The Molecular Basis of Texture in Mashed Potato

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Dedication

To my Mum and Dad, thank you for everything. I could not have wished for more wonderful parents. Without your encouragement, love and understanding I could never have accomplished all I have done. Please accept this work as a small token of my gratitude.

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I must first thank my supervisor Dr Mike Jarvis. His knowledge, patience, encouragement and humour have helped me through this from the start. Thanks must also be given to Dr Harry Duncan for his insight on life, football and science, which he has provided in equal measure.

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Without my family I would never have got close to doing a PhD thank you for everything. Thank you Gill for your patience and support, if we can get through a thesis write-up I think we will get through most things. Despite all their jokes at the expense of the mighty *Solanum tuberosum*, I'd like to thank my friends that inhabit the 'real' world for putting up with me for three and a half years 'as I did stuff with potatoes'. I apologise to those who still bear a grudge about the lack of smash in the thesis.

SUMMARY

Mash production often involves thermal pre-treatments (pre-cooks) that are designed to increase the physical strength and restrict cell separation of cooked potatoes prior to mashing. This improves the mash quality. During pre-cooking, the control of starch swelling pressures during cooking and/or the activation of pectin methylesterase (PME) prior to cooking can occur.

The aim of this thesis was to examine changes in texture of cooked potatoes caused by these pre-cook treatments prior to mashing. Cooking was carried out in laboratory conditions. During steam cooking (one-stage cooking) the potatoes quickly reached 60-64°C, as indicated by the appearance of gelatinised starch. As PME is quickly denatured at these temperatures, this suggested that PME activity was quickly denatured during steam cooking and little demethylation of cell wall pectin occurred. Three-stage cooking (cooking that includes a pre-cook and cool treatment) was shown to demethylate cell wall pectin and increase the mechanical strength of the potato compared to one-stage cooked potatoes. Three-stage cooking was carried out in two different ways. The first entailed conditions allowing maximal PME activation (Three-stage (55°C), whilst the second, (three-stage (70°C)), combined the activation of PME and gelatinisation, then cooling, of starch prior to cooking.

A number of potato cultivars were one-stage and three-stage (70°C) cooked and their texture was evaluated. One-stage cooked potatoes, when fully cooked all had a similar mechanical strengths, but cultivars with a higher starch content had a greater degree of cell separation than cultivars with lower starch contents. Three-stage cooking reduced cell separation compared to one-stage cooking, and this was more pronounced in potatoes with higher starch contents.

The distribution of calcium in the tissues of two cultivars was observed using EELS microscopy. Tissue preparation resulted in a loss of calcium from most regions of the wall. However, calcium was retained in tricellular junctions and at the corners of intercellular airspaces. These areas of the wall have important

implications regarding cell separation. More calcium was retained in these regions in cv. Cara, which exhibited less cell separation when cooked, than the mealy cv. Golden Wonder.

Boiling thin slices of potatoes in ethanol and ethylene glycol restricted water availability during boiling and thus reduced starch gelatinisation. Little, or no, cell separation was induced but subsequent incubations in water at 75°C for a short period of time caused near complete cell separation. This showed that swelling pressures generated by starch gelatinisation can only separate cells after weakening of the cell wall during cooking.

Calcium was shown to retard softening processes caused by cooking, leaving greater mechanical strength and restricted cell separation of cooked potatoes. Adding calcium to the 70°C pre-cook water saturated potato slices in calcium and almost completely stopped softening from occurring. When magnesium was added to potatoes, this was also observed, but to a lesser extent.

The mechanical strength of three-stage (55°C) and three-stage (70°C) cooking was found to be the same and both had a greater mechanical strength than one-stage cooked potatoes. A reduction in cell separation was observed, more so when using a 70°C pre-cook. If pre-cooking was carried out below PME activation temperatures, no effect on the texture of cooked potatoes was observed. No firming effect was observed, for both pre-cook treatments when the intermediate cooling step was omitted. Less cell separation occurred in 70°C pre-cook treatments, which included a cooling step, but no differences were observed between the two 55°C pre-cooks. These experiments indicated that manipulating starch gelatinisation, as well as activation of PME activity was responsible for altering the texture of cooked potatoes and the cooling step was an essential step to increase the mechanical strength and extent of cell separation of the potato during cooking.

The pattern of pectin deesterification caused by PME activity during pre-cooking was studied. Pre-cooking at 55°C caused slightly more deesterification than

pre-cooking at 70°C. The pattern of deesterification during the two pre-cooks was followed using JIM 5 and JIM 7 monoclonal antibodies. This study indicated that pectin in the cell wall of cell corners of 55°C pre-cooked cell walls may have been less de-esterified than the 70°C pre-cooked samples.

One and three-stage cooking was repeated on a pilot plant scale. The textural changes of pilot plant cooked potatoes were similar to those observed under laboratory conditions, except that in all cases the cooked potatoes had greater mechanical strength. This was possibly due to the storage conditions used between cooking and texture assessment. Microscopic examination indicated that three-stage cooking allowed cell separation without rupture when a force was applied but the wall structure remained more intact than in one-stage cooked tissues.

Small changes in the pre-cook conditions altered the texture of the subsequently cooked potatoes. Changes in pectin methyl esterification, the extent and location of calcium bound unesterified pectin and the whether starch had been gelatinised prior to cooking were associated with these differences.

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List of Abbreviations

Abbreviation	Meaning
AAS	Atomic absorption spectroscopy
CDTA	Cyclohexandiamine tetra-acetic acid
cv.	Cultivar variety
Des	Desiree
DIC	Differential interference contrast microscopy
DMSO	Dimethyl sulphoxide
EELS	Electron energy loss spectroscopy
ESI	Elemental selective imaging
EtOH	Ethanol
EFTEM	Electron filtering transmission microscopy
FES	Flame emission spectroscopy
GW	Golden Wonder
JIM 5	John Innes Monoclonal 5
JIM 7	John Innes Monoclonal 7
KP	Kerr's Pink
LM	Light microscopy
LM 5	Leeds Monoclonal 5
LM 6	Leeds Monoclonal 6
LSD	Least significant difference
MAb	Monoclonal antibody
MCB	Mixed cation buffer
MHDP	meta-Hydroxydiphenyl
MHR	Modified hairy region
MP	Maris Piper
Nad	Nadine
NIAB	National Institute of Agricultural Botany
PBS	Phosphate buffered saline
PME	Pectin methylesterase
PMT	Pectin methyltransferase

RG I	Rhamnogalacturonan I
RG II	Rhamnogalacturonan II
s.d.	Standard deviation
SEM	Scanning electron microscopy
SMS	Stable micro systems
SIMS	Secondary ion mass spectroscopy
UB	United Biscuits
VICS	Vortex induced cell separation

List of Cooking Abbreviations

Abbreviation	Meaning
One-stage	Potatoes steam cooked for twenty minutes
Ca-one-stage	Potato slices washed in 5g/l calcium prior to one-stage cooking
Mg-one-stage	Potato slices washed in 5g/l magnesium prior to one-stage cooking
Two-stage (55°C)	Potatoes pre-cooked at 55°C for forty minutes in deionised water, then cooled for twenty minutes in 3l of deionised water
Ca-two-stage (55°C)	Potatoes two-stage (55°C) cooked in 5g/l calcium, then cooled for twenty minutes in 3l of the calcium salt solution
Mg-two-stage (55°C)	Potatoes two-stage (55°C) cooked in 5g/l magnesium, then cooled for twenty minutes in 3l of the magnesium salt solution
Two-stage (70°C)	Potatoes two-stage (70°C) for fifteen minutes in deionised water, then cooled for twenty minutes in 3l of deionised water
Ca-two-stage (70°C)	Potatoes two-stage (70°C) cooking in 5g/l calcium,

	then cooled for twenty minutes in 3l of the calcium salt solution
Mg-two-stage (70°C)	Potatoes two-stage (70°C) cooking in 5g/l magnesium, then cooled for twenty minutes in 3l of the magnesium salt solution
Three-stage (55°C)	Two-stage (55°C) cooking followed by steam cooking for twenty minutes
Ca-three-stage (55°C)	Ca-two-stage (55°C) cooking followed by steam cooking for twenty minutes
Mg-three-stage (55°C)	Ca-two-stage (55°C) cooking followed by steam cooking for twenty minutes
Three-stage (70°C)	Two-stage (70°C) cooking followed by steam cooking for twenty minutes
Ca-three-stage (70°C)	Ca-two-stage (70°C) cooking followed by steam cooking for twenty minutes
Mg-three-stage (70°C)	Ca-two-stage (70°C) followed by steam cooking for twenty minutes
Two-stage (55°C)*	Pre-cooking at 55°C for forty minutes followed by steam cooking for twenty minutes, with the intermediate cooling step omitted
Two-stage (70°C)*	Pre-cooking at 70°C for fifteen minutes followed by steam cooking for twenty minutes, with the intermediate cooling step omitted

CHAPTER ONE

INTRODUCTION

1.1. A Brief History of the Potato

Potato cultivation by Inca Indians is thought to predate the Spanish conquest by two thousand years. Potatoes were introduced into Europe by Spanish and English explorers in the 16th century (Willard, 1993). They first became a major food crop in Ireland, then spread throughout the rest of Europe and North America (Burton, 1989). Potatoes have become one of the world's major food crops because they produce heavy yields and are relatively inexpensive. They can be grown in a wide variety of soils and climates and are now an important part of peoples' diet in a large segment of the world (Woolfe, 1989). Today, potatoes underpin a huge section of the snack food and convenience food markets (Willard, 1993).

1.2. Anatomy of Potato Tuber

Figure 1.1 is a diagrammatic representation of the tuber. The structure of potato tubers has been studied using scanning electron microscopy (SEM) (Fedec *et al.*, 1977). In the immature tuber the outer layer consists of an epidermis which is replaced by the periderm at maturity. The periderm, depending on maturity, is a

layer of cells of approximately ten to fifteen cells deep. These cells are dead, contain no starch or protein grains and the walls are much thicker than those of the underlying parenchyma tissues. Going in from the periderm to the centre of the tuber the next layer is the cortex, which is abundant in storage parenchyma cells. These contain numerous round and oval shaped starch grains. Phloem cells are also present in the cortex giving it its other name, the outer phloem region.

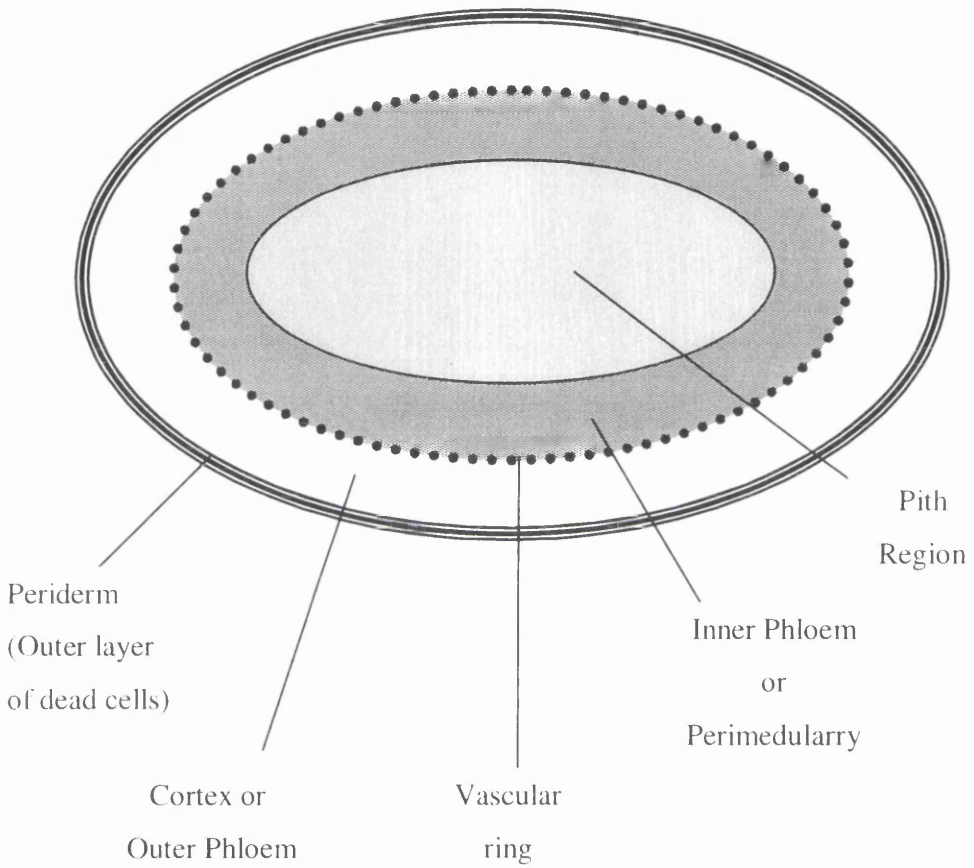


Figure 1.1. Schematic diagram of a potato tuber cut through its transverse axis. Drawing not to scale.

Beneath the cortex is the vascular ring that has vascular bundles interspersed within it. Vascular bundles consist of 30-40 xylem tracheids, which are involved in water transport. Parenchyma cells adjacent to these bundles generally have smaller sized starch granules, which are round in shape; cells located only three

cells away have large oval shaped spheres. The vascular ring separates the outer phloem region from the internal phloem region (or perimedullary region). The storage parenchyma cells here are slightly larger than those in the cortex region. The last section of the tuber is the pith (medulla or water core). It has tentacles, called pith rays, which extend through the tuber to the periderm. The cells in the pith are smaller and have a lower starch content. (Fedec *et al.*, 1977) also observed that starch grain abundance and size correlated well with the dry mater content of the tuber.

1.3. Cell Wall Structure.

The cell wall serves many purposes in the plant. Plants maintain high turgor pressures within its cells to resist compressive strengths during growth (Jarvis, 1999). Plant cells in isolation are spherical in shape (e.g. protoplasts), as this provides the most volume for surface area. In plant tissues, cells are attached to one another preventing cells from becoming spherical in shape. As a compromise cells have flat faces and angular corners. Turgor pressures generated within a cell push outward in an attempt to produce the most energy efficient shape. Cell walls must have sufficient strength to withstand such pressures. (Jarvis, 1999). As well as providing strength and shape, the cell wall also forms a physical barrier to pathogen attack and participates in cell-cell communications (Brett and Waldron, 1996).

The primary plant cell wall is a three-dimensional composite structure (Carpita and 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 and Waldron, 1996).

McCann and Roberts (1991) presented a simple model of the onion cell wall based on microscopy observations (Figure 1.2). Cellulose and xyloglucan form a network, whereby cellulose microfibrils are interlocked by xyloglucan polymers. The lengths of the xyloglucan polymers are sufficient to bind along the surface of one microfibril and span across to another preventing their aggregation. This rigid microfibril matrix is embedded in a more or less independent amorphous pectin matrix.

Cellulose is a linear β-D-linked-(1-4) glucan polymer and is the most abundant polysaccharide of the plant cell wall. Cellulose microfibrils are arranged in parallel layers, their diameter depends on variety but in onions they are in the range of 5-12nm. (McCann and Roberts, 1991). 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 in dicots, comprising 20% of the cell wall on a dry weight Basis (Bauer *et al.*, 1973). It consists of a β(1-4)-D-glucose linked backbone chain, with α(1-6)-xylose attached as side chains. The xylose residues can be further substituted with β(1-2)-D-galactose residues. Many of the galactosyl units are substituted with α(1-2)-L-fucose or occasionally α(1-2)-L-arabinose to form trisaccharide side-chains (Bauer *et al.*, 1973). Terminal fucose residues are thought to assist in the binding of xyloglucan to cellulose microfibrils, although they are not essential for this to happen (Levy *et al.*, 1991). Cellulose in onions has been shown to have an open microfibril arrangement, which allows xyloglucan to attach on to the surface of the individual subunits via hydrogen bonding (Brett and Waldron, 1996, Ha *et al.*, 1998).

One of the roles of the pectic matrix in celery collenchyma is to hold the cellulose microfibrillar layers of the cell wall together across their thickness (Jarvis, 1992). Cell wall thickness is controlled 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.

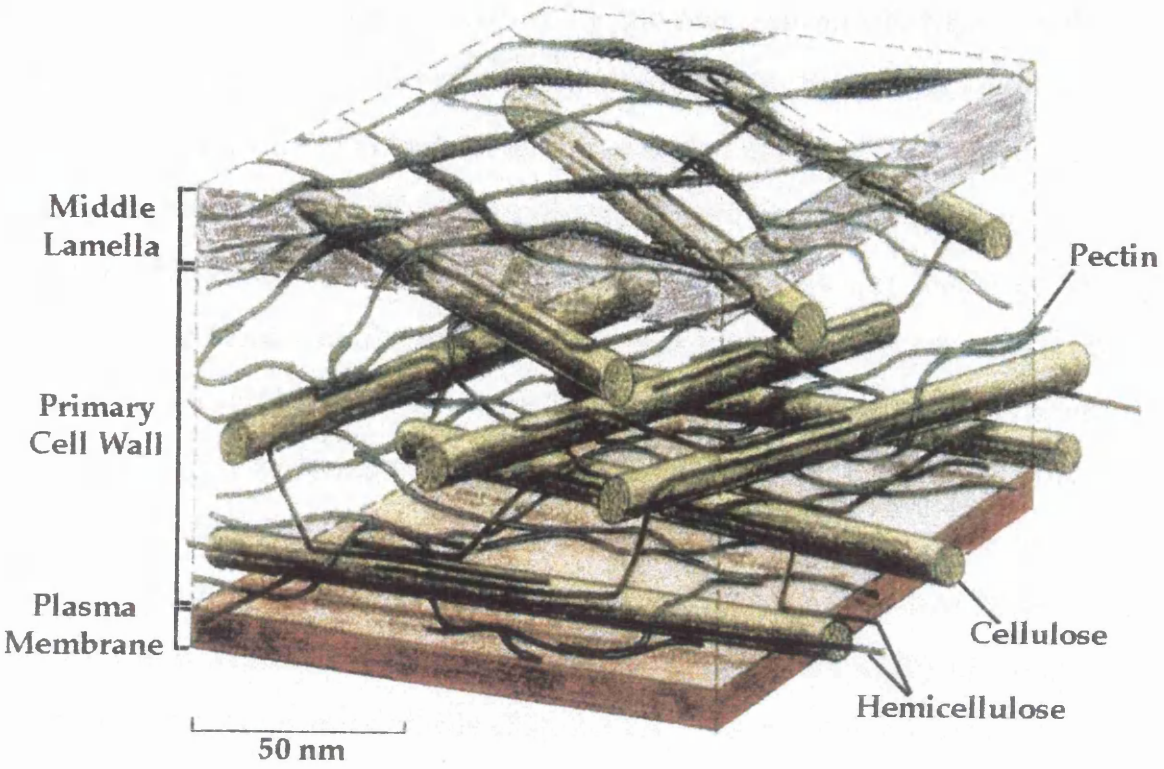


Figure 1.2. A model of the onion cell wall representing how the three broad classes of polymers are arranged (taken from McCann and Roberts, 1991). Although simplistic, the sizes and spacing of the polymers are based on direct measurements of native cell walls (McCann et al., 1990) and are drawn to scale

Transport of materials between cells must include passage through the cell wall. The porosity of the cell wall is principally determined by the porosity of the pectic matrix (Baron-Epel *et al.*, 1988, McCann and Roberts, 1991). 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 and 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 (Shomer *et al.*, 1995).

As starch grains are kept within cells, when starch gelatinises it cannot escape from within cells, producing a pressure similar to turgor pressures that are lost during cooking. As the cell wall is weakened, during cooking starch gelatinisation can act as a cell separation force (Jarvis *et al.*, 1992). Cell walls are weakened during cooking by degradation of the pectin network. The extent and location of pectin degradation will govern cell separation or rupture (Jarvis and Duncan, 1992). As the pectin structure is of importance in texture development, it is discussed in more detail below.

1.4. Pectin Structure

The pectin network is found both in the cell wall and middle lamella of plants. The middle lamella is the area between adjacent cell walls (Bret and Waldron, 1996) and is the area where cell separation occurs (Jarvis and Duncan, 1992). Tricellular junctions are where three cells meet (Goldberg *et al.*, 1996). A schematic diagram of the cell, middle lamella and tricellular junction is shown in Figure 1.3.

1.4.1. Pectin Polymers

The term pectin is used to describe a group of closely related polysaccharides rich in galacturonic acid, rhamnose, galactose and arabinose (Brett and Waldron, 1996). The main constituent of pectin is a linear chain of α-(1-4)-D-linked galacturonic acid known as homogalacturonan (Fig 1.4 B). This polymer has 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) (fig 1.4 C) (Lau *et al.*, 1985). Zhan *et al.* (1998) showed that the RG I fraction is composed of rhamnose/galacturonic acid 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 and Waldron, 1996). In potatoes the dominant neutral carbohydrate in the side chains are linear chains of D-galactose (Jarvis *et al.*, 1981). L-arabinose and D-xylose are also present (Jarvis, *et al.*, 1981; Ng and Waldron, 1997a; van Marle *et al.*, 1997b).

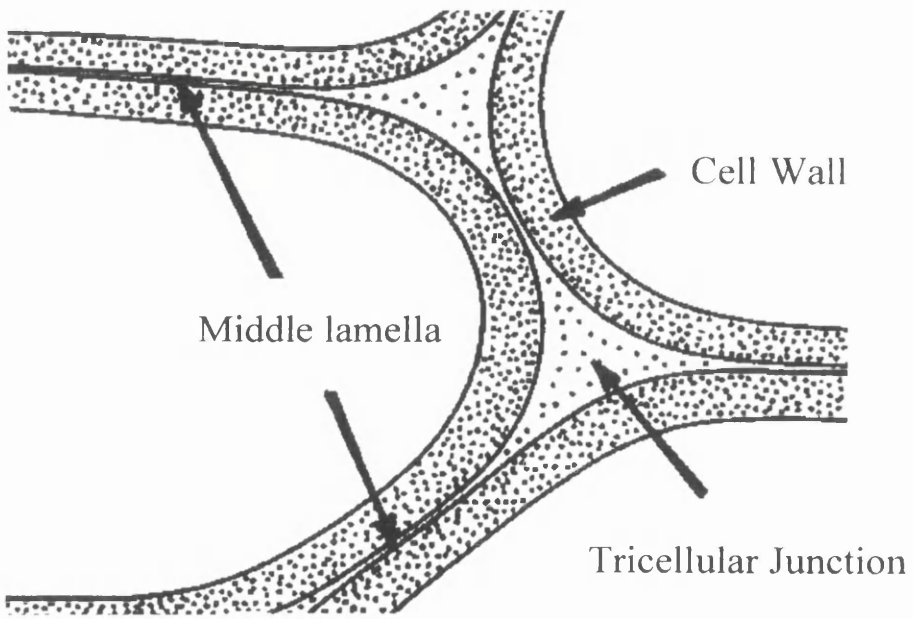


Figure 1.3 Arrangement of the cell wall, middle lamella and tricellular junction. From Jarvis and Daud (1995).

Schols (1995) extracted a portion of the pectin network from apple after extraction with rhamnogalacturonase, which was referred to as modified hairy region (MHR). It is a high molecular weight fraction that is characterised as a highly branched oligomer rich in arabinose. It also contains galactose, xylose, rhamnose and galacturonic acid monosaccharide. Schols (1995) proposed that the MHR was composed of three sub units. The first subunit, xylogalacturonan, consists of linear regions of galacturonic acid units with β(1-3)-D linked xylose units attached to each galacturonic acid. The second subunit consists of a rhamnogalacturonan core with α(1-5)-L-linked arabinan side chains stubs attached. The third consists of a rhamnogalacturonan core with α(1-4)-D-linked galactans, some of which had α(1-5)-L-linked arabinans attached to the galactose residue. MHR differs in composition from RG I, as xylogalcturonans are not present in RGI (Schols, 1995).

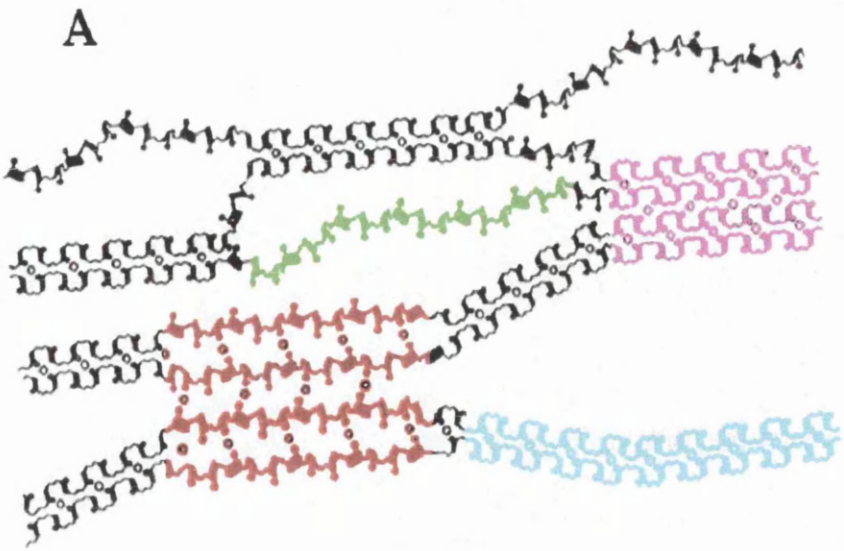
Rhamnogalacturonan II (RG II) is a minor, but highly complex, component of the primary cell wall (Figure 1.5). It is composed of galacturonic acid, rhamnose, apiose and galactose, as well as other rare sugars (Brett and Waldron, 1996). Boron, in the form of boric acid, has been shown to link two RG II chains together (Kobayashi *et al.*, 1996).

Figure 1.4

A The cable structure (adapted from Goldberg *et al.*, 1996 by Marry, 1999) for pectic junction zones. The structure contains two levels of aggregation. First single chains (coloured green) join to give the dimeric egg-box junction zones (coloured blue) and can also form as inter-junction segments between junction zones, accounting for the rhamnosyl units of RG I and their associated sidechains. In the second level, the cable is formed by both aggregation of egg-box junction zones (pink) and Ca^{2+} junction zones forming in areas where the degree of esterification is relatively low (red)

B A unit structure if homogalacturonic acid (adapted from Olesen, 1997 by Marry, 1999). This is a helical polymer consisting of D-galacturonic acid units. Random methyl esterification is shown at the C-6 position.

C A unit structure of RG I (adapted from Olesen, 1997 by Marry, 1999). This is composed of alternating α(1-4)-linked-D-galacturonic acid (yellow) and α(1-2)-L-linked rhamnosyl residues (purple). Side chains of neutral arabinan, galctan and arabinogalactan are often attached at the C-4 of the rhamnosyl residues. O-acetylation on the C-3 of galacturonic acid residues (yellow) and random methyl esterification is shown at the C-6 position


A

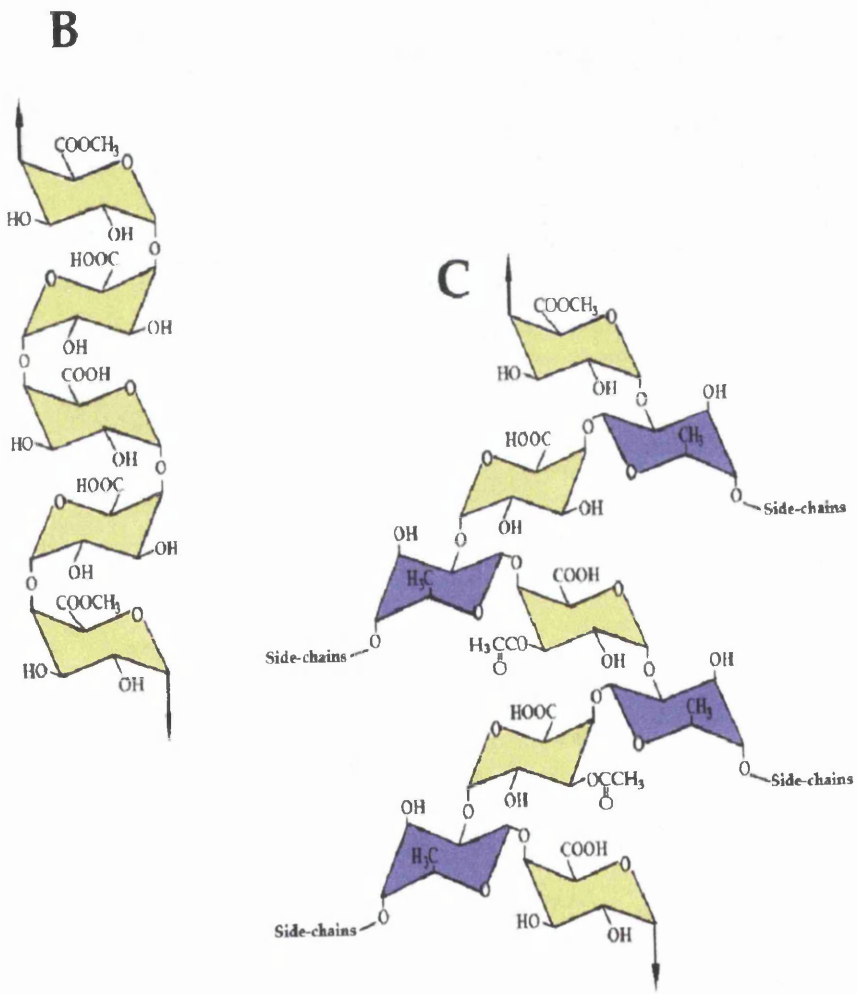
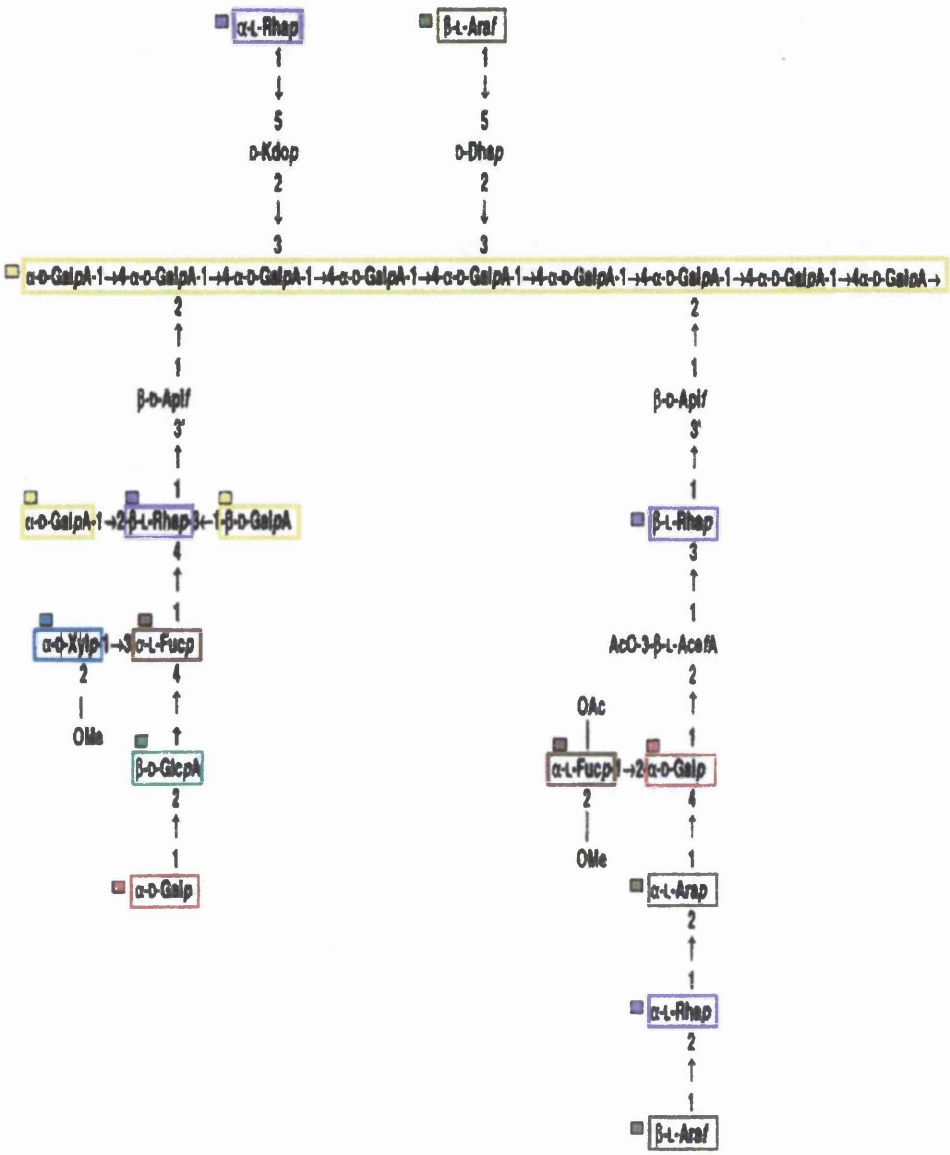

B
C
COOCH3
HO
OH
HOOC
COOH
Side-chains
H3C
H3CCO
CH3
OCCH3

Figure 1.5. A unit structure of RGII released by endopolygalacaturonase treated sycamore cell walls (adapted from O'Neill *et al.*, 1996). The backbone consists of 9 linear α(1-4)-D-linked galcturonsyl residues (yellow) to which four side chains are attached. The order of attachment is unclear, although the sequences attaching C-2 and C-3 to the backbone are known. A high proportion of rhamnose (purple) which occurs (1-3) and (1-2,3,4)-linked and as terminal units. Terminal arabinose (green), terminal galactose (red) and fucose (brown) are also present. The formations of borate diester crosslinks are thought to occur on the C-2 and C-3 of apiofuranosyl residue (Api*f*) within the RGII side-chains.

Key to Figure:

Galacturonic Acid		Rhamnose	
Glucuronic acid		Fucose	
Galactose		Arabinose	
Xylose			


α-L-Rhap
1
5
D-Kdop
2
3
β-L-Araf
1
5
D-Dhap
2
3
α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA-1→4-α-D-GalpA→
2
1
β-D-Apif
3'
1
α-D-GalpA-1→2-β-L-Rhap-3←1-β-D-GalpA
4
1
α-D-Xylp-1→3-α-L-Fucp
2
OMe
4
1
β-D-GlcpA
2
1
α-D-Galp
2
1
β-D-Apif
3'
1
β-L-Rhap
3
1
AcO-3-β-L-AcefA
2
1
OAc
α-L-Fucp-1→2-α-D-Galp
2
OMe
4
1
α-L-Araf
2
1
α-L-Rhap
2
1
β-L-Araf

1.4.2. Degree of methylation.

The degree of methyl esterification found on the galacturonan chain has important consequences for the resulting pectin characteristics. Pectin galacturonan is synthesised in the cis Golgi and methyl esterified in the medial Golgi, by the enzyme pectin methyltransferase (PMT) (Zhang and Staehlin, 1992). The esterified galacturonan is then selectively de-esterified by the wall bound enzyme pectin methyl esterase (PME) (Markovic and Kohn, 1984). However, Goldberg *et al.* (1996) 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. It is now clear that PMT transfers methyl ester groups onto galacturonic acid groups at random, and when in the cell wall the pectin molecules are at least partially deesterified in a blockwise fashion by the action of PME (Doong *et al.*, 1995)

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

1.4.3. The Role of Calcium Within the Cell Wall.

The affinity of cell wall pectin for calcium is very strong, in the same order of magnitude as proton ions (Demarty *et al.*, 1984). Calcium has the ability to form divalent cross-links 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).

Chelators of calcium such as CDTA (Jarvis *et al.*, 1981) and imidazole (Mort *et al.*, 1991) are used to extract the pectic fractions held by Ca bridging in the cell wall. Ryden and Selvendran (1990), using two CDTA extractions, which extracted 49% of pectin. This had less neutral sugar sidechains attached than the remaining cell wall pectin later extracted after saponification.

1.4.4. The Cable Structure.

Goldberg *et al.* (1996) proposed the cable structure (Figure 1.4 A). The 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. It also has calcium acting as bridging ions between the galacturonan chains in the 3_1 conformation proposed by Walkinshaw and 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 present, but sodium and magnesium are also found in large amounts.

1.4.5. Non Methyl Ester Linkages

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 (Jarvis, 1982). Alkaline conditions will result in the cleavage of covalent ester bonds by saponification, therefore alkali extraction of pectin material may be due to cleavage of a covalent ester linkage between a pectin molecule and another constituent of the cell wall. It has been suggested that other alcohols can substitute for methanol (Fry, 1986). Kim and Carpita (1992) found that methyl esters accounted for two thirds of the total ester groups found in maize coleoptiles. Their findings suggested the existence of

non-methyl ester groups in grasses attached to the C-6 galacturonyl group of pectin at quantities approaching 20% during some stages of growth. The novel ester groups were not extractable by chelation prior to an alkali treatment.

Non-methyl ester linkages have been found in potato cell walls, on the C-6 carboxylic acid groups (Jardine, 1998). These linkages were present on approximately 15% of the potato galacturonosyl residues. Methyl esters occupy an additional 37-53% of the potato galacturonan (see Section 1.5), so the abundance of non-methyl ester linkages found was substantial. As yet, the location or structure of these linkages has not been elucidated. Saponification of potatoes prior to boiling resulted in smaller molecular weight galacturonan fractions than non-saponified, boiled cell walls, which suggested that the linkages had a structural role in the cell wall. As the fractions released after saponification of the cell wall were rich in substituted neutral sugars it was suggested that the linkages must be in the RGI fraction.

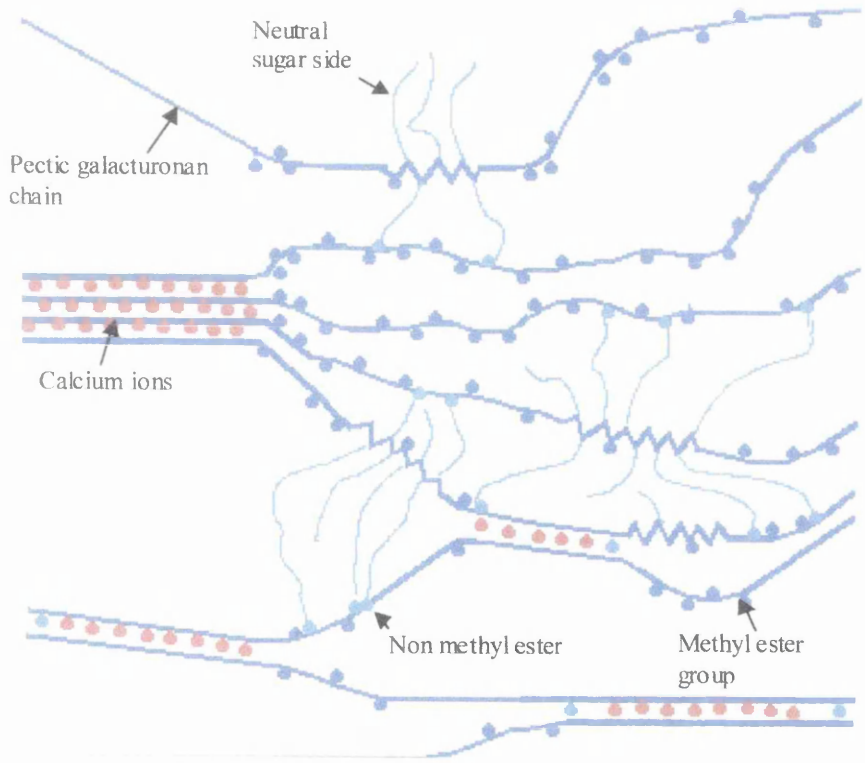


Figure 1.6 Possible structural arrangement of pectin when non-methyl esters are considered (from Jardine 1998).

A possible model of the structural arrangement of pectin with non-methyl ester linkages is shown in Figure 1.6. As rhamnose constituted only 8% of the cell wall and 15% of the galacturonosyl residues had non-methyl ester linkages, it was deduced that not all the non-methyl ester linkages could be attached to rhamnose units. Other possible sites of attachment could be arabinose and galactose neutral sugar side chains (Jardine, 1998). It is also possible that the ester linkage could be a lactone, attached to the C-2 alcohol group on an adjacent galacturonic acid unit. (Jarvis, unpublished).

1.4.6. The Spatial Distribution of Pectin.

The use of monoclonal antibodies (mAbs) has aided in the understanding of the distribution of pectic substances within the cell wall. Bush and McCann (1999) used a selection of mAbs that had binding specificity to epitopes within specific regions of the pectin matrix in potatoes. JIM 5 (John Innes monoclonal antibody 5) and JIM 7 were used to locate pectins with high and low degrees of methylation respectively. The epitope that JIM 5 recognises contains five consecutive unesterifed galacturonic acid residues; while JIM 7 recognises regions of five consecutive methyl esterified galacturonic acid residues (Knox *et al.*, 1990). Additional to JIM 5 labelling, regions of low esterified pectin were located using the mAb 2F4, which is dependent on the pectin epitope being calcium bound (Liners *et al.*, 1994). The mAbs LM 5 (Leeds monoclonal antibody) (Jones *et al.*, 1997) and LM 6 (Willats *et al.*, 1998) were used to locate the distribution of galactans and arabinans respectively.

Low and high methyl esterified pectin in potatoes was located, using JIM 5 and JIM 7, throughout the cell wall and middle lamella. Calcium bound pectin, labelled by the 2F4 mAb, was found throughout cortex tissue but no labelling was found in the primary cell walls of perimedullary parenchyma tissues. Calcium bound pectin was found in the middle lamella and especially in tricellular junctions, the corners of intercellular regions and around plasmodesmata in this region. Calcium bound pectin has also been found in tricellular regions of flax

bundles using secondary ion mass spectroscopy (SIMS) (Goldberg *et al.*, 1996) and in apples using electron energy loss spectroscopy (EELS) (Huxham *et al.*, 1999). Calcium bound pectin was also found in the cell walls around vascular tissues (Bush and McCann, 1999). Fedec *et al.* (1977) have reported that starch content in these cells was lower than in cells in surrounding tissues.

Bush and McCann (1999) postulated that calcium bound pectin was located in regions of weakness in plant tissues. Calcium bound pectin was found in the cell walls around plasmodesmata. Plasmodesmata are delicate microtubule structures that preserve cytoplasmic connections between two cells and are involved in symplastic transport within the plant (Brett and Waldron (1996). Bush and McCann postulated that calcium bound pectin was found in the cell wall around plasmodesmata to protect them during cell expansion. Waldron *et al.* (1997) suggested that, after cooking, plasmodesmata are potential regions of cell rupture. After cooking the cell wall network is disrupted and cell separation can occur (see section 1.10). As the regions around the plasmodesmata are protected by calcium-bound pectin, cells are less likely to separate at these points, therefore, it is these areas where cell rupture might occur when a force, such as chewing, is applied.

High levels of calcium bound pectin in cell walls around vascular bundles might keep the bundles together during expansion. Calcium bound pectin was also found in the cortex regions of tissues may to restrict water losses (Bush and McCann, 1999). Jarvis (1999) has shown that tricellular junctions and the corners of intercellular spaces are regions of cells that experience greatest cell separation forces from turgor pressure or starch swelling pressure. It is therefore likely that calcium bound pectin was found in such high quantities in these regions to help alleviate these pressures (Bush and McCann, 1999).

Using the monoclonals LM5 and LM 6, Bush and McCann (1999) visualised both arabinan and galactan epitopes in relatively equal quantities within the primary cell walls of potato parenchyma tissue, but not within the middle lamella. This co-localisation was not unexpected, as Jarvis *et al.* (1981) showed that nearly half

of the arabinose residues in potatoes were carried on galactan side chains. In the outer regions of the potato tissues (the cortex) arabinans were detected throughout the wall and galactans were only detected close to the plasmalemma. Galactans were also less abundant around plasmodesmata and in vascular tissues.

Jardine (1998) found that 15% of galacturonic acid residues in potatoes had non-methyl ester linkages. As yet where and in what form these linkages exist has not been discovered. Jardine (1998) suggested that they could be attached on to arabinan or galactan side chains. The fact that arabinans and galactan chains are spatially distributed in potatoes (Bush and McCann, 1999) could have significance to the spatial distribution of non-methyl ester linkages.

1.5. Potato cell walls

Many researchers have studied the chemical structure of the pectin in the potato tuber cell wall (Ishii, 1981; Jarvis *et al.*, 1981; Selvendran, 1985; Ryden and Selvendran, 1990; van Marle *et al.*, 1994, 1997a, 1997b; Schols and Voragen, 1994; Ng and Waldron, 1997a). The percentage 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 and O'Neill, 1987) and 26% (Ng and Waldron, 1997a). 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.*, 2000) to 53% for cv. Bintje (Ng and Waldron, 1997a) have been reported.

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 of 0.78 and 0.82 respectively. Potato MHR differed from other vegetables as no xylose was present in the structure. It also had a very high degree of acetylation of 90% (Schols and Voragen, 1994).

Ryden and Selvendran (1990) reported that, unlike many plant cell walls, fucose, the terminal residue in xyloglucan side chains was not present in potato.

1.6. What happens to potatoes during cooking?

The overall effect of cooking potatoes is softer tissues, which come apart very easily. A number of processes that cause softening during cooking have been reported, such as gelatinisation of cellular starch, pectin solubilisation, and membrane breakdown causing loss of turgor. The last of these processes results in the free movement of cellular ions throughout the tissues. This can cause the removal of calcium, by chelation, from pectin within the wall and middle lamella leading to pectin degradation. Conversely cations released into the cell wall that can enhance cell wall and middle lamella strength preventing degradation. Finding which of these processes is of most importance in relation to texture development has proved difficult and nothing conclusive has been reported. A huge problem with assessing potato texture is the large numbers of variables attached to the problem. Variations in potatoes arise within different tissues of a tuber, from differences in agronomic conditions, storage conditions, cooking treatments and the way in which texture was assessed (Andersson *et al.*, 1992).

1.6.1. Gelatinisation of Starch.

Non-industrial potatoes consist of 60-75% starch on a dry matter basis. The correlation between starch content and dry matter content is so strong, that specific gravity is commonly used as a guide of starch content of potatoes (Burton, 1989). Starch consists of two polymers based on α(1-4)-D-linked glucose residues. Amylose is a linear, flexible polymer of α(1-4)-D-linked glucose (Coultate, 1988). It contains approximately 4,000 glucose units. Like

amylose, amylopectin consists of α(1-4)-D-linked glucose, but some 4-5% of the glucose units have an α(1-6) linkages to other chains creating a highly branched polymer (Coultate, 1988). Potato starch contains between 12 and 20% amylose. A small amount of phosphate is present in potato starch (~0.05%) mostly in amylopectin (Burton, 1989). Starch in its native state occurs as water insoluble, roughly spherical granules with highly ordered structure (Morris, 1990).

Heating above a characteristic temperature in aqueous conditions causes an irreversible swelling or gelatinisation of starch granules (Morris, 1990). Gelatinisation of starch in potato cells was measured in the range of 58-64°C (Lamberg and Olsson, 1989). Phosphate groups within potato starch provide it with a net negative charge. On gelatinisation, the negative charge causes repulsion between chains, creating greater swelling pressures. Cell wall pores are smaller than intracellular starch molecules (Shomer *et al.*, 1995). This prevents escape of gelatinised starch, which produces an outward pressure on the surrounding cell wall (Shomer, 1995). During cooking an increase in cell size has been observed (Reeve, 1977 and 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, but only after degradation of the middle lamella and/or the cell wall had occurred.

1.6.2. Release of Cellular Constituents.

Rupture of the plasmalemma will allows free diffusion of ions from the cytoplasm to the cell wall (Bartolome and Hoff, 1972). There are two possible mechanisms by which this influences softening. The increased ionic content of the cell wall will increase the rate of β-elimination (see following section) (Keijbets and 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)

Keijbets *et al.* (1976) found that citrate and phytate will favour the solubilisation of pectin by chelation or precipitation of divalent cations (Ca^{2+} and Mg^{2+}), 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 of beans did not occur until the beans were over cooked. They found no evidence that phytic acid formed complexes with cations.

Selvendran *et al.* (1990) postulated that potatoes with a greater starch content show greater cell separation as the thicker starch matrix retards citrate from diffusing out of tissues into the cooking media. Citrate would thus remain within the cell wall and be available to chelate calcium from the pectin gel, thereby increasing the pectin matrix breakdown resulting in more cell separation.

1.6.3. Thermal degradation of the pectin matrix.

It has been shown that heating under neutral and alkaline conditions will result in cleavage of glycosidic linkages between galacturonic acid units in the pectin chain according to a β-elimination mechanism (Neukom and Deuel, 1958; Keijbets and Pilnik, 1974, Kravtchenko *et al.*, 1992). β-Elimination is a process by which the pectin galacturonan chain is cleaved next to a methyl esterified galacturonic acid residue during heat treatment (Keijbets and Pilnik, 1974). 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%, concluding that at pH 6.1 β-elimination was the primary reaction for heat degradation for pectic galacturonan.

During cooking, degradation of the plasmamembrane results in an influx of cations and anions into the cell wall. A greater salt content increases the cleavage of pectic galacturonan chains (Keijbets and Pilnik, 1974, Sajjaanantakul *et al.*, 1993). Divalent cations have a greater enhancing effect from monovalent cations at the same concentration. The larger the methyl ester content, the more extensive was the degradation of the galacturonan by β-elimination (Sajjaanantakul *et al.*, 1993). Other researchers who have studied potato texture assume the major cause of degradation of pectin during cooking is be β-elimination (Ng and Waldron, 1997a, van Marle *et al.*, 1997a).

1.6.4. Texture of Potatoes: Mealy and Firm Types

The terms mealiness and firmness are commonly used in the potato industry to distinguish textural attributes of different varieties. Differences are often varietal specific and there is a strong correlation with starch content (Jarvis and Duncan, 1992). Mealy potatoes, when cooked, easily fall apart and can be broken down into a slurry of cells. In terms of mouthfeel they have a dry texture often described as floury. Firm potatoes, when fully cooked, can be sliced intact. In the mouth they have a firm and moist texture. In the food industry, potatoes of different texture types are used for different products. Firm potatoes are preferred in canned and salad products, whilst mealy types are used for mashed potatoes (Jarvis and Duncan, 1992).

Softening of potatoes can occur by cell separation or cell rupture, caused by breakage across the middle lamella or rupture of the cell wall respectively. Both cell separation and cell wall rupture contribute to the softening of the cooked potato. The importance of the two will vary depending on the variety. The balance between cell separation and cell wall rupture will determine the dryness or moistness perceived within the mouth (Jarvis and 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 and Duncan, 1992). The extent to which cells fractured during cooking was measured by weighing the amount of water absorbed into filter papers. The recorded levels of moisture decreased in both mealy and firm varieties as cooking progressed. When fully cooked, a greater amount of moisture was released from the firm variety compared to the mealy (Jarvis and Duncan, 1992).

The texture of a potato variety after cooking is strongly correlated to its starch content (Burton, 1989). McComber *et al.* (1994) examined four potato cultivars with different texture properties as assessed by a sensory panel. The Russet

Burbank cultivar was assessed as the mealiest of the four. The four cultivars were studied by SEM, after steam cooking. Some varieties dehydrated and starch within cells shrunk in size during the alcohol dehydration step. Cv. Russet Burbank was the only cultivar to retain its shape and exhibited no starch shrinkage. The cell shape of the firm variety, cv. Pontiac had distorted and starch was shrunken into the middle of the cell. The Russet Burbank potato, when mashed had intact cell walls and the cells had not compacted. Whereas in the firm cv. Pontiac, the cells had flattened and cell rupture was observed.

Fracture planes of two potato cultivars; a mealy variety (cv. Irene) and firm variety cv. Nicola when raw and cooked were examined using cryo-SEM (van Marle *et al.*, 1992). The fracture plane of both varieties when raw went through cell walls exposing starch grains within cells. When cooked, fracturing left cells intact but the firm variety cell's had greater cellular contact than the mealy. The mealy cells had lost their angular structure and were rounded. Reeve (1977) observed this loss of angular structure of potato cells caused by starch gelatinisation. This has been referred to as 'rounding off'.

Greater cell separation and pectin solubilisation was measured in the mealy cv. Irene. The rate of citrate diffusion for cv. Irene was lower than predicted, as greater sloughing during cooking would increase cell surface area (van Marle *et al.*, 1994). Irene had thicker walls (van Marle *et al.*, 1997b) with slightly more highly branched pectin network (van Marle *et al.*, 1997a) that could restrict diffusion. Selvendran *et al.* (1990) proposed that potatoes with a higher starch content would produce a denser barrier against the diffusion of citrate ion. Thus citrate diffusion into the cooking medium would be restricted, with citrate remaining within the cell wall and middle lamella. This would mean greater calcium chelation, in turn leading to a greater amount of pectin degradation.

Sequential extraction of the raw cell walls from each cultivar indicated that cv. Nicola's middle lamella pectin was slightly more branched. Also, it had a greater amount of pectin, that was probably more branched and entangled in the cellulose network which could not be extracted with 0.5M KOH (van Marle *et al.*, 1997a).

van Marle (1997) postulated that a mealy potato has stronger cell wall, which combined with a more rigid starch gel within the cell, produces more rigid cells. These could round off to a more energetically favourable shape with less cellular contacts and are less likely to be damaged by handling. The firm potato has a weaker gel and weaker walls with cells that are in close contact with each other and are more likely to be damaged. This study was carried out only on two cultivars and it is uncertain if the differences reported were consistent for all mealy and firm types of potato.

1.7. What is mash?

Mashed potatoes and products made from it have different textural properties from other types of cooked potatoes. Mashed potato is a mass of free cells and small aggregates held loosely together by residual adhesive effects of the middle lamella, surface tension of the wet cells and starch released from cells (Faulks, 1986). Cells appear to 'round off' losing their angular corners thus reducing their cellular cohesion and packing density.

How potatoes come apart is of extreme importance in the final texture of mashed potatoes. During mashing some cell walls are ruptured releasing their starch, giving the mash a very thick and sticky texture (Hadziyev and Steele, 1979). This occurs to a greater extent if mashing is too extensive or if immature or undercooked tubers are used (Faulks, 1986). Potatoes with a firm texture are also more likely to break across cells, thus releasing more starch (Freeman *et al.*, 1992). This can be overcome by adding milk, cream or fat to the mash, reducing the stickiness and helping the cells slip over one another (Faulks, 1986).

Mashed potato is produced commercially in a dehydrated form that can be reconstituted with hot or boiling water. The processing conditions involve shear and compressive forces which potatoes would not generally experience with conventional mashing and excessive cell rupture can occur (Willard and Kluge, 1975, Talbut, 1975, Hadziyev and Steele, 1979).

1.8. Production of Mashed Potatoes on an Industrial Scale

Potatoes were being processed almost as soon as the Incas had cultivated them. Potatoes were frozen at night and thawed during the day, juice was squeezed out of the potato by treading on the thawed potatoes. This cycle of freezing and thawing was continued until the potato was dehydrated sufficiently to preserve it as a product called *chuno* (Willard, 1993). *Tunta* was another dehydrated product made by the Incas, which helped maintain military expeditions, allowing the expansion of the Inca Empire and is still made in the South American Andes today (Willard, 1993).

After their introduction to Europe and with the onset of the Industrial Revolution potatoes began to be processed industrially for rations during military campaigns, being very important during World War One. Processing involved methods of dehydrating the potatoes to preserve them. Attempts to develop an instant cooking dried mashed potato began in the 1920's. The concept was the same as the Inca Indians' production of *chuno.* Potatoes were frozen, thawed, water was expressed mechanically and the residue was dried. During World War Two, granules became a very important military ration. The higher bulk density and their storage stability made them preferable to canned or fresh potatoes. (Willard 1993).

Following the end of the war, social and economic changes in Europe and the USA lead to the creation of convenience foods (Willard, 1993). The methods used until then had limited how much of the annual crop could be used for instant mash potato production (Willard and Cording, 1957). Unless tubers of high specific gravity were used cell damage occurred during dehydrating steps, which on reconstitution, lead to a product of inferior texture compared to fresh potatoes. Also, potatoes stored at low temperatures had to be reconditioned for

approximately two weeks before they could be used (Willard and Cording, 1957). Willard and Cording (1957) patented a process of pre-cooking and cooling potatoes prior to cooking that improved the resilience of potatoes to the mechanical stresses involved in industrial cooking, mashing and dehydration on a drum dryer.

The patent was based on microscopy observations of potato during heating. Reeve (1954) observed that heating potatoes to temperatures that caused starch gelatisation, then cooling them prior to cooking, reduced the amounts of iodine-stain starch released during cooking. A minimal amount of starch release is desirable in mashed potatoes. It was also observed that less sloughing (cell separation) during cooking occurred if the potatoes were heated and cooled prior to cooking (Reeve 1953). Potatoes that exhibit less cell separation would have a greater mechanical strength and are less likely to be damaged during the rigours of industrial cooking, resulting in a better quality mash (Willard and Cording (1957). Later literature has attributed the firming effect to the cell wall degrading enzyme pectin methyl esterase (PME) (Bartolome and Hoff, 1972).

Cooking and mashing methods are described below, while the processes that firm potatoes are discussed later. Washed, peeled and sliced potatoes are pre-cooked at temperatures ranging from 50 to 70°C in a large volume of water. Most literature on industrial cooking discusses pre-cooking at 70°C to allow starch gelatinisation in the potatoes. During the 70°C pre-cook it is very important that complete gelatinisation of the starch occurs so that retrogradation is complete during cooling (Willard and Kluge, 1975). After pre-cooking the texture of the potato is more pliable, but if broken or sliced, a stringy material (gelatinised starch) is released (Willard and Kluge, 1975).

After pre-cooking the potatoes are cooled in water. In a commercial plant potatoes are passed through a counter current flow of cold water until the minimum internal temperatures required are reached (Willard and Kluge, 1975). The Blue Value Index (BVI) is a colorimetric determination with iodine to measure free starch. Measuring the BVI in the cooling water is used to measure

the reduction in soluble amylose (Willard and Kluge, 1975, Jankowski, 1992). It was found the higher the starch content, the quicker the drop in the BVI. Cooling water at lower temperatures also decreased the time for the BVI to drop. The maximum internal temperature needed for retrogradation differs with the specific gravity of the potatoes. For higher specific gravity potatoes (>1.080) the temperature is ~20°C, while potatoes with lower specific gravities require temperatures of ~10°C. To produce properly cooked potatoes, lower specific gravity potatoes require longer cooking times at the next stage of cooking. Although lower specific gravity potatoes can be used for processing, the shorter cooking and cooling times and the reduced cooling temperatures required for potatoes of higher specific gravities make them preferred for mash production (Willard and Kluge, 1975).

After cooling, potatoes are steam-cooked. Steam cooking is preferred as it causes less physical abrasion than boiling and reduces the chances of sloughing damage (Hadziyev and Steele, 1979). Reeve (1954) observed that less starch was released from steam cooked compared to boiled potatoes. Steam cooking of potatoes also reduces the amount of sloughing compared to boiling as no uptake of water occurs during cooking. Starch within cells is, therefore, unable to gelatinise to the same extent, which reduces starch gelatinisation pressures and thus, reduces the extent of cell separation (Wilson W. and Jarvis M.C., unpublished results). Cooking must allow the separation of potato tissues into single cells with a minimum amount of damage to the cells. Undercooking causes cell rupture releasing starch during, which produces an inferior product. Differences in specific gravities within and between potatoes and unknown factors probably relating to storage conditions, lead to variation in the extent of cooking. This problem is accentuated when potatoes of different varieties are cooked together (Willard and Kluge, 1975).

Cell rupture is the most detrimental factor during mash potato production. After the potatoes are steam-cooked they are mashed hot. Mashing at temperatures close to cooking temperatures separates cells easily with minimal cell rupture (Hadziyev and Steele, 1979). Most commercial mashers are operated at minimum

speed to minimise cell rupture. Mashing is done by passing the mash through a perforated plate or a set of rods (Willard and Kluge 1975). During or after mashing, additives are added to the potato such as anti-oxidants (to prevent rancidity), chelating agents (to prevent iron discolouration) and sulphite (to prevent non-enzymic browning). Emulsifiers such as 0.5% monoglyceride are also added to counter the effects of starch released from cell rupture.

The mashed potato is either used fresh or frozen or can be dehydrated for instant mash or as an ingredient in other products. There are two processes by which mashed potatoes are dehydrated: flake and granule production. This thesis concentrates only on the texture of freshly cooked potatoes, but to give an idea of the forces potatoes endure through the whole industrial process, dehydration techniques are also discussed below.

1.8.1. Flake Production

In flake production, the mashed potatoes are heated on large heated drums with a series of rollers to squeeze and separate apart mash particles to produce a more uniform consistency and improved bulk density (Willard and Kluge, 1975). The flake is then cut with a reciprocating blade, and the flake is collected as a dry sheet (moisture content of ~7%) onto a collecting screw conveyor. The flake is then ground up. A balance has to be met between a sufficiently high bulk density and excessive cell damage, flakes made from lower solid content potatoes when ground generally release more starch (Willard and Kluge, 1975). Flake production can cause a lot of cellular damage and to avoid a sticky, undesirable product emulsifiers, such as monoglycerides, are added to complex the starch (Hadziyev and Steele, 1979).

1.8.2. Granule Production

Granules are produced commercially by a process known as the add-back system (Hadziyev and Steele, 1975). Previously dehydrated granules are mixed in with potatoes after the steam cooking process. The repeated mild pressing and

shearing action of the granules against the cooked tissues causes separation into individual cells without cell damage whilst reducing the dry matter of the mix (Hadziyev and Steele, 1979).

