. (1987) suggest that some oil enters the slice during frying, while most absorption occurs upon removal from the oil. Whereas, Ufheil & Escher (1996) state that absorption only occurs upon removal from the oil.

The resulting oil content can also be reduced by partial dehydration prior to frying, or by removal from the oil before dehydration is complete and using microwave or super heated steam to complete the task (Gamble et al., 1987; Lisinska & Leszczynski, 1989). The role of the oil temperature is less certain. Temperature has been shown to decrease oil absorption (Saguy & Pinthus, 1995; Baumann & Escher, 1995); whereas Gamble et al. (1987) showed that, for potato crisps, the oil content is independent of the fry temperature.

The viscosity of the oil will affect the quantity of oil that will coat the slice as it is removed from the frier (Guilaumin, 1988). The oil has a finite suitability time. Upon repeated usage the oil will undergo autooxidation, thermal oxidation and polymerisation, all of which will result in an increased viscosity. As the viscosity of the oil increases the fat content of the slice also increases. Unsaturated glycerides are less stable to the air and temperature, hence degrade faster (Guilaumin, 1988).

Fat penetration

The oil does not penetrate through the whole slice (Reeve & Neel, 1960; Baumann & Escher, 1995; Strock et al., 1966) due to the pressure of the escaping steam and the hindrance of the surface crust (Strock et al. 1966). The oil is absorbed into distinct areas which represent the capillary holes and voids, where the slice was most damaged during frying (Gamble et al. 1987; Saguy & Pinthus, 1995). The oil enters the slice upon removal from the oil, due to the vacuum caused by the condensation of the steam (Gamble et al. 1987). Consequently, the surface area of the slice is a major determinant of oil absorption. The condition of the slice upon removal from the oil is the decisive factor concerning fat uptake (Ufheil & Escher, 1996). Slices cut using blunt blades will have a greater surface area, due to greater disruption of the slice surface, resulting in tearing (Selman & Hopkins, 1989). The slice thickness will also play a part, due to a reduced surface area to volume ratio (Baumann & Escher, 1995); however, this relationship is not linear.

Reduced oil uptake has also been observed in reconstituted potato crisps. Rubnov & Saguy (1997) incorporated fructose (0-10% w/w) into the potato mix. As the water boiled off the surface the fructose formed a crust over the slice. However, this crust may also act as a barrier to oil absorption. When the surface of slice was examined it was shown that the fructose had had a smoothing effect on the slice.

Reeve & Neel (1960) examined the cellular structure of the crisp. They found that the cells were shrunken and the cell wall was convoluted around the dried gelatinised starch. When the crisp was rehydrated, the cells swelled and the gelatinised starch remained within the cell, indicating that the cell wall had not been ruptured. Therefore, the voids through which the vapour was lost formed between cells and not by rupture of the cells. If steam generation occurs at a rate faster than it is able to escape from the slice then blisters will develop on the crisp surface (Mottur, 1989). The leathery dehydrated surface impedes the venting of the escaping steam as does blockage of the channels by oil absorption (Mottur, 1989). The formation of blisters occurs between cells (Reeve & Neel,

1960), and is more common in thicker slices where the ratio of surface area to volume is reduced. The middle of the slice will still be soggy and cooking while the outside edges will be dehydrated and hardened.

As a result of the dehydration process, the crisp will be thinner (65%) than the raw slice (Mottur, 1989). Cells from tubers with a higher specific gravity will shrink less upon crisping; consequently, the low starch centre of the slice will shrink more than the higher starch cells on the circumference (Mottur, 1989). This problem, accentuated by the dehydration process, prevents full swelling of the gelatinised starch (Reeve & Neel, 1960). This distribution of uneven shrinkage is responsible for the typical saddle shape of the crisp (Mottur, 1989).

The texture of potato crisps is an important quality aspect and will affect consumer acceptability. The texture of the crisp will be determined by the architecture of the crisp. The architecture of the crisp will be determined by the changes of the potato slice during the frying process. The distribution of the voids - channels- and their occurrence will be governed by the behaviour of the cell wall during frying. If the cells separate during the initial frying stage, then the void channels for the escape of the venting steam will form between the cells. If the cells rupture then the channels will form in a different direction. The strength of the pectic matrix in the cell wall and middle lamella and the extent to which they can withstand the forces applied to them will play a crucial role in the development of the morphology and the resulting crisp texture. Their strength will be dependent on the degree to which they are degraded during the cooking processes, which is dependent on their original structure.

Cell Wall Introduction.

The primary plant cell wall is a three-dimensional composite structure (Carpita & Gibeaut, 1993). The network is built up from a series of layers. Following cell division, additional layers are deposited between the plasma membrane and the earlier layers (Brett & Waldron, 1990). Each layer is composed of a

microfibrillar phase that is embedded into a matrix of pectin, hemicelluloses and proteins (Brett & Waldron, 1990, McCann & Roberts, 1991). There are many functions performed by the cell wall. It dictates the shape of the cell; provides structure and rigidity by withstanding turgor pressure; forms a physical barrier to pathogen attack and participates in cell-cell communications (Brett & Waldron, 1990).

Cellulose, a linear β(1-4)glucan, exist as microfibril chains of diameter 5-12nm, depending on variety. The microfibril chains are arranged as parallel layers (McCann & Roberts, 1991). The cellulose microfibrils in onion are made up of subunits of diameter 2nm arranged in an open packed hexagonal arrangement (Ha et al., 1998).

Xyloglucan is the principal hemicellulose of primary cell walls, making up between 20-25% (dry weight) of the cell wall in dicotyledons. It consists of β(1-4)-linked glucose backbone chains, many of which will have an α(1-6)xylose attached as sidechains. Xyloglucan can hydrogen bond to the cellulose microfibrils (Fry, 1989; Brett & Waldron, 1991). The open cellulosic microfibril arrangement allows xyloglucan to be attached on the surface of the individual subunits via hydrogen bonding (Ha et al., 1998). The xyloglucan can also span between microfibrils and may be responsible for holding adjacent microfibrils together (Fry, 1989; McCann et al., 1995).

The area between adjacent cell walls is the middle lamella (figure 1.1) and consists entirely of pectic substances (Selvendran, 1985). Tricellular junctions are where three cells meet

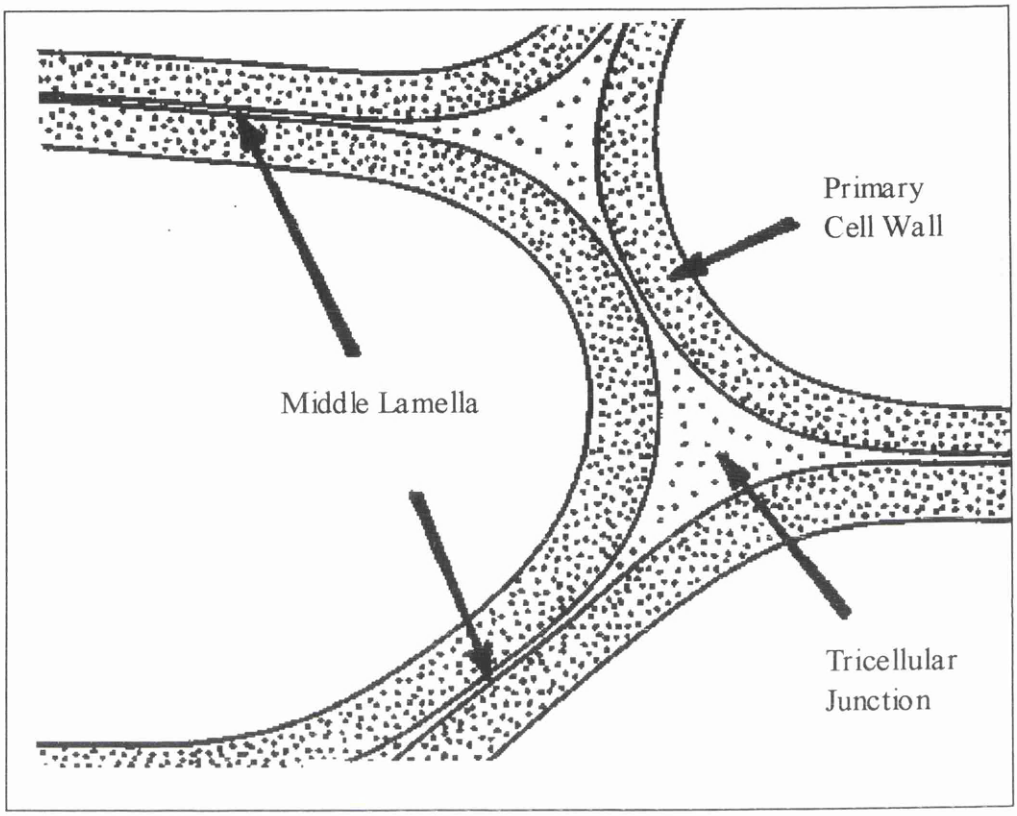


Figure 1.1 Arrangement of the cell wall, middle lamella and tricellular junction. From Jarvis and Daud (1995).

One of the roles of the pectic matrix in celery is to hold the cellulose microfibrillar layers of the cell wall together across their thickness (Jarvis, 1992a). This control of the thickness of the cell wall is achieved by the cohesion and swelling potential of the pectin. The exact mechanism, either ionic or covalent will be dependent on the structure of the pectin in the local environment.

Substances that enter and exit the cell need to pass through the cell wall and the plasma membrane. The porosity of the cell wall is principally determined by the porosity of the pectic matrix (Baron-Epel et al., 1988; Brett & Waldron, 1990). Most pores are smaller than 10nm, with a few larger ones of 20nm. Removal of pectin increases the pore size range to 20-40nm (McCann & Roberts, 1991). Macromolecules with a Stokes radii of <3.3nm penetrate the soybean cell wall unimpeded, those with Stokes radii of 3.3-4.6nm experience hindrance during

transport, while molecules of a Stokes Radius >4.6nm will not pass through pores within the cell wall (Baron-Epel et al., 1988). Thus, sugars, growth substances and most amino acids will pass easily; whereas, starch molecules are too large (Burton, 1989; Shomer et al., 1995).

Pectic substances have an important role in the expression of texture in fruits and vegetables during growth, ripening and storage (Voragen et al., 1995).

Pectic Structure

The term pectin is used to describe a group of closely related polysaccharides rich in galacturonic acid, rhamnose, galactose and arabinose (Brett & Waldron, 1990). The main constituent of pectin is a linear chain of α(1-4)linked D-galacturonic acid known as homogalacturonan (figure 1.2). This polymer may have regions where some of the C-6 is methyl esterified. There are regions where the α-D-galacturonic acid is followed by an α(1-2) L-linked rhamnose unit, this sequence is referred to as rhamnogalacturonan I (RG I) (figure 1.3) (Lau et al., 1985). Zhan et al. (1998) showed that the RGI fraction is composed of rhamnose/galacturonan dimer repeating units. The 1-2 linkage of the rhamnose has the potential to introduce kinks into the polymer chain when an odd number of dimers occur in the chain (Powell et al., 1982). The C-4 sites on the rhamnose units are also points of attachment for neutral sugar chains of varying lengths (Brett & Waldron, 1990; Voragen et al., 1995). In potatoes the dominant neutral sugar in the side chains are linear chains of D-galactose (Jarvis, 1981). L-arabinose, and D-xylose are also present (Jarvis, 1981;Ng & Waldron, 1997; van Marle 1997a). Homogalacturonan is often referred to as the smooth region; while, RG I is referred to the hairy region as a result of the neutral sugar side chains (Lau et al. 1985). Arabinans, galactans and arabinogalactans are also present. D-glucose and D-mannose are also present in pectic extractions but form part of the hemicellose network (Jarvis et al. 1981). Rhamnogalacturonan II is a

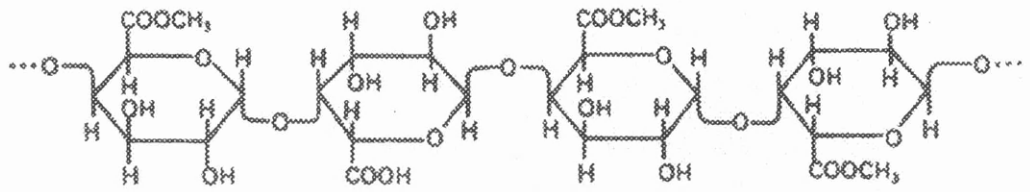


Figure 1.2 Homogalactoronan, α(1-4)linked D-galacturonic acid showing regions of methyl esterification. From Coultate (1989)

minor but highly complex component in the primary cell wall composed of galacturonic acid, rhamnose, arabinose and galactose, as well as other rare sugars (Brett & Waldron, 1990; Voragen et al., 1995). Boron, in the form of boric acid, has been shown to link two RG II chains together (Kobayashi et al., 1996)

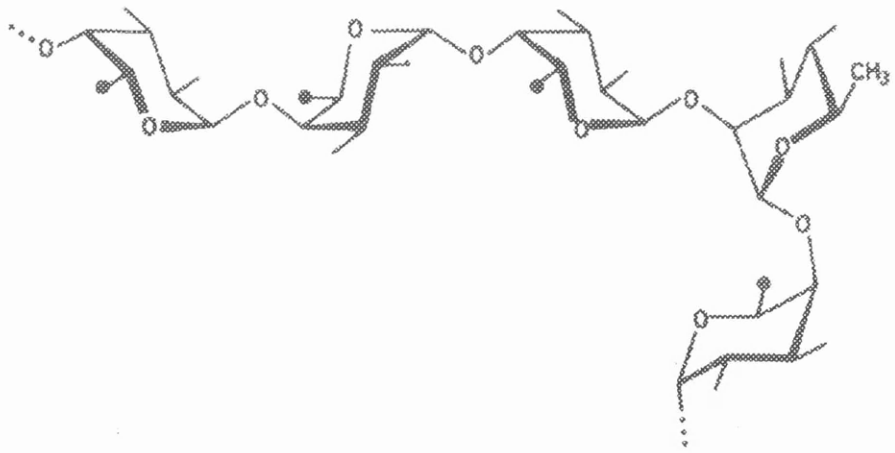


Figure 1.3 Rhamnogalacturonan I. α(1-4) galacturonic acid followed by an α(1-2) rhamnose unit. From Coultate (1989)

The degree of methyl esterification found on the galacturonan chain has important consequences for the resulting pectic characteristics. Pectic galacturonan is synthesised in the cis golgi and methylesterified in the medial golgi, by the enzyme pectin methyltransferase (PMT) (Zhang & Staehlin, 1992).

The esterified galacturonan is then selectively de-esterified by the wall bound enzyme pectin methyl esterase (PME) (Markovic & Kohn, 1984). However, Goldberg et al. (1996), suggests that this is an over simplification. They suggest that PME and PMT are heterogeneous enzymes, the level and pattern of the resulting methyl esterification is controlled by a balance between both enzymes. The PME will de-esterify in a blockwise fashion, while the PMT activity is random.

The level of methyl esterification will affect the charge density and binding capacity of the cell wall. A decrease in the methylesterification from 80% to 60% doubles the mean charge density and the cation-binding capacity (Goldberg et al., 1996).

Calcium has the ability to form divalent crosslinks between adjacent acidified galacturonan chains (Jarvis, 1984). This calcium bridging results in the formation of junction zones between adjacent regions of non-methylated homogalacturonan (Jarvis, 1984), of 15-20 units in length (Kohn, 1975). The occurrence of an odd number of rhamnose/galacturonic acid dimer units in the chain will result in cessation of a junction zone due to the kink in the chain (Powell et al., 1982). The presence of neutral sugar side chains will also prevent association. These regions known as interjunction zones will also form in regions of methyl esterification (Jarvis, 1984). If calcium is in excess then dimerised junction zones can aggregate together to form a 4 chain association (Jarvis, 1984).

Goldberg et al. (1996) proposed the cable structure. This model maintains the egg-box structure first suggested by Morris et al. (1982), whereby the calcium bridges between the galacturonan chain in the 2_1 conformation. The cable model also has calcium acting as the bridging ion between the galacturonan chain in the 3_1 conformation as proposed by Walkinshaw & Arnott (1981). At low calcium concentrations single chains aggregate to form galacturonan dimers, while higher calcium concentrations results in aggregation of the dimers to form interjunction segments built up from four or more chains. The gel can change conformation depending on the cations present. Calcium is the main cation bound, but sodium and magnesium are also bound in large amounts.

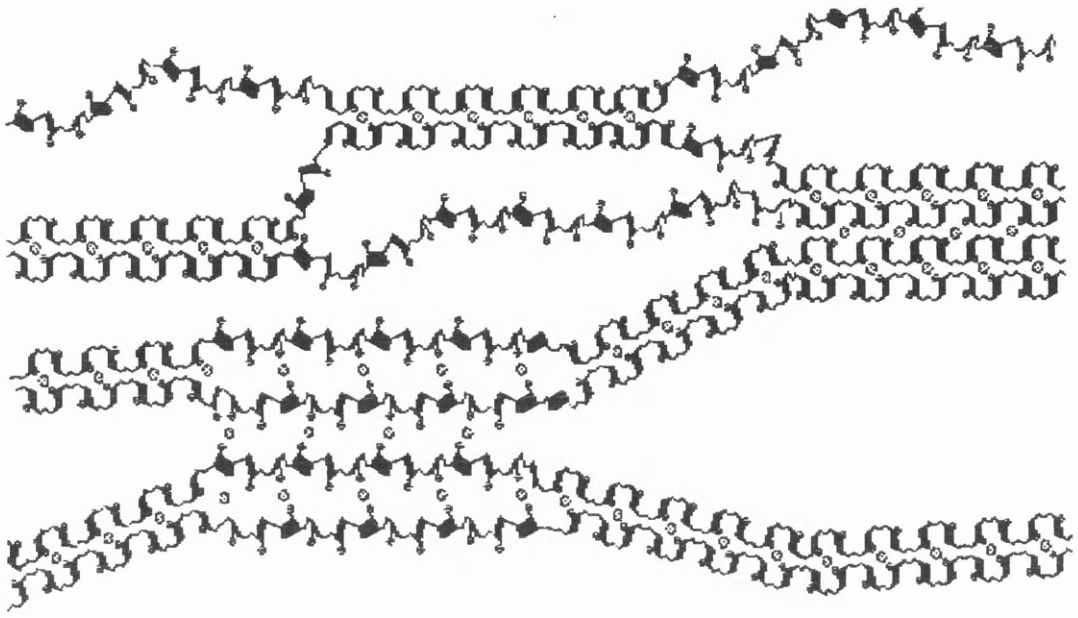


Figure 1.4 Cable structure for concentrated calcium gels. From Goldberg et al (1996)

Voragen et al. (1995) suggested that further stabilisation of the junction zones was possible due to hydrogen bonding between undissociated carboxyl groups and secondary alcohol groups, as well as hydrophobic interactions between methoxy groups.

SIMS microscopy of flax has shown that the Ca is located principally in the cell wall. There is also an accumulation of Ca in the tricellular junction (Goldberg et al., 1996). Huxham et al (1998) showed using EELS on potato, calcium bound pectins are dominant in the middle lamella. Chelators of calcium such as CDTA (Jarvis, 1981) and imadazole (Mort et al., 1991) are used to extract the pectic fractions held by Ca bridging in the cell wall. Van Marle et al. (1997a) extracted 20% of the cell wall material for cv. Nicola and cv. Irene using CDTA followed by cold Na_2CO_3.

The idea of non-methyl ester groups originated from observations that further extraction of CDTA treated pectin from the cell wall was obtainable under alkali conditions during sequential extraction (Selvendran, 1985). The alkali would result in the cleavage of ester bonds. It has been suggested that other alcohols can

substitute for methanol (Fry, 1986). Kim & Carpita (1992) found that methyl ester values could only account for two thirds of the total ester groups found in maize coleoptiles. Their findings suggested the existence of non-methyl ester groups in grasses, at quantities approaching 20% during some stages of growth. The novel ester groups were not extractable by chelation prior to an alkali treatment. No such ester groupings have been reported in potatoes.

Spatial arrangement

The use of monoclonal antibodies has aided in our understanding of the distribution of pectic substances within the cell wall. Knox et al. (1990) used the antibodies JIM5 and JIM7 to label the low ester and high ester regions of pectin in the root apex of carrot. They found that pectin is evenly distributed across the cell wall. The high-methyl esterified galacturonan was shown to be uniformly distributed throughout the cell wall while the low-methyl esterified galacturonan was principally located in the middle lamella.

The middle lamella pectins are predominantly homogalacturonan, with the branched RGI found deeper within the cell wall (Selvendran, 1985; Jones et al. 1997). The structure of the pectin in the middle lamella and the tricellular junction will play a crucial role in determining the strength of attachment between individual pectic chains and the attachment between adjacent cells. These pectins are predominantly homogalacturonan, with a cable structure (figure 1.4) close to the linear commercial galacturonan (Goldberg et al., 1996). The cable structure involves two levels of aggregation; single chains joining together to form dimeric egg box junctions, while these dimer junctions can further aggregate together with 4 or more chains (Ha et al., 1997).

The pectic matrix in the cell wall contains many elements of the cable model; however, the levels of branching and methyl esterification will affect the closeness of aggregation (Goldberg et al., 1996). The neutral sugar side chains of the galacturonan will prevent aggregation of the hairy pectic chains (Jarvis, 1984). There is no evidence that the branched regions can act as sites for Ca

crosslinking (Goldberg et al., 1996). Sites of methyl esterification will also reduce the opportunity for Ca crosslinking.

Pectin in potato cell walls

Many researchers have studied the chemical structure of the pectin in the potato cell wall (Ishii, 1981; Jarvis et al., 1981; Selvendran, 1985; Ryden & Selvendran, 1990; van Marle et al., 1994, 1997a, 1997b; Schols & Voragen, 1994; Ng & Waldron, 1997). The amount of pectic galacturonan found in the cell wall has been shown to be dependent on variety, within the range 17.3-26%; 17.3% (Selvendran & O'Neill, 1987), 18.8% for cv. Irene and 17.4% for cv. Nicola (van Marle, 1997a), 26% for cv. Bintje (Ng & Waldron, 1997) and 25.2% cv. King Edward (Binner et al., in press). However, some of the variation may be due to differences in measurement. The degree of methyl esterification of the whole cell wall is less often reported, due to cleavage of the ester groups with alkali -KOH or Na_2CO_3- during sequential extraction. There is also the additional problem of PME activity during isolation or extraction. However, degrees of methyl esterification from 37% for cv. King Edward (Binner et al., submitted) to 53% for cv. Bintje (Ng & Waldron, 1997) have been reported. Ryden & Selvendran (1990) found that the first CDTA extract, extracted galacturonan with the highest degree of methylation (51%), compared to the second CDTA extract (42%).

Analysis of the isolated modified hairy regions of potato pectin showed that fractions containing the highest degree of methyl esterification (32%) also had the greatest percentage of linear galacturonan, with a rhamnose/galacturonic acid ratio of 0.40. Whereas, fractions with lower degrees of methyl esterification, 3 and 8%, had higher rhamnose/galacturonic acid ratios, 0.78 and 0.82 respectively (Schols & Voragen, 1994)

Ishii (1981) extracted 95% of the galacturonans using a polygalacturonase. The soluble fraction was fractionated by gel filtration. The lowest molecular weight fraction collected, which had the greatest degree of methyl esterification (30%) also contained the most linear fraction.

Controlling Factors in Texture

Texture is a multidimensional property that cannot be described by one descriptor (Verlinden et al., 1995), the resultant texture is determined by the integrity of the cell wall and middle lamella components as well as the turgor pressure (Jackman & Stanley, 1995). Texture loss upon processing is due to both loss of turgor pressure, caused by rupture of the cell membrane, as well as a breakdown in cell wall components (Mittal, 1994). The pectin content plays a decisive role in the cooking behaviour of root vegetables (Harada & Paulus, 1987). Solubilisation or cleavage of the pectin will result in softening of potatoes (van Buren, 1970; Hughes et al., 1975), the key processes taking place in the middle lamella (Harada & Paulus, 1987). The early stages of the frying process are similar to the boiling processes (Selman & Hopkins, 1989). Pectic substances may also play an important role in determining the structure and oil uptake of the crisp (Selman & Hopkins, 1989) by controlling the degree of cell separation and consequently the morphology of the crisp.

Heat Induced Softening

The cooking behaviour of root vegetables is dependent on several rather than one single parameter (Harada & Paulus, 1987). Softening can occur by cell separation or cell rupture caused by breakage of the middle lamella or rupture of the cell wall respectively. Both cell separation and cell wall rupture contribute to the softening of cooked potato. The importance of the two will vary depending on variety (Freeman et al., 1992). The balance between cell separation and cell wall rupture will determine the dryness or moistness (Jarvis & Duncan, 1992). Potatoes whose main softening mechanism is cell separation will have a mealy dry mouthfeel; conversely, when the main softening mechanism is cell wall rupture they will have a moist, waxy mouthfeel (Jarvis et al., 1992).

The tricellular junction plays an important role in textural determination. Softening resulting from cell separation requires the degradation of the middle lamella. Initiation of the separation is dependent on the force by which the

tricellular junction pectins can bear before separation. The only polymers present in the tricellular junction zone are the pectic substances (Goldberg et al., 1996; Jarvis, in press).

Parallels have been drawn between the composition of Chinese water chestnuts and potatoes. However the behaviour upon cooking is very different. The Chinese water chestnut will maintain its textural properties when cooked; whereas the potato will soften (Loh & Breene, 1982).

The textural stability of the Chinese water chestnuts has been ascribed to the occurrence of cell wall phenolics. The cell wall showed considerable autofluoresence originating from ferulic acids (Parker & Waldron, 1995). Parr et al. (1996) showed that 40% of the ferulic acid existed as dimers, which could potentially form heat stable dimers between the polysaccharides. Electron dense areas were shown to occur in the tricellular junction (Parker & Waldron, 1995). Initiation of any cell separation originates in the tricellular junction (Jarvis, in press). Thus in the load bearing role the middle lamella pectins have the additional support of the ferulic acid dimers.

The ferulic acid dimers found in Chinese water chestnuts are not found in potato tubers. This may be part of the explanation why potato cells are more susceptible to cell separation. The cell separation forces are spread over the reinforcing zone (Jarvis, in press), therefore the structure in the immediate area will play a crucial role in the textural development.

Gelatinisation of Starch.

Cell wall pores are smaller than intracellular starch molecules (Shomer et al., 1995). Upon cooking however, the plant cell membrane will burst and the porosity will increase. The extent to which this occurs will depend on the microfibrillar lattice of cellulose and the nondegraded hemicellulose and pectin (Shomer et al., 1995). At temperatures of 70°C and above starch gelatinisation will occur distending the surrounding cell wall (Shomer, 1995) and causing an

increase in cell size (Harada and Paulus, 1986,1987; Jarvis et al., 1992). Starch swelling pressure has been measured (Jarvis et al., 1992) at 10^2 kPa which was thought to be sufficient to separate the potato cells, only once degradation of the middle lamella has occurred. The combination of increased porosity and gelatinisation results in leakage of starch from the cell (Anderson et al., 1994).

Rupture of the plasmalemma occurs at the same time as gelatinisation, thus allowing diffusion of citrate, phytate and Ca into the cell wall. Keijbets et al. (1976) found that citrate and phytate will favour the solubilisation of pectin by chelation and precipitation, thus preventing the formation of divalent bridges. Citrate can also promote β-elimination due to an increased ion content. However, Bernal-Lugo et al. (1991) showed in beans, that diffusion of phytic acid into the cooking water did not occur until the beans were over cooked. They found no evidence that phytic acid formed complexes with cations. Nevertheless, phytic acid may have a role in texture development as an acceptor of protons produced during β-elimination, thus maintaining a suitable pH for β-elimination.

Solubilisation of pectin

Removal or hydrolysis of pectin considerably weakens cell cell adhesion and strength (van Buren, 1970), the rate at which this happens will depend on maturity, pectic galacturonan structure, ions present and the cooking conditions (Hughes et al., 1975). With cv. Bintje Ng & Waldron (1997) observed softening upon steaming, this was accompanied by a reduction in the pectic galacturonan of the cell wall. Changes occurred in all areas of the cell wall as shown by changes in the sequential extraction of the raw and cooked material (Ng & Waldron, 1997). Solubilisation is achieved at a faster rate when the tissue is cooked in NaCl salt solution possibly due to displacement of divalent cations or enhancement of β- elimination (Hughes et al., 1975).

