Characterization of changes in potato tissue during cooking in relation to texture development

Promotor : dr.ir. A.G.J. Voragen
hoogleraar in de levensmiddelenchemie

Co-promotoren: dr.ir. C. van Dijk
hoofd afdeling biochemische verwerking van voedselprodukten
ATO-DLO

dr.ing. K. Recourt
hoofd sectie voedselgenetica
ATO-DLO

Netty van Marle

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Stellingen

1. De conclusie van Shomer et al. dat het zwelgedrag van gedroogde, gekookte aardappelcellen afhankelijk is van de diëlektrische constante van de vloeistof waarin de cellen gesuspendeerd worden, is voorbarig, zolang de invloed van andere eigenschappen van de vloeistof (zoals oplossend vermogen) niet uitgesloten kunnen worden.
   - Shomer, I., R. Vasiliver and P. Lindner, 1995. *Carbohydrate Polymers*, 26, 55-59.

2. Als het zwelgedrag van aardappelcellen in diverse vloeistoffen bestudeerd wordt, omdat dit gedrag een rol kan spelen bij het bepalen van de textuur, is het logisch dat dit (ook) gedaan wordt in vloeistoffen die representatief zijn voor de celvloeistof in aardappelen met een verschillende textuur.
   - Shomer, I., R. Vasiliver and P. Lindner, 1995. *Carbohydrate Polymers*, 26, 55-59.

3. De hypothese van Liu dat de hittestabiliteit en oplosbaarheid van eiwitten van verschillende aardappelrassen zodanig verschillen dat de rol van eiwitten in textuurontwikkeling vergelijkbaar wordt met die in het "hard-to-cook" defect in "legume seeds", lijkt niet aannemelijk.
   - Liu, K., 1995. *Critical Reviews in Food Science and Nutrition*, 35, 263-298.

4. Het belang van een éénduidige definitie van een textuurdescriptor wordt door Baier en Pichert niet onderkend, gezien het feit dat zij "körnigkeit" gebruiken als een maat voor de grootteverdeling van zetmeelkorrels in niet-gekookt aardappelweefsel en als een omschrijving voor de aanwezigheid van losse cellen in gekookt aardappelweefsel.
   - Baier, E.D. and H. Pichert, 1988. *Potato Research*, 31, 335-342.

5. Het is noodzakelijk aardappelweefsel zowel (bio-)chemisch als fysisch te karakteriseren om verschillen in textuurontwikkeling tijdens het koken van aardappelen te kunnen verklaren.
   - dit proefschrift.

6. Zowel de stevigheid van de intercellulaire contacten (pektine) als de stevigheid van het cellulose-xyloglucaannetwerk zijn van belang bij het verklaren van textuurveranderingen tijdens het rijpen en verwerken van aardappelen, groente en fruit.
   - dit proefschrift.
   - J.-P. Vincken, 1996. Enzymic modification of cellulose-xyloglucan networks. Ph.D. thesis, Wageningen, Agricultural University.

7. De laatste jaren wordt er terecht meer aandacht geschonken aan die karakteristieken van verschillende aardappelrassen die van belang zijn bij de verwerking.
   - dit proefschrift.
   - *Allerhande*, 1996, 1, 27-29.

8. De uitdrukking "je bent maar een aardappel" zal een "aardappelonderzoeker" niet snel in de mond nemen.

9. Het afwijzen van asielzoekers, die vervolgens om technische of beleidsmatige reden niet uitgezet kunnen worden naar het land van herkomst, is onrechtvaardig en inhumaan.
   - Voor een humaan en rechtvaardig asielbeleid. Oecumenische bezinning, 1995, 9.
   - Asielzoekers, laat ze niet zitten. Vluchtelingenwerk, Stichting Pharos, 1996.

10. In Benin (West-Afrika) is snelheidsbeperking geen probleem dankzij de gaten in het wegdek. Ook in Nederland zou het verval van wegen als verkeersremmende maatregel kunnen worden overwogen in plaats van aanbrengen van drempels, "punaises", plantenbakken, etc.

11. Het risico bestaat dat na verloop van tijd in het Groene Hart het boezemgebied volledig ingenomen wordt door kamers.

12. Veel dier- en plantensoorten zijn voor hun overleving in de stad afhankelijk van natuurlijke begroeiing. Omdat deze in particuliere tuinen veelal ontbreekt, ligt hier een taak voor gemeenten om daarin te voorzien.

13. Het "studiehuis" in de "tweede fase" van het voortgezet onderwijs zou voor een deel van de leerlingen wel eens een "sterfhuis" en een "eindfase" kunnen betekenen.

14. Het feit dat in de nieuwe spelling de tussen-s naar believen mag worden toegevoegd danwel weggelaten, betekent dat ook de nieuwe spelling nog spelling(s)problemen kent.

15. Het verdient aanbeveling tempobeursstudenten een OV-jaarkaart te geven op de hogesnelheidslijn.

16. Voor het grootbrengen van een tweeling in combinatie met het schrijven van een proefschrift zijn flexibele werktijden noodzakelijk.

Stellingen behorend bij het proefschrift, getiteld "Characterization of changes in potato tissue during cooking in relation to texture development" door Netty van Marle

Wageningen, 5 februari 1997

Aan mijn ouders

Aan Roel, Renske en Arjan

Abstract

Texture of cooked potatoes is an important quality aspect. The diversity in texture types was sensory evaluated. Most of the differences between texture types could be explained by differences between mealy and non-mealy characteristics. Furthermore, cultivars with similar mealy/non-mealy characteristics could be discriminated on basis of firmness of cooked potato tissue.

Cryo-scanning electron microscopy showed differences in intercellular contact and appearance of cell walls between fracture planes of cooked tissue from mealy and non-mealy cooking potato cultivars. Therefore, further research was focused on structure and composition of cell walls from the mealy cooking cv. Irene and the non-mealy cooking cv. Nicola.

The degradation of middle lamellae during cooking was determined by recording the release of pectic material in cooking media. It was found that a given percentage release of pectic material results in more cell sloughing for cv. Irene than for cv. Nicola.

Firstly, the effect of differences in ionic conditions in cell walls and middle lamellae on the degradation of pectic material was studied by recording the transfer of calcium, potassium and citrate during cooking of potato tissue. The transfer rates of potassium and citrate for cv. Irene are lower than expected in comparison with cv. Nicola. Calcium showed a deviant behaviour, since 80% of the calium initially present remains in tissue during cooking.

Furthermore, composition and structure of the pectic polysaccharides in the cell walls and middle lamellae of both cultivars were studied. Although, isolated cell wall material has a comparable molar composition for both cultivars, different types of pectic polysaccharides are solubilized during cooking.

The structure of pectic polysaccharides was further elucidated using chemical fractionation and enzymic degradation. It is proposed that the pectin matrix in the primary cell wall of cv. Irene had a thicker and/or less porous structure than the matrix in the cell wall of cv. Nicola. Additionally, the primary cell wall of cv. Irene has a more dense and/or thicker cellulose-xyloglucan network than the primary cell wall of cv. Nicola.

Contents

Chapter 1

General introduction

Potatoes (*Solanum tuberosum* L.) are a major agricultural crop in The Netherlands. In 1993/1994 7,800,000 tons potatoes were produced. 3,400,000 tons were destined for consumption, either as table potatoes or as potato products. In the Netherlands about 1,400,000 tons potatoes are consumed each year (fresh and processed) (Landbouwcijfers, 1995).
To make potatoes suitable for consumption, a heat treatment is required. Blanching (Andersson, 1994; Califano and Calvelo, 1983; Kozempel et al., 1981; Lamberg and Hallström, 1986), cooking (Burton, 1989; Harada et al., 1985a; Harada et al., 1985b; Harada and Paulus, 1987; Kozempel, 1988; Verlinden et al., 1995) and frying are the most common processing steps.
The texture of potato tissue after heating is an important quality parameter. The choice of a potato cultivar by either processors or consumers is among other factors based on the textural properties of potato tissue after cooking. For instance, potatoes with a waxy texture are suitable for processing in potato salads. On the other hand, a more mealy texture is preferred for the production of mashed potatoes.

Potato texture

Sensory evaluation of potato texture

To describe the texture of cooked potatoes a large vocabulary of descriptors is given in literature. These descriptors are not always unambiguous, because the same descriptor can be defined or measured in different ways by different authors. As a result, sometimes correlations were found between different texture descriptors, but not always. Furthermore, correlations between texture descriptors and chemical, physical and morphological characteristics respectively are various.
A widely used classification system for cooked potato texture is given by the European Association of Potato Research (EAPR) (Böhler et al., 1986; Winiger and Ludwig, 1974). Four types of cooking behaviour (A, B, C, D respectively) are distinguished based on a sensory evaluation using the following descriptors:

- *mealiness*: the easiness of cooked potato tissue to disintegrate (either with a fork or during consumption) into clusters of cells, without fracturing cells.
- *consistency*: the resistance of cooked potato tissue to mashing/disintegration (either with a fork or during consumption).
- *sloughing*: the loosening of the outer layers of the cooked potato.
- *moistness*: the moistness/dryness of potato tissue (perceived during consumption).

- *structure*: the presence of granules and vascular bundles in cooked potato tissue (perceived during consumption).

Relation between texture descriptors and potato characteristics

Starting from the descriptors defined by the classification system of the EAPR, the following correlations between texture descriptors and potato characteristics were found in literature.

Mealiness. Crumbliness and dry appearance of cooked potato tissue were observed as manifestation of mealiness during sensory evaluation (Gray and Hughes, 1978; Leung et al., 1983; Ludwig, 1972; McComber et al., 1988). According to Woodman and Warren (1972) mealiness was primarily controlled by the solids content of potatoes. Also, a positive correlation between mealiness and specific gravity (s.g.) was found (Böhler et al., 1986; Freeman et al., 1992; Gray and Hughes, 1978; Linehan and Hughes, 1969a; McComber et al., 1988; Woodman and Warren, 1972). S.g. is commonly used as a measure for the variation in the amount of dry matter in potatoes (a sample of potatoes is dipped in a series of brine-baths with ascending s.g.). Linehan and Hughes (1969a) and McComber et al. (1988) reported a positive correlation between mealiness and starch content. McComber et al. (1988) studied the influence of starch on potato texture extensively. They concluded that the effects of amylose, amylopectin and the size of starch granules were not unambiguous and no correlation was found between texture and temperature of gelatinization, swelling pressure and viscosity of isolated potato starch. Another approach was used by Gray and Hughes (1978), who stated that the degree of crumbliness was the result of the amount of intercellular adhesion in potato tissue.

Consistency. The descriptors firmness, hardness and softness were also used to describe comparable characteristics of cooked potato tissue as consistency. Böhler et al. (1986) and Leung et al. (1983) found no correlation between mealiness and consistency of cooked potato tissue. Positive relations with s.g. and starch content respectively were reported (Gray and Hughes, 1978; Leung et al., 1983; Linehan and Hughes, 1969a). This can be explained by the fact that a higher starch content results in a increased viscosity of the gelatinized starch leading to a higher consistency of potato tissue (Warren and Woodman, 1974).

Sloughing. A weak correlation was found between mealiness and sloughing (Böhler et al., 1986). In general, s.g. and sloughing were positively correlated (Ludwig, 1972). Sloughing is determined by the amount of intercellular adhesion within cooked potato tissue (Woodman and Warren, 1972). Swelling of cell wall material (Warren and Woodman, 1974), amount of pectin release and the presence of ions (H^+, Ca^{2+}, Na^+) were mentioned to influence the amount of sloughing (Ludwig, 1972; Keijbets, 1974;

Woodman and Warren, 1972).
Moistness. Moistness was highly correlated with mealiness (Böhler et al, 1986) and was even used as a sensory descriptor for mealiness (Gray and Hughes, 1978).

Instrumental measurements of potato texture
A lot of efforts were made to measure the different sensory descriptors instrumentally. Mealiness was measured using a number of cutting, compression, puncture, tensile, stress, relaxation and extrusion measurements (Davis and Leung, 1987; Gray and Hughes, 1978; Jarvis and Duncan, 1992; McComber et al., 1988; Woodman and Warren, 1972). The simple but efficient CPW (cooked potato weight)-test was used to measure mealiness (Gray and Hughes, 1978) and sloughing (Ludwig, 1972). During this test, cubes of potato tissue are cooked and subsequently sieved under standard conditions and the weight of the cooked potato tissue on the sieve is recorded. In fact this test determines the amount of cell separation. Different variants on this test are described in literature (Freeman et al., 1992; Jarvis and Duncan, 1992). Instrumental measurements of consistency were exerted with a penetrometer, tenderometer (Harada and Paulus, 1986) and with an Instron using texture profile analysis (TPA) (Leung et al., 1983).
It appeared quite complicated to relate measured characteristics such as elasticity, viscosity and cohesiveness of potato tissue to certain potato constituents. Therefore, more insight in the composition and structure of potato tissue and potato cells and the changes taking place during cooking will provide information about processes which are important in determining texture.

Histology of the potato tuber

In a mature potato tuber different tissue types can be distinguished (Fig. 1.1), from which the histological development was clearly described by Reeve et al. (1969). The skin is a corky layer (periderm) (Burton, 1989). It contributes about 4% to the total fresh weight and contains 3% of the total solids of a whole potato tuber (Reeve et al., 1970). The cortex, adjoining the periderm, is a layer of parenchyma tissue (Burton, 1989). The average cell size of this tissue type is smaller than for the vascular storage parenchyma tissue and pith, although these cells contain the highest number of starch granules. The cortex accounts for about 35 to 50% of the total fresh weight and about 40 to 50% of the total solids of a whole potato tuber (Reeve et al., 1971). Beneath the cortex lies the vascular storage parenchyma at both sides of the vascular ring (Talburt et al., 1987). In this storage parenchyma numerous phloem groups are embedded

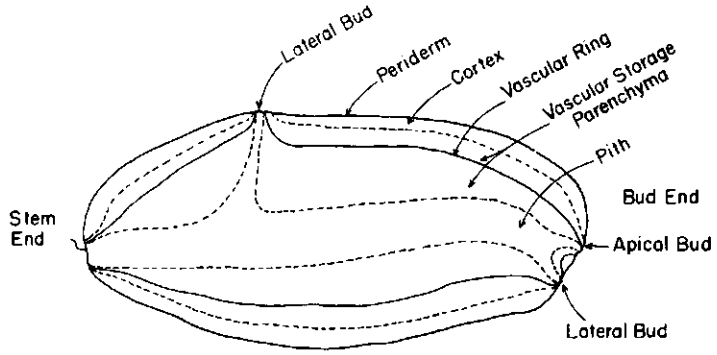

Fig. 1.1 Longitudinal section of a Russet Burbank potato showing different types of tissue (Talburt et al., 1987).

(Burton, 1989). Having the highest average cell size (Reeve et al., 1973), this tissue type takes up about 45 to 60% of both fresh weight and total solids of a whole potato tuber (Reeve et al., 1971). The storage parenchyma tissue inside the vascular ring (internal storage phloem parenchyma) was used for the experiments described in the Chapters 3, 4, 5 and 6. The pith forms the central core of the potato tuber with branches through the internal storage parenchyma tissue to the buds (Talburt et al., 1987). The cells of the pith contain the lowest number of starch granules. The pith accounts for 2 to 7% of total fresh weight and 1 to 4% of total solids of a whole potato (Reeve et al., 1970). The mentioned variation in percentages are due to differences between cultivars. Within a potato tuber there is also variation between stem end, middle and bud end (Reeve et al., 1970; Reeve et al., 1971).

Potato tissue

The cortex, storage parenchyma and pith are composed of roughly isodiametric cells (Burton, 1989). Since the cells are tightly packed, the intercellular spaces occupy only 0.3% to 0.9% of the tuber volume (Es and Hartmans, 1981). The cell size is dependent on tissue type, tuber size, cultivar and conditions during growth (Baier and Pichert, 1988; Fedec et al., 1977; Reeve et al., 1970; Reeve et al., 1971; Reeve et al., 1973).

During heating potato cells obtain a more round shape, which is favourable with respect to the amount of surface energy, because the ratio of surface to volume is at its minimum (Burton, 1989). The potential thermal expansion of cell contents during cooking may amount to about 4% of the cell volume (Burton, 1989). However,

Bartolomé and Hoff (1972), Bretzloff (1970) and Nonaka (1980) found no experimental evidence for an increase in cell volume. Jarvis et al. (1992) reported that upon cooking cylinders of potato tissue initially shrank due to a rapid loss of turgor, but on extended boiling a slight expansion of the tissue was observed as compared with the non-cooked tissue.

Non-cooked tissue fractures predominantly across cells (cell cleavage), but during cooking cell separation becomes more important (Andersson et al., 1994; Faulks, 1986). Cell cleavage results in cells which are broken open, whereas cell separation results in "intact" cells which are separated from each other. Jarvis and co-workers (Jarvis and Duncan, 1992; Jarvis et al., 1992; Freeman et al., 1992) analyzed the texture of cooked potatoes with respect to cell cleavage and cell separation and postulated that upon cooking cell wall softening facilitated cleavage of cells by teeth or knife. Subsequent release of the cell contents might be responsible for a moist texture. On the other hand, the middle lamellae were degraded resulting in cell separation. By measuring the fluid released from a fractured cylinder of potato tissue they found that cell cleavage diminished during cooking. Furthermore, a mealy cooking cultivar released less fluid after cooking than a non-mealy cooking cultivar.

Structure and function of plant cell walls

Polysaccharide composition and structure of plant cell walls

Much research has been performed to elucidate the plant cell wall structure. Chemical fractionation and analysis of cell wall carbohydrates provided information concerning sugar composition and presence of covalent and non-covalent linkages (Bacic et al., 1988; McNeil et al., 1984; Selvendran et al., 1985; Selvendran and O'Neill, 1987). More specific information about glycosyl linkages was obtained by methylation analysis (Ryden and Selvendran, 1990; Selvendran and O'Neill, 1987). Furthermore, specific enzymes were applied to unravel the structure of the different carbohydrates (Rouau and Thibault, 1984; Schols et al., 1990; Schols and Voragen, 1994; Schols et al., 1994; Tucker and Mitchell, 1993; Voragen et al., 1980; Voragen et al., 1993; Voragen and Schols, 1997; Vries et al., 1982).

Combining composition, linkage and structure data, several cell wall models have been proposed. In the early model of Keegstra et al. (1973) the cell wall is visualized as a network in which all components are interconnected by covalent bonds (except the non-covalent hydrogen binding between cellulose and xyloglucans). However, no experimental evidence was found for the postulated interpolysaccharide glycosidic bonds and therefore the co-existence of different networks within cell walls was proposed. Wilson and Fry (1986) described in their review the warp-weft model of

Lamport in which cellulose microfibrils and extensin (a glycoprotein rich in hydroxyproline) form a net-like structure suspended in a pectin-hemicellulose-gel. In their study on onion cell walls McCann et al. (1990) raised their objections to this model, because onion cell walls contained only 2% of hydroxyproline-poor protein, which was quite low with respect to the postulated central role of extensin in the cell wall. Similar objections can be made for potato cell walls, because Ryden and Selvendran (1990) reported that cell wall material from potato parenchyma also contained only 1.7% protein with a low content of hydroxyproline. In the model proposed by Talbott and Ray (1992) cellulose microfibrils are coated by a hemicellulose sheath composed of xyloglucan and free arabinogalactan polymers respectively. The interstices between the microfibrils are occupied by a pectin gel. The pectic and hemicellulosic components of the matrix are regarded as nearly separate phases. In the models of McCann and Roberts (1997) and Carpita and Gibeaut (1993) the cell walls are composed of a cellulose-xyloglucan network and a pectic polysaccharide network. A schematic representation of the cell wall of onion parenchyma tissue (McCann and Roberts, 1991) is given in Fig. 1.2. Cellulose and xyloglucan form a framework, whereby the cellulose microfibrils are interlocked by xyloglucans, which are able to bind tightly to the surface of the cellulose microfibrils and which are able to bridge the space between the microfibrils. This framework is embedded in a more or less independent pectin matrix.