The mash mix is dried in two stages: air lift drying and fluid bed drying, both are designed for minimal cell damage. Moist mash is fed near the bottom and lifted up by a flow of hot air, thereby avoiding aggregation. The are dried from approximately 30% to 12% moisture content. The potatoes are then further dried to 6% in a fluidised bed drier. This is a porous ceramic plate or very fine mesh through which hot air is passed. The granules, suspended in air, behave like a fluid moving from one end of the bed to the other. The cooled granules are then sieved; those of particle size >80 mesh being collected as end product or add back material

Ooraikul (1978) described an alternative cooking process for granule production. After cooking and mash mixing, potatoes were frozen, thawed, granulated and then dried. Starch release was prevented by retrogradation by freezing and thawing prior to mashing. In their method, pre-cooking was omitted as greater forces were required to break down tissues into smaller aggregates causing excessive cell damage and making granulation difficult. This method does not seem to have been adopted commercially. Its end product did have a lower bulk density than conventional granules, perhaps making it less desirable for packing material. Problems with transfer of heat when freezing the mash, development of off-flavours and problems with sanitation may also have prevented the method from being used commercially (Willard and Kluge 1975).

1.9. Processes that cause firming of cooked potatoes.

As discussed previously, cooking and mashing potatoes in a factory is very different process from that carried out in the kitchen or in laboratory studies. Potatoes cooked in factories encounter greater shear and compressive forces that can lead to tissue damage. This can cause rupturing of the cell wall and an

excessive release of starch that results in a sticky undesirable texture when mashed. To avoid this, potatoes are cooked in a way which reduces the chances of damage to their structure. Prior to cooking, potatoes are pre-cooked and cooled to increase the mechanical strength and reduce cell separation (Willard and Kluge, 1975, Hadziyev and Steele, 1979).

As the cooking procedure is done over three stages, pre-cooking, cooling and then steam cooking, in this thesis it is referred to as three-stage cooking. A number of possible reasons for the increase in mechanical strength and reduction in cell separation have been reported.

1.9.1. PME activation.

Bartolome and Hoff (1972) showed that pre-cook temperatures of 50-70°C activated pectin methyl esterase (PME). PME is a wall bound enzyme that will cleave the methyl ester group from the C-6 position of galacturonan residues, resulting in an acidified galacturonan (Moledina *et al.*, 1981). PME activity in non-growing celery collenchyma and enzmically active cucumber hypocotyls caused a reduction in pectin mobility (Fenwick *et al*, 1997, Fenwick *et al*, 1999). Activating PME prior to cooking potatoes causes a restriction in softening and cell separation (Bartolome and Hoff, 1972).

PME has a two fold effect on firming; firstly, by reducing 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 (Moledina *et al.*, 1981, Ng and Waldron, 1997a). Hudson and Buescher (1986) have suggested that excessive demethylation alters the configuration of the pectic galacturonan, which contributes to loosening of the middle lamella and cell wall components, resulting in softening.

Optimisation of PME activity in potato tubers will occurs at 55°C and pH 7.6 (Puri *et al.*, 1982). Below pH 6.1 little desorption of PME from the cell wall will occur (Moledina *et al.*, 1981). However, other workers state that PME's activity

is optimal at 50°C. Denaturation of the enzyme is temperature dependent; 10 minutes at 50°C and 1 minute at 70°C (Tijskens *et al.*, 1997). Ng and Waldron (1997a) pre-cooked potatoes at 50°C for three hours and observed a drop in methylation of approximately 23% with little solubilisation of pectin.

PME activity is also dependent on the ionic conditions. Optimum PME activity was obtained with a NaCl concentration 1-1.6%; increasing this concentration has an inhibitory effect on PME activity (Moledina *et al.*, 1981). At pre-cook temperatures the integrity of the plasma membranes within cells is lost. This will allow the free diffusion of cellular constituents throughout the tissues and into the cell wall. Cations released into the cell wall, principally potassium, resulted in an increase in activity of PME. Calcium and magnesium released into the walls are able to cross-link with newly formed acid groups thereby increasing the strength of the cell wall (Bartolome and Hoff, 1972).

Ng and Waldron (1997a) pre-cooked potatoes (cv. Binjte) for three hours at 50°C to prior steam cooking. As mentioned above, pre-cooking caused little solubilisation of pectin to occur, but the degree of methylation fell from 53% to 31%. After pre-cooking, the potatoes were steam cooked and compared to steam cooked potatoes without a pre-cook treatment. A small amount of demethylation occurred during steam cooking. The dimethly sulphoxide (DMSO) soluble pectin from potatoes that had been pre-cooked prior to steaming was smaller in quantity and showed a reduction in the molecular size compared to steam cooked potatoes that had not had a prior pre-cook treatment. Despite the restriction in pectin solubilisation there was no difference in firmness of the potatoes cooked by the different treatments. The lack of firming was attributed to a lack of calcium in the cell walls to cross-link newly formed acid groups. Pre-soaking potato tissues prior to pre-cooking in 3mM calcium resulted in a 30% increase in firmness compared to steam cooked tissues (Ng and Waldron, 1997a).

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, possibly by the

formation of physical barriers that would limit the access of the PME (Hudson and Buescher, 1986).

1.9.2. Introduction of Cations into the Cell Wall.

It has been well established that calcium has an important effect on the texture of cooked potatoes. The addition of calcium into pre-cooking or cooking water results in potatoes with a firmer texture, less cell separation (Andersson *et al.*, 1992; Ng and Waldron, 1997a) and a reduction in the quantities of pectin solubilised (Moledina *et al.*, 1981). Steamed potatoes, following calcium pre-treatments, are firmer than boiled potatoes even if boiling is done in calcium, as no leaching of calcium can occur (Moledina *et al.*, 1981).

Calcium has the ability to form divalent crosslinks between acidified pectic galacturonan chains (Jarvis, 1984). Calcium cross-linked pectin is less likely to be solubilised during cooking and this will result in a stronger cell wall that which is more capable of resisting cell separation or rupturing processes. There is insufficient calcium present in cell wall 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).

Calcium has an affinity for pectin as strong as protons (Demarty *et al.*, 1984). Its small ionic radius allows it to fit into the egg-box structure, whereas ions with bigger ionic radii such as magnesium or monovalent ions, sodium and potassium, cause a repulsion of pectin chains from one another. Magnesium though, is a better competitor for pectin binding than monovalent cations (Demarty *et al.*, 1984)

Magnesium is also released into the cell wall during pre-cooking (Bartolome and Hoff, 1972, Moledina *et al.*, 1980). Conflicting reports have been published concerning the role of magnesium in texture development. Davis and Le Torneau (1967) reported that magnesium did not restrict sloughing of cooked potatoes whereas Zaehringer and Cunningham (1971) showed that magnesium restricted

cell separation, but to a lesser extent than calcium. Other research, (Haydar *et al.*, 1980 and Moledina *et al.*, 1981) suggests that magnesium restricts starch swelling by neutralising phosphate charges in the amylopectin component, but has no effect on restricting pectin solubilisation. They also showed that gelatinisation of starch during the pre-cook was the main source of cations released into the wall.

1.9.3. Restriction of Starch Swelling Pressure

Reeve (1954) observed that heating potatoes at starch gelatinisation temperatures, then cooling them prior to cooking, resulted in less starch release than when potatoes were just cooked. The potatoes showed less cell separation and were described as 'tougher' or more 'leathery' in texture. This has often been assumed to be due to retrogradation of starch during the cooling stage. During the subsequent cooking stage starch exerts less of a swelling pressure on the cell walls. This will lead to less cell separation and thus cells have a greater cohesive strength (Reeve 1972, Willard and Kluge, 1977, Hadziyev and Steele, 1979)

Starch retrogradation is the separation and crystallisation of the amylopectin and amylose phases. Retrogradation occurs on a time scale of hours and days. The lower the temperature the greater the extent of retrogradation. (Morris, 1990, Jankowski, 1992, Keetels *et al.*, 1993).

Freezing, then thawing potatoes during the production of granules, as detailed by Ooraikul (1978) would lead to retrogradation of starch. Prior to mashing, potatoes were frozen to -29°C, thawed to ~5°C, mashed and dried to produce granules. Retrograded starch released from mash resulted in less aggregation of particles, producing a better quality of granules (Ooraikul, 1977). Pre-cooking with the freeze thaw method was not required as it caused excessive damage and rupture of cells, resulting in an inferior mash (Molidina *et al.*, 1978).

Normal cooking of potatoes during the production of mash does not include a freeze-thaw step. Instead, a cooling stage of 20-30 minutes is carried out after the pre-cook step (Willard and Kluge, 1975). Hoff (1972) observed, the short cooling

time after pre-cooking left starch in a gelatinous state, so it was unlikely to have retrograded. The initial retrogradation of amylose and starch gels is accompanied by development of turbidity and increase in shear modulus, followed by longer term polymer crystallisation (Miles *et al.*, 1986). During the initial stages of cooling of potato starch a small decrease in the amount of soluble amylose occurs, which continues to fall as time progresses (Jankowski, 1992). Both these observations of the initial stages of retrogradation were attributed to aggregation, but not crystallisation of amylose polymers.

This was referred to in earlier literature as retrogradation of starch, during the cooling stage of the three-stage cooking process (Reeve 1971, Willard and Kluge, 1977, Hadziyev and Steele, 1979), but is in fact most likely to have been aggregation of amylose chains.

1.9.4. An Increase in Non Methyl Ester Linkages?

An increase in the percentage of non-methyl ester linkages in the cell walls of pea sprouts after pre-cooking has been reported (Hou and Chang, 1996). As well as demetyhlation, PME might lead to the formation of new non-methyl ester linkages by a trans-esterification reaction. It should be noted though that they utilised a non-specific method for total ester determination and the possibility that the increase in ester linkages may not have been to C-6 galacturonyl acid groups.

Quinn and 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. Jardine (1998) suggested that non-methyl ester linkages are possibly located on neutral sugars. An increase in non-methyl ester linkages during pre-cooking of potatoes could have a very important role in the firming mechanisms that occur during three-stage cooking. No evidence of an increase in non-methyl ester linkages in potatoes caused by PME has been reported.

1.10. Cell Separation during Cooking.

Softening of potatoes can occur by cell separation or cell rupture caused by breakage of the middle lamella or rupture of the cell wall respectively. Cell separation with minimal rupture is desired during mash production. During cooking, the pectin gel in the cell wall is degraded by a number of processes including β-elimination and chelation of divalent cations by organic acids (van Marle *et al.*, 1994). It is not always the case that cell separation after weakening of the wall will occur. In vegetables that contain starch, such as potatoes and peas, the turgor pressure lost during cooking is replaced by starch swelling pressure that allows separation of cells between weakened cell walls (Jarvis, 1999). Both cell wall degradation and the driving of pressures generated from starch gelatinisation are required for separation (Jarvis *et al.*, 1992).

The tricellular junction is a key area in cell separation. A single isolated cell would be spherical in shape as it allows maximum volume for surface area. In plant tissues, cells are very closely packed and a compromise prismatic shape is observed. Turgor pressure or starch swelling pressure from within cells push outwards in an attempt to form a spherical shape. This creates separation pressures at tricellular junctions. Introduction of intercellular spaces reduces cell separation forces and redistributes them to space corners (Jarvis, 1999). Examination of cooked potatoes, by TEM microscopy, showed that cell separation was initiated at tricellular junctions (van Marle et al. 1997 b).

Until recently it was thought that the only polymers present in potato tricellular junction zones were pectic substances (Goldberg et al., 1996; Jarvis, 1999). However, a recent study of potato stolons by EELS suggests that nitrogen, which is most likely protein, is very abundant in tricellular junction zones of potatoes (Marry M. unpublished results). The chemistry of pectin within these regions plays an important role in texture determination. Softening resulting from cell

separation requires the degradation of the middle lamella. Initiation of separation is dependent on the forces that pectins in the tricellular junction can bear before separation (Jarvis, 1999). Therefore, the structure in the immediate area will play a crucial role in the textural development. Bush and McCann (1999), using the 2F4 monoclonal antibody, found calcium bound pectin was located only in the middle lamella and most predominantly in the tricellular junctions of potatoes. The same has been found in apples using EELS (Huxham et al., 1999).

Calcium bound pectin, is probably predominantly found in tricellular junctions, as these are the areas where stresses from turgor pressures are greatest (Goldberg et al., 1996, Bush and McCann, 1999). Pre-cooking causes the activation of PME and the release of calcium into the cell wall (Bartolome and Hoff, 1972). An increase of calcium bound pectin in the tricellular junction would result in greater resistance to cell separation forces from within the cell.

In Chinese water chestnuts and other related vegetables adjacent cell walls are cross-linked by dimeric feruloyl esters between arabinosyl or xylose residues of arbinoxylans (Parker and Waldron, 1995). Parr et al. (1996) showed that 40% of the ferulic acid existed as dimers, which could potentially form heat stable dimers cross-links between polysaccharides. This would prevent cell separation during cooking and produces a very different texture to potatoes (Waldron et al., 1997). Ferulic acid shows considerable autofluorescence under UV light. Autofluorescent areas in Chinese water chestnuts were most abundant in the tricellular junctions of cells (Parker and Waldron, 1995). Thus the regions where cell separation forces are at their greatest in Chinese water chestnuts, the tricellular junctions, have the additional support of diferulic acid ester linkages.

In sugar beet, diferulic ester linkages are attached mainly to arabinose side chains of RG I (Marry *et al.,* 2000). The authors suggest that the feruloyl cross links are located near or in the middle lamella of sugar beet walls, further strengthening cells against separation forces further strengthening cells against separation forces.

1.11. Aims and Objectives.

The aim of this thesis was to characterise changes in the potato cell wall during pre-cooking and cooking under varied conditions and relate this to the texture of the cooked potato. These studies may potentially improve the processing conditions used in the production of industrial mashed potato.

CHAPTER TWO

MATERIALS AND METHODS

All glassware used for physical, chemical or microscipal examination was washed and cleaned in 2-5% decon, then rinsed five times in deionised water and dried. All chemicals, unless otherwise stated, were Analar grade.

2.1. Storage and Characterisation of Potatoes.

2.1.1. Storage in Glasgow.

Potatoes were stored in brown paper bags in a cold storage room at 4-5°C.

2.1.2. Storage in High Wycombe

Potatoes (cv. Maris Piper) were grown in Cambridgeshire. On arrival at High Wycombe, potatoes were transferred from brown sacks to nets. The potatoes were stored alongside potatoes used for crisping and were therefore kept at the relatively high temperature of 8-10°C. Some of these potatoes (three sacks) were transferred to Glasgow, which were then stored at 4-5°C in a cold storage room.

2.1.3. Specific Gravity Measurements Made in the Laboratory

Potatoes were taken from the cold room, washed and equilibrated to room temperature overnight. Whole potatoes were pierced and hung underneath a digital scale by a piece of string. The weight of the tuber in the air was recorded. A large beaker of deionised water was raised from below until the tuber was just fully immersed in the water and the weight of the tuber in water recorded. The specific gravity of potato was calculated from the weight of the tuber in air and water using Equation 2.1 from Burton (1989). From the specific gravity of the tuber, the percentage starch content and dry mater were calculated using Equations 2.2 and 2.3 from Burton (1989).

$$\text{Specific Gravity}_{(\text{tuber})} = \text{Specific Gravity}_{(\text{water})} + \frac{\text{(Weight of tuber in water)}}{\text{(Weight of tuber in air)}}$$

Equation 2.1. Determination of potato specific gravity.

$$\% \text{ Starch of Fresh potatoes} = 17.55 + 199\,(SG - 1.098)$$

Equation 2.2. The percentage starch content of fresh potatoes calculated from its specific gravity.

$$\% \text{ Dry matter} = 24.18 + 211\,(SG - 1.098)$$

Equation 2.3. The dry matter content of potatoes calculated from its specific gravity.

2.1.4. Specific Gravity of Potatoes used in Pilot Plant.

The specific gravity of the potatoes was measured by weighing 3.2 kg of cleaned, unpeeled potatoes into a basket with a handheld scale attached to it. The potatoes were immersed in a tank of water and the weight taken again. The handheld scale

had a scale of specific gravity calculated from Equation 2.1 giving an immediate value for specific gravity. The specific gravity was measured this way in triplicate.

2.2. Cooking of Potatoes in the Laboratory.

2.2.1. Apparatus Used.

Sliced potatoes were steam cooked in a domestic steamer that consisted of three separate units. The first was a boiling pan where water was boiled producing steam. The 'boiling unit' was filled to a depth of 1000ml with deionised water and boiled with a Bunsen burner. Above this potato slices were steam cooked on a tray that fitted on top of the boiling pan. The cooker came with a lid that prevented steam escaping.

Pre-cooking was carried out in a beaker of 300ml of deionised water that was heated in a water bath to the required temperature. The volume of water in the bath was kept constant so as that rate of temperature equilibration between the surrounding water and the pre-cook water was kept constant. After pre-cooking, the potatoes were removed from the pre-cook water and cooled in a pan containing 3L of deionised water.

2.2.2. Preparation of Potatoes.

For each cooking experiment four to five potato tubers (depending on their size) were used. Potatoes of roughly the same size were chosen, hand-peeled and sliced. To minimise enzymatic activity, potatoes were prepared immediately after being taken from cold storage. Slices were cut to a uniform thickness of 5mm (+/- 0.5mm). One hundred grams, the amount used for cell wall preparations, was sub-sampled for cooking and rinsed with deionised water to remove starch from their outer surface. Between preparation and cooking, the slices were immersed in

deionised water to prevent dehydration or enzymic degradation. The time between preparation and cooking was kept to a minimum.

2.2.3. Protocol for One-Stage and Three-Stage Cooking.

2.2.3.1. One-stage cooking.

A tray holding 100g of potatoes were placed in the second compartment above the boiling water, the lid was replaced and the potatoes were steamed for twenty minutes. After cooking the potatoes were allowed to cool to room temperature and analysed as required.

2.2.3.2. Three-stage cooking with a seventy degree pre-cook (three-stage (70°C) cooking).

Slices were immersed in a beaker containing 300ml of deionised water, which had been preheated to 70°C in a water bath and heated for fifteen minutes. After pre-cooking, the pre-cook water was drained off and if required for analysis quantitatively made to 500ml and frozen. The heated potatoes were then cooled in 3l of deionised water for twenty minutes, a 250ml aliquot of which was, if necessary, kept. Potatoes were then steam-cooked in the steamer as described in 2.2.3.1.

2.2.3.4. Three-stage cooking with a fifty-five degree pre-cook (three-stage (55°C) cooking).

Conditions for pre-cooking, cooling and steam cooking were as above except the pre-cook water temperature was 55°C and potatoes were pre-cooked for forty minutes.

2.2.3.5. One-stage and three-stage cooking in 5g/l Ca or Mg salt solutions.

Sample preparation, cooking times and temperatures were the same as those detailed above. In place of deionised water 5g/l Ca^{2+} or Mg^{2+} salt were solutions (from calcium or magnesium chloride salt) were used for the pre-cook and cooling waters during three-stage cooking. One-stage cooked tissues were immersed in 300ml of the appropriate ion solution at ambient temperatures for two hours prior to steam cooking.

2.2.4. Determination of One-Stage and Three-Stage Cooking Times.

2.2.4.1. One-stage cooking.

Potatoes were steam-cooked for ten, fifteen and twenty minutes. The texture was assessed by wedge test measurements. Twenty minutes of cooking resulted in the mechanical strength of slices to almost half in comparison to undercooked potatoes cooked for ten or fifteen minutes.

2.2.4.2. Three-Stage (70°C) cooking times.

The pre-cook time was evaluated by measuring the texture of the subsequently steam-cooked potato. Potato slices were pre-cooked for ten, fifteen or twenty minutes. The potatoes were then cooled for twenty minutes and then steam cooked for either ten or twenty minutes. The pre-cook time was chosen as the time required to produce potatoes that needed the greatest cutting force compared to one-stage cooked potatoes. This was assessed by wedge test measurements.

2.2.4.3. Three-stage (55°C) cooking times.

Potato slices were pre-cooked for twenty or forty minutes then cooled and steam cooked. The length of both the cooling and steam-cooking steps was twenty

minutes. The texture of three-stage (55°C) potatoes was evaluated by wedge test and cell separation measurements.

2.2.5. Measuring the Pre-Cook Water Temperature Before and After the Addition of Slices.

A mercury thermometer was placed into the pre-cook water and the temperature of the water was measured in increments of thirty seconds before and after the addition of 100g of potato slices.

2.2.7. Measurements of the Starch Gelatinisation Front of Slices During Steam Cooking.

Measuring the extent of starch gelatinisation over time was used to follow the temperature within a steam cooked potato slice. Potato starch gelatinises between 58 and 64°C (Lamberg and Olsson, 1989), thus when starch had gelatinised the internal temperature of the potato must have reached this temperature range. When starch gelatinises, it is easily identified as it has a translucent appearance. Slices were steamed for up to two and a half minutes then cooled. The tissues were split apart and the extent of starch gelatinisation was observed.

2.2.8. Determination of the Role of the Cooling step in Three-Stage Cooking.

Three-stage (55°C) and three-stage (70°C) cooking was carried out with or without an intermediate cooling step. If no cooling step was included the potatoes were referred to as two-stage* (55°C) or two-stage* (70°C) cooked potatoes. The texture of the cooked potatoes was evaluated by wedge test and cell separation measurements.

2.3. Pilot Plant Cooking.

2.3.1. Preparation of Potatoes.

Potatoes were fed into a rotating drum. The sides of the drum were lined with coarse sandpaper that removed the bulk of the peel. A hose supplying water into the drum washed the potatoes free of peel and dirt. Any remaining peel or eyes were removed by hand peeling.

Potatoes were fed into a revolving drum which threw the potatoes through a set of fixed blades producing chips with an average cross sectional area of 8x8mm, though size could vary somewhat. To restrict enzymic activity and dehydration, peeled and chipped potatoes were kept immersed in cold tap water until used.

2.3.2. The Cooking Apparatus.

Pilot plant cooking was carried out using the Polar System cooking apparatus. This consists of three large stainless steel tanks, one for each stage of cooking connected together as tank I, II and III (Figure 2.1). For three-stage cooking potatoes were loaded into tank I and pre-cooked at either 55°C or 70°C. After pre-cooking the potatoes were transferred into tank II to cool. After cooling the potatoes were finally transferred into tank III where they were steam-cooked. One-stage cooking involved just steam cooking the potatoes in tank III (Figure 2.2).

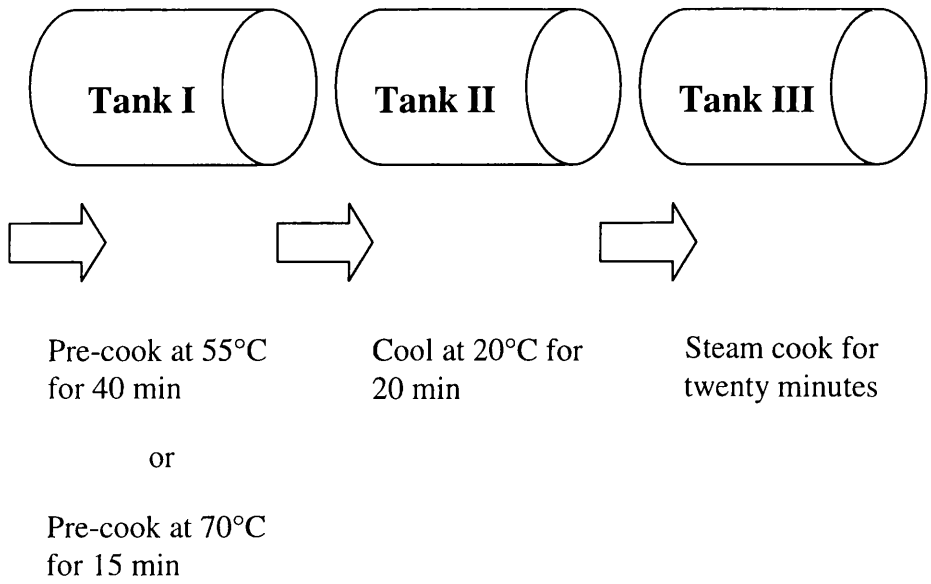


Figure 2.1. Three-Stage cooking using the pilot plant. Chips were fed into tank I pre-cooked at 55°C or 70°C, cooled in tank II before being steam cooked in tank III.

Tanks I and II (the pre-cook and cool tanks) both held 1,600l of water. Pre-cooking in tank I was carried out at 55°C or 70°C; steam at 3.2 bar was delivered to the bottom of the tank heating the water to the required temperature. The temperature of the tanks was thermostatically controlled. The third tank where steam cooking took place was smaller than the previous two tanks and was only half filled with water during cooking. Like tank I, it was heated by a steam source at 3.2 bar delivered into the water at the bottom of the tank. As the tank was only half filled the potatoes were not immersed in water and were cooked by the steam rising out of the water.

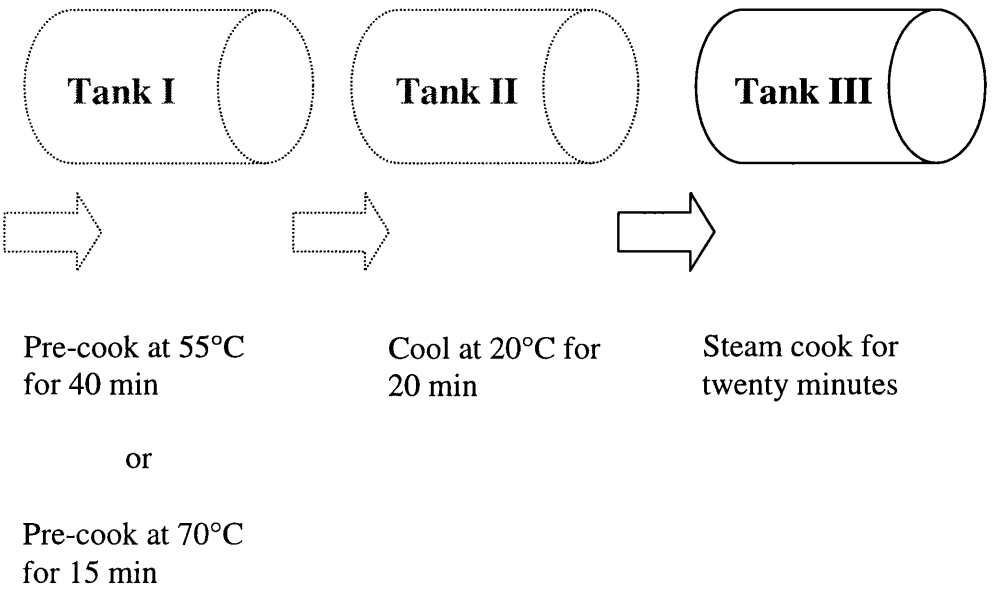


Figure 2.2. One-Stage cooking using the pilot plant. Chips were fed into tank III and steam cooked.

2.3.3. The Pilot Plant Cooking Protocol.

One-stage and three-stage cooking was carried out using the same cooking times as the lab-scale cooking process (Section 2.2.3). The Polar cooking apparatus was designed for potatoes to be cooked in a continuous manner. Within each tank was a rotating series of drums. Each drum had a set of 'shelves' attached to it. Approximately one kilo of chipped potatoes was fed on to each shelf as the drum rotated. Controlling the rate of the drums' rotation, controlled the residence time for the chips in each tank and thereby the overall cooking time. Unfortunately the mechanism to control the rate of the drums' rotation in tank III malfunctioned at high temperatures expelling the chips immediately. As a result of this potatoes had to be cooked in a batch process.

The first tank to be used (either tank I or III) was filled as quickly as possible with chips as the drums ran continuously. The shelves were loaded with chips until the first batch came out the other end of the tank. The drums were then turned off for

the cooking time required before being turned on again to empty the potatoes from the tank. For three-stage cooking, the chips were then transferred from tank I to tank II. With the drums running continuously, the chips from tank I were transferred to tank II, where they were cooled. After cooling the potatoes were then transferred, in the same manner, to tank III where they were steam-cooked.

Approximately 2.5kg of potatoes were used for each cooking treatment. The bulk of the cooked potatoes were mashed and used for sensory and physical measurements of texture. Some cooked chips and sub-sampled pre-cooked and cooled chips were used for physical texture measurements and examination of the tissue structure by microscopy.

2.3.4. Mashing of the Cooked Potatoes.

Cooked potatoes were mashed, whilst hot, by gently forcing the potatoes through a 3mm perforated plate by a motor driven piston.

2.3.5. Storage of Cooked Material.

Raw, pre-cooked/cooled and cooked chips were placed on plastic trays and cooled to room temperature if required. The chips were placed in foil bags, sealed and stored at 4°C. The mashed potatoes were stored in plastic containers (ice cream tubs) at 4°C. Physical texture of the chips and mash was measured the following day. The sensory panel also assessed the mashed potatoes from each treatment the following morning. Microscopy was carried out two days after cooking.

2.4. Points where Analysis of One-Stage and Three-Stage Cooked Potatoes Occurred.

Potatoes, cooked in the laboratory or pilot plant, were used to investigate physical and chemical changes that occur during one-stage and three-stage cooking. Measurements were made at four strategic points during cooking. The first was

without any heat treatment i.e. uncooked potatoes (raw). The second was after the pre-cooking and cooling of the potato at 55°C or 70°C (two-stage (55°C) or (two-stage (70°C) cooking). The third was after three-stage cooking with either a pre-cook at 55°C or 70°C (three-stage (55°C) or (three-stage (70°C) cooking), and the fourth was after one-stage cooking (one-stage cooking).

2.5. Texture Measurements.

The texture of potatoes was measured by methods slightly adapted from Freeman *et al.* (1992). Two parameters were measured, the mechanical strength of the potatoes when cutting with a wedge cutter and the degree of cell separation.

Measurements were done on the central pith area of slices. Results were statistically analysed by ANOVA (TUKEY) with the Minitab 10 statistical software package. Analyses with large differences between data populations were logged to the base 10 before statistical analysis.

2.5.1. Manual Wedge Test

The Freeman *et al.* (1992) method measured mechanical strength by cutting a potato with a taut wire. Raw and pre-cooked and cooled slices were too tough to be cut with a wire. Instead a wedge shaped cutter was used instead. Cutting with a wedge shaped blade records the force required to propagate a stable crack within a potato (Vincent *et al.*, 1990). The angle of the wedge was 18°. Unlike the Freeman *et al.* (1992) method, which recorded the average point during cutting, it was felt that recording the average was force too subjective a measurement especially when cutting cooked material, which was a rather rapid process. Instead the peak force during cutting was recorded. Apart from that, the apparatus was the same as described in Freeman *et al.* (1992).

Slices after different stages of cooking were cut to a width of 2cm and placed on a microscope slide. A 1mm deep notch was cut into the surface of the raw and two-stage cooked tissue to reduce the initial force of penetration. The slice was

then placed on an analogue weighing scale. A jack slowly pushed up the slice until contact was made with a sharp metal wedge. The upward movement was maintained forcing the slice to be cut by the wedge. The maximum weight recorded during cutting the slice was converted into Newtons (N) using Equation 2.4 and was recorded as the maximum force required whilst cutting through the tissue.

$$\text{Maximum Force Recorded} = \frac{\text{Weight recorded in grams x } 1.02}{100}$$

Equation 2.4. Conversion of grams into Newtons (N).

2.5.2. Cell Separation.

The cell separation in the Freeman *et al.* (1992) paper was devised so that cell separation could be measured as a parameter independent of firmness measurements. The method used here was slightly adapted for ease and reproducibility but the underlying principle behind it was the same.

A 2cm diameter core was cut from cooked slices and crushed to half its original height. Crushing in this way is independent of the physical force applied and therefore the degree of cell separation can be measured as an independent parameter (Freeman *et al.*, 1992). The separated cells were washed with a gentle flow of deionised water through a 1mm sieve into an adapted graduated centrifuge tubes that had a filter funnel attached to its neck. The separated tissues were allowed to settle for half an hour. The degree of cell separation was determined as the volume of separated cells at the bottom of the tube.

2.5.3. Evaluation of the Role of Starch Swelling Pressure in Cell Separation During Cooking.

Potatoes were boiled in different concentrations of ethanol or ethylene glycol. This was done to restrict the amount of available water during cooking, which in turn restricted the amount of water available for starch gelatinisation during

cooking. The effect of subsequently gelatinising the starch on cell walls, weakened by cooking, was investigated.

One centimetre square potato discs of 1mm thickness were boiled for up to an hour in differing concentrations of ethanol (0%, 50%, 70%, 80% and 100 %) or ethylene glycol (100%). The discs were then cooled and transferred in to 5ml of deionised water. The discs were then incubated for twelve minutes at 75°C or at room temperature. The extent of cell separation was evaluated by Vortex Induced Cell Separation method (VICS), Parker and Waldron (1995). The discs were vortexed for one minute and then shaken to a count to ten; the scoring system used was slightly different from the published Parker and Waldron (1995) method.

2.5.4. Texture Measurements Made on Pilot Plant Cooked Potatoes Using the Stable Micro Systems (SMS) Device.

Chips and mashed potato samples, prepared as described in section 2.3, were brought out of the fridge and left to stand for a minimum of an hour to equilibrate to room temperature before being measured. Measurements were made using a Stable Micro Systems (SMS) TA.XT2*i* tensile testing apparatus. The data was recorded using SMS Texture Expert 2.0 software package.

2.5.4.1. Penetrative force to cut through raw, two-stage, and three-stage cooked potato.

Chips from each cooking treatment were cut with a wedge shaped blade at a rate of 10mm/s to a depth of 6mm (average chip depth was 8mm). The angle of the wedge was 30°. Due to the speed of analysis, the large population size and rather imprecise cooking conditions, approximately 60 replicates were measured for each treatment. The maximum force of the initial peak, the mean plateau force and the total energy required to cut through raw and two-stage cooked slices were recorded. The maximum cutting force of one-stage and three-stage cooked potatoes was measured.

2.5.4.2. Penetrative force to plunge through mashed potato.

A 10mm core of mash was gently placed in a small beaker of (width and depth both 10mm). A small metal cylinder of 8mm diameter was plunged though the mash to a depth of 8mm at a rate of 10mm/s. Again, approximately 60 replicates were taken for each mash treatment. Then average force of the probe passing through each mash was recorded.

2.5.4.3. Sensory analysis of mashed potatoes.

Sensory analysis was carried out and the data analysed by the sensory research section at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe, headed by Dr. Zoe Cselik. Samples of the three mash treatments were assessed twice on consecutive weeks and under code.

The taste panel consisted of a trained and experienced panel of part time assessors. Samples stored at 4°C were re-heated prior to being presented to the taste panel. Approximately 500g of mash was placed into a 23x18x5 cm Pyrex dish and spread evenly over the base of the dish and the dish was covered with a lid. The sample was re-heated in a 650W microwave oven (Panasonic NN-500) for two minutes on full power. The mash was stirred, heated for a further minute, allowed to stand for a minute and then served to the panel.

2.6. Isolation of Isolated Cell Walls and Solubilised Pectin.

Cell walls were isolated from raw and cooked tissues using the Jardine (1998) method. The method was designed for the isolation of uncooked and freshly cooked/processed potatoes. In chapters four and seven the isolation was carried out on frozen cooked materials. This required slight alterations to the original procedure.

2.6.1. The original protocol.

The purpose of this method is to extract the primary cell walls of plants in as intact a form as possible. The method was developed primarily for use on potatoes, though it can be used to isolate other primary cell walls. An integral part of this procedure is the removal of starch. The method allows for the recovery of solubilised pectin, the removal of starch and prevention of enzymic degradation during extraction. To prevent enzymic degradation, the initial steps were carried out in ice cold conditions if the plant material had not been enzymically denatured e.g. by heat treatment.

The whole procedure was carried out in a mixed cation buffer (MCB) that was intended to mimic the ionic conditions of the apoplast. MCB was used to reduce swelling of cell wall and starch polysaccharides and to restrict solubilisation of cell wall polymers due to rapid changes in ionic conditions. The MCB salt concentrations were derived from Goldberg *et al.* (1996) and are listed in Table 2.1. The pH of the MCB buffer had previously been measured as between 6.8 and 7.2 (Jardine W.G. unpublished results).

Salt	Conc. (mM)
Sodium Acetate	10
Potassium Chloride	3
Magnesium Chloride	3
Calcium Chloride	1

Table 2.1. Salt concentrations of the Mixed Cation Buffer (MCB).

One hundred grams of tissue was blended in 300ml of MCB-triton X-100 detergent to disrupt the cell walls and solubilise cytoplasmic material. To prevent the detergent foaming a small amount of octanol (approximately 5ml) was added prior to blending. The tissue was then filtered through a 54μm sieve, which retained primary cell walls, and the washings were collected to retain any

solubilised pectin. Washings were collected in two fractions. The first 500ml of washing comprised mainly of the MCB-triton mixture and washings. The second was the remaining collected washings. Washing was carried out until the filtrate ran clear. One and a half litres, in total, was usually sufficient.

If the original tissues were enzymically active after filtering the samples were placed in saturated phenol (~80%) to denature their enzymes (Newman *et al.*, 1994). The material was stirred for a minimum of 30 minutes in phenol, then washed through a grade 3 glass sintered glass funnel.

To allow access for starch removing enzymes in the subsequent starch removal step, the material was frozen in liquid nitrogen and milled in a domestic coffee grinder (Braun KSM 2) This was done to break up the physical structure of the cell wall and was referred to as cryo-milling. In uncooked potatoes, starch is in a crystalline form and is difficult for enzymic hydrolysis to occur. Prior to incubation uncooked material was stirred in ~60ml of boiling MCB for approximately 40 seconds and then immediately transferred to ~600ml of chilled MCB. This was done to gelatinise starch with minimal heat degradation of cell wall polymers.

Material with gelatinised starch was then incubated with α-amylase, (Sigma typeVI-B from porcine pancreas). Excess α-amylase was used (approximately 1g which contains 13,400 units, where 1 unit will liberate 1mg of maltose from dissolved starch in 3 minutes at 20°C), as the exact amount of starch, which was to be removed, was unknown. Pullulanase (*Klebsiella pneumonia* sigma type P-5420) was used to remove the amylopectin branch points (Selvendran and O'Neill, 1987). 0.03• l was added, which contains 0.4 units, where 1 unit will liberate 1• mole of maltotriose from pullulan in one minute at 25°C. Approximately 4-5ml of toluene was added to prevent microbial degradation. The enzymic incubation was usually carried out at 25°C overnight on an orbital shaker. Enzymic removal was found to be a more rapid method than incubating in 90% DMSO (Jardine 1998). Removal of starch was verified by staining a small

aliquot with KI/I_2 (1%KI, 0.5%I_2). If starch was still observed, then additional α-amylase and pullulanase were added

After starch removal was complete, the material was centrifuged. An aliquot of the supernatant was retained and the pellet re-suspended in 50% acetone and filtered through grade 3 glass sinter funnel. Water was removed from the cell wall structure by with acetone in a sequential series of increasingly concentrated solutions of acetone (2x50%, 70°C%, 90% then 3x100%). The material was then stored in a desiccator.

2.6.1.1. Collection of solubilised pectin.

Washings from uncooked samples were centrifuged to remove starch grains. Aliquots from cooked samples were incubated until starch removal was complete. After starch was removed an aliquot was precipitated in 80% ethanol (Jarvis 1988). 40ml aliquots were precipitated from the first 500mls of washing and 200ml from the second washing fraction and enzyme liquor. The precipitated material was re-suspended in deionised water to a volume of 25ml. The material was then frozen with a layer of toluene covering it to prevent microbial degradation.

2.6.2. Modification of the protocol for isolating cell walls from a large number of raw samples (chapter 4).

Samples were blended in MCB-triton detergent and filtered through the 54μm sieve. Enzymes were denatured in saturated phenol and then the cell walls' physical structure was disrupted by cryo-grinding. Samples were then stored in a deep-freeze. At a later date, selected samples were removed from the deep freeze and starch gelatinisation, starch removal and sequential drying of samples was carried out.

One and three-stage cooked samples, which had been used for wedge test measurements, were also stored in the deep freeze. Cell walls were isolated from

selected samples at a later date. An ultra pure deionised water extraction was attempted in three-stage cooked samples to quantify methanol released by PME activation. This proved unsuccessful and was not attempted on one-stage cooked samples. Removal of starch from three-stage cooked samples had been very difficult. To aid starch removal from one-stage cooked samples, a boiling step was included to gelatinise starch prior to incubation with α-amylase and pullulanase.

2.6.3. Modification of the protocol for frozen samples (chapter 7).

After cooking samples were immediately frozen in liquid nitrogen to restrict starch retrogradation. The samples' cell walls were then isolated as discussed in section 2.6.1. A starch gelatinisation step was included for all samples to improve starch removal efficiency.

Washings from raw and pre-cooked samples were heated for two minutes in a microwave then immediately chilled in a bucket of iced-water. Starch was then removed using α-amylase and pullulanase, as discussed above.

2.7. Chemical Characterisation of Isolated Cell Walls.

2.7.1. Residual Starch of the MCB-insoluble material.

The protocol was based on the enzymic method used by Batey (1982) and Karkalas (1985). The starch is simultaneously gelatinised at 94°C and hydrolysed by a thermostable α-amylase. The maltose released is then hydrolysed to form glucose using amyloglucosidase. The free glucose released is oxidised/ perooxidised and forms a coloured compound with o-dianisidine.

To a screw cap pyrex tube, 25mg of cell wall, 5ml water, 0.1ml of tris buffer and 0.1ml of thermostable α-amlyase was added and boiled for one hour. After

cooling the tubes were centrifuged. 1ml of the supernatant (aliquot was diluted to 1ml if necessary) and 0.2ml amyloglucosidase from *Aspergillus niger* (diluted to 45 units/ml, where 1 unit liberates 1mg glucose from starch in 3 minutes at pH 4.5 at 55°C) were incubated for 2.5 hour to produce glucose. Glucose standards of 10, 20, 30, 40, 50, 50, 60, 70°C, and 80mg/l were prepared. To the samples and standards 3.5ml of glucose oxidase/peroxidase/o-dianisidine mixture was added and incubated at room temperature in the dark for fifty minutes.

The mixture contained:

65 mg (containing 15,000 units) of Glucose oxidase type II from *Aspergillus niger*, Sigma, where 1 unit will liberate 1mg of glucose from starch in 1 minutes at pH 5.1 at 35°C.

3.5 mg (containing 300 units), of Peroxidase Type 1, from horseradish, Sigma. One unit will liberate purpogallin from purpogallin in 20 seconds at 20°C

50mg of O-dianisidine

The glucose is oxidised to gluconic acid and hydrogen peroxide produced. In the presence of peroxidase the o-dianisidine oxidised to produce a red colour. The absorbance of the samples was measured at 455nm. Samples were measured in triplicate in 4.5ml disposable along with appropriate blanks.

2.7.2. Dry Matter Content of the MCB-insoluble material

30mg of MCB-insoluble material was weighed (to the nearest 0.1 of a milligram) into an oven dried pre-weighed porcelain crucible. The material was then dried in an oven at 100°C for a minimum of twenty-four hours. The material was weighed, then replaced in the oven for another three hours and reweighed. This was repeated until the reading was stable.

2.7.3. Galacturonic acid determination.

Pectic galacturonic acid was measured using the Jardine (1998) method, which was slightly modified from the Filsetti-Cozzi and Carpita (1991) method.

2.7.3.1. Cell wall hydrolysis.

Cell walls were hydrolysed by Saeman hydrolysis according to the Englyst and Cummings (1984) method. 10mg of MCB-insoluble material were hydrolysed in triplicate in 0.2ml of 72% sulphuric acid for 1hr at room temperature. 2.4ml of deionised water was added to the material, which was then heated for 3hr at 100°C.

2.7.3.2. Measurement of galacturonic acid.

To a wide mouth boiling tube, 1ml of solubilised cell wall was added. 5ml of concentrated sulphuric acid (containing 0.0125M sodium tetraborate) 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 pipetted into a series of tubes. Care was taken to ensure that the acid hit the centre of the liquid so that the heat of dilution would drive the reaction. Once cool, 0.2ml of 0.15% (w/v) m-hydroxydiphenyl (MHDP) in 0.5%(w/v) NaOH was added and the sample was vortexed. Blank samples were used to correct for neutral sugar interference, 0.2ml of 0.5%NaOH was added minus MHDP. After the colour had developed but no longer than 20 minutes after the addition of MHDP the absorbance was measured at 525nm in 4.5ml cuvettes with the appropriate blanks.

Determination of soluble pectin was carried out by pipetting 1ml of the redissolved pectin into boiling tubes. The procedure was then continued as described above.

2.7.4. Methyl ester determination.

The degree of methylation was measured using a slightly modified version of Klavons and Bennett (1986) method. Methyl ester groups were cleaved from pectic chains by saponification releasing methanol. Alcohol oxidase was used to oxidise methanol to formaldehyde, which then forms a coloured complex with pentandione.

MCB insoluble material was weighed (12mg) into centrifuge tubes. 6.25ml of water and 1ml of 1M KOH was added and the walls were saponified for an hour at room temperature. The sample was then neutralised with 1ml of 0.49M H_2PO_4. Blank samples were carried through the experiment to correct for any free methanol contamination. As controls, H_2PO_4 and KOH were added in the opposite order from the other samples hence maintaining the same ionic conditions as true samples without cleavage of ester groups. Methanol standards in the range of 2, 4, 6, 8, 10, 12, 14, 16μM were made up in a phosphate buffer pH 7.5 (0.2M monobasic and 0.2M dibasic sodium phosphate).

The samples were centrifuged for five minutes and 1ml of supernatant containing methanol released. Any required dilutions were made to the supernatant prior to incubation in alcohol oxidase. The sample, blanks, and standards were then incubated at 25°C with 1ml of alcohol oxidase (1unit/ml) from *Pichia pastoris*, (Sigma, where 1 unit oxidises 1μmole of methanol to formaldehyde at pH 7.5 at 25°C). 2ml of 0.02M 2,4-pentandione (in 2.0M ammonium acetate and 0.05 acetic acid) was added and samples, blanks, and standards were incubated 60°C for 15 minutes. The solutions were cooled and the absorbance was measured at 412nm in 4.5 ml disposable cuvettes along with appropriate blanks.

2.7.5. Non-methyl ester linkages determination.

Non-methyl esters were measured by the Keijbets and Pilnik (1974) method with slight modifications. Copper chemically bound to galacturonic acid groups on pectin are removed with excess acid. The copper content of the filtrate gives a

value of galacturonic groups present in the cell wall. Copper bound to unsaponified cell walls gave a value for galacturonic acid units with a free uronic acid group i.e. the non-esterified galacturonic acid content. Saponification of the walls removes all ester groups from the cell wall. Copper bound to saponified galacturonic acid gave a total value of galacturonic acid groups within the cell wall.

Using these two values the total esterified galacturonic acid content can be calculated using Equation 2.5. Using this value and the methyl ester content from the Klavons and Bennett method the non-methyl ester value can be calculated (Equation 2.6).

75mg of MCB-insoluble material were weighed into a small grade 3 glass sintered funnel in triplicate. To this was added 30ml of 2% copper sulphate. After 30 minutes the copper was filtered off and another 30ml of 2% copper sulphate was added. Physically bound copper was washed away with deionised water. Removal was ensured by measuring the conductivity of the filtrate. When the filtrate had a reading of < 20μS the washing was considered complete. Chemically bound copper was subsequently removed with 1M nitric acid in 60% ethanol (the filtrate was collected into clean glassware). The filtrate was made to 50ml in a volumetric flask and stored.

Total Esterified Gal A = Total Gal A – Non-Esterified Gal A

Equation 2.5. Total ester content of cell wall galacturonic acid

Non-Methyl Esterified Gal A = Total Esterified Gal A - Methyl Esterified Gal A

Equation 2.6. Non-methyl esterified galacturonic acid content

The MCB-insoluble material was then saponified with 30ml of 0.1M NaOH in 60% ethanol for one hour and then neutralised in acetic acid buffered to pH 5.6 in 60% ethanol. The process of copper additions, washing away physically bound

copper, then chemically bound copper was then repeated. The chemically bound copper was again filtered into clean glassware and made up to volume in 50ml volumetric flasks.

The filtrates were analysed for copper using atomic absorption spectroscopy (AAS). The samples were given a 1 in 10 dilution; thus standards were made up in 4% ethanol to ensure samples and standards were of the same matrix.

2.8. Chemical measurements of total ion levels in potato tissues.

Cooked and raw potatoes for analysis were stored in a freezer at –18°C. 10g of frozen tissues were weighed into long Kjeldahl digestion tubes; 10ml of concentrated nitric acid was added and samples were left overnight. The following day the samples were boiled at 120°C for 3hrs, cooled and quantitatively filtered through Whatman no.50 filter papers. Samples were made up to 100ml in volumetric flasks and diluted to fit in 0-1 ppm Ca or 0-5 ppm Mg concentration range. Samples were measured by AAS using a nitrous oxide flame. KCl solution was added to samples and standards, at the dilution stage to ensure a concentration of 0.01% KCl in the matrix to avoid interference from phosphates.

2.9. Microscopy Examination of Raw and One-Stage Cooked Cara and Golden Wonder Potatoes

2.9.1. Fixation and Resin Embedding

Cores of 2mm diameter were taken from raw and one-stage cooked Cara and Golden Wonder potatoes. The tissues were fixed for two hours in 2.5%

gluteraldehyde in 0.1M phosphate buffer saline (PBS) (40.5 ml of 0.2 M solution of dibasic sodium phosphate with 9.5 ml of 0.2 M solution of monobasic sodium phosphate diluted to 100 ml with deionised water) The tissues were then dehydrated in a consecutive series of incubations of 30%, 50%, 70%, 90% and 100% ethanol (EtOH). The tissues were then left overnight in 100% EtOH and LR White (1:1) (Wells 1985) resin in a rotator. The following day the tissues were placed in fresh LR White and left on the rotator. At the end of the day, the sections were placed in fresh LR White and again left on the rotator overnight. The following morning, the tissues were embedded in polypropylene capsules and polymerised. As the sections were not intended for immnocytochemical work, polymerisation was carried out in an oven at 60°C for twenty hours.

2.9.2 Sectioning and Examination

Sections of approximately 60nm thickness were cut on a LKB III ultramicrotome and picked up on a 100 hexagonal mesh nickel grids. Thicker sections of 1µm sections were taken for light microscopy examination and stained with toludine blue. The 60nm sections were examined at zero-loss energy, which is when elastic electrons are not viewed in the final image plane (Sorber, 1993), which improves the contrast of the image. The microscope used was a Zeiss (LEO) TEM 902 operating at 80 kV and at 12,000 times magnification with a beam current of 125 x 10^{-8}A in a vacuum of 7 x 10^{-7} Torr.

2.10. Electron Energy Loss Microscopy (EELS)

EELS involves the analysis of the energy distribution of initially monoenergetic electrons after they have interacted with the specimen (Egerton, 1986). Electrons, when passing through a specimen, can interact with the specimen's atoms in a number of different ways. Most electrons pass though the specimen without interacting at all and are registered beyond the specimen as zero-loss electrons. Some interact with the nucleus and are deflected over large angles with virtually no loss of energy (Sorber, 1993). This is referred to as elastic scattering.

If the electron interacts with a specimen atom's shell electron, the incident electron is scattered over a smaller angle and is subject to energy loss. This second type of interaction is referred to as inelastic scattering. The energy losses encountered by the incident electrons inelastically scattered are element specific. This specificity is used for EELS-analysis (Sorber, 1993). Inelastically scattered electrons are selected by increasing the energy of the incident beam by the exact energy-loss of the electron that interacted with the element to be analysed. This allows specific inelastically scattered electrons to pass though an electron filter. The intensity of the electrons at this energy is then recorded. The electron beam goes through an energy range producing a spectrum of electron intensity over that range. Due to other elements having energy edges a range of energies were selected and the energy signal at each step were recorded (Egerton, 1986). The data was recorded as image EELS.

Using EELS, the distribution of calcium in cell walls was analysed. Images prior to and after the energy sequences was complete were taken and used as pre- and post-acquisition reference images, which were used to correct any electron beam drift while the sequence was taken (Egerton, 1986). The energy loss spectrum was then used to produce an elemental map of the image. By saturating specimens in calcium prior to analysis the distribution of galacturonic acid units could also be detected. Specimens were 'doped' with calcium by placing grids, section side down, onto droplets of 5mM calcium acetate for one hour. The calcium acetate had been centrifuged to remove any insoluble salt crystals. After the hour incubation, the specimens were washed by placing the grid on three successive droplets of deionised water.

Electron-spectroscopic analysis of the doped and undoped specimens was carried out using a Zeiss (LEO) TEM 902. The operating conditions were the same as those detailed in Section 2.9.2. Spectroscopic image sequences containing a 3-eV energy slice over the energy-loss range (ΔE = 305-375 eV) were collected from LR White resin-embedded sections on nickel grids. The electron-energy loss regions of organic compounds of interest in a plant cell wall have been previously characterised Huxham *et al.* (1999), including the carbon K-edge (E = 285 eV)

and the calcium L2, 3 edge (E = 345 eV). Sequences were analysed off-line using ESI vision analysis software to produce elemental maps for calcium distribution.

2.11. Imunogold Labelling and Imaging of Samples.

The monoclonal antibodies JIM 5 and JIM 7 recognise pectin epitopes of relatively low and high methyl esterification respectively (Knox *et al.*, 1990). They were used to elucidate demethylation patterns caused by PME activity during pre-cooking. Dr Mazz Marry kindly donated the monoclonal antibodies used. Resin embedded grids were labelled with both antibodies and 10nm gold conjugate secondary antibodies (British Biocell) and visualised under the electron microscope, conventional zero-loss and energy filtered images were collected.

2.11.1. Low Temperature Embedding of Raw and Pre-cooked Isolated Cell Walls

Dr. Mazz Marry kindly carried out the resin embedding detailed below. MCB-insoluble cell wall material was fixed overnight with 2.5% gluteraldehyde in 0.05M sodium cacodylate, adjusted to pH7.2, prior to embedding in agarose gel. The blocks of agarose-embedded material were cut into small cubes (92mm x 2mm x2mm) and fixed for 1hr as before. The fixed blocks were placed in tissue handling devices to allow easy transfer from one solution to another (Wells, 1985). Samples were processed within a low temperature-embedding box, in which the temperature was regulated by electronic control of a flow of liquid nitrogen. Samples were dehydrated with consecutive 1hr incubations in 30%, 40%, 70%, 90% and 100% ethanol. Dehydration was conducted at 4°C for the first step, -20°C for the second and then at -35°C for the remaining steps. Samples were then infiltrated in LR White resin containing 0.5% benzoin methyl ether using a series of 1hr incubations in resin:ethanol mixes of 1:1, 2:1 and 3:1, each at -20°C. Fresh resin was then added and incubated for a further 8hrs at -20°C. The samples were then transferred to pre-cooled resin filled BEEM

capsules at -20°C and polymerised under UV light for 24hrs and then for 16hrs at room temperature.

2.11.2. Sample Preparation.

Sections of approximately 60nm thickness were cut on a LKB III ultramicrotome and picked up on 100 hexagonal mesh nickel grids. The sections were floated, section side down in droplets of 0.05M glycine in PBS for fifteen minutes, then transferred to droplets of PBS for 3 x 5 minutes. The grids were then floated section side down in drops of primary antibody diluted 1:20 in PBS for two hours. The grids were washed on drops of PBS for 5 x 10 minutes and then transferred to drops of gold anti-rat 10nm gold labelled secondary antibody (British Biocell) diluted 1:20 with PBS for two hours. The grids were then washed in PBS (5 x 19 minutes), then in distilled water and dried.

2.11.3. Zero loss imaging.

Images of electron density were viewed with Zeiss (LEO) TEM902 operating at 80 kV and at 12,000 times magnification with a beam current of 125 x 10^{-8}A in a vacuum of 7 x 10^{-7} Torr. The operating conditions were the same as those detailed in Section 2.9.2.

2.11.4. Electron Filtering TEM (EFTEM) Imaging of samples.

As was mentioned in section 2.10, the energy loss of inelastically scattered electrons' is element specific allowing specific inelastically scattered electrons to be filtered through an electromagnet (Egerton, 1986). The electrons that interacted with gold atoms (150 KeV) in the specimen were selected and used to finely focus the electron beam onto the specimen. This resulted in improved contrast of the images. Gold particles that appeared black using conventional imaging showed up white.

2.12. X-Ray Microanalysis

X-ray microanalysis depends upon the interaction between the outer shell electrons from the surface atoms of a sample and an electron beam. This results in the excitation and emission of X-rays, the energy of which is elementally specific. This specificity allows the distribution of elements within a sample to be mapped. The sequence of X-ray signals from different elements is detected with an X-ray detector and a spectrum of energy emitted is recorded (Morgan, 1986). In section 2.10, the EELS methodology used for the location of calcium in the cell wall was detailed. Both X-ray microanalysis and EELS can be used as tools for mapping the elemental distribution in specimens (Eggerton, 1986). Both methods have pros and cons. EELS has the benefit of better resolution and that pre- and post-image drift correction can be done. The cost of the equipment, though, is expensive and the technique is initially difficult to master. X-ray microanalysis, on the other hand is relatively inexpensive and large areas of the specimen can be analysed simultaneously.

X-Ray microanalysis was used to gain insight into the calcium distribution of potato slices before and after two-stage (70°C) cooking in a 5g/l calcium salt solution. This was compared to raw and two-stage (70°C) cooking in deionised water. Tissues were prepared and cooked as detailed in section 2.2 for two-stage (70°C) cooking in either deionised water or a 5g/l salt solution. This work was done at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe, with the assistance of Drs Sue Gedney and Helen White.

Cooled potato slices were fractured through the transverse axis by cutting a small notch along the surface of the slice and applying a gentle force to separate the tissues. Chips were not cut with a knife to avoid smearing of the fracture plane. A small section (approximately 3 x 3 x 5mm) was cut from the fracture plane and placed on a SEM (ISI WB6) sledge, with the fracture face uppermost. Six pieces of fractured potato were stuck onto each sledge, with duplicate specimens were taken from each cooking treatment. To reduce charging effects by the electron

beam, the specimen, apart from the fracture plane, was pasted in conductive carbon paste. The blocks were then transferred into de-gassed liquid nitrogen and rapidly frozen under vacuum to -196°C. The frozen block was then transferred under vacuum to the cryo-preparation chamber (Hexland Cryo Trans CT 1000) and examined for signs of physical damage.

Water was removed from the potatoes by freeze etching, which involves subliming the water out of the structure by warming to -55°C. The specimens were transferred to the cold stage (-186°C) and warmed to -55°C. The specimens were examined at low magnification and electron beam (x1000 at 3kv), heating was continued until water crystals had disappeared from the structure. After etching, the heater was turned off and the specimens were quickly placed back into the cryopreparation chamber at -186°C.

A qualitative X-ray spectrum was run at 15kV. Mapping the elemental distribution of pre-cooked tissues was not possible. As gelatinisation of starch during the pre-cook had 'trapped' free water, preventing satisfactory removal of water making concentrations in the specimens too low to map. As an alternative to mapping, five consecutive spectra were taken going down the profile of the fracture plane and the ratio of elemental peaks relative to one another was calculated.

After a spectrum was recorded, the sample was returned to the cryo-preparation chamber where it was gold coated. The sample was returned to the SEM and representative areas were photographed.

2.13. Scanning Electron Microscopy (SEM).

The fracture planes of chips cooked using the polar cooking apparatus were examined using an ISI WB6 SEM. Potatoes were examined at the stages of

cooking described in section 2.4. Chips were fractured and prepared for SEM examination as detailed in the previous section (2.12) when slices were examined by X-ray microanalysis.

A segment from the fracture plane was then cut into a small (2-3mm^2) block and with the fracture plane facing upwards was stuck onto a copper SEM sledge with carbon cement. The sledge and all but the fracture planes of the potato segments were coated in carbon cement. If the etching had been successful, the specimens were gold coated and representative areas from each sample were photographed. This work was done United Biscuits (UK) Ltd, Group Research and Development, High Wycombe with the assistance of Drs. Sue Gedney and Helen White.

2.14. Cryo-Stat Prepared Light Microscopy.

Potato samples were fractured in the same way as for SEM examination. The potato section was placed on a block with the fracture plane perpendicular to the block. This was to ensure that when the specimen was sectioned the fracture face would be perpendicular to the blade and would remain intact with sectioning. The specimen was covered in Tissue-Tek O.C.T. compound cryo-protectant material (Sakura Finetek Europe) and frozen in liquid nitrogen. The specimen was then warmed to –30°C, sections of 7μm thickness were cut with a microtome and placed on a microscope slide. Sections were either stained with iodine vapour or toludine blue. Representative photographs were taken from each sample. This work was done at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe with the assistance of Drs Sue Gedney and Helen White.

2.14.1. Differential Interference Contrast Microscopy (DIC).

Specimens of potatoes pre-cooked and cooled at 70°C after cooking were sectioned as described above and examined under the DIC microscope. Thin

sections of raw and potatoes pre-cooked and cooled at 55°C were taken by hand using a scalpel. Representative photographs were taken from each sample.

2.14. Statistical Analysis of Results

Statistical analysis of chemical and textural results were analysed using the Tukey LSD program on the Minitab 4.1 statistical software package. Results obtained that had a 95% certainty of being from different populations were denoted by different letters in parenthesis beside each result.

CHAPTER THREE

CONSTRUCTION AND OBSERVATIONS OF THE LAB-SCALE COOKING PROCESS.

3.1. Introduction.

The objective of this thesis was to understand how three-stage cooking effects the mechanical strength and cell separation of potatoes. Controlling starch swelling pressure (Reeve, 1977) and PME activity (Bartolome and Hoff, 1972, Ng and Waldron, 1997a) have both been suggested as the cause of firming during three-stage cooking. Attempting this on either an industrial or pilot plant scale would have been very difficult.