Vollmert observed that the alkaline conditions used for de-esterification at room temperature caused cleavage of the glycosidic linkages between galacturonic acid units (Kravtchenko et al., 1992). It has been shown that heating under

neutral and alkaline conditions will result in cleavage of the pectic chain according to a β-elimination mechanism (Neukom & Deuel, 1958; Keijbets & Pilnik, 1974). The β-elimination reaction requires the presence of a methyl ester group on the C-6. β-elimination is a process by which the pectic galacturonan chain is cleaved next to a methyl esterified galacturonic acid residue (Keijbets & Pilnik, 1974) during heat treatment. Sajjaanantakul et al. (1989) measured the extent of β-elimination at 100°C and pH 6.1 after 4 hours and found it was less than 2%. They concluded that at pH 6.1 β-elimination was the primary reaction for heat degradation for pectic galacturonan. If the salt content is increased greater cleavage of pectic galacturonan occurs (Keijbets & Pilnik, 1974, Sajjaanantakul et al., 1993). Divalent cations have a greater enhancing effect than monovalent cations at the same concentration. However, Paoletti suggested that the solvation of Mg^{2+} and NH_4^+ may restrict their interaction, thus reducing the enhancement (Sajjaanantakul et al., 1993). The methyl ester content was the dominant effect on β-elimination . The larger the ester content the smaller the molecular sizes the pectic galacturonan was degraded to (Sajjaanantakul et al., 1993).

The calcium ion can act as a bridge between adjacent galacturonan chains (Jarvis, 1984). Rupture of the plasmalemma will occur at the same temperature as starch gelatinisation, thus allowing free diffusion of ions from the cytoplasm to the cell wall (Bartolome & Hoff, 1972). There are two possible mechanisms by which this would influence softening. The increased ionic content of the cell wall will increase the rate of β-elimination (Keijbets & Pilnik, 1974, Keijbets et al., 1976). Increased galacturonan solubilisation has also been attributed to calcium chelation by citrate, phytate and malate (Keijbets et al., 1976).

Heat Induced Firming

The addition of calcium into cooking water, either as part of a pre-treatment or as the main cooking, has been shown to have a firming effect on the resultant texture (Hudson and Buescher, 1986; Ng & Waldron, 1997) and a reduction in the quantities of pectin solubilised (Moledina et al., 1981). This firming effect is

most effective between pH 6 and 6.5 (Stanley et al., 1995). Steamed potatoes, following calcium pretreatments, are firmer than those boiled even if they are boiled in calcium, as no leaching of calcium can occur (Moledina et al., 1981). Calcium achieves this firming effect by a number of possible mechanisms.

Calcium has the ability to form divalent crosslinks between acidified pectic galacturonan chains (Jarvis, 1984). Native cell wall calcium is present in insufficient quantities to saturate all the acidified galacturonan units (Moledina et al., 1981). Gelatinisation of potato starch will supply the cell wall with calcium (van Buren, 1970; Moledina et al., 1981). A cooling step following a pre-treatment of over 70°C will allow stabilisation of calcium pectate bridges, thus rendering the pectic galacturonan resistant to further solubilisation during subsequent cooking (Moledina et al., 1981). However, Krall & McFeeter (1998) propose that the rate of formation of calcium crosslinks would be too slow to explain the firming effect. They also ruled out inhibition of acid hydrolysis as a possible firming mechanism.

Additional calcium may act by altering the electrostatic repulsion- attraction fields so that there is a greater possibility of the formation of stable configurations held by non covalent forces van Buren (1970). Monovalent cations can also contract the electrostatic repulsion field but not as successfully as divalent cations.

Quinn & Schafer (1994) proposed cross-links between pectic neutral sugar chains to other cell wall components of potato. The cross-links would provide support to the cell wall. The greater the frequency of side chains and cross-links, then the greater the support offered to the cell wall, thus minimising the galacturonan solubilisation during cooking.

The quantity of methyl esters may play a crucial role in determining the resultant quantity of pectic galacturonan solubilised during the blanching treatment. β-elimination will cleave the pectic chain adjacent to a methyl esterified galacturonic acid residue, (Keijbets & Pilnik, 1974) resulting in softening and

solubilisation of pectic galacturonan, (Ng & Waldron, 1997). Concurrently, pectin methyl esterase will have become activated, (Moledina et al., 1981; Puri et al., 1982). Pectin methyl esterase is a wall bound enzyme that will cleave the methyl ester group resulting in an acidified galacturonan (Moledina et al., 1981). Pectin methyl esterase has a two fold firming effect; firstly, by removing the potential for β-elimination (Sajjaanantakul et al., 1989) and secondly by providing potential sites for calcium cross links to occur between adjacent pectic chains. However, Hudson & Buescher (1986) suggested that excessive demethylation changes the configuration of the pectic galacturonan, which contributes to loosening of the middle lamella and cell wall components, resulting in softening.

Optimisation of pectin methyl esterase activity will occur at 60°C and pH 7 (Moledina et al., 1981). Below a pH of 6.1 little desorption of PME from its product will occur. Denaturation of PME is temperature dependent; 10 minutes at 50°C and 1 minute at 70°C (Tijskens et al., 1997). During a 30 minutes 60°C pre-cook of potato negligible pectin was solubilised; while the degree of methylation fell from 55% to 52% (Moledina et al., 1981).

The PME activity is also dependent on the ionic conditions. Optimum PME activity was obtained with a NaCl concentration 1-1.6%; increasing the concentration has an inhibitory effect on PME activity (Moledina et al., 1981). It has also been reported that addition of 0.3% $CaCl_2$ reduces demethylation. This reduction could be achieved by reducing the activity of PME. One possibility is by the formation of physical barriers that would limit the access of the PME (Hudson & Buescher, 1986).

The reduction in pH due to PME activity however does not stop β-elimination, but a reduction in methyl esters will lessen the opportunity and rate (Sajjaanantakul et al., 1989).

Aims and objectives of the thesis

The original aim of the thesis was to characterise the chemistry of the pectin in the raw and processed cell walls of two potato varieties used in the crisping industry, relating any differences in pectin content or structure to the different textural attributes of the crisps.

An additional aim adopted at a later stage was to modify the raw cell wall prior to processing and note what effect this had on the behaviour of the pectic substances and the texture of the crisps.

Chapter 2

Sample Preparation and Procedures for Isolation and Characterisation of Potato Plant Cell Walls

Sample Preparation

Preparation of Cold and Hot wash slices and Cold and Hot wash crisp

Two common potato varieties used in the crisping process are cv. Record and cv. Saturna. These varieties were chosen for study, as the textural parameters of their crisps were located at the extremities of the range.

Crisping and their respective pre-treatments were all carried out by staff of United Biscuits (UK) Ltd in their pilot frying plant at the Group Research & Development Centre, High Wycombe.

The potato tubers were washed, peeled with an abrasive peeler and left in tap water until required for slicing. Batches of potato (2kg) were sliced to 1.4mm using a commercial slicer (Urschell) then agitated in cold water (20°C) for 2 minutes If hot washing was required, then further agitation was carried out in a hot water bath at 75°C (50kg water / 1kg slices) for 2 minutes. Cold and hot slices were next fed into a pilot scale continuous fryer and fried at 175°C to a moisture content of 1.5% $^{+}/_{-}$ 0.3%. To produce frozen samples of cold wash and hot wash potato, slices were frozen in large amounts of liquid nitrogen before being stored in a freezer at -20°C.

Crisping of Saponified Slices

Prior to the cold wash treatment slices of cv. Saturna were saponified overnight in chilled 0.1 M KOH / 70 % EtOH at 4°C. This was decanted off and the slices

neutralised by immersion in chilled salt buffer /70 % EtOH (10mM NaOAC, 3mM KCl, 2mM $MgCl_2$, 1mM $CaCl_2$ /70% EtOH). The salt buffer solution is based on the measured ionic conditions of the apoplast (Jarvis, 1990) and is an attempt to maintain the ionic environment of cell wall in near to natural conditions, thus minimising the solubilisation of the pectic material. Following neutralisation the slices were fried in a batch fryer.

Control slices were treated in salt buffer / 70% EtOH overnight at 4°C, instead of undergoing saponification, and were subsequently treated in the same way.

Boiling of potato slices

Potato tubers of cv. Maris Piper were sliced to thickness of 1.6mm using a Braun Multipractic food processor. The slices were immediately immersed in boiling water for 2.5minutes (10ml water/ 1g slice). Saponified and salt buffer treated slices as described previously were also boiled in the same way. The cooked slices were frozen in liquid nitrogen and stored at -20°C. The cooking water was also frozen.

Crisps for Taste Panel Trials

Saponified and non-saponified control slices were prepared as described. Following neutralisation the raw slices were cold and hot washed prior to frying. Cold wash and hot wash slices were also fried, including treatments with the hot wash blanch in different solutions:- 0.15, 1 and $5gl^{-1}$ $CaCl_2$ and 2M sucrose.

All chemicals used in the preparation of the crisps intended for the taste panel were suitable for human consumption. No flavourings or salt were added to the crisps.

Cell Wall Isolation

Standardised Protocol

Total Cell Wall Isolation from the Raw Tuber and Cold Wash Slices

The salt buffer solution - 10mM NaOAC, 3mM KCl, 2mM $MgCl_2$ and 1mM $CaCl_2$- was prepared the day prior to the cell wall isolation and chilled at 4°C overnight. The salt buffer solution was used throughout the procedure. A detergent solution with the same ionic composition as the salt buffer as well as Triton 100 (2mg/ml) was also prepared and chilled the previous day.

Three potato tubers were peeled, diced and a known weight - approximately 100g - was added to a Waring blender. (With the cold wash slices 100g of the frozen slices were used.) Chilled detergent (300ml) and 200g ice were also added. The detergent was intended to remove the cytoplasmic material and the ice kept the temperature low thus minimising enzymatic degradation. A few drops of octanol were also added to prevent frothing during blending. The mixture was then blended for six successive bursts of 15 seconds.

Following blending the mixture was immediately placed on a 53μm sieve. The detergent was removed by washing through the sieve using the chilled salt buffer until no Triton/ octanol smell could be detected. Additional ice was added as necessary. The washings were collected in two stages; the first 500ml and then the remaining washings. The washed residue was removed from the sieve and stirred in 50% acetone. Upon filtration the sample was weighed into a large beaker and five times the sample weight of phenol was added to achieve a saturated phenol solution. The saturated phenol solution was intended to denature cell wall enzymes and act as a protein extractant. Once the solution had been stirring for 30 minutes the saturated phenol was removed by suction and the residue was washed with the salt buffer solution on a grade 3 sintered glass funnel.

Aliquots of sample after the phenol washing were dropped into a metal beaker containing liquid nitrogen. Once all the nitrogen had boiled off the frozen pellets were added to a Braun KSM 2 coffee grinder and cryomilled for 15 seconds. A small quantity of liquid nitrogen - enough to cover the base of the grinder - was added and once it had evaporated the sample was ground for a further 15 seconds. This broke open the cellular structure allowing greater penetration of the α-amylase, which was used to break down the starch to maltose. After cryomilling and defrosting the sample was mixed into a paste using the cell wall preparation salt buffer. This paste was stirred into a larger volume of boiling salt buffer and boiled for 30 seconds. Immediately following the 30 second boil the mixture was decanted into an excess of chilled salt buffer, resulting in immediate cooling. Starch removal was achieved using α-amylase - from porcine pancreas, Sigma type VI-B. One unit of α-amylase will liberate 1mg of maltose from dissolved starch in 3 minutes at 20°C. As the exact quantity of starch in the isolated cell wall was unknown an excess of α-amylase -13,400 units in 1g, 1 unit liberates 1mg of maltose in 3 minutes at pH 6.9 at 20°C- was added, with further additions as necessary. The α-amylase was dissolved in the salt buffer used during cell wall isolation. The dissolved α-amylase, pullulanase -0.4 units in 0.02ml *Klebsiella pneumoniae*, Sigma type P-5420. One unit liberates 1μmole of maltotriose from pullulan in one minute at pH 5 at 25°C.- and a few drops of toluene to prevent microbial growth were added to the sample. The sample was then agitated in an orbital shaker at 20°C overnight. If starch was still remaining additional α-amylase was added and incubation continued. Hydrolysis of starch in the gelatinised form was quicker than removal of starch from the intact starch grain, thus minimising the opportunity for microbial growth during the α-amylase incubation. The presence of starch was detected using KI indicator - 1% KI, 0.5% I_2 - the sample was examined under the light microscope.

Once starch removal was seen to be complete- as assessed by KI- the sample was filtered on a grade 3 sinter and sequentially dried in 50, 75, 90 and 100% acetone. The salt buffer insoluble residual cell wall was stored in a desiccator until analysis.

Total Cell Wall Isolation from the Hot Wash Slices

The procedure here was almost identical to previously described. The only omission was the gelatinisation step. The slices had already been gelatinised during the cooking process.

Total Cell Wall Isolation from Crisps

The first step was defatting the crisps. Approximately 30g of crisps were immersed in diethyl ether overnight followed by four hours of soxhlet extraction in fresh diethyl ether the following day. The extraction thimble containing the defatted crisps was removed from the soxhlet and the remaining diethyl ether allowed to evaporate.

Approximately 15g of the fat free crisps were then used for the cell wall isolation. The cold conditions used in the raw tuber were not necessary during isolation of the crisp cell wall as the heat during crisping would have destroyed all enzymic activity. However, 100ml of salt buffer was also added to the blender which made the detergent mixture easier to filter. Once the detergent had been removed α-amylase was added and the sample was incubated overnight and treated as the raw sample.

Salt buffer Soluble Pectins

The washings from the cell wall preparation and enzymatic incubation were collected and used to monitor pectic solubilisation during the isolation procedure.

The detergent washings from the raw and cold-wash samples were centrifuged to separate the starch grains and a 40ml aliquot of the supernatant was removed and added to 160ml of ethanol. The volume of ethanol needed was four times the aqueous volume thus resulting in an 80% ethanol solution (Jarvis et al, 1988). The solubilised pectic polymers precipitated in the ethanol, which was centrifuged and the precipitated pellet was re-dissolved in salt buffer and frozen.

Aliquots of the detergent washings (40ml of the first 500 ml of washings and 200ml of the remaining washings) from the hot-wash slices and crisp cell wall isolations were treated with α-amylase prior to ethanol precipitation. A 200ml aliquot of the pectin solubilised during enzyme incubation was also used for pectin precipitation.

Development of the Cell Wall Isolation

The original aim was to develop a cell wall isolation protocol suitable for monitoring the chemical changes undergone by potato cell walls during various heat treatments. This involves recovery of the total cell wall and of any pectic substances solubilised during the cell wall preparation and - where possible - during the cooking process itself. During the cell wall isolation process it is important to inactivate potentially degradative enzymes and to minimise any chemical alterations as well as removing the starch.

Removal of cytoplasmic material

The first procedure started with homogenisation of the chopped tuber in chilled 0.2% Triton X-100 in the presence of ice and octan-1-ol. Detergents have long been used to isolate plant cell walls from forages. The first was van Soest (1963). Salt buffered Triton X-100 detergent has been used to remove ionically bound material (Ridge & Osborne, 1970) (Esquerre-Tugaye & Mazau, 1974). Gordon et al (1977) used a 0.1% Triton X-100 for isolation of mesophyll cells in grasses. The ice and chilled detergent were to maintain the temperature below 4°C thus minimising the potential degradative effects of enzymes released during homogenisation. The octan-1-ol was added as an antifoaming agent, (Harris, 1983). Pith cells are the smallest potato cells in the range 70 x 132μm to 96 x 158μm (Burton, 1989) and are retained in the sieve with mesh size of 53μm; smaller starch grains which have been released by rupture of some of the cells are lost.

Denaturation of enzymes

Once the detergent had been removed the sample was transferred to a beaker and phenol added. To obtain the phenol saturated with water 5x the sample weight of phenol needed to be added (Newman et al. 1994). Complete removal of the sample from the sieve required washing; consequently, this increased the quantity of phenol needed. However, 40g was the maximum quantity permitted in the fumehood at any time, by our COSSH regulations. Thus, once the sample had been removed from the sieve an approximately equal volume of chilled acetone was added. This removed excess liquid, consequently reducing the quantity of phenol needed.

Physical disruption of cell wall

Homogenisation breaks up the tuber into clumps of cells rather then individual cells - as observed under the light microscope. Further physical disruption is needed for efficient removal of starch. Initially ball milling using ceramic balls at a speed of 60rpm was employed. While some disruption was observed there were still many clusters remaining. Intact starch grains could be observed inside the clusters, whereas outer cells, showed reduced starch content. Even with ball milling times of 5.5 hours complete separation did not occur. Cryomilling using a dental amalgamator was more successful. However only small amounts could be treated at a time. Cryomilling in the Braun coffee grinder proved to be most successful at breaking the cellular structure. The greater the sample volume in the grinder the greater the physical disruption, probably due to a greater number of collisions of the brittle material thus breaking it down further.

Starch removal

To compare the different methods of starch removal a trial was set up using a batch of cell walls that had undergone the same cryomilling. The sample was subsectioned and aliquots treated with either 90% DMSO, α-amylase or both - 2 days in DMSO, followed by 2 days in α-amylase. The samples were spotted onto

microscope slides and the progress of starch removal monitored. The samples were sonicated daily. The α-amylase sample gave a negative reaction to the KI at the 4th day while the DMSO was not clear until day 6. The combined treatment was clear at day 5. The method chosen was α-amylase. A few drops of toluene were added to each solution to prevent microbial infection.

Heat treated samples

Defatting of the crisps was carried out according by two methods. Firstly by homogenising the crisps according to Ritchie et al. (1983) with hexane, hexane : diethyl ether 10:1 and then hexane : diethyl ether 10:1.5. Secondly by soxhlet extraction in diethyl ether for four hours. Both methods were successful but refluxing was adopted as being the more efficient.

Cell wall isolation from crisps did not need to be carried out under 4°C as the heat treatment during frying would have denatured all cellular enzymes. The defatted crisps were also dry thus the 0.2% Triton extract had not been diluted by ice or by water from the potato and proved to be very viscous to sieve. This was partly due to the viscosity of the Triton but mostly due to the gelatinised starch proving difficult to filter. Robertson and Horvath (1993) also noted this. To aid filtration 100 ml of salt buffer was added to the blender.

Starch removal from the crisp samples only took an overnight incubation as opposed to four days for the raw samples. To aid the starch removal from the raw sample a gelatinisation step was introduced into the preparation. Following cryogrinding the cell wall preparation was mixed into a paste with a small amount the salt buffer. As this paste is stirred into a larger volume of boiling salt buffer and the sample has been homogenised heat transfer is rapid. The longer the enzyme incubation period the greater the potential for microbial infection and increased pectic solubilisation. This rapid gelatinisation step removes the necessity for a lengthy enzyme incubation. Pullulanase (0.02ml) was also added to digest the branch points of the amylopectin (Selvendran & O'Neill, 1987).

Starch that has been gelatinised can be hydrolysed faster than intact starch grains, possibly through providing a greater surface area for the α-amylase to work on.

Sample Characterisation

Methyl Ester Determination

The degree of methyl esterification was measured by modification of the Klavons & Bennett (1986) method, using alcohol oxidase to oxidise methanol to formaldehyde which then forms a coloured complex with pentanedione.

The first stage is to cleave the ester groups from the cell wall. Cell walls (12g) were weighed into centrifuge tubes in triplicate, to which 6.25ml of water and 1ml of 1M KOH were added. The sample was saponified for an hour at room temperature, followed by neutralisation using 1ml of 0.94M phosphoric acid. Methanol standards in the range 0, 2, 4, 6, 8, 10, 12, 14 and 16μmml^{-1} were made up in a phosphate buffer pH7.5 (0.2M monobasic and 0.2M dibasic sodium phosphate).

The sample was centrifuged at 3000rpm for 3 minutes and 1ml of the supernatant containing the released methanol was pipetted into a centrifuge tube. (Any dilutions required could only be carried out prior to addition of the alcohol oxidase.) This was incubated at 25°C for 20 minutes with 1ml of alcohol oxidase (1unit/ml) *Pichia pastoris*, Sigma. One unit oxidises 1μmole of methanol to formaldehyde in 1 minute at pH 7.5 at 25°C. The standard solutions were treated in the same way.

A stock solution of 0.02M 2,4-pentanedione was prepared in 2.0M ammonium acetate and 0.05M acetic acid. Then pentanedione solution (2ml) was added to the incubated samples and the samples were vortexed on an orbital incubator at 60°C for 15minutes. The solutions were cooled and their absorbance values measured at 412nm.

Neutral Sugar Composition by GLC as Alditol Acetates

Neutral sugars were determined according to Englyst & Cummings (1984). The first step is hydrolysis using the Saeman method, whereby 10mg of cell wall are hydrolysed in triplicate in 0.2ml of 72 % (w/w) sulphuric acid for 1hr at room temperature. Inositol was used as an internal standard and 0.5ml - 2mgml^{-1} - were added together with 1.9ml of deionised water. This mixture was then heated at 100°C for 3 hours. A standard solution of neutral sugars was subjected to the same acidic conditions and heated for three hours.

Reduction was carried out by sodium borohydride and acetylation using acetic anhydride with a 1-methylimidazole catalyst.

The sample (0.5µl) was injected onto a DB-225 column (15m x 0.53mm) via a split injection (split ratio 2/1, splitter flow rate 12ml minute^{-1}, 250°C) using helium as the carrier gas (11.3ml minute^{-1}). The separation was at 190-220°C programmed at 15°C minute^{-1}. The method of detection was flame ionisation (250°C).

Pectic Galacturonan Determination:- Colorimetry

Standardised Protocol

Cell walls solubilised by the Saeman hydrolysis were also used for measurement of the pectic galacturonan. To a wide mouth boiling tube 1ml of the triplicated solubilised cell walls was added. To this 5ml of concentrated sulphuric acid containing sodium tetraborate (0.0125M) was dispensed into the centre of the tube. Standards (1ml) containing 0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6mM monohydrate D-galacturonic acid were also pipetted into a series of wide mouth boiling tubes. It is important to hit the centre of the liquid and not the side of the tube as the heat of dilution is used to drive the reaction. Once cool, 0.2ml of 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH was added and the sample vortexed. Blank samples were used to correct for neutral sugar interference

where 0.2ml of 0.5% (w/v) NaOH alone was added. Once full colour development had occurred, within 20 minutes, the absorbance was measured on the spectrophotometer at 525nm.

Determination of the soluble pectins was carried out by pipetting 1ml of the redissolved pectins into the boiling tubes and the procedure was continued as described.

Development of the pectic galacturonan protocol

The major constituent of pectin is D-galacturonic acid, thus attempts to quantify the pectin content colorimetrically generally rely on galacturonic acid standards. One of the largest cause of interference is the non specific browning caused by the neutral sugar side chains. Attempts have been made to optimise the method to minimise neutral sugar interference or to quantify the neutral sugars and correct for them. Initially quantification of the pectic galacturonan was attempted by the carbazole method (Dische, 1947). This was modified by Galambos (1967) who noted that borate depressed and sulphamate eliminated non-specific browning.

It is widely reported that neutral sugars interfere with colorimetric determination of pectic substances. There have also been many modifications to the protocols first postulated. The most common procedure today involves m-hydroxydiphenol (MHDP) which was first introduced by Blumenkrantz and Asboe-Hansen (1973), either by a fully automated procedure (Thibault, 1979) or manually (Filisetti-Cozzi & Carpita, 1991).

Initially the carbazole method using a phenol-sulphuric (Dubois et al., 1956) correction as described by Mankarios et al (1979) was carried out. However, as the MHDP is less affected by non specific browning a smaller correction factor would be required. Thus attempts to measure a correction value for the (Filisetti-Cozzi & Carpita, 1991) MHDP method were employed.

There were several problems encountered with this approach. The absorbance of the galacturonic acid standards in the MHDP was less than in the carbazole. This

could have been due to the possibility of heating upon addition of the sulphuric acid prior to the boiling step. Thus the heat of dilution conditions used in the carbazole were employed but using the MHDP reagents. The ratio of the sample, sulphamate and MHDP were maintained. The first step was to determine the need for a correction factor for neutral sugars in the MHDP and if necessary calculate it. The absorbance of 10mM solutions of arabinose, galactose and glucose were measured according to the MHDP protocol (table 2.1).

Neutral sugar	sugar concn mM	measured equivalent Gal UA concn mM
arabinose	10	0.10
galactose	10	0.25
glucose	10	0.19

Table 2.1 The absorbance of 10mM solutions of arabinose, galactose and glucose using MHDP

Resulting from this, attempts were made to correct for neutral sugars in the MHDP procedure. Galactose standards of range 0-50, 0-10 and 0-1mM were all carried through the MHDP process. The standard graphs were found not to be linear but to be polynomial.

Other problems with the method included a cloudy precipitate formed by precipitation of the sulphamic acid with the sodium tetraborate. This problem has also been reported by Blumenkrantz & Asboe-Hansen (1973)

The aim was then to modify the method by

- elimination of the sulphamic acid
- correction of the neutral sugar browning within one determination thus preventing the need for the phenol-sulphuric measurement

Thus the possibility of removing the sulphamic acid and the need for a separate neutral sugar correction was investigated.

The effect on measured galacturonic acid of 1) removal of sulphamic acid and 2) sugar correction

Method

A 0.6mM galacturonic acid sample was chosen from the middle of the standard graph to represent a typical sample concentration. Galactose (1.8mM) was chosen as the neutral sugar to determine if any colour would develop with the sugar alone on the addition of MHDP. Colour development caused by neutral sugars occurs when the acid is first added to the sample and does not require any developing. A mixture containing both galactose (1.8mM) and galacturonic acid (0.6mM) was prepared to determine. 1) If upon omission of the sulphamic acid browning occurred 2) If subtraction of a NaOH blank as in Ahmed & Labavitch (1977) would be a sufficient correction for the neutral sugars or whether a correction factor derived from the Dubois phenol sulphuric method would be necessary.

Initially the ratios of all the reagents were maintained, with the sulphamic acid (0.1ml) replaced with deionised water (0.1ml). This water was added to the samples before addition of the sulphuric acid as the total aqueous volume will determine the resultant temperature attained from the heat of dilution. The volume of the sample being analysed was constant at 1ml. Six replicates were carried through each treatment.

The temperature of the sample was measured by dispensing the sample into a wide mouth tube containing the sample and a thermometer. The maximum temperature was noted, as well as regular measurements over the following 10minutes.

Results

The concentration of the galacturonic acid in the samples was obtained from the equation of the standard graph. The standard graph - absorbance against concentration - using a zero and the six 0.6mM galacturonic acid samples as the

standards was plotted. The aim was to subtract the absorbance of the NaOH blank from the MHDP absorbance leaving the absorbance from the galacturonic acid alone.

Table no.	Concn of Galacturonic acid (mM)	Additional Substances		Molarity tetraborate
		Vol. (ml)	Material	
2.3	0.6	0.1	Water	0.0125
2.4	0.6	0.3	Water	0.0125
2.5	0.6	0.1	MHDP	0.0125
2.6	0.6	0.1	Water	-
2.7	0.6	-	-	0.0125
2.8	0.4	-	-	0.0125

Table 2.2 Description of effects examined during omission of sulphamic acid and the neutral sugar correction in analysis of pectic galacturonan.

2.3	MHDP absorbance	NaOH blank absorbance	measured concn	s.d.
0.6mM GalUA	0.706	0.003	0.600	0.015
1.8 mM GAL	0.084	0.015	0.059	0.004
Both Gal UA & GAL	0706	0.030	0.577	0.040

Table 2.3 Replacement of sulphamic acid with water (0.1ml).

2.4	MHDP absorbance	NaOH blank absorbance	measured concn	s.d.
0.6mM GalUA	0.320	0.003	0.597	0.014
1.8 mM GAL	0.132	0.039	0.175	0.012
Both Gal UA & GAL	0.473	0.049	0.794	0.035

Table 2.4 Replacement of sulphamic acid with water (0.3ml).