Polysaccharide composition and structure of potato cell walls

Parenchyma cells from potato tissue are surrounded by primary walls (Bacic et al., 1988; Selvendran and O'Neill, 1987; Tucker and Mitchell, 1993). The main constituents of these walls are water (70%), cellulose (10%), pectic substances (12%), hemicelluloses (6%) and glycoproteins (2%) (Burton, 1989).

The composition and structure of the cellulose-xyloglucan network in potatoes was studied by Ryden and Selvendran (1990). The xyloglucans still present in the cell walls after extraction with Na_2CO_3 at 20°C, were extracted with KOH (0.5 M KOH, M KOH (1°C), M KOH (20°C), 4 M KOH, 4 M KOH + H_3BO_3 respectively). Two types of xyloglucans were detected that had different degrees of branching. The more easily extractable xyloglucans were more branched and probably less strongly associated with cellulose. Together with the xyloglucans some arabinose and galactose-rich pectic material was extracted. The KOH-fractions constituted 9.5% of the potato cell wall material. The remaining cell wall residue (49.2% of cell wall material) consisted mainly of cellulose and highly branched pectic material rich in arabinose and galactose.

The structure of the cellulose-xyloglucan network in potatoes was further elucidated using endoglucanases (Vincken, 1996). Xyloglucan from potatoes (and tomatoes) has

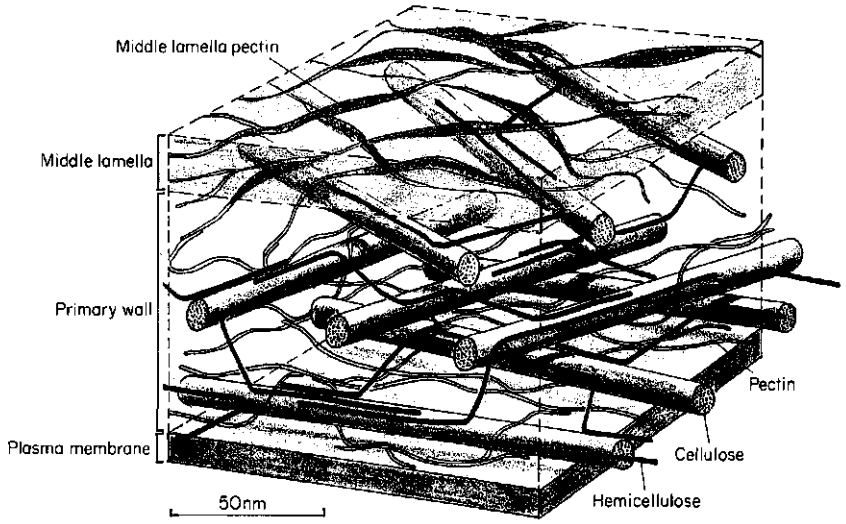

Fig. 1.2 Schematic representation of the cell wall of onion parenchyma cells (McCann and Roberts, 1991).

another branching pattern than the xyloglucans from other plants known. The potato xyloglucan is built up from repeating clusters of four ß-D-(1→4)-linked glucose residues, from which two adjacent residues are unsubstituted and two adjacent residues are substituted with α-D-(1→6) xylose (side chains).

Plant cell wall function

Primary cell walls define size and shape of cells during growth (McNeil et al., 1984) and provide cell and tissue strength (Carpita and Gibeaut, 1993). Several efforts have been made to determine the specific role of the different cell wall components with respect to the load-bearing, stress-resisting functions of the cell wall. One purpose for this type of research may be to elucidate the regulation of cell wall mechanical properties with respect to plant cell expansion during growth as reviewed by Taiz (1984).

However, knowledge concerning the physical function of the cell wall components is also useful to understand the changes in tissue and cell wall structure during processing of plant tissue and subsequent understanding of differences in texture development. Cellulose microfibrils, which have a high tensile strength, are one of the reinforcing elements of the cell wall (Bacic et al., 1988). Due to the orientation of the cellulose microfibrils and the presence of non-cellulosic polymers that interlock the

microfibrils, cell walls are capable to resist turgor pressure (Carpita and Gibeaut, 1993). The function of interlocking microfibrils is generally ascribed to the xyloglucans (Bacic et al., 1988; Cassab and Varner, 1988; McCann et al., 1990; McNeil et al., 1984; Vincken, 1996). This function was visualized when removal of xyloglucan polymers in onion cell walls led to association of cellulose microfibrils (McCann et al., 1990).

Carpita and Gibeaut (1993) calculated that turgor pressure inside a cell generates a considerable tension within the relative thin cell wall. Based on this principle, Carpita (1985) generated a high pressure within cells and was able to calculate the maximum feasible tension in cell walls from the measured breaking pressure of the walls. This technique was also used by Iraki et al. (1989) and Shedletsky et al. (1992), who studied the cell wall function of Dicotyledonae. Both concluded that the cellulose-xyloglucan network is the major load-bearing system in these cell walls.

The pectic polysaccharide network is thought to perform several functions. The presence of the gel-forming pectic polysaccharides (Bacic et al., 1988; Jarvis, 1984) limits the porosity of the cell wall (Bacic et al., 1988; McCann et al., 1990; Carpita and Gibeaut, 1993) and controls cell wall thickness (Jarvis, 1992; McCann and Roberts, 19975). Jarvis (1984) suggested that the pectin gel also has a load-bearing role, because a re-orientation of junction zones and interjunction segments was observed upon mechanical extension of collenchyma cell walls of celery. The middle lamella region is rich in relatively unesterified pectins (Bacic et al., 1988; McCann and Roberts, 1997; Cutsem and Messiaen, 1997; Talbott and Ray, 1992). These pectins play an important role in maintaining tissue integrity by connecting adjacent cells. Jarvis (1992) suggested that these middle lamella pectins must be anchored into the outer layers of the cell wall, which are formed by pectic polysaccharides.

Potato cell wall function

The function of the cellulose-xyloglucan and pectic polysaccharide networks in potato tissue was demonstrated by the work of Shomer and Levy (1988) and Shomer et al. (1993). Incubation of potato tissue with pectinase resulted in removal of the middle lamellae and exposure of the microfibrillar network of the cell walls. This treatment only slightly reduced the volume of the macerate, while incubation with cellulase resulted in a large reduction of the macerate volume. Similar observations were made for tomatoes (Shomer et al., 1984).

Cooking of potatoes led to solubilization of pectic material and swelling of the cell wall, eventually resulting into cell separation (Freeman et al., 1992; Hughes et al., 1975a; Hughes et al., 1975b; Jarvis and Duncan, 1992; Keijbets, 1974; Linehan and Hughes, 1969a; Linehan and Hughes, 1969b; Shomer, 1995; Shomer et al., 1995; Warren and

Woodman, 1974). Therefore, the composition and structure of the pectic polysaccharide network will be described in some more detail.

Composition and structure of pectic polysaccharides

Composition and structure of the pectic polysaccharide network in plant cell walls

The backbone of pectic polysaccharides consists of α-(1→4)-linked galacturonosyl units interrupted by α-L-(1→2)-linked rhamnosyl units. Long sequences of galacturonic acid residues are referred to as homogalacturonan or polygalacturonan. The backbone of rhamnogalacturonan I is composed of alternating rhamnose and galacturonic acid residues. Arabinans, galactans and highly branched arabinogalactans are present as side chains of rhamnogalacturonan I and are attached to the O-4 of the rhamnose residues. Approximately half of the rhamnose residues contain side chains (Bacic et al., 1988; Carpita and Gibeaut, 1993; McNeil et al., 1984). Another small group of pectic polysaccharides is referred to as rhamnogalacturonan II and can be distinguished by the presence of unusual linkages, such as 3 and 3,4-linked rhamnose residues and 2,4 and 3,4-linked galacturonic acid, and the presence of rare sugars like 2-O-methyl-fucose, 2-O-methyl-xylose, apiose, aceric acid, 2-keto-3-deoxy-D-manno-octulosonic acid and 3-deoxy-D-lyxo-2-heptulosaric acid (McNeil et al., 1984; Schols, 1995).

Since rhamnose can also occur as a single residue between long sequences of galacturonic acid (Jarvis, 1984), Schols (1995) proposed to use the general name rhamnogalacturonan for pectic polysaccharides with a rhamnose to galacturonic acid ratio varying between 0.05 and 1.

Beside the above mentioned classification for pectic polysaccharides, the subdivision in unbranched and branched blocks (Jarvis, 1984) or smooth and hairy regions (Vries et al., 1982; Thibault and Rinaudo, 1986) is also used. The unbranched blocks or smooth regions are composed of galacturonic acid residues and rhamnose residues may be absent or may interrupt sequences of 72-100 galacturonic acid residues (Voragen et al., 1995). The branched blocks or hairy regions, obtained after chemical or enzymic degradation of various fruits and vegetables, have a rhamnose to galacturonic acid ratio lower than 1 (Jarvis, 1984; Schols, 1995). Almost all neutral sugars and up to 10% of the galacturonic acid residues were suggested to be present in these regions (Schols, 1995; Vries et al., 1982).

Other relevant properties of pectic polysaccharides are the degree of methylation (DM) and the presence of acetyl groups. Part of the galacturonic acid residues have methyl esterified carboxyl groups. Acetyl groups are found as substituents of pectic

polysaccharides and xyloglucans. More than 30% of the galacturonic acid residues of rhamnogalacturonan are acetylated at the O-3 position. Whereas in xyloglucan the acetyl groups are bound to the galactose residues present in the side chains (Carpita and Gibeaut, 1993). However, Selvendran and O'Neill (1987) reported that the type of sugar residues which are acetylated and subsequently which carbon atom of this sugar is acetylated, is not well known.

Pectic polysaccharides are potential gelforming polymers (Bacic et al., 1988). Highly methyl esterified pectins (DM > 50%) form a gel under conditions of low water activity and low pH (Davis et al., 1980; Garnier et al., 1993; McCann et al., 1994). Interchain aggregations are stabilized by hydrogen bonding between undissociated carboxyl and secondary alcohol groups and by hydrophobic interactions between methoxyl groups (Axelos et al., 1994; McCann et al., 1994; Morris et al., 1980; Voragen et al., 1995). Low methyl esterified pectins, pectates, (DM < 50%) form gels in the presence of divalent cations, in particular calcium. The junction zones are composed of unbranched, nonesterified galacturonan chains linked together by non-covalently bonded calcium ions (Garnier et al., 1993; Jarvis, 1984; Powell et al., 1982). About fourteen neighbouring carboxyl groups are required to form stable dimers, the so-called egg-box-structure (Powell et al., 1982; Jarvis, 1984; Thibault and Rinaudo, 1986). However, recent studies with NMR and x-ray diffraction raised objections to the presence of these egg-box-structures in plant cell walls (Jarvis et al., 1997; Voragen et al., 1995). The interchain aggregations present in gels of highly methyl esterified pectins were also reported to be present in calcium-pectate-gels (Gidley et al., 1980). Both types of gel may be present in cell walls depending on the structure of the pectic polysaccharides and their location in the cell walls (Jarvis, 1984; McCann et al., 1994).

Potato pectic polysaccharides

Information about composition and structure of pectic polysaccharides in potato cell walls is obtained by chemical fractionation studies (Jarvis et al., 1981; Ryden and Selvendran, 1990) and enzymic degradation studies (Ishii, 1978; Ishii, 1981). A clear feature of potato pectic polysaccharides is the high galactose content varying between 22% and 29% of the cell wall material (on dry matter base) (Ishii, 1981; Jarvis et al., 1981; Ryden and Selvendran, 1990; Sasaki and Nagahashi, 1989).

Chemical fractionation

Calcium-chelating solvents were used to extract the pectic polysaccharides which are bound in the cell walls by calcium ions, probably originating from the middle lamella regions. Jarvis et al. (1981) extracted 19.2% of their cell wall material with hot oxalate-citrate-buffer and this fraction has a galacturonic acid to neutral sugar ratio of 3.4.

Ryden and Selvendran (1990) extracted 22.2% of their cell wall material with cyclohexane-trans-1,2-diaminetetra-acetate (CDTA), this material has a galacturonic acid to neutral sugar ratio of 1.9 and a DM of 47%. Na_2CO_3 was used to extract more branched pectic material (Ryden and Selvendran, 1990). Using this solvent at low temperatures brings about deesterification of pectic material with a minimal risk of ß-elimination (Selvendran et al., 1985). Jarvis et al. (1981) and Ryden and Selvendran (1990) extracted 35.7% and 14% cell wall material respectively under these conditions. In both cases the galacturonic acid to neutral sugar ratio was 0.6. Ryden and Selvendran (1990) solubilized an additional amount of 5% cell wall material, probably originating from the primary wall, using Na_2CO_3 at room temperature with a slightly lower ratio of 0.4, . This pectic material was thought to be associated with the xyloglucans (Selvendran et al., 1985). After removal of xyloglucan, Ryden and Selvendran (1990) found in the residual cell wall material a highly branched pectic polysaccharide next to cellulose.

Sasaki et al. (1987) and Sasaki and Nagashi (1989) reported that about 10% of potato cell wall material, composed of pectic polysaccharides, was released during incubation in water (20 hours, 35°C) in reaction to a brief exposure to EDTA during cell wall isolation. The released pectic polysaccharides contained 40% of the galacturonic acid from the initial cell wall material and were present as long chains. This material originated from the primary cell wall adjacent to the plasma membrane. The release was due to chelation of calcium ions.

Enzymic degradation

Ishii (1978; 1981) used purified pectolytic enzymes to solubilize the pectic polysaccharides from potato cell walls. He reported that most galacturonic acid residues are found to be present as homogalacturonan in potato cell walls. Furthermore, the sugar composition of material released after 10 minutes enzymic incubation (15% of uronic acid) and after exhaustive enzymic degradation (21 hours; 95% of uronic acid) were comparable. He concluded that it is quite difficult to distinguish between pectic substances from the middle lamella (first fraction) and primary wall (second fraction). Ishii (1981) also isolated a pectic fraction which was highly branched and contained 3 and 3,4-linked rhamnose residues and 3,4-linked galacturonic acid. Although no rare sugars were found, the structure resembled that of rhamnogalacturonan II.

Schols and Voragen (1994) and Schols et al. (1994) studied the modified hairy regions (MHR) from potato (0.04% f.w.). The structure of this MHR was elucidated studying the degradation of MHR with the enzyme rhamnogalacturonase beside analysis of the sugar composition and the linkage type composition. The main sugars of potato MHR

were rhamnose, arabinose, galactose and galacturonic acid, while xylose was almost absent. The rhamnose to galacturonic acid ratio (0.46) was high. Potato MHR had a low DM (13%) and a high DA (90%), assuming that the acetyl groups were only attached to galacturonic acid residues. Schols and Voragen (1994) assumed that only part of the MHR backbone was composed of alternating rhamnose and galacturonic acid residues, while these residues were present in other sequences in the other segments of the backbone. These results agreed with previous results found for apple MHR. Apple MHR contained at least three different repeating units: a xylogalacturonan, an arabinan-rich stub of the rhamnogalacturonan and units in which the RGase oligomers were dominantly present (Voragen et al., 1993). Although, xylose was almost absent in potato MHR, analogous subunits with various rhamnose to galacturonic acid ratios might be present (Schols and Voragen, 1994). Also Ryden and Selvendran (1990) reported the presence of an arabinose-rich polymer in addition to xyloglucan.

Effect of heating on potato pectic polysaccharides

Heating of potato tissue results in solubilization of pectic material. The elevated temperatures and a tissue pH which is higher than 5 result in degradation of the pectic backbone according to the ß-eliminative mechanism (Keijbets, 1974; Selvendran and O'Neill, 1987). Cleavage takes place next to a methyl esterified galacturonic acid residue. Furthermore, temperatures above 60°C increase the permeability of the cell membrane (Andersson et al., 1994; Bartolomé and Hoff, 1972) resulting in the transfer of ions from the cytoplasm into and/or through the cell wall. Keijbets and Pilnik (1974) reported that ß-elimination was stimulated by high amounts of cations and anions. The effect of ion transport on calcium-pectate will be discussed in the next section.

Interaction between pectic polysaccharides and ions

Ionic and electrical properties of the plant cell wall

Measurements of the ionic and electrical properties of cell walls indicate that polygalacturonic acids are responsible for the main charge of the walls (Grignon and Sentenac, 1991). To describe the ionic behaviour of cell walls the Donnan model and the Gouy-Chapman model are frequently used (Grignon and Sentenac, 1991; Guern et al., 1991; Morvan et al., 1985; Tu et al., 1988). In the Donnan model the galacturonic acids are viewed as a solution of free anions which are restricted to the so-called Donnan free space. In this space free cations are accumulated and free anions excluded due to the electrostatic field exerted by the polygalacturonic acids. The dimension of the Donnan free space depends on polymer structure, pH, type of

cations and ionic strength. The dimension can be estimated by the water content of the cell wall, which is generally 1-2 ml/g dry matter. In the model described by Gouy-Chapman the cell walls are treated as charged surfaces with a diffuse electric double layer (Grignon and Sentenac, 1991). The mean concentration of polymer charges in the cell wall of Dicotyledonae range between 0.2 M and 1 M. However, this charge is not equally distributed along the cell wall. Usually the charge density is found to be higher in the middle lamella region than in the region near the plasma membrane (Bacic et al., 1988; Cutsem and Messiaen, 1997; Grignon and Sentenac, 1991; McCann and Roberts, 1997; Ryden and Selvendran, 1990; Talbott and Ray, 1992). Most cell walls have a pH between 5 and 6.5 and have a significant buffering capacity (Demarty et al., 1984; Grignon and Sentenac, 1991).
It must be kept in mind that the ideal system, which is described by the Donnan and Gouy-Chapman models explain not completely all properties of plant cell walls. For instance, differences in binding of cations (with similar valency) by polygalacturonic acids are observed and can not be explained by the models (Grignon and Sentenac, 1991).

Cell wall porosity

Another feature of pectic polysaccharides is the ability to affect diffusion through the cell wall by determining cell wall porosity (Bacic et al., 1988; Carpita and Gibeaut, 1993; Grignon and Sentenac, 1991; McCann et al., 1990) and by acting as ion exchangers (Gemeiner et al., 1991; Grignon and Sentenac, 1991; Schlemmer, 1986; Schlemmer, 1989). Generally, the apparent diffusion coefficients in cell walls are one order of magnitude lower than in free solution for monovalent ions and still lower for multivalent ions (Grignon and Sentenac, 1991). However, some cations, in particular calcium, are more strongly bound than others, eventually resulting in masking part of the galacturonic acid charge (Demarty et al., 1984; Garnier et al., 1993; Grignon and Sentenac, 1991; Kohn and Luknar, 1977; Morvan et al., 1985).
With regard to this phenomenon the properties of calcium pectate have been extensively studied. The structure of a calcium-pectate-gel was described previously. The stability of this gel is influenced by the degree of methylation (Garnier et al., 1993; Garnier et al., 1994; Kohn and Furda, 1967), the distribution of the free carboxyl groups or linear charge density (Garnier et al., 1994; Kohn and Furda, 1967; Kohn and Larsen, 1972), degree of acetylation (Kohn, 1971), pH (Garnier et al., 1993; Garnier et al., 1994), temperature (Garnier et al., 1993) and ionic strength of the medium (Garnier et al., 1993; Garnier et al., 1994; Kohn and Furda, 1967).

Heating of potato tissue

Upon heating of potatoes the stability of the calcium-pectate-gel in the cell walls is affected by the transfer of ions from the cytoplasm. The main mineral and acid constituents in potatoes are given in Table 1.1.

An increased ionic strength decreases the stability constant of the calcium-pectate-gel (Garnier et al., 1994; Kohn and Furda, 1967). Also Keijbets (1974) reported that more pectic polysaccharides were solubilized by increasing buffer concentrations from 0.02 M to 0.1 M. Keijbets (1974) studied the specific effect of cations and anions on potato cell walls during boiling. Calcium, copper and iron inhibited solubilization of pectic polysaccharides when they were added in a cation to carboxylic acid ratio lower than 1. Reduced cell sloughing or pectin solubilization due to calcium was also reported by Zaehringer and Cunningham (1971), Davis and Le Tourneau (1967) and Hughes et al. (1975c). In the presence of an excess of divalent cations or the presence of monovalent ions, the probability of dimer formation is smaller. Calcium added in a ratio higher than 1 increased the solubilization of pectic polysaccharides (Keijbets, 1974). Also the presence of monovalent cations (sodium, potassium) results in increased pectin release (Davis and Le Tourneau, 1967; Hughes et al., 1975c; Keijbets, 1974). According to most studies (Davis and Le Tourneau, 1967; Haydar, 1980; Keijbets,

Table 1.1 Mineral and acid constituents in potatoes (Burton, 1989).