In chapter eight, where potatoes were cooked in a pilot plant set-up, approximately 2.5kg of chipped potatoes were used for each cooking batch, which is the equivalent of approximately 50-60 potatoes. Preparing such a relativity large quantity of potatoes was labour intensive, time consuming and the cooking procedures were rather inflexible. A simple, reproducible and flexible way of cooking was needed.

This chapter outlines the construction of a simple lab-scale cooking apparatus that mimics industrial three-stage cooking. A method of cooking was required that would allow observations to be made about the roles of PME activation or starch in firming and restricting cell separation in cooked potatoes. Using it, certain

aspects of texture changes that occur with one-stage and three-stage cooking were examined. In later chapters much of the cooking studies were carried out using this apparatus.

3.2. Results.

3.2.1. Description of the Lab-Scale Cooking Apparatus.

The lab-model was designed to mimic that of industrial cooking of potatoes for mashing, (Willard and Kluge, 1975, Hadziyev and Steele, 1977). Cooking this way incorporates pre-cooking and cooling steps prior to steam cooking. This is referred to in this chapter as three-stage cooking. The apparatus was designed to cook 100g of fresh potato; the quantity used for cell wall isolation as detailed in Section 2.6. Approximately 500g of potatoes (4-5 tubers) were washed, peeled and sliced through the longitudinal axis to a thickness of 5mm (+/-0.5mm). One hundred grams were sub-sampled and used for cooking. Slices were stored in deionised water prior to cooking to prevent dehydration or enzymic degradation.

The cooking apparatus is shown in Figure 3.1. Pre-cooking was carried out in a beaker of 300ml of deionised water heated in a water bath. The volume of water in the bath was kept constant so that temperature equilibration between the bath and heater would be constant between different pre-cooks. After the pre-cook step the potatoes were removed from the pre-cook water into a metal sieve and cooled in a pan of 3L of deionised water.

After cooling, potatoes were drained from the cooling water and cooked in a domestic steamer. The steamer consisted of two compartments and a lid. It was heated using a Bunsen burner. The bottom compartment held water which when boiled produced steam, the upper compartment, where potato slices were cooked, had a perforated base that allowed the steam to enter. Potatoes were placed one

layer thick on to the sieve and placed in the upper compartment. The time of cooking was measured from the moment the lid was replaced on the steamer.

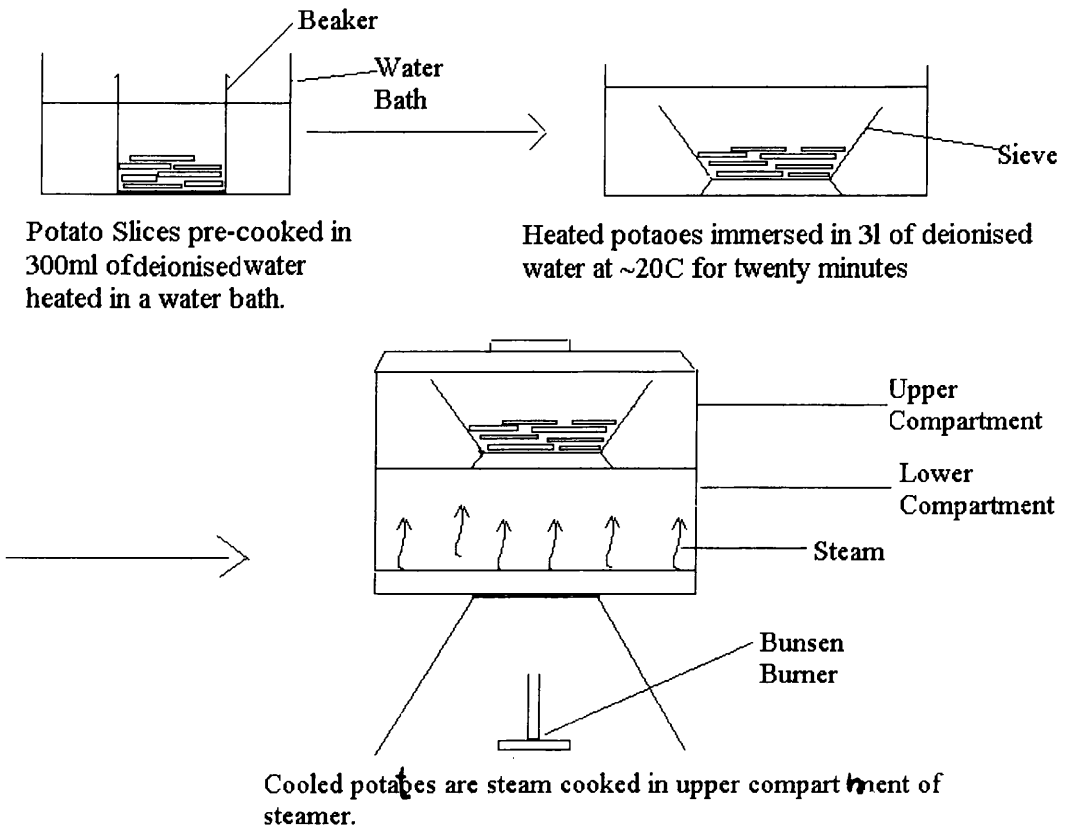


Figure 3.1. Diagram of the lab-scale cooking apparatus.

Part of the cell wall isolation protocol is the collection and measurement of any solubilised pectins released during the washing steps. During three-stage cooking pectin could be solubilised in the pre-cooking or cooling steps and aliquots from each were kept and frozen for future analysis. The pre-cook water was cooled and made to 500ml, 150ml of which was frozen. A 200ml aliquot was taken from the cooling water and also frozen.

3.2.2. Pectin Changes During Three-Stage Cooking Using the Apparatus.

cv. Maris Piper slices were three-stage cooked using the assembled cooking apparatus. The principal reason for doing this was to gain experience of cooking with the apparatus and using the cell wall isolation protocol to prepare cell walls from raw and processed potato tissues. If the cooking procedure was analogous to three-stage cooking then demethylation of pectic material should occur (Bartolome and Hoff, 1972, Ng and Waldron, 1997a). The temperature of the pre-cook water bath was set at 70°C so that PME activation and starch gelatinisation could both occur.

Potato slices were pre-cooked for twenty minutes and then cooled for twenty minutes (two-stage (70°C) cooked). Two-stage (70°C) cooked potatoes were then steam cooked for a further twenty minutes (three-stage (70°C) cooking). The cooking times were chosen arbitrarily. Mixed Cation Buffer (MCB) insoluble material was isolated, as outlined in Section 2.6, from the raw, two-stage (70°C) and three-stage (70°C) cooked potatoes. Using this material, evidence of PME activation was sought by measuring the pectic galacturonan content and its degree of methyl esterification after different cooking treatments.

Cook Treatment	**% Starch in Residue**	**Assumed % Starch Value**	**Dry Matter % Residue**	**Conversion Factor**
Raw	n/a	5	1.95	0.931
2-stage cooked	n/a	5	1.42	0.936
3-stage cooked	n/a	5	4.95	0.900

Table 3.1 Dry matter of MCB-insoluble cell walls of raw, two-stage and three stage cooked potatoes.

The dry matter content of the MCB material from each treatment was quantified (Table 3.1). Residual starch measurements were not made, as there was insufficient MCB insoluble material to do this. An assumed value of five percent was given, as this was the maximum found in MCB insoluble material found by Jardine (1998). A conversion factor was derived from the assumed residual starch value and the dry matter content, which was used to express cell wall measurements on a dry cell wall weight basis (Table 3.1).

The amount of pectic galacturonan was measured as detailed in Section 2.7.3 using the Jardine (1998) method (Table 3.2). In raw tissues, 20.6% of the cell wall was composed of galacturonic acid units. This was in close agreement with measurements made by Jardine (19.6% of the cell wall) on the same batch of potatoes and with other published values (Ryden and Selvendran, 1990, van Marle *et al.*, 1997). After two-stage (70°C) cooking for twenty minutes the galaturonan content of the cell wall fell by 5%. This was a greater loss than cv. Bintje potatoes that were pre-cooked for at 50°C for three hours (Ng and Waldron, 1997a). The higher temperatures during pre-cooking at 70°C most probably caused the cell wall to degrade, causing a release of pectic material from cell walls. A further loss of pectic material occurred after three-stage cooking which left only 12% of the cell wall being composed of pectin galacturonan.

Cook Treatment	Gal UA (µmol/ g)	Gal UA (% cell wall)	*LSD*
Raw	1146.84	20.6	(a)
2-stage cooked	904.21	15.8	(b)
3-stage cooked	684.51	12.2	(c)

Table 3.2. The galacturonic acid content of cv. Maris Piper after three-stage cooking. The pectin galacturonan content of isolated cell wall was expressed as µmol Gal A/ gram of dried cell wall or as percentage of galacturonan per gram of dried cell wall. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$).

The degree of methyl esterification of pectic galacturonan was measured by the Klavons and Bennett method (1986), as detailed in Section 2.7.4. The percentage methyl ester content remaining in the cell wall after pre-cooking fell by 15%; this is likely to be due to PME activity (Table 3.3). A further drop of 10% in methyl esterification was observed when pre-cooked tissues were then steam-cooked. It was unclear whether this latter drop was due to further PME activity or solubilisation of methyl esterified material. It is more likely due to solubilisation as PME activity is rapidly destroyed at 70°C so there would be very little PME activity after the pre-cook.

Cook Treatment	MeOH (μmol/ g)	% Degree of Methylation	*LSD*
Raw	475.90	40.7	a
2-stage	238.97	26.4	b
3-stage	115.34	16.9	c

Table 3.3. Degree of methyl esterification of cv. Maris Piper after three-stage cooking. Expressed as either μmol of methanol per gram of dry cell wall or as a percentage galacturonic acid residues methyl esterified. Treatments with different letters are significantly different based on a Tukey LSD ($p<0.05$)

The main objective of this experiment was to ensure that PME activation had occurred during the pre-cooking at sufficiently high temperatures to allow starch gelatinisation to also occur. The above results reveal very little, apart from establishing that PME activation was likely to have occurred along with some solubilisation of pectic material during two-stage (70°C) and especially three-stage (70°C) cooking. Changes in the cell wall chemistry of three-stage cooked tissues need to be compared to changes in cell walls isolated from one-stage cooked potatoes. Additionally these changes have to be related to texture changes in the tissue as a whole.

3.2.2. Optimisation of Three-stage (70°C) Cooking Times

Potatoes, cv. Maris Piper from harvest 1996-1997 with a specific gravity of 1.085 (Wilson W. and Jarvis M.C., unpublished results), were one-stage and three-stage cooked for different lengths of time. As described in Section 3.2.3 a second method was developed that involved a pre-cook that only activated PME.

The resultant texture of the potatoes after different cooking lengths was evaluated using wedge test measurements (Section 2.5.1). The length of time required for potatoes to be fully cooked during one-stage cooking was measured. Secondly, the pre-cook time required for three-stage (70°C) cooking to increase the mechanical strength of the cooked potato was evaluated.

3.2.2.1 One-stage cooking times.

Potatoes were steam-cooked for ten, fifteen and twenty minutes and the texture of the potatoes was measured using the wedge test method (Figure 3.2). The mechanical strength of raw tissues raw tissues (10.75N (Figure 3.4)) was greatly reduced after ten minutes of cooking but no further softening occurred when the potatoes were cooked for fifteen minutes and they still appeared undercooked. After twenty minutes of steam cooking the potatoes had softened further and appeared cooked. Twenty minutes was chosen as the time used to steam cook potatoes during one-stage and three-stage cooking.

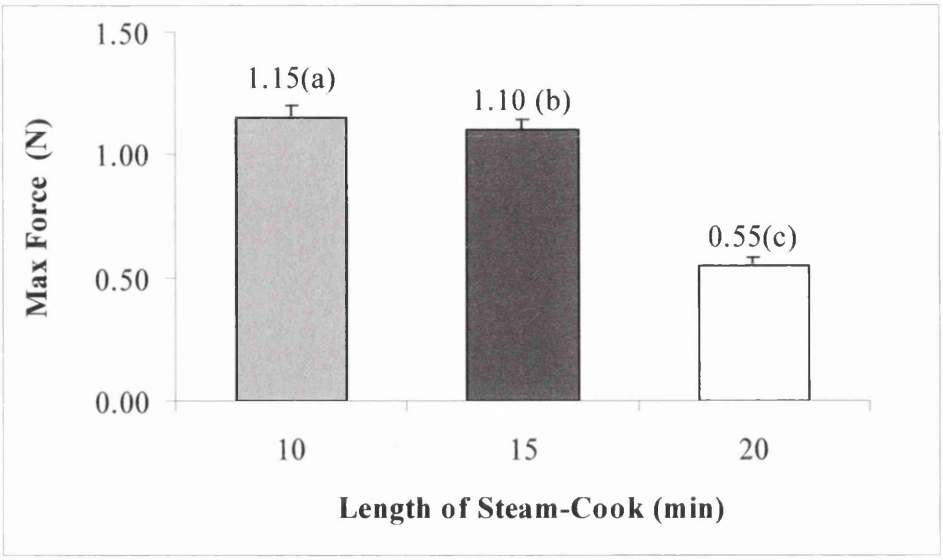


Figure 3.2. The maximum force to cut through cv. Maris Piper tissues with increasing steam cooking time using a wedge cutter. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

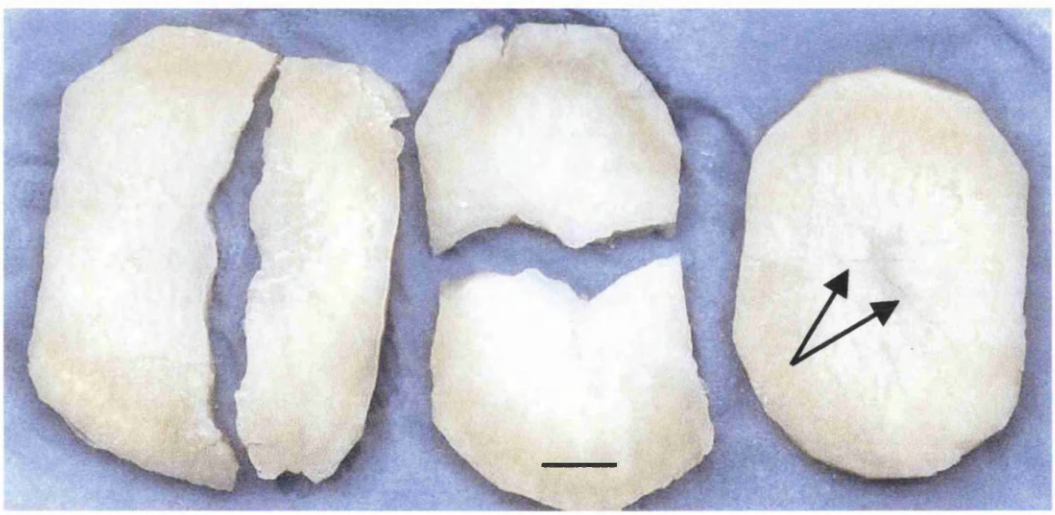


Figure 3.3. Cracking of tissue in steam cooked potatoes (in this case cv. Maris Piper) slices. The slice on the right has a small crack in pith area in the middle of the slice, which can lead to the slice splitting in two as has happened in the other two slices. The two arrows in the diagram indicate the start and end point of the crack. Scale Bar is 1cm.

When potatoes were steam-cooked, quite often cracking was observed in the potato slices (Figure 3.3). The outer regions of potatoes have a larger starch content than the inner pith region (Burton, 1989). A possible reason for this cracking was that when steam cooked, the potatoes swell more in the outer regions than the inner pulling the potatoes apart.

3.2.2.2. Two-stage (70°C) cooking times.

Tissues were heated in 300ml of water for ten, fifteen and twenty minutes at 70°C then cooled for twenty minutes. The firmness of the pre-cooked and cooled tissues was measured using the wedge test method (Figure 3.4). Potatoes softened with increasing time and it would appear that the tissues were slowly cooking. This agrees with the fact that tissues two-stage (70°C) cooked for twenty minutes, released pectin material from the wall when cell walls were isolated (Table 3.2). The firming effect that occurs with three-stage cooking was not evident after the pre-cook and cool step and the optimum duration of the pre-cook step could not be determined until after the third stage of cooking.

3.2.2.3. Three-stage (70°C) cooking times.

Slices were three-stage cooked with different pre-cooking times of ten, fifteen and twenty minutes. After a twenty-minute cooling period, slices were either partially cooked for ten minutes or fully cooked for twenty. The resultant texture was evaluated by wedge test method (Figure 3.4). One-stage cooked wedge test results for ten and twenty-minute cooking was included as a control

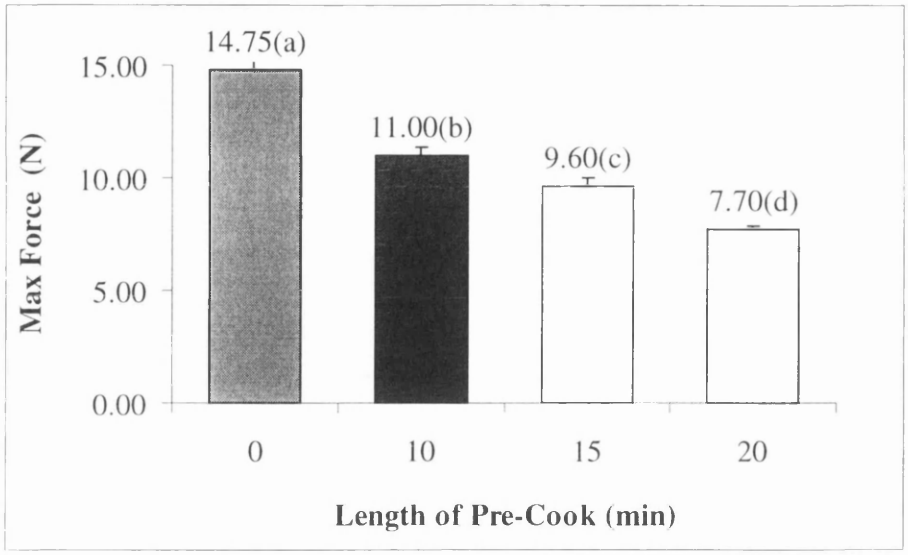


Figure 3.4. The mechanical strength of cv. Maris Piper tissues pre-cooked at 70°C and cooled using a wedge cutter. The length of pre-cooking increasing over time. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

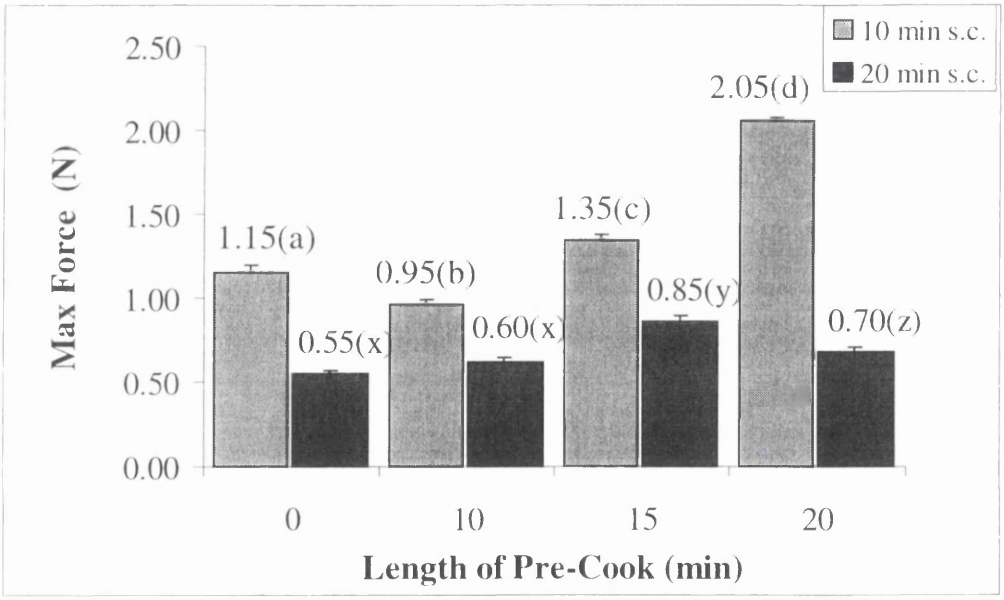


Figure 3.5. The mechanical strength of cv. Maris Piper tissues after three-stage cooking with a ten or twenty minute final steam-cook step, while varying the lengths of the pre-cook time. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

A ten-minute pre-cook at 70°C caused a slight softening of under-cooked tissues compared to the one-stage undercooked control, but this was not observed in the fully cooked treatment (Figure 3.4). A fifteen-minute pre-cook gave a significant increase in mechanical strength over both the partially cooked and the fully cooked potatoes. When the pre-cook was extended to twenty minutes the extent of strengthening greatly increased in the ten minute cooked potatoes. In the fully cooked tissues though, the maximum cutting force needed was less than that of pre-cooking for fifteen minutes. Hou and Chang (1996) have also observed this loss of firming by pre-cooking for too long a period, when cooking snap beans. As PME is quickly denatured at temperatures around 70°C, (Tijskens *et al.*, 1997) it could be possible that extended heating at this temperature slowly cooks the potato rather than imparting firmness on the subsequently cooked potato.

As a fifteen minute pre-cook produced the greatest increase in mechanical strength, it was chosen as the pre-cook time during three-stage (70°C) cooking.

3.2.3 Optimisation of the Cooking Times for Three-Stage (55°C) Cooking.

The cooking process was originally to be used with a 70°C pre-cook. During a 70°C pre-cook a number of different processes occur within a potato including the breakdown of cellular membranes, starch gelatinisation and pectin methyl esterase activation. One or all of these processes could have caused firming in the cooked potatoes (Reeve, 1953, Bartolome and Hoff, 1972, Hadziyev and Steele, 1979, Ng and Waldron, 1997a).

In an attempt to distinguish between the two effects an alternative three-stage cooking process was developed. In this process the pre-cook temperature was lowered to 55°C, which is within the range where PME is most active (Puri *et al.*, 1982) but is well below the starch gelatinisation point of 64-68°C (Lamberg and Olsson, 1986).

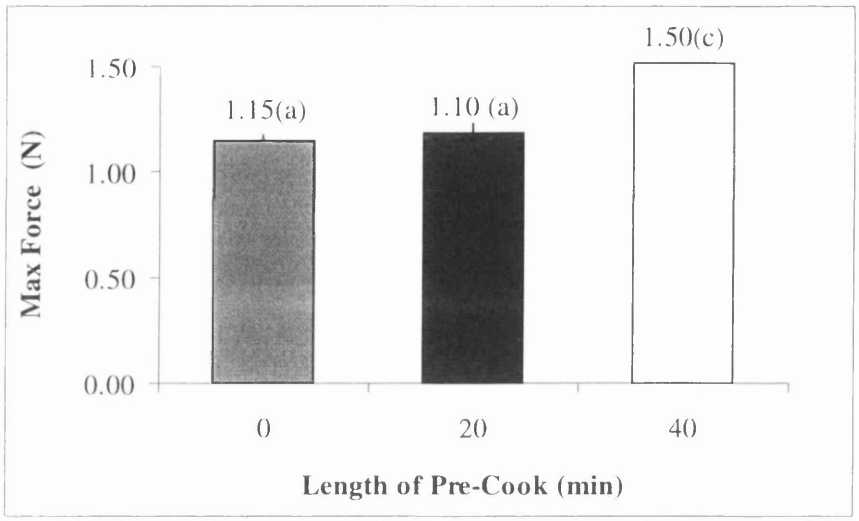


Figure 3.6 The mechanical strength of cv. Maris Piper tissues after three-stage (55°C) cooking with different pre-cook times. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

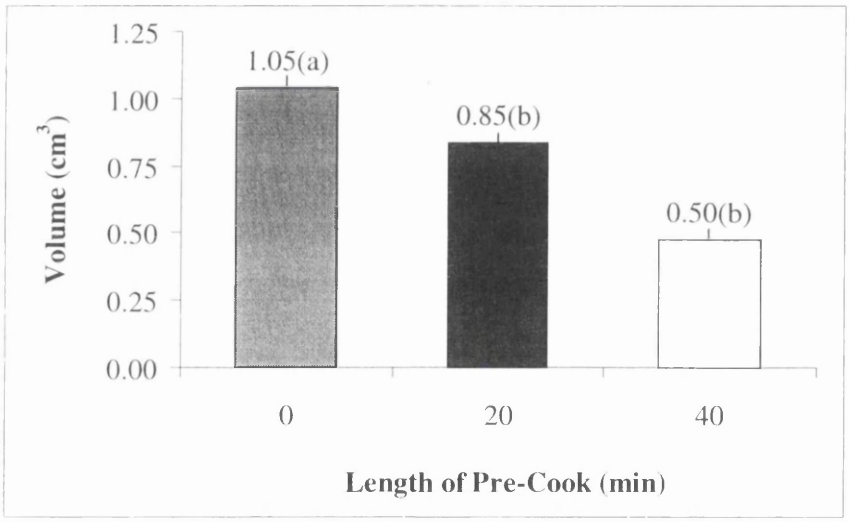


Figure 3.7. The volume of separated cells of cv. Maris Piper tissues after three-stage (55°C) cooking with different pre-cooking times. Columns 79 6.94 11.39with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

Potatoes, cv. Maris Piper from harvest 1997-1998, were prepared and cooked using the normal cooking protocol except that pre-cooking was carried out for different lengths of a time at 55°C. The texture of the cooked tissues was evaluated using the wedge test and cell separation methods adapted from Freeman *et al.* (1992). The texture of the three-stage (55°C) cooked potatoes was compared to one-stage cooked tissues. Texture changes comparable to three-stage (70°C) cooking were desired.

An initial pre-cook time of twenty minutes was carried out and the resultant mechanical strength of the cooked potatoes was no greater than one-stage cook equivalent, but there was a reduction in cell separation (Figures 3.6 and 3.7). The pre-cook time was increased to forty minutes where there was an increase in mechanical strength and a further decrease in cell separation (Figures 3.6 and 3.7). Ng and Waldron (1997a) carried out a three-stage cooking process with a 50°C pre-cook for three hours and despite causing a reduction in methyl ester content no firming effect was observed. This was assumed to be as a result of insufficient calcium within cv. Bintje potato cell walls. Pre-cooking at 55°C for forty minutes, cooling, then steam cooking produced a comparable firming effect to three-stage cooking with a 70°C pre-cook for fifteen minutes. The volume of separated cells was also comparable to three-stage (70°C) cooking measurements made earlier that year (see Section 4.2.3).

The wedge test measurements made in Section 3.2.2 for determining the cooking times for the one-stage and three-stage (70°C) cooking were much lower than the results in this section. A twenty-minute steam cook required a maximum force of 0.58N to cut through slices in the previous section, while a force of 1.08N was recorded above. The texture measurements in Section 3.2.2 were made at the very end of the 96-97 harvest, and storage times are known to have an effect on the texture of cooked potatoes (Wilson W. and Jarvis M.C., unpublished results). Another possible explanation is that Maris Piper potatoes from harvest 1996-1997 had different cooking properties to later harvests used during this Ph.D. Although other specific gravity measurements, made on the same Maris Piper potatoes

showed no differences between harvests (Wilson W. and Jarvis M.C., unpublished results).

3.2.4. Temperature Profile of the Pre-Cook Water.

The temperature of the water during pre-cooking has a large influence on the processes that occur within the tissues. Prior to pre-cooking, slices were washed and kept in deionised water at room temperature. Addition of 100g of this cool material into 300ml of water at either 55°C or 70°C would be likely to cool the temperature of the pre-cook water. To what extent the temperature dropped and how quickly it returned to its original temperature would have a bearing on the processes that occur during pre-cooking and thus on final texture of the cooked potato.

The temperature of the pre-cook water, at 55°C and 70°C, was measured before and after addition of 100g of potato slices (Figure 3.8). The addition of potatoes to both pre-cooks caused a drop of approximately 8°C and it took between ten and fifteen minutes to return the temperature in the beaker to the original value.

In the 55°C pre-cook, the temperature fell to approximately 47°C but quickly rose to 50°C where pectin esterase is activated. Thus, for the majority of the pre-cook the temperature is close to the maximum for PME activation (Puri *et* al., 1982, Tijskens *et al.*, 1997). Zhang *et al.* (1993) measured a drop in the capacitance of potato cells as a method of measuring membrane integrity. The capacitance, and therefore membrane integrity, fell sharply at temperatures 45-50°C. Therefore during a two-stage (55°C) pre-cook it is also likely that membrane integrity is lost. There is also very little chance of starch gelatinisation within the potatoes occurring at temperatures of 55°C (Lamberg and Olsson, 1989).

The fifteen-minute pre-cook at 70°C was not long enough for the temperature to stabilise at or near 70°C. Significantly, addition of potatoes into the beaker brought the temperature into a region where PME is more stable (Tijskens *et al.*,

1997). The temperature in the beaker rose steadily through the pre-cook and after about 8 minutes the temperature was approximately 65°C, the temperature at which starch gelatinisation occurs (Lamberg and Olsson, 1989) and also where PME activity declines rapidly (Puri *et al.*, 1982, Tijskens *et al.*, 1997). Based on the assumption that the temperatures during the pre-cook allowed both these events to occur, conclusions can be drawn as to what processes happen during the 70°C pre-cook. In the first half of the pre-cook, the dominant process occurring was likely to be cell wall strengthening, by PME removing methyl esters from galacturonic acid residues; while the second half of the pre-cook starch gelatinisation could take place.

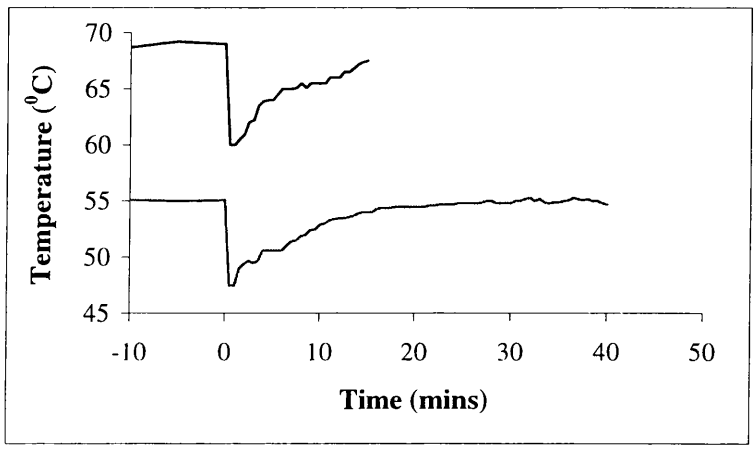


Figure 3.8. Temperature profile of the pre-cook water for both 55°C and 70°C pre-cooks before and after addition of 100g of potato slices.

Apart from the temperature profile of the two-stage (70°C) pre-cook, another indication that starch gelatinisation had occurred was that two-stage (70°C) cooked slices were far more pliable than two-stage (55°C) cooked slices. The cause of this was probably a loss of turgor during the two-stage (70°C) pre-cook. If the slices were broken apart, two-stage (70°C) cooked potatoes were full of gelatinous starch, which was not present in the two-stage (55°C) cooked tissues.

3.2.5. Pre-cooking below PME Activation Temperatures.

PME is activated at temperatures above 50°C (Puri *et al.*, 1981,Tijskens *et al.*, 1997) and the destruction of cellular membranes in potatoes has been reported to occur at 45 and 50°C (Zhang *et al.*, 1993). Destruction of cellular membranes results in the release of chelating ions citrate and phytate from the vacuole into the cell wall. Release of these ions is responsible, in part, for the softening processes that occur during cooking (Keijbets and Pilnik, 1976). So in theory it should be possible to cause a 'softening' effect on cooked tissues by pre-cooking slices at temperatures below PME activation, however doing it at temperatures sufficiently high to destroy cellular membranes that should release citrate and phytate in to the cell wall prior to steam cooking.

To test this hypothesis, shop-bought cv. Maris Piper potatoes, harvest 1999-2000, were three-stage cooked with the pre-cook carried out at either 52°C for forty minutes or 47°C for fifty minutes. The resultant texture of the cooked material was measured using the wedge test and cell separation methods and compared to one-stage cooked potatoes (Figure 3.9 and 3.10). As was expected, pre-cooking at 52°C caused a reduction in cell separation and a firming of the cooked potatoes but reducing the temperature by 5°C to 47°C resulted in a texture comparable to one-stage cooked tissues.

Further softening with a 47°C pre-cook, as postulated above, was not observed, possibly due to the wide spread of results the texture measurements produced. This experiment was slightly flawed as all three cooking treatments would result in the release of citrate and phytate into the cell wall, either during pre-cooking or steam cooking. However the experiment did provide evidence that the firming effect observed was temperature dependant and that pre-cooking must be carried out at temperatures where PME is activated.

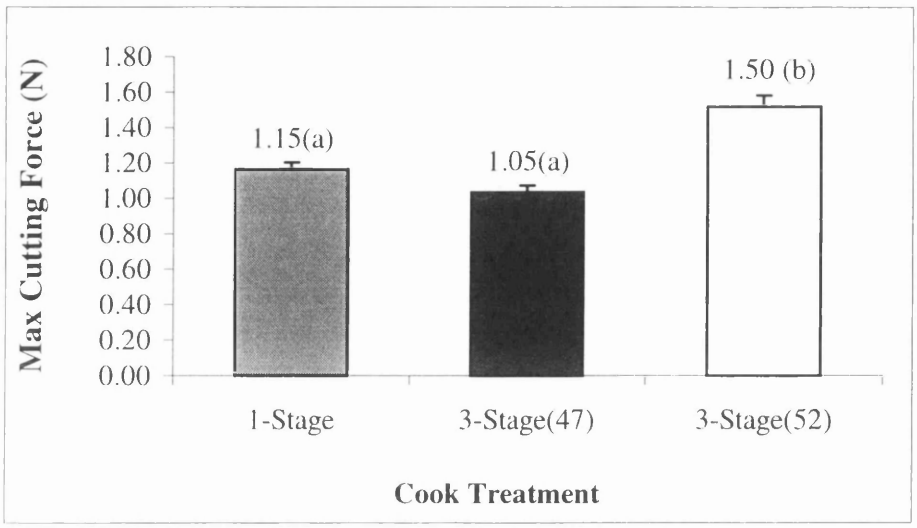


Figure 3.9. The mechanical strength of cv. Maris Piper tissues after one-stage cooking or three-stage cooking with pre-cooks at either 47°C or 52°C. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

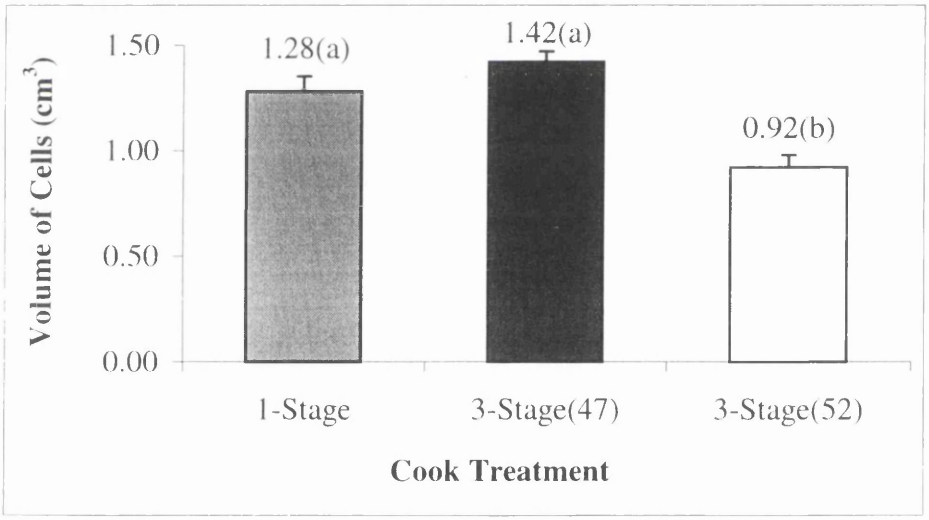


Figure 3.10. The volume of separated cells of cv. Maris Piper tissues after one-stage cooking or three-stage cooking with pre-cooks at either 47°C or 52°C. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

3.2.6. The Role of the Cooling Step.

An integral part of the three-stage cooking process is the cooling step between the pre-cook and the final steam cooking stage. A short cooling time causes a reduction in soluble amylose (Miles *et al.*, 1986, Jankowski, 1992) and doing this prior to cooking results in a greater number of intact cells (Reeve, 1953). Bartolome and Hoff (1972) discounted this theory, as after the pre-cooking and cooling steps gelatinous starch was still present, i.e. the starch had not retrograded during cooling. Moledina *et al.* (1981) postulated that the cooling step was necessary to allow the stabilisation of calcium-pectate bridges within the cell wall imparting mechanical strength to the potato.

Potatoes, cv. Maris Piper harvest 1998-1999, were cooked with or without an intermediate cooling step between the pre-cook and the steam-cook steps (three-stage or two-stage* cooking). The texture of the cooked potatoes was measured using the wedge test and cell separation methods (Figure 3.11 and 3.12).

Two-stage* cooked potatoes were no firmer than the one-stage control (Figure 3.11). Therefore with both three-stage cooking processes, unless the cooling step was included the mechanical strength of potatoes did not increase.

The amount of cooked tissues that separated after three-stage (55°C) treatment was less than after one-stage cooking (Figure 3.12). Omitting the cooling step (two-stage* (55°C) cooking) had no effect on the volume of cells separated. The greatest reduction in cell separation was observed in three-stage (70°C) cooked tissues, but when the cooling step was omitted (two-stage (70°C)) there was still a reduction in cell separation compared to one-stage cooked tissues, but to a lesser extent than when slices were cooled prior to cooking (Figure 3.12).

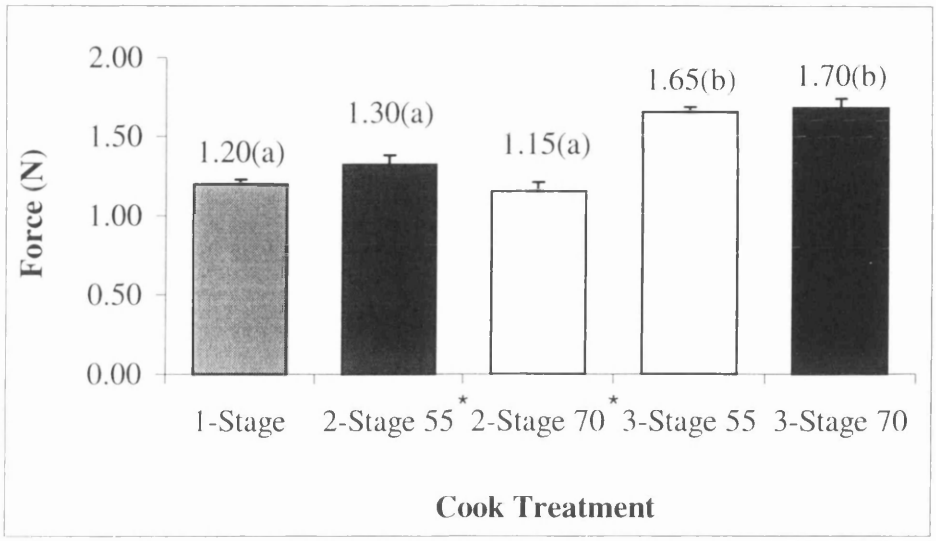


Figure 3.11. The mechanical strength of one, two* and three-stage cooked cv. Maris Piper potatoes. Where the two-stage* cooking refers to pre-cooking then steam cooking without an intermediate cooling step. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p < 0.05$).

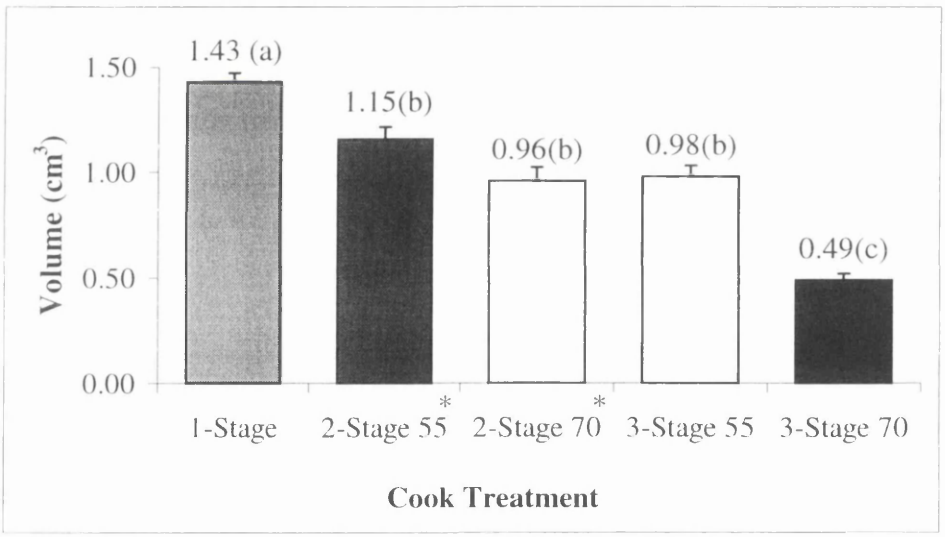


Figure 3.12. The volume of separated cells (cm^3) of cv. Maris Piper tissues after one, two* and three-stage cooking. Where the two-stage* cooking refers to pre-cooking then steam cooking without an intermediate cooling step. Columns with different letters in parenthesis are significantly different based on a Tukey LSD ($p<0.05$).

To clarify, it would appear that PME activity without a cooling step, in both three-stage cooking processes, reduced cell separation to a certain extent, but no increase in mechanical strength was evident. When a cooling step was included after pre-cooking at 55°C, the volume of cooked cells that separated was unchanged compared to two-stage* (55°C) cooked tissues, but an increase in mechanical strength was seen. When potatoes were cooled after the 70°C pre-cook a reduction in the volume of separated cells occurred along with an increase in mechanical strength, compared to the two-stage (70°C) cooked tissues.

Unless one considers that wedge test and cell separation measurements may be measuring two different but related texture parameters, the results seem to be contradictory. The wedge test measures maximum resistance required to cut through a slice and is therefore a measurement of mechanical strength. The degree of cell separation measures the volume of separated cells after crushing a potato slice to half its original thickness. The force applied to crush the potatoes is not a factor in these measurements. It is therefore a measurement of the extent that cell separation processes have occurred during cooking (Freeman *et al.*, 1992). The obvious difference between the two pre-cooks was starch gelatinisation in the 70°C pre-cook.

Therefore, manipulating the properties of starch, by gelatinising it then cooling it prior to cooking, along with activation of PME during the pre-cook, affects cell separation processes to a greater extent than PME activation alone. It was also clear that a cooling step was required to increase the mechanical strength of pre-cooked then cooked potatoes. Moledina *et al.* (1981) have postulated that a cooling step is required to allow stabilisation of calcium-pectate bridges, though no conclusive proof that this occurs has ever been produced.

This set of experiments allowed direct comparison of the textures produced by the two different three-stage cooking processes. Three-stage (70°C) and three-stage (55°C) both produced the same firming effect compared to one-stage cooked potatoes. Three-stage (55°C) also showed a reduction in cell separation compared

to the one-stage treatment, but the reduction was not as great as the three-stage (70°C).

3.2.7 Starch Gelatinisation and Cell Separation

Researchers of potato texture have in the past presented evidence for and against starch gelatinisation having a role in controlling potato texture (Hoff, 1972, Reeve, 1972). Jarvis *et al.* (1992) found starch swelling pressure of approximately 10^2KPa, which was equal to turgor pressures in uncooked tissues. Therefore, the pressures generated from starch gelatinisation could only separate cells once other cooking processes had weakened cell-cell adhesion.

If starch gelatinisation is the driving force of cell separation then heating potatoes at boiling temperatures (i.e. 100°C) but preventing starch gelatinisation would result in no cell separation. Further more, if potato tissues cooked in this way were subsequently heated at 75°C and starch allowed to gelatinise, walls that have been weakened by the previous boiling treatment, should separate from one another.

VICS Score.	Extent of Cell Separation Observed.
− − − − −	Tissues intact
+ − − − −	Some sloughing, free cells visible
+ + − − −	Break up of tissues into 2-3 lumps. Free cells also present.
+ + + − −	Large numbers of lumps present, lots of free cells present.
+ + + + −	Large degree of sloughing. Lumps greater than 2mm still present
+ + + + +	Complete disruption of tissues.

Table 3.4. Modified VICS Scoring System

To test this hypothesis thin slices of potatoes were heated in differing solvent concentrations that would reduce the amount of available water present. Sections of potatoes of approximately 1mm thickness were cut into $1cm^2$ discs from the

central pith area of the tuber but avoiding pith rays. The discs were placed in screwtop pyrex tubes with 5ml of differing concentrations of ethanol/water solutions. After boiling discs were cooled and removed from the solvent and placed in 3ml of water and either heated at 75°C or left at room temperature for twelve minutes. The discs were cooled and vortex induced cell separation (VICS) was used to evaluate the extent of cell separation in the tissues (Parker and Waldron, 1995). The scoring system to evaluate the extent of cell separation was modified slightly to describe what was observed more closely (Table 3.4).

1st Treatment	2nd Treatment (in water)	VICS Score.
100% Water 100°C 20 minutes	Room temperature	+++++
100% Water 75°C 20 minutes	Room temperature	-----
100% EtOH boiling for; twenty minutes	Room temperature	-----
	75°C 12 Minutes	++---
100% EtOH boiling for; sixty minutes.	Room temperature	-----
	75°C 12 Minutes	+++--
80% EtOH boiling for sixty minutes.	Room temperature	+----
	75°C 12 Minutes	++++- / +++++
70% EtOH boiling for sixty minutes.	Room temperature	++--- / +++--
	75°C 12 Minutes	+++++
70% EtOH 100°C boiling for twenty minutes.	Room temperature	+----
	75°C 12 minutes	+++--

Table 3.5. VICS scores for potato discs heated in different ethanol solutions, then at different temperatures in deionised water. Results are the average score of five discs, for VICS scores refer to Table 3.5

Tissues slices that were boiled for twenty minutes in 5ml of water were completely disintegrated when vortexed but if the tissues were heated at 75°C for twelve minutes, no disintegration occurred (Table 3.5). Thus starch gelatinisation without degradation of the cell walls causes no cell separation.

When slices were heated for twenty minutes in ethanol, no cell separation occurred when the cooled sections were placed in water at room temperature and vortexed, but some separated cells were visible when heated at 75°C and vortexed. The same effect was observed when the tissues were heated for an hour in ethanol except slightly more cells separated after the starch gelatinisation step (Table 3.5).

It was possible that in 100% ethanol, there was insufficient water for cell wall degradative processes. When potatoes were heated in 80% ethanol, little cell separation occurred when vortexed after the second incubation at room temperature, but after the starch gelatinisation step at 75°C for twelve minutes, the VICS score was very high. When the ethanol concentration was lowered to 70% the VICS score was the same as 80% solution after the gelatinisation step, but VICS of tissues that were not gelatinised was also quite high. When the cooking time was reduced to twenty minutes, tissues without an incubation at 75°C showed little sign of cell separation, but those with starch gelatinisation separated more (Tables 3.5).

1st treatment	2nd Treatment	VICS Score.
100% Ethylene Glycol for 1 hour	Room temperature	−−−−+
	75°C, 12 Minutes	+++++− / +++++

Table 3.6. VICS scores for potato discs heated in different ethylene glycol then at different temperatures in deionised water. Results are the average score of five disc, for VICS scores refer to Table 3.5

Ethanol has a boiling temperature of 78°C, so increasing the water content would increase the boiling temperature towards 100°C and this could provide an explanation of what was observed. To eliminate this possibility an alternative solvent, ethylene glycol, which has a boiling temperature of 109° C, was used. Potato discs were heated for one hour in 100% ethylene glycol and then either

incubated in deionised water at room temperature or 75°C for twelve minutes, at which time the discs were cooled and then assessed by VICS (Table 3.6). Again without a starch gelatinisation step very little cell separation occurred but after gelatinisation cell separation was almost complete.

These experiments indicate that starch gelatinisation has an integral part to play in cell separation processes but only when cooking has degraded cell walls.

3.2.8 Starch Gelatinisation During Steam Cooking.

The point at which starch gelatinises has a number of important effects on the cooking process. Principally it is the point were starch gelatinisation forces start exerting pressure on the cell walls (Jarvis *et al.*, 1992) and the rate of PME activity rapidly disappears (Tijskens *et al.*, 1997). Therefore, the time it takes for starch gelatinisation to occur is effectively the time tissue toughening stops and softening processes begin. Thus the rate of heat transfer between steam and the potato during steam cooking will influence the extent to which these processes occur. When starch gelatinisation occurs is easily identifiable as the potato tissue takes on a translucent appearance. Wilson W. and Jarvis M.C. (unpublished) used the rate of progress of the starch gelatinisation front in baked and microwaved potatoes as an indicator of when the internal temperature reaches 58-64°C.

Time (s)	Description of Potatoes.
60	No gelatinisation present.
90	Slight stickiness at edges of some tissues.
110	Mixture of gelatinised, partially gelatinised and ungelatinised tissues.
120	Complete gelatinisation of some tissues, others only partially.
150	Complete gelatinisation. Some more sticky than others

Table 3.7. Description of progress of starch gelatinisation with time. Cooking was repeated twice for each time, six slices were used for each measurement.

To establish the time when starch gelatinised, sliced potatoes were cooked for increasing times in the steamer. Six slices were placed in the sieve, two at the bottom and four 'stacked' around the edges, cooking was repeated twice. After each cooking time the slices were taken out and fractured and the internal tissues were examined for evidence of starch gelatinisation. However, no clear gelatinisation front was detectable in the slices due to their small size. Table 3.7 outlines the extent of starch gelatinisation during steam cooking.

Heat transfer was quite rapid between steam and potatoes and signs of gelatinisation at the edges of some slices was apparent after one and a half minutes. After two minutes all potatoes showed signs of gelatinisation, some completely so, after two and a half minutes all slices were gelatinised. Thus it is fair to say that firming processes brought about by PME occur for only a short time during steam cooking and very little demethylation of cell wall pectin would occur during one-stage cooking.

The objective of this chapter was to construct a laboratory-scale process that would mimic the conditions of an industrial three-stage cooking process. Three-stage cooking is intended to increase the mechanical strength of potatoes prior to mashing. With the apparatus constructed, optimisation of two cooking methods was carried out to produce three-stage cooked potatoes with a greater mechanical strength than one-stage cooked potatoes (Sections 3.2.3 and 3.24). These methods will be used throughout the rest of this thesis to investigate the texture of one and three stage cooked potatoes.

In the rest of the chapter, how different parts of the cooking protocol affected the texture of potatoes was investigated. Evidence that PME was in part responsible for the firming effects observed during three-stage cooking was found by pre-cooking at temperatures below which PME activation occurs (Section 3.2.5). Omitting the cooling step between pre-cooking and cooking (Section 3.2.6) showed that it was an integral part of the cooking procedure and that cooling gelatinised starch before cooking reduces cell separation additional to the effect of PME activation. In the last section (Section 3.2.8), it was deduced that little PME

activation during the steam cooking step occurs, as the potato quickly reached temperatures where PME is denatured. This would suggest that little demethylation of pectin occurs during one-stage cooking. The relevance of these observations will become clearer in other chapters and will be discussed in more detail in chapter nine.

CHAPTER FOUR

LAB-SCALE COOKING OF SIX POTATO CULTIVARS

4.1. Introduction

Most studies on the effect of three-stage cooking have been carried out on one or two varieties (Moledina *et al.*, 1981, Anderson *et al.*, 1992, Ng and Waldron, 1997a). Ng and Waldron (1997a) characterised changes in pectin chemistry during steam cooking and the effect of heating potatoes for three hours at 50°C prior to steam cooking. They characterised the changes caused by PME activity in potatoes before and after pre-cooking. Sequential extraction of the cell walls of cooked tissues with or without a pre-cook showed that the pre-cook reduced the amount of pectin released in all the cell wall fractions. A drop in the degree of methylation ofthe CDTA fractions of pre-cooked tissues, but no obvious differences in the neutral sugar content of the different fractions were observed.

It has already been shown in Section 3.2.6 that three-stage (70°C) cooking, which involves gelatinising starch, then cooling it prior to cooking, along with PME activation causes a greater reduction in cell separation than PME activation alone during the pre-cook (three-stage 55°C cooking). In this chapter, three-stage (70°C) cooking was studied in more detail by investigating the changes in texture that occur in a number of cultivars when one-stage and three-stage (70°C) cooked. As an indication of the changes that had occurred to cell wall pectin during one-stage and three-stage (70°C) cooking, changes in the galacturonic acid

content and the degree of pectin methylation of three cultivars were evaluated. The neutral sugar content of the cell walls was not investigated, as Ng and Waldron (1997a) had found that pre-cooking caused no major differences.

Six potato varieties from harvest 1997-1998 were chosen from twelve available in the laboratory, based on their texture properties in a baked potato study carried out within the laboratory (Wilson W. and Jarvis M.C., unpublished results). The potatoes used were cvs Golden Wonder (GW), Maris Piper (MP), Kerr's Pink (KP), Cara, Desiree (Des) and Nadine (Nad).

The texture properties of the six varieties assigned by the National Institute of Agricultural Botany (NIAB) (1998) are listed in Table 4.1. The NIAB state that Golden Wonder easily disintegrates with cooking resulting in a very mealy texture type. Maris Piper and Kerr's Pink were mealy; whereas Cara and Desiree were waxy and Nadine was very waxy.

Cultivar	Dry Matter Rating.	Disintegration Rating
Golden Wonder	9	3
Maris Piper	7	5
Kerrs Pink	6	5
Cara	3	7
Desiree	5	7
Nadine	2	6

Table 4.1. NIAB (1998) texture ratings of the six cultivars used. Dry matter is defined as 'the portion which is not water' measured on fresh material when oven dried, 9 is rated as high dry matter. Disintegration is defined as the 'amount of breakdown of flesh during cooking', 9 is rated as low disintegration.

4.2. Results

4.2.1. Specific Gravity of Six Cultivars.

The specific gravity of a potato is strongly correlated with both the starch content and dry matter content and is often used as a to the texture of a potato (Burton, 1989). The specific gravity for each cultivar was calculated using the weight of tubers in air and water using Equation 2.1 (Table 4.2). Golden Wonder had a very high specific gravity; Maris Piper and Kerr's Pink both had a high specific gravity; Cara and Desiree had a low specific gravity and Nadine the lowest specific gravity.

Cultivar	Specific Gravity	LSD	%Dry Matter	% Starch (fresh wt)	% Starch (dm basis)
GW	1.105	a	25.7	19.0	73.9
MP	1.084	b	21.3	14.9	69.7
KP	1.084	b	21.3	14.8	69.7
Cara	1.075	c	19.4	13.0	67.2
Des	1.073	c	18.9	12.6	66.5
Nad	1.061	d	16.3	10.1	62.1

Table 4.2. The specific gravity and starch content of six cultivars. Specific gravity was calculated from the weight of individual tubers in air and immersed in water using Equation 2.1. Specific gravity values labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$). The dry mater and starch content were calculated from the specific gravity values using Equations 2.2 and 2.3; starch content was then expressed on a dry matter (dm) basis.

The starch content on a fresh weight basis and the dry matter content of each cultivar were determined using Equations 2.2 and 2.3 and are shown in Table 4.2. The starch content followed the same pattern as the specific gravity values. The

calculated dry matter content agreed well with the ratings assigned in the NIAB handbook (1999) (Table 4.1).

4.2.2. Texture of Six Potato Cultivars after Two-Stage Cooking.

Potato slices were prepared and cooked as described in Section 2.2. Cell separation measurements were attempted on raw and pre-cooked tissues but the slices were too hard to crush. A similar problem was reported by Aglbor and Scanlon (1998) when they measured cell separation of potatoes steam-cooked for two minutes with or without a pre-cook treatment.

The texture of the raw slices and pre-cooked and cooled slices were measured using the wedge test method (Section 2.5.1). The maximum force required to cut through the varieties is shown in Figure 4.1. Four out of the six varieties; Maris Piper, Cara, Desiree and Nadine required a force of approximately 15N to cut through the raw tissues. Pre-cooking reduced the cutting force required by approximately 5N to 10N. The other two varieties, cv. Golden Wonder and cv. Kerr's Pink, when raw, were much firmer than the others. After pre-cooking and cooling, the decrease in mechanical strength of these two cultivars was larger (~10N) than that observed in the other varieties (~5N).

When cv. Maris Piper tissues were pre-cooked at 70°C for fifteen minutes (Section 3.2.2.2), a decrease in mechanical strength of 5N was observed, in agrees with the results above. In that experiment the pre-cook time was found to have a role in the resultant texture, as the length of the pre-cook increased, the tissues got increasingly softer. A number of reasons could be responsible for the reduction in mechanical strength during two-stage (70°C) cooking. One is a loss of turgor during pre-cooking, which is partially replaced by starch gelatinisation pressure. Secondly, softening could have been caused by release of citrate and phytate into the cell wall removing calcium from within calcium-pectate bridges (Keijbets *et al.*, 1976). Thirdly, degradation of the cell wall by β-elimination would cause softening to occur (Keijbets *et al.*, 1976).

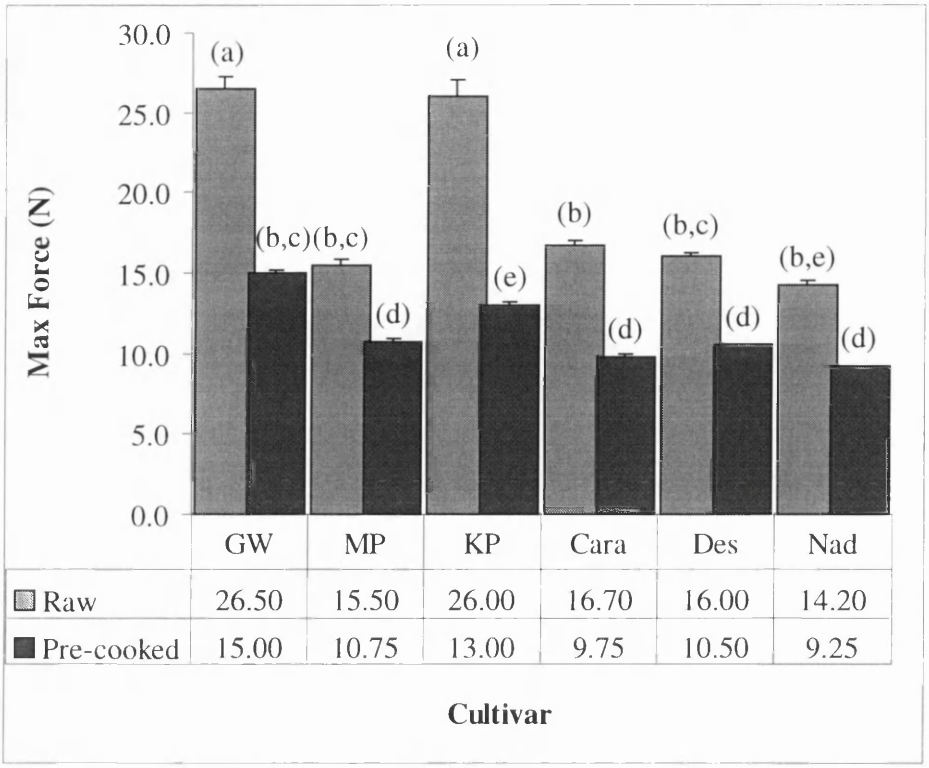


Figure 4.1. The mechanical strength of six different varieties when raw and pre-cooked (two-stage cooked). Values are mean cutting force in Newtons (N); bars on columns are standard error of the mean where n = 20, 34, 19, 20, 34 and 35 for raw tissues and n = 14,17, 16, 20, 21, and 35 for two-stage cooked tissues. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

4.2.3. Texture of Six Cultivars after One-Stage and Three-Stage Cooking.

Potatoes from each cultivar were one-stage or three-stage cooked and the texture of the potatoes was measured by wedge test and cell separation measurements (Section 2.5.1 and 2.5.2). The results for mechanical strength and cell separation for all six varieties were compared. The high number of degrees of freedom and the inherent variability in texture measurements meant that statistical analysis was difficult and some trends may have been masked (Figures 4.2 and 4.3).

The experiment was set up to answer two questions. Firstly, when under constant cooking conditions, can differences in cultivar texture be detected with the wedge test and cell separation texture measurements? Secondly, what effect does three-stage cooking have on each of the six varieties compared to one-stage cooking?