2.5	MHDP absorbance	NaOH blank absorbance	measured concn	s.d.
0.6mM GalUA	0.114	0.006	0.600	0.134
1.8 mM GAL	0.081	0.023	0.316	0.260
Both Gal UA & GAL	0.195	0.039	0.862	0.078

Table 2.5 Replacement of sulphamic acid with MHDP.

2.6	MHDP absorbance	NaOH blank absorbance	measured concn	s.d.
0.6mM GalUA	0.846	0.003	0.600	0.036
1.8 mM GAL	0.080	0.017	0.045	0.002
Both Gal UA & GAL	0.890	0.026	0.613	0.080

Table 2.6 Replacement of sulphamic acid with water (0.1ml) and omission of sodium tetraborate from concentrated sulphuric acid.

2.7	MHDP absorbance	NaOH blank absorbance	measured concn	s.d.
0.6mM GalUA	1.148	0.001	0.601	0.019
1.8 mM GAL	0.059	0.009	0.026	0.002
Both Gal UA & GAL	1.088	0.021	0.558	0.008

Table 2.7 Omission of sulphamic acid (0.6mM galacturonic acid)

2.8	MHDP absorbance	NaOH blank absorbance	measured concn	s.d.
0.6mM GalUA	0.712	0.008	0.400	0.013
1.8 mM GAL	0.042	0.006	0.019	0.002
Both Gal UA & GAL	0.757	0.015	0.396	0.015

Table 2.8 Omission of sulphamic acid (0.4mM galacturonic acid)

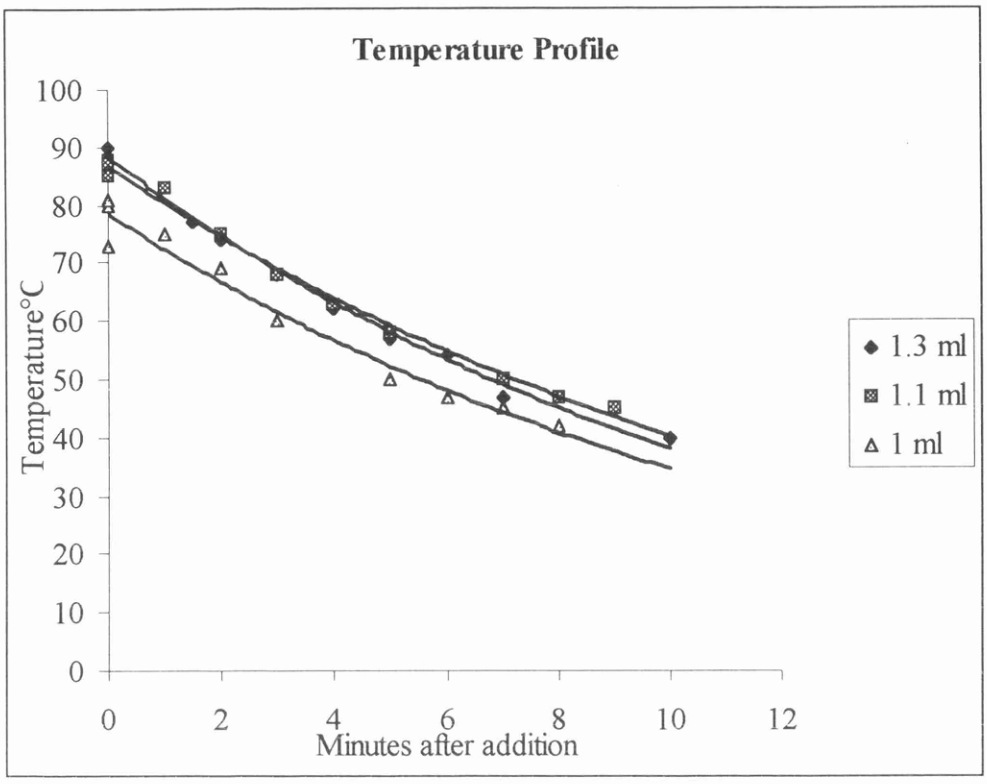


Figure 2.1 Temperature of the water mixture following addition of sulphuric acid

Discussion

Effect on replacement of sulphamic acid with water.

The subtraction of the blank lowered the measured concentration (table 2.3); whereas, the absorbance without subtraction of the blank agreed with the absorbance of the pure Gal UA.

The volume of added water was increased to 0.3ml to examine the effect caused by increasing the temperature. The response to galacturonic acid (table 2.4) was reduced while the response to galactose was increased, relative to the addition of the 0.1ml water. Subtraction of the NaOH blank was not sufficient to correct for this.

The conditions that cause increased response from the galactose also lowered the Gal UA response. Thus their absorbencies were equal (table 2.3) due to the

galactose increasing the absorbance by the same amount as the Gal UA was suppressed.

Effect on replacement of sulphamic with MHDP

Applying the MHDP prior to addition of the sulphuric acid (table 2.5) prevented formation of the colour complex.

Effect of omission of sodium tetraborate from the concentrated sulphuric acid.

Elimination of the tetraborate increased the interference from galactose in the mixed samples (table 2.6) to a greater extent than the NaOH blank could correct for. This resulted in an over estimate of the galacturonic acid concentration, there was also a greater spread of the measured concentration.

Effect of omission of the sulphamic acid without replacement.

Initially addition of sulphuric acid to the sample (table 2.7) increased the response of the MHDP to the galacturonic acid to an absorbance of greater than one. Consequently the concentration was lowered to 0.4mM (table 2.8) to obtain an absorbance reading less than one. The galactose concentration was maintained at 1.8mM. These results gave closest agreement between the actual concentration of the pure galacturonic acid and the measured concentration of the galacturonic acid mix. This was achieved when galactose concentration was 4.5x greater than the uronic acid. Greatest reproducibility was also obtained. The lower response from the neutral sugars could also be explained by the reduced temperature of the mixture. Galambos (1967) also noted higher interference at higher temperatures. On addition of the acid the temperature rose to 80°C as opposed to 90°C with other addition mixtures (figure 2.1)

The method chosen was to dispense the sulphuric acid directly onto 1ml of sample. Upon cooling the MHDP was added and the absorbance measured at 525nm. The absorbance of the NaOH blank was subtracted from the MHDP

absorbance. This method gave greatest sensitivity and speed as no separate correction experiment is needed. This method is suitable when the neutral sugar content is not greater than 5x the galacturonic acid.

Enzymic Starch Measurement

Standardised Protocol

The protocol is based on the enzymic starch analysis used by Batey (1982) and Karkalas (1985). The starch is simultaneously gelatinised at 94°C and hydrolysed by a thermostable α-amylase. The free glucose released by starch hydrolysis is measured by oxidation/ peroxidation and forms a coloured compound with o-dianisidine.

To a screw cap Pyrex tube 25mg cell wall, 5ml water, 0.1ml tris buffer and 0.1ml heat stable α-amylase -4000 units in 0.1ml, Sigma, 1 unit liberates 1 mg maltose in 3 minutes at pH 6.9 at 20°C - were added and boiled for 60 minutes. Once cool the mixture was centrifuged and 1ml of the supernatant, 0.3ml acetate buffer and 0.2ml amyloglucosidase - dilute to 45 units ml^{-1}, *Aspergillus niger,* Sigma, 1 unit liberates 1mg glucose from starch in 3 minutes at pH 4.5 at 55°C- were incubated at 60°C for 2.5 hours. The oligomers generated by the α-amylase are hydrolysed to glucose by the amyloglucosidase. Glucose standards of 10, 20, 30, 40, 50, 60, 70 and 80mgl^{-1} were prepared. To the samples and standards 3.5ml of a glucose oxidase/ peroxidase/ o-dianisidine mixture was added and kept in darkness for 50 minutes.

The mixture contained

- Glucose oxidase type II from *Aspergillus niger*, Sigma, 1500 units in 65mg. One unit oxidises 1μmole of β-D-glucose to D-gluconolactone and H_2O_2 in 1 minute at pH 5.1 at 35°C.
- Peroxidase, Sigma, 300 units in 3.5mg. One unit liberates 1 mg purpurogallin from pyrogallol in 20 seconds at pH 6 at 20°C.
- O-dianisidine, 50mg.

The glucose is oxidised to gluconic acid and hydrogen peroxide is produced, in the presence of peroxidase the o-dianisidine is oxidised to produce a red colour. The absorbance of the sample was measured at 455nm. The analysis was carried out in triplicate.

Development of the Enzymic Starch Protocol

Initially starch was measured as described by Watson & Jarvis (1995) by colorimetry. The dissolved starch reacts with an iodine solution containing potassium iodide. This method responds predominantly to amylose. Once the neutral sugar determinations by alditol acetates were calculated the glucose figures were much higher than expected than the KI / starch figures could explain. Thus an enzymic starch determination was developed.

Measurement of β-Elimination

Upon heating chemical degradation of the pectic chain takes place according to the β-elimination mechanism Keijebts & Pilnik (1974) This results in cleavage of the pectic chain next to a methyl esterified galacturonic acid residue.

A number of methods are available for estimating the unsaturated non-reducing terminal units resulting from β-elimination. However all existing methods are only suitable for soluble pectins. As it may not be assumed that all pectic fragments cleaved by β-elimination were solubilised attempts were made to find a method that could be applied to the whole cell wall.

Standardised Protocol

Raw potato cell walls (25mg) were boiled in 2ml phosphate buffer (pH6.8) for an hour. Following this 2ml of pectic extractant (0.01M CDTA, 0.1M $NaHCO_3$ or 0.1M Na_2CO_3) was added . The sample was then centrifuged and either a 1ml or 0.1 ml supernatant aliquot removed and added to 2.5ml H_2O and 1ml of 0.36M HCl. The absorbance was measured at 235nm. The extent of β-elimination was

calculated using the molar extinction coefficient (5100) quantified by Voragen (1972).

Development of β-elimination Protocol

The aim was to achieve maximum β-elimination and then solubilise those pectins and quantify the extent of β-elimination. Two approaches were considered: reaction with periodate-thiobarbituric acid (TBA) and UV absorbance. The unsaturated uronide resulting from β-elimination absorbs in the UV at 235 nm.

Periodate-thiobarbituric acid

Cell walls (25 mg) were added to a thick walled Reactivial and heated in H_2O (1.5ml) at 100°C for 20 minutes. Extraction of the β-eliminated pectin was a two step process, firstly by acid hydrolysis with HCl (1.5ml, 0.5M) for 1 hour. This was neutralised and further extracted by addition of 2.8M KOH/ 0.2M CDTA (0.3ml) Jarvis et al. (1988). The solutions were filtered and made up to 5 ml volumetrically. The thiobarbituric acid test was carried out according to Weissbach & Hurwitz (1959) utilising the prolonged oxidation step of Waravdekar & Saslaw (1959).

This resulted in an excessively dilute colour. Thus the Reactivials were weighed empty and at the end of the experiment to give the sample volume and centrifuged, consequently removing the need for diluting the sample. Problems with blanks occurred. A cell wall sample that had not undergone the heating was treated with the HCl and neutralised. This sample had a similar absorbance to the β-eliminated samples.

The negligible difference between the β-eliminated samples and the control could result from either the level of β-elimination being below the level of detection or interference from other heat extractable substances.

When the acid hydrolysed samples were neutralised the resultant solution was a faint yellow colour, which absorbed at 550nm. To maximise the opportunity for β-elimination the cell wall was boiled in a phosphate buffer at pH 6.8.

UV Test

Quantification of β-elimination was measured using the UV absorbance of the C_4-C_5 double bond at 235nm (Rombouts, 1972) and 5100 as the molar extinction coefficient (Voragen, 1972). The absorbance of the cuvette containing: 0.1ml of sample, 2.5ml water and 1ml of 0.36M HCl was measured at 235nm. Raw cell wall (25mg) was heated and 2ml of 0.1M CDTA was added to solubilise the β-eliminated pectin. The samples were treated as described by Rombouts (1972) where 0.1ml sample, 2.5ml water and 1ml of 0.36M HCl are added to a centrifuge tube and the absorbance measured at 235nm.

However, the absorbances of the samples were low (table 2.9) with little difference between the β-eliminated and the raw cell wall samples. The absorbance of the blank was also high.

Treatment	Absorbance at 235nm
β-eliminated	0.153
raw cell wall	0.118
blank	0.090

Table 2.9 Absorbance of samples at 235nm using 0.1ml of the sample

The volume of the sample added was increased to 1ml and the absorbance measured (table 2.10).

Treatment	Absorbance at 235nm
β-eliminated	1.337
raw cell wall	1.055
blank	0.952

Table 2.10 Absorbance of samples at 235nm using 1ml of the sample

Again there is little difference between the cell wall sample absorbances as well as a very high blank. Resulting from the high absorbance of the blank, the absorbance values of the solutions used in the method were measured independently (table 2.11). It can clearly be seem that the CDTA absorbance is responsible for the relatively high absorbance of the blank tubes.

Solution	Absorbance at 235nm
Water	0.000
0.1M CDTA	1.909
Phosphate buffer	0.008

Table 2.11 Absorbance at 235nm of protocol solutions

Blank tubes containing the proportions of extractant to water to be found in the sample tubes were measured (table 2.12). Extractants giving lower absorbance readings would remove the high blank problem.

Absorbance at 235nm	0.1ml sample	1ml sample
0.1M CDTA	0.230	1.865
0.02M CDTA	0.055	0.374
0.01M CDTA	0.035	0.194
0.1M Citrate	0.035	0.194
0.1M $NaHCO_3$	0.005	0.007
0.1M Na_2CO_3	0.011	0.023

Table 2.12 Absorbance at 235nm of different pectic extractants.

Three different extractant solutions (0.01M CDTA, 0.1M $NaHCO_3$ and 0.1M Na_2CO_3) were chosen to reduce the extractant effect. Also both volumes of 0.1ml and 1ml were used to minimise any effects from the change in ratios of sample to the water:HCl ratio.

Pectic Galacturonan Determination:- Titration

The cell walls were acidified in 10mM HCl/ 70 % EtOH at 4°C overnight, the low temperature and pH minimising β-elimination while the 70% EtOH preventing pectic solubilisation. The acid was removed by filtration and the sample washed with 70 % EtOH. The acid remaining in the washings was detected by precipitation of the chloride ions in silver nitrate. Washing was continued until no precipitate was observed. Following acetone drying 30mg of the cell wall was transferred into a round bottom three neck flask with 50ml of degassed 0.1M NaCl and a stirrer bar. A pH electrode was inserted through one of the screw thread arms, a screw thread septum was fixed onto the centre neck and a nitrogen cylinder connected to the third arm (figure 2.2).

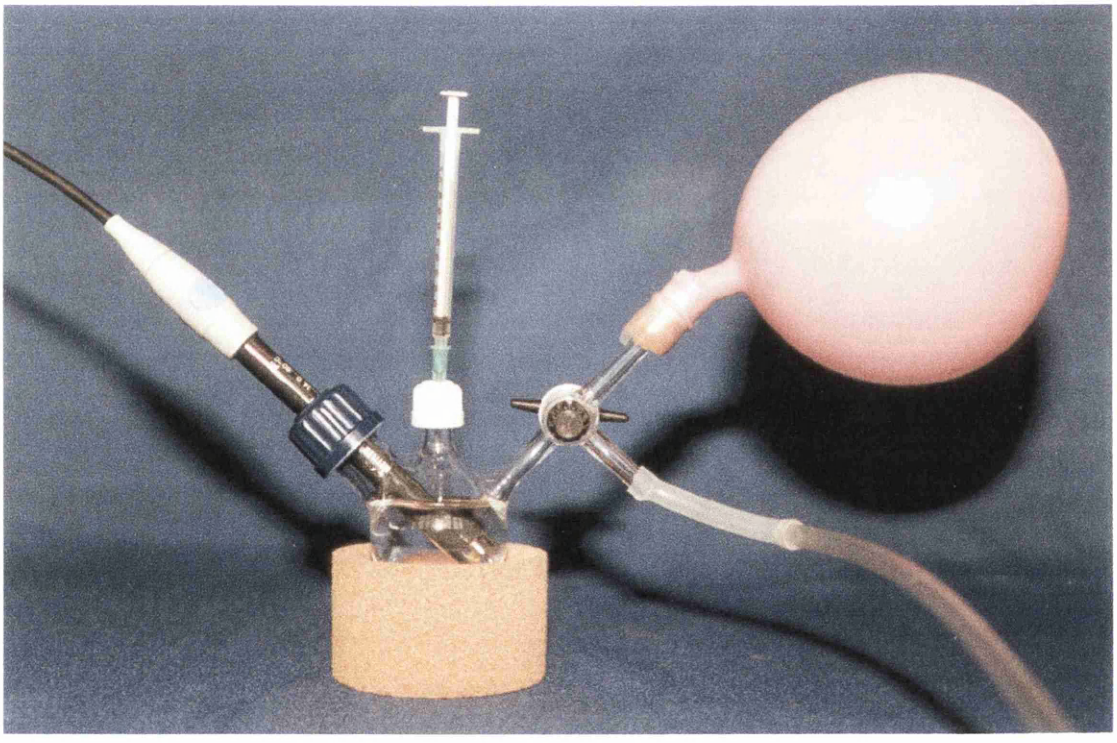


Figure 2.2 Titration apparatus

All solutions used during the titration were degassed and the titration carried out under nitrogen, as atmospheric CO_2 would dissolve to form carbonic acid. The cell wall was titrated with 10mM NaOH via a syringe inserted through the septum and the pH noted. Additions were initially of 0.1ml, once the pH began to climb additions were reduced to 0.05ml. This gave a measurement of non

esterified pectic galacturonan, assuming that only the non-esterified galacturonan contributes to the cation exchange capacity of the cell wall.

Total pectic galacturonan was measured by first saponifying the cell wall in 0.1M NaOH/ 70 % EtOH. This was subsequently removed and the residue was washed with 70% EtOH. The sample was then acidified and the process continued as before. The washing steps prior to titration removed interfering acetyl groupings cleaved by saponification.

Pectic Galacturonan Determination:- Copper Ion Exchange

This procedure was developed by Keijbets & Pilnik (1974) whereby the chemically bound copper is removed by addition of excess acid. The only difference from the published method was measurement of the released Cu by atomic absorption spectroscopy.

Molecular Sieve Chromatography

The pectic galacturonan solubilised from the boiled cv. Maris Piper during the cooking process and cell wall preparation was recovered by precipitation in ethanol as described earlier. The re-dissolved pectic galacturonan was applied to the sepharose 6B column (1.8 x 91cm). The column flow rate was set at $0.5 ml min^{-1}$ in 0.1M acetate/ 0.05M CDTA at pH 4.6. Fraction volumes between 4-5ml were collected. The pectic galacturonan was determined with MHDP as described earlier, without the NaOH blank or duplication. The neutral sugars were measured by the phenol-sulphuric method (Dubois et al., 1956) using a correction factor (Mankerios et al., 1979) to correct for absorbance of the galacturonan in the phenol-sulphuric method.

Neutral Sugar abs = Phenol-Sulphuric abs - MHDP abs(0.089)

The galacturonan and neutral sugar concentration results were plotted against K_{av}.

$$K_{av} = \frac{V_{1/2} - V_0}{V_t - V_0}$$

$$V_{1/2} = \sum_{i=0}^{i=n-1} V_i + 1/2 V_n$$

Where

V_0= Void volume (blue dextran)

V_t= Total volume (fluorescein)

V_n=Volume of fraction n

V_i =Volume of fraction i

Column packing.

The bottom of a 1m x 2cm column was sealed with a rubber bung containing the outlet pipe. Above the rubber bung was a teflon guard to prevent degradation of the rubber bung and a nylon mesh filter above the teflon. The outlet pipe penetrated through the teflon but not above. A funnel was attached to the top of the column. The column was double clamped in a vertical position with the aid of a plumb line.

The packing material was stored at 4°C in 20% ethanol. The ethanol was removed by mixing with acetate buffer, allowed to settle, then the supernatant was removed by siphon. This was repeated several times. Additional acetate buffer was added to the packing material so as to achieve a slurry which could be poured. The packing slurry was degassed for 4 hours. The column was filled with acetate buffer to approximately $^1/_3$ its height. The packing material was poured into the column in one continuous motion, a glass stirring rod was used to slow the flow thus reducing the opportunity of air bubble formation. The column was overfilled with packing material, to the point of half filling the filter funnel. The

material was left to settle for 24 hours. The excess packing material was siphoned off and returned to 20% ethanol and the column left to settle for a further 24 hours. The column was slowly eluted with acetate buffer overnight. The column flow rate was set at 0.5ml minutes^{-1} in 0.1M acetate / 0.05M CDTA (pH4.6) by adjusting the height of the elutant reservoir. Blue dextran and fluorescein were applied to the column. Fraction volumes between 4-5ml were collected to give the void and column volumes respectively.

Dry Matter Determination

Dry matter was determined by drying samples overnight at 100°C. The samples were cooled in a desiccator before weighing.

Potassium Mapping

Potassium mapping was carried out by Sue Gedney at the United Biscuits Group Research & Development centre, High Wycombe. Slices of cv. Saturna, following saponification/neutralisation were placed on a SEM (ISI WB6) sledge in a conductive carbon paste. The sample was plunged into liquid nitrogen slush for rapid freezing, then transferred under vacuum into the cryopreparation chamber (Hexland Cryo Trans CT1000). The sample was fractured using the blunt edge of a scalpel blade.

Following fracture, the sample was transferred to the SEM stage where the sample was etched, whereby the surface water was removed by heating until the cellular structure was visible. A qualitative X-ray spectrum was run at 15kV and the potassium peak was selected for mapping. A bitmap image of the relative concentrations was obtained. Following mapping the sample was returned to the cryopreparation chamber where it was gold coated. The sample was returned to the SEM and the mapped areas were photographed.

NMR Methods

Standardised Protocol

Solid state NMR has the advantage of being non-destructive. The NMR spectra were run by David Apperley at Durham University on cv. Saturna raw potato cell walls and hot wash crisp cell walls on a Varian VXR-300 spectrophotometer, operating at 75.34 MHz for ^{13}C, with magic angle spinning (MAS) rates of 2200Hz. Cell walls were hydrated to a 1/0.5 (g/cm^3) ratio of cell wall/ H_2O, with no water loss during the run. Ha et al. (1997) showed that the moisture content of the cell wall sample affects the resolution. An increase in water content increases the resolution. Five spectra were collected with cross polarisation-MAS contact times of 0.05, 0.2, 1, 3, and 20ms. The contact spin between the protons and the ^{13}C was delayed by 2 and 19ms, allowing the protons to decay by the $T_{1\rho}$ process, prior to a 1ms contact. A short recycle (100ms) single pulse ^{13}C spectrum was also run.

Background on NMR

Atomic nuclei can have a nuclear spin. This spin makes them behave like a magnet. Upon the application of a magnetic field they orientate themselves. Protons and ^{13}C both have odd number of nucleons so they can only take up one of two orientations. The spin orientations precess around an equilibrium orientation, a low energy orientation aligned with the applied field and a high energy orientation is opposed to the applied field. Upon application of a radio frequency the distribution of nuclei in the low energy state will change if the frequency matches the frequency at which the nuclei naturally precess. Following magnetisation the individual nuclei decay back to their equilibrium state in a complex wave pattern. This decay is known as free induction decay (FID) and is monitored by a receiver coil. The FIDs are added together and Fourier transformations are carried out to convert the superimposed wave patterns in the FID into a spectrum (Williams & Fleming, 1989)

The chemical environment determines the frequency at which the ^{13}C comes into resonance. The difference between the frequency at which this occurs for the different signals is referred to as the chemical shift.

Single pulse excitation comprises of a single pulse applied at the ^{13}C frequency. However, due to a 1% the natural abundance of ^{13}C the excitation/relaxation is repeated thousands of times to achieve a representative spectrum. The recycle time will be chosen according to the spin-lattice relaxation times T_1 of the ^{13}C nuclei, which is the time required for the nuclei to return to a spin equilibrium (McBrierty & Packer, 1993). The relaxation time T_1 of the ^{13}C can be tens of seconds. The time scale of the experiment can be reduced if the relaxation times can be reduced. One method of overcoming the long ^{13}C T_1 is to polarise the protons and transfer their polarisation to the ^{13}C, a method known as cross polarisation (CP) (Homans, 1989). The recycle time is then controlled by the much shorter ^{1}H T_1. The improvement in signal to noise ratio is in the order of thousands (Homans, 1989).

In a rigid environment the ^{13}C will cross polarise faster than those in a more mobile environment will. Conversely the ^{13}C CP-MAS signal will decay rapidly by the ^{1}H $T_{1\rho}$ process. As a consequence of the slow CP and rapid $T_{1\rho}$ processes the mobile component is not always visible.

Single pulse experiments can used be to preferentially visualise the more mobile regions of a polymer. The mobile phases have very much shorter ^{13}C relaxation times, while the rigid components have relaxation times of tens of seconds (McBrierty & Packer, 1993). If the recycle time is reduced to 1 second the rigid components will be saturated and show no signal allowing measurement of the free induction decay from the most mobile fractions of the polymer without interference from the rigid components (Foster et al., 1996).

In CP-MAS experiments delayed spin locking will give smaller signals from the ^{13}C associated with regions of local mobility within the polymer (McBrierty & Packer, 1993). The CP-MAS 20ms contact and the 19ms delay with the 1ms

contact spectra will have the same amount of ^1H $T_{1\rho}$ decay. However, there will be a greater amount of CP with the 20ms contact spectra. The rigid component has a fast CP rate; whereas, the mobile component has a slow rate of CP. Both spectra will show the rigid component, while only the 20ms contact time spectrum will show both the mobile and rigid components. Consequently, if the delayed contact spectra is subtracted from the 20ms contact spectra, then a spectra indicative of the mobile components will be achieved (Ha et al., 1996). The same principles apply to the 3ms contact and the 2ms delay 1ms contact experiment.

Solid state NMR spectra can be run on cell walls that have been kept hydrated and thus are close to their state *in muro* (Koh et al. 1997). Consequently it can be used to probe the molecular rigidity and mobility of individual polymers within the cell wall (Foster et al., 1996). Hydration makes little difference to the rigidity of cellulose and most of the xyloglucans but the pectic fraction shows greater mobility (Ha et al. 1997). Thus, the level of hydration is important, as this will affect the motion of the mobile polymer fraction. Ha et al. (1996) found that they could observe polymer chains which were too rigid for solution state NMR, but too mobile for CP-MAS NMR under normal conditions, using CP-MAS NMR with extended contact times. The pectic galacturonan and the galactose side chains are amongst the most mobile components in onion cell walls (Jarvis et al. 1996; Ha et al. 1996).

Mechanical Strength

Tubers of cv. Saturna harvested in 1996 were hand sliced and rectangular strips of diameter 26mm were cut perpendicular to the pith. Incisions of 5mm parallel to the edge were made on both sides midway down the strip. The strip was clamped at both ends to a tensiometer and the strip pulled apart at a speed of 10mm s^{-1}. The force (N) required to break the strip was noted.

The force required to break cold and hot-wash slices which had had the same incisions as the raw was measured while the slice was in the aqueous medium.

Aluminium tabs were glued onto the potato strip prior to the immersion in the wash water. The strips were anchored to the bottom of the container and the wash water added. After 2 minutes treatment the strip was stretched.

Cold and hot-wash strips that had been fried were defatted then rehydrated and stretched in the same manner as the cold-wash strips.

Sensory Evaluation of Crisps

The taste panel was organised at Strathclyde University, Food Sciences Department and was run with the aid of John Piggott. The descriptors are used to describe the texture of the crisp during eating. The initial descriptors were based on previous sensory crisp analysis carried out by United Biscuits in High Wycombe. The panellists had previous experience in the sensory analysis of foods. Two days were spent training the panel to familiarising them with the use of the descriptors to describe the crisp texture. A variety of ready salted crisps

Descriptor	Description	10%	90%
Hardness	Force required to bite through the crisp with incisors.	soft	hard
Crispness	Tendency to crack or shatter without substantial prior deformation	nil	crisp
Crunchy	Crumbly:-breaks down to a powder Brittle:- shatters with force	crumbly	brittle
Particle Angularity	Jaggedness after 4 chews	round	sharp
Adhesiveness	Sticks to teeth and roof of mouth	nil	sticky
Greaseiness/ Oiliness	Mouthfeel	nil	greasy
Rate of breakdown	Rate of breakdown	slow	rapid

Table 2.12 Explanation of descriptors used in sensory analysis.

were used during the training period. Following discussions with the panellists, the final descriptors (table 2.12) were chosen: hardness, crispness, crunchiness, particle angularity, adhesiveness, greasiness/oiliness and the rate of breakdown.