Constituent	Approximate normal range or main value (mg/100g dry matter)	(% fresh weight)
Aluminium	3 - 9	
Calcium	30 - 90	
Chlorine	100 - 500	
Copper	0.4 - 1	
Iron	2.5 - 10	
Magnesium	60 - 140	
Phosphorus	150 - 300	
Potassium	2800	
Sodium	20 - 300	
Zinc	1.8	
Ascorbic acid		0.015 - 0.025
Citric acid		0.277 - 0.514
Malic acid		0.046 - 0.139
Oxalic acid		0.013 - 0.026
Phosphoric acid		0.037 - 0.061

1974; Thibault and Rinaudo, 1986) magnesium was not able to inhibit solubilization of pectic polysaccharides, although Zaehringer and Cunningham (1971) reported less cell sloughing due to magnesium.
The organic acids are present in the anionic form ($citrate^{2-}$, $malate^{2-}$, $phytate^{12-}$) and enhanced solubilization of pectic polysaccharides due to chelation capacities with calcium ions (Keijbets, 1974). Davis and Le Tourneau (1967) and Zaehringer and Cunningham (1971) reported an increase in cell sloughing due to citrate. Selvendran et al. (1990) proposed that enhanced middle lamella breakdown in a mealy cooking cultivar may be the result of retarded citric acid leakage compared with the leakage for a non-mealy cultivar.

Starch

Potato starch

In potato, starch contributes for 60% to 80% to the dry matter (Gray and Hughes, 1978). The distribution of starch within a tuber is tissue dependent (Burton, 1989). Each potato cell contains starch granules of different sizes. The granule size distribution is also tissue dependent, whereby parenchyma tissue contained larger granules than for instance pith tissue. It is not clear if the granule size distribution is cultivar dependent (Burton, 1989). The ratio of amylose and amylopectin within the starch granules is comparable for the different tissue types within a tuber. For mature tubers this ratio is cultivar dependent (Weaver, 1978). With maturation of potato tubers both the size of starch granules and the ratio of amylose and amylopectin increase (Burton, 1989).

Starch gelatinization

During heating potato starch starts to gelatinize at a temperature between 50° and 70°C (Liu, 1990; Reeve, 1977), which depends on the ratio of amylose and amylopectin (Burton, 1989). Lamberg and Olsson (1989) reported that in potato tissue gelatinization starts at a temperature between 58° and 64°C (at an ambient temperature of 85°C). Cooking time, temperature and the relative amounts of starch and water present determine the properties of the gelatinized starch. In potatoes the dry matter content normally varies between 16% and 26% (Burton, 1989). Based on empiric relations, it can be calculated that this dry matter range corresponds with a starch content between 10% and 20% (Simmonds, 1977). The starch gels in potato cells have a concentration between 12 wt% and 27 wt%. Few literature deals with the properties of starch gels with these concentrations.
Upon heating in the presence of water the starch granules absorb water and swell. In

potato starch gels with a concentration higher than 4%, the swollen granules fill the whole available volume. The starch gels which occupy the potato cells after cooking have a structure which consists of partly swollen, irregularly shaped granules with a thin amylose matrix gel in between. The degree of swelling of the granules and the amount of amylose leakage depend on the starch concentration. At higher concentrations starch granules swell less, are stiffer and less amylose is leaking out of the granules (Keetels, 1995; Ring, 1985; Steeneken, 1987). Keetels (1995) also studied the deformation properties of 10%, 20%, 30% and 40% potato starch gels. She found that the Young modulus (a measure for stiffness of the gel) increased with about a factor 10, when the concentration increased from 10% to 30% (measured at 90°C). Furthermore, the fracture properties were measured after cooling the gels to 20°C and storing them for minimal 4 hours. It was found that the relative deformation necessary for fracture was higher at lower concentrations. Whereas the fracture stress increased with increasing concentrations (fracture occurred not until one day storage of the gels). She proposed that in 10% potato starch gels the properties of the amylose matrix gel may be important, whereas in 30% starch gels the stiffness of the granules mainly determines the fracture stress. This study indicates that the starch gels in the cells of cooked potatoes with high and low dry matter content respectively have different properties. Directly after cooking the gels in cells of potatoes with high dry matter are more elastic compared with the gels in cells of potatoes with low dry matter. Furthermore, less relative deformation and higher stress were necessary to fracture more concentrated starch gels. Therefore, cooked potato cells with high dry matter content are more rigid than those with low dry matter with respect to their starch gel properties.

Leakage of starch, in particular amylose, during cooking was reported and may be due to breakdown of starch polymers and increased pore size of the cell wall (Andersson et al., 1994; Fedec et al., 1977; Hoover and Hadziyev, 1981; Hughes et al., 1975c; Shomer, 1995). Linehan and Hughes (1969c) reported that this amylose might increase intercellular adhesion in cooked potato.

Starch swelling pressure

Another effect of starch gelatinization is the possible development of a swelling pressure. This was proposed by several authors, but others found no experimental evidence by measuring cell volumes during heating (Bartolomé and Hoff, 1972; Bretzloff, 1970; Hoff, 1972; Nonaka, 1980; Reeve, 1977). Recently, Jarvis et al. (1992) measured directly the pressure exerted by starch suspensions (concentrations of about 15% and 40%) during gelatinization. The pressure was in the order of 10^2 kPa and was significantly higher for the more concentrated starch suspensions. Shomer (1995)

and Shomer et al. (1995) reported an increase in swelling of gelatinized starch with increasing dielectric constant of the solvents. Charged phosphate groups are responsible for this phenomenon by providing the starch polymers with an electrical double layer. Furthermore, they reported that in the presence of excess water dried cooked potato cells swelled more (about 1.5 times) at 70°C than at ambient temperature.

Interaction between gelatinized starch and ions

After cooking the gelatinized starch fills the whole cell volume, thereby decreasing the apparent diffusion coefficients of solutes. Andersson (1994) reported that the dry matter content of potatoes affects this apparent diffusivity, which decreases with increasing dry matter content. On the other hand, the calcium ions which are present in potato starch become more mobile after gelatinization of starch and are able to diffuse into the cell wall and strengthen these walls by cross-linking pectin chains (Keijbets, 1974).

Ions in the cytoplasm may in turn reduce the viscosity or gel strength of potato starch gels (Mita, 1992; Muhrbeck and Eliasson, 1987; Whistler and Paschall, 1967). This is due to compression of the electric double layer around the phosphate groups (Keetels, 1995).

Aim and outline of this thesis

Texture is an important property of cooked table potatoes. Since the diversity in texture types is considerable, research is focused on the question how these different types of texture can develop. Sensory evaluation has proved to be an useful method to describe potato texture types. For an explanation of the development of different texture types, the degradation of pectic polysaccharides, the influence of ions on the solubilization of pectic polysaccharides and the role of starch were research targets. Pectic polysaccharides are the main components of the middle lamellae and are so responsible for the intercellular contacts between adjacent cells. During cooking pectic polysaccharides are solubilized, thereby decreasing intercellular contacts, eventually resulting into cell sloughing. It is not yet known which feature(s) of pectic polysaccharides (such as amount, composition or structure) are responsible for differences in texture development. Composition and structure of potato cell walls are comprehensively studied, but up till now these studies are restricted to one potato cultivar per study.

Ions are known to influence the solubilization of pectic polysaccharides. However, cultivars with different texture types should be compared to learn if the differences in

ion composition between those cultivars are large enough to explain (part of) the differences in texture development.

Starch is another potato constituent which may influence development of different texture types. The fact that (i) dry matter content is still the most practical property of potatoes for predicting cooking behaviour with respect to mealiness, (ii) starch contributes for 60 to 80% to the total dry matter content and (iii) starch content and dry matter content are positively correlated, has contributed to this. More knowledge about the possible roles of starch in texture development should be obtained. For instance, the development of a swelling pressure during gelatinization is reported.

The aim of the study described in this thesis is (i) to obtain the most relevant sensory texture descriptors, which reflect the diversity in cooked potato texture, (ii) to visualize the change in ultrastructure of potato tissue during cooking to reveal the relative importance of cell walls, middle lamellae and starch with respect to texture development, (iii) to measure the degradation of middle lamellae during cooking for potatoes with a different texture type, (iv) to determine if and/or how ions and composition and structure of potato cell walls contribute to texture development.

Firstly, the diversity in texture types will be screened by sensory evaluation. Ten table potato cultivars are chosen which are known to represent most of the observed differences in texture (Chapter 2). The data are analyzed by Principle Component Analysis (PCA), which has the advantages that the relative contribution of texture descriptors in discriminating between cultivars and the correlations between the descriptors will be known. It is observed that the differences between mealy and non-mealy (waxy) texture types are responsible for most of the diversity that is found in potato texture of different cultivars.

Next the ultrastructure of four cultivars which are representative for mealy and non-mealy texture types are subject of a cryo-Scanning Electron Microscopy (cryo-SEM) study (Chapter 3). Cryo-fixation is used to preserve the original hydrated structures of (cooked) potato tissue. The three dimensional observations of the fracture planes of cooked potato tissue reveal different organizations of the cellular structure with respect to texture type.

To study the role of cell walls and middle lamellae, two approaches are chosen and in both cases potatoes from cv. Irene (mealy cooking) and cv. Nicola (non-mealy cooking) are used.

Firstly, cell sloughing, solubilization of pectic polysaccharides and the transfer of ions (citrate, calcium, potassium) during cooking of potato disks from both cultivars in distilled water will be recorded (Chapter 4).

Secondly, the composition (Chapter 5) and the structure (Chapter 6) of cell walls from both cultivars will be studied, to elucidate their contribution to the difference in release

of pectic material.

Finally, the conclusions presented in this thesis will be discussed with emphasis on the relative contribution of the studied potato properties to texture development.

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Chapter 2

Sensory evaluation of the texture of steam-cooked table potatoes

J.T. van Marle, R. van der Vuurst de Vries, E.C. Wilkinson and D. Yuksel

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Summary

Trained panelists evaluated the texture of steam-cooked table potatoes. Quantitative Descriptive Analysis (QDA) was used to generate twelve sensory descriptors referring to appearance and mouthfeel. Ten potato cultivars were evaluated after two, four and nine months storage for three consecutive years. The sensory data were analyzed using Principal Component Analysis (PCA) and regression analysis. PCA revealed that the first two principal components explained 95% or more of the variance between the data. The first principal component was dominated by the descriptors *mealy (M)/crumbly (A/M)* on the positive side and the descriptors *waxy (A/M)* on the negative side. The descriptor *firm (M)* had a high positive loading on the second principle component and had very low correlations with the descriptors dominating the first principal component. Regression analysis showed that cultivar effects far dominated storage effects. Based on these data, a proposal is made to divide the ten cultivars in four groups which differ for the descriptors *mealy (M)/crumbly (A/M)*, *waxy (A/M)* and *firm (M)*.

The dry matter content of all potato samples was determined before each sensory evaluation. Correlations were found between dry matter content and the dominant descriptors for the first principal component, whereas correlations with the descriptors dominating the second axis were very low.

During storage, potato texture became more waxy and sticky with respect to both appearance and mouthfeel. Other changes in texture as a result of storage were strongly cultivar dependent.

Introduction

Cooked potatoes are not a uniform product, but exhibit a great deal of diversity. Cultivar and conditions during growth and storage (Faulks and Griffiths, 1983; Burton, 1989) affect the texture of cooked potatoes. This diversity in texture has enabled the potato to meet specific preferences of consumers, producers and processors (Niederhauser, 1993). However, it has also led to numerous investigations into describing cooked potato texture based on sensory perception and into explaining the development of different texture types using histological, biochemical and physical approaches.

Usually four main types of cooking behaviour (A, B, C, D) are distinguished among cooked potatoes. This classification system is based on a sensory evaluation using five main descriptors, namely "sloughing", "consistency", "mealiness", "moisture" and "structure", as established by the European Association of Potato Research (EAPR) (Böhler et al., 1986; Winiger and Ludwig, 1974). In the literature a large vocabulary of descriptors is used with respect to cooked potato texture. The meaning of these descriptors is not always unambiguous, because the same descriptor can be defined or measured in different ways by different authors (Gray and Hughes, 1978; Leung et al., 1983; Ludwig, 1972; McComber et al., 1988).

Research into an explanation for the different texture types has concentrated on correlations between texture descriptors and potato constituents, in particular dry matter content (or starch content or specific gravity) (Böhler et al., 1986; Freeman et al., 1992; Gray and Hughes, 1978; Leung et al., 1983; Linehan and Hughes, 1969; Ludwig, 1972; McComber et al., 1988; Warren and Woodman, 1974; Woodman and Warren, 1972). Up till now, dry matter content has proved the most practical property of potatoes for predicting cooking behaviour with respect to mealiness (Burton, 1989).

Efforts were made to establish the effect of storage on potato texture. Davies and Dixon (1976) evaluated the texture of nine potato cultivars during four months of storage. They observed no significant storage effect, probably due to the fact that for each descriptor the mean score of all cultivars for different storage periods was compared. Faulks and Griffiths (1983) studied eight cultivars during 24 weeks storage and observed that the correlation between sensory and physical parameters was suddenly lower after 24 weeks. Keijbets (1974) determined the influence of storage on some potato constituents and intercellular cohesion for different specific gravity-classes of cv. Bintje.

For this study, a range of cultivars was selected exhibiting different texture types and maturity types as to reflect the diversity present between cultivars. The texture descriptors were generated using quantitative descriptive method. In this study, the

Table 2.1 Cooking classification and maturity type of ten potato cultivars (Parlevliet et al., 1991)

cultivar	type of cooking behaviour			
	A	B	C	D
First early				
Accent	———			
Doré		———		
Eersteling	———	———		
Second early				
Bintje		———	———	
Eigenheimer		———	———	———
Early maincrop				
Agria		———	———	
Bildtstar		———	———	
Eba		———	———	
Irene			———	———
Nicola	———	———		

- type A: an especially firm, non-mealy potato with a fine structure;
- type B: a firm, slightly mealy potato with a fine or rather fine structure;
- type C: a rather loose, mealy potato;
- type D: a loose, very mealy potato.

relative contribution of a descriptor in discriminating between different cultivars and the possible correlations between the descriptors were determined, these aspects have received little attention in the literature. Furthermore, the correlations between dry matter content and the texture descriptors were established. Finally, much attention is given to the effect of storage on the sensory perception of potato texture, taking into account that individual cultivars can behave differently and in particular may react differently to storage.

Materials and methods

Potatoes

Ten potato cultivars, namely Accent (Acc), Agria (Agr), Bildtstar (Bil), Bintje (Bin), Doré (Dor), Eba, Eersteling (Eer), Eigenheimer (Eig), Irene (Ire) and Nicola (Nic), were chosen representing nearly all occurring combinations of the four types of cooking behaviour and different maturity types (Table 2.1) (Parlevliet et al., 1991). The cultivars were grown in 1991, 1992 and 1993 on clay soil at the experimental station of ATO-

DLO in the North East Polder, The Netherlands. In the first year the cultivars Accent and Bildtstar were not available from the experimental station. Mature potatoes were harvested and potatoes of size 45-55 mm were stored at 6°C and 95% relative humidity for nine months. During each of the three storage seasons (1991/1992, 1992/1993, 1993/1994), samples for sensory evaluation were taken in December (two months storage; during dormancy), February (four months storage; after loss of dormancy) and June (nine months storage; after prolonged storage).
At the beginning of the storage season for each cultivar, the weight distribution was estimated by weighing 150 individual potatoes. To exclude very small or large potatoes, potatoes with a weight within the central 67%-interval of the weight distribution were selected for the samples used for the sensory evaluations. In practice, this is a single-tuber-weight between about 80 g and 120 g depending on cultivar.

Dry matter

The dry matter content of potatoes was calculated from the under-water-weight (Simmonds, 1977; Ludwig, 1988). The under-water-weight of the samples was measured each time prior to sensory evaluation.

Cooking

Potatoes were hand-peeled (leading to about 18% weight loss) and steam-cooked (100°C; 10^5 Pa) for 30-35 minutes. After cooking, potatoes were cut lengthwise into two halves. Each panelist evaluated one half.

Table 2.2 Average dry matter content of ten potato cultivars calculated from the measured under-water-weight (SD(n-1)). Cultivars with different superscript(s) are significantly different ($P<0.05$).

Cultivar	Dry matter (% f.w.)
Accent	18.4 ± 0.3^{a}
Agria	19.2 ± 0.8^{b}
Nicola	20.0 ± 0.3^{c}
Eersteling	20.5 ± 0.3^{cd}
Bintje	21.2 ± 0.4^{de}
Bildtstar	21.9 ± 1.4^{ef}
Doré	22.5 ± 0.7^{f}
Eba	23.6 ± 0.9^{g}
Irene	24.7 ± 1.2^{h}
Eigenheimer	24.8 ± 0.9^{h}

Table 2.3 Correlation between dry matter content and the twelve sensory descriptors for the ten potato cultivars used.

Descriptor	Correlation coefficient
Waxy (A)	-0.76
Waxy (M)	-0.83
Sticky (A)	-0.80
Sticky (M)	-0.73
Crumbly (A)	0.76
Crumbly (M)	0.79
Mealy (M)	0.81
Grainy (M)	0.86
Firm (M)	-0.33
Breakable (A)	0.61
Mashable (A)	0.56
Moist (M)	-0.91

A = appearance; M = mouthfeel.

Sensory evaluation

Sensory evaluation of the steam-cooked potato texture was carried out using a panel of 15 trained panelists. A quantitative descriptive method was used for sensory analysis (Quantitative Descriptive Analysis (QDA), Stone et al., 1974). The panel members individually generated descriptors, based on a range of five cultivars (Accent, Bildtstar, Bintje, Eigenheimer, Nicola) representing the four main cooking types (Parlevliet et al., 1991). Group discussions and a checklist were used to derive a consensus vocabulary.

Using this vocabulary the panelists rated the samples for the perceived intensity of the respective descriptors on a five-point category scale (1=none/very slightly; 2=slightly; 3=moderately; 4=very; 5=extremely).

During each session, five samples were presented in randomized order. Each single sample was evaluated twice. For data collection PSA-SYSTEM (OP&P, Utrecht, The Netherlands) was used. Data were processed using a multivariate analysis program (Unscrambler II, version 5.5 , CAMO, Trondheim, Norway) and Genstat (1993).

Data analysis

Because of the multi-dimensional character of the data-set (each descriptor provides a dimension) and the numerous interrelationships between the descriptors, a dimension-reducing analysis technique was needed to analyze the dataset (Smith, 1988). Accordingly Principle Component Analysis (PCA) was used. The purpose of

PCA is to transform the set of original correlated descriptors into a new set of principle components, which are linear combinations of the original descriptors and which are not correlated with each other. The number of principal components is equal to the number of original descriptors. However, they are ranked so that the variation in the data-set explained by the successive principle components decreases. Usually the first few components account for most of the variance in the data and this makes PCA a dimension-reducing technique (Smith, 1988).

Regression analysis (Genstat, 1993) was used to test for the presence of significant differences between cultivars and significant changes during the storage season for individual cultivars.

The explanatory variables cultivar, storage and panelist together with their interactions were used in regression analysis as indicator variables (Montgomery and Peck, 1982). The response variables, the descriptors, were categories with a lower limit of 1 and an upper limit of 5 and therefore could not be assumed to follow the normal distribution, which has no limits. Instead generalized linear regression models were fitted (McCullagh and Nelder, 1989) in which the response variable was assumed to follow a binomial distribution. The binomial distribution incorporates upper and lower limits. The deviance ratios calculated with this model for each explanatory variable were compared with the F-statistics, which test the null-hypothesis of no effect for that explanatory variable.