Figure 4.2. (Next page) The mechanical strength of six potato varieties after one-stage and three-stage cooking (where one-stage cooking is defined as steam cooking slices for twenty minutes and three-stage cooking as pre-cooking, cooling, then steam cooking of slices. Values are the peak cutting force in Newtons (N); bars are standard error of the mean of replicates. Where for one-stage cooked tissues n = 13, 20, 22, 29,16 and 14 and for three-stage cooked tissues n = 13, 20, 22, 29,16 and 14. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 4.3. (Next page) The volume of cells separated in six different varieties when one-stage and three-stage cooked. Values are volume of separated cells in cm^3; bars on columns are standard error of the mean. For one-stage cooked tissues n = 10, 10, 9, 10, 10 and 9 and for three-stage cooked tissues n = 10 in all cases. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

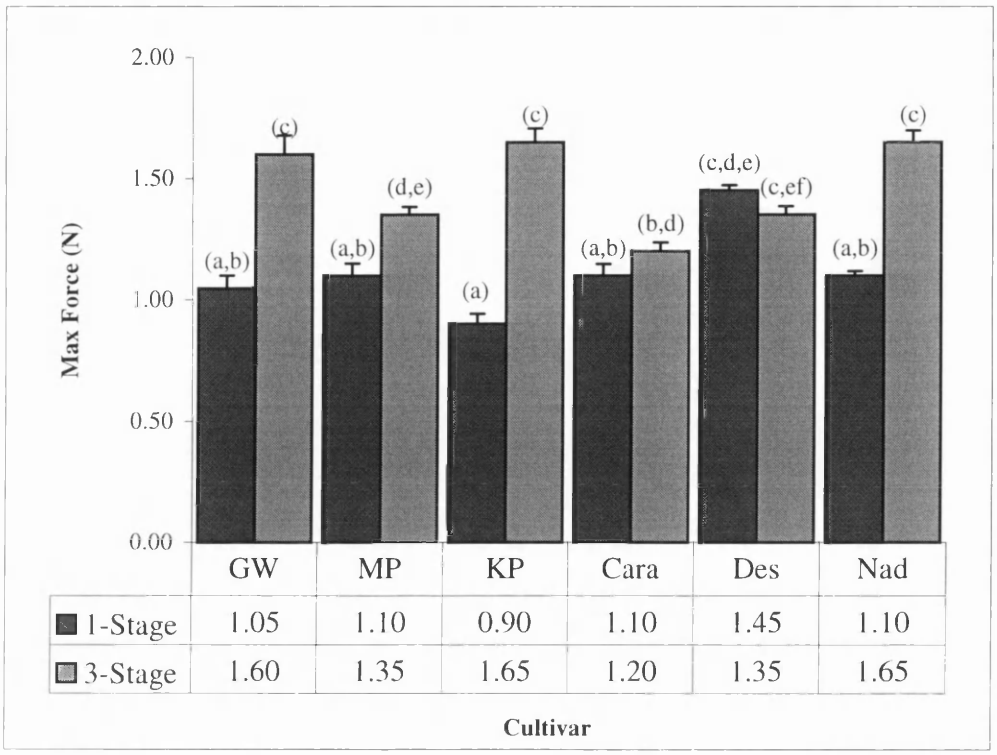


Figure 4.2

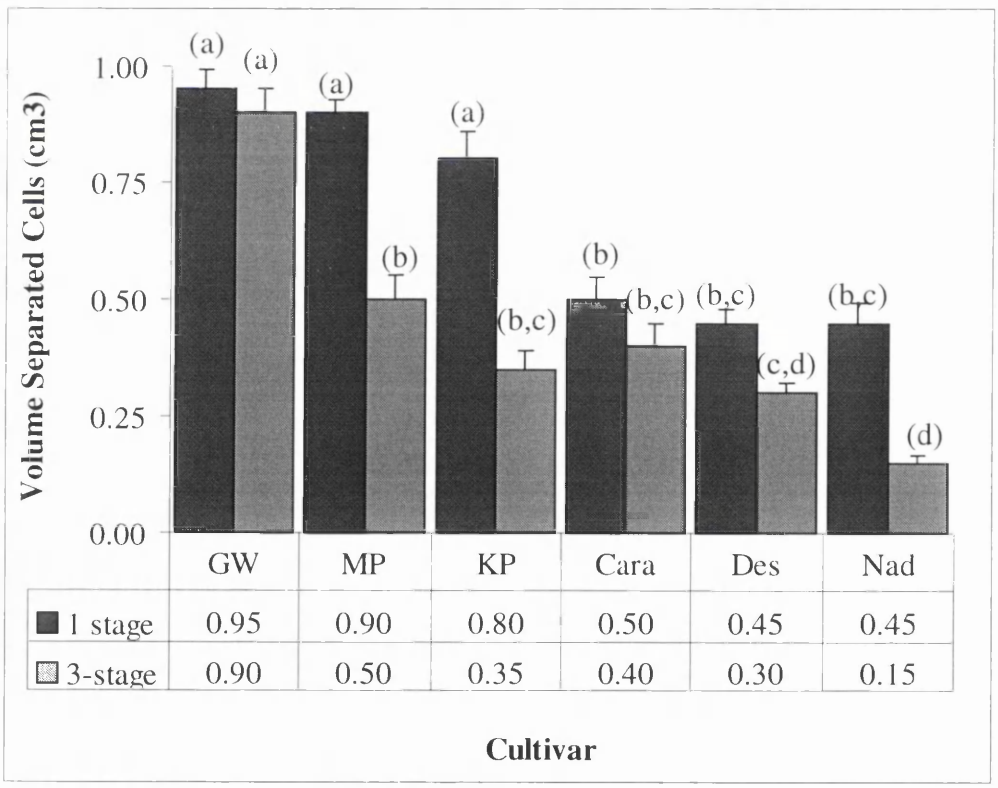


Figure 4.3

4.2.3.1. Effect of cooking treatment on texture of six varieties.

When all six varieties were one-stage cooked, five of the tissues had similar firmness except for Desiree, which was much firmer than the others (Figure 4.4). It is possible that a twenty minute steam cook was too short a time to fully cook the Desiree slices. All the other varieties had the same mechanical strength and were considered sufficiently cooked.

How tissues separate when fully cooked is often used to classify the texture into mealy or waxy (Jarvis and Duncan, 1992). The volume of separated cells after one-stage cooking fell into two distinct ranges (Figure 4.5); the high specific gravity varieties had high volumes, whereas the low specific gravity varieties had lower volumes of separated cells. Classifying the potatoes into mealy and waxy texture types by the cell separation results; the higher specific gravity potatoes would be mealy and the lower specific gravities would be waxy.

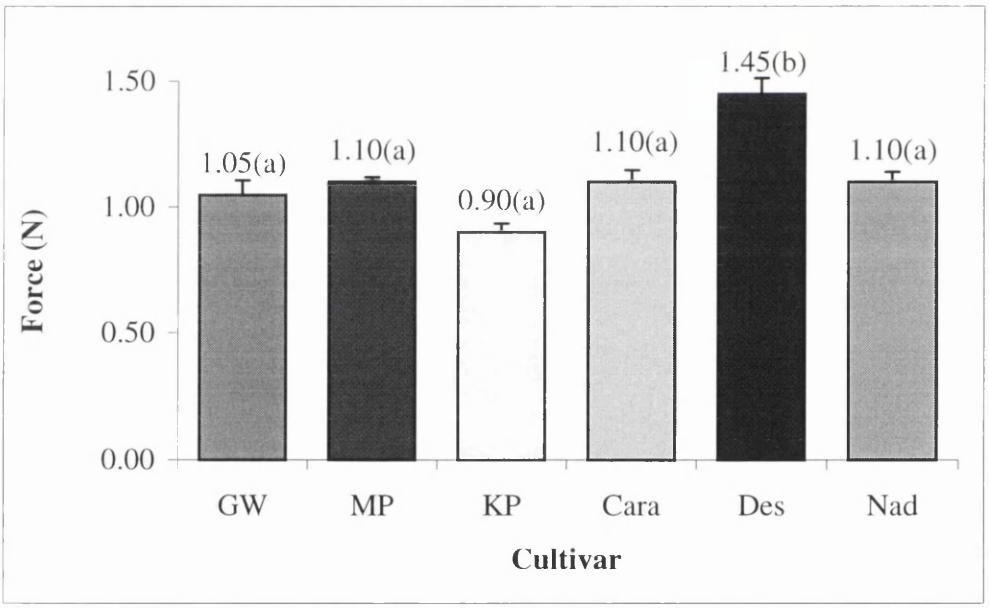


Figure 4.4. The mechanical strength of six different varieties when one-stage cooked. Values are mean cutting force in Newtons (N); bars on columns are standard error of the mean where n = 13, 20, 22, 29,16 and 14. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

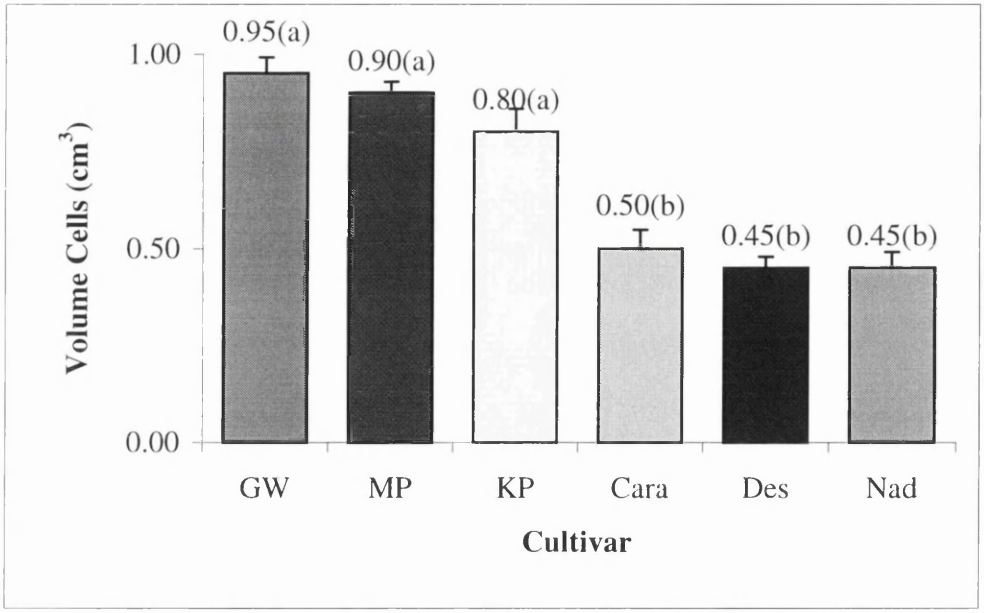


Figure 4.5. The volume of cells separated in six different varieties when one-stage cooked. Values are volume of separated cells in cm^3; bars on columns are standard error of the mean where n = 10, 10, 9, 10, 10 and 9. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

The results agreed with the NIAB (1999) Table 4.1 disintegration ratings. The NIAB (1999) distinguished more differences between varieties than the results presented here, but it was unclear how they had measured disintegration. Therefore, differences in texture could be detected in one-stage cooked potatoes using the cell separation method. However, as there might be some dubiety as to whether twenty minutes was sufficient to cook Desiree slices, classification of its texture from these results would have to be done with care.

When the varieties were three-stage cooked the mechanical strength results were not as clear cut as the one-stage results (Figure 4.6). Three-stage cooking is designed to produce potatoes with a greater mechanical strength (Hadziyev and Steele, 1979), but the firming effect varied between different cultivars used in this study. Three of the varieties (cvs. Golden Wonder, Kerr's Pink and Nadine) had a similar mechanical strength (1.6-1.65N), while the mechanical srtength of

theother three varieties, (cvs. Maris Piper and Desiree and Cara), mechanical strength was slightly less (1.20-1.40N).

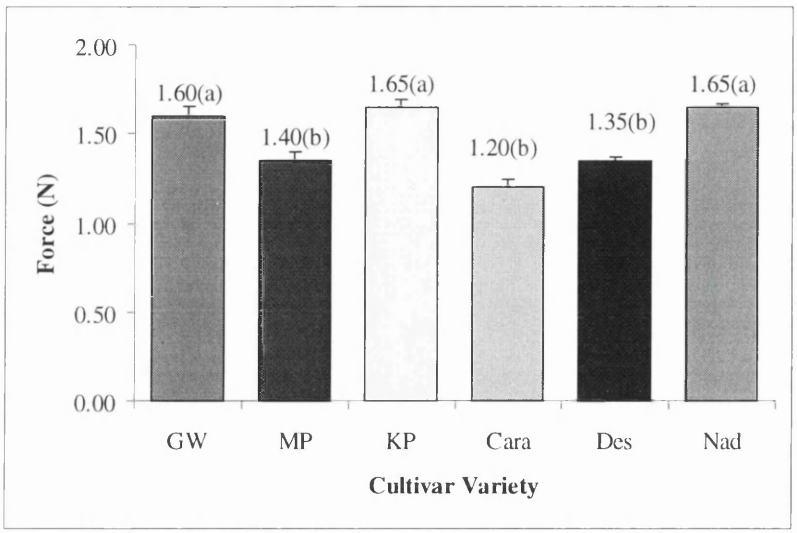


Figure 4.6. The mechanical strength of six different varieties when three-stage cooked. Values are mean cutting force in Newtons (N); bars on columns are standard error of the mean where n = 13, 20, 22, 29,16 and 14. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

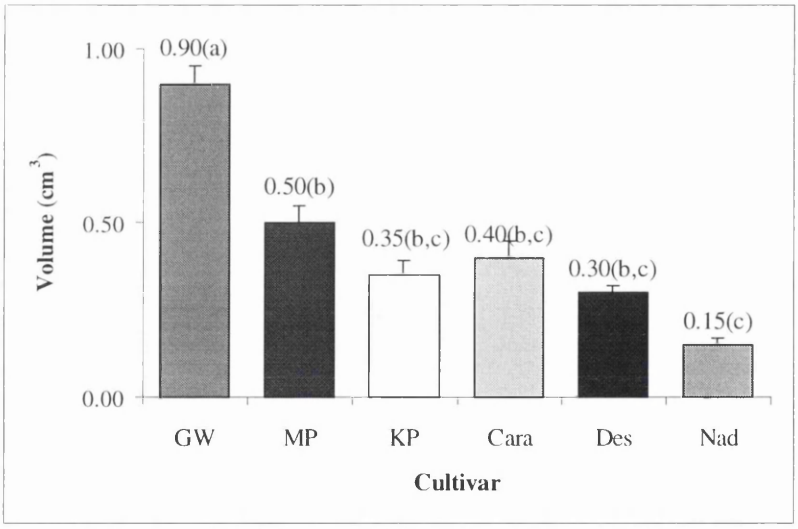


Figure 4.7. The volume of cells separated in six different varieties when three-stage cooked. Values are volume of separated cells in cm^3; bars on columns are standard error of the mean where n = 10 in all cases. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

As well as firming potatoes, three-stage cooking may reduce sloughing prior to mashing (Hadziyev and Steele, 1979). When the six varieties were three-stage cooked, the extremely mealy variety (cv. Golden Wonder) had much higher cell separation than the other varieties and the extremely firm variety, cv. Nadine, had lower cell separation than the five other varieties (Figure 4.7). The moderately mealy and firm potatoes (cvs Maris Piper, Kerr's Pink, Cara and Desiree) all had similar volumes of separated cells. This would suggest that, apart from potatoes at the extreme range of texture, three-stage cooking produces potatoes which have similar residual cell adhesion.

4.2.3.2. Effect of one-stage and three-stage cooking on six varieties.

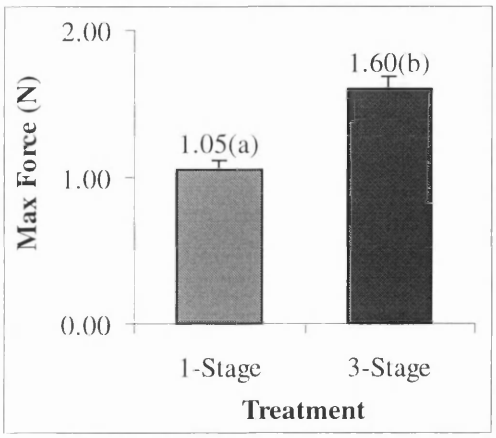
The texture results were reassessed to compare one-stage and three-stage cooked tissues for each variety (Figures 4.8-4.13). Three of the varieties (cvs Maris Piper, Kerr's Pink and Nadine) after three-stage cooking, produced potato slices that were both firmer and showed less cell separation than one-stage cooked tissues (Figure 4.9, 4.10 and 4.13).

Golden Wonder showed an increase in firmness when three-stage rather than one-stage cooked, but no reduction in cell separation was observed (Figure 4.8). Golden Wonder has extreme textural properties, which have been attributed to its high starch content. For example when boiled, the tissues quickly disintegrate into a soupy-slurry of separated cells (Wilson W. and Jarvis M.C., unpublished results). Three-stage cooking involves an intermediate step between pre-cooking and steam cooking where the potato tissue is cooled after pre-cooking. In experiments using cv. Maris Piper (Section 3.2.6), the effect of omitting this cooling step was tested. These experiments showed that pre-cooking at 70°C influenced both PME activation and starch properties prior to cooking. It is possible that the texture of potato varieties with very high starch contents, such as Golden Wonder, is unaffected by cooling gelatinised starch prior to cooking (as occurs in a 2-stage (70°C) pre-cook) and this type of pre-cook is not sufficient to reduce cell separation.

Figure 4.8. Texture of one-stage and three-stage cooked cv. Golden Wonder. a) mechanical strength in Newtons (N). b.) Volume of separated cells in cm^3. In both figures bars on columns are standard error of the mean where n = 13 and 14 for figure a). and n = 10 and 10 for figure b). Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 4.9. Texture of one-stage and three-stage cooked cv. Maris Piper. a) mechanical strength in Newtons (N). b.) Volume of separated cells in cm^3. In both figures bars on columns are standard error of the mean where n = 20 and 22 for figure a). and n = 10 and 10 for figure b). Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 4.10. Texture of one and three-stage cooked cv. Kerr's Pink. a) mechanical strength in Newtons (N). b.) Volume of separated cells in cm^3. In both figures bars on columns are standard error of the mean where n = 22 and 23 for figure a). and n = 9 and 10 for figure b). Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).



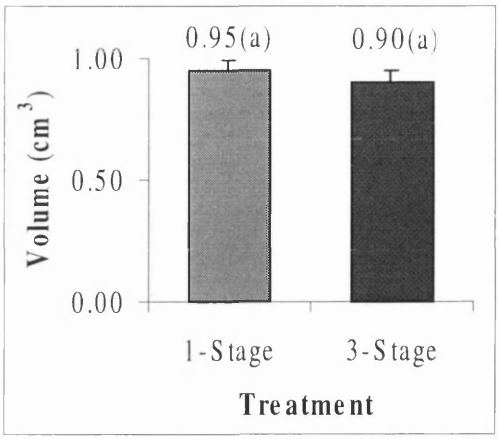
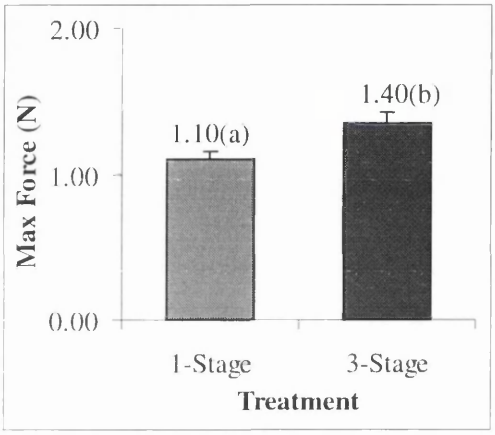


Figure 4.8 a.) Wedge Test b.) Cell Separation



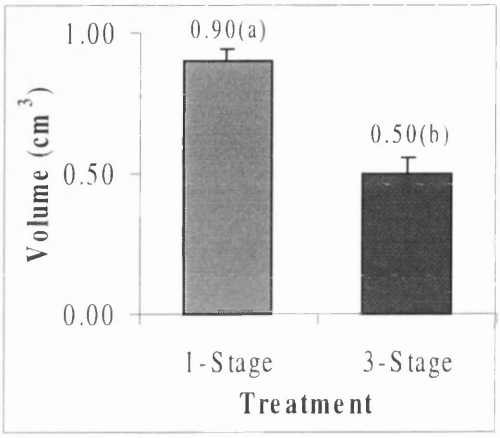
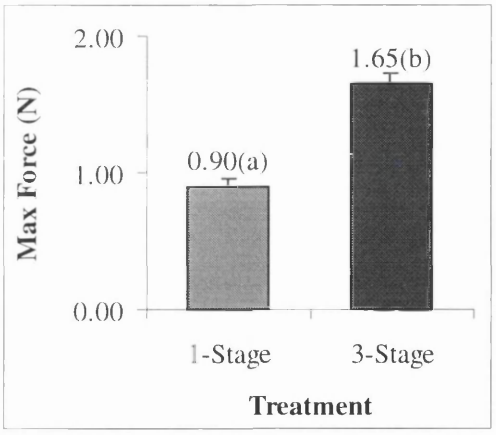


Figure 4.9 a.) Wedge Test b.) Cell Separation



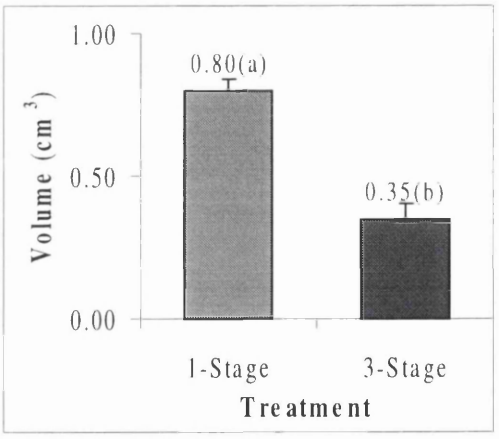
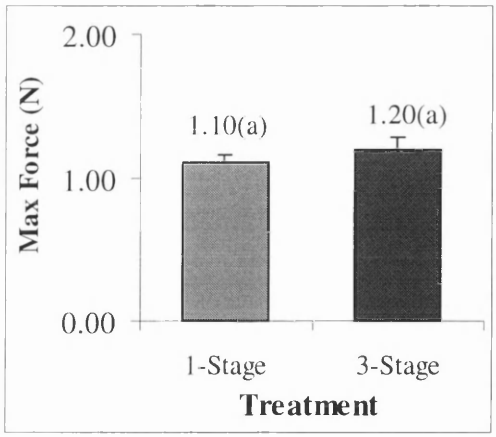


Figure 4.10 a.) Wedge Test b.) Cell Separation

Figure 4.11. Texture of one-stage and three-stage cooked cv. Cara. a) mechanical strength in Newtons (N). b.) Volume of separated cells in cm^3. In both figures bars on columns are standard error of the mean where n = 19 and 16 for figure a). and n = 10 and 10 for figure b). Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 4.12. Texture of one-stage and three-stage cooked cv. Desiree. a) mechanical strength measured in Newtons (N). b.) Volume of separated cells in cm^3. In both figures bars on columns are standard error of the mean where n = 20 and 18 for figure a). and n = 10 and 10 for figure b). Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 4.13. Texture of one-stage and three-stage cooked cv. Nadine. a) mechanical strength measured in Newtons (N). b.) Volume of separated cells in cm^3. In both figures bars on columns are standard error of the mean where n = 20 and 22 for figure a). and n = 10 and 10 for figure b). Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).



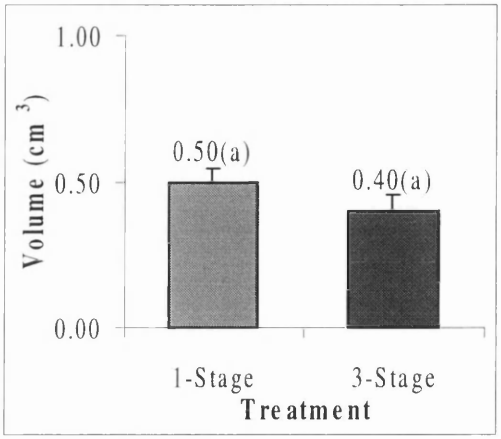
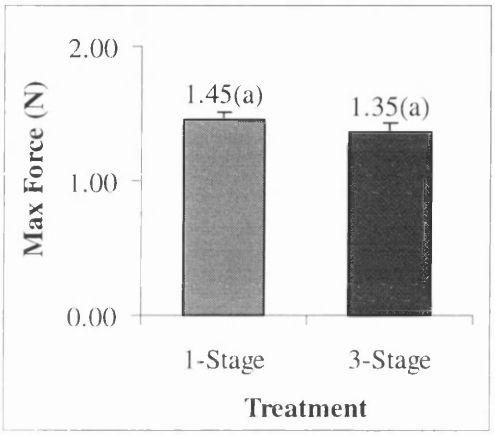


Figure 4.11 a.) Wedge Test b.) Cell Separation



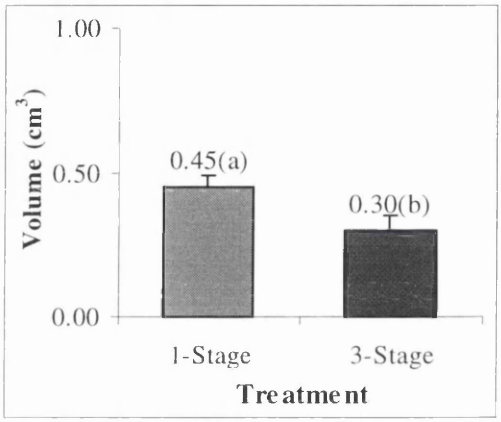
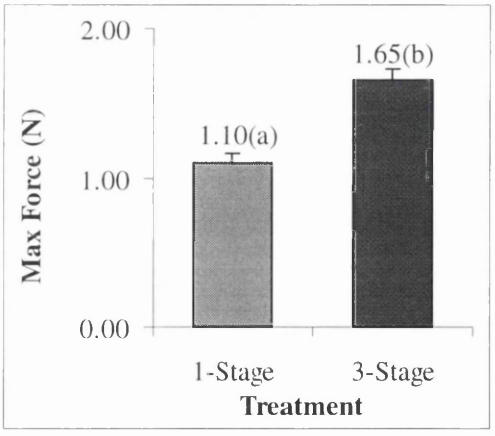


Figure 4.12 a.) Wedge Test b.) Cell Separation



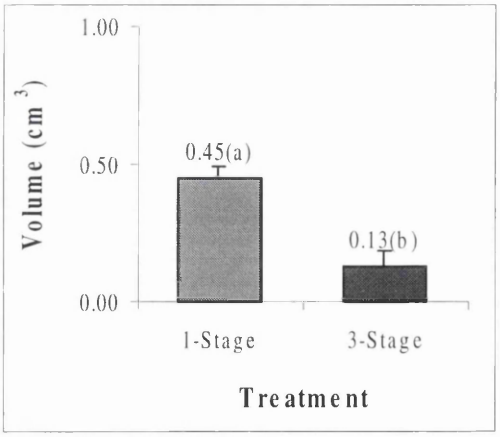


Figure 4.13 a.) Wedge Test b.) Cell Separation

The texture of Cara and Desiree is shown in Figures 4.11 and 4.12. For both cultivars, three-stage cooking resulted in no greater mechanical strength compared to one-stage cooked tissues. Cara also showed no difference in cell separation when three-stage cooked compared to one-stage cooked tissues, but Desiree did show a decrease in the volume of cells that separated when the tissues had been three-stage cooked. Apart from the cell separation result, the lack of an effect is difficult to explain, especially as the mechanical strength results suggested that Desiree was undercooked when one-stage cooked (Figure 4.3). Both varieties' cells did not separate to any great extent when one-stage cooked (Figure 4.5). If the level of cell separation in one-stage cooking is small, then small further reductions due to three-stage cooking may not be apparent. This however, does not explain the lack of difference in mechanical strength between the two cooking processes (Figures 4.12 and 4.13).

Cv. Nadine had the lowest starch content of all the varieties assessed (Table 4.2). When one-stage cooked, little cell separation was observed (Figure 4.13). Low starch content may produce only a small swelling pressure, resulting in little cell separation. When three-stage cooked, the volume of separated cells in Nadine was less than after one-stage cooking and there was an increase in mechanical strength (Figure 4.13). It was unclear whether this was due to a cell wall or a starch swelling effect.

In the following section, the cell walls of the cooked tissues were isolated and analysed to see if changes in cell wall chemistry could further explain changes in texture observed. Isolating the cell walls of all six varieties would have been extremely time consuming. It was, therefore, decided to isolate cell walls from only three varieties. The choice of cultivar was made on the basis of the textural properties when one-stage and three-stage cooked. The varieties chosen were Golden Wonder, Kerr's Pink and Cara. The effect that three-stage cooking had on the texture of the potatoes compared to one-stage cooked tissues is summarised in Table 4.3.

The texture of Cara and Desiree could not be fully explained by their starch content so both were suitable candidates for further investigation. As Desiree was

possibly undercooked, Cara was chosen. Maris Piper and Kerr's Pink both showed an increase in firmness and decrease in cell separation. Kerr's Pink was chosen, as its mechanical strength was greater when three-stage cooked, which was not explained by its starch content. Golden Wonder was the third choice as it showed firming but no difference in cell separation between one and three-stage cooking.

Variety	Mechanical Strength	Degree of cell separation
GW	Increased	One-stage cell separation was high but three-stage had no effect
KP	Increased	One-stage cell separation was high but reduced with three-stage cooking
Cara	No difference	One-stage cell separation was low but three-stage had no effect

Table 4.3. The change in texture of Golden Wonder, Kerr's Pink and Cara potatoes after three-stage cooking compared to one-stage cooked potatoes.

4.2.4. Chemical Characterisation of Isolated Cell Walls of Three Cultivars after One-stage and Three-stage Cooking.

4.2.4.1. Isolation and characterisation of MCB-insoluble material from raw, one-stage and three-stage cooked tissues.

The protocol for cell wall isolation and the purpose of each step in the procedure is described in Section 2.6. The procedure was designed for the isolation of fresh potato tissues either when raw or after a cooking process (Jardine, 1998). The tissues used for cell wall isolation of one-stage and three-stage cooked tissues had previously been used for wedge test measurements. After cooking the tissues were cooled and wedge test measurements were made. The tissues were then frozen to -18°C and cell walls isolated at a later date. This meant that there had to be slight alterations to the cell wall isolation protocol described in Section 2.6. Figures 4.14-4.16 outline these differences and the reasons for them.

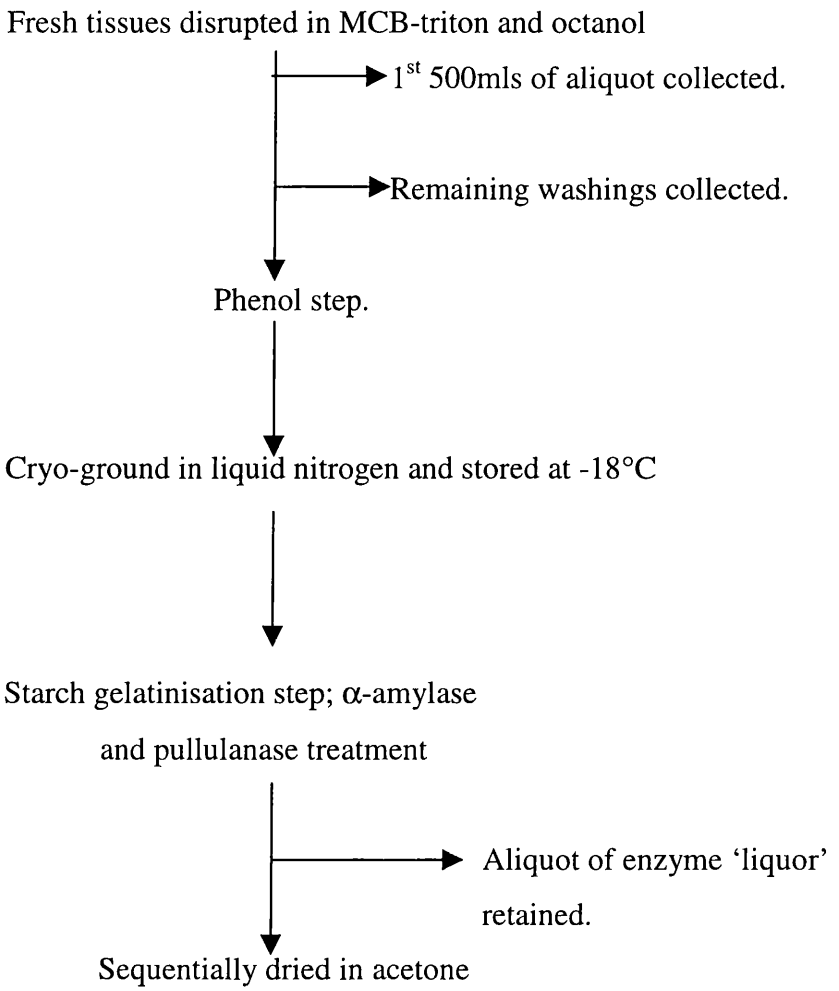


Figure 4.14. Flow chart of cell wall isolation method for raw tissues. As, discussed in section 2.6, before the cooking experiment began it was unclear which of the six varieties would be chosen for cell wall analysis. Therefore cell walls from all six varieties's were partially isolated. Cytoplasmic material was removed by blending in MCB-triton solution then filtered through a 54μm sieve. Proteins were denatured in saturated phenol and the cells were broken up by cryo-grinding in liquid nitrogen. After cryo-grinding the cell wall materials was stored at -18°C until the three varieties were selected. Subsequently, the chosen walls were heated to gelatinise starch, which was then removed using α-amylase and pullulanase. The walls were then sequentially dried in acetone.

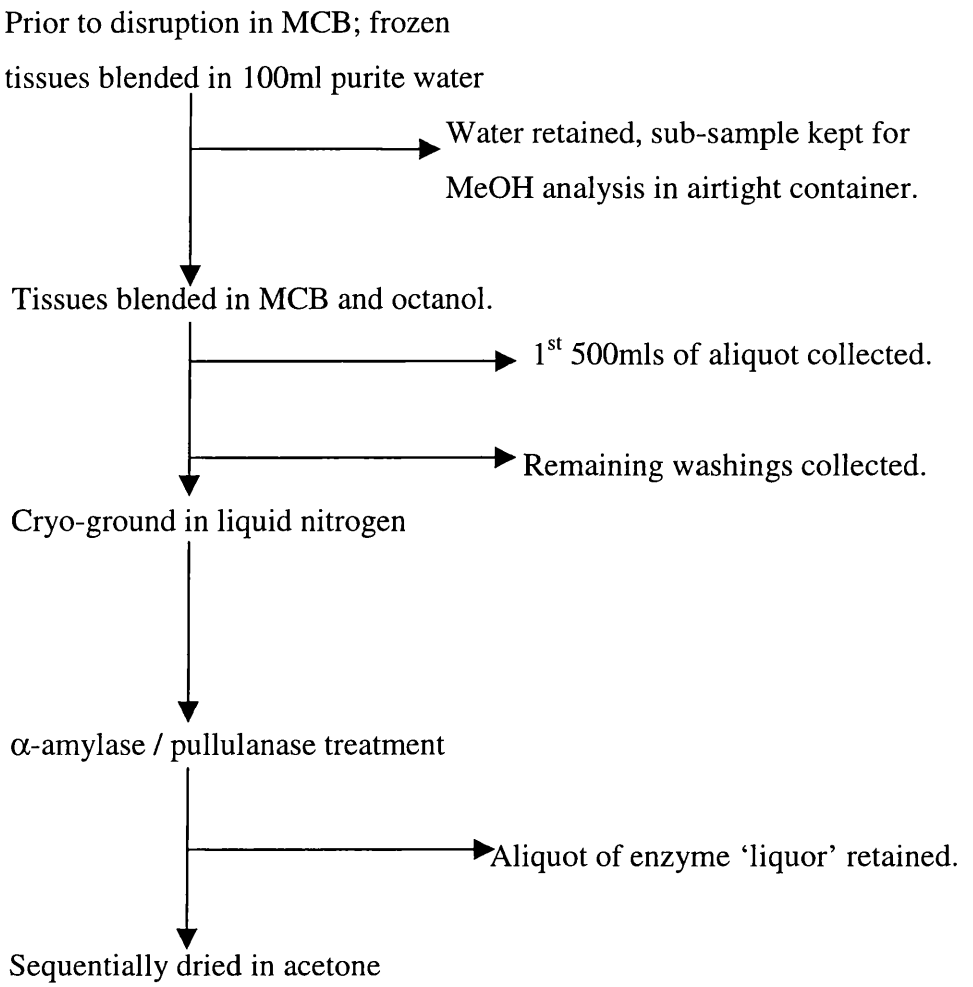


Figure 4.15 Flow Chart of cell wall isolation method for three-stage cooked tissues. In an attempt to quantify any released methanol due to PME activity, prior to disruption in MCB, tissues were blended in ultra-pure deionised water. Any methanol released would be in this fraction and could be measured. The outcome of this is discussed in Section 4.2.4.3. The tissues, after disruption in water, were blended in triton-MCB and the cell wall procedure was carried out as normal. Removal of starch during the α-amylase/pullulanase step was extremely difficult and resulted in a long incubation period.

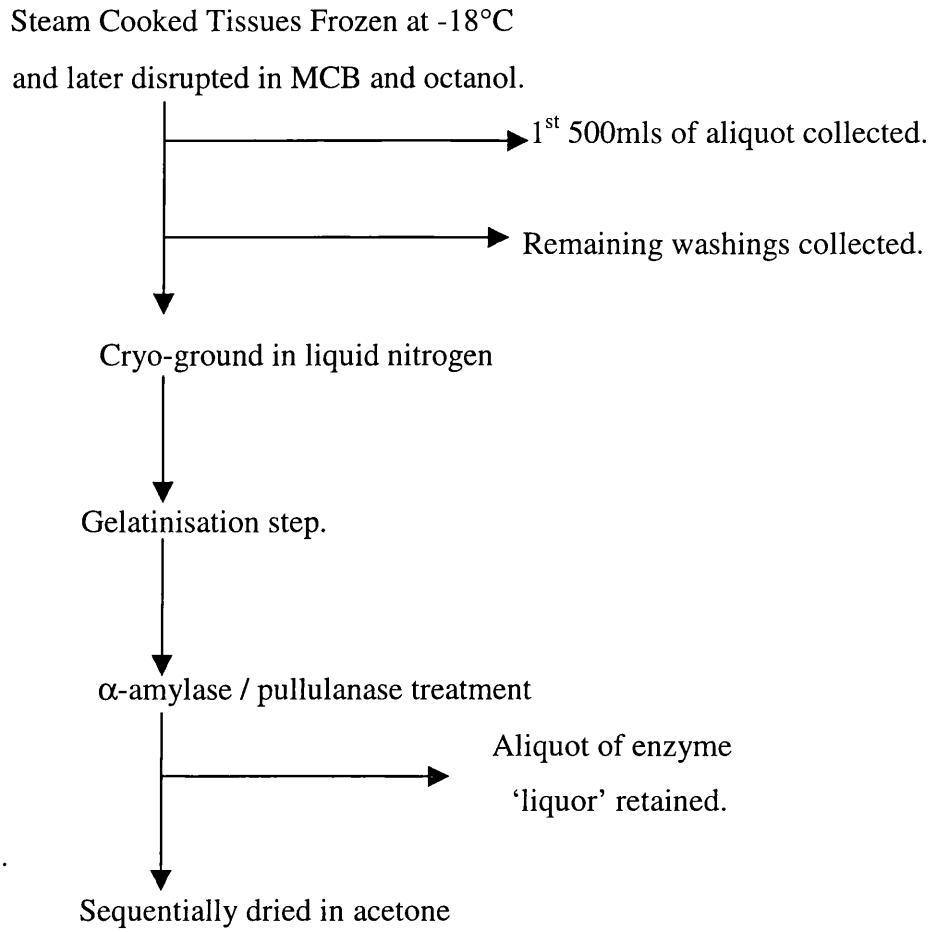


Figure 4.16. Flow Chart of cell wall isolation method for one-stage cooked tissues. The water extraction was not carried out with one-stage cooked tissues as it had not proven successful with three-stage cooked tissues. Instead frozen tissues were immediately disrupted in triton-MCB. A starch gelatinisation step was included to hopefully aid starch removal by α-amylase and pullulanase. This appeared to work as no KI/I_2 staining was visible under the microscope after a 24 hour incubation. Quantification by the enzymic method (Section 2.7.1) proved otherwise (Table 4.4)

Changes in pectin chemistry are expressed on a dry cell wall basis. The dried MCB-insoluble material contained both cell wall polysaccharides and residual starch not removed by α-amylase/pullulanase treatment (Jardine, 1998). Therefore, to ensure the results were expressed correctly, the residual starch content and dry matters were quantified (Section 2.7.1 and 2.7.2) and are shown in Table 4.4. A conversion factor, used for later calculations, was calculated from the two values and is shown in Table 4.4.

Variety	Cook Treatment	Starch % Residue	Dry Matter % Residue	Conversion Factor
Golden Wonder	Raw	5.13	4.72	0.90
	One-Stage	36.10	12.42	0.51
	Three-Stage	44.36	11.88	0.44
Kerr's Pink	Raw	9.87	6.17	0.84
	One-Stage	21.56	9.58	0.69
	Three-Stage	41.18	11.90	0.47
Cara	Raw	12.62	6.47	0.81
	One-Stage	20.95	11.35	0.68
	Three-Stage	44.41	10.02	0.56

Table 4.4. Residual starch and dry matter contents of MCB-insoluble material isolated from the three cultivars after different cooking treatments. The conversion factor was calculated by summing the percentage moisture content and residual starch content then subtracting this value from 100. The value obtained was then divided by 100 giving the conversion factor. This factor could then be multiplied by the weight of MCB-insoluble material, which allowed results to be expressed on a cell wall weight basis.

The starch contents (Table 4.4) of the uncooked MCB-insoluble materials were considerably lower than the cooked materials and were in the same region as found by Jardine (1998). As starch had already been gelatinised when the potatoes were cooked, it was presumed that no gelatinisation step was needed when cell walls were isolated from frozen cooked material.

Cell walls were isolated from three-stage cooked samples before one-stage cooked samples. After an overnight incubation with α-amylase and pullulanase, large amounts of KI/I_2 stained starch remained. Even after three days of incubation significant amounts were still present. At this point MCB-insoluble material was removed from the enzyme liquor, as it was highly likely that prolonged incubation in buffer would lead to losses of pectin from the cell wall by solubilisation. Quantification of the residual starch in the MCB-insoluble material, by the enzymic method, showed that starch constituted 40-45% of the MCB-insoluble material (Table 4.4).

The probable explanation of this was starch retrogradation during freezing. When starch, gelatinised during cooking was frozen, the separation of the amylose and amylopectin phases allowed crystallisation (i.e retrogradation) of the amylose (Morris, 1990). This would restrict access of α-amylase and pullulanase enzymes to amylose during the starch removal process, making it very difficult. It was highly likely that starch removal had been successful in raw samples (Table 4.4), as starch had not been gelatinised prior to freezing. This meant that no retrogradation could occur during storage, so when the material was heated to gelatinise starch, all the starch was accessible to the enzymes.

It might have been possible to re-gelatinise the starch in the three-stage cooked samples and re-incubate it with α-amylase and pullulanase enzymes. As they had already had a three-day incubation, there was a possibility of pectin solubilisation during a further incubation so it was decided to use the MCB-insoluble residues even with their high starch contents.

A starch gelatinisation step was, however, included to aid starch removal from cell walls isolated from one-stage cooked samples. Starch gelatinisation appeared to have worked, as an overnight incubation with α-amylase and pullulanase removed all KI/I_2-stainable starch when observed under the light microscope. Quantification by the enzymic method proved otherwise as there were still large amounts present in the MCB-insoluble materials (Table 4.4). The Golden Wonder samples still had 35% starch present in the MCB-insoluble material.

Incubating Kerr's Pink and Cara material after the gelatinisation step resulted in the starch content being halved to around 21% of the MCB-insoluble material (Table 4.4).

The difference in starch content predicted by KI/I_2 staining and enzymic determination was considerable. Jardine (1998) initially quantified starch levels in the MCB-insoluble material by a KI/I_2 colorimetric method. Jardine (1998) found that the KI/I_2 method did not correlate well with the enzymic method (Karkalas, 1985) used. He also observed that the lack of staining with KI/I_2 was not always indicative of complete starch removal (Jardine, unpublished work).

The obvious conclusion from these results is that starch removal is far easier if it is carried out on samples that have not been previously frozen. When designing this experiment, problems with starch removal had not been anticipated. Freezing all the samples, then isolating cell walls from samples of interest at a later date was felt to be the most convenient and practical method. The results also show that staining with KI/I_2 should not be used to monitor complete starch removal from cell walls and that quantification of residual starch and dry matter of isolated cell walls should be done so that results can be interpreted with more confidence.

The problem of starch removal in these samples was exacerbated further by the method of freezing. The tissues were slowly frozen to -18°C, which increase the time for starch retrogradation. In chapter seven to try to minimise this problem, cooked tissues from which cell walls were isolated, were frozen in liquid nitrogen immediately after cooking to restrict starch retrogradation.

4.2.4.2. Pectic galacturonan content of MCB-insoluble cell walls and pectin released during cell wall isolation.

The pectic galacturonan content of the MCB-insoluble cell walls was measured as described in Section 2.4.2. The results were statistically tested by Tukey's LSD in two ways. The first was to study the effect of cooking on galacturonic acid levels

for each individual variety (Table 4.5). Secondly, the differences between the three cultivars after each cooking treatment were compared (Table 4.6). These comparisons were considered to be intra-variety and inter-variety comparisons respectively.

The results were compared with the quantities of galacturonic acid solubilised into the pre-cook and cool waters and during the cell wall isolation procedure (Table 4.7). The quantities of galacturonic acid measured are probably an over estimation, as the neutral sugar correction did not fully compensate for high starch content in some of the samples. This would be more likely for three-stage cooked samples, in particular, due to the problems of starch removal. The results are expressed on a recovered cell wall basis, though it was unlikely that 100% of the cell wall was recovered, which would also lead to over-estimation.

Variety	Cook Treatment	Gal A (μmol/ g)	% Gal A Cell Wall.	*Varietal LSD*
Golden Wonder	Raw	903	16.60	a
	One-Stage	530	9.33	b
	Three-Stage	280	4.94	c
Kerr's Pink	Raw	769	13.55	l
	One-Stage	651	11.47	m
	Three-Stage	542	9.55	n
Cara	Raw	772	13.60	x
	One-Stage	631	11.93	y
	Three-Stage	677	11.11	z

Table 4.5. Galacturonic acid content of three cultivars when raw, one-stage and three-stage cooked. LSD comparison is of intra-varietal results after different cook treatments e.g. Gal A content of Cara cell walls when raw after one-stage or three-stage cooking.

The galacturonic acid content remaining after cell wall isolation, in the cell walls of raw tissues was lower than published values, which range from 17% to 25%

(Jardine, 1998, Binner *et al.*, 2000) (Table 4.5). Of the three varieties, Golden Wonder had the highest amount of galacturonic acid remaining in the cell wall. Only 13-14% of the MCB-insoluble wall of Kerr's Pink and Cara was pectic galacturonan (table 4.6).

The likely cause of the low levels remaining in the cell walls was solubilisation of pectin during isolation of the cell walls. The amount of galacturonic acid detected in the washings retained during cell wall isolation (Table 4.7) was higher than that found by Jardine (1998) when isolating cell walls from uncooked tissues. For all three varieties, very little pectin was released in the first 500ml washing of raw potatoes, though more came out in the second washing. The highest amounts of galacturonic acid were found in the enzyme fractions, especially for cvs Cara and Kerr's Pink. It is likely that the heating step used to gelatinise starch caused some thermal degradation of the cell wall.

Cook Treatment	Variety	Gal A (μmol/g)	% Gal A Cell Wall	*Cook LSD*
Raw	Golden Wonder	903	16.60	a
	Kerr's Pink	769	13.55	b
	Cara	772	13.60	b
One-Stage	Golden Wonder	280	4.94	l
	Kerr's Pink	542	9.55	m
	Cara	677	11.93	n
Three-Stage	Golden Wonder	530	9.33	x
	Kerr's Pink	651	11.47	y
	Cara	631	11.11	y

Table 4.6. Galacturonic acid content in raw, one-stage or three-stage cooked potatoes of three different varieties. LSD comparison is of inter-varietal results after different cook treatments e.g. Gal A of raw Golden Wonder, Kerr's Pink and Cara.

Cook Treatement	Cultivar	Pre-cook Water	Water Extract	1st Wash	2nd Wash	Enzyme	Total
Raw	Golden Wonder	-	-	3.63	60.4	37.6	101.6
	Kerr's Pink	-	-	7.46	88.3	109.1	204.9
	Cara	-	-	1.82	91.4	98.0	191.2
One-Stage	Golden Wonder	-	-	383.3	36.2	280.3	699.5
	Kerr's Pink	-	-	181.9	21.8	228.3	432.0
	Cara	-	-	130.0	24.5	132.9	287.4
Three-Stage	Golden Wonder	7.5	11.0	176.8	43.8	218.0	457.0
	Kerr's Pink	21.2	4.7	89.4	30.9	190.9	337.2
	Cara	2.6	2.2	73.4	17.4	120.2	215.8

Table 4.7 Solubilised galacturonic acid released from the three cultivars during cell wall isolation expressed as µmol/g dry cell wall. Raw tissues released the least amount of pectin. One-Stage cooking released the most pectin of all three cooking treatments. Three-stage cooking treatments had reduced amounts of pectin solubilised. For all cultivars the cooked potatoes had substantial amounts of pectin released into the enzyme liquor.

Unlike the protocol of Jardine (1998), all the MCB insoluble material was heated so that solubilisation data could be expressed on a cell wall basis. As there was more material to be heated, this required a longer boiling step to gelatinise all the starch; therefore heating was increased from 40 to 100 seconds. After the cell walls had been heated for 100 seconds, they were decanted into approximately 500ml of chilled MCB buffer to prevent heat degradation of the cell wall. As more cell wall material was heated than in the Jardine (1998) method, the ratio of chilled buffer to hot buffer would be less and therefore the length of time to cool the material might have been slightly longer. Both these factors could have lead to solubilisation of pectin by heat degradation during the starch gelatinisation step.

Cell walls isolated from each variety after one-stage cooking had lower galacturonic acid contents than uncooked cell walls (Table 4.5). The amount remaining in one-stage cooked cell walls for each variety, though, was different; Golden Wonder had the least amount, followed by Kerr's Pink, while Cara had retained the most galacturonic acid (Table 4.6). Galacturonic acid loss during cooking is a well-known phenomenon (Andersson *et al.*, 1992, van Marle, 1997, Ng and Waldron, 1997a, Jardine, 1998) however, the losses found, especially in Golden Wonder, were higher than previously reported.

The lower amounts of galacturonic acid present in the MCB-insoluble walls of one-stage cooked samples was reflected in the large amounts of galacturonic acid present in the MCB-soluble fractions (Table 4.7). The first and second washes reflect the amount of pectin solubilised by degradative cooking processes. Higher amounts of galacturonic acid were present in the first wash fractions of all three one-stage cooked samples compared to raw samples (Table 4.7). Therefore it is likely that processes that occur during cooking of potatoes were partially responsible for the losses of pectin from cell walls. Large amounts of galacturonic acid were also released into the enzyme liquor of one-stage cooked potatoes (Table 4.7). As the incubation period for one-stage cooked samples was only 24 hours, prolonged incubation in MCB was not the cause of the large amounts present. Presumably, the losses of galacturonic acid into the enzyme liquor were caused by boiling the samples to gelatinise starch causing pectin depolymerisation, which was then released out of the cell wall.

As with one-stage cooked samples, cell walls isolated from three-stage cooked tissues had less galacturonic acid than raw samples (Table 4.5). For cvs. Golden Wonder and Kerr's Pink, more galacturonic acid remained in the MCB-insoluble cell walls of the three-stage cooked samples than one-stage cooked samples (Table 4.5); Golden Wonder having less galacturonic acid remaining in the cell walls than Kerr's Pink (Table 4.6). Ng and Waldron (1997a) have made similar observations when they studied changes that occurred in cv Binjte, when steam cooked with or without a pre-cook. The opposite was true for cv. Cara; more galacturonic acid remained in the MCB-insoluble walls of the one-stage cooked samples than three-stage cooked samples (Table 4.5), though the difference between the two was quite small.

The amount of galacturonic acid measured in the soluble fractions during cell wall isolation of three-stage cooked samples was less than for one-stage cooked samples (Table 4.7). This is agrees with the levels retained in the MCB-insoluble material (Table 4.5). There was only a small amount of galacturonic acid in the pre-cook water and none was detected in the cooling water (Table 4.7). There was also little galacturonic acid in water extracts. To minimise pectin losses, blending and filtering had been done as quickly as possible in an attempt to keep the potatoes frozen whilst breaking up the tissue structure sufficiently to release any methanol into the water. The majority of pectin solubilisation was during the lengthy α-amylase/pullulanase treatment (Table 4.7). The losses of galacturonic acid during the gelatinisation step were not as great for three-stage cooked samples as for the one-stage cooked samples. As no gelatinisation step was included with three-stage cooked samples, losses most likely occurred because of the long incubation in α-amylase and pullulanase.

4.2.4.3. Degree of Methylation of MCB-insoluble cell wall pectin

The degree of methylation of the galacturonic acid was measured as detailed in Section 2.7.4. As with the galacturonic acid content of the MCB-insoluble material, the degree of methylation results were compared statistically in two

ways. The first was the differences within a cultivar after different cooking treatments i.e. intra-variety comparison (Table 4.8). The second was the degree of methylation of each cultivar after the three different cooking treatments i.e. inter-variety comparisons (Table 4.9). Measuring the degree of methylation of the MCB-soluble galacturonic acid could not be done. High levels of methanol were found in blank assays, so measurements could not be made with confidence. Samples had been stored with toluene to prevent microbial degradation and trace levels of methanol in the toluene had contaminated samples resulting in extremely high blanks. As significant amounts of pectin were released during cell wall isolation, the results might not reflect the true degree of methylation in the cell wall prior to isolation.

Variety	Cook Treatment	MeOH (μmol/g)	% Degree of Methylation of Pectin	*Varietal LSD*
Golden Wonder	Raw	379.1	40.2	a
	One-Stage	70.9	25.3	b
	Three-Stage	85.2	16.1	c
Kerr's Pink	Raw	302.5	39.3	l
	One-Stage	138.0	25.5	m
	Three-Stage	104.5	16.1	n
Cara	Raw	251.3	34.0	x
	One-Stage	140.6	20.8	y
	Three-Stage	117.9	18.7	y

Table 4.8. The degree of methyl esterification of pectic galacturonan remaining in the cell wall after isolation of the three cultivars after different cooking treatments. The concentration of methanol released after saponification was expressed in μmol/g of cell wall. This value was also divided by the galacturonic acid content, also in μmol/g of cell wall, and expressed as a percentage degree of methyl esterification of pectic galacturonic acid. The LSD comparison is of intra-varietal percentage degree of methylation results after different cook treatments eg degree of pectin methyl esterification of Cara cell walls when raw and after one-stage or three-stage cooking.

Variety	Cook Treatment	MeOH (μmol/g)	% Degree of Methylation of Pectin	*Cook LSD*
Raw	Golden Wonder	379.1	40.2	a
	Kerr's Pink	302.5	39.3	a
	Cara	251.3	34.0	b
One-Stage	Golden Wonder	70.9	25.3	l
	Kerr's Pink	138.0	25.5	m
	Cara	140.6	20.8	m
Three-Stage	Golden Wonder	85.2	16.1	x
	Kerr's Pink	104.5	16.1	x
	Cara	117.9	18.7	y

Table 4.9. The degree of methyl esterification of pectic galacturonan remaining in the cell wall after isolation of the three cultivars after different cooking treatments. How the results were expressed is detailed in the legend of Table 4.8. The LSD comparison is of inter-varietal results after the same cook treatment e.g. the degree of pectin methyl esterification of raw Golden Wonder, Kerr's Pink and Cara

Cell wall pectin from uncooked cell walls from Cvs Golden Wonder and Kerr's Pink had the same degree of methylation, while cell wall pectin from cv.s Cara's was less methylated (Table 4.9). After one-stage cooking the degree of methylation was reduced in all three varieties by approximately 15%. In Section 3.5, the temperature profile of the steam cooked potato was followed by observing when starch gelatinisation commenced. Starch gelatinisation in potatoes occurs between 58 and 64°C (Lamberg and Olsson, 1989). At these temperatures PME activity rapidly declines (Tijskin *et al.*, 1997). Starch gelatinisation was complete after two and a half minutes hence PME would have been rapidly denatured in one-stage cooked tissues and its remaining activity could be considered minimal. Therefore, it can be assumed that the decrease in methyl esterification of MCB-insoluble pectin was due to the release of highly methylated pectin during cooking and the cell wall isolation procedure.

The degree of methylation of three-stage cooked MCB-insoluble material for Golden Wonder and Kerr's Pink was lower than one-stage cooked tissues, while no significant difference was found for cv. Cara (Table 4.8). As the extent of pectin demethylation during steam cooking was unclear, the extent of demethylation caused by the pre-cook could not be evaluated from these results. Presumably the lower degree of methylation was due to PME activation during the pre-cook.

Despite the difficulties caused by losses of pectin during cell wall isolation, the changes in pectin chemistry do provide some information as to why the three varieties had slightly different textures. In both Golden Wonder and Kerr's Pink pre-cooking caused a reduction in solubilisation of pectin during steam cooking (Table 4.5), and the pectin remaining in the cell wall was less methylated (Table 4.8).

It has been suggested that cell walls containing more pectin with a lower degree of methyl esterification would be more able to resist cell separation (Linehan and Hughes, 1969). Relative to the amounts of galacturonic acid remaining in one-stage cooked cell walls, Golden Wonder when three-stage cooked retained more galacturonic acid than Kerr's Pink (Table 4.8). Despite this, Golden Wonder's degree of cell separation was unaffected by three-stage cooking whilst Kerr's Pink degree of cell separation was reduced (Figures 4.8 and 4.10). Therefore factors other than total cell wall pectin such as distribution of low ester pectin (see Chapter 5) ot the effects of higher starch content may control the texture of cooked Golden Wonder.

Golden Wonder had the highest starch content of the six cultivars (Table 4.1) and released the largest volume of separated cells (Figure 4.5). In texture studies that compared twelve cultivars, in terms of dryness, Golden Wonder readily broke down into a slurry of free cells when boiled (Wilson W. and Jarvis M.C., unpublished results). It is highly likely that gelatinisation and cooling starch during the pre-cook and cool steps of Golden Wonder was not sufficient to reduce the starch swelling pressure on cell walls during cooking and therefore there was no reduction in cell separation.

Like Golden Wonder, the degree of cell separation in cv. Cara was unaffected by three-stage cooking (Figure 4.11). The galacturonic acid content and degree of methylation of cell walls of cv. Cara's was the same after one-stage or three-stage cooking. After cooking, very little pectin was lost from the cell wall (Tables 4.5 and 4.8). More pectin in the cell wall after cooking may maintain greater cell adhesion, resulting in a small amount of cell separation being observed. As both one and three-stage cooked samples retained the same amount of pectin within walls, which had the same degree of methyl esterification, in the case of cv Cara there was no contradiction with the lack of textural differences when one or three-stage cooked.

It was hoped that general observations about changes in galacturonan chemistry caused by one-stage and three-stage cooking in the three varieties chosen for analysis could explain differences in texture of all the varieties used. This did not prove to be possible because the results obtained with cv Golden Wonder could not be reconciled with results from the other two cvs. With only three varieties, starch effects could not be separated from effects of cell-wall architecture.

In terms of texture measurements, when cultivars were one-stage cooked the extent of cell separation broadly agreed with the NIAB results and could be explained by their starch content. Problems arose when trying to interpret the texture of the cultivars when a pre-cooking step was included. Interpreting the results was not helped by the fact that the two-stage pre-cook used with three-stage (70°C) cooking involves PME activation and gelatinising then cooling of starch. In chapters six, seven and eight the texture of three-stage (55°C) and three-stage (70°C) cooked samples were compared in just one variety, cv Maris Piper, in an attempt to distinguish between cell wall and starch effects.

CHAPTER FIVE

MICROSCOPY EXAMINATION OF TWO CULTIVARS WHEN RAW AND ONE-STAGE COOKED

5.1. Introduction

Two out of the six varieties were studied by light microscopy (LM), transmission electron microscopy (TEM) and electron energy loss spectroscopy (EELS). The choice of cultivars was based on their cell separation properties when one-stage cooked. The cv. Golden Wonder had the highest starch content of the six and the greatest cell separation values whereas cv. Cara's cell separation was comparable to the other low starch content varieties

van Marle *et al.* (1997 b) used TEM to examine changes in a mealy and a firm variety, when raw, partially boiled and after fifteen minutes of boiling. The raw cell walls of both cultivars were held tightly together, with the mealy variety appearing to have a more electron-dense cell wall. Upon cooking the cell had separated, with separation initiated at tricellular junctions of cell walls.

Bush and McCann (1999) showed that calcium bound pectin lay predominantly in tricellular junctions and at the corner of air spaces in potato parenchyma tissues. Calcium has also been found to be preferentially distributed in the tricellular junctions of developing flax hypocotyls (Goldberg *et al.*, 1996) and apples

(Huxham *et al.*, 1999). The tricellular junctions and corners of cell spaces are under most tensile stress from turgor and starch swelling pressure, (Jarvis, 1999). Calcium cross-linked pectin within these regions increase the strength of the cell wall to combat separation pressures (Goldberg *et al.*, 1996, Jarvis 1999, Bush and McCann, 1999).

In this chapter, changes caused by one stage cooking of cvs Cara and Golden Wonder were examined by LM and TEM microscopy techniques to evaluate changes in the cell wall caused by steam cooking. The distribution in the cell wall of low esterified pectin and calcium bound pectin was evaluated by EELS microscopy.

5.2. Results

5.2.1. Morphological Examination by light microscopy and transmission electron microscopy.

A 3mm core was taken from the pith region of the raw or cooked potato was sequentially dehydrated in ethanol and embedded in LR White resin. General differences were examined between raw and cooked potatoes but varietal differences were not looked at. The study focused on examination by TEM but some sections were examined by light microscopy.

Sectioning was especially difficult with raw samples, as the resin had not infiltrated into the starch grains. During sectioning, the grains would fall out leaving large holes in the specimens, which led to tearing of the sections (Figure 5.1). The remnants of the plasma membrane were visible as small thin strands within the cells.