The crisps (~30g) were presented to the panel in isolation booths, under red lighting, in a randomised order. The descriptors were scored by clicking the mouse on a line on the screen of known dimensions with an explanation of the descriptors at either end. The results were analysed by principal components analysis (Piggott, 1986).

Chapter 3

Monitoring the Chemical Changes during the Crisping Process and its Pre-treatments: 1995

Introduction

Much of the research into crisps has been concerned with fat uptake; (Baumann & Escher, 1995; Gamble et al., 1987; Keller et al., 1986) and the affects of sugars on the resultant crisp colour (Sowokinos, 1978; Sinha et al 1992). No data have been published concerning the chemical changes occurring during the frying processes. The chemical structure of the cell wall may be able to explain the different textural attributes found in crisps from different potato varieties or the differences between the cold wash crisp and the hot wash crisp.

The potato varieties cv. Record and cv. Saturna are used for the manufacture of potato crisps in the UK. Cell walls were isolated from the raw tubers of cv. Saturna as described in Chapter 2. Slices of cv. Saturna and cv. Record after both the cold wash and hot wash pre-treatments were frozen and subsequently underwent cell wall isolation. Cell walls were also isolated from crisps fried after a cold wash as well as after the cold wash followed by a hot wash. The starch, dry matter, galacturonic acid, methyl ester, and neutral sugars were all determined for the buffer insoluble residual cell wall. Pectin solubilised during the isolation process was also retained and quantified.

Results and Discussion

The starch and dry matter content of cv. Record (table 3.1) and cv. Saturna (table 3.2) residual cell wall preparations were quantified. A conversion factor was used to correct the residual cell wall weights for starch and dry matter, thus allowing expression of results on a cell wall basis.

Record	Starch % Residue	Dry matter % Residue	Conversion factor
Cold wash	4.9	92.7	0.878
Hot wash	15.0	93	0.780
Cold wash crisp	4.7	91.6	0.869
Hot wash crisp	2.7	90.1	0.874

Table 3.1 Starch, dry matter and conversion factor for cv. Record

Saturna	Starch % Residue	Dry matter % cell wall	Conversion factor
Raw	3.1	93.7	0.906
Cold wash	8.2	93	0.848
Hot wash	13.6	83.6	0.700
Cold wash crisp	20.3	93	0.727
Hot wash crisp	2.8	94.2	0.914

Table 3.2 Starch, dry matter and conversion factor for cv. Saturna

The effect of processing on the pectic galacturonan content of the cell wall

Pectic galacturonans remaining in the cell wall and those solubilised by the isolation procedure were measured. Any changes in pectic structure or solubility would be due to the cooking process itself. Sequential extraction is a method for measuring the extractability of pectic galacturonan; however, this may result in degradation of the cell wall itself (Hughes et al., 1975; Selvendran, 1985) independent of the cooking process.

The value of 18.6% (table 3.3) for pectic galacturonan in the raw cell wall of cv. Saturna is in agreement with published pectin values of 17.3% (Selvendran & O'Neill, 1987), 18.8% for cv. Irene and 17.4% for cv. Nicola (van Marle, 1997a).

The purpose of the 2 minute cold wash is to remove starch grains from the cut surface of the strips thus preventing the strips from sticking together upon starch gelatinisation, which would result in reduced crispness. Washing will also reduce

Galacturonic acid (% cell wall)	Record		Saturna	
Raw	-	-	18.6	a
Cold wash	19.9	a	19.5	a
Hot wash	18.9	a	17.8	a
Cold wash crisp	10.7	b	11.4	b, c
Hot wash crisp	12.6	c	10.3	b

Table 3.3 Pectic galacturonan of isolated cell wall from cv. Record and cv. Saturna, expressed as the percentage of pectic galacturonan units (176.2) per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

Galacturonic acid (μmol/g)	Record		Saturna	
Raw			1057	a
Cold wash	1132	a	1105	a
Hot wash	1073	a	1010	a
Cold wash crisp	607	b	647	b, c
Hot wash crisp	717	c	584	b

Table 3.4 Pectic galacturonan of isolated cell wall from cv. Record and cv. Saturna, expressed as μmol of pectic galacturonan units (176.2) per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

the sugar content giving improved crisp colour (Lisinska & Leszczynski, 1989). There was no solubilisation of pectin at this stage (table3.4).

During storage, build up of reducing sugars could potentially lead to dark crisps when fried; however, hot washing can be undertaken to further increase the quantities of reducing sugar leached from the slice (Lisinska & Leszczynski, 1989). Varietal differences in storage behaviour will determine from what point into storage this would be required. The two minute hot wash at 75°C would have resulted in starch gelatinisation and rupture of the plasmalemma. No

difference in the total cell wall pectin was found either between the cold or hot wash treatments or between the varieties (table 3.3 & 3.4).

Despite there being no detectable differences in total pectic galacturonan in the buffer insoluble cell wall residue there were differences in the quantities solubilised during the cell wall isolation between the cold and hot wash treatments of cv. Record and cv. Saturna (tables 3.5 and 3.6 respectively). The quantity of pectin solubilised into the salt buffer used in the cell wall isolation increased following the heat treatment. A hot wash of 2 minutes is unlikely to be sufficient for the degraded pectin to be lost into the washing water by diffusion; but, upon homogenisation the soluble pectin was able to pass into the triton/ salt buffer mix, from which it was recovered. The solubilisation was most probably due to heat induced degradation of the pectic galacturonan. While there was greater pectic galacturonan solubilisation following the hot wash the amounts are too small to cause a significant difference to the measurement of the cell wall pectic galacturonan content. Heat treatments (50°C, 3h) on cv. Bintje (Ng & Waldron, 1997) also showed no difference in total pectic galacturonan of the cell wall material from the raw tissue. As washing progressed the quantity of pectic galacturonan also decreased showing that there was not continuous solubilisation of the same concentration. Both cv. Record and cv. Saturna showed the same trend with little difference from each other.

Solubilised Gal UA (mg/g) Record	Cold wash	Hot wash	Cold wash crisp	Hot wash crisp
1st washings	0.14	0.33	3.60	9.86
2nd washings	0.09	0.11	2.02	2.13

Table 3.5 Recovered pectic galacturonan of cv. Record solubilised during cell wall isolation. Total galacturonan (mg) recovered from the cell wall isolation washings from a dry weight (g) of the sample.

Solubilised Gal UA (mg/g) Saturna	Cold wash	Hot wash	Cold crisp wash	Hot crisp wash
1st washings	0.07	0.29	2.42	8.57
2nd washings	0.03	0.14	1.38	1.86

Table 3.6 Recovered pectic galacturonan of cv. Saturna solubilised during cell wall isolation. Total galacturonan (mg) recovered from the cell wall isolation washings from a dry weight (g) of the sample.

The washed strips were fried for 2 minutes at 175°C. The net result of frying is dehydration of the strips (Selman & Hopkins, 1989). There is also an influx of oil into the surface air spaces with limited penetration into the deeper air spaces (Gamble et al., 1987; Saguy & Pinthus, 1995).

Obviously as a consequence of the greater solubility of the pectin in the cell wall after frying the total pectin remaining in the isolated cell wall was reduced (table 3.3 & 3.4). Approximately two fifths of the pectic galacturonan was solubilised. An exception was cv. Record hot wash crisp where the overall loss was less (33% reduction). Heat treatments (50°C, 3h) on cv. Bintje (Ng & Waldron, 1997) prior to steaming reduced the effects caused by steaming alone.

There was little opportunity for the pectin degraded during the frying process to become solubilised. Thus, during the subsequent cell wall isolation procedure the broken segments of pectin were released into the Triton and washed out by the salt buffer.

Both cv. Record (table 3.5) and cv. Saturna (table 3.6) crisps had significantly increased solubility of pectin after frying. Pectic galacturonan solubilisation increased by factors in the range of 25 to 29 during the Triton wash. There were also differences in solubilisation between treatments. Hot washing prior to frying resulted in greater solubility with both varieties.

However, while the quantities of pectic solubilisation fell on washing there were still significant amounts being lost as the washing was continued. Consequently the values obtained can not be looked upon as representing total solubilisation, as further pectin could have been removed by continued washing or during the enzyme incubation.

The effect of processing on the methyl ester content of the cell wall

The quantity of methyl esters may play a crucial role in determining the resulting quantity of pectic galacturonan solubilised during the blanching treatment. β-elimination will cleave the pectic chain adjacent to a methyl esterified galacturonic acid residue (Keijbets & Pilnik, 1974), resulting in tissue softening and solubilisation of pectic galacturonan (Ng & Waldron, 1997). Concurrently, pectin methyl esterase will have become activated (Moledina et al., 1981; Puri et al., 1982). Pectin methyl esterase is a wall bound enzyme (Moledina et al., 1981) which will cleave the methyl ester group resulting in acidified pectic galacturonan units. Thus, pectin methyl esterase has a two fold firming effect; firstly, removing the potential for β-elimination (Sajjaanantakul et al., 1989) and secondly providing potential sites for calcium cross links to occur between adjacent pectic chains.

MeOH μmol/g	Record		Saturna	
Cold wash	453	a	477	e
Hot wash	337	b	419	f
Cold wash crisp	138	c	140	c
Hot wash crisp	200	d	156	c

Table 3.7 Methanol content of the cell wall of cv. Record and cv. Saturna, expressed as μmol of methanol per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

MeOH % Galacturonan	Record		Saturna	
Cold wash	40.1	a	43.1	e
Hot wash	31.5	b	41.4	a, e
Cold wash crisp	22.7	c	21.6	c
Hot wash crisp	28	d	26.7	d

Table 3.8 Percentage methyl esterification of the cell wall of cv. Record and cv. Saturna. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The methyl ester content of the cell wall of both cv. Record and cv. Saturna fell on processing (table 3.7). This reduction could originate from either pectin methyl esterase activity or preferential solubilisation of methylated pectic galacturonan. As only small quantities of galacturonan were solubilised during the wash stage then the pectin that was solubilised must have been highly esterified to explain the reduction in methylation observed. With cv. Saturna there was no significant reduction in the percentage methyl esterification (table 3.8) of the pectic galacturonan from cold to hot wash, suggesting that methylated pectin had not been preferentially solubilised during the hot wash or the cell wall isolation. However, cv. Record showed a significant reduction in percentage methyl esterification. It is not possible to say whether this is due to pectin methyl esterase or preferential solubilisation due to β-elimination.

The methyl ester content of the cell walls isolated from crisps (table 3.7) and the percentage methylation (table 3.8) of the pectic galacturonan of the crisps fell when compared to the respective methyl ester content of the washed slices prior to frying. This effect was more pronounced in the cold wash fry treatment than in the hot wash fry treatment. At temperatures of 175°C the pectin methyl esterase will have become denatured; thus, the reduction in methyl esters is likely to be due to the preferential solubilisation of the methylated pectic galacturonan. This is in agreement with the hypothesis of β-elimination.

The effect of processing on the neutral sugar content of the cell wall

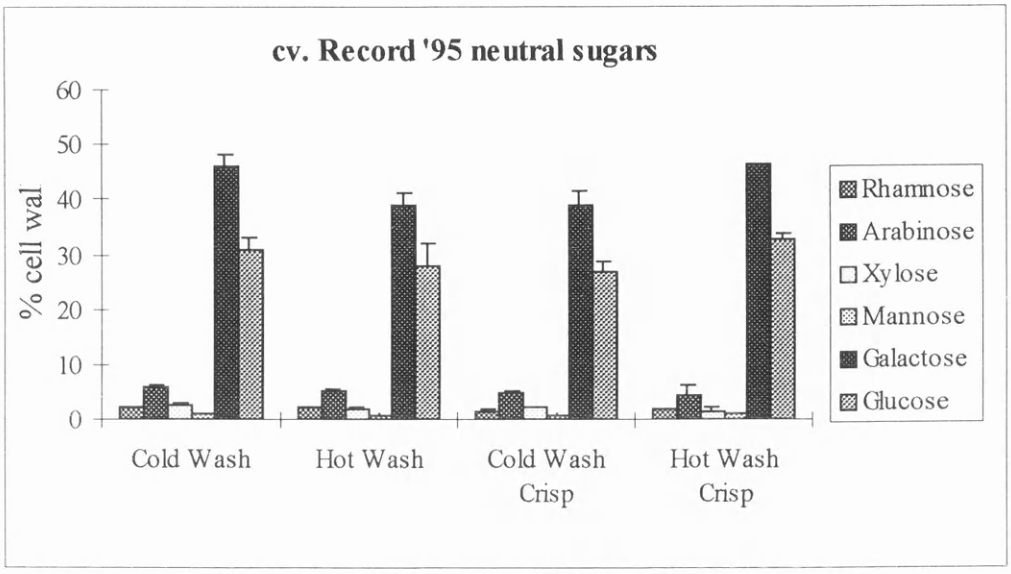


Figure 3.1 Neutral sugar analysis of isolated cell wall from cv. Record, expressed as the percentage of dry isolated cell wall. Error bars indicate the standard error.

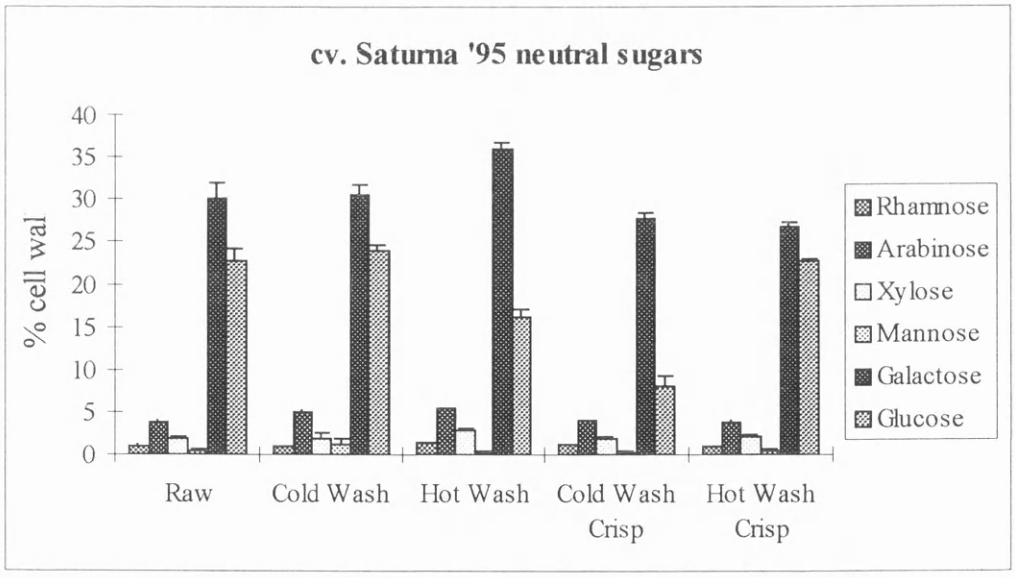


Figure 3.2 Neutral sugar analysis of isolated cell wall from cv. Saturna, expressed as the percentage of dry isolated cell wall. Error bars indicate the standard error.

Analysis of the neutral sugar content of cv. Record (figure 3.1) and cv. Saturna cell walls (figure 3.2) showed that the percentage rhamnose, arabinose, xylose, mannose and galactose of the cell wall was not altered during the washing process. The cell wall-glucose values were corrected for starch-glucose values.

The o-dianisidine starch-glucose value was subtracted from the alditol acetate total-glucose value, resulting in the cell wall-glucose figure. This has resulted in an underestimation of the cell wall glucose, which was more pronounced in samples with higher starch values. The reason behind the effect was not clear. However, starch may not have been hydrolysed into glucose during the Seaman hydrolysis, therefore would not be quantifiable after alditol acetate derivatisation. Thus little information concerning cell wall glucose can be gained.

While the residual pectic galacturonan fell significantly on frying the neutral branched chains, when expressed as percentage of the cell wall, of cv. Record (figure 3.1) remained unchanged. Thus, the pectin chains solubilised as a result of the crisping process were linear and more heavily methylated than those that remained.

The neutral sugars remaining in the isolated crisp cell wall of cv. Saturna (figure 3.2) were not significantly different from the raw, cold wash or hot wash treatments with one exception. There was a reduction in the cell wall galactose content between the hot wash slices and the hot wash crisps.

Characterisation of soluble pectin	Solubilisation as a result of:-	Record	Saturna
Gal UA μmol/g cell wall	Cold wash crisp	525	458
	Hot wash crisp	356	426
MeOH μmol/g cell wall	Cold wash crisp	315	337
	Hot wash crisp	137	263
% Methyl esterification	Cold wash crisp	60	74
	Hot wash crisp	38	62

Table 3.9 Characterisation of the pectic galacturonan solubilised as a result of the frying process. The concentrations of the Gal UA and the MeOH solubilised by the crisping process were obtained by subtraction of the crisp concentrations from their respective wash concentrations. The % methyl esterification was obtained using the MeOH and Gal UA values given in the table.

Frying solubilised 33-46 % of the pectic galacturonan. This solubilised fraction consisted of linear galacturonan, which was proportionately more heavily methylated (table 3.9), than the pectin in either the raw tuber or the residual crisp cell wall. The same was found for both cv. Record and cv. Saturna.

The aim of these experiments was to elucidate of the role of pectin in the texture development of potato crisps, by monitoring structural changes as the cooking process proceeded. Comparison of the pectic galacturonan in the raw with the pectic galacturonan in the cooked tissue would indicate the type of galacturonan solubilised as a result of processing. This in turn would indicate the type of pectin and pectic bonds that are important in crisp processing.

Following CDTA and Na_2CO_3 (1°C and 20 °C) Ryden and Selvendran (1990) solubilised 52% of the uronic acid while leaving the neutral sugar in the cell wall. The CDTA extract itself was highly methylated.

Using a polygalacturonase Ishii (1981) extracted 95 % of the galacturonans. The soluble fraction was fractionated by gel filtration. The lowest molecular weight fraction collected that had the greatest degree of methyl esterification (30%) also contained the most linear fraction.

The isolated modified hairy regions of potato (Schols & Voragen, 1994) showed that the fractions containing the highest degree of methylation also had the greatest degree of linearity.

Using ammonium acetate buffer, 0.05 M CDTA and then cold 0.05 M Na_2CO_3 van Marle et al. (1997a) extracted 20% of the pectic material with a degree of methylation of 12 %. During the isolation of the cell wall material all temperatures were maintained below 4°C; however, denaturing of enzymic activity was not reported. The initial fractionation steps involved suspension in ammonium acetate and CDTA both at 20°C, during this time there is the possibility of pectin methyl esterase activity as a result of incomplete denaturation of enzymic activity.

The amount of extractable pectin is dependent on both the extraction technique and the variety (Selvendran 1985; van Marle et al., 1997a). It has been discussed that the most easily extractable pectic material is the linear methylated fraction. The crisping process preferentially solubilised the linear methylated galacturonan.

The pectin content plays a decisive role in the cooking behaviour of root vegetables (Harada & Paulus, 1987). The early stages of the frying process are similar to the boiling processes (Selman & Hopkins, 1989). Pectic substances may also play an important role in controlling the degree of cell separation and consequently the morphology of the crisp (Selman & Hopkins, 1989). Reeve & Neel (1960) showed that the void formation had occurred between the potato cells. Therefore it can be suggested that cleavage of the methylated homogalacturonan chain resulted in solubilisation and cell separation. This implies that the methylated homogalacturonan was the pectic fraction that played the decisive process in crisping.

Chapter 4

Monitoring the Chemical Changes during the Crisping Process and its Pre-treatments: 1996

Introduction

Following on from the harvest and crisping of cv. Record and cv. Saturna in 1995 (chapter 3) the same varieties from the following year's harvest (1996) were also collected, stored and crisped.

Cell walls were isolated from the raw tubers of cv. Record and cv. Saturna as described in chapter 2, with the omission of the pullulanase from the enzymic starch removal stage. Slices of cv. Saturna and cv. Record after both the cold and hot wash pre-treatments were frozen and subsequently underwent cell wall isolation. Cell walls were also isolated from crisps fried after a cold wash as well as after the cold wash followed by a hot wash. The starch, dry matter, galacturonic acid, methyl ester, and neutral sugars were all determined for the buffer insoluble residual cell wall. Pectin solubilised during the isolation process was also retained and quantified.

Results and Discussion

The starch and dry matter content of the cv. Record (table 4.1) and cv. Saturna (table 4.2) residual cell wall preparations were quantified. A conversion factor was used to correct the residual cell wall weights for starch and dry matter.

Record	Starch % Residue	Dry matter % Residue	Conversion factor
Raw	5.1	93.0	0.879
Cold wash	11.3	94.9	0.836
Hot wash	45.7	95.1	0.494
Cold wash crisp	2.3	95.5	0.932
Hot wash crisp	4.4	93.9	0.895

Table 4.1 Starch, dry matter and conversion factor for cv. Record.

Saturna	Starch % Residue	Dry matter % cell wall	Conversion factor
Raw	4.7	96.3	0.916
Cold wash	10.6	94.8	0.842
Hot wash	53.4	94.1	0.407
Cold wash crisp	4.7	92.4	0.877
Hot wash crisp	4.1	93.4	0.893

Table 4.2 Starch, dry matter and conversion factor for cv. Saturna.

The effect of processing on the pectic galacturonan content of the cell wall

The pectic galacturonan content of 1036 μmol/g for cv. Saturna 96 (table 4.3) is in agreement with the value of 1057 μmol/g found with the raw cv. Saturna from the 1995 harvest. However the total levels of pectic galacturonan in the raw cell walls for both cv. Record (17.7%) and cv. Saturna (18.2%) were not significantly different from each other (table 4.4). Nevertheless the values are in agreement with published pectic values of 17.3% (Selvendran & O'Neill, 1987), 18.8% and 17.4% for cv. Irene and cv. Nicola respectively (vanMarle, 1997a).

The pattern shown in the 1995 harvest (Chapter 3) where there were no detectable differences between the cold and hot wash pretreatments were also shown with the 1996 harvest.

Galacturonic acid (μmol/g)	Record		Saturna	
Raw	1004	a	1036	a
Cold wash	956	a	964	a
Hot wash	978	a	935	a
Cold wash crisp	566	b	592	b
Hot wash Crisp	568	b	570	b

Table 4.3 Pectic galacturonan of isolated cell wall from cv. Record and cv. Saturna, expressed as μmol of pectic galacturonan units (FW 176.2) per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

Galacturonic acid (% cell wall)	Record		Saturna	
Raw	17.7	a	18.2	a
Cold wash	16.8	a	17.0	a
Hot wash	17.2	a	16.5	a
Cold wash crisp	10.0	b	10.4	b
Hot wash Crisp	10.0	b	10.1	b

Table 4.4 Pectic galacturonan of isolated cell wall from cv. Record and cv. Saturna, expressed as the percentage of pectic galacturonan units (FW 176.2) per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

While there were no detectable differences in the total pectic galacturonan of the isolated cell wall between the treatments there were differences in the amount of soluble galacturonan recovered. The soluble pectic galacturonan was recovered as described in chapter 2. The first washings (500ml) contained most of the Triton detergent as well as the salt buffer washings. The second washings consisted of all the remaining washings and the enzyme incubation wash contained any pectic galacturonan solubilised during the α-amylase incubation.

Solubilised Gal UA (mg/g) Record	Raw	Cold wash	Hot wash	Cold wash crisp	Hot wash crisp
1st washings	0.04	1.14	0.11	7.22	6.53
2nd washings	-	1.95	0.10	0.62	0.93
Enzyme incubation	0.51	1.10	0.60	0.54	0.68
Total	0.55	4.19	0.81	8.38	8.14

Table 4.5 Recovered pectic galacturonan of cv. Record solubilised during cell wall isolation. Galacturonate (mg) recovered from the cell wall isolation washings from a dry weight (g) of the sample.

Solubilised Gal UA (mg/g) Saturna	Raw	Cold wash	Hot wash	Cold wash crisp	Hot wash crisp
1st washings	0	1.35	0.08	5.82	6.91
2nd washings	-	0.94	0.26	0.96	0.71
Enzyme incubation	0.37	1.49	0.50	0.86	0.62
Total	0.37	3.78	0.84	7.64	8.24

Table 4.6 Recovered pectic galacturonan of cv. Saturna solubilised during cell wall isolation. Total galacturonate (mg) recovered from the cell wall isolation washings from a dry weight (g) of the sample.

There were trace amounts of galacturonan solubilised during the initial washing from the raw cell walls in both cv. Record (table 4.5) and cv. Saturna (table 4.6). Further solubilisation during the wash stage was not detectable, indicated by an absence of a precipitate in ethanol, in either cv. Record or cv. Saturna. During α-amylase incubation there was continued solubilisation of the pectin galacturonan.

Solubilised pectin recovery from the washing stages of the cold wash slices required centrifugation of the starch granules prior to ethanol precipitation. Enzymic removal of starch was not carried out, as the sample had not yet undergone the phenol stage. However, centrifugation as a means to remove the starch was not completely successful and the retrograded starch also precipitated

with the ethanol. The NaOH blank used in the galacturonan determination was not sufficient to correct for the starch interference, as the ratio of pectic galacturonan to total sugar was small. Consequently the figures for solubilised galacturonan for the cold wash slices are an over estimation.

The recovered pectic galacturonan was expressed as mg/g dry matter starting material rather than as a percentage of the isolated cell wall as complete transfer of the cell wall through the isolation procedure did not occur. The starch in the raw, cold and hot wash samples was gelatinised prior to enzyme incubation. This necessitated stirring the sample in boiling buffer. It was important to obtain rapid heat transfer through the sample, thus ensuring complete gelatinisation, whilst minimising the total heating time to minimise any heating effect on the cell wall. Following gelatinisation the sample was decanted into a large volume of chilled salt buffer to ensure rapid cooling. With every addition of gelatinised sample into the chilled salt buffer the temperature would rise. To limit the temperature increase and ensure rapid cooling only three additions of gelatinised sample occurred. Thus, sample that was not gelatinised during the three gelatinisation stages was discarded.

It can be seen that the main source of solubilisation of the pectic chain was the crisping process. As the washing was continued the remaining broken molecules were washed out, while some additional solubilisation may occur during the enzymic incubation

The effect of processing on the methyl ester content of the cell wall

The methyl ester content of the isolated cell wall of both cv. Record and cv. Saturna fell during processing (table 4.7) while the pectic galacturonan content was unchanged (table 4.3). There was a drop in methyl esterification between the raw and the cold wash isolated cell wall for both cv. Record and cv. Saturna. This could result from pectin methyl esterase activity upon the slicing of the tuber prior and during the cold wash process. Following the cold wash the slices were frozen in liquid nitrogen. The frozen slices were dark in colour and had frozen into large lumps. The darkness is an indication of polyphenol oxidase

activity. As the slices had frozen as aggregates, instead of single slices, freezing would not have been instantaneous. Thus allowing the possibility of pectin methyl esterase activity during the slow freeze. The total pectic galacturonan was not affected by this as the activity of polygalacturonase is much less than the activity of pectin methyl esterase.

MeOH μmol/g	Record		Saturna	
Raw	468	a	431	d
Cold wash	398	b	342	e
Hot wash	378	b	319	f
Cold wash crisp	207	c	163	g
Hot wash crisp	187	c, g	182	g

Table 4.7 Methanol content of the cell wall of cv. Record and cv. Saturna expressed as μmol of methanol per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

MeOH % Galacturonan	Record		Saturna	
Raw	46	a	41	b
Cold wash	42	b	35	c, d, e
Hot wash	38	b, c	34	c, d, e
Cold wash crisp	36	c, d	27	f
Hot wash crisp	33	d	31	d, e

Table 4.8 Percentage methyl esterification of the pectic galacturonan of cv. Record and cv. Saturna. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The methyl ester content of the hot wash cell wall was less than the raw cell wall, but not significantly different from the cold wash cell wall. There was no detectable fall in pectic galacturonan. Ng & Waldron (1997) also found a drop in methyl ester content while there was no significant drop in pectic galacturonan in cell walls isolated from cv. Bintje following a 3hr pretreatment at 50°C. Thus the reduction in methyl esters could be due, in part, to activation of pectin methyl

esterase during the hot wash process. However, the same problems involved in the freezing of the samples also occurred with the hot wash slices. Thus it is not possible to determine how much of the reduction in methyl esters is due to the hot wash process itself and how much to activation of PME during a slow freeze in each sample.