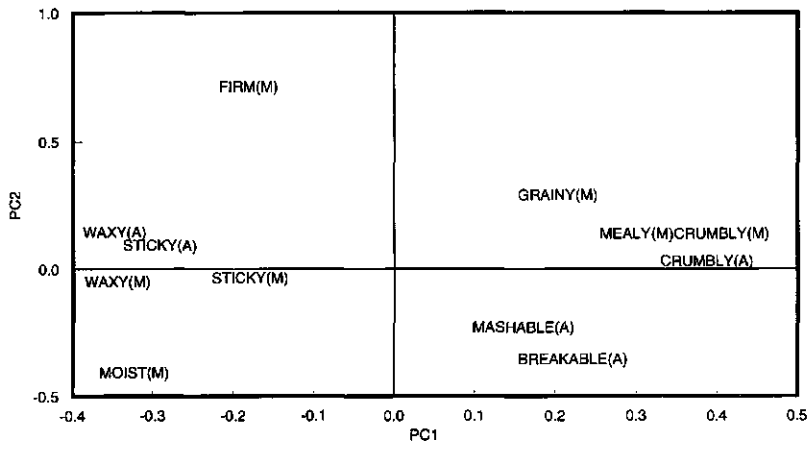

Fig. 2.1 Loadings of sensory descriptors on the first and second principal component (PC), example of storage season 1993/1994.

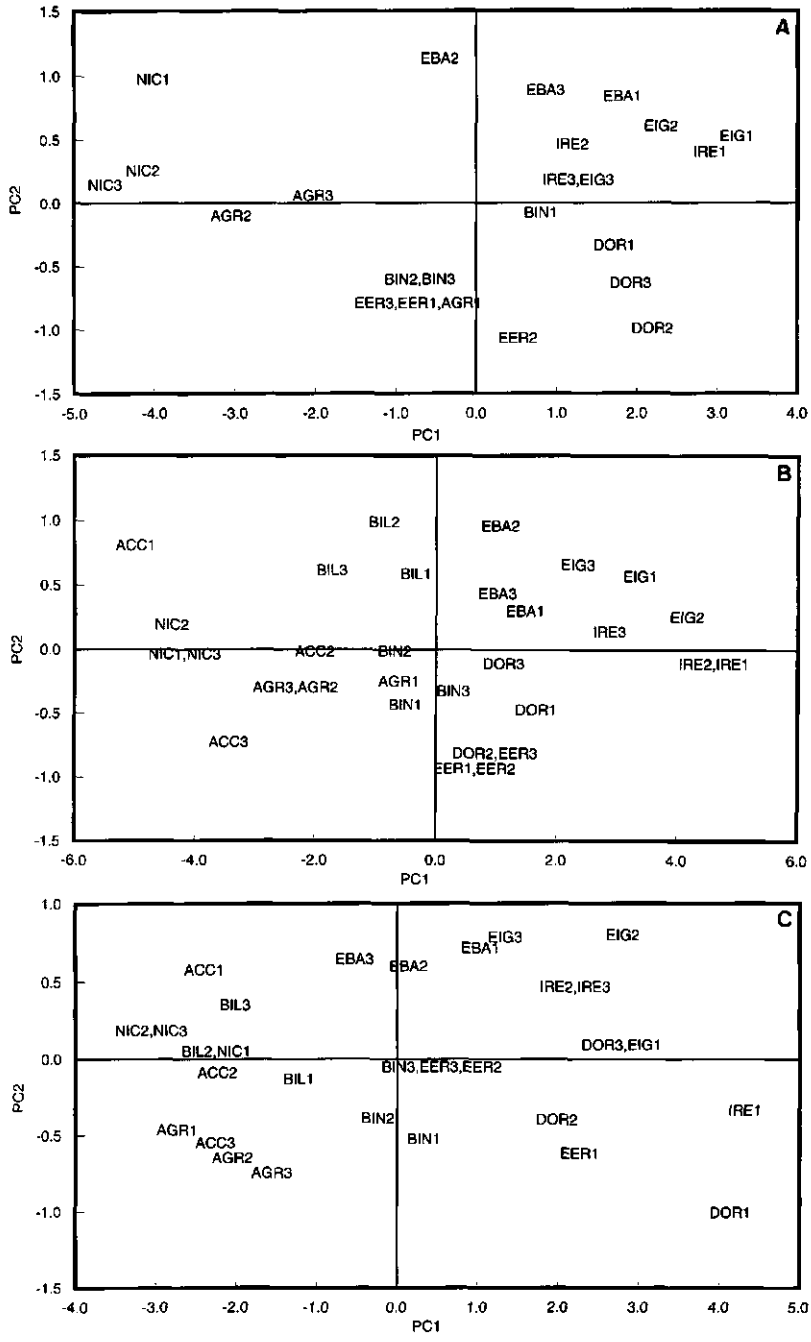

Fig. 2.2 Sample scores of potato cultivars on the first and second principal component evaluated in December (1), February (2) and June (3) for the three years studied. A. 1991/1992; B. 1992/1993; C. 1993/1994.

Results

Sensory evaluation

Following set of descriptors was developed and used to evaluate the texture of cooked potatoes:

- referring to appearance (A) (tested using a fork): *waxy (A), crumbly (A), sticky (A), breakable (A), mashable (A);*
- referring to mouthfeel (M): *waxy (M), crumbly (M), sticky (M), firm (M), moist (M), grainy (M), mealy (M).*

Dry matter

The average dry matter content for each cultivar is given in Table 2.2. The correlations between dry matter content and the texture descriptors are given in Table 2.3.

Principle Component Analysis

PCA was applied to the combined data of the three storage periods for each of the three years. The first two principal components explained 95% or more of the variation in the data for each of the three years studied (the first principal component alone accounted for 90% of the variation).

The descriptors *mealy (M)* and *crumbly (A/M)* had high positive loadings and the descriptors *waxy (A/M)*, *sticky (A)* and *moist (M)* had high negative loadings for the first principal component (Fig. 2.1). The second principal component had a high positive loading for the descriptor *firm (M)* and negative loadings for *moist (M)*, *breakable (A)* and *mashable (A)* (Fig. 2.1). Original descriptors with comparable loadings on the first two principal components are highly correlated. For instance, the descriptors *waxy (A/M)* and *sticky (A/M)* and the descriptors *mealy (M)* and *crumbly (A/M)* respectively are highly correlated. Only the plot for the storage season 1993/1994 is shown, since for each of the three years studied, the original descriptors had comparable loadings for the first two principal components.

The scores of the ten potato cultivars for the first and second principal components are shown in Fig. 2.2. The distribution of the cultivars along the first principal component reflects their mealy/non-mealy characteristics. Whereas the cultivars Irene and Eigenheimer have high positive scores for the first principal component indicating their mealy and crumbly character, cv. Nicola represents the non-mealy type of potatoes having high negative scores for the same principal component.

Furthermore, the distribution of the cultivars along the second principal component reflects their firm and moist character.

Each cultivar is mentioned three times (marked with 1, 2, 3) referring to the three

Table 2.4 Deviance ratio of panel, cultivar and storage on sensory descriptors of ten potato cultivars for three years.

Descriptor	1991/1992				1992/1993				1993/1994			
	P	C	S	C.S	P	C	S	C.S	P	C	S	C.S
Waxy (A)	8.2***	46.4***	18.4***	1.4	9.8***	72.6***	7.2***	2.2***	4.1***	49.6***	9.3***	1.6
Waxy (M)	7.3***	55.3***	5.3**	2.2**	6.9***	73.7***	2.1	2.6***	3.0***	46.3***	9.5***	1.2
Sticky (A)	10.8***	22.9***	10.3***	1.5	16.3***	38.6***	2.5	1.0	14.3***	32.4***	11.2***	1.8*
Sticky (M)	12.7***	19.6***	11.6***	1.5	16.7***	14.5***	7.5***	1.1	14.6***	12.8***	5.7**	1.1
Crumbly (A)	5.0***	59.9***	11.0***	2.6***	13.7***	79.6***	2.5	2.7***	8.6***	61.7***	9.3***	1.8*
Crumbly (M)	5.9***	60.3***	11.2***	2.4***	11.6***	78.8***	0.2	2.6***	10.1***	51.7***	10.1***	1.7*
Mealy (M)	3.6***	39.7***	8.4***	2.1**	7.8***	67.1***	2.8	2.5***	11.2***	46.8***	3.1*	1.0
Grainy (M)	14.9***	39.6***	1.2	1.6*	24.8***	31.1***	3.4*	1.2	16.0***	19.7***	1.8	0.6
Firm (M)	6.0***	14.3***	0.1	2.5***	11.0***	20.8***	2.9	2.2**	8.6***	18.0***	5.0**	2.4**
Breakable (A)	6.8***	13.5***	0.6	3.2***	7.6***	31.2***	1.5	2.3**	9.8***	19.0***	5.3**	2.9***
Mashable (A)	4.2***	9.5***	1.0	1.4	9.0***	25.4***	1.2	1.6*	10.6***	10.0***	2.5	1.3
Moist (M)	8.3***	47.8***	8.1***	1.7*	8.9***	66.9***	3.9*	1.3	9.0***	48.9***	1.3	1.0

P = panel; C = cultivar; S = storage; C.S = interaction between cultivar and storage; A = appearance; M = mouthfeel.
***, **, * Significantly different using F test at P<0.001, P<0.01, P<0.05, respectively.

storage periods respectively. There is no obvious shift of all cultivars upon storage, indicating that the storage effect is less pronounced than the cultivar effect.

Regression analysis

The influence of panelist, cultivar and storage on the twelve descriptors used to describe the texture of cooked potatoes is shown in Table 2.4. Only the cultivar.storage-interaction is shown as interactions involving panelists were found not to be significant. The differences between the cultivars were highly significant ($P<0.001$) for all descriptors.

Regression analysis revealed that the effect of cultivar was considerably more pronounced than the effect of storage (Table 2.4). Therefore the cultivars can be arranged regardless of storage duration in order of their scores for the twelve descriptors for each individual year, without loss of information (Table 2.5). For each descriptor significant differences between cultivars are marked and were comparable for the three consecutive years.

The effect of storage on texture was far less than the effect of cultivar and varied for the three years. The effect was most pronounced between the sensory evaluations of December and February. For some descriptors interactions between cultivar and

Table 2.5 Differences between potato cultivars for twelve sensory descriptors as found by regression analysis. The cultivars are listed in a decreasing (<-) or increasing (->) order. Cultivars with different superscript(s) within columns are significantly different ($P<0.05$).

1991/1992

Waxy (A)	->	Eig^{a}	Dor^{ab}	Ire^{b}	Eba^{c}	Bin^{c}	Eer^{c}	Agr^{d}	Nic^{e}
Waxy (M)	->	Eig^{a}	Dor^{a}	Ire^{a}	Eba^{b}	Bin^{c}	Eer^{d}	Agr^{e}	Nic^{f}
Sticky (A)	->	Eig^{a}	Ire^{a}	Eba^{a}	Dor^{a}	Eer^{b}	Bin^{b}	Agr^{c}	Nic^{d}
Sticky (M)	->	Eig^{a}	Dor^{a}	Ire^{a}	Eba^{ab}	Bin^{bc}	Eer^{cd}	Agr^{d}	Nic^{e}
Crumbly (A)	<-	Eig^{d}	Dor^{d}	Ire^{d}	Eba^{c}	Bin^{c}	Eer^{c}	Agr^{b}	Nic^{a}
Crumbly (M)	<-	Eig^{e}	Ire^{e}	Dor^{e}	Eba^{d}	Bin^{cd}	Eer^{c}	Agr^{b}	Nic^{a}
Mealy (M)	<-	Eig^{f}	Ire^{ef}	Dor^{ef}	Eba^{de}	Bin^{cd}	Eer^{c}	Agr^{b}	Nic^{a}
Grainy (M)	<-	Eig^{f}	Ire^{ef}	Dor^{ef}	Eba^{de}	Bin^{d}	Eer^{c}	Agr^{b}	Nic^{a}
Firm (M)	->	Dor^{a}	Eer^{ab}	Bin^{bc}	Ire^{cd}	Agr^{cd}	Eig^{de}	Eba^{ef}	Nic^{f}
Breakable (A)	<-	Dor^{e}	Ire^{de}	Eig^{de}	Eer^{de}	Bin^{cd}	Eba^{bc}	Agr^{b}	Nic^{a}
Mashable (A)	<-	Dor^{d}	Eer^{cd}	Bin^{c}	Eig^{c}	Ire^{bc}	Agr^{bc}	Eba^{b}	Nic^{a}
Moist (M)	->	Eig^{a}	Ire^{a}	Eba^{a}	Dor^{b}	Bin^{e}	Eer^{f}	Agr^{f}	Nic^{g}

1992/1993

Waxy (A)	->	Ire^{a}	Eig^{b}	Eer^{c}	Dor^{c}	Eba^{c}	Bin^{d}	Bil^{de}	Agr^{e}	Acc^{f}	Nic^{f}
Waxy (M)	->	Ire^{a}	Eig^{a}	Eba^{b}	Dor^{b}	Eer^{c}	Bin^{cd}	Bil^{de}	Agr^{e}	Acc^{f}	Nic^{f}
Sticky (A)	->	Ire^{a}	Eig^{a}	Eba^{b}	Dor^{b}	Eer^{b}	Bin^{c}	Bil^{c}	Agr^{c}	Acc^{d}	Nic^{e}
Sticky (M)	->	Eig^{a}	Ire^{a}	Eba^{b}	Dor^{b}	Eer^{bc}	Bin^{c}	Bil^{c}	Acc^{c}	Agr^{c}	Nic^{d}
Crumbly (A)	<-	Ire^{f}	Eig^{e}	Dor^{d}	Eer^{d}	Eba^{d}	Bin^{e}	Bil^{b}	Agr^{b}	Acc^{a}	Nic^{a}
Crumbly (M)	<-	Ire^{f}	Eig^{e}	Eba^{d}	Dor^{d}	Eer^{cd}	Bin^{c}	Bil^{b}	Agr^{b}	Acc^{a}	Nic^{a}
Mealy (M)	<-	Ire^{e}	Eig^{e}	Dor^{d}	Eba^{d}	Eer^{d}	Bin^{c}	Bil^{bc}	Agr^{b}	Acc^{a}	Nic^{a}
Grainy (M)	<-	Eig^{f}	Ire^{f}	Eba^{f}	Dor^{e}	Eer^{d}	Bil^{d}	Bin^{cd}	Agr^{bc}	Acc^{b}	Nic^{a}
Firm (M)	->	Eer^{a}	Ire^{a}	Dor^{ab}	Eig^{bc}	Bin^{cd}	Agr^{d}	Eba^{d}	Bil^{e}	Acc^{e}	Nic^{e}
Breakable (A)	<-	Ire^{f}	Eig^{ef}	Eer^{e}	Dor^{e}	Eba^{d}	Bin^{d}	Agr^{cd}	Bil^{bc}	Acc^{ab}	Nic^{a}
Mashable (A)	<-	Ire^{f}	Eer^{e}	Eig^{e}	Dor^{de}	Eba^{c}	Bin^{c}	Agr^{bc}	Bil^{b}	Acc^{a}	Nic^{a}
Moist (M)	->	Eig^{a}	Ire^{a}	Eba^{b}	Dor^{c}	Bil^{c}	Bin^{d}	Eer^{d}	Agr^{d}	Acc^{e}	Nic^{e}

1993/1994

Waxy (A)	->	Dor^{a}	Ire^{ab}	Eig^{b}	Eer^{c}	Eba^{d}	Bin^{d}	Bil^{e}	Agr^{e}	Acc^{ef}	Nic^{f}
Waxy (M)	->	Ire^{a}	Dor^{a}	Eig^{a}	Eer^{b}	Eba^{b}	Bin^{c}	Bil^{d}	Acc^{d}	Agr^{d}	Nic^{d}
Sticky (A)	->	Ire^{a}	Dor^{a}	Eig^{a}	Eer^{b}	Eba^{b}	Bin^{bc}	Bil^{cd}	Acc^{de}	Agr^{de}	Nic^{e}
Sticky (M)	->	Ire^{a}	Eig^{a}	Dor^{a}	Eba^{b}	Eer^{b}	Bin^{bc}	Acc^{c}	Bil^{c}	Agr^{c}	Nic^{c}
Crumbly (A)	<-	Dor^{e}	Ire^{e}	Eig^{d}	Eer^{d}	Eba^{c}	Bin^{c}	Bil^{b}	Acc^{ab}	Agr^{ab}	Nic^{a}
Crumbly (M)	<-	Ire^{e}	Dor^{e}	Eig^{e}	Eer^{d}	Eba^{cd}	Bin^{c}	Bil^{b}	Agr^{b}	Acc^{b}	Nic^{a}
Mealy (M)	<-	Ire^{c}	Dor^{c}	Eig^{c}	Eer^{b}	Eba^{b}	Bin^{b}	Bil^{a}	Agr^{a}	Acc^{e}	Nic^{a}
Grainy (M)	<-	Dor^{e}	Eig^{e}	Ire^{de}	Eba^{d}	Eer^{d}	Bin^{c}	Acc^{bc}	Bil^{abc}	Nic^{ab}	Agr^{a}
Firm (M)	->	Dor^{a}	Ire^{a}	Eer^{bc}	Bin^{bc}	Eig^{c}	Agr^{c}	Eba^{de}	Bil^{ef}	Acc^{ef}	Nic^{f}
Breakable (A)	<-	Dor^{f}	Ire^{f}	Eer^{e}	Eig^{e}	Bin^{de}	Eba^{cd}	Agr^{bc}	Bil^{ab}	Acc^{ab}	Nic^{a}
Mashable (A)	<-	Dor^{e}	Ire^{de}	Eig^{d}	Eer^{cd}	Bin^{cd}	Eba^{bc}	Agr^{b}	Acc^{ab}	Bil^{ab}	Nic^{a}
Moist (M)	->	Ire^{a}	Eig^{ab}	Dor^{bc}	Eba^{c}	Eer^{d}	Bin^{d}	Bil^{e}	Acc^{f}	Agr^{f}	Nic^{f}

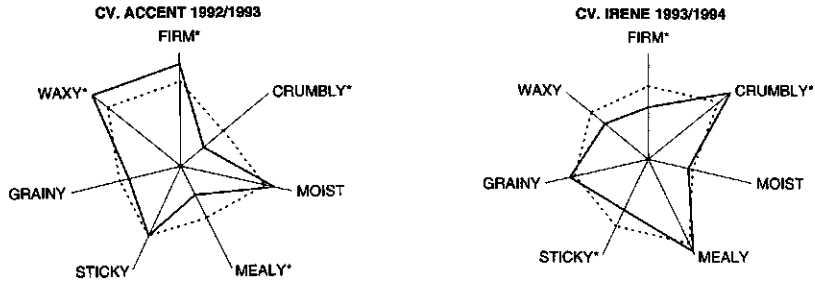

Fig. 2.3 Storage effect on mouthfeel descriptors between the first (solid line) and second (dashed line) sensory evaluation. The descriptors marked with an asterisk changed significantly ($P<0.05$).

storage were significant, indicating that the cultivars behaved differently with respect to those descriptors during storage.

In general, the scores for the descriptors *waxy (A/M)* and *sticky (A/M)* tend to increase during storage and showed no or small interaction with cultivar for the three years studied (Table 2.4). For the first (1991/1992) and third (1993/1994) storage season, a decrease in the scores for the descriptors *crumbly (A/M)* and *mealy (M)* was observed during storage. On the other hand, the descriptor *mashable (A)* was not affected by storage at all.

Furthermore, the cultivars could be distinguished by the degree of change in texture during storage. The texture of the cultivars Nicola and Bintje did not change during storage for the three consecutive years. Depending on the year the texture of the cultivars Doré, Eba, Eersteling and Bildtstar was also quite stable during storage. The cultivars Irene and Accent had the least stable texture during storage, which is visualized for the mouthfeel descriptors in spread-pattern diagrams (Fig. 2.3) (Stone and Sidel, 1993).

Discussion

In the literature, different sensory descriptors are used to describe the same primary product parameters (Stone and Sidel, 1993) such as hardness, cohesiveness and adhesiveness, moisture content and particle size. In this study, QDA was used to generate those descriptors which were considered most important in describing steam-cooked potato texture according to the perceptions of the panelists.