After cooking, the starch had gelatinised and during alcohol dehydration it had shrunk into the middle of the cells (Figure 5.2). Starch shrinkage in cooked potatoes, when studied by TEM, has been observed before (McComber *et al.*, 1994 and van Marle *et al.*, 1997 b). The shape of the cooked cells was different to the raw cells (Figures 5.1 a + b and 5.2 a + b). Alcohol dehydration during the resin embedding procedure caused shrinkage in both. In the raw tissues this caused the tissues to shrink as a whole, causing the cell shape to distort. After cooking, the strength of adhesion between cells had weakened allowing cells to separate during shrinking. This resulted in cells keeping their shape. In the light micrographs of the cooked sections, there were areas where the cell walls were 'pinched' together and had not separated. In some tricellular junctions there appeared to be deposited material (Figure 5.2).

The observations made under the light microscope were seen in more detail in 100nm thick sections observed with the TEM. The middle lamellae of raw cell walls were clearly visible as an electron dense region between the two walls (figures 5.3-5.6). A material of high electron density on the inside of cell walls was present which was likely to be remnants of the plasma membrane degraded during the alcohol dehydration. At the corners of tricellular junctions the walls seemed thicker, possibly to impart greater physical strength (Figure 5.4).

Compared to the raw cell walls, cell walls of cooked tissues were less compact and ordered (figures 5.7-5.10), this was also observed by van Marle *et al.* (1997 b). The walls of cooked tissues appeared much wider than raw cell walls and it could be assumed that cooking caused a swelling of the cell wall. The electron dense region in the middle lamella was no longer present, the probable cause of this being pectin solubilisation during cooking (van Marle *et al.*, 1997b). In other regions, cell walls had separated completely (Figure 5.8). van Marle *et al.* (1997b) had also dehydrated the tissues in ethanol as part of their fixation process. As with this work, alcohol dehydration had caused cooked cells to separate, whilst the raw remained together.

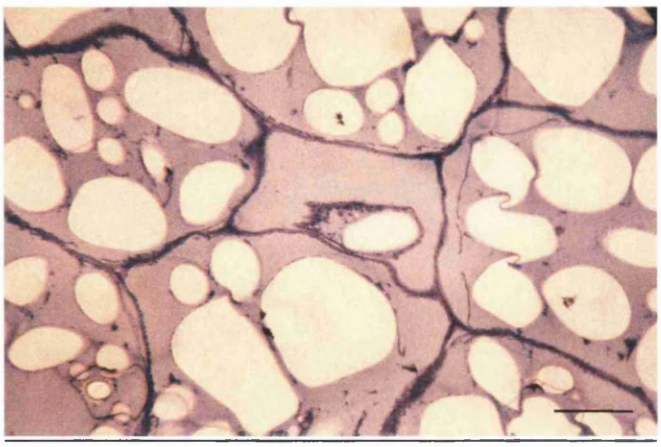


Figure 5.1 a. Raw Golden Wonder sections.

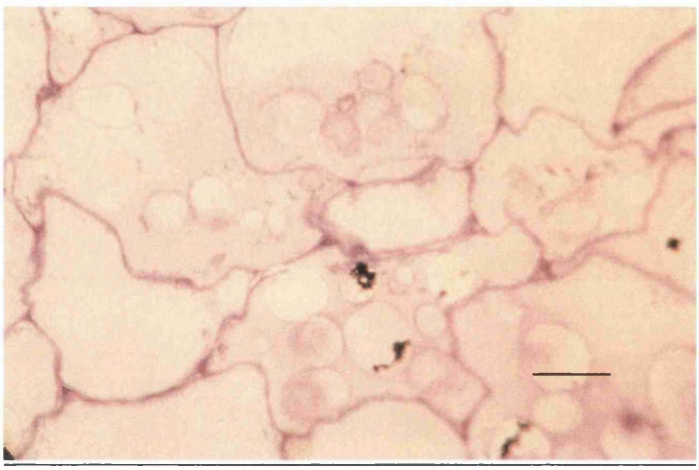


Figure 5.1 b. Raw Cara sections.

Figure 5.1. Raw Golden Wonder (figure a) and Cara (figure b) alcohol dehydrated, LR White embedded specimens. Starch grains that did not get embedded in resin fell out during sectioning which caused the large holes in the sections. During alcohol dehydration the tissues shrunk leading to cellular distortion. Remnants of the plasma membrane were visible as small thin strands within the cells. Scale bar are 137.5μm.

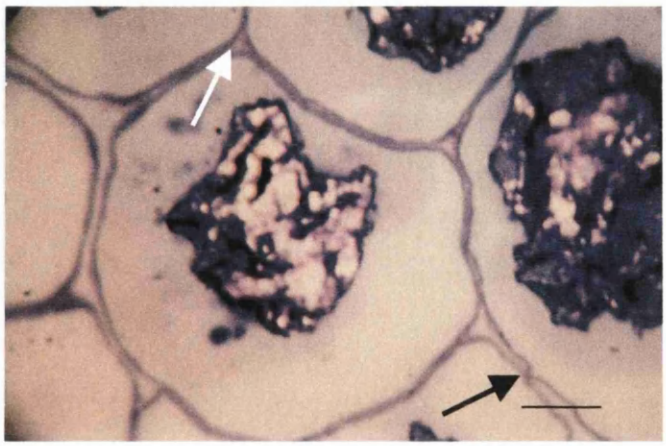


Figure 5.2 a. One-stage cooked Golden Wonder sections.

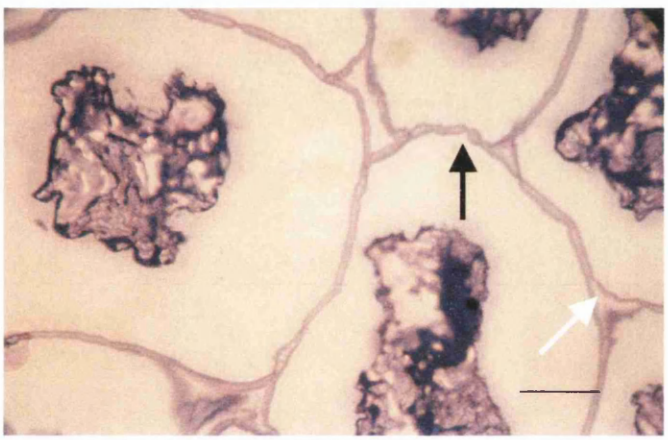


Figure 5.2 b. One-stage cooked Cara sections.

Figure 5.2. Light micrographs of one-stage cooked Golden Wonder (graph a) and Cara (figure b). The shape of the cooked cells differed from raw tissues. Alcohol dehydration during resin embedding procedure caused shrinkage in both. In the raw tissues this caused the tissues as a whole to shrink causing the cell shape to distort. During cooking the strength of adhesion between cells had weakened, allowing cells to separate during alcohol dehydration shrinking, thus allowing the cells to keep their shape. Around the walls there were areas where the cells walls were 'pinched' together and had not separated (as indicated by black arrows). Evidence from electron micrographs (Figures 5.3-5.10) identified these regions as plasmodesmata. Material appeared to be deposited in some intercellular air spaces (white arrows). Scale bar in pictures is 137.5μm.

The remnants of the plasma membrane observed in raw tissues were also no longer present (figures 5.7-5.10), as membranes were rapidly destroyed by heat (Zhang *et al.*, 1993). The air spaces of the steam cooked slices were filled with material, which may be solubilised pectin (Figure5.9). The tricellular junctions of boiled tissues examined by TEM did not appear to have any deposits in the tricellular junctions (Marle *et al.* 1997b). This was probably because boiling could allow the escape of solubilised pectin, whilst in steam cooked potatoes pectin would remain trapped.

Light microscopy images indicated that certain regions of the cooked walls appeared 'pinched'. On closer examination using TEM, pinching was seen to be caused by plasmodesmata. Plasmodesmata are protein structures involved in cell messenger transfer and can cover up to 50% of the cell wall (Brett and Waldron, 1996). In raw samples plasmodesmata appeared unaffected by cooking (Figures 5.5 and 5.6.). In cooked samples, as mentioned above, cell walls had swollen in width except in regions where plasmodesmata were located (Figure 5.10).

During cooking, the walls around them had not weakened and therefore the wall had not swollen. Pectin around plasmodesmata, have low degrees of methyl esterification and are bound by calcium (Bush and McCann, 1999). A region of wall with pectin of this type would be less prone to degradation processes during cooking. It has been postulated that these regions, which are not weakened by cooking, could be the place where cell rupture might occur during chewing (Waldron *et al.*, 1997).

Figure 5.3. TEM micrograph of raw cv Cara cell walls. The middle lamella is clearly visible as an electron dense region between the two walls. The black material on the inside of cell walls is likely to be remnants of the plasma membrane degraded during the alcohol dehydration. Scale bar on picture is 1.2μm.

Figure 5.4. Micrograph of a intercellular airspace from cv. Golden Wonder. The darker area around the corners of each cell wall is likely to be thickening of the wall. Scale bar on picture is 1.2μm.

Figure 5.5. Plasmodesmata from cv. Golden Wonder cut near the top of the cell wall. Scale bar on picture is 1.2μm .

Figure 5.6. Plasmodesmata from cv .Golden Wonder cut through the middle of the cell wall. Scale bar on picture is 1.2μm.

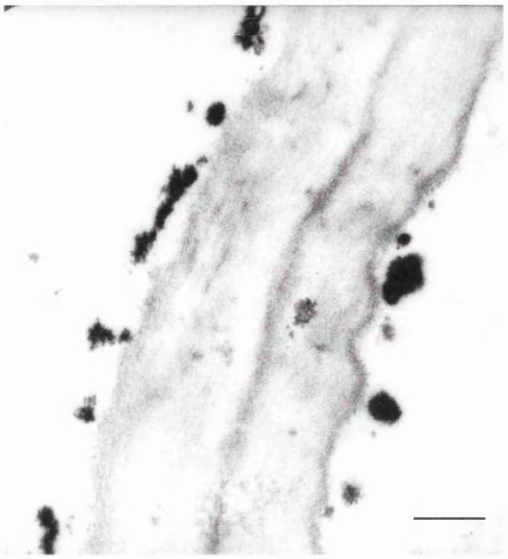


Figure 5.3

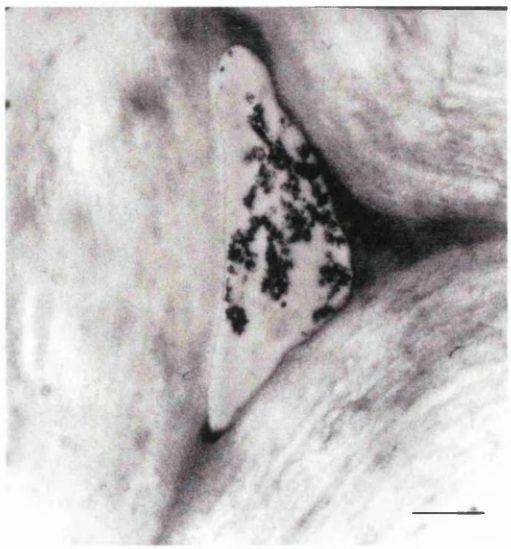


Figure 5.4

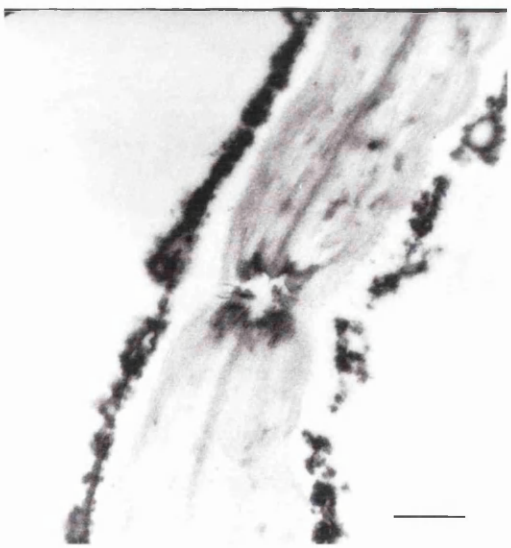


Figure 5.5

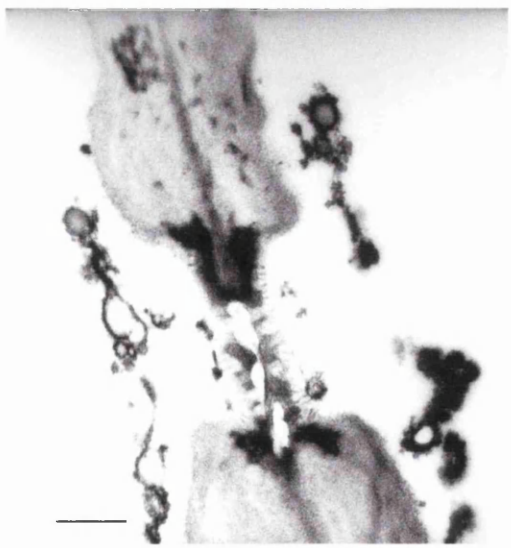


Figure 5.6

Figure 5.7. Cell wall from cooked Cara in a region where cell had not separated. Compared to the raw cell walls the walls were less compact and ordered. The remnants of the plasma membrane observed in raw tissues were also no longer present as these membranes were rapidly destroyed by heat (Zhang *et al.*, 1993). Scale bar on picture is 1.2μm.

Figure 5.8. Cell wall from cooked Golden wonder. This figure shows the separation during alcohol dehydration of two adjacent walls caused by weakening of the cell walls by cooking. Scale bar on picture is 1.2μm.

Figure 5.9. An intercellular airspace from cooked Cara. The tri-cellular junction (arrow) was filled with material, most likely pectin solubilised during cooking. Scale bar on picture is 1.2μm.

Figure 5.10. Region of cooked Cara cell wall where plasmodesmata was unaffected by cooking. The pinching caused by the plasmodesmata is visible in the picture. Scale bar on picture is 1.2μm.

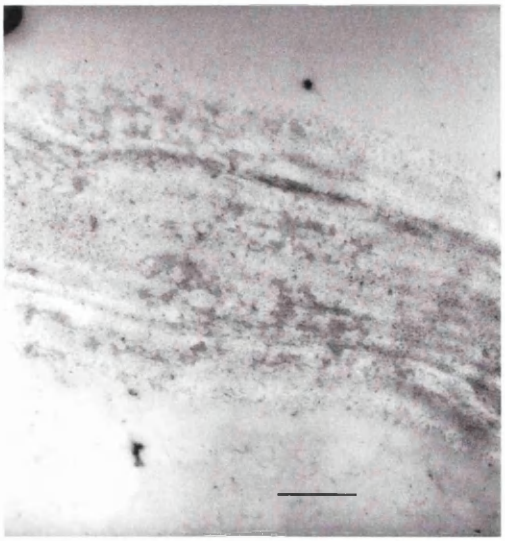


Figure 5.7

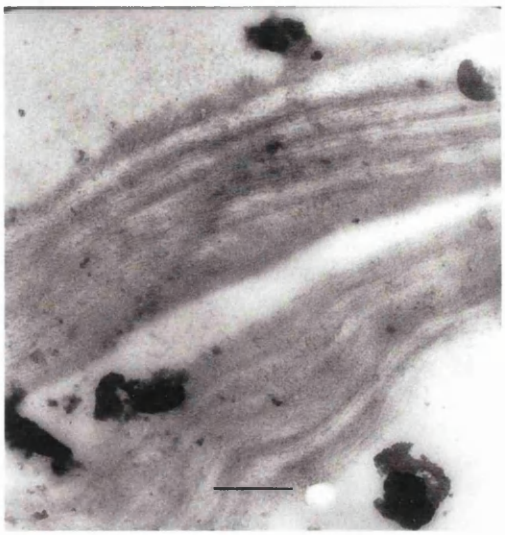


Figure 5.8

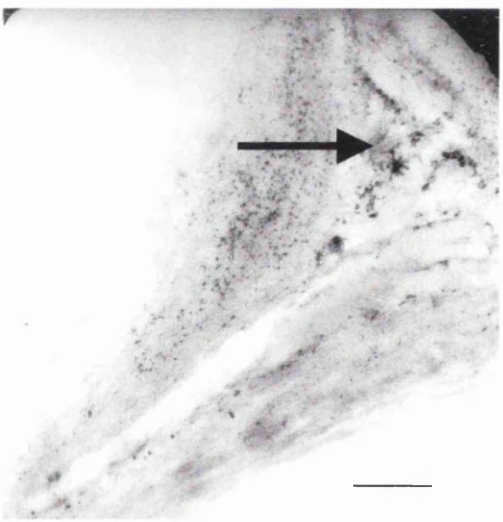


Figure 5.9

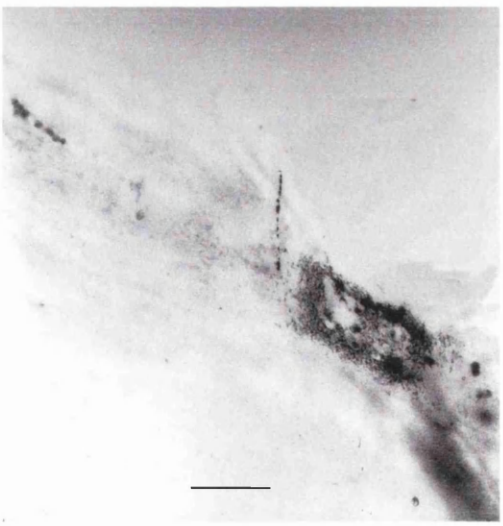


Figure 5.10

5.2.2. Location of Calcium and Galacturonic Acid Groups Within Raw and Cooked Cell Walls Using EELS.

The cell walls of cvs. Cara and Golden Wonder were mapped for calcium by EELS microscopy before and after doping with 5mM calcium acetate. Undoped maps provide information on where regions of calcium bound pectin is located within the cell wall. Doping cut section in 5mM calcium acetate, then washing the grids to remove unbound calcium provides information on the location of low esterified regions of pectin within the wall.

In certain cases, the elemental maps of some samples suggested the presence of calcium ions in regions outside the cell wall. The cause of this was likely to be elemental signal bleaching (Marry, personal communication). In samples with very little or no detectable elemental signal, elastic back-scattering can occur, causing the production of weak digital artefacts. These are produced by a lack of threshold control over the computer algorithms employed in generating the elemental maps.

5.2.2.1. Examination of undoped samples

No calcium was detected in the cooked samples even after the doping (results not shown). As mentioned previously, the cooked walls had swollen in size. There was the possibility that pectic material had been lost during the fixation process. During cooking, the amylose fraction of starch has been observed to leach into the cell wall (Linehan and Hughes, 1969). Amylose within the cell wall could mask potential calcium binding sites.

In the uncooked tissues of both varieties, calcium was not evenly distributed throughout the cell wall (Figure 5.11-5.14). Little calcium was found in the straight parts of walls (Figure 5.11 and 5.13), but it was found in the corners of air spaces (Figure 5.12 and 5.14). EELS work carried out on Cara and Golden Wonder tissues that were freeze-slammed, freeze dried material, and then

embedded in LR White had calcium throughout the walls (Wilson W. and Jarvis M.C., unpublished results). So it is likely that during the alcohol embedding procedure, used to prepare these cell walls, calcium was lost from the cell walls except in specific regions of the walls, which had a higher affinity for calcium.

Calcium has previously been reported to be located in tricellular junctions and at the corners of airspaces. Goldberg *et al.*, (1996) localised it in flax hypocotyls using secondary ion mass spectrometry (SIMS). Bush and McCann, (1999) used the 2F4 monoclonal antibody, which recognises calcium-bound pectic regions, in potatoes showed that calcium was located in primarily in tricellular junctions. The low degree of pectin esterification in these regions of the wall must have allowed the retention of calcium whilst it was lost in other parts of the wall.

More calcium was retained in raw Cara than raw Golden Wonder. This has important implications for their texture when cooked. Tricellular junction zones are regions than withstand separation stresses brought on by turgor or starch swelling pressures (Jarvis, 1999). A greater number of calcium crosslinks within this region would impart greater strength to cells to withstand cell separation processes. In chapter four, cv. Golden Wonder had higher cell separation values than Cara when one-stage cooked. It was hypothesised that Golden Wonder's degree of cell separation could be either a starch effect or a cell wall effect. The fact that greater calcium cross-linking was found in Cara compared to Golden Wonder meant that cell wall architecture is a factor that influences the extent of cell separation that occurs. Other factors are likely to be starch content of the varieties and the degree of pectin degradation during cooking (Jarvis 1992). Cara had a lower starch content and less solubilisation of pectin compared to Golden Wonder (Chapter four). The regions where low esterified pectin are located in the cell wall could be of potential importance in terms of texture. In the next section, doping experiments, which indicate potential ionic binding domains, are discussed.

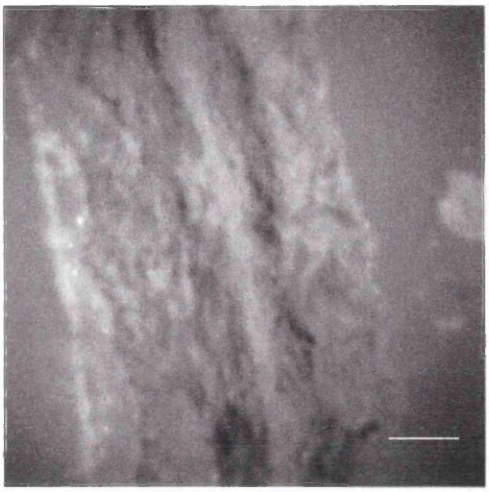


Figure a

b

Figure 5.11 Reference image of undoped Cara cell walls (figure a) and the elemental map of calcium (figure b). Little calcium was found in straight regions of the wall. (scale bar on reference image is 0.5μm)

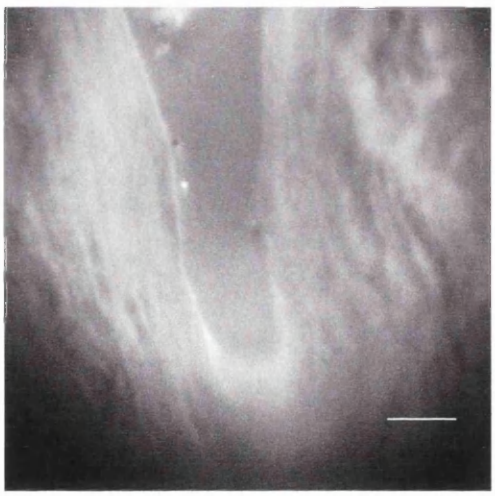
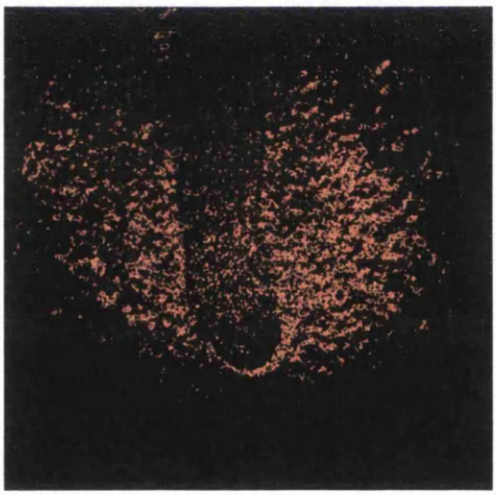
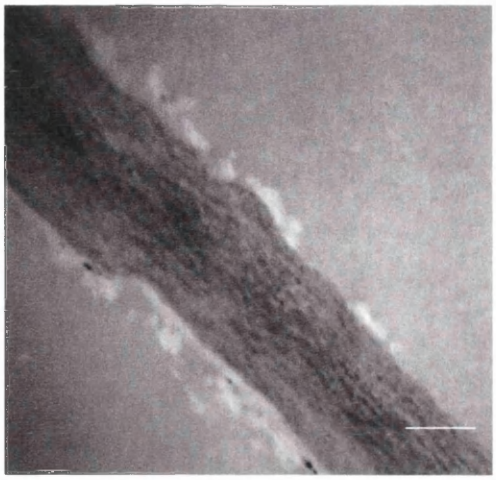


Figure a



b

Figure 5.12 Reference image of undoped Cara cell walls at the corner of an air space (figure a) and the elemental map of calcium (figure b). Calcium bound pectin was located within these regions (scale bar on reference image is 0.5μm)



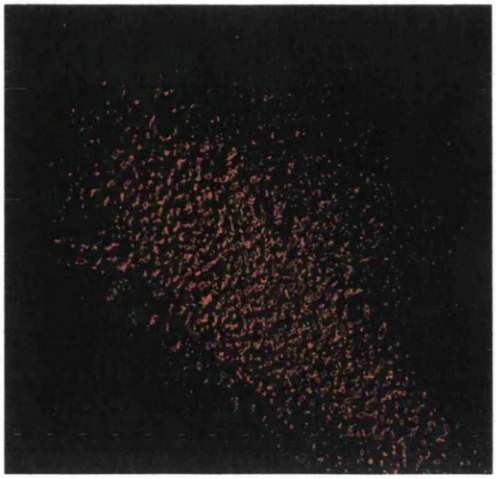


Figure a b

Figure 5.13 Reference image of undoped dense cell wall of Golden Wonder (figure a) and the elemental map of calcium (figure b). In most case the calcium distribution was similar to Cara. This cell wall had higher levels than other regions mapped. scale bar on reference image is 0.5μm)

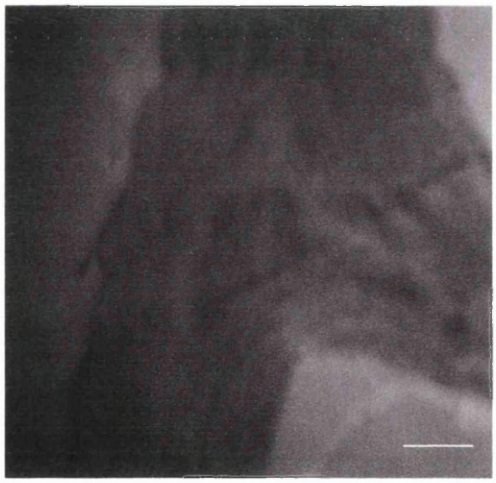


Figure a b

Figure 5.14. Reference image of Golden Wonder intercellular cell space cell walls at the corner of an intercellular air space (figure a) and its elemental map of calcium (figure b). Calcium was located in the corner but it was not as great as similar Cara regions (scale bar on reference image is 0.5μm)

5.2.2.2. Examination of doped samples.

After doping, only slightly more calcium was found in cell walls and the corners of air spaces Cara sections (figures 5.15 and 5.16). More calcium was found in Golden Wonder cell walls especially at the corners of cell spaces (Figure 5.17 and 5.18). This would suggest that there were a great deal more regions of unesterified pectin in Golden Wonder, especially at cell wall corners, compared to Cara. The results also suggest that apart from the corners of intercellular spaces of cv. Cara no unesterified pectin was present within the cell wall. This was not the case as chemical characterisation of isolated cell walls gave a value of 34% methylation of the cell wall.

These results have to be interpreted with caution. In chapter seven, doping experiments carried out on raw and pre-cooked walls of cv. Maris piper were unsuccessful. The results from that experiment suggested that the washing stage after doping had removed pectin from the grid. This also appeared true for raw sections of Cara but not Golden Wonder. Why doping appeared to work for cv. Golden Wonder but not cvs. Cara and Maris Piper is discussed later, in conjunction with the results from chapter seven.

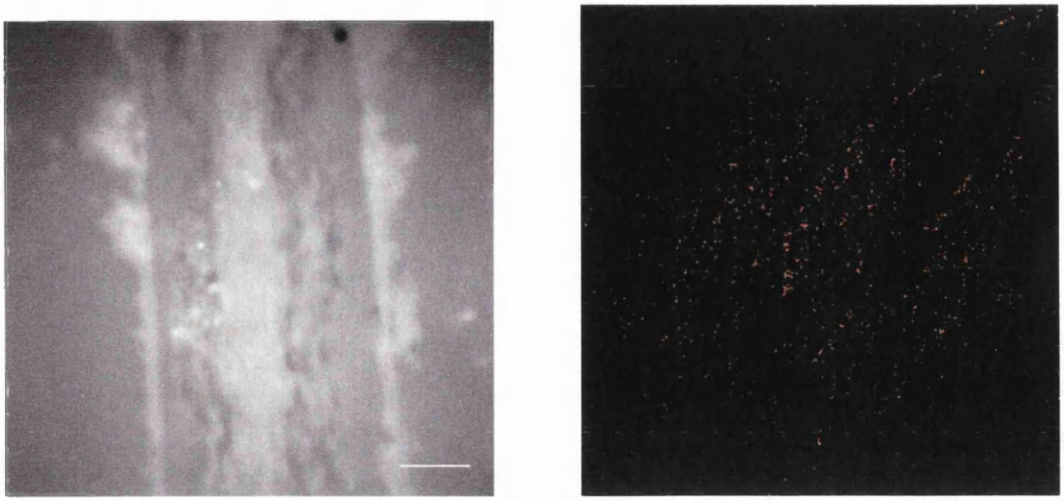


Figure a b

Figure 5.15 Reference image of doped Cara cell walls (figure a) and its elemental map of calcium (figure b). Levels of calcium were no greater in doped samples than undoped samples. (Scale bar on reference image is 0.5μm)

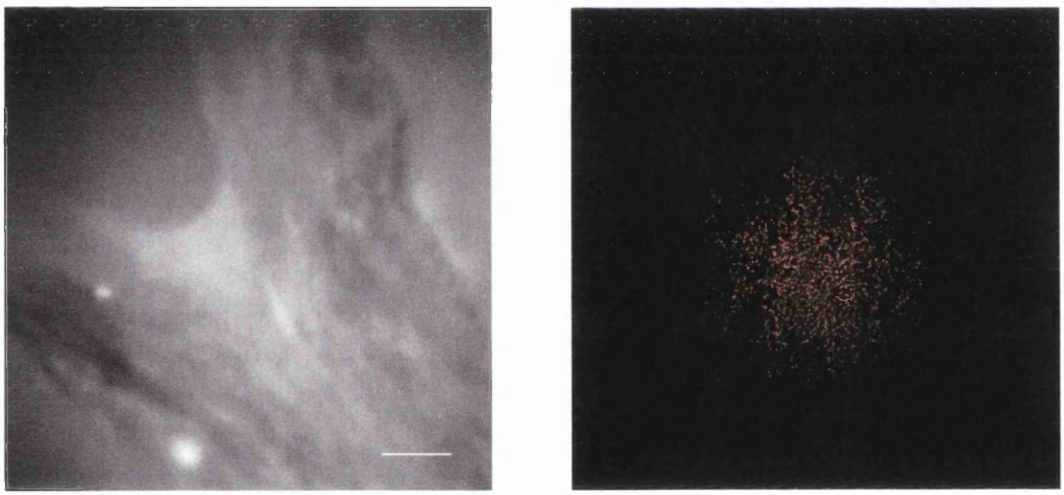


Figure a b

Figure 5.16. Reference image of doped Cara cell walls at the corner of an air space (figure a) and the elemental map of calcium (figure b). (Scale bar on reference image is 0.5μm)

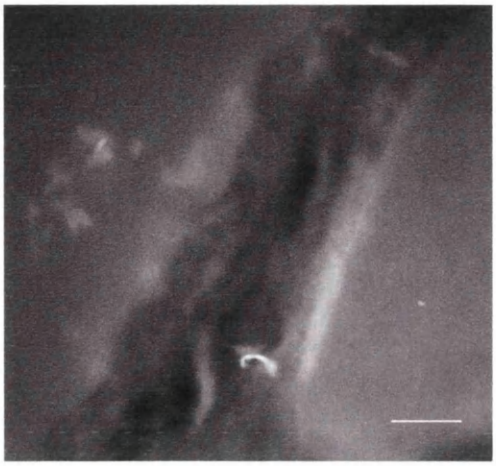


Figure a

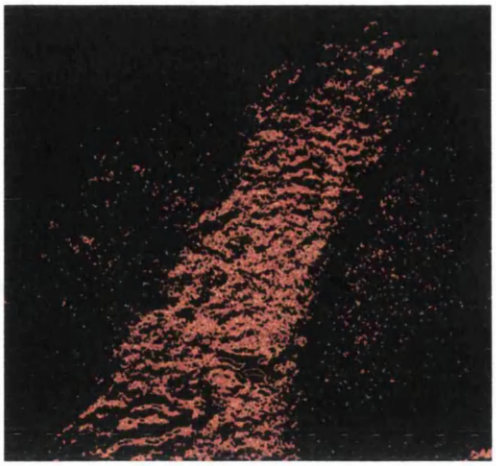


figure b

Figure 5.17. Reference image of doped Golden Wonder (figure a) and the elemental map of calcium (figure b). Greater calcium levels were found in doped Golden Wonder wall than undoped samples. (Scale bar on reference image is 0.5μm)

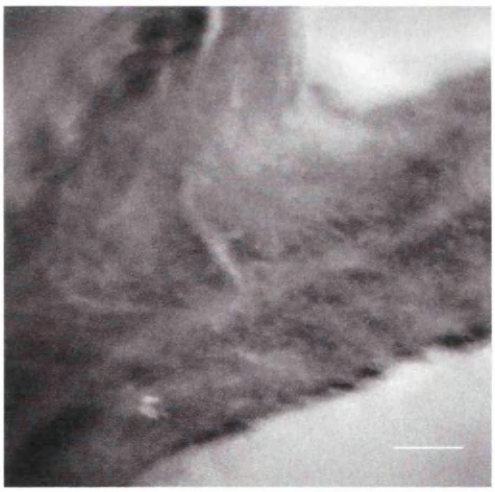


Figure a

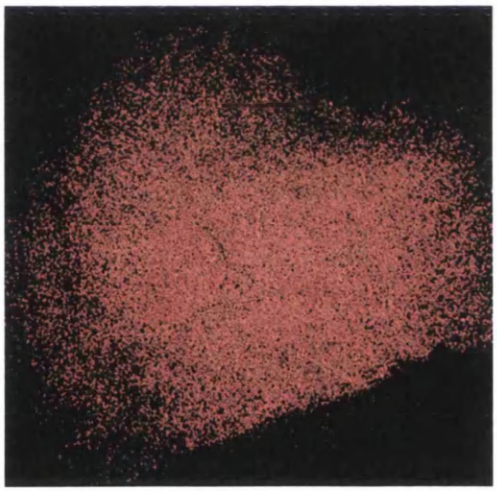


figure b

Figure 5.18. Reference image of a corner of Golden Wonder intercellular air space doped with calcium (figure a) and its elemental map of calcium (figure b). More calcium was located in the tricellular region than straight parts of the wall. (Scale bar on reference image is 0.5μm)

CHAPTER SIX.

LAB-SCALE ONE-STAGE AND THREE-STAGE COOKING IN DIFFERENT IONIC CONDITIONS.

6.1. Introduction

Calcium ions play an integral role in texture formation during cooking and pre-cooking Anderson *et al.* (1992). Moledina *et al.* (1981) postulated that after demethylation by PME during the pre-cook, calcium diffuses into the cell wall and stabilises the newly formed pectic bridges. Ng and Waldron (1997a) showed that calcium ions reduced vortex induced cell separation in potato discs. Magnesium ions also cause a restriction of cell sloughing but to a lesser extent than calcium (Zaehringer and Cunningham, 1971). Haydar *et al.* (1980) showed that magnesium restricted the swelling of starch during cooking, but had no effect on restricting pectin solubilisation. Magnesium though, does bind to cell walls (Demarty *et al.*, 1984), but less strongly than calcium. Nakajima *et al.* (1981) that magnesium and calcium bound to cell walls of pea epicotyls, although the mechanical strength imparted on the tissues by magnesium was less than calcium.

The following work was carried out to investigate the role of calcium and magnesium on the texture of one-stage and three-stage cooked potatoes. X-ray microanalysis and staining with potassium iodide was used to evaluate the extent and homogeneity of ion diffusion through Maris Piper potatoes during pre-cooking. From this, a cooking method to allow ionic diffusion into potatoes

was developed. Potatoes were pre-cooked and cooled in solutions of 5g/l calcium, 5g/l magnesium or deionised water then steam cooked. One-stage cooked potatoes were soaked in calcium or magnesium solutions for two hours prior to cooking. Cooked tissues were frozen and at a later date the tissues were digested in concentrated nitric acid. Calcium and magnesium levels in the tissues were measured by atomic absorption spectroscopy (AAS); sodium was measured by flame emission spectroscopy (FES). Prior to freezing, wedge tests and cell separation measurements were carried out.

6.2 Results

6.2.1. Ion Diffusion Measurement

The aim of these experiments was to infiltrate potatoes with different ions prior to cooking and to observe what effect this would have on the texture of the cooked potatoes. Before this could be done, one had to know whether ion infiltration was possible and to what extent it occurred.

6.2.1.1. Feasibility of ionic infiltration into raw tissues at low temperatures.

Jardine (1998) had successfully infiltrated potassium ions into thin 1.4mm potato slices by incubating in 80% ethanol overnight. As a preliminary experiment, 5mm slices of cv. Maris Piper tissues were incubated at 4°C overnight in 1% KI/I_2 (1% KI +0.5% I_2) in 70% ethanol. Any ion infiltration into the tissues would be observable by blue-black staining of starch by iodine molecules. After a 24 hour incubation, the outside edges of the slices was stained completely, fracturing of the tissues showed that the iodine had infiltrated less than 1mm, the remainder of the tissue was totally unstained. Slices were incubated for a further week and no

further infiltration was seen. This indicated that ions could not be infiltrated into raw potato tissues prior to a heat treatment.

6.2.1.2. X-Ray microanalysis study of potatoes pre-cooked at 70°C in a 5g/l calcium solution.

It was hoped that pre-cooking in an ionic solution might be a method to get ions into tissues prior to cooking. Destruction of plant cell membranes occurs when heated above 47°C (Zhang *et al.*, 1993). With membranes removed, ions might infiltrate more easily into tissues. Slices of cv. Maris Piper tissues were two-stage (70°C) cooked in deionised water or in 5g/l calcium (Ca-two-stage (70°C) cooked). A 5g/l calcium solution was used as it had previously been shown to have clear firming effect on the texture of crisps pre-bathed in such a solution prior to frying (Jardine, 1998). The potatoes used in the remaining of this chapter were the same as those used in the pilot plant (chapter eight). Their specific gravity was recorded as 1.085. The potatoes were stored at 8-10°C at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe, while potatoes were stored in Glasgow at 2-5°C.

Using X-ray microanalysis, the extent and homogeneity of calcium diffusion into slices was evaluated, it was presumed that calcium and magnesium diffusion into slices would be the same. This work was done at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe with the assistance of Drs Sue Gedney and Helen White of the Microscopy Unit.

After pre-cooking, tissues were fractured through the transverse axis to expose a plane going though the middle of the slice from one face to the next (Figure 6.1). Mapping the elemental distribution of pre-cooked tissues was not possible. Gelatinisation of starch during the pre-cook had 'trapped' free water, preventing freeze etching, therefore the elemental concentrations in the specimens were too low to map. As an alternative to mapping five spectra consecutive spectra were taken going down the profile of the fracture plane.

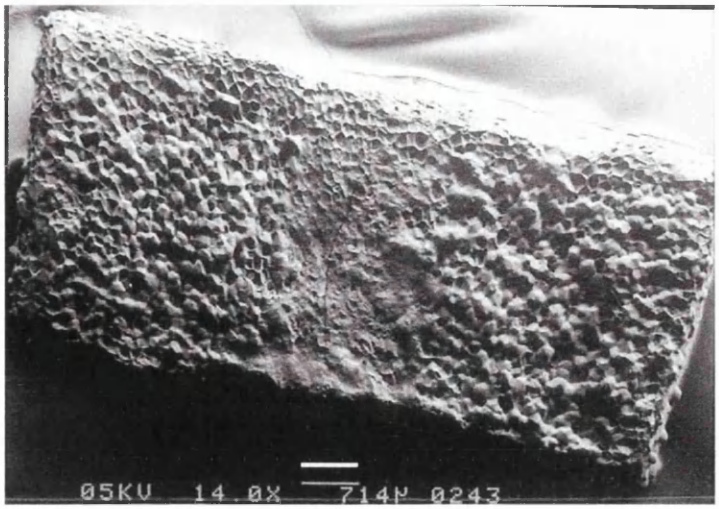


Figure 6.1 (a) Fracture plane of two-stage (70°C) cooked tissues (scale bar is 714µm).

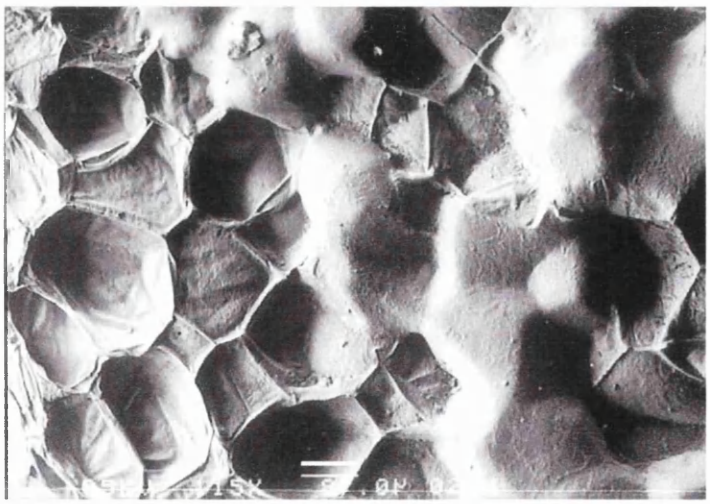


Figure 6.1 (b). Scale bar is (84 µm).

Figure 6.1. SEM Micrographs of fracture planes of potatoes two-stage (70°C) cooked, analysed by X-ray microanalysis. a). Fracture profile from which spectra were taken. b). Representative area from which spectra were taken

In Figure 6.2 representative spectra from raw, two-stage (70°C) and Ca-two-stage (70°C) cooked slices are shown. To gain a qualitative idea of gains and losses of calcium though the respective cooking treatments, the peak height of the calcium peak from each spectrum was recorded. To standardise measurements between spectra, the calcium peak height was ratioed to the peak heights of potassium and chlorine (the salt used to make up the salt solution was calcium chloride) (Figure 6.3 a and 6.3 c). The ratio of chlorine to potassium was also recorded (Figure 6.3 b).

The ratios showed that no losses of chloride or calcium, relative to potassium concentrations occurred during two-stage (70°C) cooking. When slices were Ca-two-stage (70°C) cooked, the calcium to potassium ratio was approximately doubled and the distribution was almost uniform throughout the slice (Figure 6.3a). The chlorine to potassium ratio also increased (Figure 6.3b), while the calcium to chlorine ratio remained the same (Figure 6.3b). It was clear that the increase in calcium was caused by calcium chloride in the pre-cook water entering into the slice during the pre-cook.

The results from the X-ray microanalysis suggested that Ca-two-stage (70°C) cooked tissues caused a doubling of calcium levels compared to raw tissues. This increase was fairly homogenous through the slice.

The fact that chlorine concentrations did not decrease with pre-cooking may provide information on the behaviour of other ions whilst pre-cooking. After the loss of cellular integrity, chelating anions such as citrate are released into the cell wall and decrease cellular integrity by removal of calcium bridging ions (Keijbets *et al.*, 1976). Selvendran *et al.* (1990) proposed that citrate loss from potato tissues into the cooking medium is impeded by having to pass through a thick starch gel. This could result in a greater loss of cell adhesion as more calcium ions could potentially be removed from pectin molecules by citrate. If citrate ions were to behave in a similar fashion to chloride ions, then during pre-cooking at least, little citrate would escape from cells, which would be consistent with the hypothesis suggested by Selvendran *et al.* (1990).

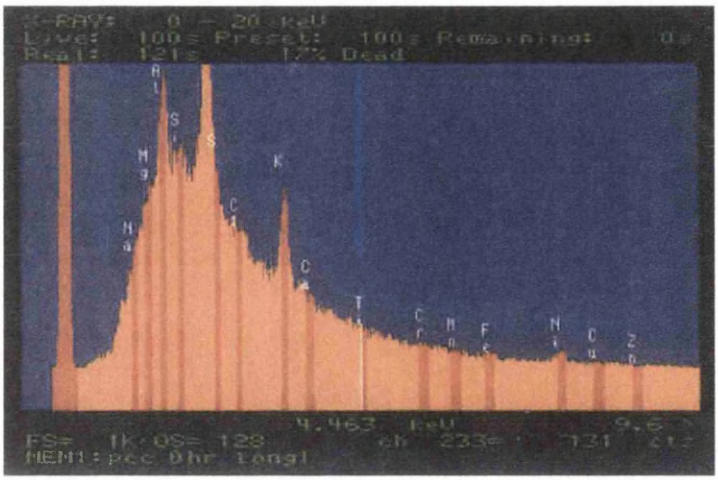


Figure 6.2 a) Representative spectra from raw slice.

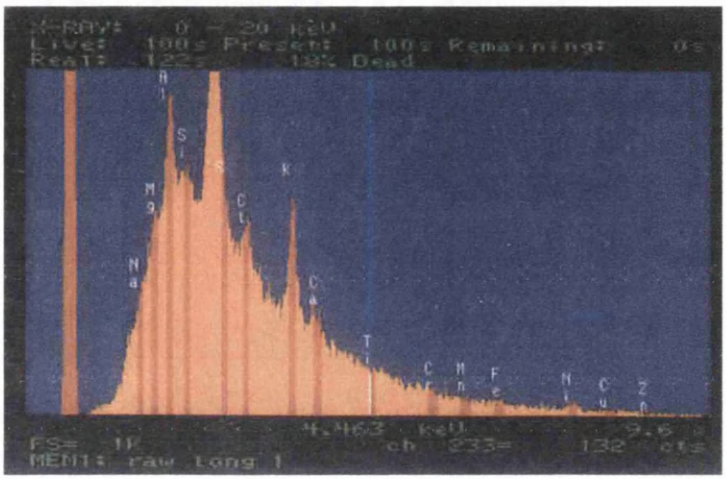


Figure 6.2 b) Representative spectra of a two-stage (70°C) cooked slice

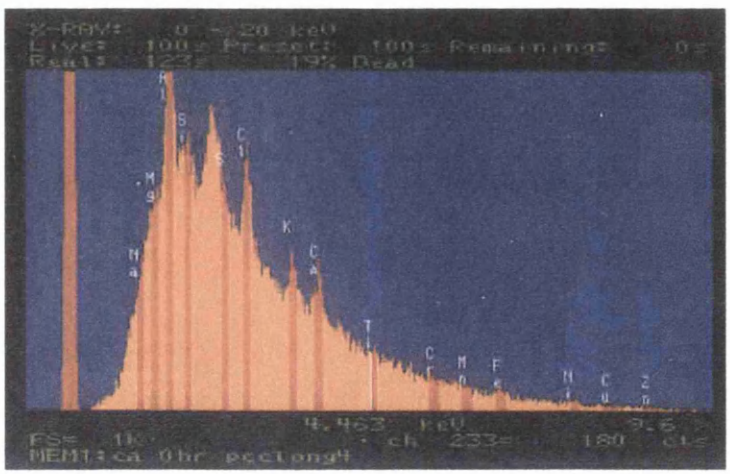


Figure 6.2 c) Representative spectra of a Ca-two-stage (70°C) cooked slice.

Figure 6.2. Representative X-ray microanalysis spectra obtained from cv. Maris Piper fracture planes from. a) raw, b) two-stage (70°C) and c) Ca-two-stage (70°C) cooked slices.

Figure 6.3 a). The Ca:K ratio.

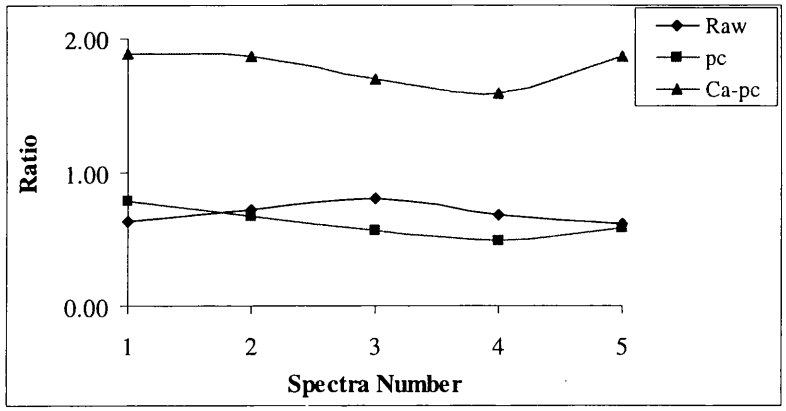


Figure 6.3 b). The Cl:K ratio

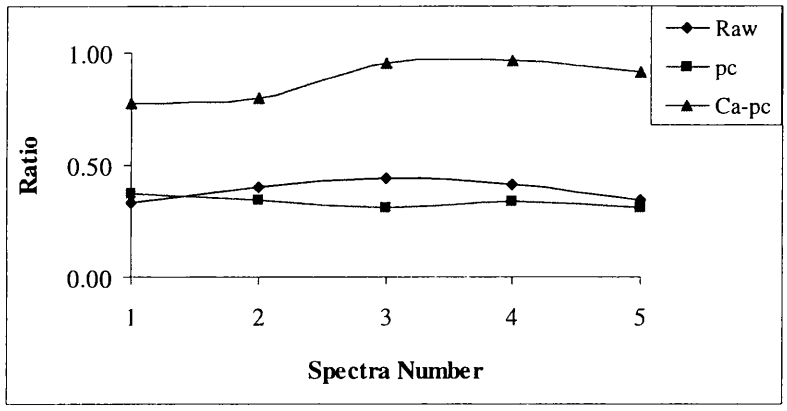


Figure 6.3 c). The Ca:Cl ratio.

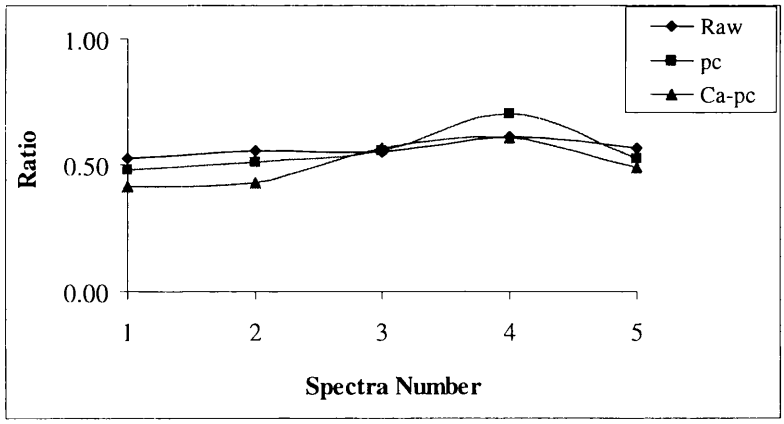


Figure 6.3. Spectra Ratios of a) the calcium to potassium (Ca:K) ratio, b) the chlorine to potassium (Cl:K) ratio and c).the calcium to chlorine (Ca:Cl) ratio. Where 'pc' is the two-stage (70°C) slice and 'Ca-pc' denotes Ca-two-stage (70°C) cooked slices. The spectra numbers along the X-axis indicate the position in the fracture plane where the spectrum was recorded i.e. spectrum number 1, was recorded at the top of each slice, and spectrum number 5 at the bottom.

6.2.2.3. 2-stage (55°C) cooking in 5g/l Iodine.

At a later date, after returning from High Wycombe, it was decided to extend the to 3-stage (55°C) cooking in different ionic conditions. To gain an insight into ion infiltration when pre-cooking at this temperature cv. Maris Piper potatoes were pre-cooked and cooled in a 5g/l iodide/2.5 g/l iodine solution. Examination of fractured tissues suggested that ions penetrated only 1mm into the tissues (Figure 6.4), though a small amount of iodide might have been transported through vascular tissues into the inner part of the slice. It appeared that ion diffusion into tissues was less effective at 55°C compared to 70°C.

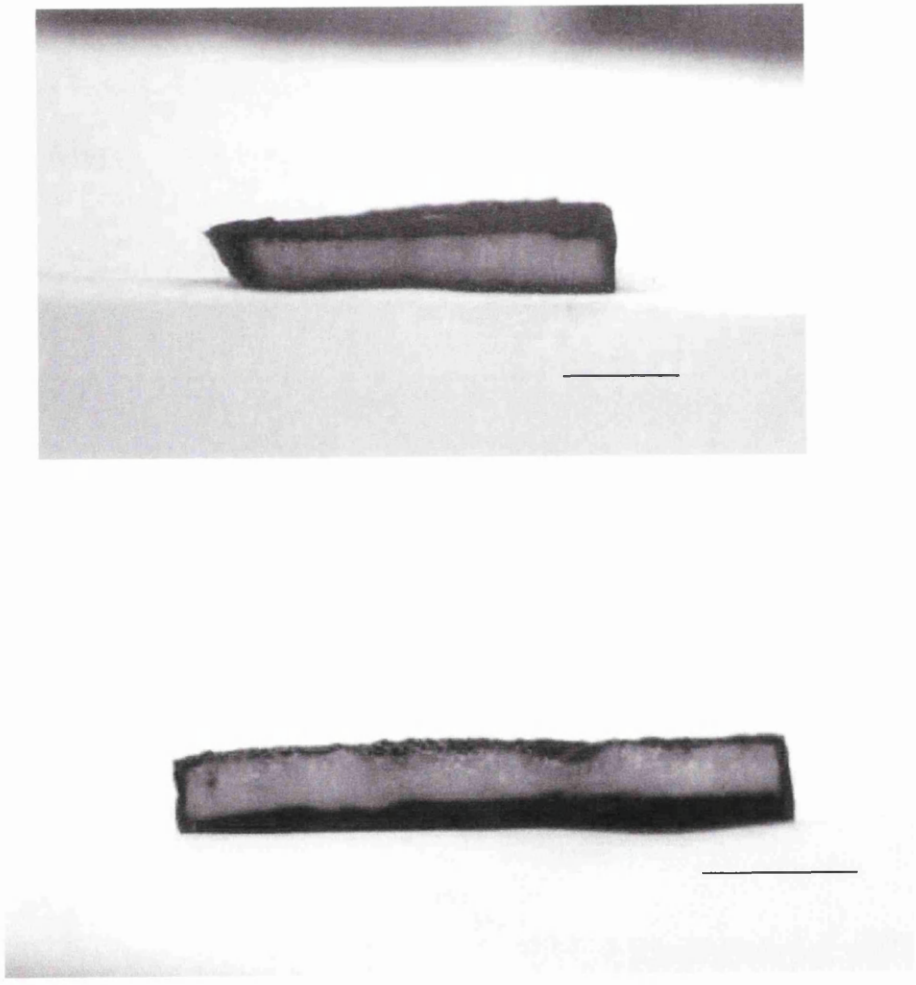


Figure 6.4. Fracture planes of cv. Maris Piper tissues pre-cooked at 55°C for forty minutes in 5g/l KI. Black regions are areas where iodide/iodine molecules have stained the potato. Staining is only obvious on the outer surface and first millimetre into the slice. Scale bar is 1cm.

6.2.2. One-Stage and Three-Stage Cooking in Different Ionic Conditions.

Using the lab-cooking apparatus and protocols, cv. Maris Piper tissues were one-stage and three-stage cooked in 5g/l calcium or 5g/l magnesium. As a control, three-stage cooking using deionised water in the pre-cook and cool steps was also done. Additionally, one-stage cooking with potatoes soaked in 5g/l calcium or 5g/l magnesium for two hours prior to steam cooking was carried out as a further control.

Although it appeared that two-stage (55°C) cooking would not let ions into the slice, it did appear to leave a surface layer of ions on the slices. During steam cooking, when cellular integrity is lost, these ions could infiltrate into tissues. Therefore, three-stage (55°C) cooking was done to establish if this occurred.

Two-stage (70°C) cooking allowed a uniform distribution of the salt through the slices, so tissues were steam cooked after a two-stage (70°C) pre-cook (three-stage (70°C) cooking) in the different ionic solutions.

After cooking, the tissues' texture was measured and frozen. At a later date the frozen tissues were digested and metal concentrations were measured. In the next two sections the total calcium, magnesium and sodium ions are reported. The texture of the cooked tissues is discussed in the Section 6.24 in conjunction with the total, calcium, magnesium and sodium concentrations.

6.2.3. Total Ion Levels of the Cooked Tissues.

The experiment produced a large number of results that were split up to allow trends to be more easily interpreted. Changes in calcium, magnesium and sodium concentrations were made for each ionic pre-cook treatment. To keep matters simple, statistical comparisons between different elements and different pre-cook treatments were not made but general trends were observed.

6.2.3.1. Deionised water pre-cooked samples.

One-stage cooking resulted in no loss of calcium or sodium, though some magnesium was lost (Table 6.1). Blanching potatoes resulted in a loss of all three ions, though the losses were less in the two-stage (55°C) and three-stage (70°C) treatments. If the results were expressed as Normals (N) then sodium would have a similar concentration to calcium and would therefore occupy as many exchange sites as calcium within the potato.

Cooking Treatment	Calcium (µMol/g)	Magnesium (µMol/g)	Sodium (µMol/g)
Raw	1.82 (a)	8.88 (a)	3.57 (a,b)
1-stage	1.92 (a)	6.12 (b)	3.88 (a)
2-stage (55°C)	1.51 (b)	4.76 (c,d)	2.90 (b,c)
2-stage (70°C)	1.39 (b,c)	3.88 (e)	2.51 (c)
3-stage (55°C)	1.25 (c)	4.42 (d,e)	2.81 (b,c)
3-stage(70°C)	1.51 (b)	5.74 (b,c)	3.22 (a,b,c)

Table 6.1. Calcium, magnesium and sodium levels in cv. Maris Piper potato slices pre-cooked or bathed in deionised water. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

6.2.3.2. 5g/l Calcium pre-cooked samples.

It had been established that ions would not enter the potato slices by just bathing them in a salt solution. Soaking slices in 5g/l calcium prior to steaming (Ca-one-stage cooking) would leave a surface residue of calcium on the slices that might be incorporated into the tissues during cooking. Tissues pre-cooked at 55°C in 5g/l calcium (Ca-two-stage 55°C cooking) would also be unlikely to have calcium enter the slices during the pre-cook. As with Ca-one-stage cooking, any

ions left on the surface of slices after the cooling step may be incorporated into the tissues during steam cooking.

Cooking Treatment	Calcium (μMol/g)	Magnesium (μMol/g)	Sodium (μMol/g)
Raw	1.82 (a)	8.88 (a)	3.57 (a,b)
1-stage	5.16 (b)	4.89 (b)	3.73 (a,b)
2-stage (55°C)	6.34 (b)	7.51 (a)	4.38 (a,b)
2-stage (70°C)	61.63 (c)	5.17 (b)	3.14 (a)
3-stage (55°C)	9.29 (b)	3.57 (b)	3.83 (a,b)
3-stage (70°C)	58.23 (b)	4.98 (b)	5.42 (b)

Table 6.2 Calcium, magnesium and sodium levels in cv. Maris Piper potato slices pre-cooked or bathed in 5g/l calcium salt solution. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$). Calcium values were logged to the base 10 prior to analysis to compensate for large differences between some values.

As can be seen in Table 6.2, Ca-one-stage cooking resulted in a significantly greater amount of calcium in slices compared to raw tissues. Ca-two-stage (55°C) cooked tissues also had higher levels of calcium than raw tissues, but the levels were no greater than Ca-one-stage tissues. Ca-three-stage (55°C) cooked samples had higher levels than raw, Ca-one-stage and Ca-two-stage (55°C) tissues (Table 6.2). As was presumed, a surface layer of calcium ions was left on the surface of the slices during a pre-cook at 55°C or by just bathing slices in the salt solution, which was absorbed into the tissues during cooking.

When tissues were pre-cooked in 5g/l calcium at 70°C (Ca-two-stage (70°C) cooked) there was a huge influx of calcium causing a 30-fold increase in calcium concentration compared to the raw tissue. High levels of calcium were also present in the Ca-three-stage (70°C) slices (Table 6.2). Compared to the levels of

calcium found by AAS, X-ray microanalysis results hugely underestimated the calcium levels of tissues pre-cooked in 5g/l calcium. The microanalysis results, though, did indicate a homogenous distribution of calcium through the slice.

Apart from changing the calcium concentration in the potato tissues, calcium treatments had little effect on magnesium or sodium in the slices during cooking apart from preventing a loss of magnesium in the two-stage (55°C) treatment.

6.2.3.3. 5g/l Magnesium treated samples.

Bathing slices in magnesium (Mg-one-stage) prior to steaming caused a small but significant increase in magnesium levels compared to raw tissues (Table 6.3). Though the rise in magnesium levels was not as great as the increase in the Ca-one-stage cooked samples (Table 6.2).

The Mg-two-stage (55°C) cooked slices had high levels of magnesium. In Section 6.2.1.3, slices pre-cooked at 55°C in potassium iodide showed little infiltration of iodide into the slices and very little calcium entered into the slices in Ca-two-stage (55°C). As there were such large amounts of magnesium in the Mg-two-stage (55°C) cooked sample, magnesium could not have encountered any resistance in entering the potato tissues during the pre-cook. In all the magnesium cooked samples, sodium levels were lower than the raw tissue (Table 6.3).

It has been shown that calcium ions prevent senescence in cabbage leafs by stabilising membrane lipids (Cheour *et al.*, 1992). Additionally, correlations between calcium levels and resistance to necrosis in potatoes have been observed, which was most likely due to stabilisation of membranes by calcium (Tzeng *et al.*, 1986). It is conceivable, therefore, that during pre-cooking in magnesium destabilisation of the potato membranes occurred by displacement of calcium by magnesium. This resulted in the loss of cellular integrity, which in turn resulted in the loss of sodium ions from cells, which then diffused into the pre-cook/cool or bathing salt solutions.