The cell walls isolated from the cv. Record crisps were less methylated than the raw cell wall. There was also a decrease in methylesterification from the crisp cell wall when compared to its respective wash treatment. While there may be some doubt as to the true methyl ester value for the wash treatments, it still follows that the crisping process has resulted in solubilisation of methyl esterified galacturonan as the true wash values could only have been higher. The temperatures involved in frying would denature any PME activity (Tijskens et al., 1997).

There were no detectable differences between the cv. Record and cv. Saturna hot wash crisps. There were also no detectable differences between the cold and hot wash percentage methyl esterification for cv. Record. Nevertheless, cv. Saturna cold wash was significantly less methyl esterified than the hot wash crisp. This trend was also shown in the 1995 harvest where the cv. Saturna cold wash crisp had a significantly reduced percentage methyl esterification.

The effect of processing on the neutral sugar content of the cell wall

The neutral sugar content of the cell wall of cv. Record (figure 4.1) and cv. Saturna (figure 4.2) shows that the percentage of rhamnose, arabinose, xylose and mannose in the cell wall was not altered during the washing or crisping processes. However, the percentage galactose in the cell wall fell during the cold and hot wash treatments. This could be due to the solubilisation of an RG I fragment containing long galactose chains. The solubilisation could have occurred either during the wash processes themselves or during the cell wall isolation.

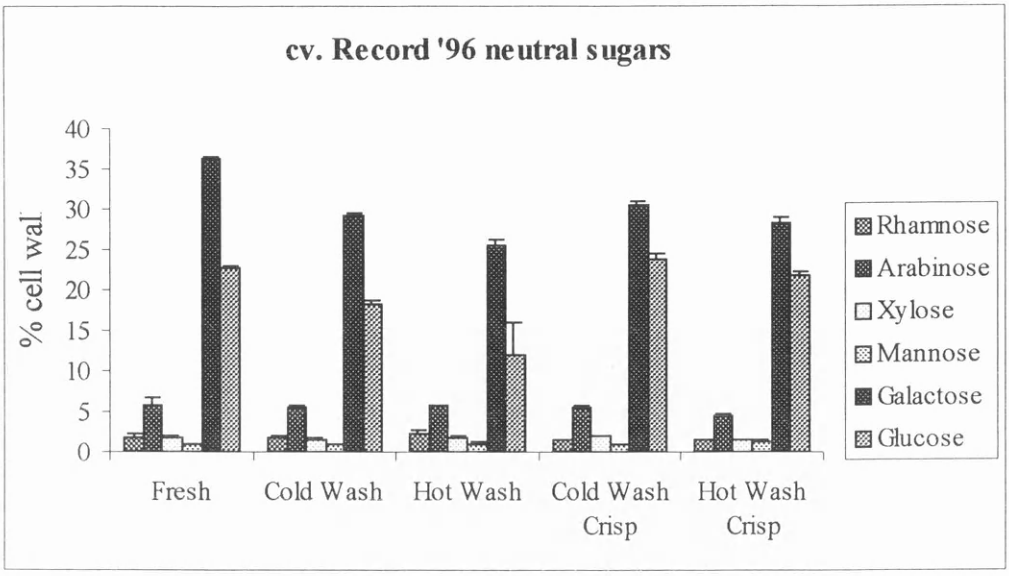


Figure 4.1 Neutral sugar analysis of isolated cell wall from cv. Record, expressed as the percentage of dry isolated cell wall. Error bars indicate the standard error.

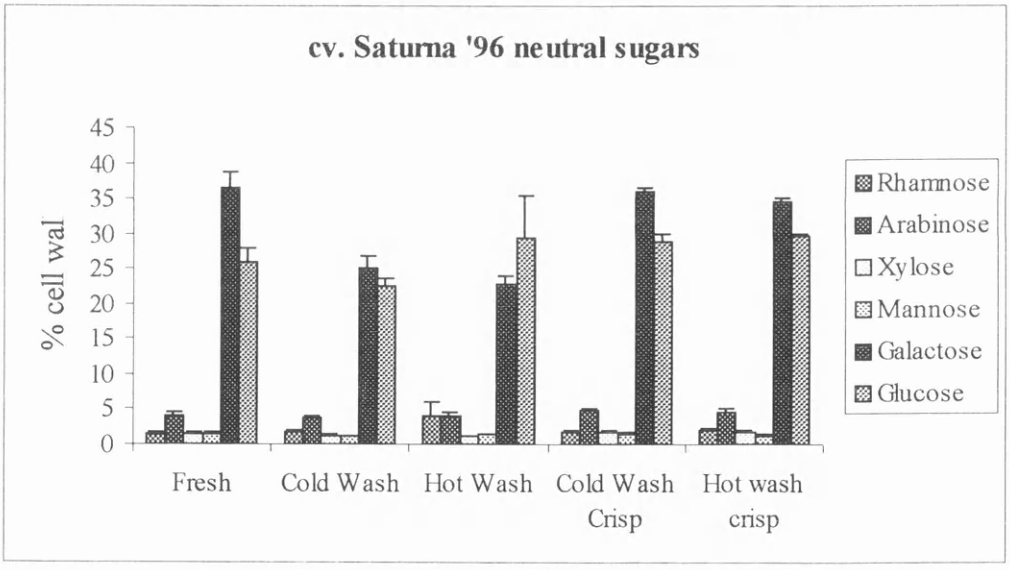


Figure 4.2 Neutral sugar analysis of isolated cell wall from cv. Saturna, expressed as the percentage of dry isolated cell wall. Error bars indicate the standard error.

The percentage galactose of the isolated cell wall of both cold and hot wash crisps was greater than both the wash pretreatments. The pectic galacturonan solubilised as a result of the crisping process was homogalacturonan; thus, the percentage of the galactose expressed as the remaining cell wall is greater. The same trend was also observed from the 1995 harvest.

Saturna mg/g dm	Cold wash crisp		Hot wash crisp	
	1st wash	Enzyme wash	1st wash	Enzyme wash
Rhamnose	0.14	0.10	0.25	0.06
Arabinose	0.30	0.27	0.32	0.12
Xylose	0.44	0.28	0.57	0.16
Mannose	0.14	0.03	0.24	0.02
Galactose	1.43	0.71	2.80	0.42
Pectic galacturonan	5.6	0.8	6.7	0.6

Table 4.9 Characterisation of the pectic galacturonan solubilised during the isolation process of cv. Saturna. Expressed as neutral sugar/galacturonan (mg) recovered from the cell wall isolation washings from a dry weight (g) of the sample.

Saturna	1st wash	Enzyme wash	Cell wall residue
Raw	-	-	2.2
Cold wash crisp	0.3	1.2	3.9
Hot wash crisp	0.5	0.9	3.9

Table 4.10 Ratio of arabinose plus galactose to the pectic galacturonan.

The recovered galacturonan solubilised during the cell wall isolation– 1st wash and the enzyme incubation- of cv. Saturna cold and hot wash crisps was freeze dried. This was then solubilised (Saeman hydrolysed) and the neutral sugar content (table 4.9) was measured as alditol acetates as described in Chapter 2. The concentration of neutral sugars was greatest during the first washings for both the cold and hot wash crisps. When the ratio of the branch chains is compared to the pectic galacturonan content (table 4.10) it can be seen that the galacturonan solubilised initially contained fewer or shorter side chains. During the enzyme incubation there was continued solubilisation. The galacturonans solubilised during the enzyme incubation were proportionately hairier than those initially solubilised, but were still proportionately smoother than those in the original cell wall.

The pectic galacturonan solubilised as a result of frying was predominantly homogalacturonan.

Force required for tensile fracture of the strips

The force required to break the strips was measured to act as a guide to the forces required for cell separation during the crisping process.

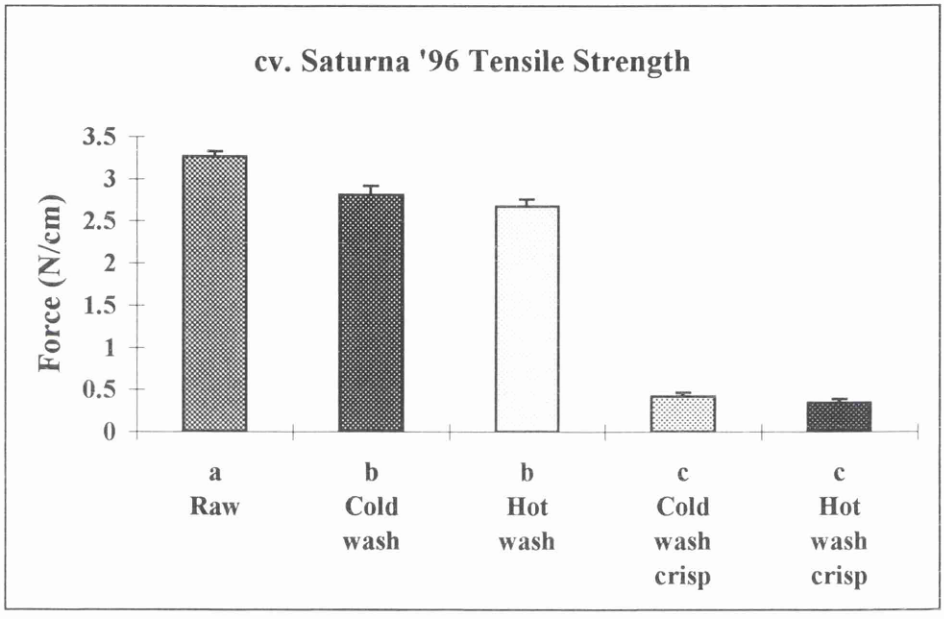


Figure 4.3 Tensiometer data for cv. Saturna.

The maximum force required to break the strip of potato tissue was measured as described in Chapter 2. Both the cv. Saturna cold and hot wash strips required less force than the raw to cause breakage (figure 4.3). Although there were no detectable differences in total pectic galacturonan between the raw, cold and hot wash slices there was a measurable decrease in strength. The cold wash resulted in 14% reduction in strength while the hot wash had an 18% reduction in strength when compared to the raw. The crisping process further reduced the strength (figure 4.3). The defatted rehydrated cold wash crisps have lost 87% of their original strength, while the hot wash crisps have lost 89% of their original strength. There were no measurable differences between the cold and hot wash slices or crisps.

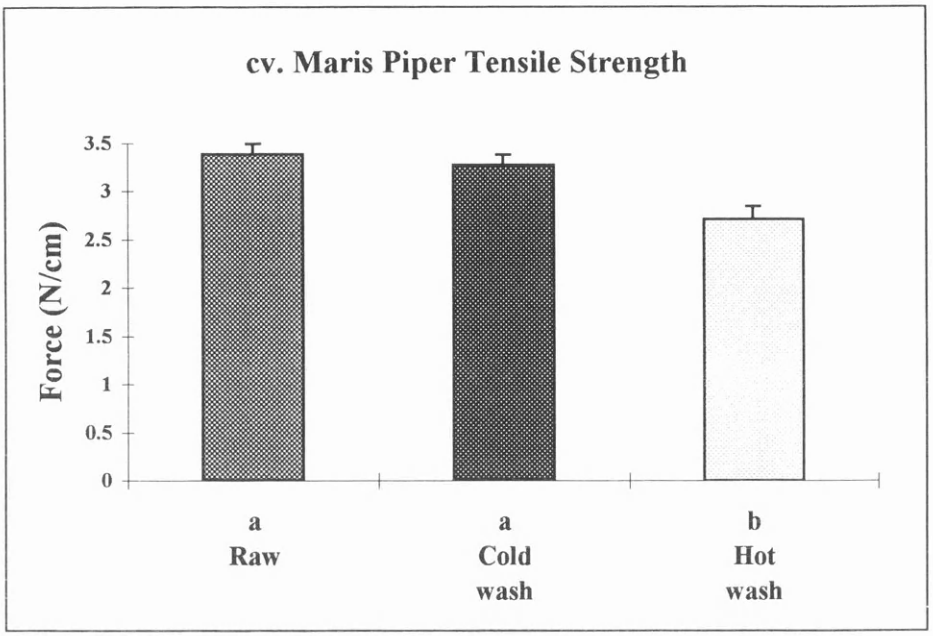


Figure 4.4 Tensiometer data for cv. Maris Piper.

Tensile tests on raw, cold-wash and hot-wash strips of cv. Maris Piper were also carried out (figure 4.4); however, when fried the reducing sugar levels were too high and produced black and burnt crisps. Nevertheless, the pre-fry treatments showed that the hot wash process had caused a 20% reduction in strength when compared with the raw tuber.

NMR analysis of cv. Saturna cell walls

Raw cv. Saturna cell wall

The CP-MAS spectra of cell walls from raw (fig 4.5) cv. Saturna is shown with varied and delayed contact times. The maximum intensity was observed with a 1ms contact time after zero decay, also shown for beet cell walls by Renard & Jarvis (1999). The main peaks visible in the raw potato cell wall spectra after 1ms contact time are cellulose C-1 (105ppm) and the C-2, C-3 and C-5 (72-75ppm). The cellulosic C-4 and C-6, both interior and exterior subunits of cellulose, were visible at 89, 84 and 65, 62ppm respectively. The pectic

galacturonan was also visible; C-1, 100-101ppm; C-2 and C-3 at 69ppm and less obviously the methyl ester peak at 54ppm.

In this experiment all signals in the spectrum decayed evenly as the contact time was increased. With other cell walls, delaying the contact for ^{1}H $T_{1\rho}$ relaxation to occur reduced the intensity of pectic signals more than cellulose signals (Renard & Jarvis, 1999; Newman et al. 1996). The greater mobility of pectins shortens the ^{1}H $T_{1\rho}$. Extending the contact time had the same effect in the experiments of McCann et al. (1995); however, Fenwick et al. (1996) and Ha et al. (1996) found the opposite as slow cross polarisation of the pectic galactan signals outweighed the effect of rapid ^{1}H $T_{1\rho}$ decay. The absence of any such effect in the experiment described here may be due to the averaging effect of the proton spin diffusion.

The difference between the variable contact and the delayed contact spectra (Ha et al., 1996) was plotted (fig 4.6). The differences are small due to the averaging effect, but the largest difference is between the C-4 pectic galacturonan at 80ppm. The difference in signal intensity between the 2 and 19ms from the 1 and 20ms was plotted as a difference spectrum (fig 4.7). Despite a high signal to noise ratio the spectrum of the mobile galactose component was visible; C-1, 104.4 ppm; C-2, 71.7 ppm; C-3, 73.7 ppm; C-4, 69.6 ppm; C-5, 75.9 ppm and C-6, 61.8 ppm. While the pectic galacturonan itself was not visible, the methylated galacturonan peak at 54ppm was, showing that it is more mobile and can spin.

The short recycle pulse spectra (fig 4.8) profiles the mobile component. Due to the increased motion, narrow bands were obtained. The galactose signal peaks are clearly visible, with smaller arabinose peaks visible.

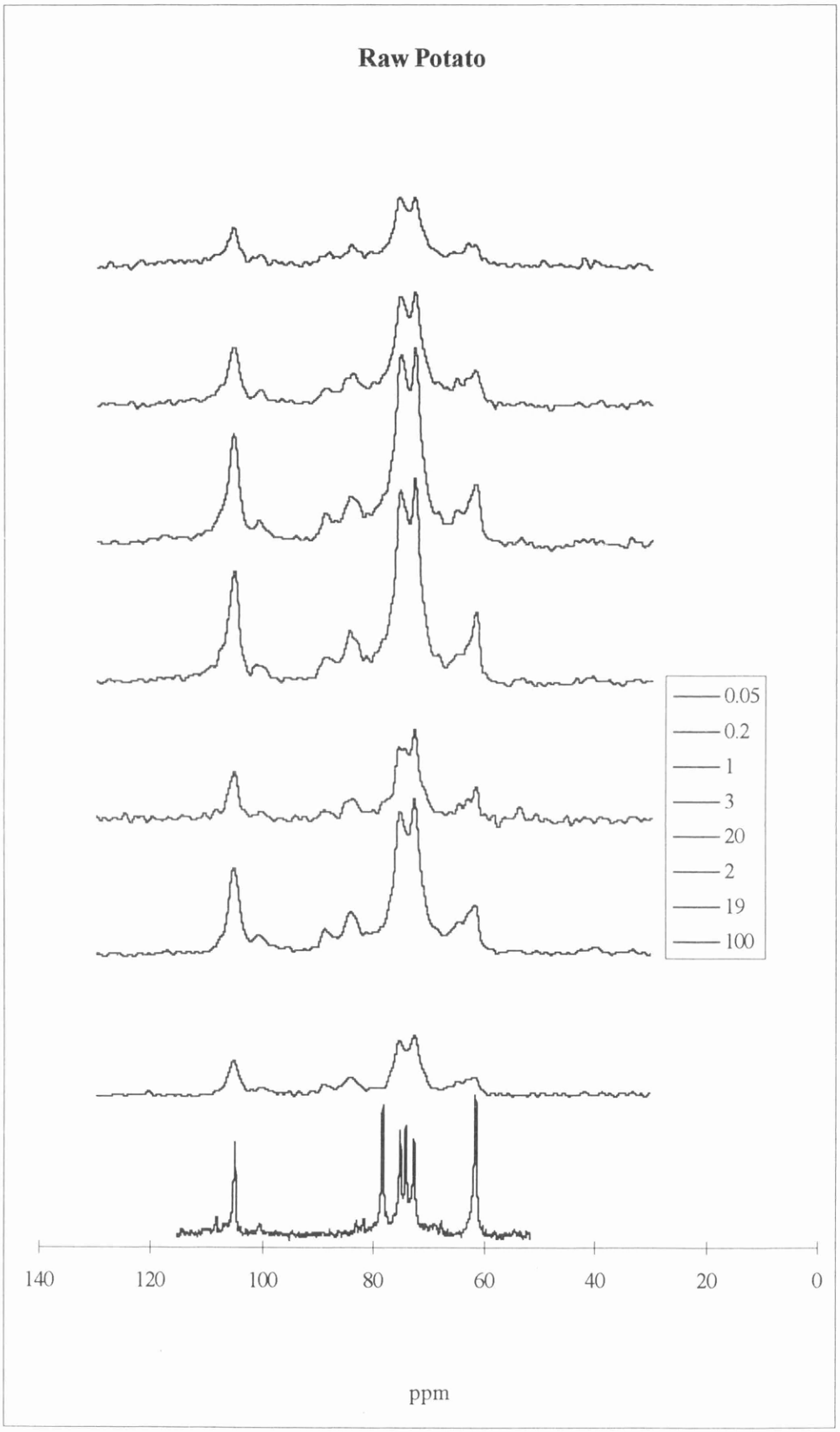
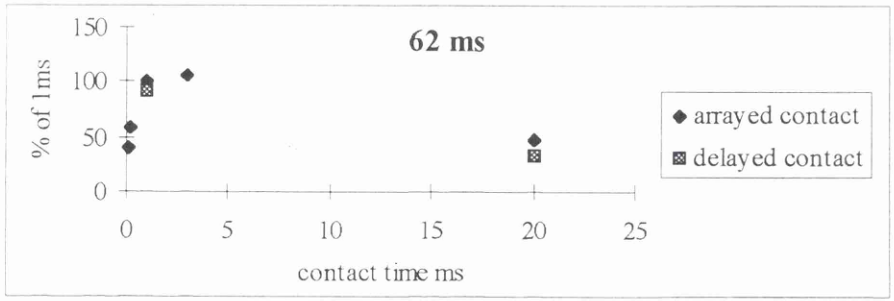
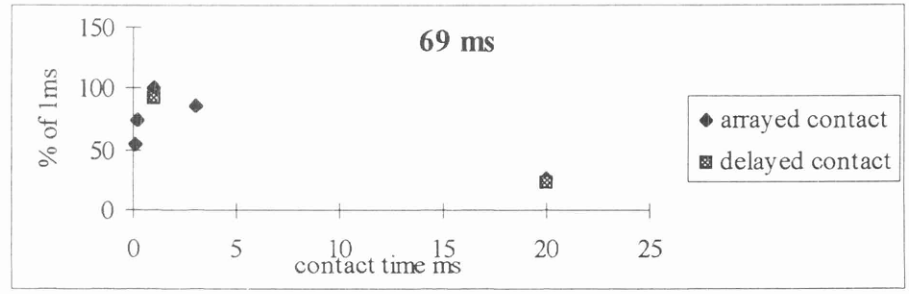
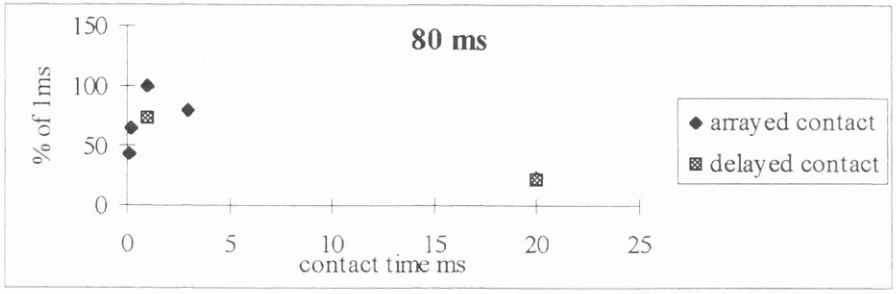
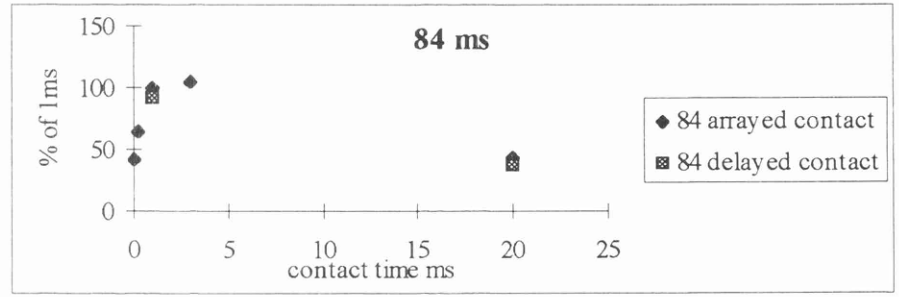


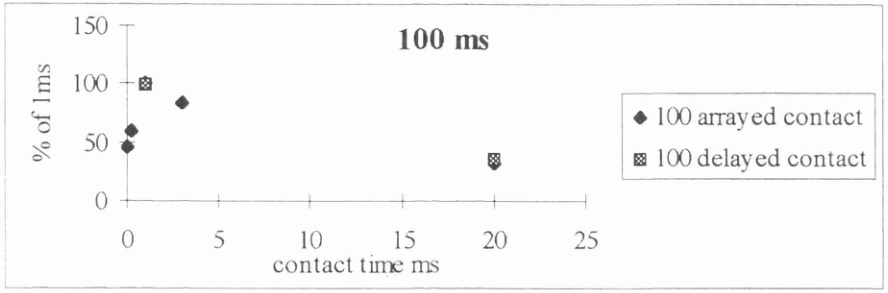
Figure 4.5 Raw potato cell wall: CP-MAS spectra with varied and delayed contact.











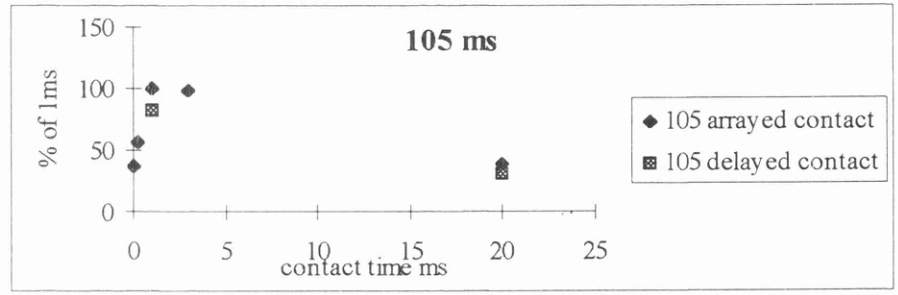


Figure 4.6 Signal intensities from the varaiable and delayed contact experiments.

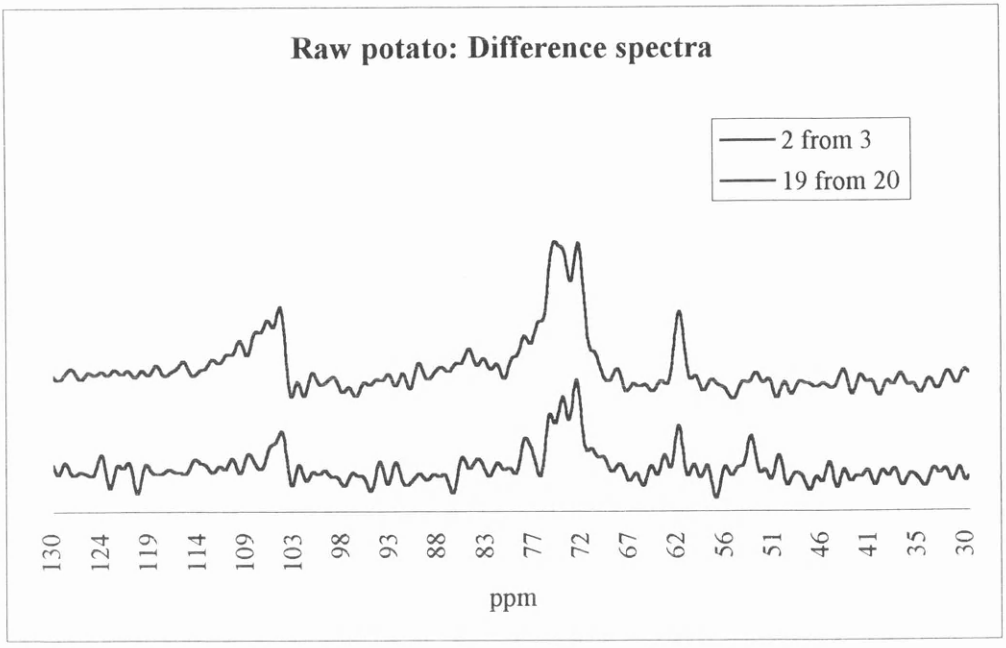


Figure 4.7 Raw potato cell wall: Difference spectra

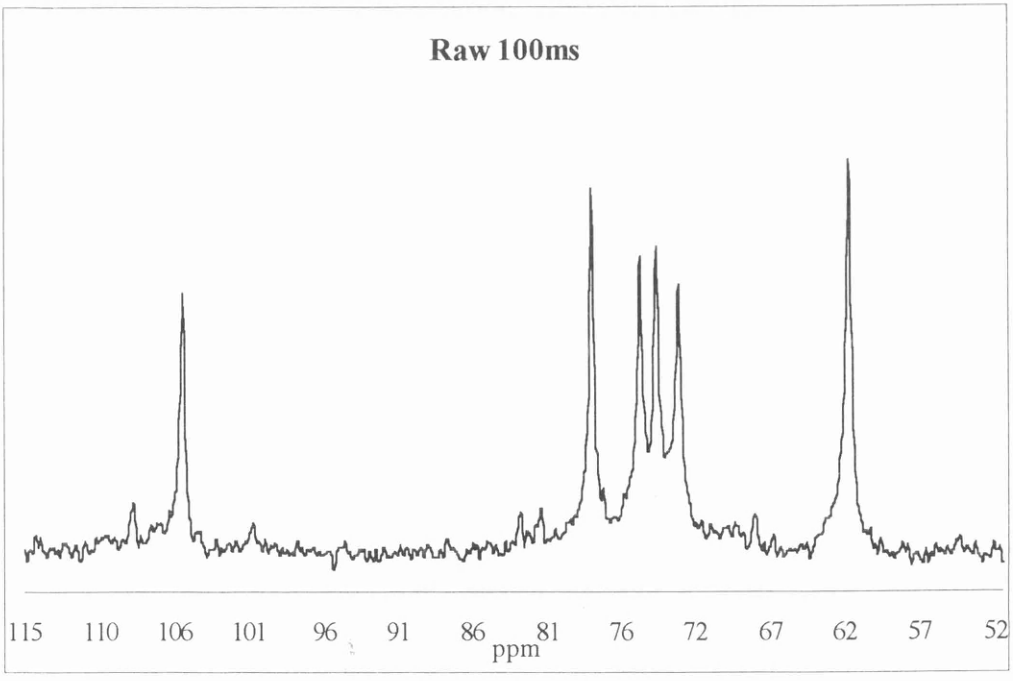


Figure 4.8 Raw potato cell wall: Short spin recycle pulse spectra

Hot wash crisp cv. Saturna cell wall

The cv. Saturna hot-wash crisp cell wall, variable and delayed contact spectra (fig 4.9) show the same trend as the raw potato cell wall. The greatest spectral intensity was shown at a contact time of 1ms with zero decay followed by a general decrease in signal intensity with increasing contact time.