Sensory evaluation and subsequent data analysis by PCA revealed the interrelationships between the descriptors and their relative importance. It was found that the first two principal components explained most of the variance between different texture types. The first principal component was dominated by the mealy/non-mealy characteristics at the positive and negative side respectively (Fig. 2.1). A mealy texture becomes manifest by a mealy and crumbly appearance and mouthfeel, while a non-mealy texture is characterized by a waxy and sticky appearance and a waxy and moist mouthfeel (Fig. 2.1). The differences between these two texture types are responsible for most of the diversity that is found in potato texture of different cultivars. Representative for these differences in texture are for instance the cultivars Irene (mealy) and Nicola (non-mealy) (Fig. 2.2).

The descriptor *firm (M)* had a high positive loading on the second principal component. Firmness had very low correlations with all of the other descriptors, apart from some correlations with *breakable (A)* and *mashable (A)* (Fig. 2.1). Böhler et al. (1986) and Leung et al. (1983) also found no correlation between mealiness and consistency, while in the classification system used by the EAPR firm and non-mealy are both used as descriptors for cooking type A (Böhler et al.,1988; Winiger and Ludwig, 1974). Results in this study indicated that firmness could be used to differentiate cultivars which have similar mealy/non-mealy characteristics. For instance, the cultivars Bildtstar, Doré, Eersteling and Eba have comparable characteristics with respect to mealiness, but they can be discriminated on basis of firmness (Fig. 2.2).

Based on the results obtained by regression analysis (Table 2.5) and PCA (Fig. 2.2) the cultivars studied can be divided in four groups with respect to the descriptors *mealy (M)/crumbly (A/M)*, *waxy (A/M)* and *firm (M)*:

- group 1: *waxy*: Nicola, Accent, Agria;
- group 2: *mealy/crumbly*: Irene, Eigenheimer;
- group 3: moderately *waxy* and *mealy/crumbly*, not *firm*: Eersteling, Bintje, Doré;
- group 4: moderately *waxy* and *mealy/crumbly*, *firm*: Eba, Bildtstar.

In order to find an explanation for the different texture types, many attempts have been made to find correlations between potato constituents and texture properties. In this study, a correlation is found between dry matter content and the texture descriptors dominating the first principal component (Table 2.3). Since this first principal component explains most of the variance in potato texture, the observed correlation corresponded well with the fact that dry matter content is still the most practical property to predict potato texture with respect to mealiness (Burton, 1989). The descriptors *firm (M)* in particular and also *breakable (A)* and *mashable (A)* are poorly correlated with dry matter content. This result was also reported by Jarvis and Duncan (1992). This poor correlation is clearly illustrated by the difference in firmness

of the cultivars Bildtstar and Doré (Fig. 2.2; Table 2.5) which have comparable dry matter contents (Table 2.3). Andersson et al. (1994) reported in their review that within a given cultivar dry matter and mealiness are correlated, but between different cultivars different or no correlations are found between those properties. This is in agreement with our results, because dry matter and mealiness are correlated comparing cultivars from group 1 and 2 respectively, while no or low correlations are found comparing cultivars from group 3 and 4.

The causal relationship between texture properties and dry matter or starch content is not very clear. Several factors may influence texture, including composition of cell wall and middle lamella, starch content and ionic composition (Faulks and Griffiths, 1983; Jarvis et al., 1992; Marle et al., 1994).

The mealy and crumbly mouthfeel is brought about by individual cells. This particular mouthfeel arises when, during cooking, intercellular contacts are broken, while the cell walls are left more or less intact. On the other hand, for non-mealy cooking potatoes, fracturing also takes place alongside cells. In this case the cell walls are more easily ruptured upon fracturing or mastication. Leakage of gelatinized starch out of the cells brings about the waxy/sticky/moist mouthfeel (Burton, 1989; Marle et al., 1992; Andersson et al., 1994). Thus, structure and composition of cell walls play an important role in determining texture (Jarvis and Duncan, 1992; Marle et al., 1994). At the same time, starch may play a role by producing a swelling pressure during gelatinization facilitating cell separation (Jarvis et al., 1992) and after cooking by giving the individual cells some rigidity (Burton, 1989; Marle et al., 1992; Warren and Woodman, 1974). In fact texture properties may have better correlations with starch swelling pressure (Jarvis et al., 1992) or starch gel rigidity (Keetels, 1995).

The descriptor *firm (M)* was found to give an extra differentiation. Firm cooked potato tissue can be characterized by the presence of intercellular contacts and strong cell walls, which are preserved to a large extent upon fracturing or mastication. Cell wall strength and intercellular contacts seemed to be important parameters in determining tissue firmness. This was also postulated by Jarvis and Duncan (1992).

The effect of storage on cooked potato texture is cultivar dependent. Changes in texture with respect to the evaluated descriptors were most pronounced comparing the results of the sensory evaluations in December and February. During this period dormancy was lost. Faulks and Griffiths (1983) observed a change in correlation between sensory and physical measurements of potato texture after 24 weeks storage. They found no causal explanation, except that this change coincided with break of dormancy. However, the effect of storage and break of dormancy, in particular, on potato constituents, which are important in determining texture is not well known. Keijbets (1974) reported the presence of slightly more pectic galacturonan and

an increase in free carboxyl groups comparing cell wall material from potatoes which were stored for two and six months respectively. However, Faulks and Griffiths (1983) did not observe a change in non-polysaccharide composition during 24 weeks storage. They reported shrinkage of the mean cell size. Burton (1989) reported that changes in pectin solubility were found for potatoes stored in tropical climates (25°-30°C). Changes in total starch content during storage are negligible with respect to an effect on starch gel rigidity (Keijbets, 1974; Burton, 1989).

In conclusion, data analysis with PCA revealed the relative contribution of each descriptor in discriminating between different cultivars and revealed the correlations between the descriptors. Based on the results obtained by PCA and the fact that the effect of cultivar was more pronounced than the effect of storage, the cultivars studied were divided in four groups with different texture types.

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Chapter 3

Cryo-scanning electron microscopy investigation of the texture of cooked potatoes

J.T. van Marle, A.C.M. Clerkx[1] and A. Boekestein[2]

Food Structure, 1992, 11, 209-216

Summary

The texture of steam-cooked potatoes was investigated by examining the fracture planes of four different cultivars, using cryo-scanning electron microscopy (cryo-SEM), which yielded a good preservation of the hydrated structures in potato tissue. For all cultivars, fracturing after steam-cooking took place between cells preferentially alongside the cell walls. However, textural difference appeared from the degree of intercellular contact, the cell shape and the appearance of cell surfaces. Cells in the fracture planes of firm potatoes had large intercellular contacts. In this case most of the cells were flat and cell surfaces showed folds and cracks. For mealy potatoes, it appeared that cells in the fracture planes had little intercellular contacts. The cells were round and turgid and had smooth surfaces. In conclusion, the structure of cell wall and middle lamella and the starch content, appear to be important parameters to distinguish firm and mealy cultivars.

[1] Technical and Physical Engineering Research Service (TFDL-DLO), Wageningen, The Netherlands

[2] State Institute for Quality Control of Agricultural Products (RIKILT-DLO), Wageningen, The Netherlands

Introduction

Texture is a quality parameter of cooked potatoes which varies from firm to mealy. The choice of a potato cultivar for particular processing may be based on textural properties of the potato tissue after cooking. Firm potatoes are suitable for processing into products in which pieces of tissue should be recognizable. For instance, in Germany this type of potatoes is used for the production of salads. On the other hand, a mealy texture is preferred for mashed potatoes. For this reason, mealy table potatoes are popular in The Netherlands. Knowledge of the parameters which determine the texture of potatoes may contribute to optimizing processing conditions and selection of suitable raw material.

Scanning electron microscopy (SEM) has previously been applied to study potatoes during processing. Generally, steam-cooked potato cells remained intact, but they had wrinkled surfaces (Fedec et al., 1977; Moledina et al., 1978). However, in these studies, tissue was pretreated by chemical fixation and dehydration. These techniques may alter the highly hydrated structures, cell walls and gelatinized starch, present in steam-cooked potato-tissue (Robards and Sleytr, 1985; Sargent, 1988). Physical fixation by cryo-methods results in better preservation of hydrated structures, although it may also alter structures due to ice crystal formation. Recently, it has been shown that the combination of physical fixation by cryo-methods and freeze-drying in comparison with chemical fixation and dehydration resulted in less wrinkled cell walls (Huang et al., 1990).

During cooking, pectin is degraded from the middle lamellae and cell walls, resulting in the separation of the potato cells (Burton, 1989). The degree of cell separation was not clearly visible in a section of cooked tissue composed of cross-sections of cells (Moledina et al., 1978), but sections with cell surfaces revealed better images (Fedec et al., 1977).

Additionally, the gelatinization of starch was clearly visualized. Heated cells were filled by gelatinized starch, which had a reticulated structure, and a void space was always left between cell walls and starch gel (Huang et al., 1990).

In this study, cryo-SEM was used to distinguish steam-cooked potato tissue of four cultivars based on the textural differences at the cellular level. To preserve the original hydrated structures of the tissues, we used cryo-fixation. The results revealed a relationship between cellular organization of the fracture planes and texture of the cultivar.

Table 3.1 Cooking classification of four potato cultivars (Parlevliet et al., 1991).

cultivar	type of cooking behaviour			
	A	B	C	D
Nicola	—	—		
Eersteling		—	—	—
Irene			—	—
Eigenheimer	—	—		

- type A: an especially firm, non mealy potato with a fine structure;
- type B: a firm, slightly mealy potato with a fine or rather fine structure;
- type C: a rather loose, mealy potato;
- type D: a loose, very mealy potato.

Materials and methods

Potatoes

Four potato cultivars, Eersteling, Eigenheimer, Irene and Nicola, were grown in 1990 on clay soil at our experimental station in the North East Polder, The Netherlands. Mature potatoes were harvested and potatoes with size 45/55 mm were stored at 6°C and 90-95% relative humidity. According to the classification described by Parlevliet et al. (1991), the four cultivars represented two extreme forms of texture (Table 3.1). This classification was based upon the appearances of whole cooked potatoes. The term "fine structure" stands for a regular surface structure of the whole potato, without disturbances. Potatoes with a mealy structure have surfaces with bursts and loose cells.

Determination of starch

The starch content of the cultivars was assessed by weighing ten potatoes of each cultivar in water. A washed and dried unpeeled potato was weighed in air (dry weight = DW) and under water at 10°C (under water weight = UWW). By using the DW and UWW, we calculated the starch content of the tuber as described by Burton (1989).

Cryo-scanning electron microscopy

For cryo-SEM, five hand-peeled (about 18% weight loss) potatoes of each cultivar were steam-cooked separately with demineralized water for 30 minutes (under normal pressure) and broken into two halves.

Fracture planes were obtained before freezing by cutting pieces of the internal phloem storage parenchyma tissue (2x3x5 mm^3) from the fracture planes of the two halves.

Table 3.2 The mean starch content on fresh weight of four potato cultivars assessed by weighing in water.

cultivar	starch (% f.w.)
Nicola	12.5 ± 2.1
Eersteling	15.3 ± 1.2
Irene	18.2 ± 2.0
Eigenheimer	18.8 ± 2.0

The pieces of tissue, with the fracture plane (2x5 mm^2) up, were mounted on brass stubs using carbon cement. The stubs were immersed in nitrogen slush (about 60°K), using a Hexland CT1000/CP2000 cryo-system. The samples were etched (20-30 minutes at about 0.1 Pa and 190°K) in a Philips SEM 535, equipped with a cold stage. Sputtering with gold took place in the Hexland cryo-system (2 minutes at 6.5 Pa and 100°K). Cross-sections were obtained after freezing by fracturing the frozen tissue with a cooled razor blade in the Hexland cryo-system before etching. The samples were examined in the Philips SEM 535 at 15 kV accelerating voltage. During etching, sputtering and examination the temperature of the anticontaminator was held at 90°K (Robards and Sleytr, 1985; Sargent, 1988).

Results

Starch content

The starch content of the four cultivars was determined to investigate its correlation with texture (Linehan and Hughes, 1969). The results are given in Table 3.2.
It appeared that the mealy-cooking cultivars, Irene and Eigenheimer, had a higher starch content in comparison with the firm-cooking cultivars, Nicola and Eersteling.

Cryo-scanning electron microscopy

By using cryo-SEM, fracture planes of non-cooked and cooked potatoes were examined at the same magnification. For non-cooked potatoes, fracturing took place through cells leaving the starch granules intact. This way of fracturing appeared similar for all cultivars studied. Fig. 3.1 shows a fracture plane of the cv. Nicola. The parenchyma cells had large intercellular contacts and only a few intercellular spaces were present. The numerous starch granules present in non-cooked potato cells were clearly visible.
In contrast to non-cooked potatoes, fracturing of steam-cooked potatoes took place

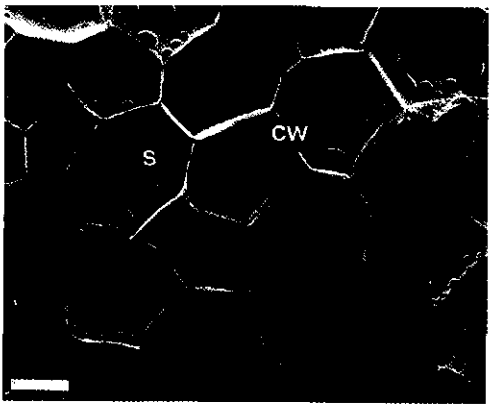


Fig. 3.1 Fracture plane of non-cooked potato tissue of the cv. Nicola. Bar=0.1 mm. s=starch granule; cw=cell wall.

between cells preferentially alongside the cell walls (Fig. 3.2). Further, significant differences could be observed between fracture planes of the firm-cooking cultivars, Nicola and Eersteling, and the mealy-cooking cultivars, Irene and Eigenheimer. The cultivars Nicola and Eersteling had fracture planes with a generally flat appearance and large intercellular contacts were visible (Fig. 3.2A and 3.2B). In contrast to the firm-cooking cultivars, the fracture planes of the cultivars Irene and Eigenheimer appeared to be rougher and intercellular contacts were small (Fig. 3.2C and 3.2D). Higher magnifications revealed more details concerning the cell surfaces which form the fracture planes. For the firm-cooking cultivars, Nicola and Eersteling, most of the cell surfaces had little folds and cracks (Fig. 3.3A and 3.3B). However, some cell surfaces appeared round and possessed many cracks with a reticulated structure. For the mealy-cooking cultivars, Irene and Eigenheimer, cells appeared round and turgid. Frequently, remnants of the shape of the non-cooked cells were recognizable (Fig. 3.3C and 3.3D). Most of the cell surfaces were smooth, but some cells had wrinkled surfaces.

Although for cooked potatoes of all cultivars fracturing took place between cells, the appearances of the cell surfaces were clearly different by comparing the firm-cooking with the mealy-cooking cultivars. To prove the fact that for firm-cooking potatoes

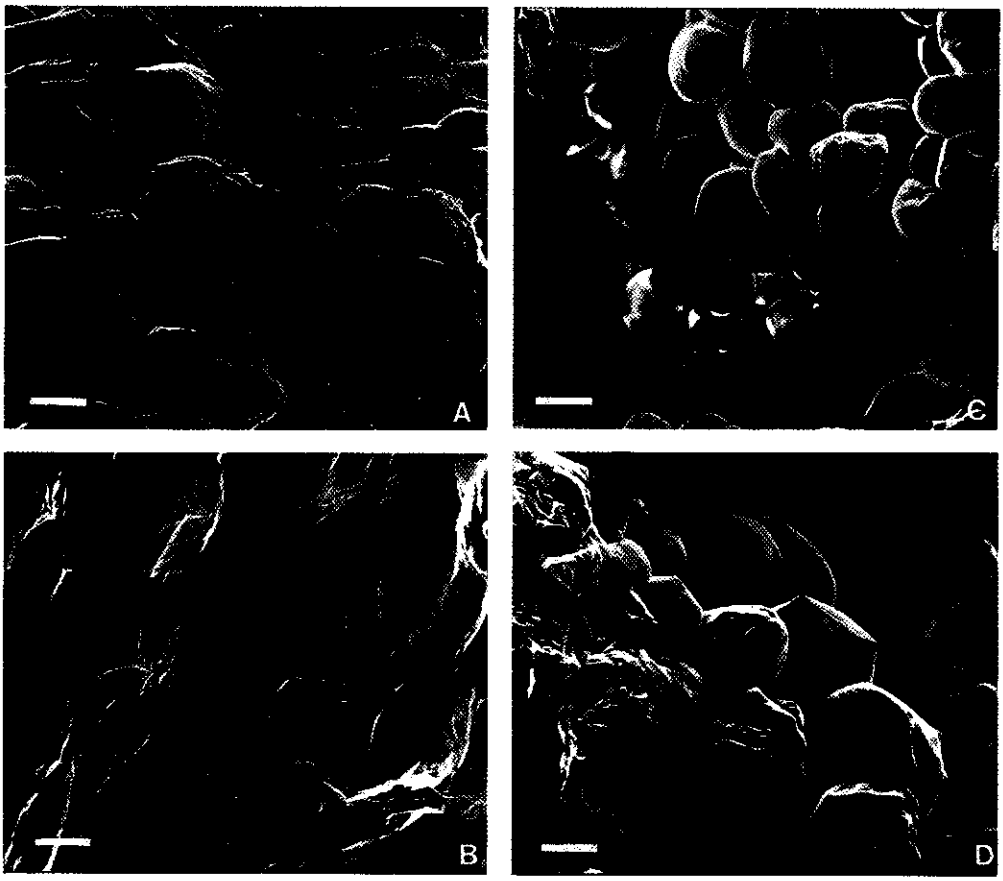
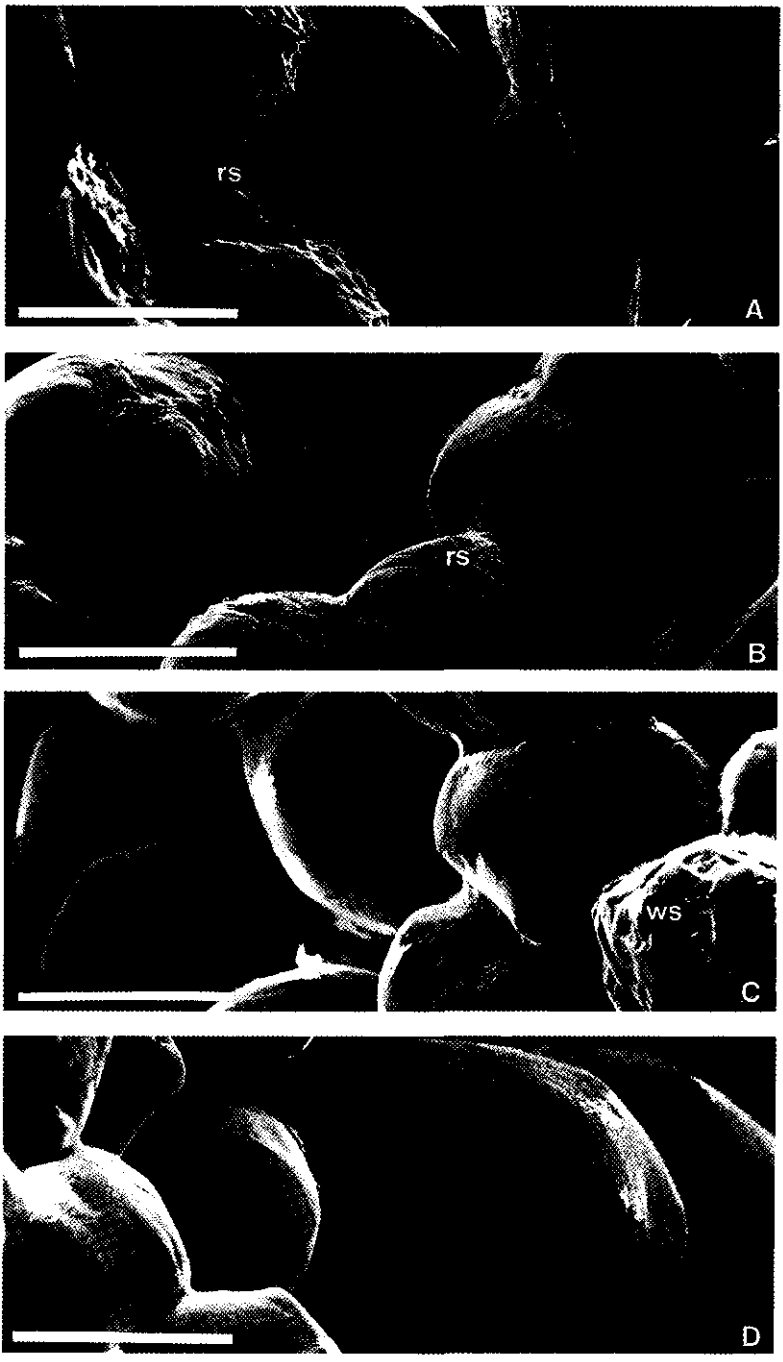


Fig. 3.2 Fracture planes of steam-cooked potato tissue of four cultivars. The fracture planes of the cultivars Nicola (A) and Eersteling (B) have a flat appearance. Those of the cultivars Irene (C) and Eigenheimer (D) have a rough appearance. Bar=0.1 mm.