Pre-cooking at 70°C in magnesium (Mg-two-stage (70°C) cooking) caused an even greater influx of magnesium into the tissues than Mg-two-stage (55°C) cooked slices. These elevated levels were still present when the pre-cooked tissues were cooked (Mg-three-stage (70°C) cooked). The greater magnesium levels in the two-stage (70°C) cooked tissues compared to the two-stage (55°C) cooked tissues could have been due to starch gelatinisation exposing phosphate exchange sites for magnesium to attach to (Haydar *et al.*, 1980). Alternatively, the high levels of magnesium present were because, like Ca-2-stage (70°C) cooked samples, the elevated temperature of the pre-cook had destroyed cellular integrity and allowed free diffusion of ions throughout the slices.

Cooking Treatment	Calcium (μMol/g)	Magnesium (μMol/g)	Sodium (μMol/g)
Raw	1.82 (a)	8.88 (a)	3.57 (a)
1-stage	1.47 (b)	11.94 (b)	1.39 (b)
2-stage (55°C)	2.28 (c)	37.59 (c)	1.80 (c)
2-stage (70°C)	1.86 (a)	68.55 (d)	1.64 (b)
3-stage (55°C)	1.39 (b)	28.67 (e)	1.66 (b)
3-stage(70°C)	1.39 (b)	65.50 (d)	1.52 (b)

Table 6.3. Calcium, magnesium and sodium levels in cv. Maris Piper potato slices pre-cooked or bathed in 5g/l magnesium salt solution. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$). Magnesium values were logged to the base 10 prior for statistical analysis to compensate for large differences between some values.

The concentration of calcium and magnesium in the respective two-stage (70°C) cooked tissues (Tables 6.2 and 6.3) was very similar. It would seem reasonable that during two-stage (70°C) cooking in 5g/l salt solution of calcium or magnesium resulted in the slices being saturated in the respective salt.

The increase in both calcium and magnesium was far greater than predicted from the X-ray microanalysis. The cells in the fracture plane of two-stage (70°C) cooked tissues (Figure 6.1) had separated when fractured. Fracturing raw tissues caused the cells to rupture, exposing the cell interior (result not shown). When the raw slices were etched, cellular fluid would have been removed, leaving the cellular constituents within the cells. The X-ray microanalysis probe detects emissions only from the surface of materials (Morgan, 1985). Therefore, the probe would have detected cellular calcium from raw tissues and cell wall calcium from both two-stage (70°C) cooked tissues. This could explain the differences between the X-ray microanalysis results and the total metal contents measured by AAS.

6.2.4. Texture of Potatoes One-Stage and Three-Stage Cooked Potatoes in Different Ionic Solutions.

Prior to acid digestion, the texture of the potatoes cooked in different ionic conditions was measured. The mechanical strength of two-stage (55°C) and two-stage (70°C) tissues cooked in the different ionic solutions was measured by the wedge test method. Wedge test and degree of cell separation measurements were used to measure the texture of deionised-water, calcium and magnesium one-stage and three-stage cooked tissues.

6.2.4.1. Raw and Two-Stage Cooked Tissues.

When tissues were two-stage (55°C) cooked, no softening occurred in any of the ionic conditions used (Figure 6.5). The cause of softening during two-stage (70°C) cooking is likely to be due to a loss of turgor pressure, degradation of the pectin matrix and gelatinisation of starch. The lack of softening during two-stage (55°C) cooking was probably due to a very low rate of β-elimination and no starch gelatinisation.

Unlike two-stage (55°C) cooked slices, two-stage (70°C) slices were reduced in mechanical strength (Figure 6.6). This was also observed in Chapter 3 and 4. A retardation of softening was observed when pre-cooking was carried out in 5g/l Ca solution, but slices were still softer than raw potato slices.

Figure 6.5 (next page). The mechanical strength of two-stage (55°C) cooked cv. Maris Piper. Slices were pre-cooked at 55°C for 40 minutes in different ionic conditions, then cooled in same ionic solution. Ionic conditions used were deionised water, 5g/l Ca^{2+} or 5g/l Mg^{2+} salt solutions. No significant softening occurs by pre-cooking and cooling in this way. Values are mean cutting force in Newtons; bars are standard error of the mean where n =30 27 31 and 28 respectively. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 6.6 (next page). The mechanical strength of two-stage (70°C) cooked cv. Maris Piper. Slices were pre-cooked at 70°C for 15 minutes in different ionic conditions, then cooled in same ionic solution. Ionic conditions used were deionised water, 5g/l Ca^{2+} or 5g/l Mg^{2+} salt solutions. Pre-cooking and cooling in deionised water caused softening of tissues; when done in 5g/l Ca^{2+} softening also occurs but to a significantly lesser extent; heating in 5g/l Mg^{2+} was no different from pre-cooking in deionised water. Values are mean cutting force in Newtons; bars are standard error of the mean where n = 30, 24, 20 and 29 respectively. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

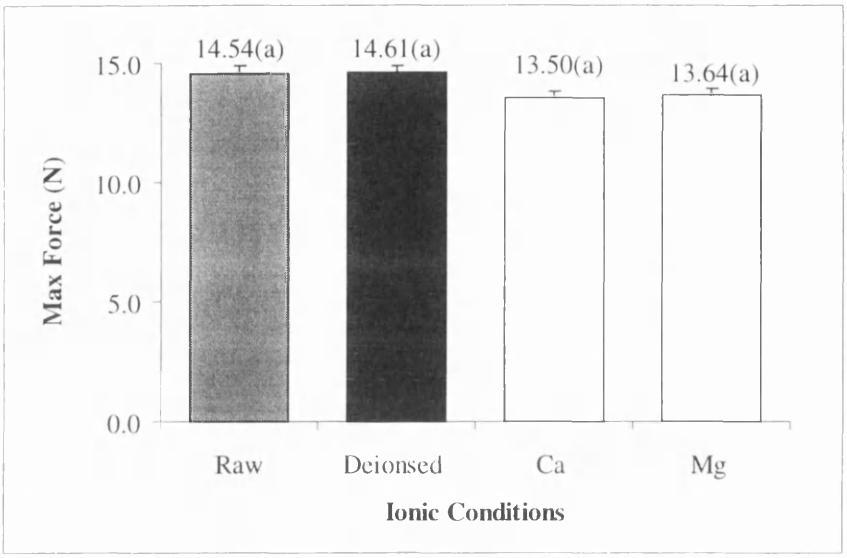


Figure 6.5. Mechanical Strength of two-stage (55°C) slices.

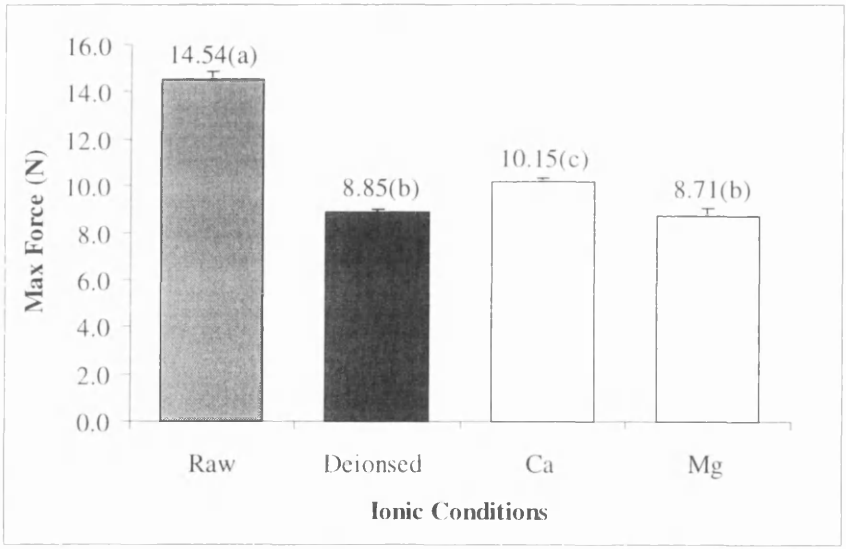


Figure 6.6. Mechanical Strength of two-stage (70°C) slices.

Pre-cooking in a 5g/l Mg solution had a similar effect to pre-cooking in deionised water (Figure 6.6). Tissues pre-cooked in magnesium at either 55°C or 70°C had elevated levels of magnesium. The same was true of tissues pre-cooked in calcium at 70°C but not at 55°C. However, pre-cooking in ionic solutions caused little or no change in the texture of the pre-cooked tissue. As with three-stage cooking in deionised water, any changes in the texture of the potato may only become apparent in the steam-cooked tissue.

6.2.5. One-Stage and Three-Stage Cooked Tissues.

The complete set of mechanical strength and cell separation results are shown in Figures 6.7 and 6.8. The cell separation results were spaced over a relatively small range of 0.13-1.40 cm^3. Some of the results, such as Ca-one-stage cooked tissues, had large standard deviations, which may have masked certain trends. To avoid this, two comparisons were made. The first was a comparison of different cooking sequences with constant ionic pre-cooking/bathing conditions (Figure 6.9). The second compared different ionic pre-cooking/bathing conditions with a constant cooking sequence (Figure 6.10). This was not done for the mechanical strength results as the trends for both comparisons were clear from Figure 6.7.

Three-stage cooking (either 55°C or 70°C) in deionised water resulted in cooked potatoes that were approximately 25% firmer than one-stage cooked tissues (Figure 6.7). A smaller volume of cells was released from these control three-stage (70°C) cooked potatoes than the control three-stage (55°C) cooked potatoes, both of which released less cells than one-stage cooked tissues. The major difference between the two pre-cooks is that in the 70°C pre-cook, starch is gelatinised along with activation of PME, then cooled prior to cooking, while in the 55°C pre-cook only the activation of PME occurs. Starch-swelling pressure is the probable driving force for cell separation (Jarvis *et al.*, 1992). The likely difference in the volumes of cell separation in the two three-stage cooking methods is due to the manipulation of starch properties prior to cooking in the 70°C pre-cook treatment.

Figure 6.7. The mechanical strength of one-stage and three-stage cooked cv. Maris Piper potato tissues cooked in different ionic conditions. Ionic conditions used were 5g/l Ca^{2+} or Mg^{2+}. Three-stage cooked potatoes were pre-cooked and cooled in either 5g/l Ca^{2+} or Mg^{2+} salt solutions then steam cooked, one-stage were bathed in relevant salt solution for 2 hrs prior to steam cooking. As a control pre-cooking and cooling was carried out in deionised water during three-stage cooking; while one-stage cooked potatoes were steam cooked after washing potatoes in deionised water. Values are mean cutting force in Newtons (N); bars are standard error of the mean where n = 20, 18, 26 for controls; n = 24,18 and 19 for Ca^{2+} treated tissues and n = 22, 20 and 28 for magnesium treated tissues. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Figure 6.8. The degree of cell separation of one-stage and three-stage cooked cv. Maris Piper tissues cooked in different ionic conditions. The ionic conditions used were 5g/l Ca^{2+} or Mg^{2+}; three-stage cooked potatoes were pre-cooked and cooled in either 5g/l Ca^{2+} or Mg^{2+} salt solutions then steam cooked, one-stage were bathed in relevant salt solution for 2 hrs prior to steam cooking. As a control the pre-cooking and cooling was carried out in deionised water during three-stage cooking; while one-stage cooked potatoes were steam cooked after washing potatoes in deionised water. Values are volume of separated cells in cm^3; bars are standard error of the mean where n = 10 in all cases. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

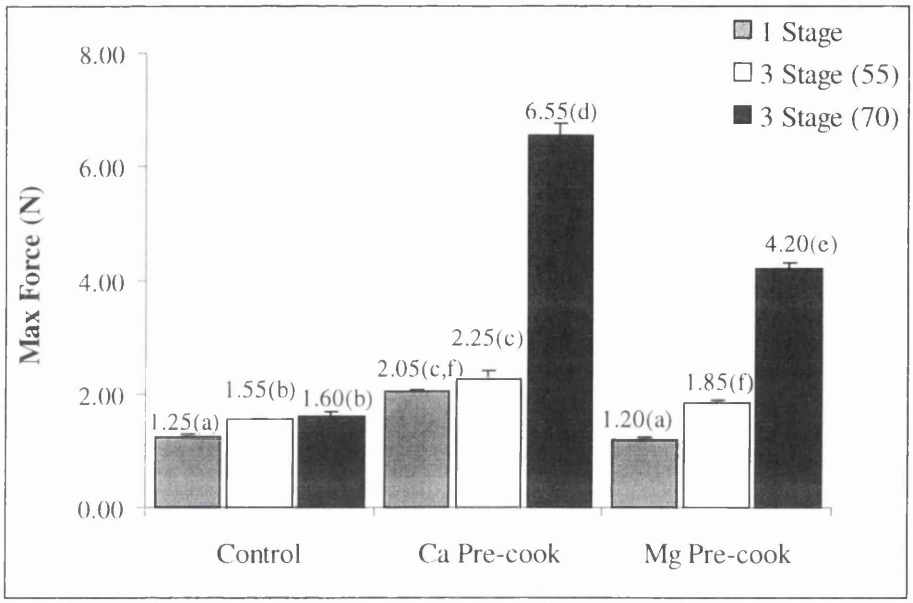


Figure 6.7. The mechanical strength of one-stage and three-stage cooked tissues in different ionic conditions.

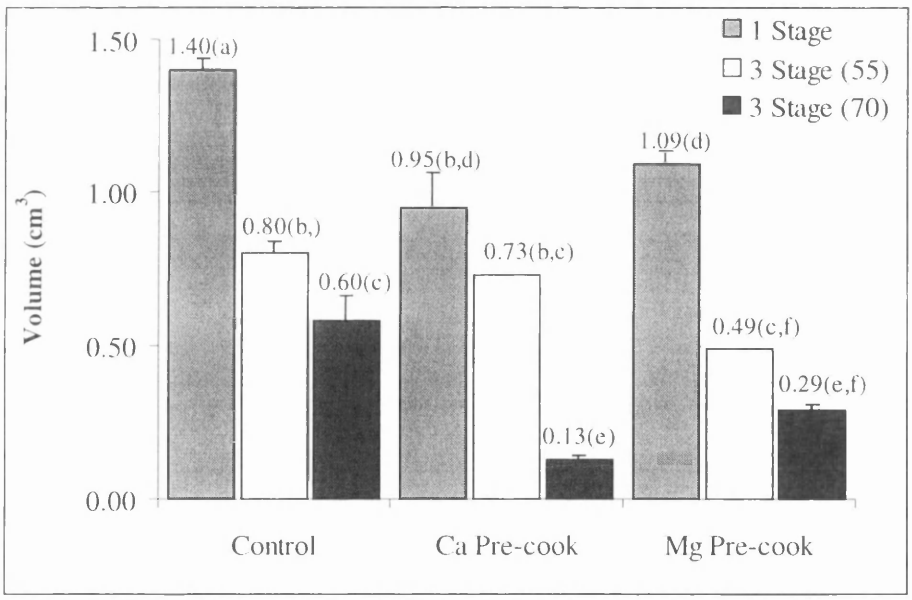


Figure 6.8. The degree of cell separation after one-stage and three-stage cooking in different ionic conditions.

Ca-one-stage cooked samples had the same calcium concentration as Ca-three-stage (55°C) cooked tissues, both of which were greater than raw tissues and control cooked tissues (Table.6.2). Ca-one-stage and Ca-three-stage (55°C) cooked slices had similar mechanical strengths, which were greater than the mechanical strength of the one-stage and three-stage cooked controls (Figure 6.7).

Comparing calcium treated samples with deionised controls, a calcium pre-treatment resulted in a smaller degree of cell separation with all cooking treatments (figure 6.10 a,b and c). This was unsurprising, as calcium's role in controlling texture has been well established (Andersson *et al.*, 1992). In terms of cooking treatment, Ca-three-stage (55°C) cooked tissue had a smaller degree of cell separation the than the Ca-one-stage cooked sample (Figure 6.9b). The difference in cell separation values between the two cooking methods could be due to PME activitation in the three-stage cooked slices.

Mg-one-stage cooked tissues had a slightly greater concentration of magnesium than the raw and cooked control slices (Table 6.3) but this did not affect its mechanical strength compared to the control one-stage cooked tissues (Figure 6.7). However there was a small decrease in cell separation (Figure 6.10a). The elevated levels of magnesium in Mg-three-stage (55°C) tissues correlated with greater mechanical strength than in the three-stage cooked controls (Figure 6.7). The mechanical strength was greater and the quantity of separated cells was less than Mg-one-stage cooked tissues (Figure 6.c), the difference between the two is likely to have been caused by PME activity during the pre-cook at 55°C.

Ca-three-stage (55°C) cooking caused a small increase in the calcium concentration in slices, whilst Mg–three-stage (55°C) cooking resulted in high amounts of magnesium entering slices. Ca-three-stage (55°C) cooked slices had greater mechanical strength than Mg-three-stage (55°C) tissues (Figure 6.7). Both treatments had less cell separation than the three-stage 55°C control. Unlike the mechanical strength measurements, magnesium had a greater effect at reducing cell separation than calcium (Figure 6.10 b). Molidina *et al.* (1980) showed that

starch swelling properties were restricted in the presence of magnesium, which could account for this observation.

Figure 6.9 (next page). The effect of cooking treatment on the extent of cell separation in one-stage, three-stage (55°C) or three-stage (70°C) cooked cv. Maris Piper slices when pre-cooked in different ionic conditions. For cooking treatments and ionic conditions, please refer to Figure 6.8.

a). Cell separation of one-stage, three-stage (55°C) or three-stage (70°C) tissues when cooked in deionised water.

b). Cell separation of one-stage, three-stage (55°C) or three-stage (70°C) tissues when cooked in 5g/l Ca^{2+} salt solution.

c). Cell separation of one-stage, three-stage (55°C) or three-stage (70°C) tissues when cooked in 5g/l Mg^{2+} salt solution.

Figure 6.10 (next page). The effect of different ionic conditions on the extent of cell separation of cv. Maris Piper tissues when one-stage, three-stage (55°C) or three-stage (70°C) cooked. For cooking treatments and ionic conditions, please refer to Figure 6.8.

a). One-stage cooked tissues in deionised water, 5 g/l Ca^{2+} or 5g/l Mg^{2+} salt solution.

b). Three-stage (55°C) in deionised water, 5 g/l Ca^{2+} or 5g/l Mg^{2+} salt solution.

c). Three-stage (70°C) in deionised water, 5 g/l Ca^{2+} or 5g/l Mg^{2+} salt solution.

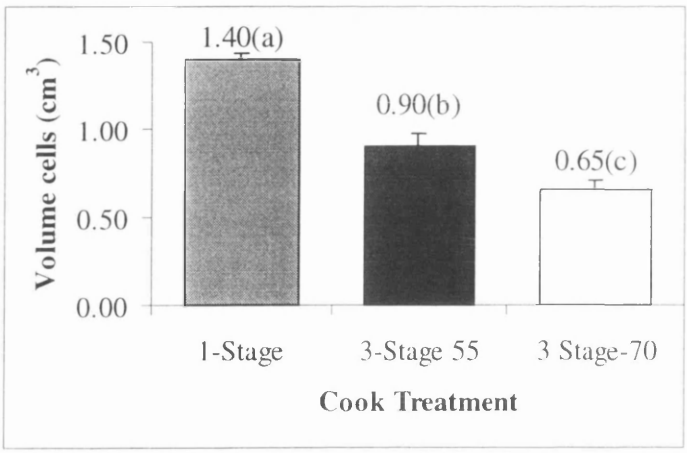


Figure 6.9 a). Cell separation of One-stage, three-stage (55°C) or three-stage (70°C) tissues when cooked in deionised water;

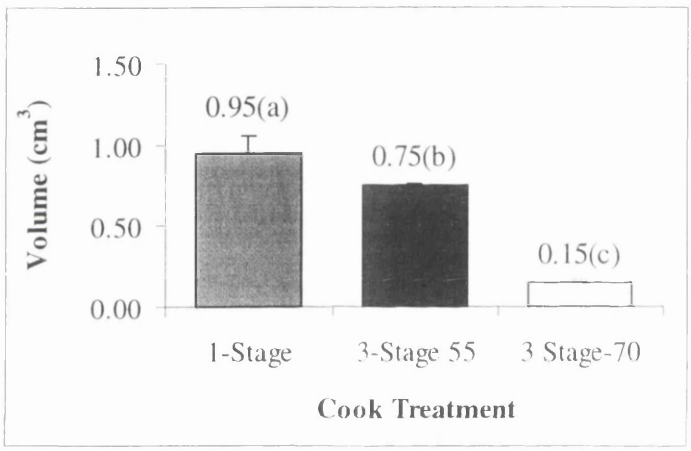


Figure 6.9 b). Cell separation of one-stage, three-stage (55°C) or three-stage (70°C) tissues when cooked in 5g/l Ca^{2+} salt solution and

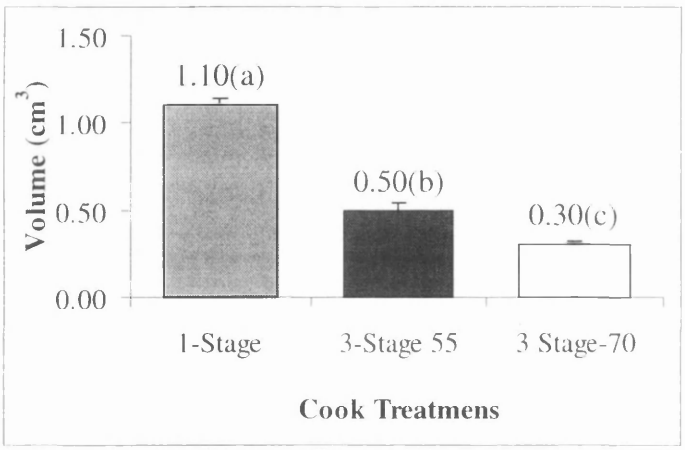


Figure 6.9 c). Cell separation of one-stage, three-stage (70°C) or three-stage (70°C) tissues when cooked in 5g/l Mg^{2+}

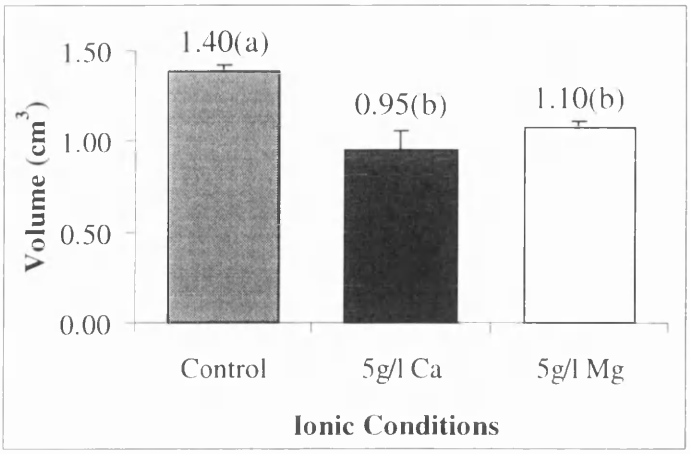


Figure 6.10 a). Cell separation of one-stage cooked tissues in deionised water, 5 g/l Ca^{2+} or 5g/l Mg^{2+} salt solution.

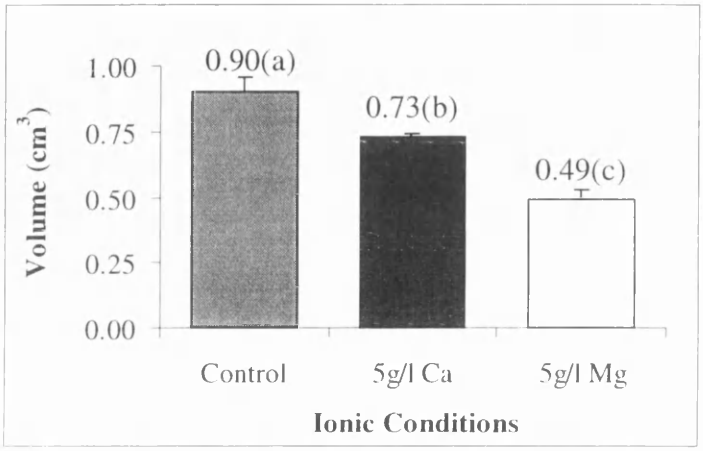


Figure 6.10 b). Three-stage (55°C) in deionised water, 5 g/l Ca^{2+} or 5g/l Mg^{2+} salt solution.

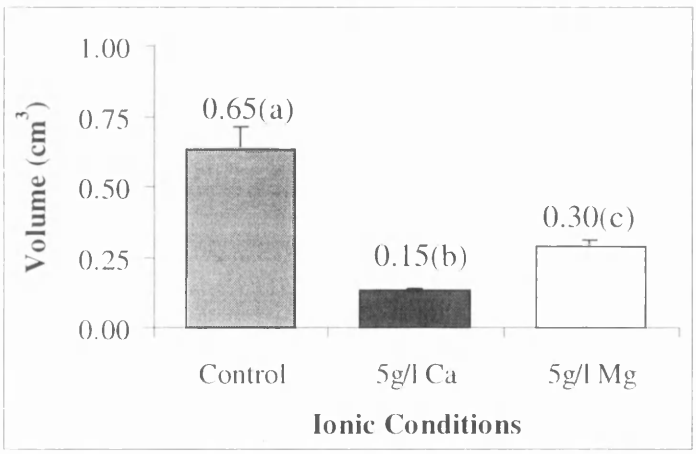


Figure 6.10c). Cell separation of three-stage (70°C) in deionised water, 5 g/l Ca^{2+} or 5g/l Mg^{2+} salt solution.

Two-stage (70°C) cooking in either 5g/l Ca or 5g/l Mg resulted in the slices being saturated in the respective ions. This had a very significant effect on the mechanical strength of the subsequently steam cooked tissues. Three-stage cooking in deionised water produced a 25% increase in firmness compared to the one-stage cooked control; Ca-three-stage (70°C) cooking caused a 525% increase in mechanical strength compared to the control. There was also an increase in mechanical strength of the magnesium treated sample by 350%, compared to the one-stage cooked control. Saturating the tissues with calcium meant that after pre-cooking the tissues were only reduced in strength by 3.5N (compare Figure 6.6 and 6.7) and that the high levels of calcium almost completely restricted the softening process. Magnesium also restricted softening processes but not to the same degree as calcium.

Three-stage (70°C) cooking caused the least cell separation all ionic cooking conditions (Figure 6.9 a, b and c). In deionised water, cell separation was probably due to a combination of starch gelatinisation and the activation of PME. Tissues pre-cooked at 70°C in calcium or magnesium salts were saturated with that salt, which led to a reduction in cell separation and greater wedge test firmness than the deionised controls. Saturating the slices in calcium caused a greater reduction in cell separation than magnesium (Figure 6.10c).

These results indicate that calcium is a far better firming agent than magnesium when concentrations were equal (three-stage (70°C) treatments) or when calcium was at a lower concentration than magnesium (three-stage (55°C) treatments) (Tables 6.2 and 6.3). Calcium has a high affinity for the cell wall (Demarty *et al.*, 1984); an increase of calcium would impart mechanical strength by forming crosslinks with pectin galacturonan acid sites (Moledina *et al.*, 1981).

When potato slices were not saturated in either ion (one stage or three-stage 55°C treatments) elevated magnesium concentrations had a greater effect on cell separation than mechanical strength measurements on the cooked potato (Table 6.3 and Figures 6.7 and 6.9c). Haydar *et al.* (1980) reported that magnesium retarded texture development by restricting starch gelatinisation. The deionised

water results showed that manipulating starch gelatinisation as well as PME activity, caused a greater reduction in cell separation than manipulating PME activity alone. At lower levels, magnesium had a greater effect on cell separation than firming, which might support the observations of Haydar *et al.*, (1980). It could be assumed that slightly elevated levels of magnesium restricted starch swelling and therefore reduced the volume of separated cells. Magnesium has an affinity for the cell wall (Demarty *et al.*, 1984), and imparts mechanical strength to pea coleoptiles (Nakajima *et al.*, 1981). When magnesium was present at higher levels after two-stage (70°C) treatments, the increase in mechanical strength was so large that it must have been caused by strengthening the cell wall as well as restricting starch gelatinisation.

The purpose of three-stage cooking is to increase mechanical strength and reduce cell separation of cooked potatoes prior to mashing (Wilard and Kluge, 1975). The way in which this is done commercially is by activating PME and/or gelatinising then cooling starch prior to steam cooking. These experiments in this chapter illustrated that ionic conditions in which potatoes are pre-cooked prior to cooking can have a much larger effect on texture than PME or starch effects. Potentially this finding could have significant economic importance to potato processors as the temperature and residence time of pre-cooks could be drastically reduced.

There were a large number of potential texture experiments involving the manipulation of ionic conditions in the pre-cook and cool steps of three-stage cooking. Unfortunately, time restraints prevented these from being attempted. In the following chapter, it was attempted to establish calcium distribution in the walls of Ca-two-stage (70°C), two-stage (55°C) and two-stage (70°C) pre-cooked tissues by electron energy loss spectroscopy (EELS).

CHAPTER SEVEN

CHANGES WITHIN THE CELL WALL CAUSED BY PME ACTIVATION DURING PRE-COOKING.

7.1. Introduction

Pre-cooking in the temperature range of 55°C to 70°C is known to cause changes in the texture of cooked (Bartolome and Hoff, 1972) and mashed (Hadziyev and Steele, 1979) potatoes. This has been attributed to starch properties (Reeve, 1977) and PME (Bartolome and Hoff, 1972). In chapters three and six, changing the pre-cook temperature from 55°C to 70°C altered the texture of the cooked potatoes. The intention was to gelatinise starch in the latter but not the former treatment, while activating PME in both treatments. PME has different iso-enzymes activated and inactivated at different temperatures (Tijskens *et al.*, 1997) and differences in texture could have been due to different PME activities.

Hou and Chang (1996) found non-methyl ester linkages in the cell wall of pea sprouts. The levels reported were 15% of the pectin content, the same value as found by Jardine (1998) in potatoes. Hou and Chang (1996) also reported that PME activity caused an increase in the non-methyl ester content of the cell wall.

If these linkages are formed during pre-cooking of potatoes then they could have a considerable role in the texture changes observed during three-stage cooking.

PME activation during pre-cooking is well established as being an important factor influencing the texture of cooked potatoes (Bartolome and Hoff, 1972). More importantly is the location of PME activation within the cell wall and middle lamella. The middle lamella of plants is the region where adjacent cell walls meet and where solubilisation of pectin during cooking is thought to occur (Ng and Waldron, 1997a). Low methyl ester pectins were found throughout the wall, but calcium bound pectin was found only in the tricellular junctions and corners of air spaces (Bush and McCann, 1999). The tricellular junctions are the regions where cell separation forces are at their highest (Jarvis, 1999). The extent of demethylation in these two regions would influence the texture changes observed in these regions.

In this chapter the degree of methylation of pectin after pre-cooking in deionised water at 55°C and 70°C and in 5g/l calcium at 70°C were investigated. Any increase in non-methyl ester linkages after these treatments was also investigated. The pattern of de-esterification after pre-cooking was monitored in two ways. The distribution of low ester and high ester regions of isolated MCB-insoluble cell walls was monitored using JIM5 and JIM7 respectively (Knox *et al.*, 1990). This work was initially carried out on potato tissues that were alcohol embedded in the same way as cv.s Cara and Golden Wonder in chapter five using JIM5 antibodies (donated by Dr Paul Knox). This proved problematic, as little binding was observed and significant problems with phosphate precipitation was observed. This could have been due to either the antibody being stored at too high a temperature before use, or an interaction with the sections and the PBS. In order to eliminate both problems, MCB-insoluble walls were cold-resin embedded in LR White resin at the John Innes Centre (section 2.11) and were labelled with fresh JIM 5 and JIM 7 antibodies donated by Dr. Mazz Marry.

The second method involved doping raw and pre-cooked wall specimens with calcium. In principle, by locating new areas of calcium binding to pre-cooked cell walls using EELS, areas of unesterified pectin could be identified.

7.2. Results.

7.2.1. Characterisation of the MCB-Insoluble Material.

During the texture experiments in chapter six, duplicate cooking of each treatment was carried out. Immediately after cooking, the second batch of potato slices were frozen in liquid nitrogen, then stored in a freezer at –18C. At a later date, cell walls were isolated from raw, two-stage (55°C), two-stage (70°C) and Ca-two-stage (70°C) cooked tissues and used for analysis.

Cooking Treatment	% Dry Matter	% Residual Starch	Conversion factor
Raw	6.66	12.84	0.81
Two-Stage (55°C)	5.01	10.27	0.85
Two-stage (70°C)	10.13	19.23	0.71
Ca-two-stage (70°C)	11.43	23.16	0.65

Table 7.1. Dry matter and residual starch content of the four isolated MCB-insoluble materials. The conversion factor was calculated by summing the percentage moisture content and residual starch content then subtracting this value from 100. The value obtained was then divided by 100 giving the conversion factor. This factor could then be multiplied by the weight of MCB-insoluble material, which allowed results to be expressed on a cell wall weight basis.

The cell wall isolation protocol was for frozen material as detailed in section 2.6. As the materials were still enzymically active, enzymes were denatured in water-saturated phenol. During the cell wall isolation, the tissues were boiled for a minute to re-gelatinise the starch and, unlike the cell walls extracted from frozen

material in chapter four, starch removal appeared successful after an overnight incubation in α-amylase and pullulanase. The dried MCB-insoluble material was corrected for any residual starch and dry mater and the results are shown in table 7.1.

The starch removal step during isolation was far more successful than in chapter four. This was due to a combination of rapid freezing of the samples and gelatinising the material prior to incubating in α-amylase and pullulanase. No starch appeared to be present when stained with KI/I_2. Quantification by the enzymic method proved that this was not the case (Table 7.1). As discussed in chapter four, freezing potatoes after starch gelatinisation has occurred will lead to retrogradation. This makes parts of the starch fraction inaccessible to α-amylase and pullulanase, which makes starch removal very difficult.

In an attempt to avoid the problems encountered with starch removal in chapter four, tissues were rapidly frozen in liquid nitrogen and starch was re-gelatinised prior to incubation in α-amylase and pullulanase. Considering that significant levels of starch were still present in tissues that contained gelatinised starch, these steps were not entirely successful. They also strongly suggest that residual starch quantification in cell wall material by the enzymic method should always be done when isolating potato cell walls and that results that do not include a quantification of residual starch should be interpreted with care.

7.2.2. Chemical Changes in the Pectin Structure During Pre-Cooking and Cooling.

Changes in pectin content were evaluated by measuring the galacturonic acid content of the cell wall residue. This was compared to the amount of solubilised galacturonic acid during pre-cooking and isolation of the cell wall. Solubilised pectin values may not have been may true levels of solubilisation. Over-estimation due to incomplete recovery of insoluble material and neutral sugar effects would distort the exact values. However, trends in the solubilisation patterns could be interpreted. The extents of demethylation and non-methylester

linkages were measured in the insoluble material. The degree of methyl esterification of solubilised material could not be measured. Methanol contamination from the toluene solvent used to prevent microbial degradation interfered with measurements as discussed in chapter four.

7.2.2.1. Changes in pectin content during pre-cooking.

The pectin content of the insoluble cell walls from raw tissues was in agreement with other values for uncooked cv. Maris Piper pectin levels (chapter three and Jardine, 1998) (table 7.2). No loss of pectin occurred between the raw cell walls and cell walls isolated after two-stage (55°C) cooking (table 7.2). Additional proof that little pectin was solubilised is provided by the small amounts of soluble galacturonic acid recovered from the MCB-buffer used to wash the cell walls during the isolation procedure (Table 7.3).

Cooking Treatment	μMol GalA/g dry cell wall	% Gal A in cell wall	LSD
Raw	1131.33	19.52	a
Two-Stage (55°C)	1065.48	18.97	a
Two-stage (70°C)	868.71	15.46	b
Ca-two-stage (70°C)	919.06	16.20	b

Table 7.2. Galacturonic acid content of the four isolated MCB-insoluble cell walls.

The galacturonic acid remaining in the cell wall after two-stage (70°C) and Ca-two-stage (70°C) cooking was less than raw cell walls (table 7.2). The higher temperatures of a 70°C pre-cook caused a decrease in mechanical strength (chapter three, four and six) so some degradation of the cell wall structure would not have been unexpected. High levels of calcium are known to restrict pectin solubilisation (Andersson *et al.*, 1992). Due to the high levels of calcium

measured in Ca-two-stage (70°C) tissues in chapter six, the amount of galacturonic acid solubilised was unexpectedly high.

As with the cell wall material in chapter four, little can be said about pectin content changes in the cell wall during cooking until the solubilisation data is considered. Significantly more material was lost during the washing steps of two-stage (70°C) material than the Ca-two-stage (70°C) material. Most of the losses in the latter case were during the enzymic removal of starch (table 7.3). This would tend to suggest that higher levels of calcium in tissues initially restricted solubilisation. The cause of this was probably a combination of dilution of calcium content due to continual dilution with MCB-buffer as washing continued and degradation of the cell wall by boiling the material for a short time to gelatinise starch.

Cook Treatment	Pre-cook Water	1st Wash	2nd Wash	Enzyme	Total
Raw	n.d.	19.2	n.d.	50.9	70.1
Two-Stage (55°C)	n.d.	17.9	n.d.	76.2	94.2
Two-Stage (70°C)	n.d.	171.7	41.1	47.8	260.6
Ca-two-Stage (70°C)	n.d.	82.89	66.2	130.3	279.6

Table 7.3. Solubilised pectin released from the pre-cooked and cooled tissues during the cell wall isolation process, expressed as μmol Gal A /g dry cell wall (where n.d. = not detectable).

7.2.2.2. Changes in the degree of pectin methyl esterification.

The degree of methylation of raw material agreed well with other results for cv. Maris Piper (chapter three and Jardine, 1998). Two-stage (55°C) cooking of the cultivar caused a large drop in the degree of methylation of the potatoes. The drop of 17% is similar to the results found by Ng and Waldron (1997a) when they measured tissues pre-cooked at 50°C.

Cooking Treatment	µMol MeOH /g dry cell wall	% DM of Gal A	LSD
Raw	453.3	40.92	a
Two-Stage (55°C)	244.9	22.97	b
Two-stage (70°C)	228.7	26.01	c
Ca-two-stage (70°C)	267.9	29.14	d

Table 7.4. The degree of methylation of the MCB-insoluble pectin.

Two-stage (70°C) cooking resulted in less of a drop in methyl esterification than a 55°C pre-cook. This result was not unexpected, as PME is rapidly denatured at temperatures above 60°C (Puri *et al.*, 1982 and Tijskens *et al.*, 1997). Less de-esterification of the Two-stage (70°C) pre-cooked tissues was not unexpected. Although PME activity was not measured during either pre-cook method, the temperature profile of the each pre-cook (Chapter three) suggests that PME would have remained active for a shorter time in the 70°C pre-cook than the 55°C pre-cook.

The degree of methylation of Ca-two-stage (70°C) was only slightly higher than the two-stage cooked samples degree of methylation. As high levels of calcium within the wall restricts PME activity (Moledina *et al.*, 1981) less de-esterification in the Ca-two-stage (70°C) cooked material than the two-stage cooked (70°C) sample might have been expected (Table 7.4). As was seen in section 7.2.2.1 a significant amount of Ca-two-stage (70°C) pectin material was lost from the sample during cell wall isolation. Therefore, it was hard to interpret degree of methylation remaining within the insoluble cell wall material of the Ca-two-stage (70°C) sample.

7.2.2.3. Changes in the degree of non-methyl ester linkages.

The existence of non-methyl ester linkages was analysed using the copper ion exchange method, which was slightly modified from the Keijbets and Pilnik, (1974) method (section 2.7.5). The copper released from the saponified material gives a value for total galacturonic acid. This value should be the same as the MHDP values calculated. As can be seen in table 7.5, this was true for the two-stage (70°C) and the Ca-two-stage (70°C) cell walls.

Differences were found between the two methods for raw and two-stage (55°C) cooked cell walls. Losses of pectic material, especially from two-stage (55°C) cell walls must have occurred during the copper ion exchange method. The copper ion exchange method was done over two days. On the first day, the raw and two-stage (55°C) samples were prepared. The two-stage (70°C) and Ca-two-stage (70°C) were processed on a second day. As losses only occurred on the first day, it is probable that some unknown systematic error occurred on that day.

Cooking Treatment	Total Gal A			
	MHDP Method		Copper Method	
Raw	1131.1	(a)	999.8	(a,b)
Two-Stage (55°C)	1065.5	(a)	890.8	(b)
Two-stage (70°C)	868.7	(b)	902.2	(b)
Ca-two-stage (70°C)	919.1	(b)	925.3	(b)

Table 7.5 Comparison of MHDP and copper ion exchange methods for estimating galacturonic ester contents.

Cook Treatment	Unesterified (MHDP)	Non-Ester (Cu) (μmol/g cell wall)	Total Ester (μmol/g cell wall)	Methyl Ester (μmol/g cell wall)	% Total Esterified	% Methyl Esterified	Difference
Raw	1131.1	481.0	650.4	453.3	57.5	40.1	17.4
Two-Stage (55°C)	1065.5	635.0	430.5	244.9	40.4	23.0	17.4
Two-Stage (70°C)	868.7	341.2	349.5	228.7	40.2	26.3	13.9
Ca-two-Stage (70°C)	919.1	525.1	394.0	267.9	42.9	29.2	13.7

Table 7.6. Non-methyl ester content of cell wall with the figures based the MHDP results for total galcturonic acid..

Cook Treatment	Total Gal A. (Cu^{2+})	Non-Ester (Cu) (μmol/g cell wall)	Total Ester (μmol/g cell wall)	Methyl Ester (μmol/g cell wall)	% Total Esterified	% Methyl Esterified	Difference
Raw	999.8	481.0	518.8	453.3	51.9	45.3	6.5
Two-Stage (55°C)	890.8	635.0	255.8	244.9	28.7	27.5	1.2
Two-Stage (70°C)	902.2	341.2	386.0	228.7	42.6	25.3	17.4
Ca-two-Stage (70°C)	925.3	525.1	400.3	267.9	43.3	29.0	14.3

Table 7.7. Non-methyl ester content of cell wall with the figures based the copper ion exchange results for total galcturonic acid.

If the values had been statistically indistinguishable then an average value for mean value of the two methods would have been used to calculate the degree of total and methyl esterification values. As there were differences between the two total galacturonic acid values the levels of esterification were calculated separately using both total galacturonic acid values, from each method i.e. the MHDP value and the copper ion exchange value (table 7.6 and 7.7).

After pre-cooking, Jardine (1998) found non-methyl ester levels of 15% in raw potatoes. The value found in the raw cell walls was in close agreement with this value (table 7.6). After pre-cooking, the levels of total ester and methyl ester levels fell, but the differences between the two, i.e. the non-methy ester content, stayed roughly the same (table 7.6).

These results show that increase of NME found by Hou and Chang (1996) in bean sprouts does not occur in potatoes. This would suggest that the main role of PME during pre-cooking of potatoes is the removal of methyl ester groups from galacturonic acid of the pectin chain.

7.2.3. Locating the Changes in Pectin Methyl Esterification.

7.2.3.1. Immuno gold labelling

Changes in the degree of methylation of pectin within the wall during two-stage cooking were evaluated using monoclonal antibodies JIM 5 and JIM 7. JIM 5 and JIM 7 antibodies bind to regions of high and low methyl esterified pectin respectively (Knox *et al.*, 1990). The epitopes that the antibodies recognise are either five consecutive galacturonic acid residues that are methyl esterified (JIM 7) or five consecutive galacturonic acid that are unesterified (JIM 5) (Knox *et al.*, 1990). In theory, if PME activity creates a region of the pectin molecule with consecutive unesterified galacturonic acid residues, then the JIM 5 monoclonal will bind to it. Additionally, if PME removes a methyl ester group from a region of five consecutive methyl ester groups, then JIM 7 binding will not occur. Thus,

evidence of PME activity within the wall can be inferred from either an increase in JIM5 labelled regions, or a decrease in JIM 7 labelling.

Resin embedded samples were sectioned and labelled with either JIM5 or JIM7 antibodies then 10nm gold conjugated secondary antibodies. Control samples were labelled with only the secondary antibodies (figure 7.1 and 7.2). If gold labelling was observed in controls, this would indicate that the secondary antibody recognised an epitope other than JIM5 or JIM7. No gold was found in the controls (figure 7.1 and 7.2). Images were recorded with a cold stage CCP attachment to a TEM with an electron beam voltage of 80Kv instead of a 700Kv beam used when conventional images are recorded. Resolution was slightly impaired, but images were viewed immediately, allowing focusing and corrections for brightness to be carried out online.

To verify that the black dots in the images were gold conjugates, images were viewed at 150eV (figure 7.3 and 7.4). This is a process known as energy filtering TEM (EFTEM). One of the three ionisation edges of gold is at 150eV, which also provides the best energy filtering focus for resin embedded biological material. At 150eV, the gold conjugates appear as white areas as the ionisation events of the other elements have been filtered from the image beam of the microscope. Figures 7.3 and 7.4 are the same region of JIM 5 labelled raw cell walls as figures 7.7 and 7.8, which were taken at zero-loss (0eV). Black dots from figures 7.7 and 7.8 match white dots in 7.3 and 7.4, which confirms that the black dots viewed in the images were gold conjugated particles.

This set of experiments also brought up the opportunity to examine the ultrastructure of isolated MCB-insoluble cell walls. In general, the cell walls were very fragmented as a result of the cryo-milling step carried out during the isolation protocol. This made examination rather difficult as certain regions of interest, such as the wall or tricellular junctions were often not intact. Another problem was swelling, which made examination at magnifications above x 7,000 difficult. Often, it appeared that the internal regions of the wall were unaffected and maintained their rigid structure, but the outer edges were swollen and the walls

appeared 'wavy'. As this was apparent in raw tissues, it could not have been caused by pre-cooking. The most likely cause of this was that the short boiling step carried out on the walls, during cell wall isolation to gelatinise starch, caused swelling. The regions of interest in this study were the straight regions of the wall and the tricellular junctions.

The raw tissues were labelled with JIM 7 throughout the wall and tricellular junctions (figures 7.5 and 7.6) but JIM 5 labelling was confined to the middle lamella (figure 7.7) and around the corners of intercellular spaces (figure 7.8). The weakening of the pectin matrix is required for cell separation to occur (van Marle *et al.* 1994, Ng and Waldron 1997). The fact that the middle lamella pectin was mainly unesterified suggests that weakening of the pectin network in the middle lamella could occur mostly by calcium chelation. As little JIM 5 label was observed in the cell wall, calcium chelation would be less likely to have such an important role in cell wall weakening as it would in the middle lamella. Highly methyl esterified regions, which were recognised by JIM 7 were found throughout the cell wall. Therefore, degradation by β-elimination potentially could occur in both the cell wall and middle lamella.

Even if some pectic molecules in the middle lamella were not subject to β-elimination, this would not rule out the possibility of solubilisation of middle lamella pectin as a result of β-elimination processes. It is probable that that the middle lamella pectin is 'anchored' to cell wall pectin (Jarvis, 1984, McCann and Roberts, 1991), so any breakage in the cell wall network caused by β-elimination could cause a weakening of the middle lamella resulting in cell separation.

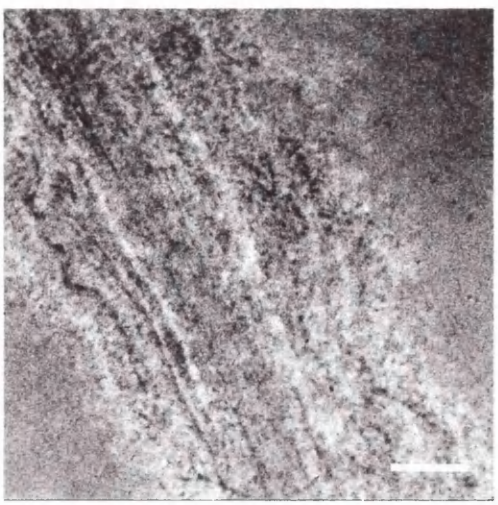
Two-stage (55°C) cooking caused a decrease in the amounts of JIM7 labelling throughout the wall, except the corners (Figures 7.9 and 7.10). However, an increase in JIM5 labelling was only observed in the middle lamella (figure711) and in the tricellular junctions (figure 7.12).

Two-stage (70°C) cooked cell walls contained very few JIM 5 reactive epitopes (figures 7.15 and 7.16). No intact tricellular junctions could be found in the

two-stage (70°) samples, but it was presumed they would label in a similar way to the Ca-two-stage (70°) cooked samples. Like two-stage (70°) samples, the Ca-two-stage (70°) cooked walls had very little JIM5 labelling in the cell wall, but it was found in the corners of tricellular junctions (Figure 7.17-7.20).

It is unclear what caused this lack of JIM 5 recognition. During the 70°C pre-cook, amylose from gelatinised starch could have diffused into the cell wall, which retrograded when frozen. Both the raw and two-stage (55°C) samples starch had not gelatinised prior to freezing. The tricellular junctions of potato walls contain low-ester pectin and are the region of the wall where calcium is predominantly found. Therefore, it is highly likely that the corners of air spaces and tricellular junctions constitute a very tightly packed pectin environment that would exclude amylose from entering it.

Figures 7.1-7.20. TEM images of isolated cell walls after different pre-cook treatments, labelled with JIM 5 or JIM 7 monoclonal antibodies (mAbs).



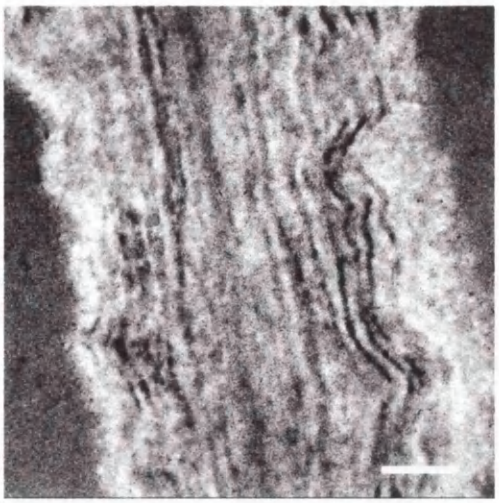
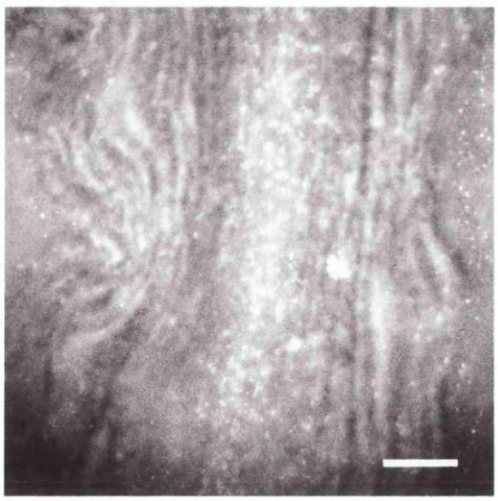


Figure 7.1 and 7.2. JIM 5 and JIM 7 controls. Raw sections were incubated in the secondary gold-conjugate without prior incubation in the primary (scale bar is 0.5μm)



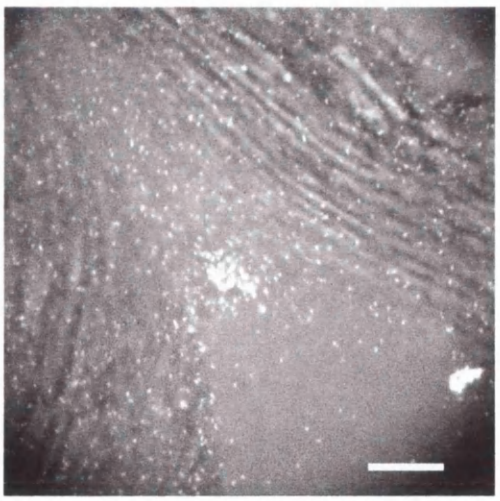


Figure 7.3 and 7.4. JIM 5 labelled raw cell walls viewed at 150Kev which shows up gold particles as white dots. Figure 7.3 is in straight part of the wall (scale bar is 0.5μm). Figure 7.4 is in the corner of an intercellular air space (scale bar is 1μm)

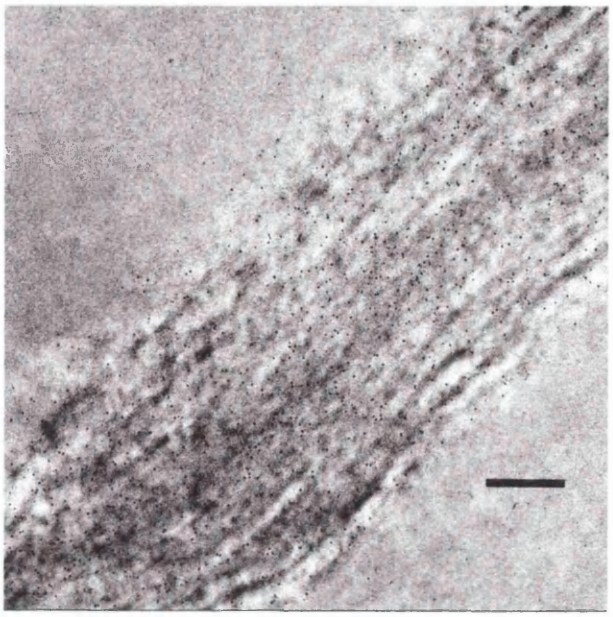


Figure 7.5. Raw cell wall labelled with JIM 7 (scale bar is 0.5μm)

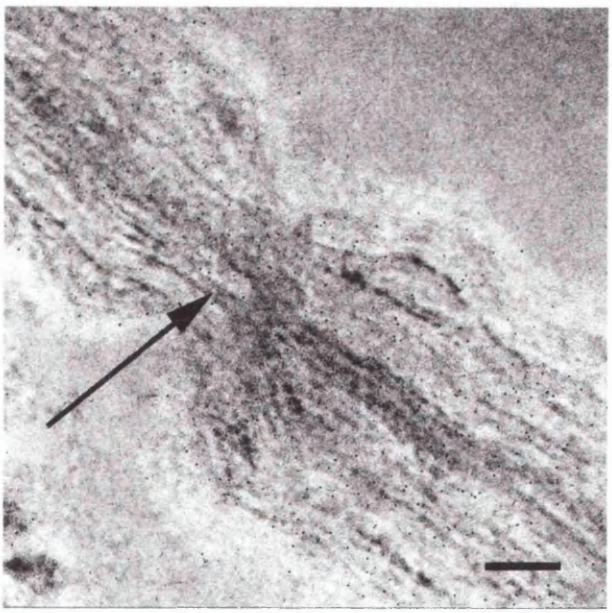


Figure 7.6. Raw cell wall labelled with JIM 7, an arrow denotes a plasmodesmata (Scale bar is 0.5μm)

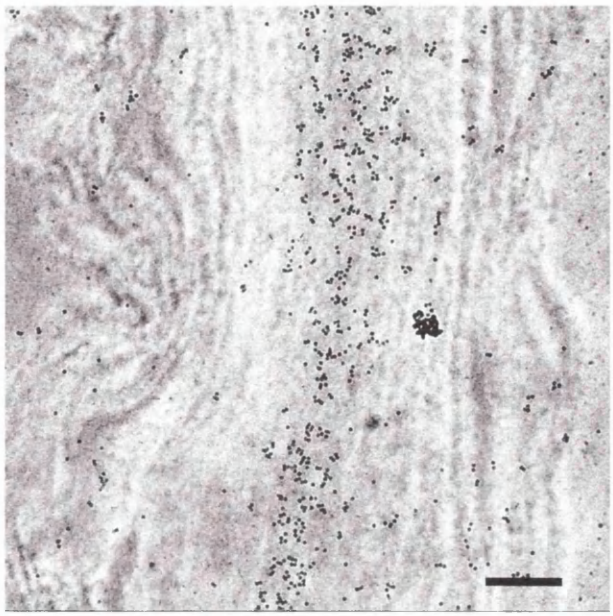


Figure 7.7. A region of a raw cell wall labelled with JIM 5 (scale bar is 0.5 μm)

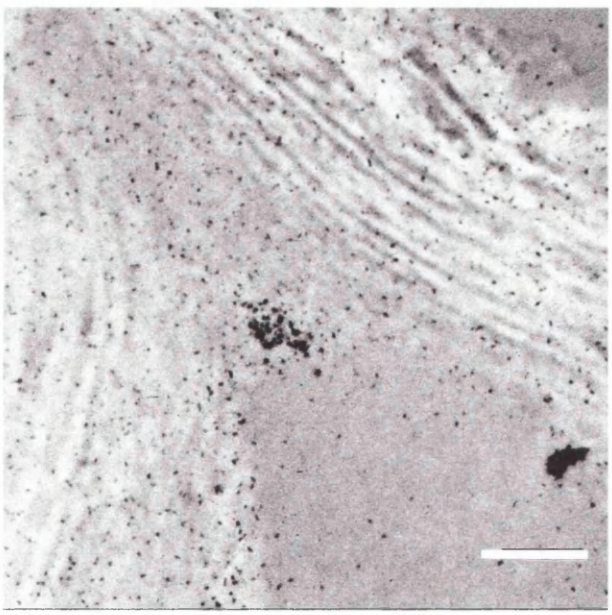


Figure 7.8. A corner of an intercellular air space, in raw cell walls, labelled with JIM 5 (scale bar is 1.0 μm)

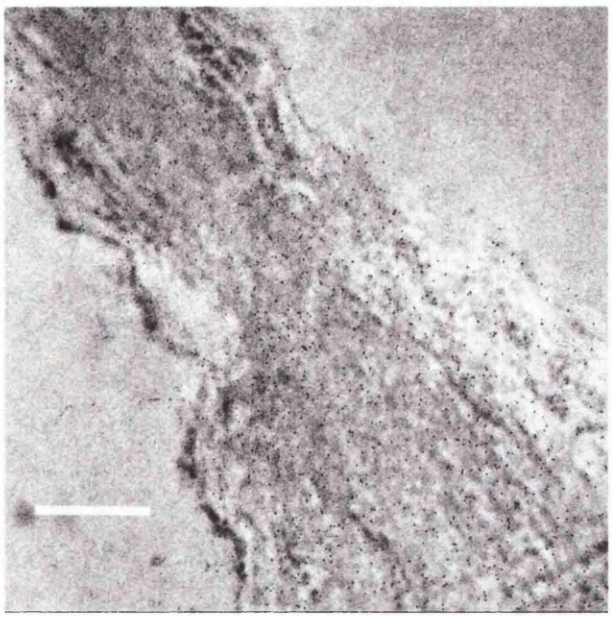


Figure 7.9. Two-stage (55°C) cooked cell wall labelled with JIM 7 (scale bar is 1.0μm)

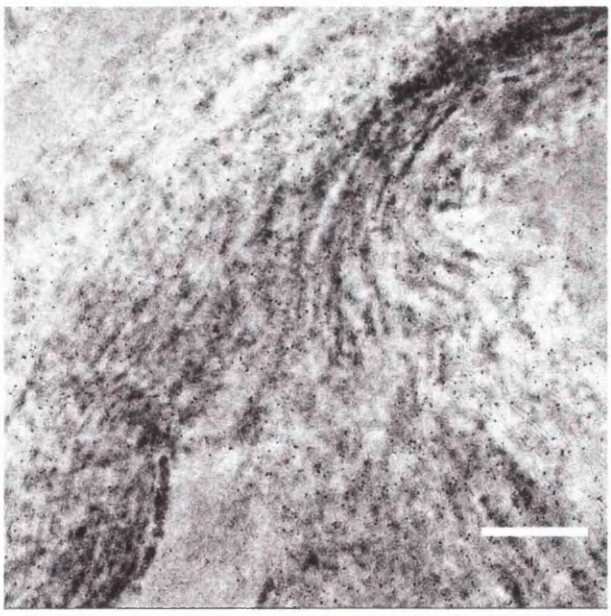


Figure 7.10. JIM 7 labelled two-stage (55°C) cooked wall, the amount of labelling appeared less than in raw samples (scale bar is 1.0μm)

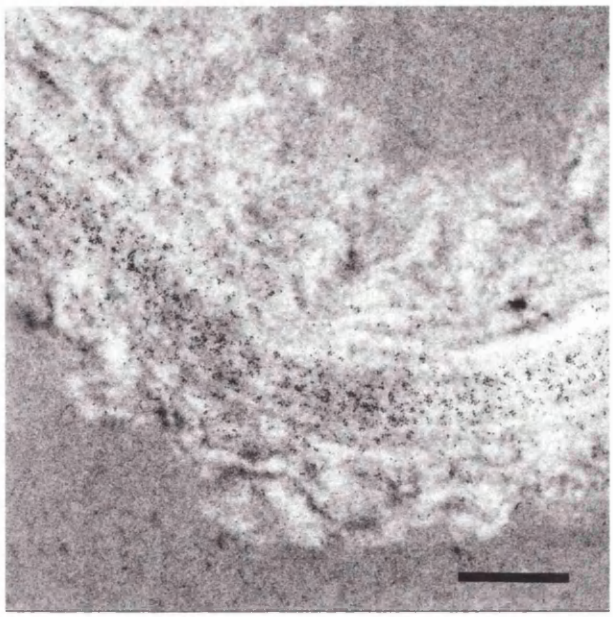


Figure 7.11. Two-stage (55°C) cell wall, labelled with JIM 5, Labelling was predominantly in the middle lamella region (scale bar is 1.0μm).