There is little remaining signal when the difference spectra between the 2ms delay/1ms contact and the 3ms contact is plotted (fig 4.10) and also with the 19ms delay/1ms contact and the 20ms contact spectra. The difference between the variable contact and the delayed contact signals were generally larger than those found in the raw potato cell wall but were still relatively small due to rapid $T_{1\rho}$ decay.

The short recycle single pulse spectra (fig 4.11) again showed a clear mobile galactose peaks, with larger arabinose peaks relative to the raw potato cell walls.

The peak heights at 1ms contact for both the raw and crisp cell walls were normalised, and consequently they have similar peak heights when overlaid (fig 4.12). When the 19ms delayed contact spectra were also overlaid (fig 4.13) the peak heights are smaller for the crisp cell walls. This could indicate that the galacturonans in the crisp cell walls, on average, have greater mobility. Foster et al. (1996) suggested that the linear pectic galacturonan restricted the movement of the galactose side chains. As previously discussed, the crisp hot-wash cell walls solubilised pectic galacturonan, while maintaining their galactose content. The increased galactose mobility is consistent with increased mobility of the RG1 fraction. This mobility may be the result of cleavage of the homogalacturonan chains between the RG1 fractions. Thus, the results agree with Foster et al (1996) that once homogalacturonan was removed, the branched segments remaining in the cell wall had greater mobility.

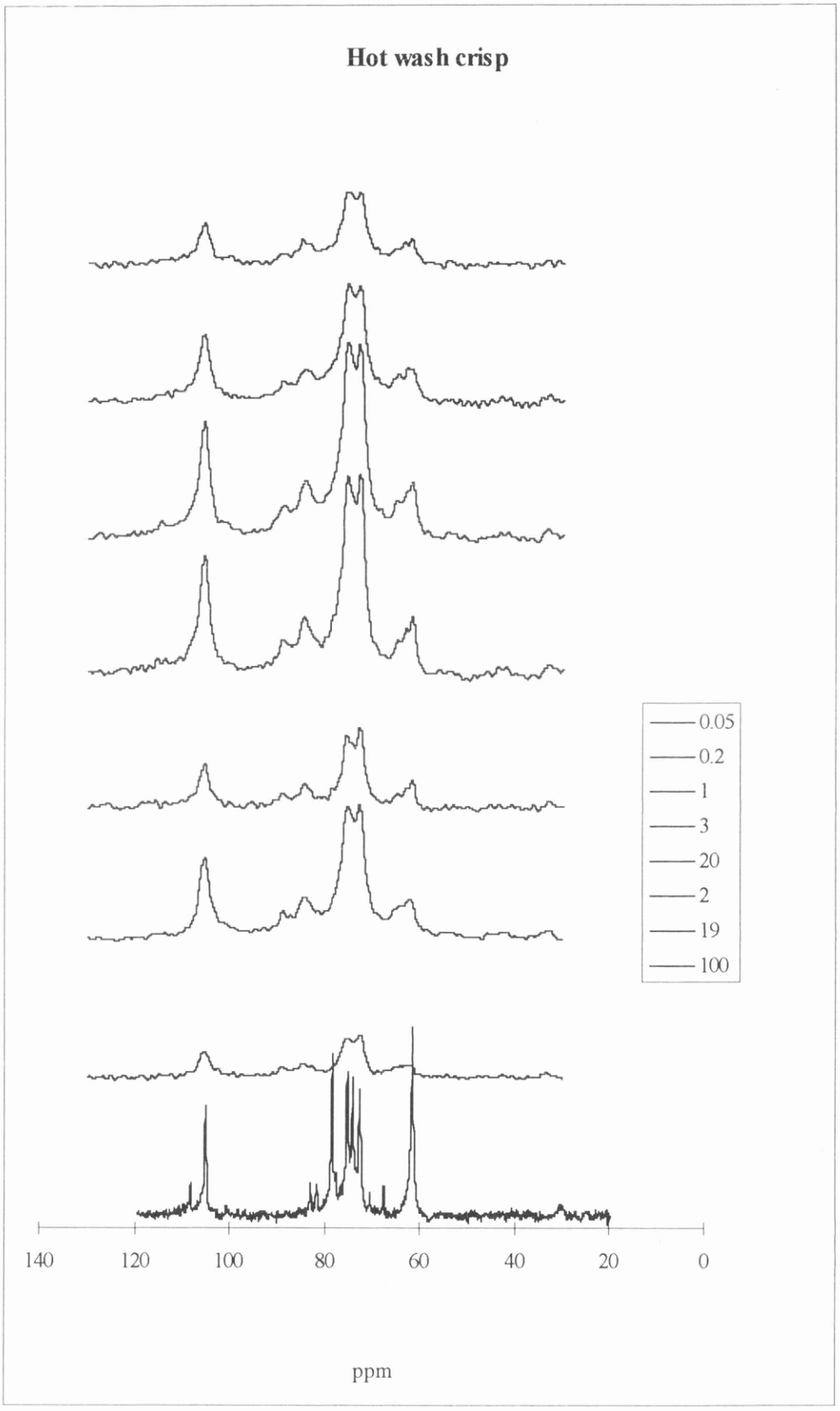


Figure 4.9 Hot wash crisp cell wall: CP-MAS spectra with varied and delayed contact.

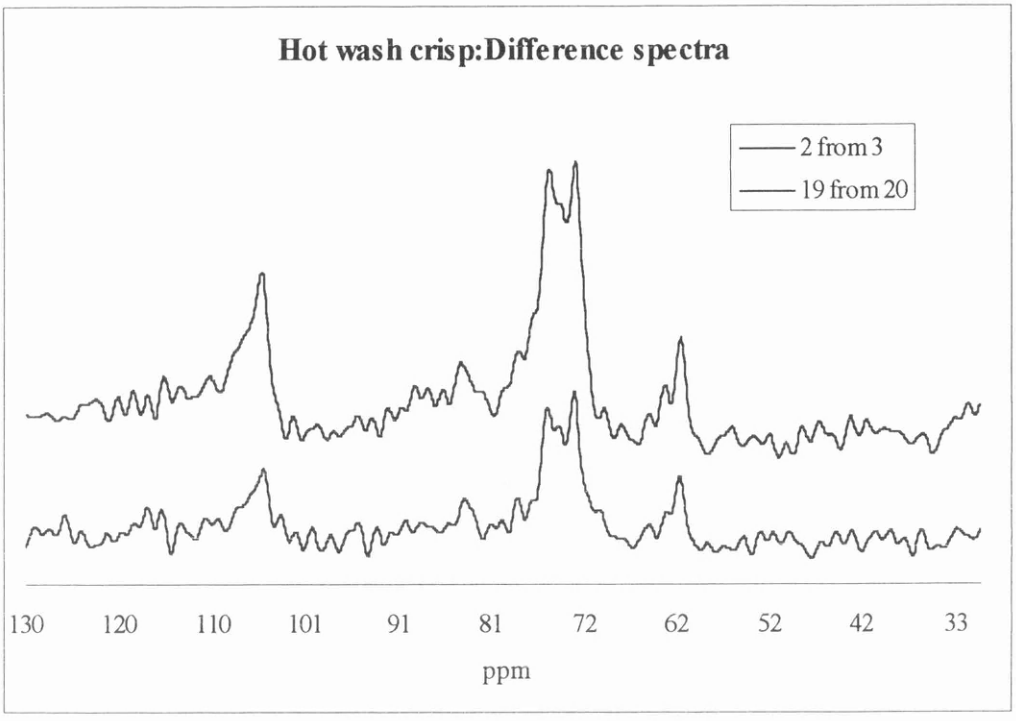


Figure 4.10 Hot wash crisp cell wall: Difference spectra

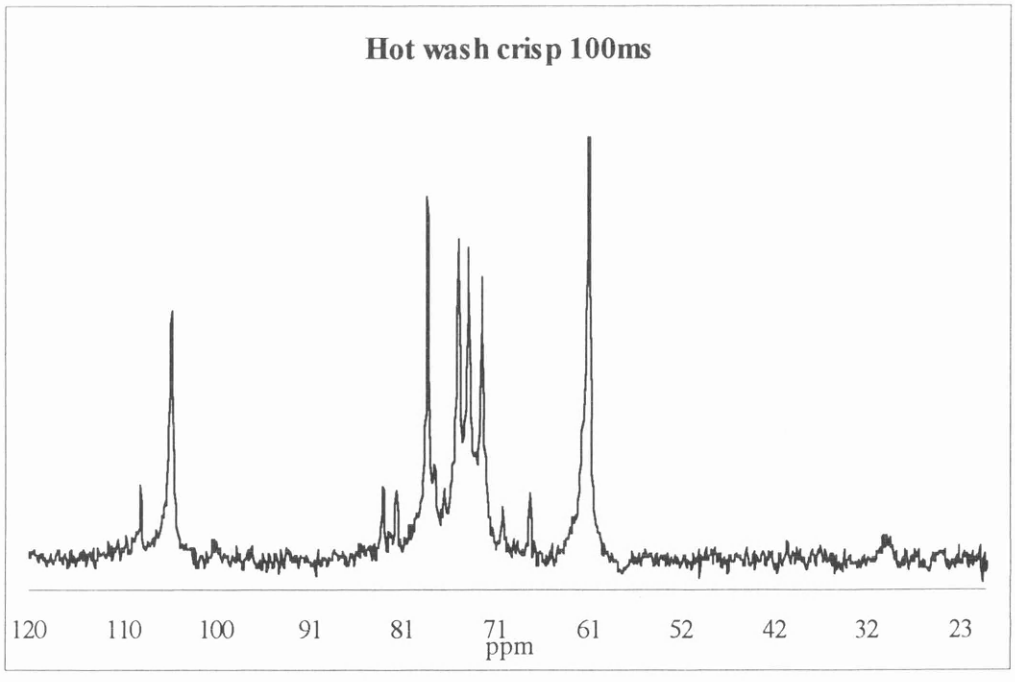


Figure 4.11 wash crisp cell wall: Short spin recycle pulse spectra

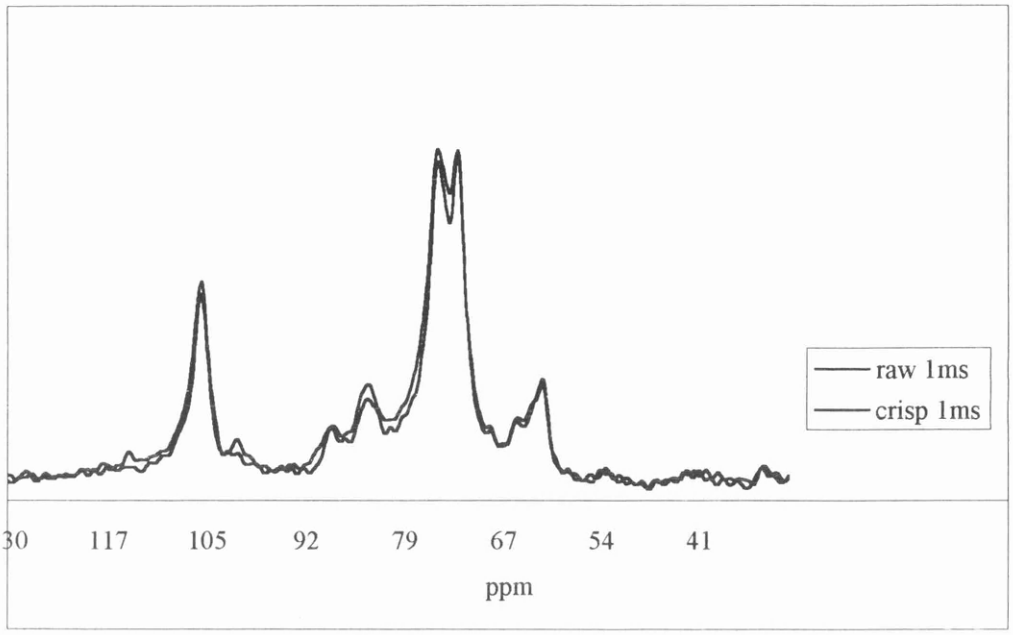


Figure 4.12 Overlay of 1ms contact for cv. Saturna, raw and hot wash crisp cell walls

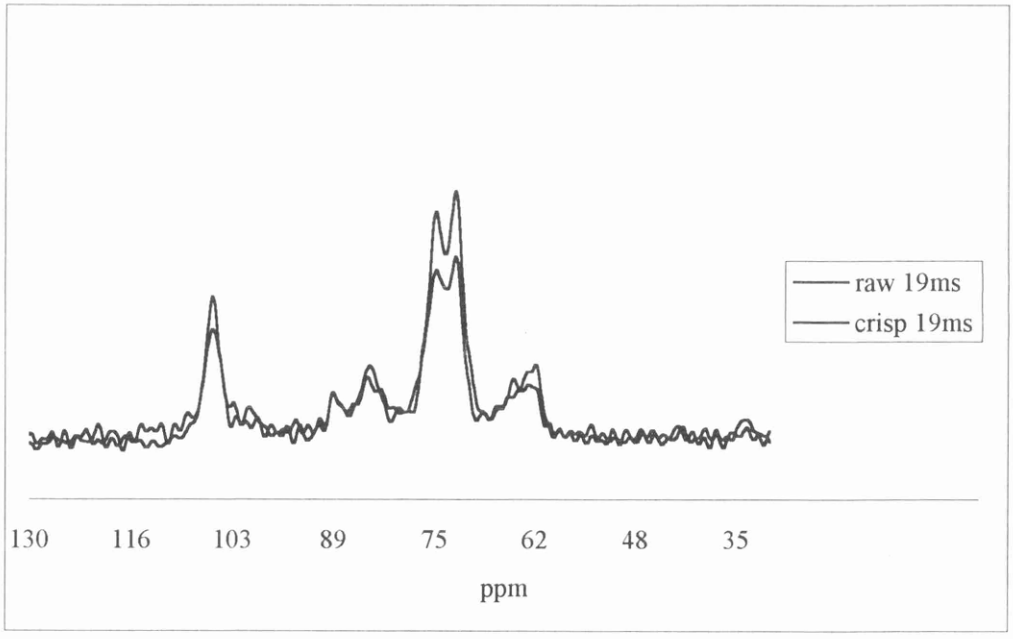


Figure 4.13 Overlay of 19ms contact for cv. Saturna, raw and hot wash crisp cell walls

Chapter 5

Chemical Changes Induced during Boiling of cv. Maris Piper

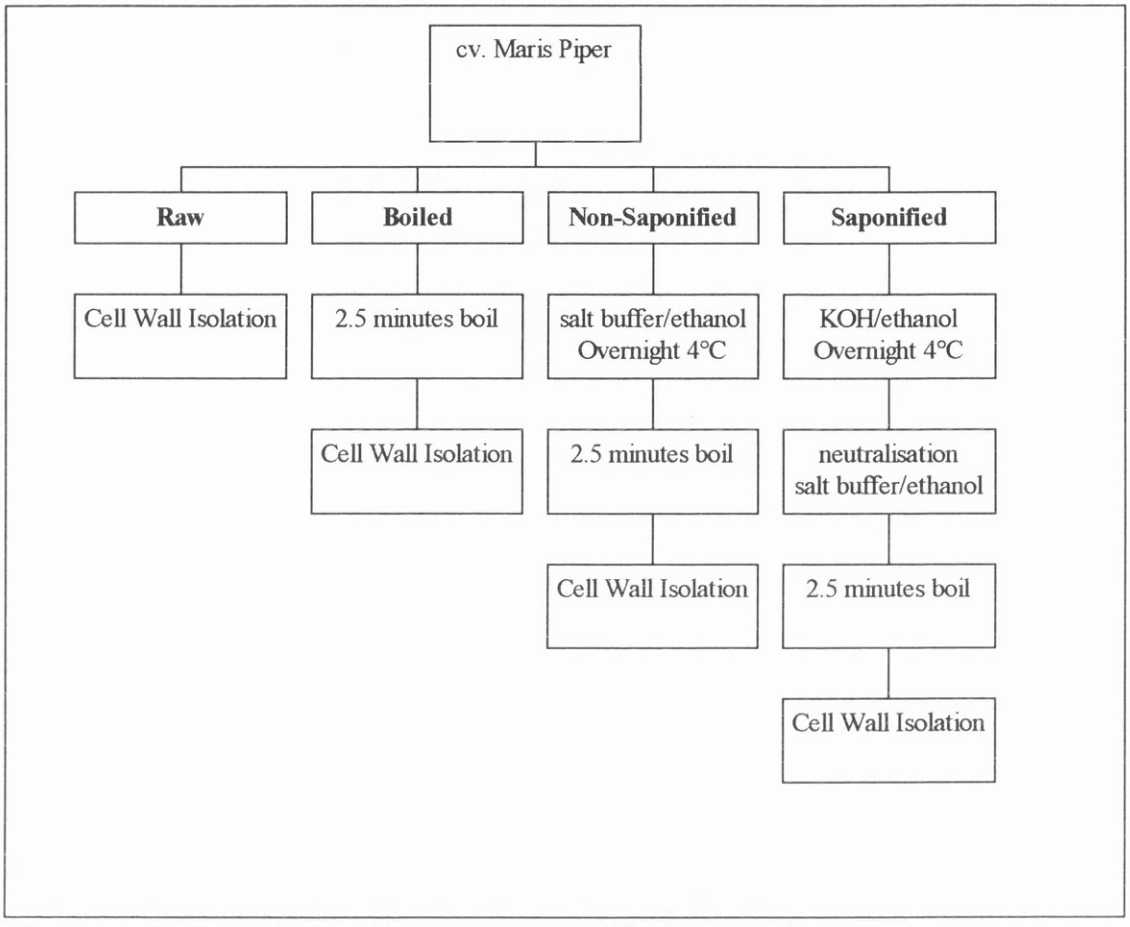
Introduction

The cooking of potato tubers makes them suitable for human consumption. The resulting texture is an important quality attribute. The composition of the cell wall as well as the starch content of the cell are often considered to be the primary attributes to texture development (Reeve, 1972; Warren et al. 1975; Jarvis et al. 1992). Previous attempts to determine the controlling reaction in texture development have proven inconclusive (chapter 3 & 4; van Marle 1997a, 1997b). Also varieties with different textural attributes have been shown to have no gross composition differences (Quinn & Schafer, 1994). Thus the aim was to chemically modify the cell wall prior to heat treatment and monitor what effects this had on the resultant cell wall structure.

β-Elimination is the cleavage of the pectic chain adjacent to methyl ester groups (Keijbets & Pilnik, 1974). This results in softening of potatoes (Ng & Waldron, 1997). If the tuber is treated with alkali this will cleave all ester groups thus removing the potential for β-eliminative cleavage.

Tubers of cv. Maris Piper were sliced to a thickness of 0.6mm. Raw, saponified and a non-saponified control slices were boiled in water.

Cell walls were isolated from the raw tubers of cv. Maris Piper as described in chapter 2. A flow chart illustrating the sample names and a brief description of the procedure used to prepare them is shown (flow chart 5.1). Tubers of cv. Maris Piper were peeled, sliced and boiled as described in chapter 2. Saponified



Flow chart 5.1 Description of sample titles and their preparation.

and non-saponified control slices were also boiled in the same way. Cell walls were isolated from the slices following the boil treatment. The starch, dry matter, galacturonic acid, methyl ester, and neutral sugars were all determined for the buffer insoluble residual cell wall. Pectin solubilised during the cooking and isolation processes were also retained and quantified. The recovered pectic material was also analysed by molecular sieve chromatography.

Results and Discussion

The tuber needed to be sliced to allow diffusion of the alkali in the saponification stage. Slices of the same thickness were used throughout the experiment to minimise the effect of heat transfer through the sample. Potassium mapping showed that diffusion of the KOH was complete.

The starch and dry matter content of cv. Maris Piper residual cell wall preparations were quantified. A conversion factor was used to correct the residual cell wall weights for starch and dry matter.

	Starch % Residue	Dry matter % Residue	Conversion factor
Raw	2.7	90.6	0.8791
Boiled	3.5	90.1	0.8658
Non-Saponified	17.5	89.9	0.7241
Saponified	28.3	90.0	0.6168

Table 5.1 Starch, dry matter and conversion factor for cv. Maris Piper.

The effect of boiling on the pectic galacturonan content of the cell wall

Pectic galacturonans remaining in the cell wall and those solubilised by the isolation procedure were measured.

Galacturonic acid	µmol/g cell wall	% cell wall	
Raw	1113	19.6	a
Boiled	1023	18.0	b
Non-Saponified	1032	18.2	b
Saponified	622	11.0	c

Table 5.2 Pectic galacturonan of isolated cell wall from cv. Maris Piper, expressed as µmol of pectic galacturonan units (176.2) per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The pectic galacturonan content in the raw isolated cell wall for cv. Maris Piper (19.6%, table 5.3) was similar to that for cv. Saturna (18.6%, chapter 3) and 17.3% (Selvendran & O'Neill, 1987), 18.8% for cv. Irene and 17.4% for cv. Nicola (van Marle, 1997a). Boiling caused a reduction in the total pectic galacturonan in the residual cell wall. The non-saponified control was assessed to remove any effects that the soaking in cold 70% ethanol may have had. There

were no differences between the pectic galacturonan content of the boiled and the non-saponified treatments. This showed that the soak in 70% ethanol had no effect on the cell wall pectic galacturonan. Saponification followed by boiling resulted in a 44% reduction in pectic galacturonan content. The saponification/ boil treatment had a greater effect than boiling alone. It is not known whether the solubilisation of the pectic material occurred as a result of saponification alone as no cell walls were isolated from a raw saponified sample.

Solubilised galacturonan (mg/ g)	Non-Saponified	Saponified
Cook water	1.1	23.3
Cell wall preparation	4.3	4.2

Table 5.3 Pectic galacturonan recovered from the cooking water, expressed as mg recovered per gram dry weight of raw tuber prior to EtOH incubation.

The cooking process solubilised more pectic galacturonan in the saponified sample than the non-saponified control.

The effect of boiling on the methyl ester content of the cell wall

As discussed earlier (chapter 1 & 3), the methyl ester content is important in providing potential sites for β-elimination and consequently cleavage of the pectic galacturonan chain. Conversely, their absence could supply a point for calcium ions to bridge adjacent pectic chains (Goldberg et al., 1996). Boiling of cv. Maris Piper slices resulted in a decreased methanol content of the cell wall (table 5.4). However, when the percent methyl esterification of the pectic galacturonan is considered there was no change between the raw and the boiled sample. This shows that the pectic galacturonan solubilised during boiling and the subsequent cell wall isolation had the same average degree of methyl esterification as that remaining in the cell wall.

The non-saponified slices showed a greater decrease (table 5.4) in the methanol content of the cell wall and of the percentage methyl esterification of the pectic

galacturonan, compared with the boiled slices. The 70% ethanol solution used as the non-saponified control also contained the salt buffer solution used during the cell wall isolation protocol -10 mM NaOAC, 3 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$ /70% EtOH. The subsequent increase in the divalent cation content of the cell wall could be responsible for increased β-elimination, causing solubilisation of a more highly methylated pectic galacturonan fraction. Another possible explanation could be enhancement of the pectin methyl esterase activity due to the increased ionic concentration. The methyl ester content of the recovered pectic galacturonan would need to have been measured to show which explanation is most likely.

MeOH	μmol/g cell wall		% pectic galacturonan	
Raw	492	a	44.2	a
Boiled	454	b	44.4	a
Non-Saponified	438	c	42.5	b
Saponified	23	d	3.6	c

Table 5.4 Methanol content of the cell wall of cv. Maris Piper, expressed as μmol of methanol per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The methyl ester content of the saponified slices was 23 μmol/g compared to 492 μmol/g for the raw slice. It can be assumed that due to complete penetration of the KOH the reduction in methyl esters was due to chemical saponification and not preferential solubilisation of a highly methylated fraction.

The effect of boiling on the neutral sugar content of the cell wall

Between the raw, boiled and non-saponified samples there were no significant differences in neutral sugar content of the cell wall (fig 5.1). Saponification followed by cooking significantly reduced both arabinose and galactose contents of the cell wall.

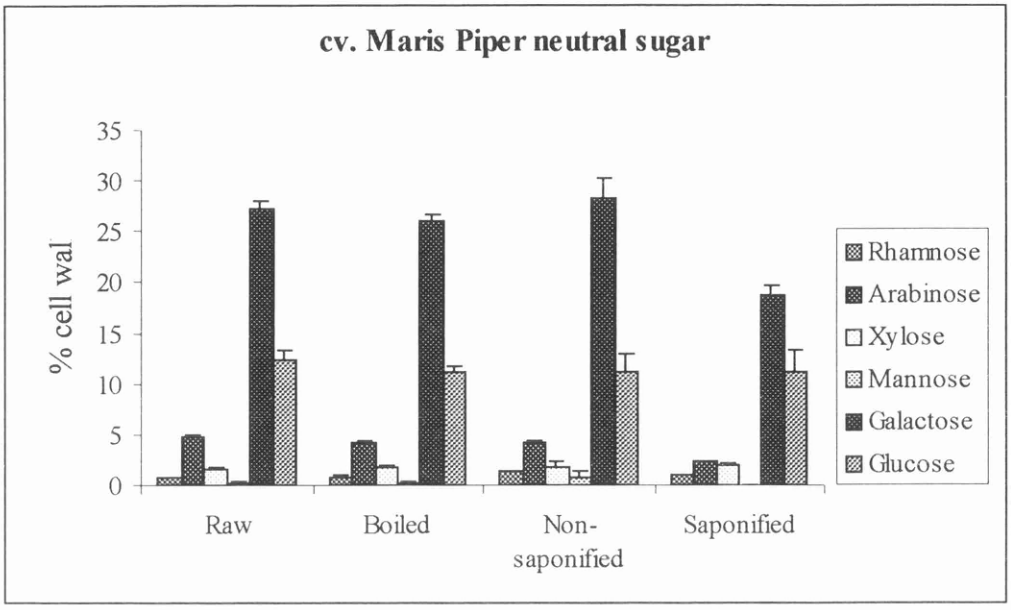


Figure 5.1 Neutral sugar analysis of isolated cell wall from cv. Maris Piper, expressed as the percentage of dry isolated cell wall. Error bars indicate the standard error.

Force N	Raw	Boiled
mean	1.5750	0.2802
s.d.	0.1181	0.0873
minimum	1.3775	0.1837
maximum	1.7347	0.4592
range	0.3571	0.2755

Table 5.5 Measurement of cutting force (N) before and after boiling.

Boiling slices of cv. Maris Piper has resulted in softer slices (table5.5). This softening on cooking is paralleled by an 8% drop in the pectic galacturonan content of the cell wall. Boiling resulted in a decrease in the methanol content of the cell wall but there was no reduction in percentage methyl esterification. There was also no reduction in the neutral sugar side chains. Boiling of cv. Maris Piper resulted in the solubilisation of linear pectic galacturonan whose methyl ester content was not significantly different from the cell walls of the raw tuber.

Saponification followed by boiling resulted in reduced methyl ester content and a greater solubilisation of pectic galacturonan, 44% compared to 8% with boiling alone. Cleavage of the pectic chains by β-elimination during the saponification stage is prevented by the low temperature (4°C) and the high pH (Kravtchenko et al., 1992). Cleavage of the pectic chains by methods other than β-elimination must have occurred.