Fig. 3.3 (facing page) Cells in the fracture planes of steam-cooked potato tissue of the four cultivars. The cultivars Nicola (A) and Eersteling (B) have cell surfaces showing cracks with reticulated structures. The cultivars Irene (C) and Eigenheimer (D) have round, turgid cells with smooth surfaces, although one cell shown has a wrinkled surface. Bar =0.1 mm. rs=reticulated structure; ws=wrinkled surface.


rs
A
rs
B
ws
C
D

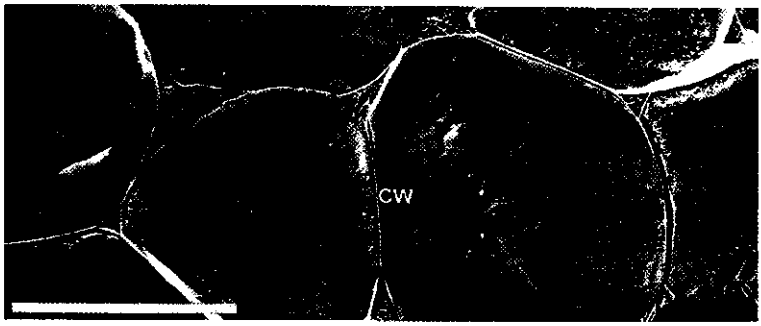


Fig. 3.4 Cells in a cross-section of steam-cooked potato tissue of the cv. Nicola. The cell walls are visible as clear lines. Bar=0.1 mm. cw=cell wall.

fracturing took place between cells, rather than through cells, fracture planes and cross-sections were compared for each piece of potato. In this case, the cross-sections revealed the structures, which would be visible if fracturing took place through cells. In Fig. 3.4, a cross-section of the cultivar Nicola is shown. The cell walls between adjacent cells were visible as clear lines. Furthermore, the individual starch granules, present in non-cooked cells, had disappeared and the cooked cells are filled up with gelatinized starch. For all cultivars, the observed differences between cell surfaces and cross-sections were clear.

Discussion

According to Parlevliet et al. (1991) cooked potatoes of the cultivars Nicola and Eersteling were firm and those of the cultivars Irene and Eigenheimer were mealy. This study showed that these cultivars could similarly be classified by comparing their tissue structures by using cryo-SEM. Moreover, this technique revealed clear differences at the cellular level between the firm and mealy potatoes.
For mealy potatoes, it appeared that the intercellular contact was clearly diminished, resulting in cells with a round and turgid appearance. Occasionally, the straight edges and corners of the fresh cells were recognizable (Fig. 3.1, 3.2C and 3.2D). Most of the cells had smooth surfaces (Fig. 3.3C and 3.3D). In contrast, in firm potatoes large intercellular contacts were preserved and most of the cells were flattened with cracks and folds in the surfaces (Fig. 3.2A and 3.2B).
Comparing details of cell surfaces in the fracture planes of the cv. Nicola (Fig. 3.3A) with cross-sections of the same tissue at the same magnification (Fig. 3.4) revealed

that the structures seen at the cell surfaces in the fracture planes were ruptured cell walls. These structures did not have the reticulated structure of the cell contents (Huang et al., 1990; Pagani et al., 1989) as was shown by cross-sections. Thus, in firm potatoes fracturing took place between cells and the cell walls appeared to be ruptured (Fig. 3.3A and 3.3B). In contrast, fracture planes of mealy potatoes showed smooth cell surfaces, which suggested that the cell walls remained intact.

Cells and cell walls visualized by cryo-SEM were less wrinkled in comparison with results of previous SEM-studies, in which chemical fixation and dehydration (Fedec et al., 1977; Moledina et al., 1978) or physical fixation (Huang et al., 1990) followed by freeze-drying was applied as a preparation-technique. The few cells with wrinkled surfaces in the fracture planes of mealy potatoes probably collapsed during preparation of the samples. In comparison with previous studies (Moledina et al.,1978; Huang et al., 1990), little void space between cell walls and gelatinized starch was visible, indicating that cryo-SEM gave less shrinkage of the gelatinized starch.

Cell surfaces of the round cells of potatoes of the cultivars Nicola and Eersteling had cracks and reticulated structures (Fig. 3.3A and 3.3B). The nature of the reticulated structure was not clear. It might be composed of amylose, because leaking of amylose was also mentioned by Hoover (1981) and Fedec et al. (1977). Secondly, these structures might be formed during breakdown of the cell wall (Haydar et al., 1980; Moledina et al., 1978).

Mealy and firm potatoes differed clearly with respect to intercellular contact, cell shape and cell surface. These differences could be explained by different breakdown of (i) the middle lamella or (ii) the cell wall or (iii) by the starch content of the potatoes.

During cooking, the conditions in potato tissue cause the breakdown of pectin (Hughes et al., 1975), the major component of the middle lamella. This process results in a decline of the intercellular adhesion (Burton, 1989). Therefore the small intercellular contacts observed between cells in mealy potatoes might be the result of an almost complete breakdown of the middle lamella. Consequently, fracturing between cells could take place easily, resulting in intact cell surfaces. In the potatoes classified as firm, large parts of the middle lamella might still be present, explaining the cracks on the cell surfaces, which occurred upon fracturing. Sterling and Aldridge (1977) also mentioned this difference in intercellular contact after cooking, depending on texture. Additionally, general cell wall degradation (Burton, 1989) will occur during cooking. The results of our study suggested that the cell walls of firm potatoes might be degraded more depending upon the original structure or on the reaction conditions in the tissue (for example the ionic strength) (Shu-I et al., 1988). The observed cracks and loosening of the cell wall structures could explain the leakage of amylose (Fedec et al., 1977). Further microscopic research (e.g., transmission electron microscopy) may give

more detailed information about breakdown of cell walls and middle lamellae.
Finally, the amount of starch may contribute to the difference in texture. A previous study indicated that starch concentrations up to 30% enhanced the rigidity of the starch gel (Ring, 1985). Since the mealy cultivars had a higher starch content than the firm cultivars (Table 3.2), one could imagine that in mealy potatoes the starch gel formed upon cooking was more rigid. This resulted in round cells with a more turgid appearance due to a higher swelling pressure (Jarvis et al., 1992) and more resistance to forces exerted during fracturing.
We concluded that differences in texture of cooked potatoes were also visible on an ultrastructural level. The results of this study suggested that the structure of the cell wall, the middle lamella and the starch content are important parameters in determining firm and mealy texture after cooking. Further research will be aimed at the (bio-)chemical characterization of cell walls and middle lamellae of different cultivars.

Acknowledgements

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Chapter 4

Comparison of the cooking behaviour of the potato cultivars Nicola and Irene with respect to pectin breakdown and the transfer of ions

J.T. van Marle, C. van Dijk, A.G.J. Voragen[1] and E.S.A. Biekman

Potato Research, 1994, 37, 183-195

Summary

The potato cultivars Nicola and Irene were investigated with respect to cell sloughing, specific cell surface area, release of pectic material and transfer of ions using a model cooking study. Both cell sloughing and the release of pectic material were higher for the mealy cooking cultivar Irene. As the respective cell size distributions measured were the same for both cultivars, the difference in release of pectic material could not be explained by a difference in the specific cell surface areas between the cultivars. Additionally, it was recorded that during cooking only 20% of the calcium present in non-cooked tissue was transferred into the cooking medium, whereas more than 70% of potassium and citrate were transferred. The ratios $D_{a,citr2-}/D_{a,K+}$ and $D_{a,Ca2+}/D_{a,K+}$ for water and cooked potato tissue revealed that in water, potassium ions diffused faster than citrate and calcium ions. However, in cooked potato tissue both citrate and calcium ions diffused faster than potassium ions.

[1] Agricultural University, Department of Food Science, Wageningen, The Netherlands

Introduction

Texture is an important quality parameter of processed potatoes. Research is being performed to understand the chemical structure and the physical behaviour of both non-cooked and cooked tissue to gain an insight into texture changes of potatoes during cooking. A previous study indicated that the structure of the cell wall and the structure of the middle lamella appeared to be important factors determining texture (Van Marle et al., 1992).

For non-cooked tissue of Dicotyledonae, the primary wall may be represented as a network of cellulose microfibrils with xyloglucans interlocking this cellulosic framework. Part of the xyloglucans form a monolayer coating the microfibrils and part will span the space between the microfibrils. The cellulose-xyloglucan framework is embedded in a pectin matrix. The pectic polysaccharides are thought to perform many functions, such as the definition of cell wall porosity and the provision of charged surfaces capable to modulate the cell wall pH and ion balance (Carpita and Gibeaut, 1993). The middle lamella is the interstitial layer of the cell walls of two adjacent cells and can be distinguished from the primary wall by its relative higher concentration of pectin (Keijbets, 1974). Jarvis et al. (1981) and Ryden and Selvendran (1990) determined the composition of isolated cell walls of potatoes. On average the cell walls are composed of 19% middle lamella components (of which 77% are uronic acids), 29% cellulose, 10% xyloglucans and 35% pectin (of which 38% are uronic acids).

Pectate generally forms calcium gels in which the junction zones are composed of unbranched, non-esterified galacturonan chains, linked together by non-covalently bonded calcium ions. The parts of the pectin chains with a higher degree of esterification and containing more side chains are supposed to be more flexible and less susceptible to aggregation (Jarvis, 1984).

During cooking, the distribution of ions within the tissue changes as a result of the increased permeability of the cell membrane above 60°C (Bartolomé and Hoff, 1972). Ions migrate from the cytoplasm into the cell wall thereby affecting the conditions for reactions in the cell wall and the middle lamella. Keijbets and Pilnik (1974) observed that the pectin breakdown was enhanced by both cations and anions. Kohn and Furda (1967) observed a decrease in the stability constant of the calcium-pectate gel as a result of increased ionic strength. Specific actions of defined ions have also been reported. Bartolomé and Hoff (1972) reported the possibility of cell wall strengthening by calcium ions migrating from the gelatinized starch into the cell wall, where calcium is necessary for the formation of a calcium-pectate gel. Selvendran et al. (1990) proposed that enhanced middle lamella breakdown in a mealy cultivar may be the result of retarded citric acid leakage. Citrate, abundantly present in potatoes (Burton,

1989), can form a stable complex with calcium and thus is a competitor of pectate for calcium.

Due to thermal treatment, pectic material is converted as a result of chemical degradation and activation of pectin esterase (PE). On heating, chemical degradation of the pectin chain in potato tissue takes place according to the ß-eliminative mechanism (Keijbets, 1974). This results in cleavage of the pectin chain next to a methyl esterified galacturonic acid residue. This depolymerisation reaction becomes less pronounced with decreasing degree of esterification. Native potato PE, which is active up to 70°C may decrease the degree of esterification thereby counteracting ß-elimination and promoting pectate gel formation.

Part of the pectic material is released into the cooking medium. This process is supposed to affect the strength of the primary wall and the intercellular cohesion, eventually resulting in cell sloughing.

From the discussion given above, it may be obvious that the influence of cooking on texture is very complex and that several distinct and mutually interactive processes are involved. In this study, research is focused on the release of pectic material and the transfer of ions (citrate, calcium, potassium) during cooking of potato tissue. Hughes et al. (1975) studied the release of pectic material for one potato cultivar. However, in this study we used two cultivars that exhibited large differences in texture characteristics to compare their behaviour under identical conditions. To estimate the influence of ion transfer on texture changes, the ratios $D_{a,citr2-}/D_{a,K+}$ and $D_{a,Ca2+}/D_{a,K+}$ were calculated for the two potato cultivars. The possible effect of cell size on texture is obvious and cell sizes were measured by microscope (Hughes et al., 1975; Baier and Pichert, 1988). To overcome the disadvantage that only very limited numbers of cells can be measured, a Coulter Counter was used to measure cell sizes of a cell suspension.

Materials and methods

Potatoes

Two potato cultivars, Nicola and Irene, were chosen because of their large differences in texture. They were grown in 1991 on clay soil at our experimental station in the North East Polder, The Netherlands. The following fertilizers were applied: organic manure (19 t/ha, August 1990), potassium (315 kg/ha, October 1990), phosphate (225 kg/ha, October 1990) and additional nitrogen (170 kg/ha, March 1991). Potatoes were planted on 3 April. Haulm was killed on 4 September and harvesting took place on 11 September. Potatoes with size 45 - 55 mm were stored at 6°C and 95% relative humidity for 2 months and samples were taken for experiments.

Model cooking study

Approximately 70 potatoes of each cultivar were randomly selected. Disks of tissue, 17 mm dia and 5 mm thick were cut from the internal phloem parenchyma. The disks were randomized and rinsed in demineralized water and blotted with tissue paper. Batches of 25 disks were transferred to 500 ml erlenmeyer flasks containing 250 ml boiling demineralized water. The flasks were equipped with a condenser to prevent water evaporation (Selvendran et al., 1990). From the time boiling recommenced, it was continued for different periods from 150 seconds to 1800 seconds. After cooking, the disks were separated from the cooking medium by sieving (mesh: 2.00 mm). Each flask was rinsed with 50 ml demineralized water and the contents of the flasks were added to the cooking medium. For each cooking period, 4 batches of 25 disks were combined. The disks were frozen in liquid nitrogen, freeze-dried and milled (Retsch-mill, Retsch GmbH, Haan, Germany). Part of the cooking medium was directly frozen; the remainder was vacuum filtered through a GF/C-glass fibre filter (Whatman, Maidstone, UK) and the filtrate was also frozen. The residue was discarded. Two batches of 70 potatoes were processed for each cultivar.

Analytical methods

Number of cells

The number of cells in the cooking medium was counted with a stereo-microscope. A 24-well plate was filled with 20 aliquots of either 50 or 100 µl unfiltered cooking medium. The cells were stained with methylene blue.

Dry matter content

Small cut, non-cooked potato (10 g) was pre-dried overnight at 70°C and drying was continued for 20 hours at 105°C. Dry matter content was determined on three replicates.

Uronic acid content

Uronic acid contents in the filtered cooking medium and in freeze-dried potato were determined using the m-hydroxydiphenyl method of Ahmed and Labavitch (1977). Samples of 0.2 ml were used and amounts of other reagents were decreased proportionally. Determinations were made on three replicates.

Freeze-dried potato (0.10 g) was pretreated with 12 M H_2SO_4 and was further hydrolysed with 1 M H_2SO_4 at 100°C for 2 hours. A factor of 1.35 for correction of the amount of starch present in the samples was determined according to Kintner and Van Buren (1982). The filtered cooking medium was centrifuged at 12,000 x g for 5 minutes and uronic acid content determined on the supernatants.

Starch content

Starch content was determined enzymatically using the Boehringer test kit (Mannheim GmbH, Mannheim, Germany) on at least two replicates.

Citrate content

Citrate content in freeze-dried potato and filtered cooking medium was determined enzymatically using the Boehringer test kit. Freeze-dried potato (1 g) was suspended in 50 ml demineralized water and mixed (Ultra-turrax T25, Janke and Kunkel GmbH, Staufen, Germany) for 30 seconds, followed by the addition of another 50 ml water. Prior to analysis, this suspension (1.5 ml) as well as the filtered cooking medium (1.5 ml) were centrifuged at 12,000 x g for 5 minutes. The supernatants were used for further analysis. At least two determinations were made on each sample.

Calcium, potassium and ash content

Freeze-dried potato (1 g) was ashed at 500°C for 4 hours and weighed. The ash was moistened with a few drops of 25% HCl-solution and dried on a steam bath. This treatment was performed twice. The residue was dissolved in 1 ml 25% HCl-solution and 1.00 g lanthanum (as $LaCl_3.7H_2O$, Sigma L6640) was added and the solution was diluted to 100 ml. After 3 hours the solution was filtered through a GF/C-glass fibre filter and then stored in a polythene container at 4°C. Three samples for ashing were taken from each batch.

The filtered cooking medium was again filtered through a GF/C-glass fibre filter and the calcium and potassium contents determined by AES (Flame Photometer PFP7, Jenway Ltd, Essex, UK). Two determinations were made on each sample.

Osmolarity

Three measurements of the osmolarity of potato juice using an Osmomat 030 (Gonotec, Berlin, Germany) were made on each sample.

Cell size measurement

For each cultivar, the internal phloem parenchyma tissue of 20 potatoes was cut into small pieces. The tissue was enzymatically macerated using Rohament P (an enzyme preparation with endo-polygalacturonase activity; Röhm GmbH, Darmstadt, Germany) (Biekman, 1992). The maceration was performed in an isotonic NaCl-solution. The cell size distribution of the cell suspension was determined using a Coulter Counter (LS130, Coulter Electronics, Mijdrecht, The Netherlands). The cell size distributions were determined on two replicates.

Theory

Based on the overall mass balance:

$$C_{T,0}*V_{T,0}=C_{T,t}*V_{T,t}+C_{L,t}*V_{L,t} \quad [1]$$

and the mass balance for the cooking medium:

$$\frac{\partial\, C_{L,t}*V_{L,t}}{\partial\, t}=k_S*A*(m*C_{T,t}-C_{L,t}) \quad [2]$$

where:
- $C_{T,0}$ = solute concentration in tissue fluid at t=0 (mol/m^3)
- $C_{T,t}$ = solute concentration in tissue fluid at time t (mol/m^3)
- $C_{L,t}$ = solute concentration in cooking medium at time t (mol/m^3)
- $V_{T,0}$ = volume of tissue fluid at t=0 (m^3)
- $V_{T,t}$ = volume of tissue fluid at time t (m^3)
- $V_{L,t}$ = volume of cooking medium at time t (m^3)
- k_S = mass transfer coefficient between potato tissue and water (m/s)
- A = disk surface (m^2)
- m = distribution coefficient of solute between water and potato tissue (-)

and assuming constant volumes V_L and V_T and m = 1, the following equation was derived after integration of Eq. [2] to describe the transport of solutes out of the tissue:

$$C_{L,t}=\frac{1}{\beta}*C_{T,0}*\left(1-\exp\left(\frac{-\beta*k_S*A*t}{V_L}\right)\right) \quad \text{with} \quad \beta=\frac{V_L}{V_T}+1 \quad [3]$$

The equilibrium solute concentration, $C_{L,\infty}$, at infinite time is given by:

$$C_{L,\infty}=\frac{1}{\beta}*C_{T,0} \quad [4]$$

The experimental data were fitted with Eq. [3] using non-linear regression (Genstat; Anon., 1987).

Table 4.1 Characterization of non-cooked parenchyma tissue of cultivars Nicola and Irene. Values are given as g/100 g fresh weight, unless mentioned otherwise. For each cultivar the mean of the values determined for the two batches is given. (SD(n-1)).

	cv. Nicola	cv. Irene
dry matter	17.9 ± 0.5	22.0 ± 0.1
starch	12.1 ± 0.4	15.3 ± 0.3
galacturonic acid	0.285 ± 0.006	0.318 ± 0.019
ash	1.00 ± 0.04	1.26 ± 0.07
calcium	0.008 ± 0.0002	0.009 ± 0.0001
potassium	0.314 ± 0.012	0.395 ± 0.022
citrate	0.385 ± 0.002	0.570 ± 0.035
cell size (μm)	215 ± 65	206 ± 54
osmolarity (kmol/m^3)	0.38 ± 0.003	0.40 ± 0.01

Results and discussion

Tissue characteristics

Chemical and physical characteristics were determined on the non-cooked parenchyma tissue of the non-mealy cooking cv. Nicola and the mealy cooking cv. Irene (Table 4.1). All the constituents analyzed occurred in significantly higher ($P<0.05$) concentrations in the cv. Irene than in the cv. Nicola. However, the average cell size did not differ significantly ($P<0.05$) between the cultivars.