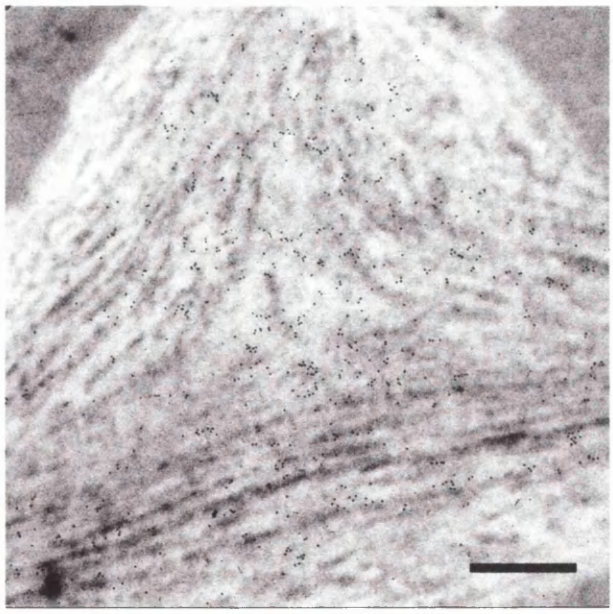


Figure 7.12. JIM 5 labelled, two-stage (55°C) tricellular junction. (scale bar is 1.0μm).

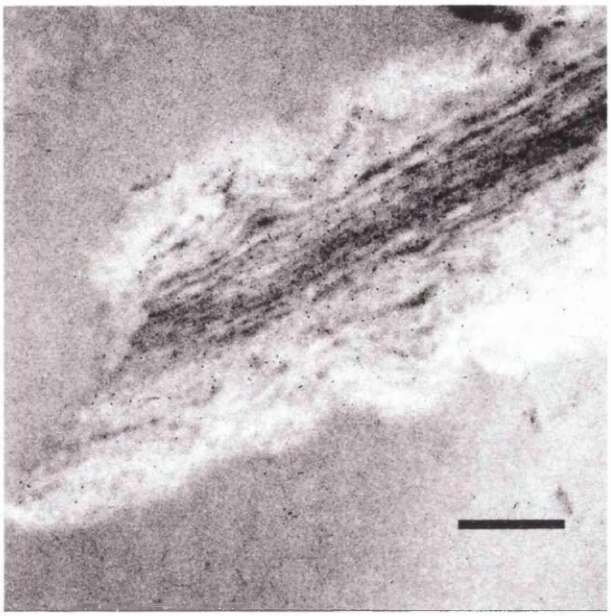


Figure 7.13. JIM 7 labelled, two-stage (70°C) cooked cell wall. Less labelling was observed, but it was throughout the cell wall (scale bar is 1.0μm).

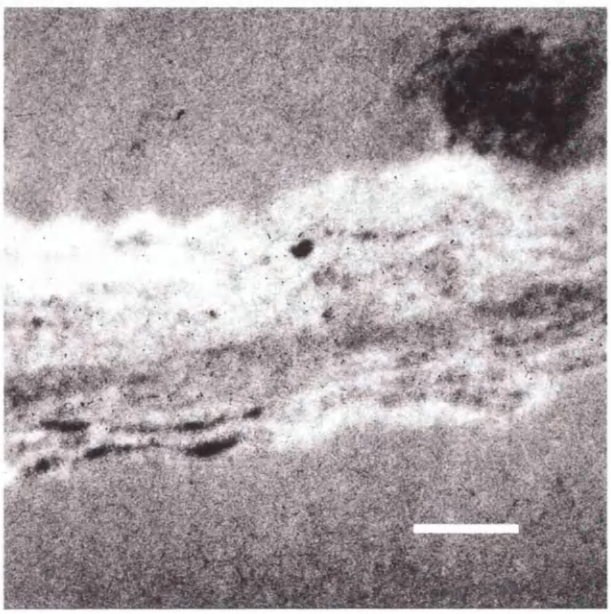


Figure 7.14. Two-stage (70°C) cooked cell wall labelled with JIM7. Little labelling was observed, but epitopes were recognised throughout the wall (scale bar is 1.0μm).

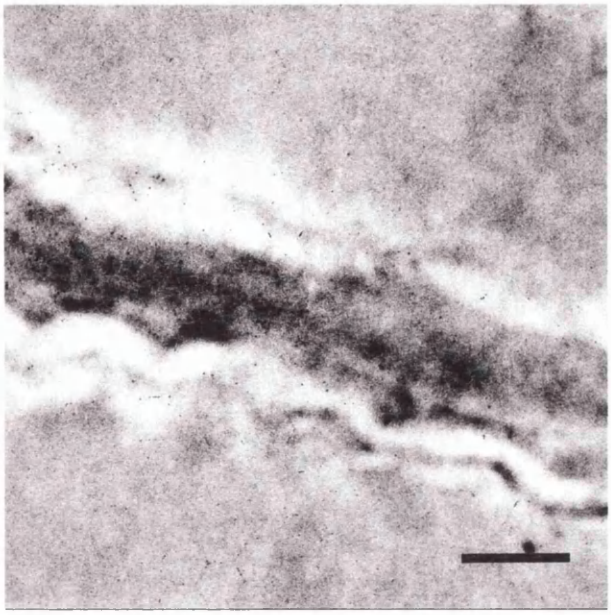


Figure 7.15. Two-stage (70°C) cooked cell walls labelled with JIM 5. Very little labelling was detected (scale bar is 1.0μm).

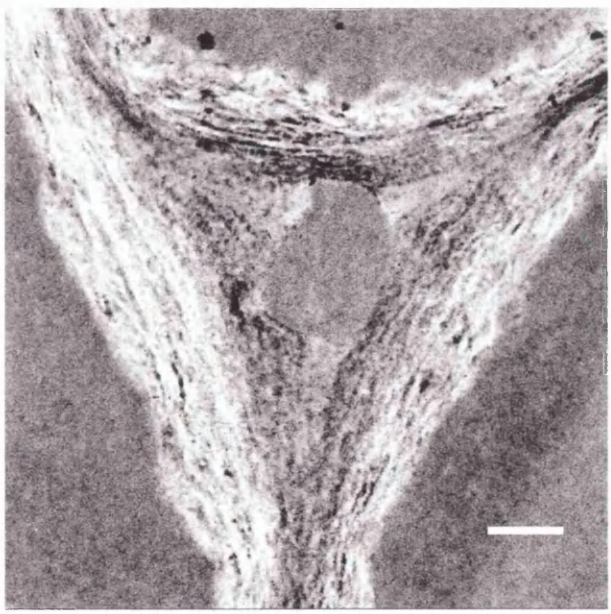


Figure 7.16. JIM 5 labelled, two-stage (70°C) cooked tricellular junction, very little labelling was observed (scale bar is 0.13μm).

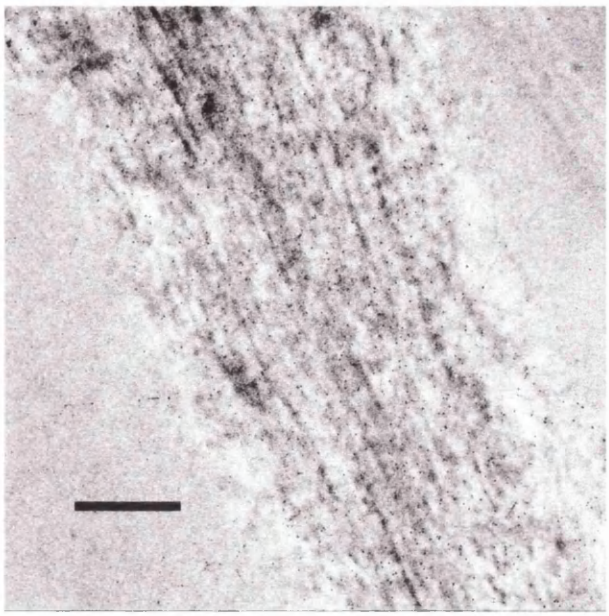


Figure 7.17. Ca-two-stage (70°C) cooked cell wall, labelled with JIM7. Labelling was observed throughout the wall, but to a lesser extent than raw samples. (scale bar is 1.0μm)

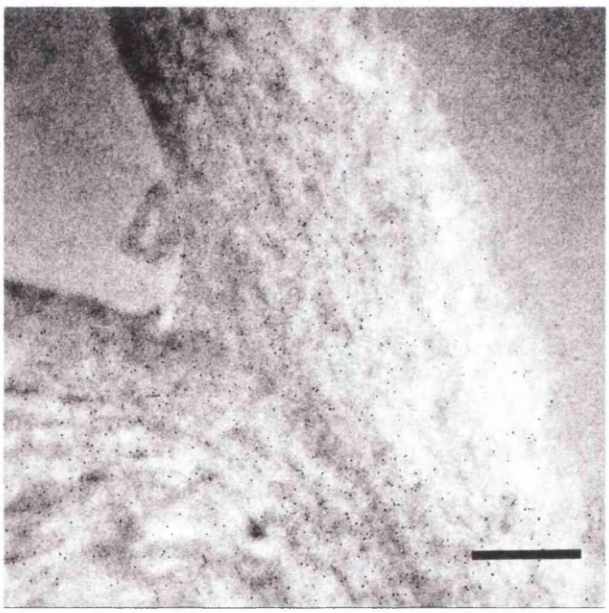


Figure 7.18. Ca-two-stage (70°C) cooked cell walls at a tricellular junction, labelled with JIM 7. Labelling appeared similar to other parts of the wall (scale bar is 1.0μm).

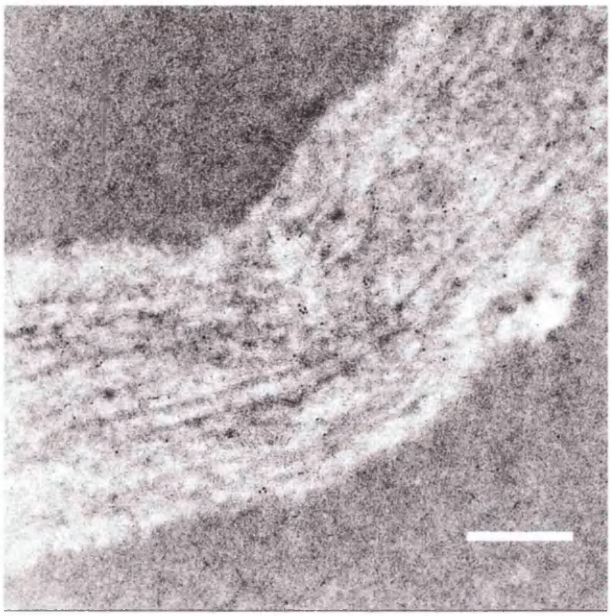


Figure 7.19. JIM 5 labelled, Ca-two-stage (70°C) cooked cell wall, little labelling was observed (scale bar is 1.0μm).

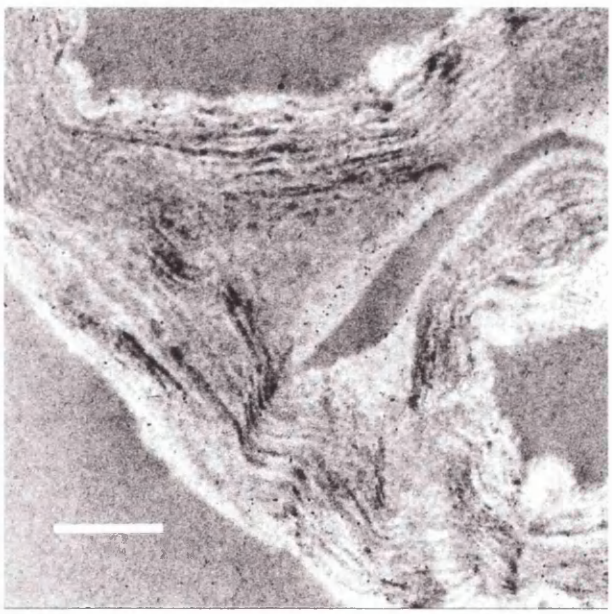


Figure 7.20. JIM 5 labelled, Ca-two-stage (70°C) cooked cell wall, at an intercellular air space. Some labelling was observed round the edges of the air space (scale bar is 1.0μm).

The location of highly esterified pectin epitopes in the two-stage (70°C) and Ca-two-stage (70°C) samples was slightly different from the two-stage (55°C) samples. JIM 7 labelling was less abundant in the two 70°C pre-cooked samples (figures 7.15, 7.16, 7.19 and 7.20) than in the 55°C pre-cooked sample (figures 7.9 and 7.10). This would indicate that less demethylation, presumably catalysed by PME, occurred in the 70°C treatment than 55°C pre-cook. Or alternatively, that highly methylated pectin in these regions had been weakened during pre-cooking at 70°C, causing solubilisation during the cell wall isolation procedure. Either of these two scenarios could have a profound effect on the texture of the subsequently cooked potatoes.

7.2.3.2 EELS mapping of calcium before and after doping.

It has been stated previously that for the pre-cooking to cause an increase in firmness and reduce cell separation, calcium entering the wall along with PME activity is required (Bartolome and Hoff, 1972, Moledina *et al.*, 1981, Ng and Waldron, 1997a). Using EELS it was hoped to locate both calcium bound pectin and newly unesterified galacturonic acid groups within the wall. A sequence of images, based on 3eV energy slices over the energy-loss range of ΔE = 305-375 eV, was used to map calcium present in the wall. Doping sections in 5mM calcium acetate saturates unesterified galacturonic acid groups in the cell wall with calcium. So in theory the distribution of calcium can be determined by EELS and thus the location of unesterified galacturonic acid groups. An increase in calcium abundance in doped walls after pre-cooking would be indicative of newly formed unesterified galacturonic acid groups. The distribution of *in muro* calcium bound to pectin was found by imaging undoped walls.

It was initially hoped to investigate this work on free-slammed, then freeze dried tissues. The initial plan was to use EELS to find out where calcium had gone in Ca-two-stage (70°C) cooked samples in comparison with two-stage (55°C) and two-stage (70°C) cooked tissues. To avoid storage differences, sample preparation had to be done as soon as possible after the texture experiments

carried out in chapter six. Unfortunately, the freeze drier was not working at the time. When it was fixed a number of months had passed from the time of the cooking in chapter six. During storage, changes that are not yet fully understood occur to the textural attributes of potatoes (Wilson, W and Jarvis, MC unpublished results). It was decided, therefore, to use resin embedded isolated cell walls rather than freshly cooked tissues that may have different cell wall attributes to those characterised earlier in this chapter.

Using isolated cell walls meant that the calcium distribution would not be the same as in the tissues characterised in chapter six. As was expected, calcium was evenly distributed throughout the cell walls of Maris Piper in all four samples (figures 7.21-7.24). During the cell wall isolation, the ionic levels within walls would have equilibrated with the MCB buffer, hence the even distribution between samples. It was still hoped to evaluate the distribution of newly formed unesterified pectin using the doping method. When doped samples were mapped for calcium, apart from raw tissues no calcium was detected. In fact, the samples appeared to have lost calcium from the cell wall structure.

Doping Procedure	Observed Effect
Doped, without washing	Calcium throughout the wall
Doped then washed	Calcium levels decreased
Washed	No calcium within wall
Washed, then doped, then washed	Some calcium left

Table 7.7. Outline and summary of results of doping/washing experiments.

To evaluate what had happened during the doping experiment, a number of different combinations of the doping and washing were carried out (table 7.7). Raw sections were used, as these had appeared less affected in terms of calcium losses during washing.

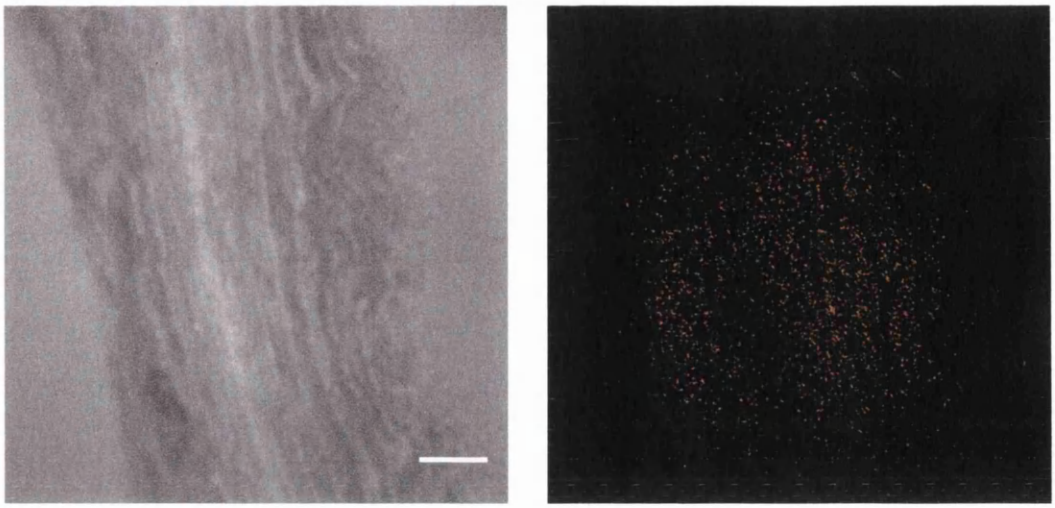
To establish if calcium could bind to cell walls, raw sections were doped but not washed prior to viewing (figure 7.25). Calcium was found throughout the wall, especially in regions that were electron dense; calcium was also present in low amounts out with walls on the resin material. Raw sections were washed after doping (figure 7.26). The calcium levels within the wall dropped and calcium was no longer present in the resin. The washing step had appeared to remove excess calcium on the resin and possibly some cell wall bound calcium. When sections were washed and then mapped, no calcium was found in the walls, the washing step removing calcium already in the wall (figure 7.27). Finally, sections were washed, doped and then washed again (figure 7.28). Calcium was detected but in lower amounts than samples doped then washed (compare with figure 7.26). The initial washing step, as well as removing calcium from the wall may have removed potential galacturonic acid calcium binding sites.

As mentioned before, this problem was accentuated in two-stage cooked material. Figure 7.29 is a representative EELS map of two-stage (70°C) sample. The washing step had removed potential binding sites and all the calcium from the wall. As the problem was less apparent in raw tissues, exposed galacturonic acid groups could have been weakened, possibly by pre-cooking. This problem was also encountered in chapter five for cv Cara but apparently not for cv. Golden Wonder. This could mean that galacturonic acid groups in Golden Wonder were held more rigidly into the wall than in cv. Cara.

Doping experiments have been used successfully on wild type tomatoes and the Cnr mutant (Thompson *et al*, 1999, Huxhum I.M., unpublished), sugarbeet (Marry M., unpublished) and flax (His I., unpublished). In the last, doping detected calcium levels binding sites in the secondary cell wall, which had previously not been reported. It was unclear, at the time of writing, whether calcium was binding to pectin or a protein component of the wall. It should be noted that nitrogen, most likely a component of proteins, has been found using EELS in the tricellular junctions and cell walls of potato stolons (Marry, unpublished). It is conceivable that acidic proteins could bind calcium. If doping experiments had been successful calcium could have bound to acidic proteins as well as unesterified

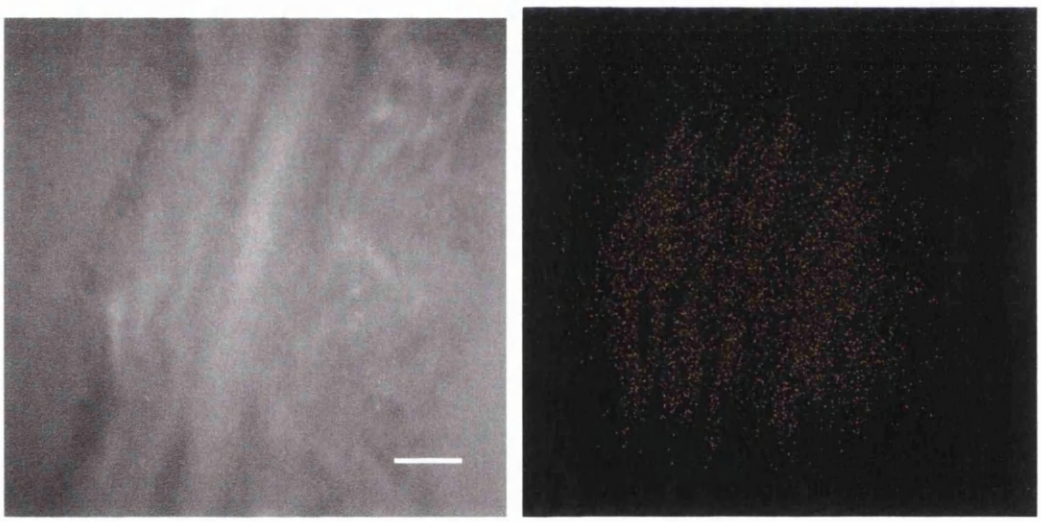
pectin. To distinguish between the two, the nitrogen content and its location in the cell wall would have been evaluated by EELS and compared to calcium levels.

The doping problem has so far only been encountered with potatoes, especially if cohesion of the cell wall network is weaker, either due to its varietal properties or by processing. These experiments were carried out at the very last stages of the thesis. Potentially they could produce some very important findings in terms of cell wall architecture and its role in texture. Due to time constraints, this work could not be properly concluded. Future experiments could entail the use of lanthanum instead of calcium as the doping ion. Lanthanum has been successfully used to dope flax cell walls (His I., unpublished) and to replace calcium in tomatoes (Marry M. unpublished). As a trivalent metal it has a stronger affinity for pectic sites. It might be the case that both it and pectic sites it has bound to, might not be removed during the washing stage.



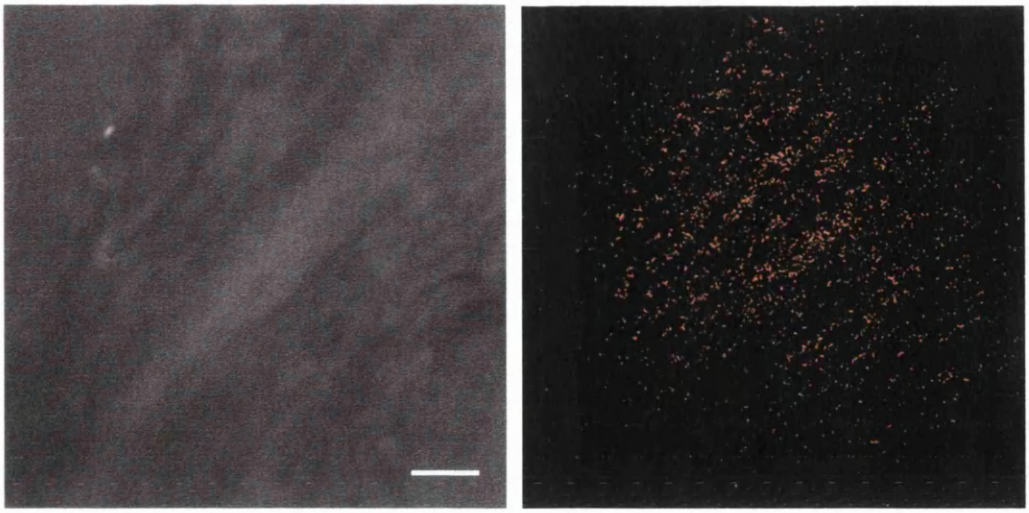
a b

Figure 7.21. Raw Maris Piper cell walls prior to doping in calcium. Figure a) is a reference image (scale bar is 0.5µm) b) a calcium map of the same area. Calcium was evenly distributed throughout the wall



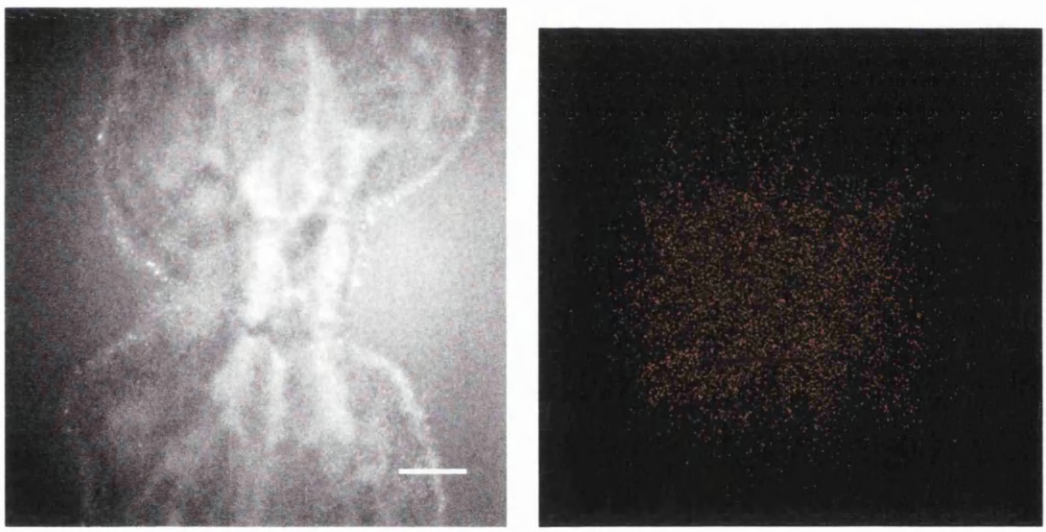
a b

Figure 7.22. Two-stage (55°C) cooked Maris Piper cell walls prior to doping in calcium. Figure a) is a reference image (scale bar is 0.5µm) b) the calcium map of the same area. Calcium was evenly distributed throughout the wall



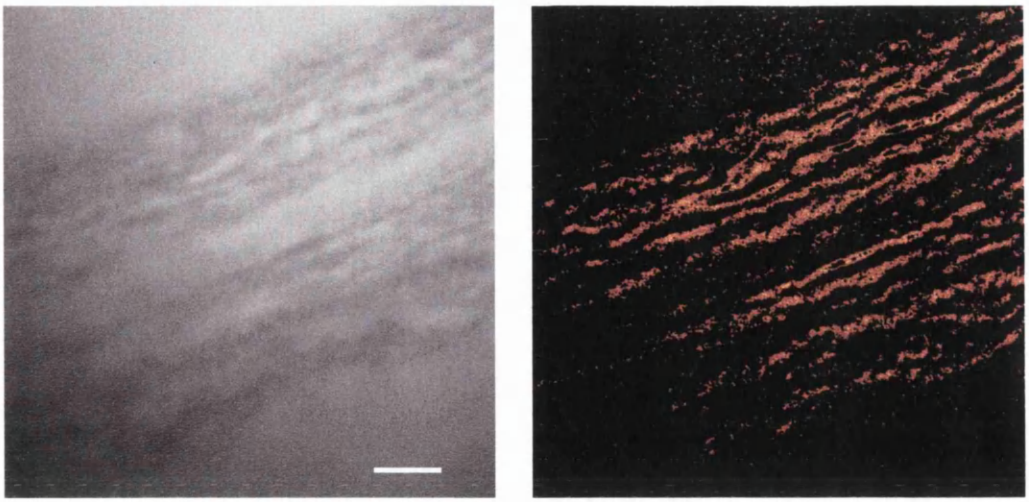
a b

Figure 7.23. Two-stage (70°C) cooked Maris Piper cell walls prior to doping in calcium. Figure a) is a reference image (scale bar is 0.5μm) b) the calcium map of the same area.



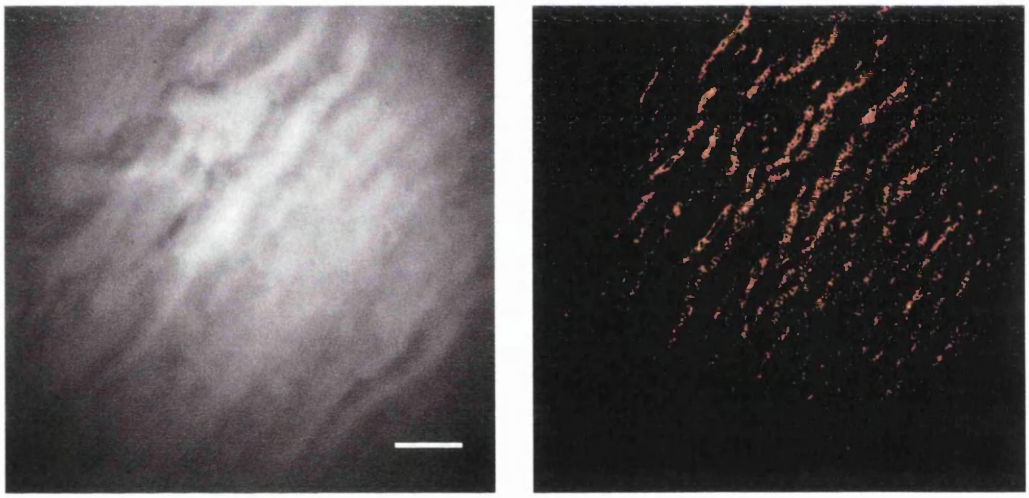
a b

Figure 7.24. Ca-two-stage (70°C) cooked Maris Piper cell wall and plasmodesmata prior to doping in calcium. Figure a) is a reference image (scale bar is 0.5μm) b) the calcium map of the same area. Calcium was evenly distributed throughout the wall



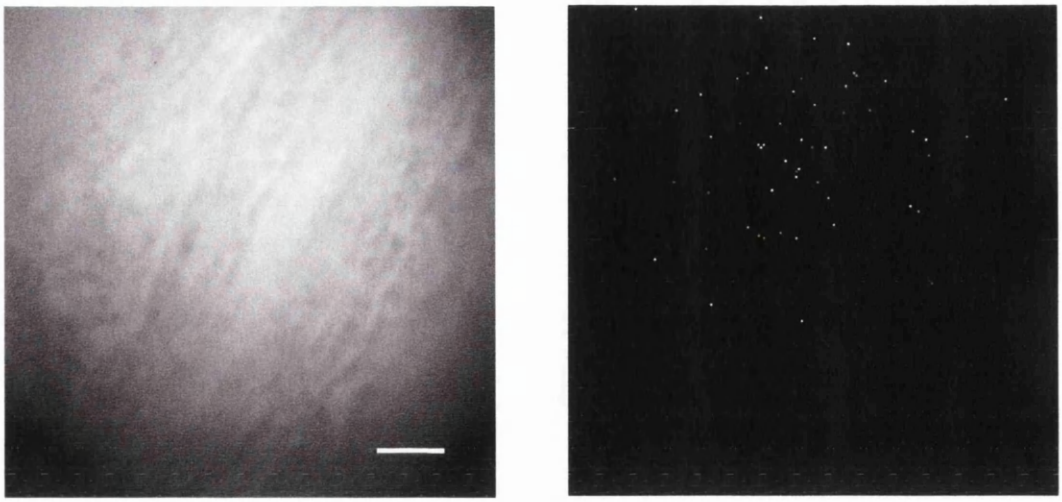
a b

Figure 7.25. Calcium doped cell wall a) reference image of raw cv Maris Piper cell wall (scale bar is 0.5µm) b.) EELS map of the region, calcium did bind to cell wall especially in the electron dense regions



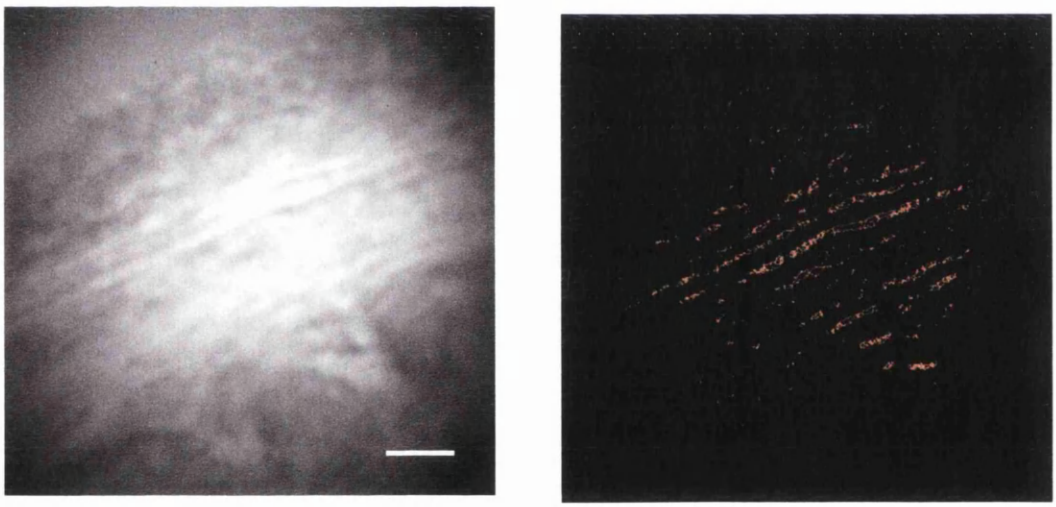
a b

Figure 7.26. Calcium doped then washed cell wall a) reference image of raw cv. Maris Piper cell wall (scale bar is 0.5µm) b.) EELS map of the region, washing caused the removal of calcium from the wall.



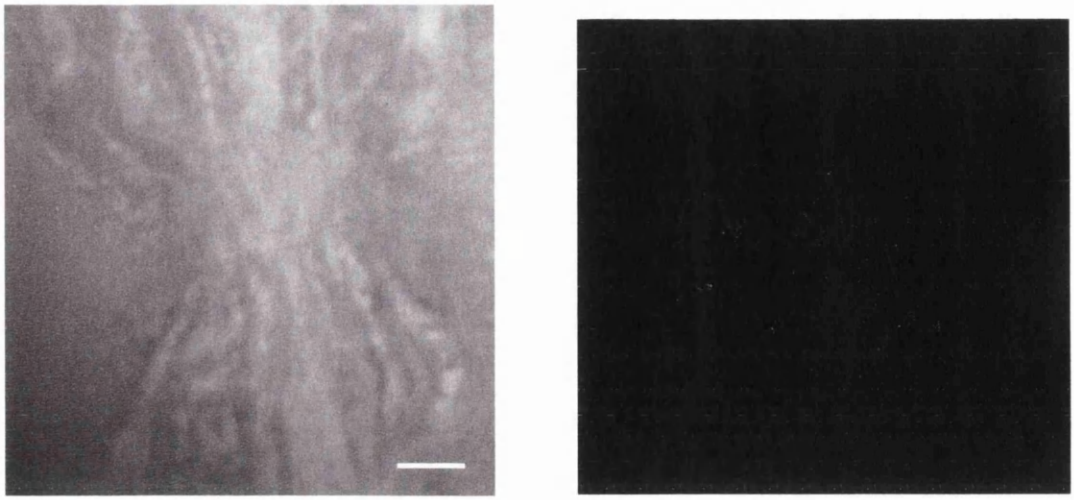
a b

Figure 7.27. Cell wall washed prior to calcium doping a) reference image of raw cv. Maris Piper cell wall. (scale bar is 0.5μm) b.) EELS map of the region, washing caused the removal of calcium from the wall.



a b

Figure 7.28. Cell wall washed doped then washed a) reference image of raw cv. Maris Piper cell wall. (scale bar is 0.5μm) b.) EELS map of the region.



a b

Figure 7.29. Cell wall washed of two-stage (70°C) cv. Maris Piper doped then washed a) reference image cell wall (scale bar is 0.5μm) b) EELS map of the region, washing caused the complete removal of calcium from the wall.

CHAPTER EIGHT

PILOT PLANT COOKING USING THE COOKING METHOD DEVELOPED IN THE LABORATORY.

8.1. Introduction

In chapters three, four and six, measurements made on laboratory cooked potatoes showed a clear difference in texture between one-stage and three-stage cooked potatoes. The following chapter describes work carried out to determine if the changes observed at the laboratory scale were reproducible on a larger pilot plant scale. The work was carried out at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe.

Potatoes were cooked with the Polar cooking apparatus using the cooking times developed for laboratory cooking (section 2.3). The physical strength of the raw, two-stage (55°C and 70°C) cooked, one-stage cooked and three-stage cooked (55°C and 70°C) potatoes was measured. The data was recorded using SMS Texture Expert 2.0 software package. Mr Harry Schimanski, from Stable Micro Systems, kindly donated this software to analyse the results.

Microscopic examination of the structure of fracture planes and inner structure was done using scanning electron microscopy (SEM). Cryostat light microscopy (LM) was also carried out at these stages of cooking to evaluate any changes in the physical structure of the potatoes. Potatoes cooked by the three different

methods were then mashed. The texture of the resultant mash was measured by physical and sensory methods.

8.2. Results.

Potatoes used were cv. Maris Piper grown in Cambridgeshire. The specific gravity of the potatoes was 1.08 (sd 0.001). Potatoes were prepared and cooked as described in section 2.3. The potatoes were cleaned and all peel and eyes were removed. The potatoes were then chipped to an 8mm thickness. An outline of the cooking regimes used for the pilot plant is shown in Figure 8.1.

Cooking of potatoes on a pilot plant scale proved to be more problematic and less precise than cooking in the laboratory. Like the lab method, cooking was carried out in a batch process. A large amount of potatoes (~ 2.5 kg) were cooked in each batch and the residence time in the cooking tanks were not identical. The first chips loaded had a slightly longer residence time than the last ones added. Large amounts of potatoes were added very quickly, resulting in the drums being heavily loaded. Heat transfer from the water to the potatoes might not have been as uniform as a continuous cooking process where smaller amounts of potatoes were added to the drums at a slower rate.

Another problem encountered due to rapid loading occurred when chips were transferred between two tanks. Quite often chips would get obstructed in the exit shoot of the first tank. This caused delays in transfer of chips between the two tanks that made cooking quite wasteful and imprecise. For example, if the transfer was between tank I and II and the exit chute of tank I was blocked half way through a transfer, the first chips coming through would pass through tank II and III immediately and out of the Polar cooker as waste. Secondly the chips still left in tank I would have a longer pre-cooking time than intended before entering tank II until the obstruction was cleared.

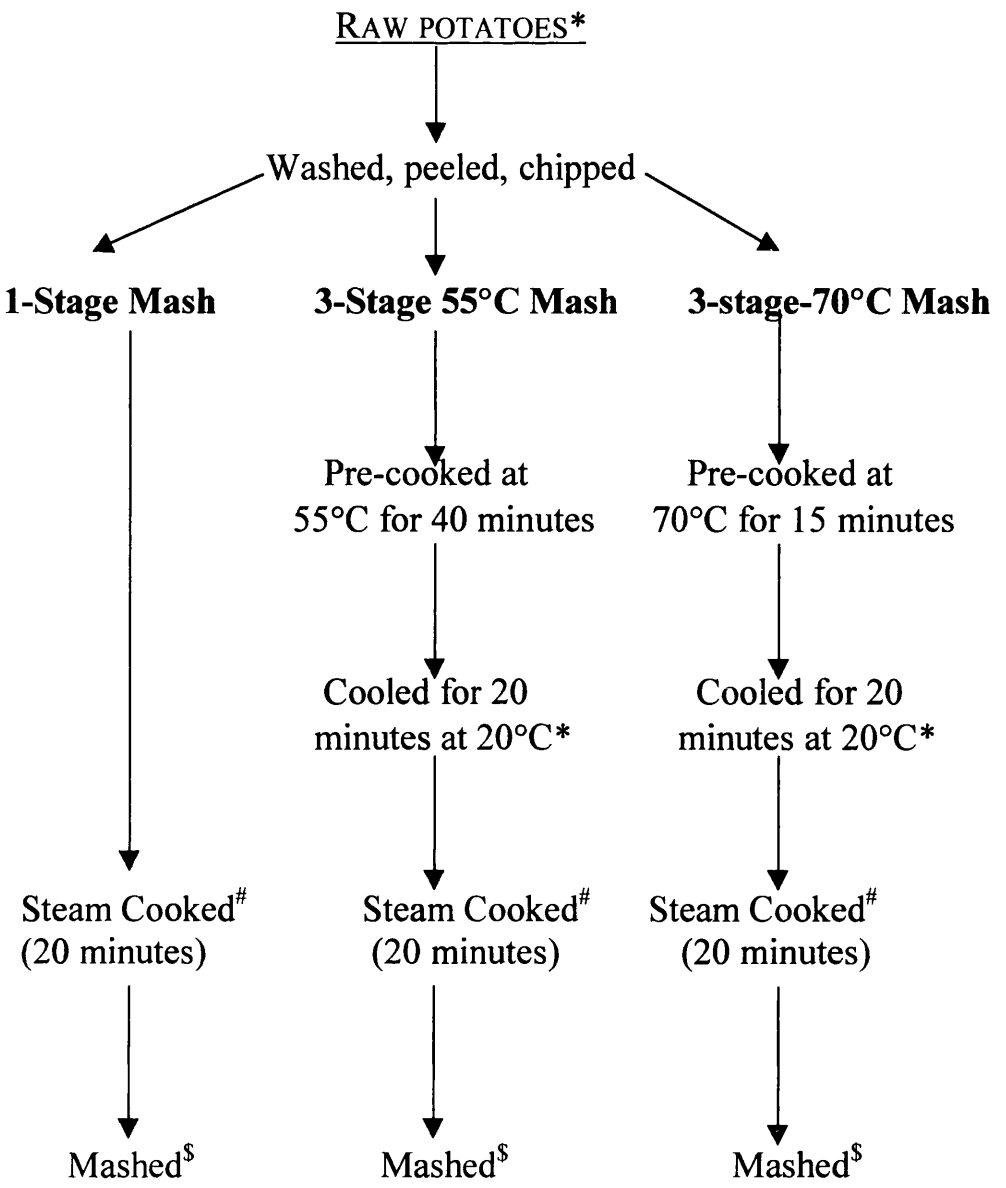


Figure 8.1. Outline of cooking methods used to prepare mashed potatoes and the measurements were carried out at each stage of cooking. The pre-cook times used were based on texture measurements made in Chapter three.

* : Raw and two-stage cooked potatoes; their physical texture and structure were examined.

: One-stage and three-stage cooked potatoes (55°C and 70°C); their physical texture and structure were examined.

$: one-stage mash and three-stage mash (55°C and 70°C); their texture was evaluated by physical and sensory methods.

8.2.1. The Physical Texture of Cooked Potatoes.

8.2.1.1. Raw and two-stage cooked potatoes.

Raw and two-stage cooked potatoes were subsampled prior to steam cooking. The chips were stored a 4°C overnight and prior to texture measurements the chips were allowed to equilibrate to room temperature. Texture measurements were made using a Stable Micro Systems (SMS) TA.XT2*i* tensile testing apparatus with data recorded and analysed using SMS Texture Expert 2.0 software package. Typical force/distance graphs for the raw and pre-cooked tissues cut with a wedge shaped cutter at a speed of 10mm/s to a depth of 6mm are shown in Figure 8.2.

Cutting with a wedge shaped cutter initiates a stable crack (Vincent *et al.*, 1990). From the shapes of the curves (Figure 8.2), it appeared that the two-stage (70°C) samples required the same force as the raw and two-stage (55°C) samples to initiate the crack in the surface of the tissues. It is likely that the temperatures of the 70°C pre-cook removed turgor pressure from cells. This made the tissues more pliable and they were compressed when the wedge came into contact with it, hence the large increase in force observed in the initial part of the curve. After the crack had been formed there was a drop in force required to cut through the two-stage (70°C) cooked material. After the crack had been initiated, less force was required to cut through the two-stage (70°C) cooked tissues as the relatively high pre-cooking temperatures had softened the tissues (Figure 8.2). After the crack was initiated in the raw and two-stage (55°C) cooked samples, the force did not decrease. In general, the force continued to rise in raw tissues as a resistance to cutting was high in the un-cooked potatoes. With the two-stage (55°C) the force required to cut through the tissues tended to plateau. Heating at 55°C would have damaged the cell membranes (Zhang *et al.*, 1993) and a slight loss of turgor may have occurred.

The maximum force and the mean plateau energy of the three samples were measured and are shown in Figure 8.3. The maximum force and plateau force of the raw and the two-stage (55°C) were the same, but there was a large difference between the two in the two-stage (70°C). This was also reflected in the total energy required to cut through the chips with the two-stage (70°C) material requiring less energy to cut through it than two-stage (55°C), which was the same as raw tissues (Figure 8.4).

A greater force to initiate a crack in the two-stage (70°C) samples than to cut through it indicates that it was a crack sensitive material (Vincent *et al.*, 1990). The fact that less energy was required to cut through the two-stage (70°C) than the two-stage (55°C) is in agreement with the results found in chapter six. This finding would suggest that at the higher pre-cook temperature, turgor loss has occurred which allowed the tissue slice to deform more before the crack is initiated, hence the large initial peak force. The overall reduced net energy in the two-stage (70°C) cooked tissues suggests that the higher pre-cook temperatures, partially cooks the slices, making them softer. This was observed previously in chapters three and six when slices were two-stage cooked in the laboratory

8.2.1.2. One-stage and three-stage cooked potatoes.

After potatoes were one-stage or three-stage cooked, they were cooled to room temperature and stored overnight at 4°C. As with raw and two-stage cooked samples, they were allowed to equilibrate to room temperature and measured in the same way as two-stage cooked tissues. To be able to compare the texture of laboratory cooked with pilot plant cooked tissues, the latter needed to be cooked fully. To ensure this, potatoes were one-stage cooked for fifteen and twenty minutes. The texture of the potatoes was assessed by organoleptic testing of the texture of the cooked potato by three pilot plant workers. The twenty minute cooked material was evaluated as fully cooked while the fifteen-minute cooked material was described as hard and undercooked. It was therefore decided to steam cook potatoes for twenty minutes.

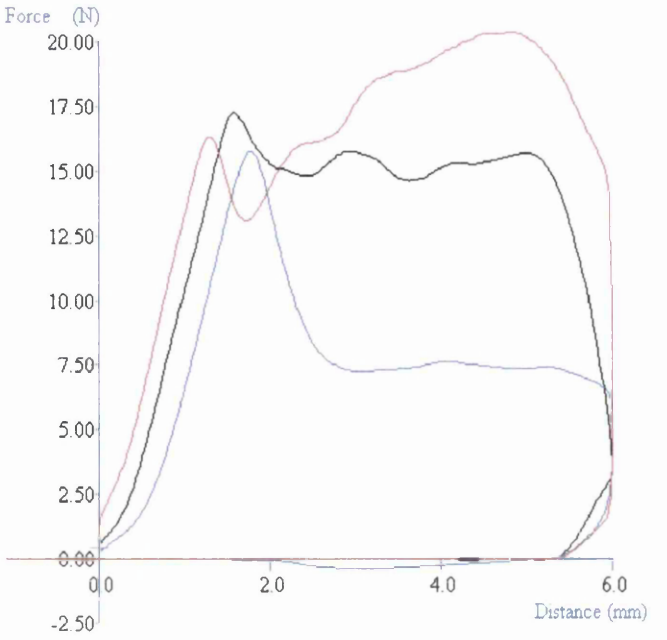


Figure 8.2. The force displacement curves of raw, two-stage (55°C) and two-stage (70°C) when cut with a wedge shaped cutter. The red curve is the raw tissue, the black curve is the two-stage (55°C) and the blue curve is the two-stage (70°C). The average peak force of each curve and the mean force after the initial peak were recorded. The area under the graph was recorded giving a value for the total energy to cut through the chips.

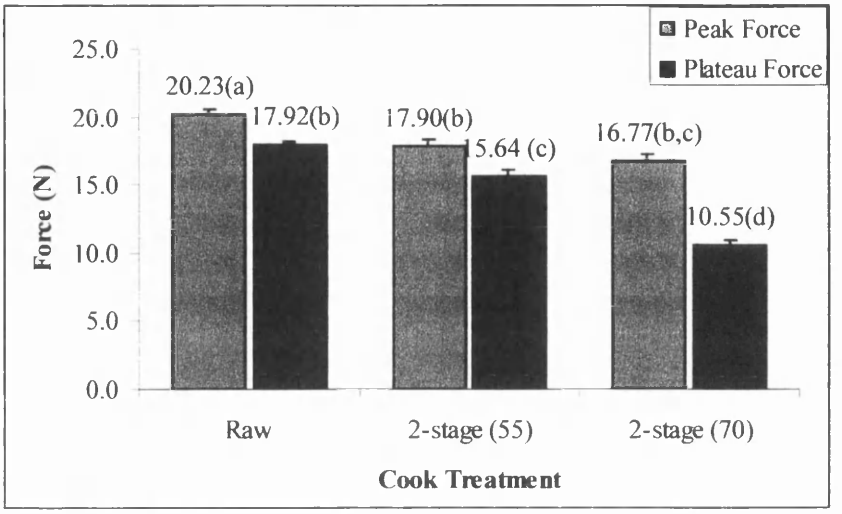


Figure 8.3. The maximum force and average plateau force recorded whilst cutting through raw and two-stage (55°C) and two-stage (70°C) cooked potatoes. Values are in Newtons; bars on columns are standard error of the mean where n = 63, 65 and 68 for both measurements. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

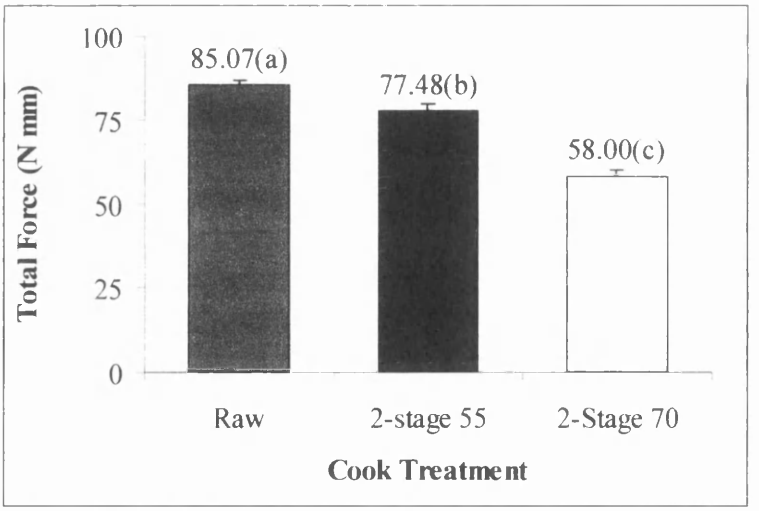


Figure 8.4. The total fracture energy recorded whilst cutting through raw and two-stage (55°C) and two-stage (70°C) cooked potatoes. Values are in Newton millimetres; bars on columns are standard error of the mean where n =64, 67 and 62. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

The force distance curves of the one-stage and three-stage cooked tissues are shown in Figure 8.5. The undercooked one-stage cooked tissues were measured along with the other three treatments. The shape of the curves did not fit the requirements for it to be considered a wedge test measurement as described by Vincent *et al* (1990). A representative curve produced by a wedge test measurement would have an initial peak, which is the force to initiate a crack, then a drop in energy as the stored force in the material is released. The force then plateaus or starts to rise. This did not occur as the cooked material was too soft to initiate a crack (Vincent *et al.*, 1990). The conditions were more analogous to a compression test so the maximum force required to cut through 6mm the tissues for each treatment was recorded and is shown in Figure 8.6.

The undercooked one-stage cooked potatoes had a greater mechanical strength than twenty-minute one-stage cooked material. This was in agreement with the informal tasting experiment carried out in the pilot plant. The three-stage cooked material was much firmer than either the undercooked one-stage and one-stage cooked tissues.

Apart from greater forces being recorded for pilot plant cooked tissues, the same trends were observed with pilot plant cooking and laboratory cooking. The forces required to cut through the pilot plant cooked tissues were much greater than the laboratory cooked tissues. This was observed at all stages of cooking, including pre-cooked tissues. There are a number of possible reasons for this. The dimensions of the pilot plant cooked tissues (6 x 6 x 8mm) were different from the laboratory tissues (20 x 20 x 5mm). The cross sectional area of the material tested is of a major factor on the results (Vincent *et al.*, 1990). As the cross-sectional area was smaller in the in the pilot plant chips, a smaller force to cut them would have been expected. An increase in shear force in potatoes that had been stored at low temperatures over a number of hours Jankowski (1992). It is possible that the greater forces required to cut the chips was due to retrogradation of starch

The times between measurements were also very different. Laboratory measurements were made immediately after the tissues had cooled to room

temperature. The pilot plant tissues were cooled to room temperature, then stored overnight at 4°C and then brought to room temperature before being measured.

Another possibility is that unlike the laboratory method, potatoes were cooked in tap water. Water in the south of England has relatively high levels of calcium. As was seen in chapter six, a small rise of calcium in potatoes can affect mechanical strength of potatoes. Consequently the calcium levels of the pilot plant cooked tissues were measured. Magnesium levels are not elevated in tap water so it was also measured as a reference to compare the calcium levels (table 8.1).

Cooking Treatment	Calcium (μMol/g)	Magnesium (μMol/g)
Raw	2.01 (a)	8.75 (x)
one-stage	2.21 (a)	7.45 (y)
Three-stage (55°C)	2.77 (b)	5.69 (z)
Three-stage (70°C)	2.89 (b)	5.62 (z)

Table 8.1. Calcium and magnesium content in cv. Maris Piper potato chips from the pilot plant. Treatments labelled with the same letter are not significantly different based in a Tukey LSD ($p<0.05$).

The calcium content of the raw potatoes was higher than the levels found in chapter six for the same potatoes. The magnesium levels were the same as those found in the Maris Piper potatoes analysed in chapter six. This would be expected as the chips, after peeling and slicing, were immersed in tap water to prevent them drying out. The higher levels of calcium in the raw potatoes compared to chapter six could have been due to residual tap water on the surface of chips which froze onto the chips when they were stored in the freezer prior to acid digestion.

After one-stage cooking the levels of calcium in raw tissues were still high but the levels of magnesium level decreased. Three-stage cooking caused an increase in the levels of calcium in the tissues while magnesium levels again decreased. The increase and losses of ions observed was caused by the tissues being immersed in

water during the pre-cook and cooling steps. Magnesium was lost into the water and calcium from the water entered the potato chips. Therefore calcium was probably, in part, responsible for the increased mechanical strength of the pilot plant cooked potatoes tissues especially in the three-stage cooked tissues.

8.2.2. Microscopic Examination of Raw and Cooked Tissues from the Pilot Plant.

Changes in potato tissue morphology caused by one-stage, two-stage and three-stage cooking were examined using scanning electron microscopy (SEM), and cryostat light microscopy (c-LM) and Differential Interference Contrast microscopy (DIC). This work was done with the assistance of Drs. Sue Gedney and Helen White of the Microscopy Unit at United Biscuits (UK) Ltd, Group Research and Development, High Wycombe. After cooking, the potato chips were cooled and stored at <5°C. Two days after cooking, the structure of the chips were studied. The fracture planes were examined by SEM and the inner tissue structure was examined by c-LM and DIC microscopy techniques.

8.2.2.1. Raw and two-stage cooked tissues.

Fracturing raw and two-stage (55°C) cooked tissues caused the cells to rupture at the fracture plane exposing their inner structure. The crystalline starch grains within the cells were visible (Figure 8.7 and 8.8). The fracture planes of the two-stage (70°C) differed from the other two treatments (Figure 8.9). It consisted of both ruptured and separated cells. Within ruptured cells, it was observed that starch had gelatinised and swollen to fill the cells (Figure 8.9b). The starch did not hold completely the cellular water it had absorbed during gelatinisation as holes in the starch were visible where water had sublimed off during etching.

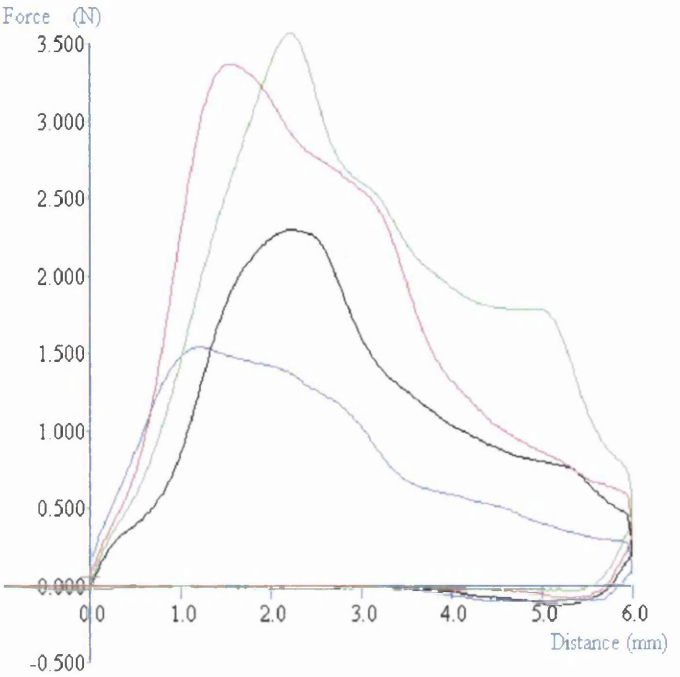


Figure 8.5. Typical force displacement curve of cooked tissues. The black curve is undercooked (15 minutes) one-stage cooked tissues, the blue is fully cooked (20 minutes) one-stage cooked potato, the red curve is three-stage (55°C) cooked and the green is three-stage (70°C) cooked potato. The peak forces were recorded and shown are in Figure 8.6

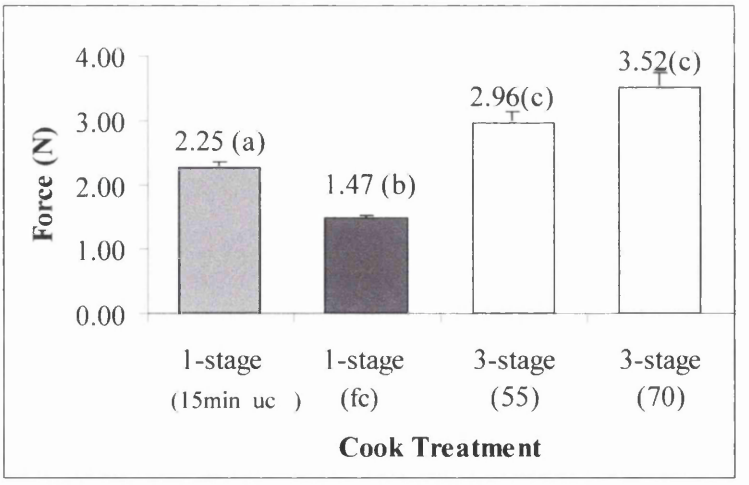


Figure 8.6. The maximum cutting force recorded whilst cutting through undercooked one-stage cooked potatoes (1-stage 15 min uc), one-stage full cook (fc), three-stage (55°C) and three-stage (70°C) cooked tissues. Values are in Newtons; bars on columns are standard error of the mean where n = 58, 40, 60 and 52. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

Examining the cellular structure of raw and two-stage (55°C) cooked tissues by c-LM was not possible. The tissues had a large amount of free water present, so during sectioning of the frozen material the structure ripped apart. Starch grains were visible (8.10a and 8.11a). To verify they had not gelatinised they were examined under cross-polarised light (8.10b and 8.11b). 'Maltese crosses', indicative of the crystalline structure of starch granules', were observed. Both the SEM and c-LM indicated that no gelatinisation had occurred during the two-stage (55°C) pre-cook treatment.

Two-stage (70°C) cooked tissues did section successfully (Figure 8.12) and staining with toludine blue was attempted but placing the sections in aqueous stains damaged the sections. Instead the tissues were stained with iodine vapour, which stained starch blue. Unfortunately this method left cell walls and intercellular spaces unstained. As was seen with the SEM images of two-stage (70°C) cooked samples, the gelatinised starch completely filled the cell structure. The cellular structure was still angular in shape and the cells were very tightly packed together (Figure 8.12).

In an attempt to observe the structure of the raw and the two-stage (55°C) hand sectioned specimens were observed with DIC microscopy. DIC microscopy is a technique that produces 3-D edges to enhance contrast under the microscope. (Lacey, 1989). As the sections were so thick, a number of different cells at different layers of the tissues were observed, but the cell outline and starch grains within were also visible (Figures 8.13 and 8.14). The two-stage (55°C) cells appeared bigger, which was probably due to the cut sections being thicker and therefore closer to the objective lens. Within the two-stage (70°C) cooked tissues the gelatinised starch had a rough structure and the outline of the cell wall was visible (Figure 8.15).

8.2.2.2. One-stage and three-stage cooked tissues.

The fracture planes of one-stage cooked material were covered in a gelatinous material (Figure 8.16). The cellular structure of the plane was only visible in small areas, but cells were intact after fracturing and appeared round in shape (Figure 8.16b). The appearance of the cells was very similar to high starch content potato varieties when steam-cooked (van Marle *et al.*, 1992) or baked (Wilson and Jarvis, unpublished results). This agrees with evidence put forward for starch swelling pressure having a role in cell separation of potatoes (Freeman *et al.*, 1992). The three-stage (55°C) potatoes were quite different from one-stage cooked potatoes (Figure 8.17). The cells were angular in appearance and no coating was observed. The three-stage (70°C) potatoes had a smooth surface but their shape was round and similar to one-stage cooked potatoes (Figure 8.18).

Compared to two-stage (70°C) cooked tissues viewed by c-LM in section 8.2.3.1, the cells of one-stage cooked potatoes appeared rounder in shape. The degree of 'roundness' differed between cells. It is conceivable that some cells rounded off at the expense of other cells (Figure 8.19). In general, cells were closely packed together but in some areas there were large spaces between the regions of iodine-stained blue regions of gelatinised starch. These were also quite abundant in three-stage (70°C) tissues but were not found in the three-stage (55°C) potatoes (Figure 8.20 and 8.21). These spaces could have been caused by starch shrinkage or cell wall swelling during cooking. As cell walls could not be stained, as aqueous dyes disrupted tissue structure, this could not be satisfactorily concluded. This matter is discussed in more detail, along with other microscopy results, in the following chapter.