The pectic material solubilised during the boiling of the saponified and non-saponified slices was recovered and run on a molecular sieve column.

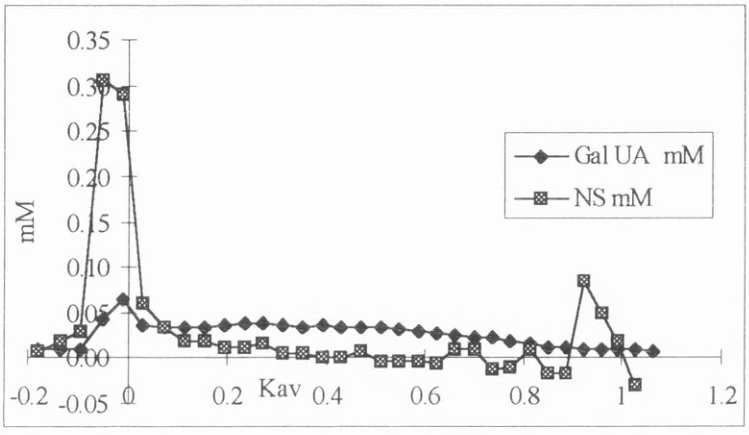


Figure 5.2 Soluble pectins recovered from cooking water of the saponified slices.

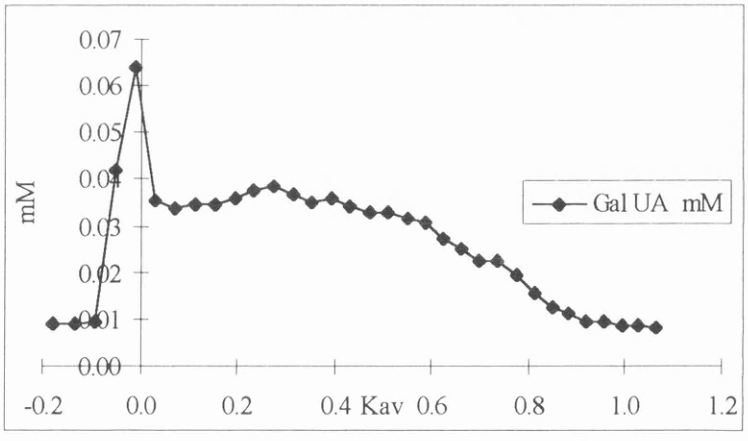


Figure 5.3 Soluble galacturonan recovered from cooking water of the saponified slices.

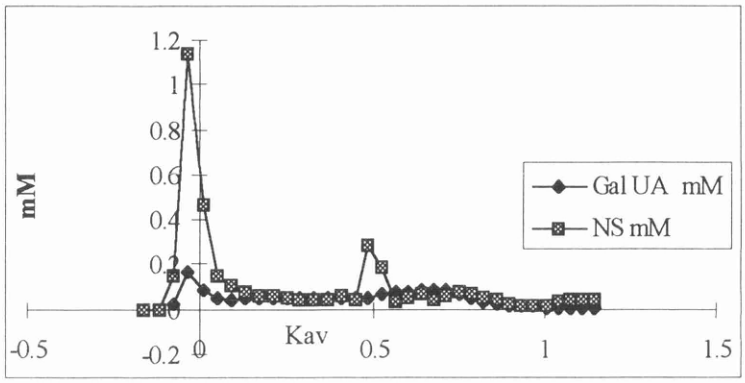


Figure 5.4 Soluble pectins recovered from the cell wall isolation salt buffer of the saponified slices.

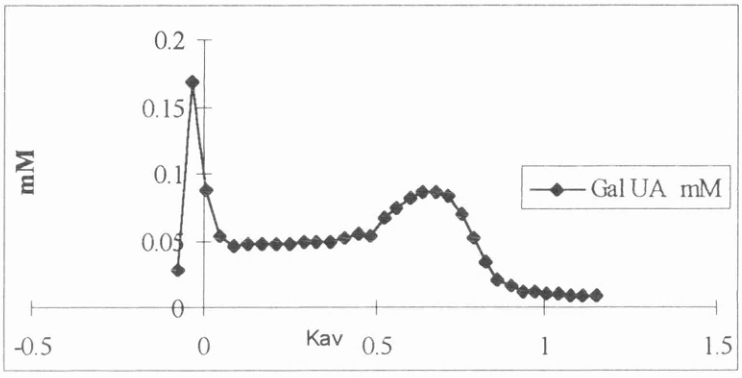


Figure 5.5 Soluble galacturonan recovered from the cell wall isolation salt buffer of the saponified slices.

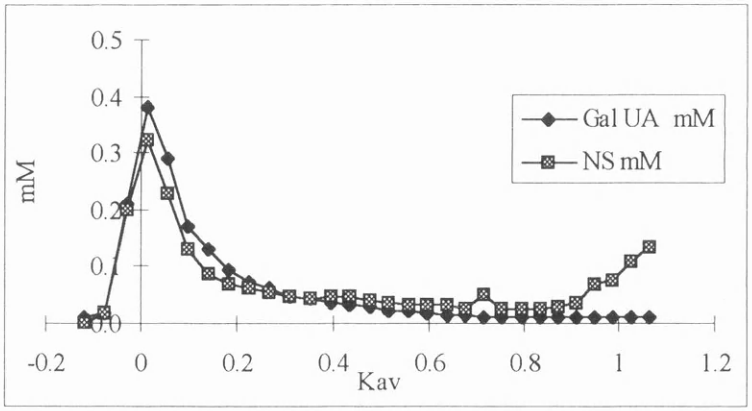


Figure 5.6 Soluble pectins recovered from the cell wall isolation salt buffer of the non-saponified slices.

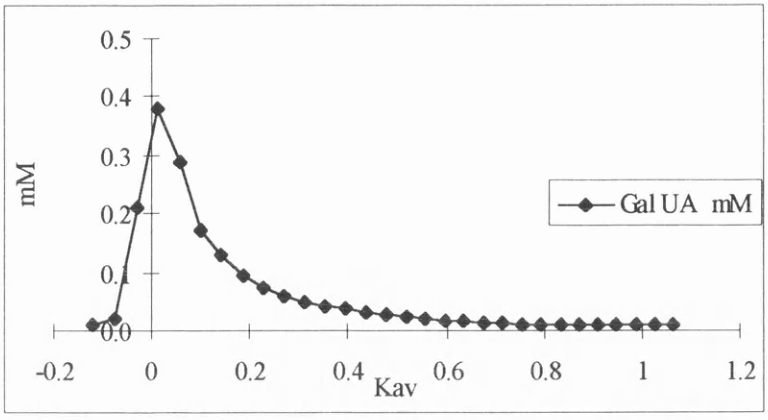


Figure 5.7 Soluble galacturonan recovered from the cell wall isolation salt buffer of the non-saponified slices.

The K_{av} values are a measure of molecular size, but no attempts were made to estimate the molecular weight of the eluted fractions. The relationship between molecular size and molecular weight of polymers depends on chain flexibility. The flexibility of the pectic chain is dependent on its structural features. A high quantity of rhamnose will lead to increased flexibility. Methylation and acetylation will also increase the flexibility (Voragen et al., 1995). Rigid molecules will have a greater apparent molecular weight than more flexible molecules.

The elution pattern of the pectic material solubilised during the cooking of the saponified slices (figure 5.2) shows that the largest pectic molecules contained the greatest proportion of neutral sugars, approximately six times as much neutral sugar as galacturonan. There was a smaller amount of neutral sugars to elute with the column volume, suggesting that some of the cleavage occurred close to branch points.

The pectic galacturonan profile was plotted alone (figure 5.3) to give a clearer picture of its elution pattern. First to be eluted would be the largest molecules. After this the profile levels off showing where molecules of intermediate size were eluted.

The pectic material solubilised during the cell wall isolation from the boiled saponified slices were also applied to the gel filtration column. The elution pattern of both the neutral sugar and the pectic galacturonan (figure 5.4) shows a similar profile to the cooking water. There was an initial peak containing the largest pectic molecules where most of the neutral sugars are located. Plotting of the galacturonan alone (figure 5.5) again shows the initial peak caused by the largest molecules excluded from the pores as well as intermediate sized galacturonan which was not as heavily substituted with neutral sugars. However, there was an additional galacturonan fraction consisting of small molecules, which were eluted just prior to the void volume.

The pectic material solubilised during the boiling of the non-saponified slices was too dilute to apply to the column. Only pectin solubilised during the initial steps of cell wall isolation were run on the column. The elution profile of the pectic material solubilised from the non-saponified slices (figure 5.6 & 5.7) was completely different from the saponified samples. The concentration of neutral sugar was approximately the same as the pectic galacturonan concentration. There may also have been some galacturonan of intermediate size solubilised.

The pectic material solubilised during the cooking and isolation of the saponified and non-saponified slices was different in structure as well as in quantity. The boiling process solubilised larger molecules whose ratio of neutral sugar to pectic galacturonan was similar. Saponification followed by boiling solubilised pectic material that was heavily substituted with neutral sugars as well as a fraction containing smaller molecules that were not heavily substituted.

Non-methyl esters

Saponification and boiling resulted in solubilisation of much greater quantities of pectic material than boiling alone (tables 5.2 & 5.3). Saponification will cleave all ester bonds within the cell wall. The cleavage of the pectic chain must have occurred by methods other than β-elimination. As cleavage of ester bonds

resulted in the solubilisation of pectic material it is possible that ester bonds other than methyl esters could be linking adjacent pectic molecules together.

Several different approaches were implemented to measure the non-esterified and total pectic galacturonan of the cell wall. Cell walls were isolated from raw tubers of cv. Marfona and cv. Fianna by Niamh O'Kennedy as described in chapter 2.

The titration of the cell wall against NaOH was carried out as described in Chapter 2. The cell wall was first acidified to ensure complete protonation of the non-esterified pectic galacturonan. The initial pH of the cell wall in NaCl was measured and the NaOH added. All solutions were maintained under nitrogen. As the NaOH reacted with the acidified carboxylate groups the pH rose. The volume required to reach the end point of pH 7 was obtained from the titration curve. To measure the total pectic content by titration the cell wall was first saponified to cleave the ester groups. The cell wall was washed with 70% ethanol to remove the excess alkali as well as the cleaved acetyl groups. The cell walls were dried in acetone and the titration carried out as before.

The copper binding experiment followed a similar principle to titration. The non-esterified galacturonan was acidified, as described in chapter 2, and copper ions were bound to it. The Cu was then removed for measurement by atomic adsorption and replaced by H^+. The cell wall was saponified then acidified and Cu bound and removed as before.

The total pectic galacturonan and methyl esters were determined spectrophotometrically using MHDP and pentanedione as described (Chapter 2).

There were no varietal differences when the individual treatment results were compared for cv. Marfona and cv. Fianna, tables 5.6 and 5.7 respectively. The measurement of the non-esterified pectic galacturonan by both titrimetry and copper binding are in agreement with each other. The total pectic galacturonan figures by titrimetry and MHDP also agree with each with each other. Both figures for total pectic galacturonan as measured by copper binding were

cv. Marfona (μmol/g)	Titration	Cu binding	MHDP	Methyl ester
Non-esterified	433 (20)	448 (22)		
Total Pectin	1062 (3)	822 (49)	1039 (44)	
Esterified Pectin	629 (21)	374		447 (10)

Table 5.6 Measurements of ester content of the cell wall of cv. Marfona. Parenthesis in brackets represents s.d..

cv. Fianna (μmol/g)	Titration	Cu binding	MHDP	Methyl ester
Non-esterified	451 (38)	457 (30)		
Total Pectin	1068 (13)	805 (21)	1045 (66)	
Esterified Pectin	616 (40)	348		456 (3)

Table 5.7 Measurements of ester content of the cell wall of cv. Fianna. Parenthesis in brackets represents s.d..

significantly lower than those measured by titrimetry and MHDP. Following the saponification step the cell walls were washed with 1N acetic acid. This would have lowered the pH of the isolated cell wall to below the pH for copper binding. Thus the lower measurement of total pectic galacturonan is due to incomplete copper saturation of the carboxylic groups on the galacturonan. Therefore the copper total galacturonan data were not used in the subsequent calculation. The first copper measurement of non-esterified galacturonan is not affected by this problem.

	cv. Marfona	cv. Fianna
Averaged esterified total galacturonan (μmol/g)	610	602
Averaged total galacturonan (μmol/g)	1050	1056
Averaged % total esterification	58	57
methyl esterification as % average galacturonan	43	43
Averaged % non-methyl esterification	15	14

Table 5.8 Calculation of non-methyl esterification.

No direct measurement of total esterified galacturonan was obtainable. Subtraction of the non-esterified from the total pectin galacturonan gives an indirect measurement of total esterification.

The measurement of the non-esterified galacturonan by titration and Cu binding was averaged (table 5.8). The measurements of total galacturonan by titration and MHDP were also averaged. A figure for total esterified galacturonan was then obtained by subtraction and expressed as a percentage of the average galacturonan. The methyl ester figure was also expressed as a percentage of the average galacturonan. The calculated total ester figure for cv. Marfona and cv. Fianna was 58% and 57% respectively compared to the methyl ester value of 43% for both varieties. Thus there was 14-15% galacturonan esterification that was unaccounted for by methyl esters alone.

These results show that non-methyl esters occurred on the pectic chain in cv. Marfona and cv. Fianna at values of 15 and 14% respectively. Saponification of the cv. Maris Piper prior to boiling would also have cleaved non-methyl esters. Cleavage of these could explain why greater pectic galacturonan solubilisation was obtained than by boiling alone. The mechanism of solubilisation is also different.

Measurement of β-elimination

The maximum extent of β-elimination during 1hour at 100°C, pH 6.8 was measured on raw cv. Saturna cell walls as described in chapter 2.

The choice of extractant or the supernatant volume made no difference to the measurement of β-elimination (table 5.9). The maximum extent of β-elimination in isolated cell walls over an hour was 1%. The cooking time of the cv. Maris Piper slices was 2.5 minutes. If it is assumed that the β-elimination is a linear reaction, then the maximum extent of galacturonan cleavage due to β-elimination would be 0.04%.

Extractant	Volume of supernatant (ml)	% β-elimination
0.01M CDTA	1	1.16
	0.1	1.05
0.1M $NaHCO_3$	1	1.00
	0.1	0.84
0.1M Na_2CO_3	1	1.18
	0.1	0.94

Table 5.9 Quantification of the rate of β-elimination.

Chapter 6

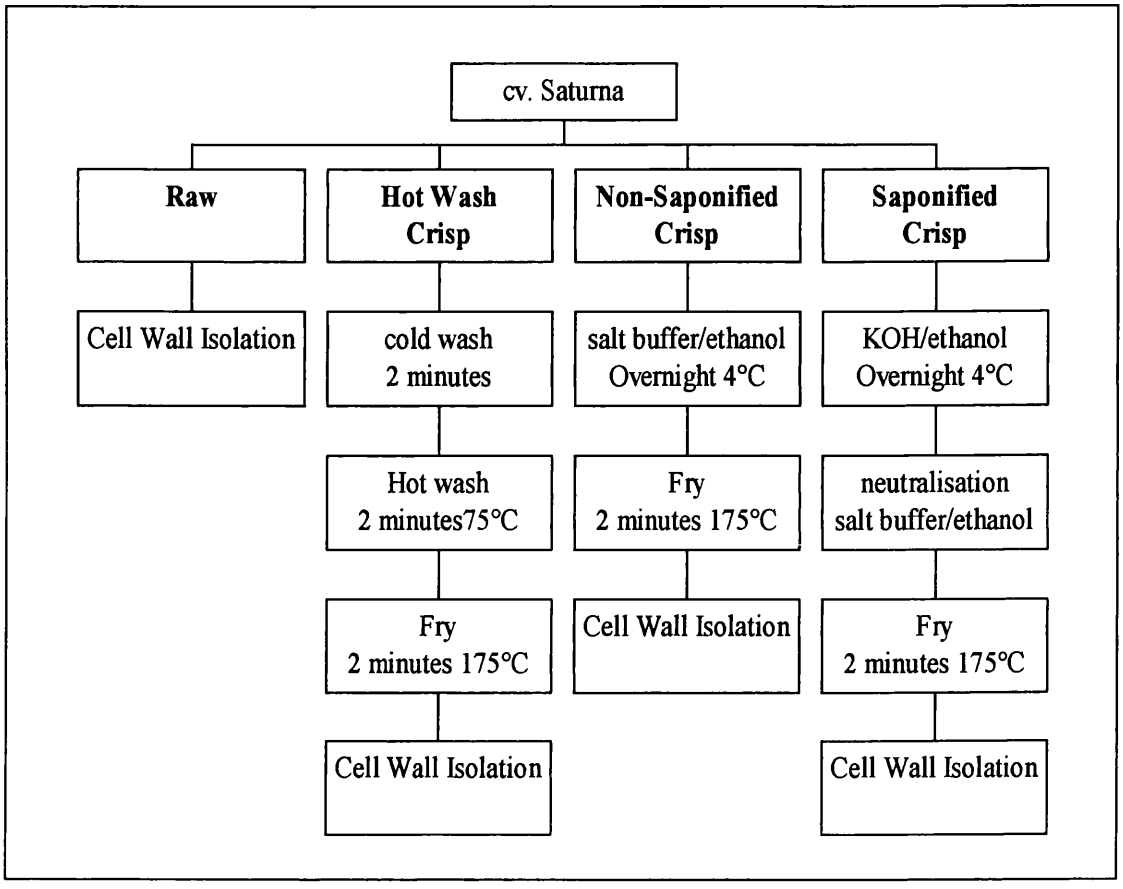
Frying of Saponified and Non-Saponified Raw Slices

Introduction

Attempts to relate textural differences in the crisps of cv. Record and cv. Saturna to the chemistry of their cell wall proved inconclusive (chapters 3 & 4). One of the main difficulties is doubt over the mechanisms that are important during the crisping process. Thus, the aim was to chemically modify the cell wall prior to processing and note what, if any, effects this had on the finished product. β-elimination is the cleavage of the pectic galacturonan chain adjacent to a methyl ester group (Keijbets & Pilnik, 1974) and is often regarded as one of the main softening mechanisms during cooking of vegetables (Sajjaanantakul et al., 1989; Ng & Waldron, 1997). Parallels between the initial stages of the crisping process and boiling have been made (Selman & Hopkins, 1989), thus β-elimination may be an important mechanism during the initial stages of crisping. Consequently, if the β-elimination reaction can be prevented, the effect this has on the resulting crisp cell wall composition can be noted. If the raw slices are treated with alkali, the ester groups will be removed, thus removing the potential for β-elimination. Slices of cv. Saturna were saponified in 70% ethanol as described in Chapter 2 prior to crisping. Saponification was carried out in 70% ethanol to prevent solubilisation of water soluble pectic material. Non-saponified control slices were treated in a similar way.

Cell walls were isolated from the saponified and non-saponified crisps as described in Chapter 2. A flow chart illustrating the sample names and a brief description of the procedure used to prepare them is shown (flow chart 6.1). The starch, dry matter, galacturonic acid, methyl ester, and neutral sugars were all determined for the buffer insoluble residual cell wall. Pectin solubilised during the isolation process were also retained and quantified. A taste panel at

Strathclyde University determined textural differences between the saponified and non-saponified crisps (chapter 7).



Flow chart 6.1 Description of sample titles and their preparation

Results and Discussion

The starch and dry matter content of cv. Saturna residual cell wall preparations were quantified. A conversion factor was used to correct the residual cell wall weights for starch and dry matter.

	Starch % Residue	Dry matter % Residue	Conversion factor
Non-saponified Crisp	9.7	99.2	0.895
Saponified Crisp	12.2	98.9	0.867

Table 6.1 Starch, dry matter and conversion factor for cv. Saturna Non-saponified and Saponified crisps.

The effect of crisping on the pectic galacturonan content on the treated cell wall

Galacturonic acid	μmol/g		% cell wall	
Raw	947	a	16.7	a
Hot wash crisp	592	b	10.4	b
Non-saponified Crisp	597	b	10.5	b
Saponified Crisp	868	c	15.3	c

Table 6.2 Pectic galacturonan of isolated cell wall from cv. Saturna, expressed as μmol and percentage of pectic galacturonan units (FW 176.2) per gram of dry isolated cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The results obtained from the cv. Saturna raw and hot wash crisp results are included for reference and are also presented in chapter 4, tables 4.3 and 4.4.

The non-saponified control slices produced crisps with the same pectic galacturonan content as the hot wash crisps, 597μmol and 592μmol (table 6.2) respectively, despite being produced in a batch frier as opposed to a continuous frier. Also the samples were prepared on different days from different batches. The saponified crisps had a greater pectic galacturonan content than the non-saponified crisps. In addition they had the greatest pectic galacturonan content when compared with all the other crisps analysed. Sodium hydroxide is used as a pectic extractant, in an aqueous environment, as part of sequential extraction during some cell wall isolation protocols (Selvendran & O'Neill, 1987). However, in this instance there was chemical modification without extraction.

As shown previously, greatest solubility of pectic galacturonan was shown in the first washings collected (table 6.3). There was greater solubility with the non-saponified crisps. There is a greater concentration of solubilised galacturonan in this experiment than previously shown. A smaller starting weight of crisps (~5g) were used, mainly due to the difficulty in crisp production. As a consequence of the smaller sample size washing was more thorough and more of the galacturonan was removed in the first washings

Solubilised Gal UA (mg/g)	Non-saponified Crisp	Saponified Crisp
1st washings	15.08	11.24
2nd washings	6.54	6.31
Enzyme incubation	8.42	3.72

Table 6.3 Recovered pectic galacturonan of cv. Saturna solubilised during cell wall isolation. Total galacturonate (mg) recovered from the cell wall isolation washings from a dry weight (g) of the sample.

The effect of crisping on the methyl ester content on the treated cell wall

MeOH	µmol/g		% galacturonan	
Raw	432	a	46	a
Hot wash crisp	163	b	31	b
Non-saponified Crisp	192	b	32	b
Saponified Crisp	17	c	2	c

Table 6.4 Methanol content of the cell wall of cv. Saturna, expressed as; µmol of methanol per gram of dry isolated cell wall and percentage methyl esterification of the pectic galacturonan of cv. Saturna. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The methyl ester content of the non-saponified cell wall fell on crisping (table 6.4). The percentage methyl esterification of the galacturonan also fell. No non-saponified sample was collected following the hot wash treatment, therefore no direct measurement of methyl ester content is possible. However, it can be assumed that the crisping process caused preferential solubilisation of a highly methylated galacturonan fraction, as has been shown with cv. Saturna hot wash crisp (chapter 3 and 4).

Saponification has not resulted in complete removal of the methyl esters, 17µmol remaining compared to 432µmol in the raw tuber; however, most of the ester groups have been removed with only 4% of the methyl ester groups remaining.

As discussed previously the reduction in methyl esters is due to the diffusion of the KOH through the raw slices resulting in cleavage of the ester bonds and not preferential solubilisation of a highly methylated fraction.

The effect of crisping on the neutral sugar content on the treated cell wall

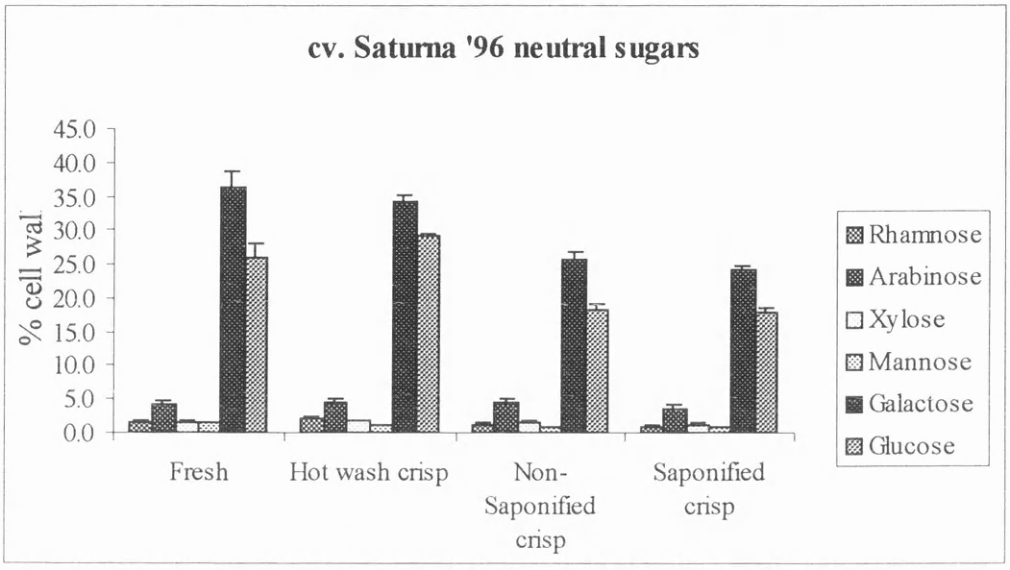


Figure 6.1 Neutral sugar analysis of isolated cell wall from cv. Saturna, expressed as the percentage of dry isolated cell wall. Error bars indicate the standard error.

The rhamnose, arabinose, xylose and mannose content of the cell wall was not changed by the crisping process (figure 6.1) nor by the saponification followed by crisping. The galactose content of the non-saponified crisp cell wall (26%) was lower than the galactose content of both the raw and hot wash crisp cell wall - 36 and 34% respectively. This drop could be due to a batch effect or possibly the different method of frying.

Saponification followed by crisping further reduced the galactose content to 22%. Saponification and boiling also resulted in reduced galactose content with cv. Maris Piper (chapter 5).

cv. Saturna mg/g dm	Hot wash crisp		Non-Saponified crisp		Saponified crisp	
	1st wash	Enzyme wash	1st wash	Enzyme wash	1st wash	Enzyme wash
Rhamnose	0.25	0.06	0.19	0.11	0.13	0.10
Arabinose	0.32	0.12	1.24	0.37	1.03	0.32
Xylose	0.57	0.16	0.50	0.39	0.40	0.30
Mannose	0.24	0.02	0.27	-	-	0.02
Galactose	2.80	0.42	2.35	1.07	3.11	1.05
Pectic galacturonan	6.7	0.6	2.8	1.6	2.1	0.7

Table 6.5 Characterisation of the pectic galacturonan solubilised during the isolation process.

Saturna	1st wash	Enzyme wash	Cell wall residue
Raw	-	-	2.0
Hot wash crisp	0.42	0.72	3.4
Non-Saponified crisp	0.8	0.68	2.45
Saponified crisp	1.50	1.54	1.44

Table 6.6 Ratio Galactose:Galacturonan.

Analysis of the galactose content of the pectin solubilised during the cell wall isolation of the non-saponified crisps showed that proportionately a larger quantity of galactose was solubilised compared to the hot wash crisps (table 6.6). Nevertheless, the pectic galacturonan remaining in the cell wall of the non-saponified crisps still had a higher galactose/galacturonan ratio than the raw cell wall showing that preferential solubilisation of homogalacturonan had occurred.

The pectin solubilised during the isolation of the saponified crisps had the highest ratio of galactose to galacturonan. The resultant ratio of galactose to

galacturonan in the isolated cell wall showed that the galactose regions had been preferentially solubilised when compared to the raw cell wall. This is the converse of the trends found with the crisping process alone.

Saponification of cv. Saturna reduced the loss in pectic material resulting from the crisping process. Saponification also resulted in a 96% reduction in the methyl ester content of the cell wall. The galacturonan solubilised as result of the saponification/fry process had a high proportion of galactose. Thus saponification prior to crisping minimised the changes to the cell wall and induced preferential solubilisation of the galacturonan containing galactose side chains.

Chapter 7

Sensory Evaluation of Crisps

Introduction

Previous chapters have focused on monitoring the changes in the chemistry of the pectic material in the plant cell wall during the crisping process. It has been shown (chapter 3 and 4) that crisping followed by cell wall isolation resulted in preferential solubilisation of a predominantly linear pectic galacturonan. Pectic substances may play an important role in controlling the degree of cell separation and consequently the morphology of the crisp (Selman & Hopkins, 1989). The morphology will determine the resulting texture of the crisp (Khan & Vincent, 1996). It was suggested that cleavage of the linear methylated fraction was responsible for cell separation (chapter 3). The aim of this chapter was to bridge the gap between the degree of cell separation and the texture of the resulting crisp. This was achieved by directly measuring the texture of the crisp following different pretreatments, which would alter the behaviour of the pectin.