Cell sloughing

The increase in the number of cells in the cooking medium as function of the cooking time was a measure of the difference in cooking behaviour between the cultivars Nicola and Irene (Fig. 4.1). For cv. Irene, the number of sloughed cells increased considerably between 300 seconds and 900 seconds and then remained almost constant. For cv. Nicola, cell sloughing started after approximately 900 seconds and remained low in comparison with cv. Irene. The observed difference in cell sloughing clearly indicated differences in texture between the two cultivars. The mealy cooking cv. Irene with its loose surface appearance (Marle et al., 1992) released more cells during cooking.

Release of pectic material

The release of pectic material, which accompanied cell sloughing, is shown in Fig. 4.2.

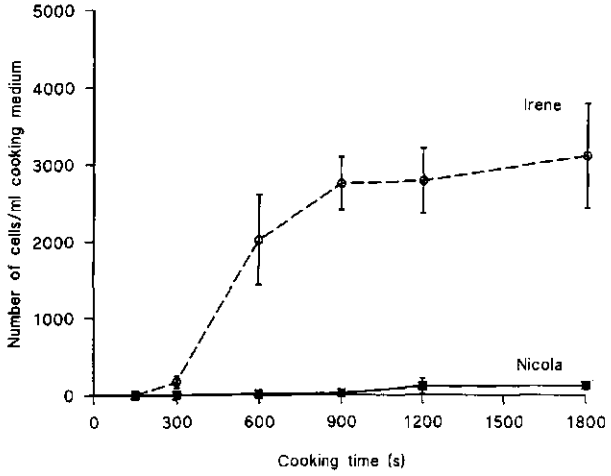

Fig. 4.1 Cell sloughing for cultivars Nicola and Irene at various cooking times. Bars indicate standard deviation (SD(n-1)) between values determined for the two batches.

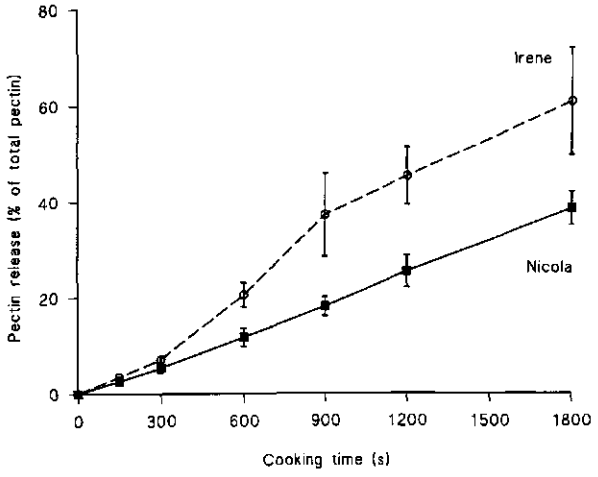

Fig. 4.2 Amounts of pectin released into the cooking medium at various cooking times, expressed as the percentage of pectin present in non-cooked tissue. Bars indicate standard deviation (SD(n-1)) between values determined for the two batches.

For cv. Irene, pectic material was released over the whole cooking period. However, the rate of release of pectic material was highest between 300 and 900 seconds and coincided with the increase in cell sloughing. For cv. Nicola, the rate of release of pectic material was almost constant up till 1800 seconds. The total amount of released

pectic material was lower over the whole cooking period in comparison with cv. Irene. From Fig. 4.1 and 4.2 it can be seen that at a given percentage release of pectic material cv. Irene showed more cell sloughing than cv. Nicola. For example, the measured percentage of pectin released after 1800 seconds cooking for cv. Nicola was comparable with that after 900 seconds cooking for cv. Irene, whereas at those cooking times the number of cells in the cooking medium was higher for cv. Irene than for cv. Nicola. Therefore we suggest that the way pectin is degraded, thereby affecting cell adhesion, is cultivar dependent.

It can be expected that the release of pectic material is affected by the cell surface area from which the pectin is transported. A cube of tissue composed of large cells has a smaller total cell surface area than a similar sized cube of small cells. Therefore differences in specific cell surface area need to be considered when calculating the release of pectic material. In potatoes, the intercellular spaces occupy 0.3-0.9% of the total volume (Es and Hartmans, 1981) and so may be disregarded in this calculation. As the cell size distributions of cultivars Nicola and Irene were similar (Fig. 4.3), this implies that both cultivars had the same specific cell surface area. Therefore the specific cell surface area is unlikely to account for the difference in release of pectic material.

Difference in the release of pectic material between the cultivars might result from differences in susceptibility of pectic material to chemical degradation, in PE-(in-) activation or in the transfer rates of ions, resulting in different ionic conditions in the cell wall and the middle lamella.

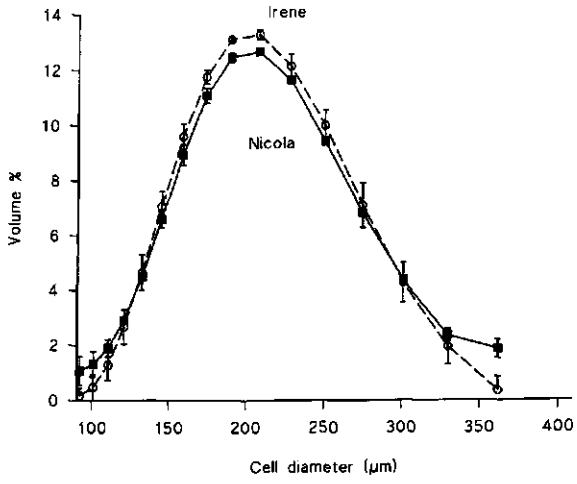

Fig. 4.3 Cell size distributions of parenchyma tissue of cultivars Nicola and Irene measured with a Coulter Counter. Bars indicate standard deviation (SD(n-1)) between the two replicates.

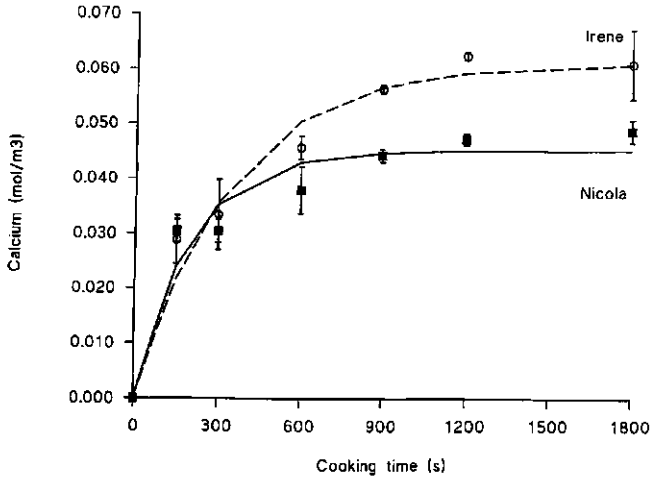

Fig. 4.4 Transfer of calcium ions into the cooking medium after various cooking times. Bars indicate standard deviation (SD(n-1)) between values determined for the two batches.

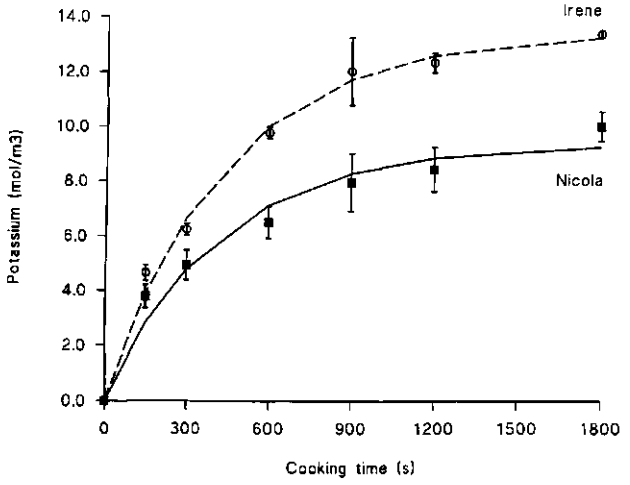

Fig. 4.5 Transfer of potassium ions into the cooking medium at various cooking times. Bars indicate standard deviation (SD(n-1)) between values determined for the two batches.

Transfer rates of ions

The transfer rates of calcium, potassium and citrate ions into the cooking medium were determined to investigate their contribution to the difference in texture between the two cultivars. Potassium, the most abundant cation in potatoes (Burton, 1989), was measured to compare its diffusion behaviour with bivalent calcium cations and bivalent

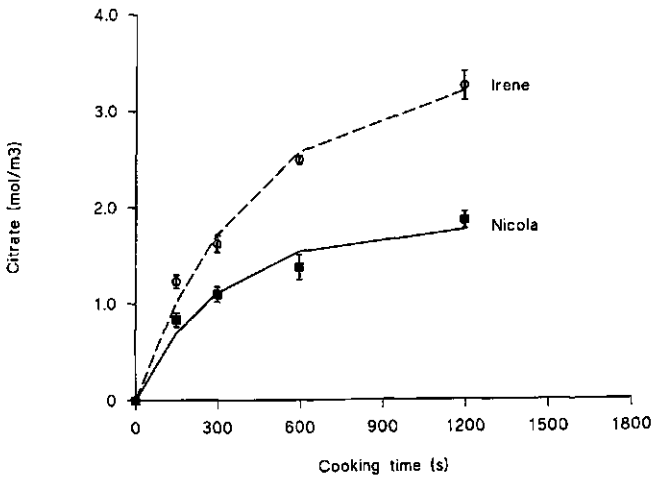

Fig. 4.6 Transfer of citrate ions into the cooking medium at various cooking times. Bars indicate standard deviation (SD(n-1)) between values determined for the two batches.

citrate anions. The increase in concentration of the three ions in the cooking medium as a function of time was fitted with Eq. [3] using non-linear regression (Fig. 4.4, 4.5 and 4.6). To prove the assumption that V_L and V_T are approximately constant, the change in weight of individual disks during cooking (up to 1200 seconds) was recorded. It was observed that disks of cv. Nicola lost up to 5% wet weight, whereas those of cv. Irene gained up to 11% wet weight (data not shown). However, the calculated values for the parameters k_S*A and $C_{T,0}$ did not change significantly when these changes in volume were taken into account. This observation justified the assumption that both V_L and V_T are constant. The calculated values for the parameters k_S*A and $C_{T,0}$ are given in Table 4.2.

From the results given in Table 4.2 it can be seen that the % variance accounted for is high for non-linear regression. k_S*A is a measure of the transfer rate and values for calcium and citrate were significantly ($P<0.05$) higher for cv. Nicola than for cv. Irene. The values for potassium were not significantly ($P<0.05$) different between cultivars. These results were unexpected because the mealy cooking cv. Irene should have shown higher k_S*A-values due to the increase in surface area during cooking as a result of sloughing.

The value for $C_{T,0}$ of each ion was determined in the non-cooked tissue and was also calculated from Eq. [3] using the measured concentrations of the respective ions in the cooking media. Fig. 4.4, 4.5 and 4.6 show that the transfer of the three ions reached

Table 4.2 Measured values for $C_{T,0}$ (mean of two batches) and calculated values for the parameters k_S*A, $C_{T,0}$ and $C_{L,-}$ from Eq. [3] and [4] for cultivars Nicola and Irene.

Solute	$C_{T,0}$ measured (mol/m^3)	k_S*A calculated *10^{-7} (m^3/s)	$C_{T,0}$ calculated (mol/m^3)	$C_{L,-}$ calculated (mol/m^3)	% variance accounted for
Nicola					
Calcium	2.42 ±0.07	4.85 ±0.81	0.47 ±0.02	0.047 ±0.002	92.6
Potassium	97.7 ±4.3	2.28 ±0.32	97.8 ±4.6	9.72 ±0.46	95.3
Citrate	24.5 ±0.3	3.08 ±0.46	18.8 ±1.0	1.83 ±0.10	96.1
Irene					
Calcium	3.02 ±0.02	2.62 ±0.37	0.68 ±0.03	0.062 ±0.003	94.8
Potassium	129.5 ±7.1	2.04 ±0.16	150.0 ±4.0	13.6 ±0.4	98.7
Citrate	38.2 ±2.3	2.11 ±0.22	38.0 ±1.7	3.4 ±0.2	98.5

an equilibrium within 1800 seconds cooking. A lower calculated than measured $C_{T,0}$-value indicates an established equilibrium different from that expected. The difference between the measured and calculated $C_{T,0}$-value is a measure of the amount of ions which are retarded in the tissue and therefore do not contribute to the equilibrium.
For cv. Irene the citrate concentration reached the equilibrium concentration after 1200 seconds cooking. However, for cv. Nicola the citrate concentration was lower than the equilibrium concentration. The potassium concentration of the cooking medium of both cultivars reached the equilibrium concentration within 1800 seconds cooking.
For both cultivars the calculated $C_{T,0}^{Ca2+}$-value was approximately 20% of the measured $C_{T,0}^{Ca2+}$-value (Table 4.2) and this implies that approximately 80% of the calcium was retarded in the tissue. Lamberg (1990) also observed different transfer behaviour of calcium ions as compared with that of citrate and potassium ions. This observation can be explained in two ways. Calcium is present in starch granules, in cell walls and in the cytoplasm and its relative distribution over the three parts is difficult to measure. In literature calcium in the cell walls ranges from 20-40% with 10% calcium in the starch grains (Keijbets, 1974) or 40% calcium in the cell walls and 40% calcium in the starch grains (Bartolomé and Hoff, 1972). However, calcium from different parts may have a different transfer behaviour during cooking. For example, calcium ions in the cytoplasm can be directly transferred through the cell walls whilst calcium ions in the starch granules can be transferred after gelatinization of the starch. Alternatively, the explanation for the retardation of calcium ions in the tissue may be

because calcium ions are able to interact with the negatively charged groups which are present both in pectin and in gelatinized starch (Bartolomé and Hoff, 1972; Haydar et al., 1980). Based on our results it can be supposed that this interaction decreases the apparent diffusion rate of calcium. In addition the potential of calcium ions to interact increases as a result of cooking. The number of binding-sites changes as more phosphate groups become accessible in gelatinized starch, the selectivity constant of pectin, K_K^{Ca}, increases with increasing ionic strength and decreasing degree of methylation (Kohn and Furda, 1967) and the activity of calcium-ions decreases with decreasing degree of esterification (Kohn and Luknár, 1975). Increasing ionic strength and decreasing degree of methylation are both favoured during cooking (unpublished results).

To estimate the hindrance exerted on the transfer of ions in cooked potato tissue, the apparent diffusion coefficient, D_a, of a specific ion can be compared with the respective diffusion coefficient, D, in water (Lamberg, 1990). From our results the D_a can be estimated from k_S*A:

$$D_a = \frac{k_S * A * 0.5 * d}{A} \qquad [5]$$

where: d = thickness of potato disk (m)

However, the surface area of the tissue disks changed during cooking as a result of sloughing. This change in surface area was different for the two cultivars. To account for this change in surface area the ratios of the apparent diffusion coefficients for citrate, $D_{a,citr2-}$, with potassium, $D_{a,K+}$, and calcium , $D_{a,Ca2+}$, with potassium were calculated (Table 4.3).

$$\frac{D_{a,x}}{D_{a,K^+}} = \frac{(k_S * A)_x}{(k_S * A)_{K^+}} \quad \text{with} \quad x = \text{citrate}, Ca^{2+} \qquad [6]$$

Potassium ions diffused faster in water than citrate ions, but citrate ions diffused faster in cooked potatoes (Table 4.3). Apparently, potassium ions were also able to interact with negatively charged groups of pectin and gelatinized starch and this decreases their apparent diffusion coefficient. In potato tissue of both cultivars, calcium ions diffused faster than potassium ions (Table 4.3), but this was only true for the 20% of calcium which was extracted from the tissue. The remaining calcium ions were not transferred, possibly because of complexation. Lamberg (1990) observed a deviant ratio for calcium and potassium (Table 4.3), but in her calculations the values for short

Table 4.3 The ratios $D_{a,citr2-}/D_{a,K+}$ and $D_{a,Ca2+}/D_{a,K+}$ calculated (Eq. [6]) for cultivars Irene and Nicola, for data obtained by Lamberg (1990) and for water of 25°C with $D_{K+} = 1.844*10^{-9}$ m²/s, $D_{Ca2+} = 1.110*10^{-9}$ m²/s and $D_{citr2-} = 0.661*10^{-9}$ m²/s (Weast, 1973).

Ratio	Water[1]	cv. Nicola	cv.Irene	Lamberg (1990)[2]
$D_{a,citr2-}/D_{a,K+}$	0.36	1.35	1.03	1.06
$D_{a,Ca2+}/D_{a,K+}$	0.60	2.13	1.28	0.77

[1] The value of D is dependent on temperature and on the equivalent conductance of the specific ion. For all ions the change in temperature is the same and the change in equivalent conductance is in the same order of magnitude in aqueous solutions (Weast, 1973). Therefore the ratio in water of 25°C can be used as comparison for the ratios in potato tissue, cooked at 100°C.

[2] The D_a-values for tissue heated for 20 minutes at 90°C were used to calculate the ratio.

extraction times (up to 8 minutes) were excluded and this may explain the difference. The transfer of calcium and citrate ions appeared to be cultivar dependent, whereas the transfer of potassium ions appeared to be cultivar independent. However, the clear difference between citric acid leakage for a mealy cultivar and a non-mealy cultivar, as reported by Selvendran et al. (1990), was not observed for the cultivars Nicola and Irene. To study the effect of ion-transfer on changes in the cell wall and the middle lamella, the structure of pectic material (degree of esterification, distribution of esterified groups along the pectin chain) should be taken into account. Furthermore, the structure of and the reaction conditions in the cell wall and the middle lamella, of which pectin is a component, will play an important role. Therefore, more information on the structure and composition of the cell walls in potatoes, including pectin, will enable a better evaluation of the influence of ion-transport.

Acknowledgements

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Chapter 5

Chemical and microscopic characterization of potato (*Solanum tuberosum* L.) cell walls during cooking

J.T. van Marle, T. Stolle-Smits, J. Donkers, C. van Dijk, A.G.J. Voragen[1] and K. Recourt

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Summary

Cell wall material (CWM) was isolated from non-cooked, 5 and 15 minutes cooked potato tissue and accompanying cooking media from the cultivars Irene and Nicola. A mass balance of the material in the different fractions obtained during isolation was made. Chemical composition of the CWM and cell size distribution of the potato tissues were analyzed. The mealy cooking cv. Irene had more CWM per unit cell surface area than the non-mealy cooking cv. Nicola. These results confirmed observations of the potato cell walls made by transmission electron microscopy (TEM). The molar composition of the CWM was comparable for both cultivars.

During cooking, for both cultivars relatively more unbranched than branched pectic polysaccharides were solubilized. However, the type of pectin solubilized after 15 minutes cooking was different for the two cultivars. This pectin was relatively more branched, more methylated and more acetylated for cv. Irene than for cv. Nicola.