As with two-stage (70°C) potatoes, gelatinised starch had trapped cellular water making cryostat sectioning possible. The sectioned potatoes were stained with iodine vapour and examined under conventional microscopy and by DIC microscopy (Figures 8.23–8.24). Sections observed by DIC microscopy confirmed the observations seen by conventional LM. The cells were rounded in shape and large gaps between cellular starch grains could be seen.

Figure 8.7. Fracture plane of raw cv. Maris Piper viewed by cryo-SEM. Cells had ruptured when fractured and cell walls were visible and within the cells, starch granules were seen.

Figure 8.8. Fracture planes of two-stage (55°C) cooked cv. Maris Piper viewed by cryo-SEM. Cells ruptured when fractured and starch had not gelatinised during the pre-cook.

Figure 8.9. Fracture planes of two-Stage (70°C) cooked cv. Maris Piper viewed by cryo-SEM. Cells both ruptured and separated when fractured. The pre-cook at 70°C had gelatinised starch which filled the cells.

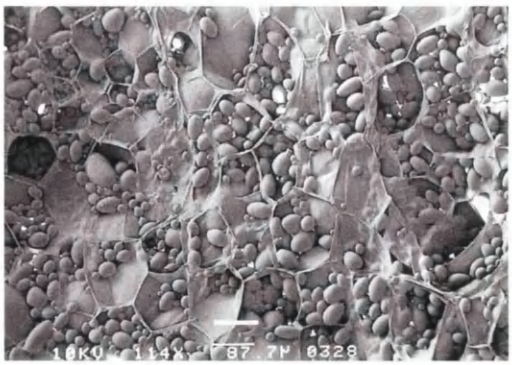


Figure 8.7a. (scale bar is 87.7μm)

Figure 8.7 b. (scale bar is 64.9μm)

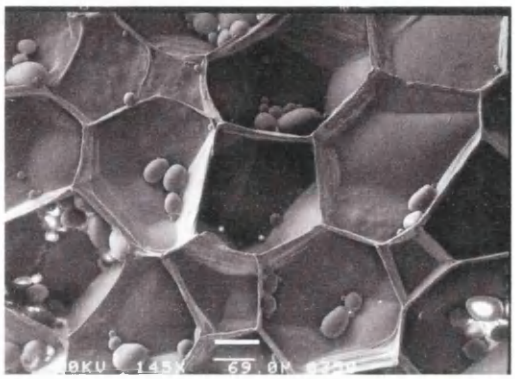


Figure 8.8.a. (scale bar is 69.0μm)

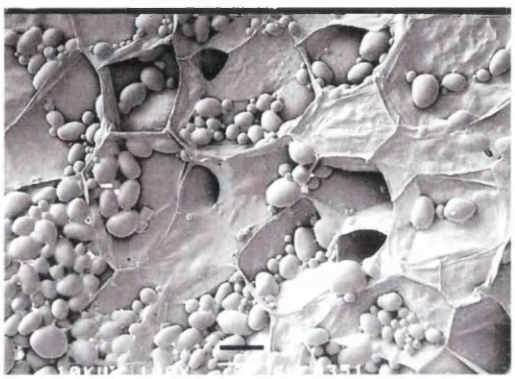


Figure 8.8 b. (scale bar is 82.6μm)

Figure 8.9.a. (scale bar is 87.0μm)

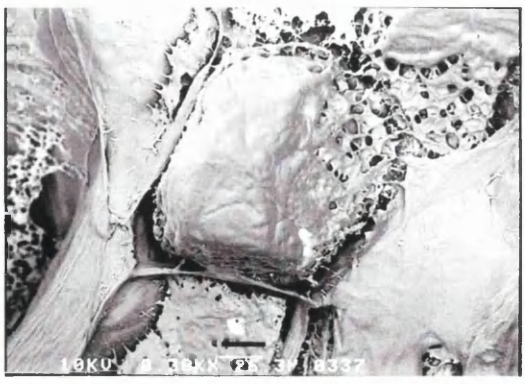


Figure 8.9. b. (scale bar is 26.3 μm)

Figure 8.10. Light microscopy sections of raw tissues. a.) As sectioning was not successful the tissue structure could not be discerned. Only crystalline starch granules were visible b). Viewed under cross-polarised light, 'Maltese crosses' were visible indicating that starch was in a crystalline state.

Figure 8.11. Light microscopy of two-stage (55°C) cooked tissues. a.) Like raw tissues, sectioning was not successful and the structure could not be discerned. All that was visible was crystalline starch granules. b). Viewed under cross-polarised light, Maltese crosses were still visible indicating that starch had not gelatinised during pre-cooking.

Figure 8.12. Light Microscopy of two-stage (70°C) cooked tissues. a). Sectioning was successful and tissue structure could be discerned after staining with iodine vapour. It was not obvious if the cells at the fracture plane were ruptured or intact. b). Cells within the tissues were full of starch but had kept their angular structure.

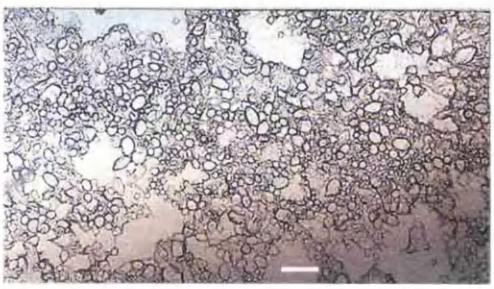


Figure 8.10. a. (scale bar is 126μm)

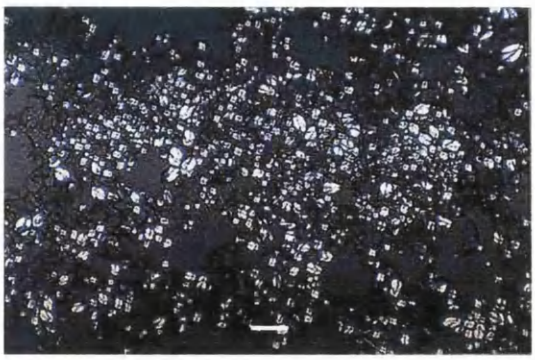


Figure 8.10 b. (scale bar is 126μm)

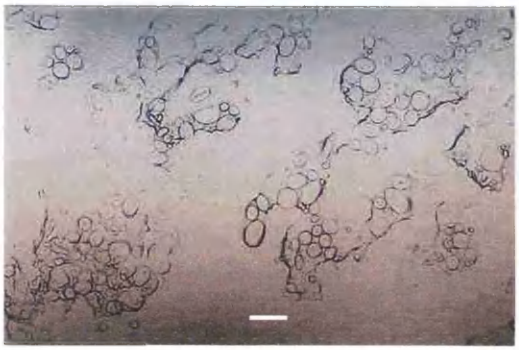


Figure 811. a. (scale bar is 126μm)

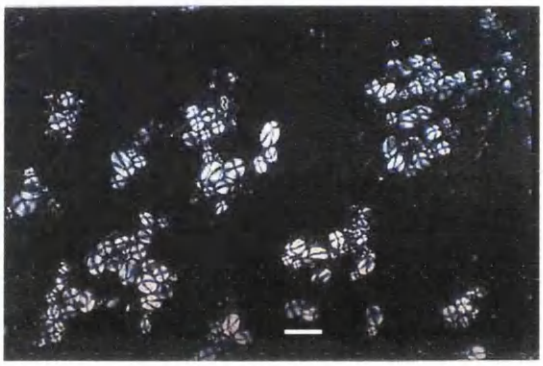


Figure 8.11.b (scale bar is 126μm)

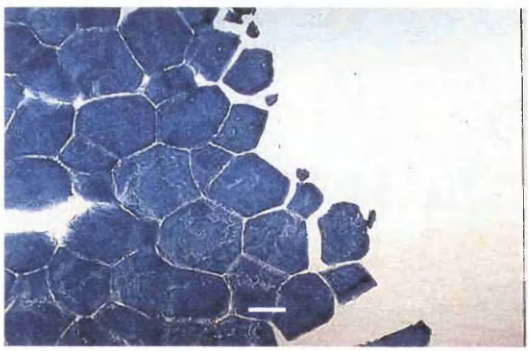


Figure 8.12. a. (scale bar is 126μm)

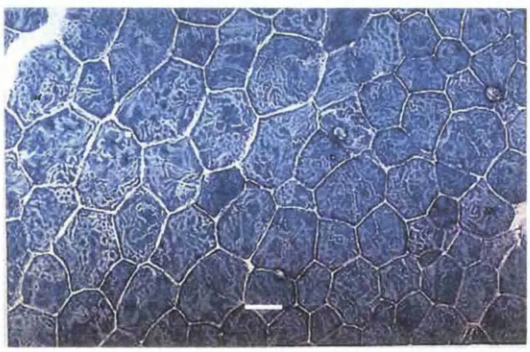


Figure 8.12.b. (scale bar is 126μm)

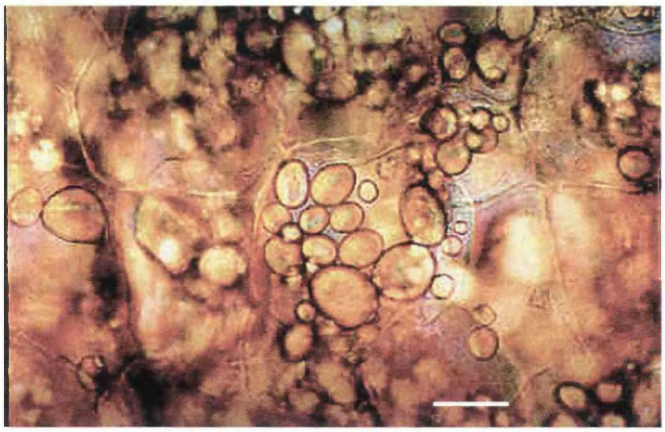


Figure 8.13. Raw cv.Maris Piper viewed under DIC microscopy. Sections were hand-cut as cryo-stat sectioning had proved to be impossible in raw specimens. Individual cells were visible, as were starch grains. (scale bar is 256μm)

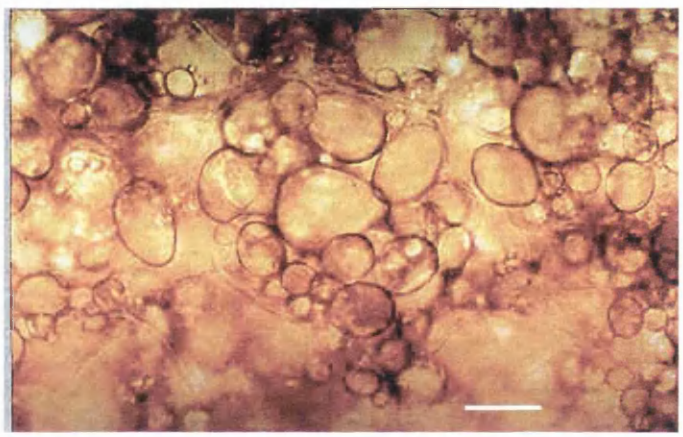


Figure 8.14. Two-Stage (55°C) cv. Maris Piper viewed under DIC microscopy. Like raw sections they were also hand sectioned. Individual cells were visible, as were starch grains. (scale bar is 256μm)

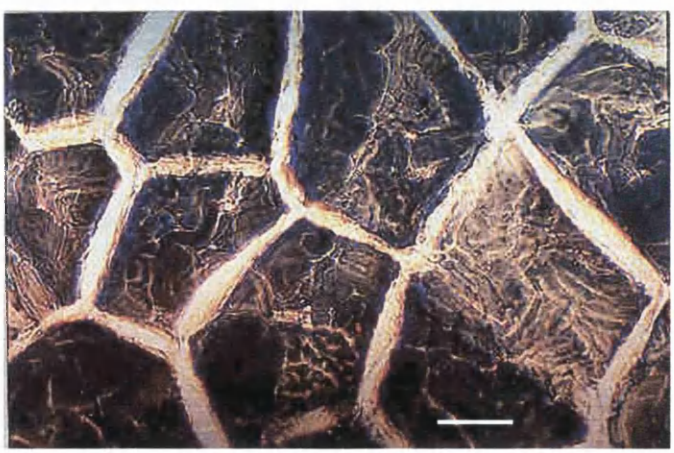


Figure 8.15 Two-stage (70°C) cv. Maris Piper stained with iodine vapour viewed under DIC microscopy. Cell walls could be distinguished and the compacted nature of the starch was also be seen. (scale bar is 256μm)

Figure 8.16. The fracture plane of one-stage cooked cv. Maris Piper viewed by cryo-SEM. a). In most parts of the fracture plane the tissues were covered with a gelatinous material, likely to be starch. b). Areas where the cells were uncovered indicated that the cells had separated intact and had lost their angular shape.

Figure 8.17. The fracture plane of three-stage (55°C) cooked cv. Maris Piper viewed by cryo-SEM. The cell structure was easy to make out as they were not covered in starch. The cells had remained intact when fractured, but the cells had a more angular appearance than one-stage cooked tissues. An arrow in Figure b shows a cell that had ruptured with gelatinous starch coming out of it.

Figure 8.18. The fracture plane of three-stage (70°C) cooked cv. Maris Piper viewed by SEM. As with three-stage (55°C) samples the specimens were not covered in starch. The cell shapes were a mixture of rounded (Figure a) and angular (Figure b).

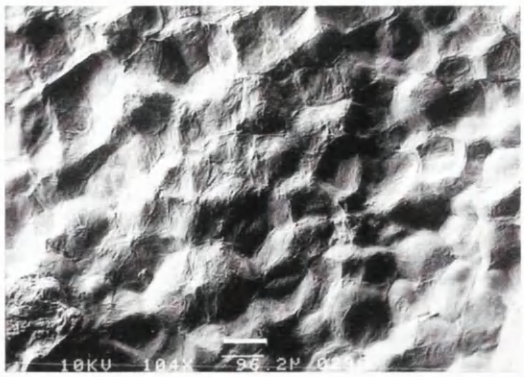


Figure 8.16 a. (scale bar is 96.2μm)

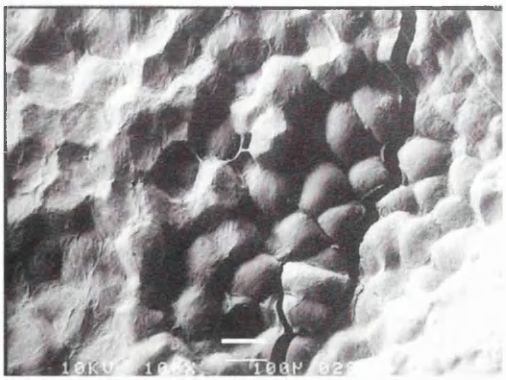


Figure 8.16 b. (scale bar is 100μm)

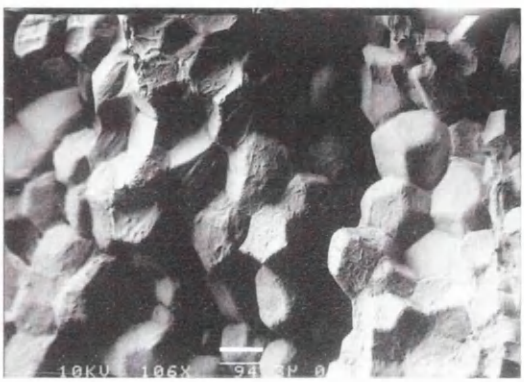


Figure 8.17.a (scale bar is 94.3.2μm).

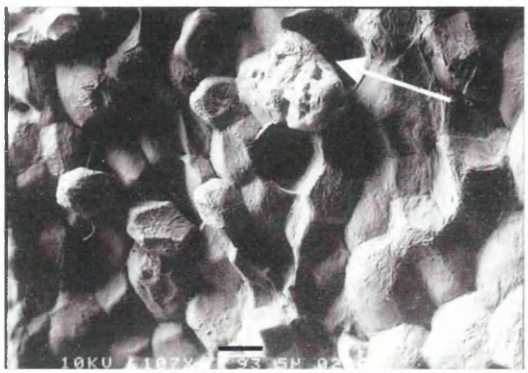


Figure 8.17.b. (scale bar is 93.0μm)

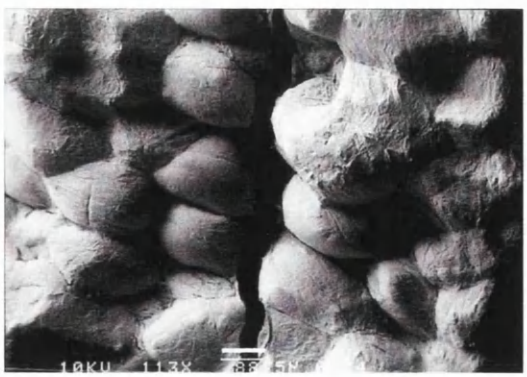


Figure 8.18.a. (scale bar is 88.5μm)

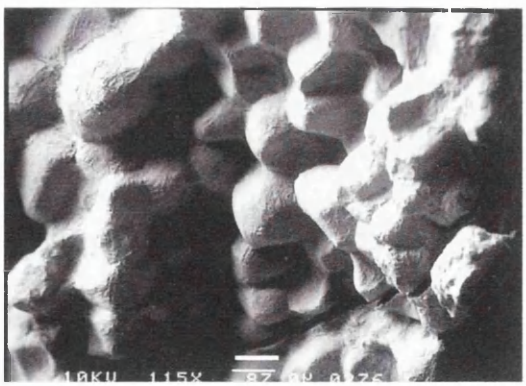


Figure 8.18.b (scale bar is 87.0μm)

Figure 8.19. Light microscopy sections of one-stage cooked cv. Maris Piper stained with iodine vapour. The cells had a more rounded shape than the two-stage (70°C) samples (Figure 8.7). There were areas present in the sections where starch completely filled the cells (Figure a) whereas in other areas, gaps between blue stained areas were visible (Figure b).

Figure 8.20. Light microscopy sections of three-stage (55°C) cooked cv. Maris Piper stained with iodine vapour. The blue stained starch filled the cells and there was very little space between cells. The shape of the cell was still quite angular.

Figure 8.21. Light microscopy section of three-stage (70°C) cooked tissues stained with iodine vapour. Large spaces between the starch and cell walls were present throughout the sections. It was not clear what had the caused this. The cell shape had rounded with cooking but not to the same extent as the one-stage cooked tissues.

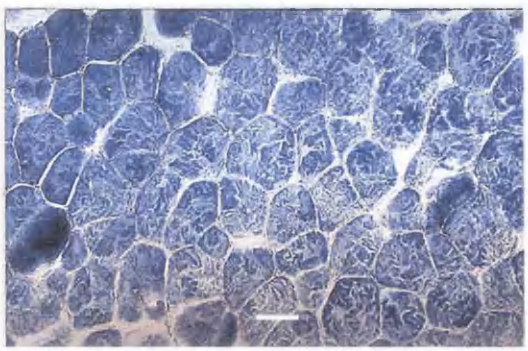


Figure 8.19.a (scale bar is 126μm)

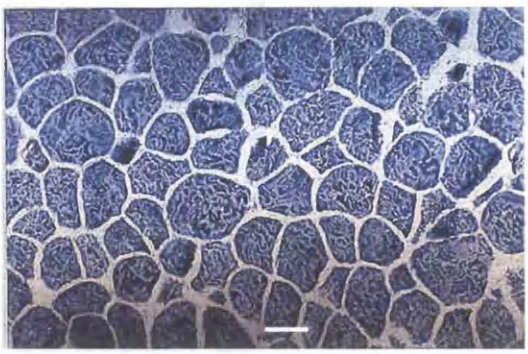


Figure 8.19.b. (scale bar is 126μm)

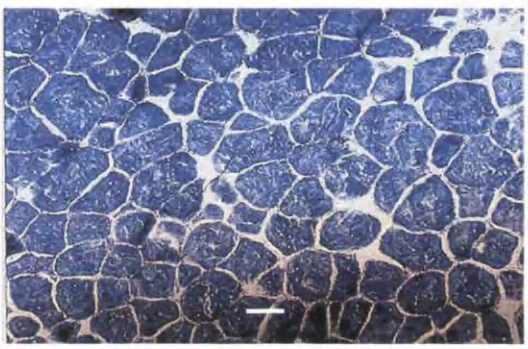


Figure 8.20 a. (scale bar is 126μm)

Figure 8.20. b. (scale bar is 126μm)

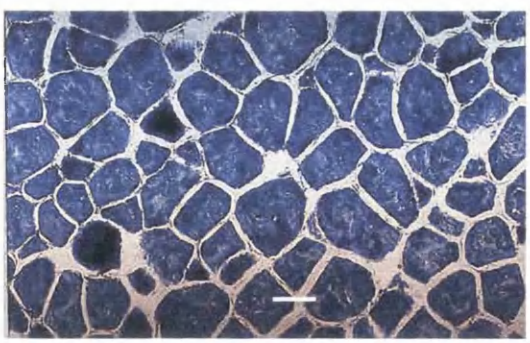


Figure 8.21. a. (scale bar is 126μm)

Figure 8.21.b. (scale bar is 126μm)

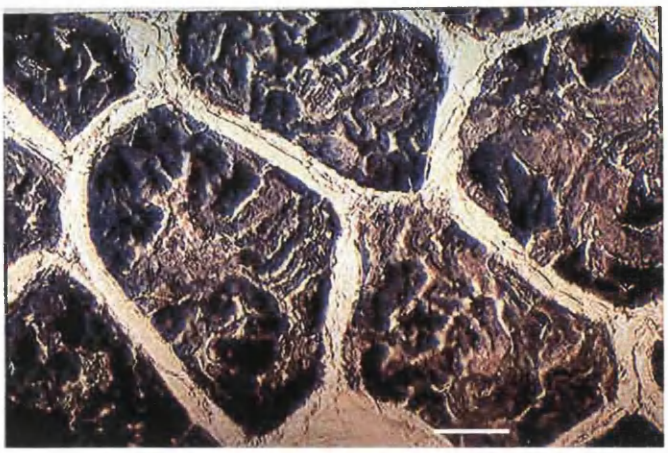


Figure 8.22. DIC microscopy image of one-stage cooked tissues stained with iodine vapour (scale bar is 256μm)

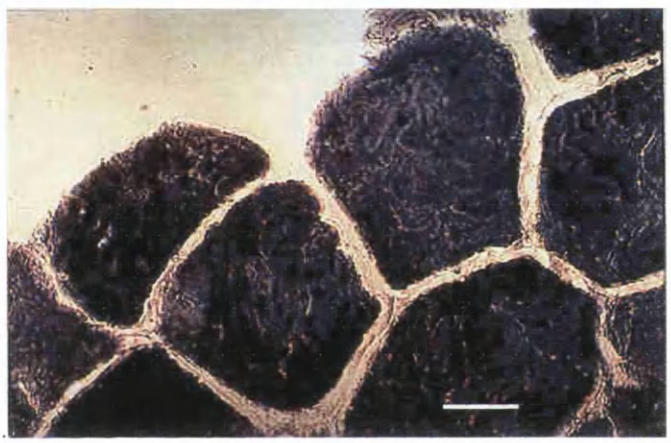


Figure 8.23 DIC image of three-stage (55°C) cooked tissues stained with iodine vapour. (scale bar is 256μm)

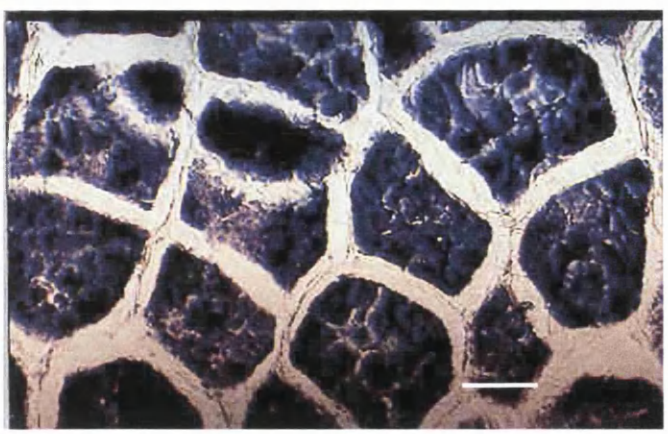


Figure 8.24 DIC image of three-stage (70°C) cooked tissues stained with iodine vapour. (scale bar is 256μm)

The gelatinous material on one-stage cooked fracture planes could have been one of two things, amylose leached out of cells or pectin released from the cell wall. Both three-stage cooked tissues fracture planes' were not coated with the gelatinous material. In three-stage (70°C) cooked material soluble amylose was likely to have decreased during the cooling step (Willard and Kluge, 1975, Jankowski, 1992) but this would not have occurred in three-stage (55°C) cooked samples. With the fact that c-LM pictures indicated no starch outside of cells it would seem that the gelatinous material was released pectin.

8.2.3. Texture of Mashed Potatoes Measured by Physical and Sensory Methods.

8.2.3.1. Physical texture.

The texture of the cold mash was assessed objectively by plunging an 8mm diameter cylinder through 10mm of mash. The typical force/distance curves produced are shown in Figure 8.25. There was an initial rapid increase in force as the cylinder came into contact with the mash. After it had entered the mash the force required to pass through the mash became roughly constant. A negative energy was encountered when the probe was brought out of the mash, which was due to the stickiness of the mash holding back the probe. The average force of the probe passing through the mash was recorded and the results are shown in Figure 8.26.

There was a small difference between undercooked and fully cooked one-stage cooked mash. The difference though was not significant. The mashes from both three-stage cooked potato chips were much firmer than the one-stage cooked potatoes but no significant difference was detected between the two. No significant difference was detected in the negative energy as the probe exited the mash. This trend of three-stage cooked material having a greater mechanical strength than one-stage materials was the same as for the cooked chips. However, the forces required to plunge through the mash were much greater than in cooked chips.

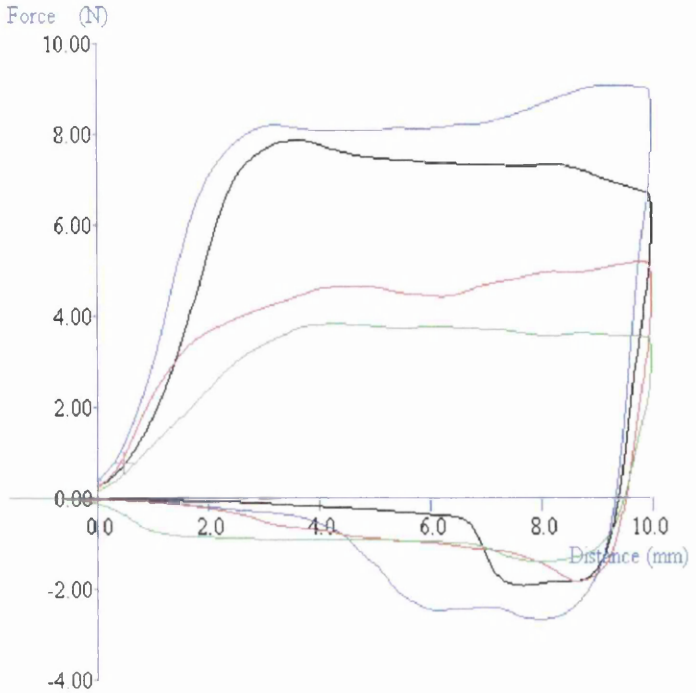


Figure 8.25. The typical shape of force distance curves of mashed potatoes produced from different cooking treatments. The red line is mash from undercooked one-stage cooked potatoes; green: fully cooked one-stage cooked potatoes, black: three-stage (55°C), and blue: three-stage (70°C) potatoes.

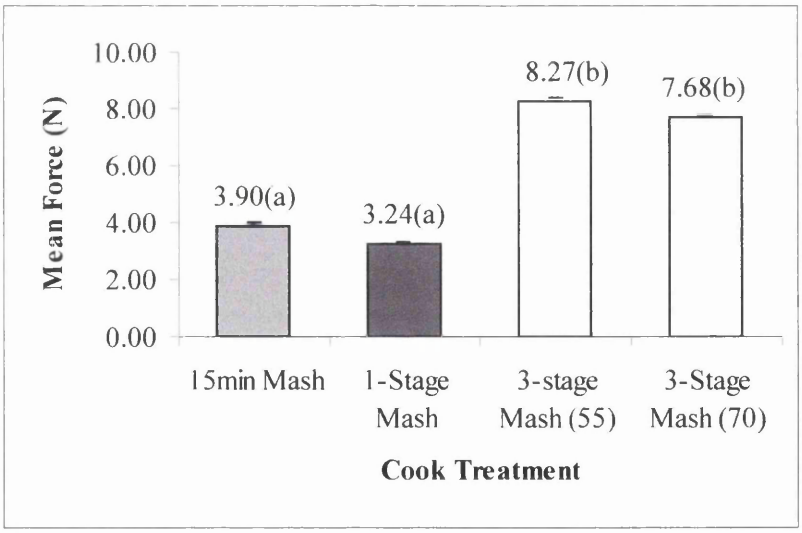


Figure 8.26. The mean plateau force of a plunger going through mash of undercooked one-stage (15 minutes) cooked, one-stage cooked, three-stage (55°C) and three-stage (70°C) cooked potatoes. Values are in Newtons; bars on columns are standard error of the mean where n = 38, 38, 55 and 60. Treatments labelled with the same letter are not significantly different based on a Tukey LSD ($p<0.05$).

8.2.3.2. Sensory evaluation of the texture.

Reheated mashed potato from each cooking treatment was given to a trained panel of part-time assessors at UB research and development. The samples were assessed for each of the parameters detailed in section 2.8.5. A report was compiled by the sensory department, the results of which are described below. Of the eleven parameters assessed, significant differences between treatments were found in only four and these parameters are reported below.

Results for each treatment were statistically compared and are shown in Figures (8.27 - 8.30). The results were also assessed for differences between batches over the two successive weeks the trial was carried out (results not shown).

Statistically significant differences were perceived in initial firmness (Figure 8.27). Three-stage (70°C) potatoes were softer than the other two mashed potato treatments.

Differences were also perceived in the assessment of the mash potato smoothness (Figure 8.28). Three-stage (70°C) mash was smoothest of the three, followed by one-stage cooked mashed potatoes while three-stage (55°C) mash was the assessed as the lumpiest. In terms of reproducibility between weeks, only three-stage (70°C) was found to be close in terms of smoothness. One-stage cooked mashed potatoes were perceived smoother in the first week, while three-stage (55°C) were perceived to be smoother in the second.

Three-stage (70°C) cooked potatoes were also perceived to be the most moist of the three and again one-stage cooked potatoes were perceived more favourably than three-stage (55°C) cooked mash (Figure 8.29).

Differences were also found in the consistency (uniformity) of the mashed potato texture. Three-Stage (70°C) received the highest score rating (Figure 8.30) and no differences between batches was perceived. The other two treatments received lower scores, three-stage (55°C) to a greater extent than one-stage cooked

potatoes (Figure 8.30). However, the second batch assessed, of both samples, received a higher score rating.

These results are in stark contrast with every other texture result of one-stage and three-stage cooked potatoes. Previous physical texture measurements in the thesis, on both cooked and mashed potatoes, showed one-stage cooked material to have a smaller mechanical strength than three-stage cooked potatoes with no difference between the two types of three-stage cooking methods. This was true of both laboratory and pilot plant cooked potatoes. An obvious difference between the sensory results and physical texture measurements was that the mash given to the taste panel was, for hygienic reasons, served hot. Physical texture measurements were made under conditions that were necessarily cold. Starch has different properties when hot and when cold, which probably resulted in the sensory tests and physical tests of mash's texture measuring different parameters.

The question remains - why should hot three-stage (70°C) mash be softer, smoother and more homogenous than the other two mashes? The SEM micrographs showed that the fracture planes of the three-stage (70°C) cooked tissues were not coated in gelatinous material (Figure 8.18), unlike the one-stage and three-stage (55°C) samples (Figures 8.16 and 8.17). The likelihood is that this was trapped pectin released from the cell wall during cooking. When the potatoes were mashed the extracellular starch would have aggregated cells together, making them lumpy and hard. It also appeared that the starch from the three-stage (70°C) cooked tissues had not fully filled the potatoes. This would have meant that there was more free water in the potatoes than the other two comparisons. When the potatoes were mashed, water released from cells could act as a lubricator. This would allow cells to slip over one another, producing a preferable mash.

The physical texture measurements described in this chapter followed the same trends as in earlier chapters even though absolute values were different. This shows that the results obtained with the cooking methods used in the laboratory were applicable to a pilot plant cooking scale.

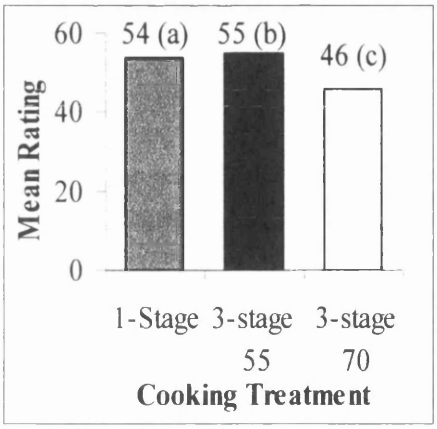


Figure 8.27. Initial firmness

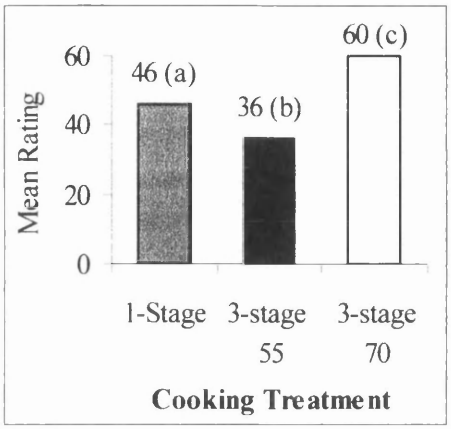


Figure 8.28. Smoothness.

Figure 8.27. The perceived initial firmness of the three mashes. Mashes were scored out of 100 where a score of 0 was classed as soft and 100 as firm.

Figure 8.28. The perceived smoothness of the three mashes. Mashes were scored out of 100 where a score of 0 was classed as lumpy and 100 as smooth.

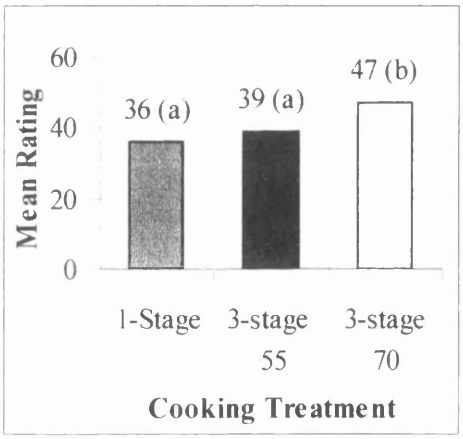


Figure 8.29. Moistness.

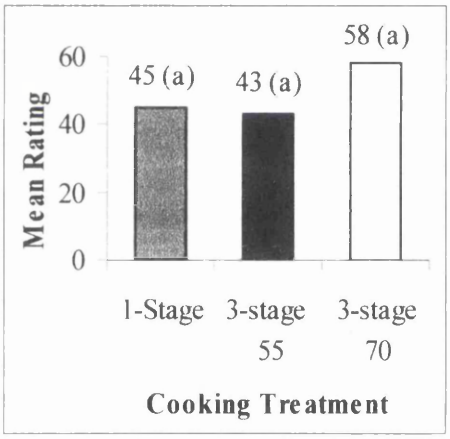


Figure 8.30. Consistency.

Figure 8.29. The perceived moistness of the three mashes. Mashes were scored out of 100 where a score of 0 was classed as dry and 100 as moist.

Figure 8.30. The perceived consistency of texture of the three mashes. Mashes were scored out of 100 where a score of 0 was classed as variable and 100 as consistent

CHAPTER NINE

GENERAL DISCUSSION AND CONCLUSIONS

9.1 Summary of Cooking Methods Used.

Steam cooking of potatoes with or without a pre-cook was used throughout this thesis. The abbreviations used and the relevant type of cooking this entailed are listed in table 9.1. Pre-cooking at 55°C was intended to incubate potatoes at temperatures where PME activity was at its maximum (Puri *et al.*, 1982, Tijskens *et al.*, 1997). The 70°C pre-cook was intended to gelatinise starch and activate PME. The temperature in the 70°C pre-cook initially fell to below 60°C, where PME is more stable then steadily rose to temperatures where starch gelatinisation was complete and PME would be denatured (chapter three).

The duration of one-stage cooking was based on the time required to produce tissues that were softer than undercooked slices. Three-stage (70°C) cooking times were based on the time required for the pre-cook to produce potato slices with firmer tissues. For three-stage (55°C) cooked samples a greater mechanical strength and a reduction in cell separation was required (chapter three).

Cooking Term	Cooking method
Raw	No cooking
Two-stage (55°C)	Pre-cooked for forty minutes in 300ml of deionised water, then cooled in 3l of deionised water for twenty minutes.
Two-stage (70°C)	Pre-cooked for fifteen minutes in 300ml of deionised water, then cooled in 3l of deionised water for twenty minutes
One-Stage	Steam cooked for twenty minutes
Three-stage (55°C)	Steam cooked for twenty minutes after a two-stage (55°C) pre-cook and cool procedure.
Three-stage (70°C)	Steam cooked for twenty minutes after a two-stage (70°C) pre-cook and cool procedure.

Table 9.1. Summary of cooking terms.

9.2. Changes to the Cell Wall and Starch in Potatoes during One-Stage, Two-Stage and Three-Stage Cooking

Changes within the cell wall and intracellular starch affect the texture of the cooked potato. Changes during one-stage, two-stage and three-stage cooking are reviewed below.

One-stage cooking resulted in large amounts of pectin being released from the cell wall during cell wall isolation. Part of this was due to cell wall losses during the isolation procedure. Starch had to be gelatinised by boiling the material for a short period of time to aid its removal by α-amylase and pullulanase and significant amounts of pectin were released into the enzyme 'liquor'. The majority of pectin released during cell wall isolation procedures was in the initial washing fractions (chapter four). The cause of these losses was likely to be heat degradative process, such as β–elimination or chelation of calcium from

unesterified pectin, weakening pectic linkages and allowing pectin to move into the MCB buffer when the cell walls were isolated.

The pectic galacturonan remaining in one-stage-cooked cell walls was less methylated than raw potatoes (chapter four). The actual degree of methylation of solubilised pectins could not be measured directly due to contamination by impurities in the toluene used to prevent microbial degradation of samples (chapters four and seven). The CDTA soluble fraction of steam cooked cv. Bintje potatoes was more methylated than the starting cell wall material, which could suggest that cooking releases highly methylated pectic material (Ng and Waldron, 1997a).

In this thesis, it was deduced indirectly that steam cooking solubilised heavily methylated pectin. Starch gelatinisation in steam cooked slices occurred within two and a half minutes of steam cooking commencing (chapter three). The starch gelatinisation point of potatoes is between 58-64°C (Lamberg and Olsson, 1989) and PME activity rapidly declines in potatoes above 60°C (Tijskens *et al.*, 1997, Puri *et al.*, 1981). It can be assumed, then, that little demethylation could have occurred due to PME activity during steam cooking. As the degree of methylation of the residual cell wall pectin was reduced, the pectin released during steam cooking must have been highly methylated. Low esterified pectin in raw Maris Piper potato cell walls was located mainly in the middle lamella by Jim 5 labelling (chapter seven). As cooking released highly methylated pectin during cell wall isolation, this pectin must have been released from throughout the cell wall and not just the middle lamella.

Although pectin cannot escape from within potatoes after steam cooking it was readily released during cell wall isolation processes. The ease with which it was released meant that the number of linkages to the wall (either ionic or covalent) was lessened. This would suggest that this pectin fraction would have no role in cell adhesion. This pectin fraction could be referred to as 'free pectin' rather than soluble pectin, as it was trapped within steam-cooked tissues and only released when tissues were disrupted during cell wall isolation with mixed cation buffer.

A gelatinous material was observed by SEM, coating cells of fracture planes of one-stage cooked tissues (chapter eight). This might have been 'free pectin' released from the cell wall but trapped in tissues. Alternatively, pectic material may have stayed within the wall and the material observed on the fracture planes may have been amylose that had leached through the cell wall.

As pectins control porosity of the cell wall (Baron-Epel *et al.*, 1988 and Shomer *et al.*, 1992), weakening the pectin matrix might be expected to increase the porosity of the wall. This could allow the diffusion of amylose, a relatively small polymer of approximately 4000 glucose units (Morris *et al.*, 1985) into the middle lamella. No coating was observed in either three-stage 55°C or 70°C cooked tissues when viewed by SEM. The probable aggregation of amylose chains during the cooling step might have been prevented amylose from diffusing through the walls of three-stage (70°C) cooked potatoes, but this would not have affected amylose diffusion in three-stage (55°C) cooked potatoes as starch properties are unaffected during pre-cooking at 55°C. Additionally, no iodine-stained amylose was present outwith cells in any of the images when viewed by cryostat light microscopy (chapter eight). This would suggest that amylose remained within cells, the material coating one-stage cooked cells was 'free pectin' released from cell walls and that 'free pectin' was not released from the cell walls of the three-stage cooked tissues.

Varietal differences were observed in the extent of pectin solubilisation during one-stage cooking. Potatoes with a higher starch content released greater amounts of pectin during cell wall isolation (chapter four). This is not conclusive evidence that starch content was responsible for the release of pectin. Differences in the cell wall architecture could have been responsible for these differences. Correlations of pectin solubility and texture have been observed in cooked potatoes, both with varietal differences (van Marle *et al.*, 1997b) and with processing conditions (Ng and Waldron, 1997a). However this does not necessarily mean that differences in pectin chemistry are responsible. Previous

research has not found conclusive evidence that differences in pectic composition in raw potatoes regulates the texture when cooked.

Quinn and Shafer (1994) found no gross compositional differences between two cultivars with different textural properties except for differences in the neutral sugar composition. van Marle et al, (1997a) found differences in the neutral sugar content of the CDTA soluble fractions between a mealy and a firm type of potato. The differences were not large enough to prove convincingly that these were the cause of differences in texture of the two varieties or provide a general theory to explain broad texture types (van Marle, 1997). Jardine (1998) found no significant differences in pectin composition between two cultivars with the same specific gravity that had different texture when crisped.

After cell wall isolation, three-stage cooked walls had more residual pectin than one-stage cooked cell walls. Changes in the potato slices to cause a restriction of pectin solubilisation must have occurred during the pre-cook and cool steps. The two different pre-cooks used caused differences in cell separation in the cooked tissues (chapters three and six), which are discussed in more detail in section 9.4. Pre-cooking at 55°C was intended to cause maximal activation of PME (Puri *et al.*, 1981, Tijiskens *et al.*, 1997). During this pre-cook treatment, the temperatures were not high enough to gelatinise starch and the starch grains remained intact within the cells (chapters three and eight).

The cells isolated from two-stage (55°C) cooked walls did not lose significant amounts of pectin during isolation but the degree of pectin methylation had been approximately halved (chapter seven). The mechanical strength of the two-stage (55°C) was no different from that of raw tissues (chapter six) and the fracture planes viewed by SEM were also very similar to raw tissues. The cells had ruptured by fracturing and starch had not gelatinised (chapter eight). These changes to the cell wall prior to cooking resulted in a reduction in cell separation and an increase in mechanical strength in the cooked tissues. This illustrates that manipulating cell wall properties, without affecting starch properties, prior to cooking affects the texture of cooked potatoes.

The effect that pre-cooking at 55°C had on the properties of pectin of subsequently steam cooked potatoes was not evaluated in the thesis. The changes in pectin during the pre-cook were very similar to those observed by Ng and Waldron (1997a) in cv. Bintje potatoes heated at 50°C. When Bintje potatoes were then steam cooked pectin solubilisation was restricted. Ng and Waldron (1997a) also found that the pectin remaining in the walls of the pre-cooked and cooked material had a lower degree of methylation than walls from steam cooked tissues. It is likely that if changes in three-stage (55°C) cooked potatoes had been studied they would have been similar to those observed by Ng and Waldron (1997a) in Bintje potatoes cooked in similar conditions.

In the 70°C pre-cook, the temperatures in the initial stages were low enough to allow PME to cause demethylation. As temperatures increased, PME would be deactivated and starch gelatinisation became the dominant process (chapter three). The higher temperatures of the pre-cook may have weakened the cell pectic matrix allowing pectin solubilisation (chapter three and seven). A reduction in mechanical strength of the two-stage (70°C) cooked tissues was also observed (chapter three, four and seven). However, the reduction may have been more to do with a loss of turgor in potatoes than weakening of the cell wall (chapter eight).

The fracture planes of the two-stage (70°C) pre-cooked tissues consisted of both intact and ruptured walls. The cells were full of gelatinised starch, which was likely to have exerted a separation pressure on walls that were being weakened by relatively high temperatures (chapter eight). The pectin remaining in the wall after cell wall isolation had been demethylated, presumably by PME, but to a lesser extent than cell walls of potatoes two-stage (55°C) cooked (chapter seven).

Changes in cell wall pectin content between two-stage and three-stage cooking in the same cultivar were not evaluated. Indirect comparisons between different cultivars, though, can give an idea of what could occur. Pectin in raw Maris Piper cell walls comprised approximately 20% of the wall and was approximately 40% methylated. Two-stage (70°C) cooking for either fifteen minutes or twenty

minutes caused some solubilisation of pectin. After pre-cooking 15% of the cell wall was comprised of galacturonic acid, which was approximately 26% methylated (chapters three and seven). Steam-cooking tissues pre-cooked at 70°C for twenty minutes (three-stage 70°C cooking) resulted in a further decrease in galacturonic acid content to twelve percent of the wall (chapter three).

In chapter four, cv. Kerr's Pink, which had a similar specific gravity to Maris Piper, was one-stage and three-stage cooked. When cooked, Kerr's Pink had similar textural properties to Maris Piper. One-stage cooking of Kerr's Pink walls left approximately 8% of the wall consisting of galacturonic acid (chapter four). Three-stage (70°C) cooking resulted in approximately twelve percent of the wall consisting of galacturonic acid. Thus it can be assumed that, like three-stage (55°C) cooking or the Ng and Waldron (1997a) method, three-stage (70°C) cooking causes a restriction in pectin solubilisation. The fact that two different three-stage cooking methods resulted in different degrees of cell separation indicates that the wall alterations that occurred during the two different pre-cooks may not have been identical.

9.3. Does starch swelling contribute to cell separation processes?

Starch swelling pressure has been an area of controversy in term of potato texture for a considerable time (Hoff, 1972, Reeve, 1972). In the following section, cell separation during cooking and the effect of pre-cooking on cell separation is discussed. Before that, it has to be decided if starch swelling pressure is a factor in cell separation. The main argument against starch swelling pressure was that evidence for it was based on visual observations and no quantitative value for the pressures generated during gelatinisation had been measured (Hoff, 1972). Jarvis *et al.* (1992) measured the swelling pressure generated by gelatinising starch as being in the magnitude of 10^2KPa. This was similar to turgor pressure in

uncooked cells and therefore cell separation could only occur after the degradation of the middle lamella and cell wall during cooking.

Sweet potatoes (*Ipomoea batatas)* are similar to potatoes, in that they are a root vegetable with a high starch content. Watson and Jarvis (1995) illustrated that by activating β-amylase in sweet potatoes by cooking at 70°C, a breakdown of starch to maltose occurred. Cooking this way compared to higher temperatures, where the enzyme was quickly denatured, produced a different texture. Tissues cooked at 70°C were harder and less likely to separate than those cooked at 100°C. This was attributed to a reduction of the starch swelling pressure by enzymic breakdown of starch at 70°C resulting in greater cell adhesion. In a later paper (Binner *et al.*, 2000), the activation of PME at 70°C was ruled out as no change in the degree of methyl esterified pectin was observed.

Evidence for starch swelling pressure being a factor also comes from SEM examinations of potatoes with high or low starch contents. High starch content potatoes are much more rounded with less intercellular adhesions than low starch content varieties (van Marle et al., 1992, Wilson, W. and Jarvis, M.C., unpublished results). Starch content within a tuber varies with tissue type, the central pith region of potatoes having much less starch in its cells than the outer cortex (Fedec et al., 1977). Agblor and Scanlon (1998) examined potato tisssues from the outer cortex and the pith region of potatoes. The cortex of the cooked potato was softer and prone to greater cell separation than the inner pith region. When blanched at 70°C, then steam cooked, the tissues from each region had a similar texture, being firmer and exhibiting less cell separation than steam cooked tissues. Aglbor and Scanlon (1999) observed that when cooked, cells from the cells in the outer cortex were rounder compared to pith cells.

Stolle-Smits *et al.* (1998) examined the internal and external regions of green beans (*Phaseolus vulgaris.*). When examined by SEM, the outer regions of the cooked beans, which have greater starch content were round in shape with very little cellular contacts. Tissues in the internal regions were much flatter and greater cellular contacts. This would be in general agreement with cells with a

greater starch content enduring greater cell separation forces. The authors, though, observed differences in the electron density of the middle lamella and postulated that the differences in cell shape were due to differences in the chemistry of the respective middle lamellae.

However, when discussing cell separation in the following section, starch swelling forces will be considered as a factor influencing cell separation during cooking. Other evidence for starch gelatinisation having a role in cell separation was presented in this thesis (chapters three, four, six and eight) and will be discussed in more detail in the following section.

9.4. The Cause of Cell Separation during Cooking.

A question concerning potato texture is what is whether pectin solubilisation during cooking is a cause or an effect of cell separation? This question can be considered to be a 'chicken and egg' type scenario, as both occur during cooking and one could be the cause of the other. Starch swelling pressure could place a strain on pectic molecules, accelerating the weakening of these linkages by processes such as β-elimination, leading to solubilisation of pectin and hence allowing cell separation to occur. Alternatively, starch swelling pressure may only become a significant cell separation 'driving force' once cell adhesion linkages have been weakened as pectin was solubilised by β-elimination. Variability in the amount of cell separation after cooking, either between varieties (chapter four) or caused by processing conditions (chapter six), was observed. These differences could be due either to variation in starch swelling pressure or in pectic degradation during cooking. Discussed below are the merits of either weakening of cell linkages or starch swelling pressure, being the dominant factor in cell separation.

The volume of separated cells from cooked potato varieties with a higher starch content was greater than from lower starch content varieties (chapter four).

Despite losses during cell wall isolation, it was clear that potatoes with higher starch contents released higher amounts of pectic material. This does not necessarily mean that starch content is the dominant cause of cell separation, but a correlation between the two was observed (chapter four). Differences in cell wall chemistry and architecture therefore influence the extent of cell separation. PME activity removes methylester groups from the C-6 position of galacturonic acid units during cooking (Bartolome and Hoff, 1972). Removal of methyl ester groups prevents β-elimination, (Keijbets and Pilnik, 1974, Sajjaanantakul *et al.*, 1989) and increases the number of calcium-pectate cross links within the wall, increasing the cell wall resistance to cell separation processes (Bartolome and Hoff, 1972, Ng and Waldron, 1997a). These observations were made on cell walls as a whole and the location of the changes due to PME was not established. In chapter 7, using JIM5 and JIM7, de-esterification appeared to be restricted to the middle lamella during 55°C pre-cooking, which is consistent with the effects on cell separation.

The one-stage cooking results do not indicate whether starch swelling or degradation of the pectin matrix, is the dominant factor in cell separation. Both three-stage cooking methods restricted cell separation compared to one-stage cooked tissues (chapters three, four and six). Three-stage (70°C) cooking restricts cell separation by a combination of PME activity and starch gelatinisation and cooling.

Earlier literature about three-stage cooking reported that a drop in the Blue Value Index (BVI) levels was required during the cooling step to reduce cell separation (Willard and Kluge, 1975, Hadziyev and Steele, 1979). The BVI is a measurement of the level of soluble amylose that stains with iodine (Nonaka, 1981) and aggregation of amylose in the initial stage of retrogradation has been observed (Miles *et al.*, 1985 and Jankowski, 1992). Transgenic potatoes with low amylose contents had different texture to the wild type, (Omeron *et al.*, 1998).

Reducing the soluble amylose content in potatoes prior to cooking could be a cause of the reduction in cell separation observed during three-stage (70°C

cooking). The purpose of the cooling step in three-stage cooking was to allow the aggregation of amylose chains to occur. The omission of a cooling stage in tissues pre-cooked at 55°C resulted in no difference in the extent of cell separation. When it was omitted after the 70°C pre-cook cell separation was greater than that observed with the cooling step included. Therefore to reduce cell separation, cooling was required (chapter three), suggesting that controlling starch properties is a key factor in controlling cell separation.

Three-stage (70°C) cooking caused a decrease in pectin solubilisation and the pectin remaining in the cell wall was less methylated than that remaining in one-stage cooked cell walls. Relative to the galacturonic acid content remaining in the walls after one-stage cooking, in Golden Wonder solubilisation was restricted to a greater extent by three-stage cooking than in Kerr's Pink (chapter four). Yet, three-stage (70°C) cooking of Golden Wonder had no effect on cell separation while Kerr's Pink's the volume of separated cells was halved when three-stage cooked (chapter four). So in Golden Wonder's case, cell wall strengthening prior to cooking, by retention of pectin, had no effect on the extent of cell separation recorded.

In separate cooking trials, Golden Wonder had extreme textural properties most likely due to its high starch content. On boiling, where cells can absorb water from the cooking media, starch swelling pressures occur to a greater extent than steam cooking and Golden Wonder rapidly disintegrated into a sticky slurry of free cells (Wilson, W. and Jarvis, M.C., unpublished results). As an extreme example, Golden Wonder's starch swelling properties during one-stage and three-stage had a larger influence on cell separation than PME activity.

Distinguishing between the effects of starch swelling and cell wall weakening for cvs. Cara and Kerr's Pink could not easily be done. The texture of Cara was unaffected by three-stage cooking and no obvious differences were observed in the pectin remaining in the cell wall after cooking. This could possibly have been due to either the lower starch content within its cells producing lower swelling pressures; or a lack of PME activity during the pre-cook.

With Kerr's Pink the volume of separated cells was halved when three-stage cooked. It is unclear whether this was a result of PME activity demethylating pectin, making it less prone to heat degradation, or whether it was an aggregation of amylose during the cooling step sufficient to reduce starch swelling pressures during cooking.

Restriction of both starch gelatinisation and PME activity during pre-cooking can control cell separation. Ooraikul, (1978) froze potatoes after mashing which caused gelatinised starch to retrograde. Granules produced after freeze-thawing were less likely to rupture and release starch than conventional granules. Pre-cooking prior to freezing was detrimental to texture as particle aggregation was too great (Molidina *et al.*, 1978). PME acvtivation combined with starch retrogradation had resulted in excessive cell adhesion.

Removing starch swelling pressure in vegetables can also restrict cell separation. When sweet potatoes were cooked at 70°C, a heat stable α-amylase was activated which hydrolysed starch into maltose reducing starch swelling pressures and restricting cell separation. When the sweet-potatoes were cooked at higher temperatures, the α-amylase enzyme was denatured and greater cell separation was observed (Watson and Jarvis, 1995). Starch gelatinisation in potatoes during cooking was prevented from occurring in chapter three by cooking in alcohol. This restricted the amount of available water, which prevented gelatinisation. Depending on the alcohol concentration used, cell separation was restricted or stopped altogether when assessed by vortex induced cell separation (VICS). However, cooking had weakened cell adhesion and when starch was gelatinised the cells disintegrated when tested by VICS.

This would suggest that without starch gelatinisation acting as a driving force cell separation does not occur. Carrots have no starch within their cells, yet SEM images of fracture planes of carrot steam cooked for twenty minutes showed separated and intact cells (Ng and Waldron, 1997b). Cooking had weakened cell-cell adhesion in carrots sufficiently that the mechanical fracture force used

had separated the cells intact. The shape of the steam cooked carrot cells were very flat (Ng and Waldron, 1997b); unlike the fracture planes, viewed by SEM in chapter eight, of potato cells that had been steam cooked. As carrots have no starch within the walls this must mean that a starch swelling pressure is not a prerequisite for cell separation. However, carrots pre-cooked prior to cooking ruptured when fractured as PME had increased cell adhesion (Ng and Waldron, 1997b). As the images of three-stage cooked fracture planes in chapter eight were of intact cells it might be that a driving force is necessary to separate cell that have stronger cellular intercellular adhesive linkages brought about by PME activity.

The results in this thesis along with other results (Jarvis *et al.*, 1992, Watson and Jarvis, 1995, Ng and Waldron, 1997b, Aglbor and Scanlon, 1998, Binner *et al.*, 2000, Wilson, W. and Jarvis, M.C., unpublished results) suggest that controlling starch swelling as well as PME activation affects cell separation. For over twenty-five years, many researchers have discounted this theory. Future research on potato texture should now take this starch swelling pressure into consideration.

The original question in this section is whether starch swelling pressure is a cause of cell wall degradation, which leads to cell separation; or does starch swelling only become a significant cell separation factor once cell wall degradation has occurred? The results produced in this thesis, in conjunction with other workers results, showed that this question does not satisfactorily apply to potatoes.

In fact, the results tended to suggest that a balance between the two affect the extent of cell separation observed. Starch swelling is not vital for cell separation to occur (SEM micrographs of steam cooked carrots, (Ng and Waldron, 1997b) and one-stage cooked potatoes (chapter eight)). If cell adhesion is still relatively strong i.e. due to PME activation during a pre-cook, then for cell separation rather than rupture to occur, starch swelling pressure is necessary (compare SEM micrographs of pre-cooked, then steam cooked carrots (Ng and Waldron, 1997b to three-stage (55°C) or 70°C cooked potatoes (Chapter eight)).

When adhesion is strengthened by PME activity (three-stage (55°C) cooking) a reduction in cell separation was observed, as the cell walls are more able to withstand the swelling pressures and heat degradation processes. When adhesion is strengthened by PME activity, combined with a reduction in soluble amylose, caused by the cooling step, the result is a greater restriction of cell separation (three-stage (70°C) cooking).

9.4.1. The Role of Tricellular Junctions in Cell Separation

The architecture of the cell wall at the middle lamella and especially at the corners of air spaces and tricellular junctions are important as this is where cell separation is initiated (Jarvis, 1999, van Marle 1997). During cooking Chinese Water Chestnuts, despite starch gelatinisation occurring do not separate. Diferulic esters linkages within Chinese Water Chestnuts walls results in very strong cellular adhesion. Ultraviolet fluorescence under the microscope indicated that diferulic ester abundance were greatest in the regions where Chinese Water Chestnut cells met one another, i.e. the middle lamella and at tricellular junctions. This prevents starch swelling pressures or cell wall degradation from causing separation upon cooking (Waldron *et al.*, 1997).

The cell adhesion linkages within these regions of the wall have to withstand tensile forces, either turgor or starch swelling pressures, which are tending to change the shape of the cell to a sphere (Jarvis, 1999). In potato parenchyma tissues, calcium bound pectin was more abundant in the middle lamella and especially in the corners of intercellular airspaces and tricellular junctions (Bush and McCann, 1999). Increasing the calcium concentration in cooked potatoes results in a decrease in cell separation (Ng and Waldron, and chapter six). When potatoes were saturated in calcium by pre-cooking in calcium at 70°C, cell separation was almost completely retarded (chapter six).

More calcium linkages were located in the corners of air spaces and in tricellular junctions of Cara than Golden Wonder (chapter five). The fact that more calcium

was retained in Cara potatoes in these regions could have important implications in regards to the extent of cell separation observed after one-stage cooking. More calcium bound pectin in these regions would increase cell adhesion in the area where cell separation forces are at their greatest. This could be an important factor as to why less cell separation was observed.

Regions of low esters and calcium bound esterified pectin could not be distinguished satisfactorily in pre-cooked walls (chapter seven). The work was carried out on isolated cell walls in which metal levels from the MCB would have equilibrated throughout the walls. While JIM 5 mAbs were not recognised satisfactorily in the two-stage (70°C) or the Ca-two-stage (70°C) cooked cell walls for definite conclusions to be made. The corners of two-stage (55°C) cell walls though, appeared more highly methylated than the rest of the wall and than two-stage (70°C) cooked walls and their tri-cellular junctions. This would mean that pectic material within these regions of three-stage (55°C) cooked walls were less able to withstand cell separation pressures during cooking, resulting in more cell separation than the three-stage (70°C) tissues. This partly explains why there was less of a reduction in cell separation in three-stage (55°C) cooked samples (chapters three and six) than three-stage (70°C) cooked potatoes.

The chemistry of these regions may play an important role in the extent of cell separation indicating that starch might not be the overriding factor in terms of cell separation. These observations were made at the very end of the project. More analysis of the pectin chemistry in these regions and the extent of calcium bound to them compared to the rest of the cell wall could very well provide further understanding as to what causes cell separation to occur during cooking.

9.5. What Significance Do These Results Have to the Texture of Mashed Potatoes?

The objective in this thesis was to gain an understanding of the factors which control cell separation during the cooking stages of mash production. Knowledge

of these changes would be of benefit during potato processing and could also give a strong foundation for studies into the texture of potatoes after mashing. The data presented here indicate that slight alterations in the temperature of the pre-cook alters the texture of the cooked potato produced prior to mashing. It was clear that controlling starch properties as well as cell wall pectin levels affects the texture of cooked potatoes.

It might have been expected that a bigger decrease in cell separation would have lead to a poorer quality mash. When a force is applied to three-stage (70°C) cooked potatoes during mashing, cells might be more likely to rupture than separate. This would lead to a greater amount of starch being released from ruptured cells, resulting in a sticky, undesirable mash. When the quality of mashed potato produced was assessed in a sensory trial the results were unexpected. One-stage and three-stage (55°C) cooked mash being less preferable than there-stage (70°C) cooked mash (chapter eight). The control of cell separation in the three-stage (70°C) cooked samples was, in part, controlled by manipulating starch gelatinisation prior to cooking. This would suggest that, in terms of mash texture, controlling starch properties rather than cell separation is more important.

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