Results and Discussion

The taste panel was arranged at Strathclyde University, Food Sciences Department and run with the aid of John Piggott. The following descriptors were used to describe the crisp texture: hardness, crispness, crunchiness, particle angularity, adhesiveness, greasiness/oiliness and the rate of breakdown. However, analysis of variance on the principal components showed that the only significant differences were with principal component 1. Hardness, crispness, crunchiness and the particle angularity were all correlated together within principal component 1, while adhesiveness, greasiness/oiliness and the rate of breakdown were all correlated together at the opposite end of the scale. In practice there was only one measurable distinction; either the crisps were

determined to be crisp, crunchy and hard or they were less crisp and broke down easier.

Saponified Crisps

The saponified and non-saponified cv. Saturna crisps analysed in chapter 6 were included in the taste panel with control hot wash crisps which were also batch fried but did not undergo the 70% ethanol treatment.

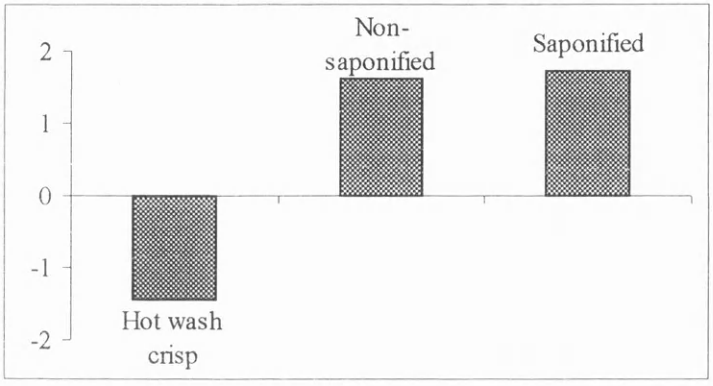


Figure 7.1 Score of principal component no. 1 for the batch fried crisps.

There were no measurable textural differences between the saponified and non-saponified crisps (figure 7.1) despite having a significantly different cell wall composition. During the saponification process the raw strips were immersed in 70% ethanol. As a consequence of this the slices were partially dehydrated prior to the crisping pretreatments. The dehydration effect of the ethanol may have had an irreversible impact, which was the dominant factor in determining the resultant crisp texture.

Calcium pretreatments

The taste panel trial was also carried out on cv. Saturna hot wash crisps, which had undergone different hot wash pretreatments. Three different calcium concentrations were chosen; low calcium (0.15g/l), which is the same concentration used in the cell wall isolation buffer; medium calcium (1g/l) and high calcium (5g/l). The aim was to monitor any effects of added calcium.

Calcium has been shown to have a firming effect on processed potatoes (van Buren, 1970; Ng & Waldron, 1997)

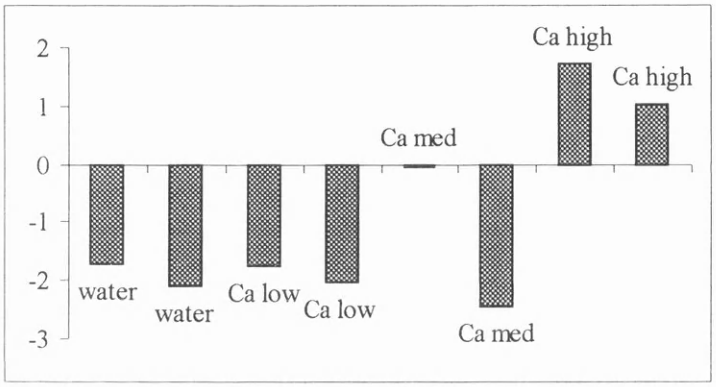


Figure 7.2 Score of principal component no. 1 for crisps following a calcium hot wash pre-treatment.

The principal component no. 1 scores for the calcium hot wash treatments were compared with a control hot wash (figure 7.2). There were no differences between the low calcium and the hot wash in water. The high calcium concentration crisps were crisper than the control, low and medium calcium crisps. The duplicate results from the middle calcium treatment were inconsistent with each other. Duplicate analyses were carried out on six of the treatments, and agreement was obtained between every other duplicated sample. Therefore it is unlikely that the difference between the medium calcium is due to the panel. Following crisping the crisps are sealed in foil bags until analysis, the bags contained approximately 500g. If the foil bag had become damaged during storage or transit, this would result in a softer product. The results from analysis of the medium calcium are consistent with this theory. However, all packaging had been discarded before the results were calculated, so this theory cannot be proven. However, if the doubtful medium calcium results are ignored, then the remaining medium results are significantly crisper than the low calcium while not as crisp as the high calcium.

When the calcium concentration in the hot wash was greater than 10 times the level found in the cell wall, the resultant crisps were crisper. The calcium acts a bridge between two carboxylic groups on the galacturonan chain (Keijbets et al.,

1976). Calcium bridging results in the formation of junction zones between non-methylated galacturonan chains (Jarvis, 1984). The concentration of native calcium ions is insufficient to satisfy all the carboxylic groups (Moledina et al., 1981). The greater the number of calcium ions then the potential for the formation of bridges is greater. At low calcium concentrations single chains aggregate to form galacturonan dimers, while higher calcium concentrations results in aggregation of the dimers to form interjunction segments built up from four or more chains (Goldberg et al., 1996). The greater the number of bridges then the stronger the association between the pectic chains within the cell wall. The increased strength of the cell wall has resulted in crisper crisps. This increased strength will lessen the degree of cell separation, due to a stronger attachment between cells. Firming mechanisms employed in the raw sample result in firmer, crisper crisps.

Cold and Hot wash Crisps

Taste panel studies at the United Biscuits research and development laboratories, High Wycombe had previously found textural differences between cold and hot wash crisp. However, in this instance, no differences were found between cold and hot wash cv. Saturna (figure 7.3).

Sucrose hot wash pretreatment

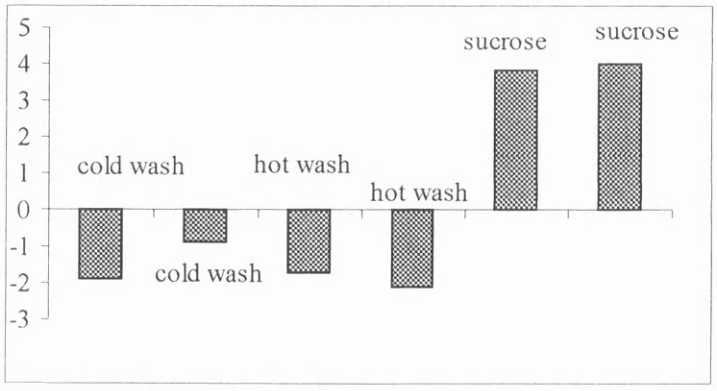


Figure 7.3 Score of principal component no. 1 from cold, hot and sucrose wash pre-treatments.

Raw slices of cv. Saturna underwent the hot wash process in a sucrose solution (2M). Following the hot wash treatment the slices were examined by polarised light under the light microscope. The Maltese cross, indicative of non-gelatinised starch grains, was visible. Thus the slices had undergone the 2 minute hot wash process at 75°C without gelatinisation of the starch grains. Gelatinisation of starch is regarded as a mechanism contributing to cell separation (Jarvis et al., 1992). The gelatinised starch will exert a swelling pressure from inside the cell. The swelling pressure aids cell separation by increasing the pressure on the middle lamellae pectins in the tricellular junctions (Jarvis, in press). If heating degraded the pectic galacturonan, this coupled with the gelatinisation pressure, would result in a softer product (Jarvis, et al. 1992). Softening to the same extent will not occur if the starch is not gelatinised as the swelling pressure will be less.

The taste panel found the sucrose treated crisps to be crisper than all the other crisps analysed (figure 7.3). The sucrose reduced the softening effect of the gelatinised starch. The sucrose may also have acted in other ways. Rubnov & Saguy (1997) observed that incorporation of fructose into a reconstituted potato mix prior to frying resulted in crisper crisps. The fructose formed a crust on the surface of the crisp that reduced the oil uptake and resulted in crisper crisps. As a consequence of the high sucrose content the pectin could have formed a gel structure similar to that used in the making of jam. The high sugar concentration creates low water activity conditions resulting in chain-chain associations instead of chain solvent associations (Voragen, 1995). This would also result in increased pectic strength, rendering it more resistant to the crisping process. Thus, while the mechanism for the increased crispness is unclear, the sucrose pretreatment did confer strength to the resulting crisp.

The pretreatments, which have reduced heat induced softening, have conferred strength to the resultant crisp.

Chapter 8

General Discussion and Conclusions

Effects of conventional frying on cell wall structure

Characterisation of the raw cell wall of cv. Record and cv. Saturna from the 1995 harvest (chapter 3) showed no significant compositional differences in pectic substances. When the cell wall in the raw tuber was compared with the cell wall in the crisp, the pectin solubilised during the cell wall isolation, as a consequence of frying, was a linear methylated galacturonan. The type of pectin solubilised and pectic bonds cleaved are indicative of the fraction of pectin that is important in crisp processing.

The following year's harvest agreed with these findings (chapter 4). Neutral sugar analysis of the fraction solubilised during cell wall isolation confirmed that the bonds anchoring homogalacturonan in the cell wall had been broken during the crisping process. Additionally, characterisation of the isolated cell wall confirmed that the methylated homogalacturonan was the fraction solubilised. The short recycle single pulse NMR spectra of cv. Saturna cell walls showed the mobility of the galactan side chains. The hot wash crisp galactans had a greater mobility than those of the raw cell wall. This was found once the homogalacturonan had been solubilised.

Effects of saponification on cell wall breakdown during processing

Crisping

Saponification followed by the crisping processes on cv. Saturna (chapter 6) reduced the quantity of galacturonan solubilised resulting from the crisping process. Less galacturonan was solubilised during the cell wall isolation from the

saponified crisps (8%), than from the non-saponified crisps (37%). Despite the saponified crisp cell wall having a greater galacturonan content its percentage galactose was less. Saponification followed by crisping resulted in solubilisation of a heavily branched fraction of rhamnogalacturonan from the cell wall.

Boiling

Saponification followed by boiling of cv. Maris Piper resulted in solubilisation of greater quantities of galacturonan than boiling alone (chapter 5). Boiling alone solubilised 8% of galacturonan found in the raw cell wall, which had an equal ratio of neutral sugar to galacturonan. The isolated cell wall showed no significant drop in percentage methyl esterification. Saponification followed by boiling solubilised 44% of the cell wall galacturonan, which was heavily branched.

Evidence for intermolecular pectic esters in potatoes

Non-methyl esterification was shown to exist in cv. Marfona and cv. Fianna at values of 15 and 14% of the galacturonan content (chapter 5). Non-methyl esters could perform a structural role by bridging adjacent pectic chains together. Saponification would result in cleavage of all ester linkages.

Non-methyl esters have also been found in pea sprouts at a third of the methyl ester value by Hou & Chang (1996). They also found that on precooking the non-methyl ester levels increased, while the methyl ester figures fell. They suggested that in addition to the removal of methyl esters, PME activity resulted in the formation of new non-methyl linkages.

Gel filtration indicated that the galacturonan fractions solubilised following the saponification/boil treatments were smaller in size than the fraction solubilised during the isolation of the non-saponified/boiled cell walls. This solubilisation could not have occurred by β-elimination, because the methyl ester groups had

previously been removed. Solubilisation of the galacturonan must have occurred by other means.

Using the evidence for non-methyl esters and the gel filtration results a model for the structural arrangement of the pectin in the cell wall is proposed (fig 8.1). The model is based in the cable model proposed by Goldberg et al. (1996) for pectin gels.

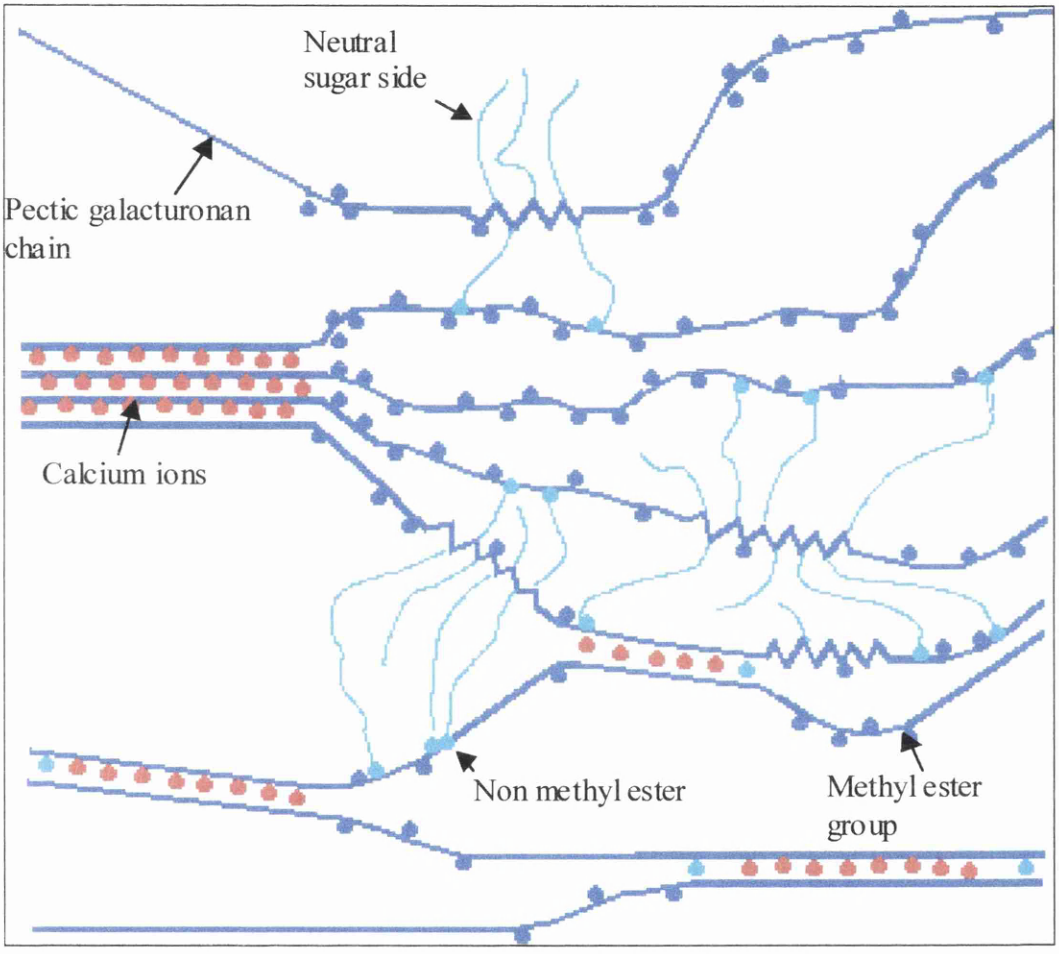


Figure 8.1 Structural arrangement of pectin in the cell wall

Neutral sugar analysis and the gel filtration data showed that saponification resulted in solubilisation of pectic material that was heavily substituted with neutral sugar side chains. It follows that cleavage of ester groups resulted in solubilisation of the RGI fraction. This suggests that ester linkages occur within the RGI fraction or in a galacturonan segment close to an RGI fraction. Methyl esters are unable to act as links between adjacent chains. This structural role could be performed by the non-methyl esters found. The galacturonan contained

15% non-methyl esters; while only 8% rhamnose. Thus, other sites of esterification within the RGI fraction may exist. The neutral sugar side chains themselves could act as sites for esterification. The side chains could be attached onto the galacturonan chain via an ester linkage from the galacturonan chain. The gel filtration patterns also support the proposal that ester linkages exist within the RGI region.

Analysis of the gel filtration graphs of the saponified sample shows that cleavage of the ester groups resulted in a reduction in chain length (chapter 5). The fraction eluted between the void and the column volume was not heavily substituted with neutral sugars. This could result from cleavage of ester bonds between galacturonan and rhamnose or a neutral sugar chain. Additionally it may be indicative of non-methyl ester links between adjacent homogalacturonan chains. Hou & Chang (1996) also suggested the occurrence of non-methyl ester intermolecular linkages.

Mechanisms of pectic network degradation during boiling

Shearing raw tissue gives rupture of the cell wall, while cooked potatoes showed rupture of the middle lamella (Sapers, 1997). Observations made in the microscopy department at United Biscuits have shown that cell separation also occurs during the crisping process. The middle lamella holds adjacent cells together and transmits the stress applied to the tuber (Jackman & Stanley, 1995). Cells will separate when it is absent. Initiation of any cell separation originates in the tricellular junction (Jarvis, in press). The cell separation forces are spread over the reinforcing zone (Jarvis, in press), therefore the structure in the immediate area will play a crucial role in the textural development. Thus the middle lamella pectins act as load bearers. Treatments that confer stability and strength on the middle lamella pectins will consequently resist the effects of processing. This applies to boiling, steaming and the crisping processes.

Following boiling, cell separation has a direct influence over the perceived texture in the mouth. The cooking time (2.5 minutes) used in the boiling

experiment was determined organoleptically. The maximum extent of β-elimination in an isolated cell wall at pH 6.8, 100°C was 1% of the galacturosyl linkages over an hour period (chapter 5). The galacturonan solubilised as a result of the boiling experiment was not preferentially methyl esterified, which would have occurred had β-elimination been an important mechanism. It would therefore seem doubtful that, over the time scale used in the boiling experiment, β-elimination was an important softening mechanism.

β-elimination has long been established as a mechanism for cleavage of methylated galacturonan chains in isolation (Neukom & Deuel, 1958; Keijbets & Pilnik, 1974). However, its role as one of dominant mechanisms for softening during the processing of vegetables which is often presumed (Ng &Waldron, 1997) needs to be re-examined.

- The rate of cleavage of galacturonan chains, due to β-elimination after an hour was 1%.
- Use of the Jim 7 antibody indicated the location of the methylesterified pectin deeper in the cell wall (Huxham et al. 1997, figure 8.2); whereas, cell separation occurs at the middle lamella.
- The role of non-methyl esters in β-elimination and the architecture of the cell wall needs to be considered.

Non-methyl ester cross links may prevent solubilisation of cleaved galacturonan chains. Cleavage due to β-elimination occurs next to esterified groups as the electron withdrawing ester group makes the hydrogen on the C5 position sufficiently acid for approach of the hydroxyl ion (Neukom and Deuel, 1958). Thus, a non-methyl ester may also be an additional site for β-elimination. The increased mobility of the galactose side chains following crisping could be due to β-elimination of the neutral sugar- galacturonan linkages.

It has been suggested that β-elimination is not the dominant softening mechanism on cooking (Loh & Breene, 1982; Qi, 1997). The rate constant of

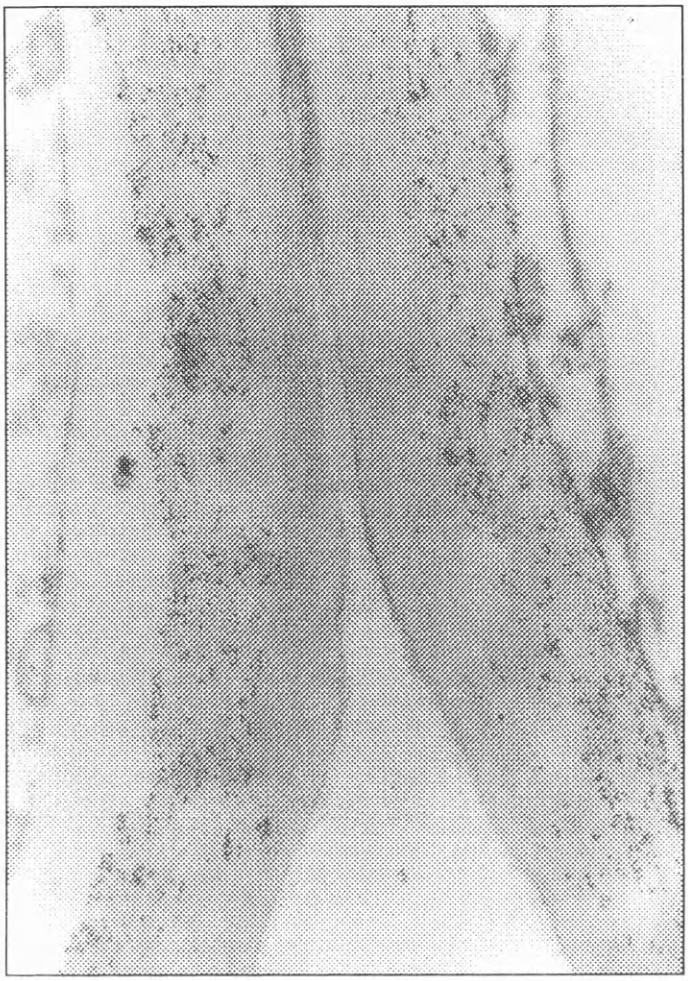


Figure 8.2 JIM 7 antibody labelling of raw potato. From Huxham et al. (1997)

hydrolysis of neutral polysaccharides is proportional to the acid concentration (Capon & Ghosh, 1965, Smidsrod et al., 1966). However, the dissociation curve for pectin did not follow this pattern. The rate of hydrolysis at pH 4 was shown to be similar to the rate of hydrolysis at pH 1(Capon & Ghosh, 1965, Smidsrod et al., 1966). This higher rate of hydrolysis was due to dissociation of the carboxyl group; protonated carboxyl groups are acid stable while dissociated carboxyl groups are acid labile (Smidsrod et al. 1966). Capon & Ghosh (1965) showed that at low pH (pH <1) the neutral glucoside reacts 45 times faster than the glucuronide; however, at pH 4.8 hydrolysis of the glucuronide occurred 35 times faster than the glucoside. At higher pH values, polyuronides are hydrolysed at much higher rates than neutral polysaccharides (Capon & Ghosh, 1965, Smidsrod et al., 1966). Constituents of RG II have also been shown to be acid labile (O'Neill et al. 1990).

Acid hydrolysis could potentially have a role in cleavage of the pectic chains, provided the local pH environment was suitable (Moustacas et al., 1991) and the carboxyl groups were dissociated. Acidification of raw potato cell walls followed by cooking at 50-75°C in Tris-Pipes buffer (pH 6.1) with a 20 minute cooling step at 25°C, showed greater pectic solubilisation (39%) than neutralised ones (2%). When the tissue was exposed to PME, which increased the available sites for acid hydrolysis, the figure rose to 86% for the acidified sample and 8% for the neutral pectin (Moledina et al., 1981). Green beans that were de-esterified and subsequently neutralised showed a 75% loss of firmness when cooked under acidic conditions. Potato tubers followed the same pattern (van Buren & Pitifer, 1992). Canned green beans and carrots both showed softening in the cooked product when they were cooked below pH 4.5, this effect was attributed to acid hydrolysis (Stanley et al., 1995). The saponified sample could not soften by β-elimination; however, acid hydrolysis is a possible mechanism due to acidified carboxyl groups resulting from saponification.

Nevertheless, Krall & McFeeters (1998) stated that acid hydrolysis will only be the dominant over β-elimination below pH 4. This pH figure is variable depending on the methyl ester figure. The higher the methyl ester figure the lower the pH where acid hydrolysis is dominant. Obviously there is a continued need for investigation into the dominant mechanisms of pectic cleavage.

In instances where cell separation is the main softening mechanism, attention should be focused on the middle lamella. During cooking the middle lamella is the point of weakness with the unzipping of the middle lamella starting at tricellular junctions (Jarvis, in press). Boiling will result in leaching of salts into the cooking water; nevertheless, the salts must first pass through the cell wall. Boiling results in full starch swelling pressures in addition to solubilisation of pectin (8%). Release of citrate, malate and possibly phytate due to starch gelatinisation would chelate calcium from acidified pectins (Keijbets et al., 1976). This chelation of calcium would have the greatest effect in the middle lamella where acidified homogalacturonan and calcium are concentrated (Goldberg et al. 1996). Thus, chelation of calcium would result in the weakening

of the attachment between adjacent cells. This chelation coupled with the starch gelatinisation pressure is the dominant softening mechanism over the time period studied.

Boiling of the saponified slices resulted in solubilisation of much larger amounts of galacturonan (44%). Saponification would have cleaved non-methyl ester linkages between the pectic chains. Chains anchored in the cell wall by ester linkages would be dependent on this divalent bridging to maintain them in the cell wall. During boiling, the divalent cations would have been chelated by citrate, malate and possibly phytate thus they became solubilised. Acid hydrolysis may also have played a part in the cleavage of the pectic chains. The galacturonan chains were dominated by acidified carboxyl groups, thus increasing the opportunity for acid hydrolysis. Acid hydrolysis may also partly explain the shorter galacturonan chain lengths found following the saponification/boil treatments.

Texture of crisps in relation to cell separation and pectin breakdown

The texture of any object will be dependent on the contributions made by the individual components and by the way in which these components are attached together. The texture of the crisp will be determined by the architecture of the crisp. A crisp with a small number of large voids with thicker void walls will be crisper than a crisp with a large number of small voids (Khan & Vincent, 1996). The architecture of the crisp will be determined by the changes of the potato slice during the frying process. The distribution of the voids - channels- and their occurrence will be governed by the behaviour of the cell wall during frying. If the cells separate during the initial frying stage, then the void channels for the escape of the venting steam will form between the cells. If the cells rupture then the channels will form in a different direction. The strength of the pectic network in the cell wall and middle lamella and the extent to which they can withstand the forces applied to them will play a crucial role in the development of the morphology and the resulting crisp texture. Their strength will be dependent on

the degree to which they are degraded during the cooking processes, which is dependent on their original structure.

The cooking processes involved in crisping and boiling are very different. Despite this, the behaviour of the pectin and its role in cell separation will influence the textural development of the crisp. Cell separation influences the structure of the crisp by directing the formation of voids. Crisping involves boiling and dehydration, which results in an increased salt content as the dehydration proceeds, followed by a rapid rise in temperature equal to the oil temperature (175°C). The occurrence of β-elimination is amplified in the presence of increased salt concentration (Keijbets et al., 1976) as well as temperature (Kravtchenko et al. 1992), both of which are experienced by a slice during frying. There are no data concerning β-elimination at 175°C. However, as β-elimination requires water the reaction rate will decrease as the slice dehydrates.

Firming mechanisms employed in boiling potatoes also result in crisper crisps (chapter 7). The calcium hot-wash pretreatment resulted in crisper crisps. This suggests that the carboxyl groups on the galacturonan C-6 play a role in the texture development of the crisp. Characterisation of the cell wall of the crisp (chapter 3 & 4) showed that the galacturonan solubilised as a result of crisping is homogalacturonan. The formation of calcium bridges between chains of homogalacturonan would have prevented their solubilisation (Goldberg et al. 1996). The firming mechanism of the sucrose pretreatment is less clear. The firmness could have been achieved by preventing starch gelatinisation and consequently starch swelling pressure. However, the formation of pectic gels similar to those in jam making is another possible firming mechanism. The high sugar concentration used in the production of jams results in low water activity conditions, which in turn produce chain-chain associations instead of chain solvent associations (Voragen et al., 1995). These associations would confer strength to the pectic substances thus making them more resistant to the crisping process.

It has been discussed that the crisping process solubilised large quantities (37%) of methylated homogalacturonan. It was not possible to relate structural differences in the raw cell wall of cv. Record and cv. Saturna to the textural attributes of the resulting crisp. However, it can be postulated that cleavage of the methylated chains within the cell wall due to β-elimination has played a role in the crisping process. The middle lamellae pectins have also been solubilised, but this may be a consequence of cleavage of the chains to which they were bonded. The addition of calcium to the hot wash process has conferred strength on all regions of the cell wall resulting in crisper crisps.

Pectin solubilisation was reduced in the saponified crisps. During the neutralisation stage, prior to frying, the raw slices were immersed in the salt buffer solution. The cations present in the salt buffer could act as bridges between the dissociated carboxyl groups. Following neutralisation the slices were crisped. Thus, pectin solubilisation was minimised due to prevention of β-elimination as well as the strengthening effects of the cations from the salt buffer.

There is a clear role for pectins in controlling the texture of the crisp; however, varietal differences can not yet be explained by differences in the raw cell wall.

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