[1] Agricultural University, Department of Food Science, Wageningen, The Netherlands

Introduction

The texture of cooked potatoes is an important quality aspect. One of the factors determining texture is the degradation of cell walls and middle lamellae as a result of heating (Jarvis et al., 1992; Andersson et al., 1994).
In general, cell walls determine the physical properties of plant tissue, which are of importance for the texture of plant products (Tucker and Mitchell, 1993). Cell walls can be presented as a cellulose-xyloglucan network embedded in a pectin matrix. The cell wall strength is established by the cellulose microfibrils and the pectin matrix acts as a "glue" that holds the microfibrils around one cell and between cells together. The two main types of pectin are linear homopolygalacturonans and rhamnogalacturonans with neutral sugar sidechains attached to the rhamnosyl-residues (Carpita and Gibeaut, 1993; Schols, 1995). For potato, this model is supported by the combined microscopic and chemical study of Shomer and Levy (1988). Potato tissue was incubated with pectinase, which resulted in removal of the middle lamella and exposure of the microfibrillar network of the cell wall. Due to this treatment, the volume of the macerate was slightly reduced. In contrast to this, incubation with cellulase resulted in a large reduction of the volumetric tissue construction.
Cooking of potatoes also affects the non-cellulosic matrix. Solubilization of pectic material has been reported (Keijbets, 1974; Moledina et al., 1981; Jaswal, 1991; Marle et al., 1994). Although the polysaccharide structure of non-cooked cell walls of single potato cultivars has been extensively studied (Ishii, 1981; Jarvis et al., 1981; Ryden and Selvendran, 1990), the polysaccharide content of cooked potatoes is reported only in relation with dietary fiber research (Englyst and Cummings, 1987; Englyst and Cummings, 1990).
In a previous study, ten potato cultivars, which represented a large part of the diversity in texture of cooked potatoes, were sensory evaluated (Chapter 2). Most of the difference in texture was explained on the basis of the differences between mealy and non-mealy (waxy) cooking potatoes. The cultivars Irene (mealy) and Nicola (non-mealy) are representative of this difference in texture. The difference in texture between potato cultivars was further investigated at an ultrastructural level by cryo-scanning electron microscopy (Marle et al., 1992). The extent of intercellular contacts and the appearance of cell surfaces were different for mealy and non-mealy cooking cultivars. Based on these observations, middle lamella breakdown, cell wall loosening and starch gelatinization were supposed to be important factors determining texture. In continuation of these studies, it was decided to investigate the influence of cell walls and middle lamellae in determining texture development. By measuring the solubilization of pectic material during cooking, it was found that the mealy cooking cv.

Irene released more pectic material, which was accompanied with more cell sloughing than the non-mealy cooking cultivar Nicola (Marle et al., 1994). More release of pectic material may be the cause or the effect of more cell sloughing. The present study was performed to elucidate whether a difference in composition of cell walls and middle lamellae resulted in the observed difference in pectin release, thereby causing more cell sloughing. Therefore, cell wall material (CWM) was isolated and its composition analyzed. Furthermore, the changes that took place in the cell wall during cooking were visualized by TEM. The mealy cooking cv. Irene and the non-mealy cooking cv. Nicola were used and examined after different time periods during cooking in order to make possible a good comparison between cultivars (with different texture types) during cooking possible.

Materials and methods

Potatoes

The potato cultivars Irene and Nicola, mealy and non-mealy cooking respectively, were grown in 1992 on clay soil at the experimental station of ATO-DLO in the North East Polder, The Netherlands. Potatoes of size 45-55 mm were stored at 6°C and 95% relative humidity for 4 months and samples were taken for experiments.

Cooking of potatoes

About 400 potatoes of each cultivar were randomly selected. Disks of tissue, 16 mm dia and 4 mm thick, were cut from the internal phloem parenchyma. The disks were randomized, rinsed in demineralized water (4°C) and blotted with tissue paper before use. A batch of 800 g of disks was directly frozen in liquid nitrogen (non-cooked tissue). For cooking, a batch of 1250 g of disks was transferred into 10 l of boiling demineralized water. After either 5 or 15 minutes, the disks were separated from the cooking medium by sieving (mesh: 2.0 mm) and subsequently frozen and disintegrated (DITO SAMA K35) in liquid nitrogen. For the 5 minutes cooked-sample three batches of 1250 g of disks were combined, while for the 15 minutes cooked-sample only one batch was used. The cooking media were frozen, freeze-dried and milled (Retsch-mill). The cooking medium of 15 minutes cooked tissue of cv. Irene was filtered through a sintered glass filter (D3), yielding a fraction of loose cells, which was also frozen in liquid nitrogen. For the other cooking media this fraction was negligible. Two batches of 400 potatoes were processed for each cultivar.

Transmission Electron Microscopy (TEM)

Small pieces of non-cooked, 5 and 15 minutes cooked potato tissue were directly

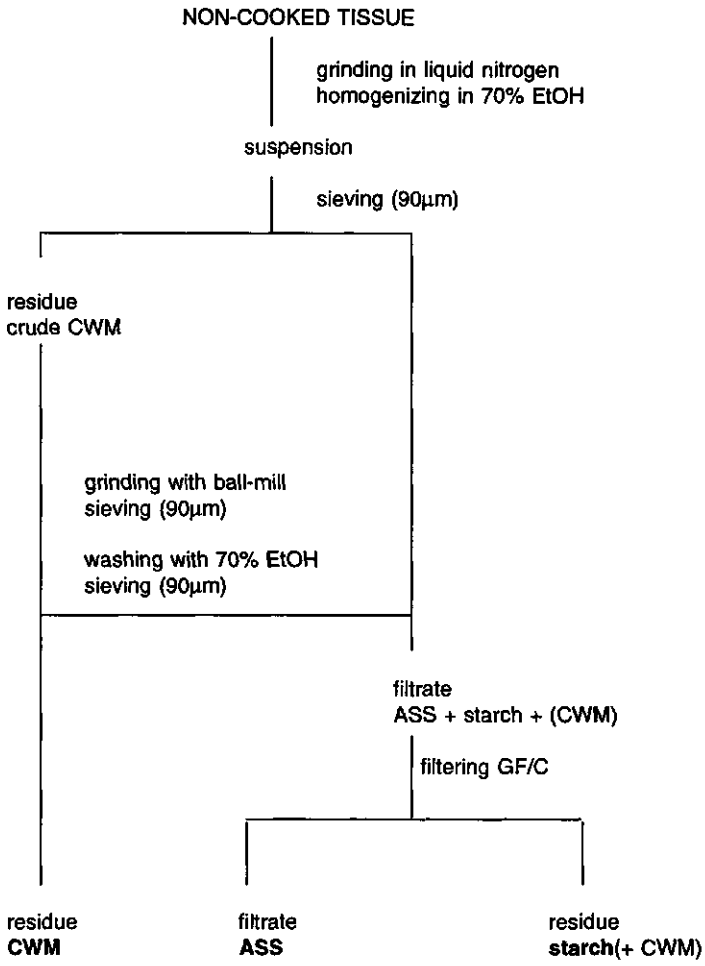

Fig. 5.1 Isolation procedure of CWM from non-cooked potato tissue.

embedded in a drop of 3% agar solution (Kaláb, 1988). Blocks of tissue embedded in agar were fixed at 4°C in 2% glutaraldehyde, buffered with 0.2 M cacodylate at pH 7.2, for 16 hours. Next, the blocks were washed in cacodylate buffer, post-fixed in 1% osmium tetroxide for 1½ hours, washed in distilled water, dehydrated in a graded series of aqueous ethanol and embedded in LR White. To obtain more contrast, sections were stained on the grid with 2% aqueous uranyl acetate for 5 minutes, followed by Reynolds lead citrate for 1 minute (Hall, 1978). Preparations were examined with a Philips EM 400 transmission electron microscope at 80 kV.

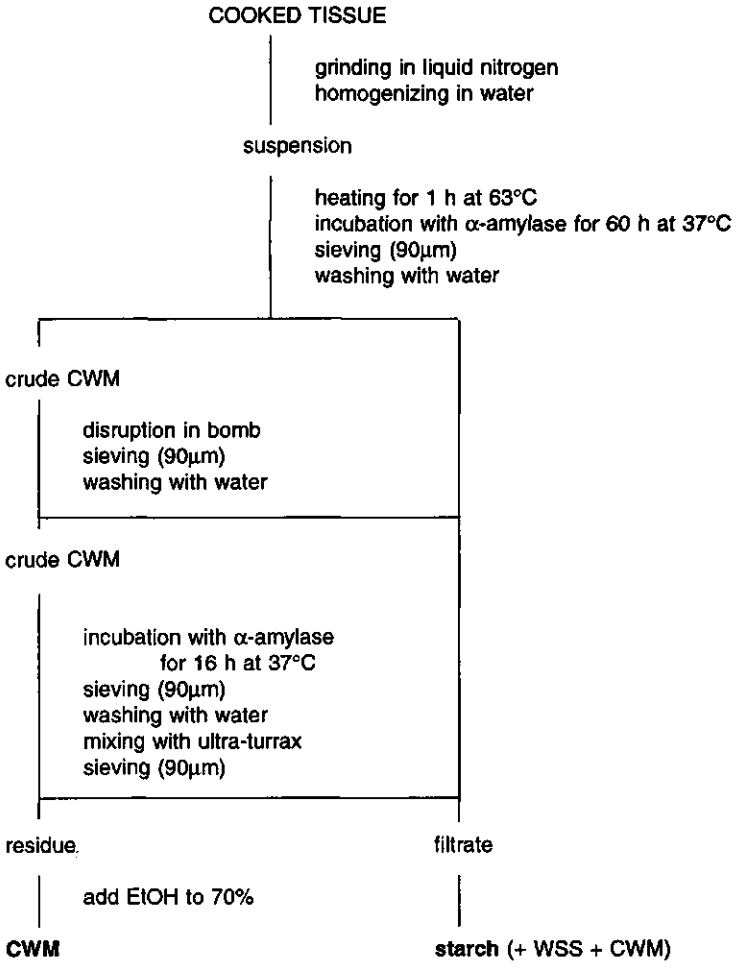

Fig. 5.2 Isolation procedure of CWM from cooked potato tissue.

Isolation of CWM from non-cooked tissue

A schematic diagram of the isolation procedure for CWM from non-cooked tissue is shown in Fig. 5.1. Cold (-20°C) ethanol was added to frozen tissue (100 g) to a final concentration of 70% (v/v). The suspension was homogenized (Ultra-turrax T25) for 5 minutes on ice and filtered through a sieve (mesh: 90 μm). Six batches, each obtained from 100 g of frozen tissue, were combined. The residue on the sieve (crude

CWM) was suspended in cold (-20°C) 70% ethanol and disintegrated with a ball-mill for 30 minutes at 4°C. The suspension was filtered through a sieve (mesh: 90 μm). The residue on the sieve was washed several times with cold 70% ethanol. Washing was continued until hardly any starch grains were observed by a light microscope. The residue was suspended in fresh, cold 70% ethanol and stored in a polythene container at 4°C (as fraction CWM).
The filtrates were combined and filtered through a GF/C-glass fiber filter (Whatman). The residue, consisting of starch and small fragments of CWM (< 90 μm), was stored in a small amount of 70% ethanol (as fraction starch). An aliquot of the filtrate was stored at 4°C (as fraction ASS).

Isolation of CWM from cooked tissue

A schematic diagram of the isolation procedure for CWM from cooked tissue is given in Fig. 5.2. Frozen tissue (100 g) was homogenized (Ultra-turrax) in demineralized water (150 ml) and after addition of 0.05% sodium azide, the suspension was heated for 1 hour in a water-bath of 63°C under gentle shaking. After the suspension cooled to 37°C, porcine α-amylase was added (Merck art. 16312, 3000 units per 100 g of tissue) and the suspension was incubated for 60 hours at 37°C. Eight batches, each obtained from 100 g of frozen tissue, were combined. The suspension was filtered through a sieve (mesh: 90 μm) and the residue was washed with demineralized water. The walls of intact cells in the residue were disrupted by decompression. For that purpose, the residue was transferred into the stainless steel chamber of the disruption bomb (Parr Instruments) and diluted with some demineralized water. The bomb was pressurized with nitrogen up to 60 atm and the suspension was equilibrated for 30 minutes on a magnetic stirrer. Decompression took place by releasing the cells from the bomb, whereby the pressure almost instantly dropped to atmospheric pressure. Subsequently, the released suspension was filtered through a sieve (mesh: 90 μm). The residue was washed with demineralized water (crude CWM) and reincubated with α-amylase (9000 units) for 16 hours at 37°C. Demineralized water was added to a volume of 800 ml and then the suspension was vigorously mixed for 5 minutes (Ultra-turrax) and filtered through a sieve (mesh: 90 μm). Mixing and sieving were repeated until hardly any starch was observed in the residue on the sieve by a light microscope. The residue was stored in 70% ethanol in a polythene container at 4°C (as fraction CWM). The filtrates, containing starch, WSS and small fragments of CWM (< 90 μm) were combined, freeze-dried and milled (Retsch-mill) (as fraction starch).

Isolation of CWM from cooking media and starch fractions

Aliquots of the cooking media and starch fractions were each suspended in

demineralized water containing 0.05% sodium azide and incubated with α-amylase (300 units/g) for 16 hours at 37°C. The cooking media were filtered (through a GF/C glass fiber filter) to remove loose cells. Ethanol (100%) was added to the filtrates of the cooking-media and to the incubated starch fractions to a final concentration of 70%. After mixing, the samples stood for 3 hours at -30°C. The samples were centrifuged (10 minutes, 2500 x g) and the residues were washed with cold 70% ethanol and centrifuged. The washing (once with 70% ethanol and once with acetone) and centrifugation were repeated. The residues were air-dried.

Analytical methods

Dry matter content

Samples were pre-dried overnight at 70°C and drying was continued for 3 hours at 105°C. Dry matter content was determined on three replicates.

Starch content

Starch content was determined enzymatically using the Boehringer test kit (Mannheim GmbH) on at least two replicates.

Neutral sugar and uronic acid content

All samples were pretreated with 72% H_2SO_4 and further hydrolyzed with 1 M H_2SO_4 at 100°C for 2 hours. The hydrolysate was filtered through a GF/C-glass fiber filter (Whatman) and neutralized with $BaCO_3$. The mixture was filtered and 10 µl of the filtrate was analyzed by HPLC (Pharmacia LKB low pressure mixer, HPLC pump 2248 and autosampler 2157) equipped with a Carbopack PA1 column (250 x 4 mm, Dionex) as described by Stolle-Smits et al. (1995). Non-starch glucose content was determined as the difference between glucose content found by HPLC and that found with the Boehringer test kit. Cellulose content was calculated as the difference between glucose contents found by HPLC in hydrolysates obtained with and without pretreatment with 72% H_2SO_4. Each sample was analyzed in duplicate.

Uronic acid content was determined in the filtrates (0.2 ml) using the m-hydroxydiphenyl method of Ahmed and Labavitch (1977). Determinations were made on three replicates.

Number of methyl esters and acetyl groups

Methyl esters and acetyl groups were determined by HPLC according to Voragen et al. (1986).

Cell size measurement

Cell suspensions of non-cooked tissue were made as described by Marle et al. (1994). Cell suspensions from tissue cooked for 15 minutes were obtained by mixing (Ultra-turrax) cooked tissue with demineralized water and after sieving (mesh: 500 μm) the filtrates were used.

The cell size distribution of a cell suspension was determined using a Coulter Counter (LS130, Coulter Electronics). The cell size distributions were determined on two replicates.

The cell size distribution was used to calculate the specific cell surface area. For each class of cells with a certain diameter (d_i) the volume percentage ($v\%_{di}$) of the total volume (V) was given.

With the volume occupied by a class of cells with diameter d_i in a total volume V,

$$V * v\%_{d_i} \qquad [1]$$

the number of cells, n_i, with diameter d_i could be calculated:

$$n_i = \frac{6 * V * v\%_{d_i}}{\pi * d_i^3} \qquad [2]$$

together with the total cell surface, A_i, of all the cells with diameter d_i:

$$A_i = \frac{6 * V * v\%_{d_i}}{d_i} \qquad [3]$$

The total cell surface, A_{tot}, per tissue volume V is:

$$A_{tot} = \Sigma \frac{6 * V * v\%_{d_i}}{d_i} \qquad [4]$$

Results

TEM

TEM visualized the changes in cell walls and middle lamellae of both cultivars during

cooking (Fig. 5.3 and 5.4). The observations give information about the changes taking place in the cell wall structure and also give indications about differences between the cultivars with respect to their cell walls.

Non-cooked tissue of cv. Irene seemed to have denser cell walls than non-cooked tissue of cv. Nicola. Upon cooking, cell walls of both cultivars expanded, resulting in a less dense and rigid appearance compared to non-cooked tissue. The intercellular contact between cells was reduced, which was initiated at the intercellular spaces. After 5 minutes cooking most of the intercellular contact was still intact and the difference in compactness of the cell walls between both cultivars was still visible. However, the cell walls of cv. Irene seemed to be more expanded than those of cv. Nicola. After 15 minutes cooking the intercellular contact was greatly reduced for both cultivars. Large intercellular spaces were visible between the cells and small remnants of solubilized cell wall and middle lamellae structures were present. It seemed that more middle lamellae material was solubilized for cv. Irene than for cv. Nicola. In the cell walls of cv. Irene chainlike structures (parallel with the cell surface) were more clearly visible than in the cell walls of cv. Nicola. Moreover, the cells of both cultivars were still surrounded by cell walls, which had a loose, porous structure, but which were not fractured.

Isolation of CWM

Due to the different states in which starch and the middle lamellae were present in non-cooked and cooked potato tissue, different isolation procedures were used to isolate CWM from both types of tissue.

The CWM isolated from non-cooked potato tissue contained 3.4 to 7.8% starch. During isolation of CWM, the presence of residual starch was monitored by light microscopy and it was observed that some large starch grains (dia > 90 μm) were left in the residue together with starch grains in some small cells, which were not broken during ball milling. The residual starch content of CWM isolated from cooked potato tissue ranged between 1.4 and 4.7%.

Table 5.1 shows the distribution of dry matter over the fractions after isolation of CWM

Fig. 5.3 (page 80) TEM photographs of cell walls in non-cooked and cooked potato tissue from cv. Irene: non-cooked (A) 1000x (B) 3600x (C) 13,000x; 5 minutes cooked (D) 1000x (E) 3600x (F) 13,000x; 15 minutes cooked (G) 1000x (H) 3600x (I) 13,000x.

Fig. 5.4 (page 81) TEM photographs of cell walls in non-cooked and cooked potato tissue from cv. Nicola: non-cooked (A) 1000x (B) 3600x (C) 13,000x; 5 minutes cooked (D) 1000x (E) 3600x (F) 13,000x; 15 minutes cooked (G) 1000x (H) 3600x (I) 13,000x.

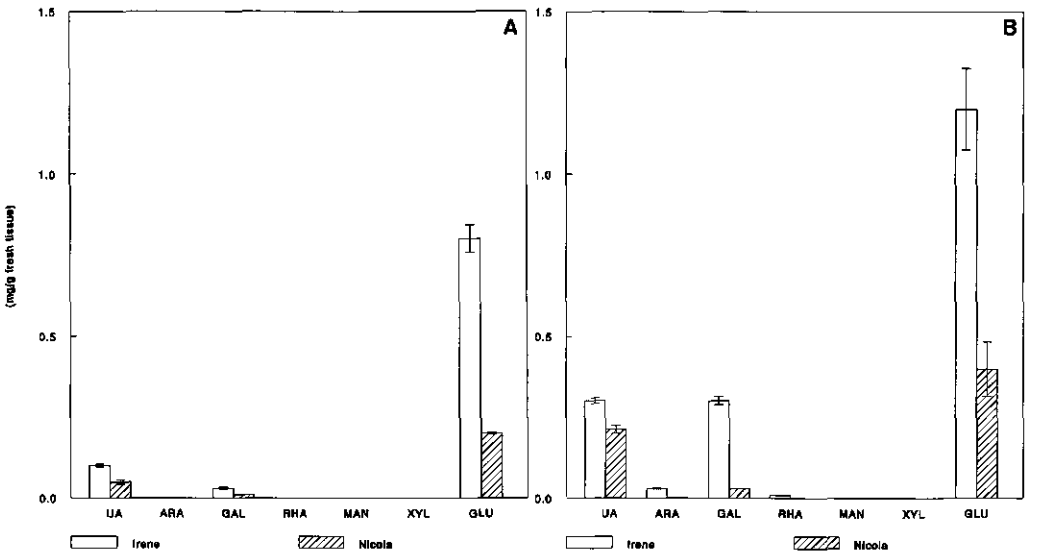

Fig. 5.8 Sugar composition of CWM solubilized into the cooking media during (A) 5 and (B) 15 minutes cooking of potato tissue from the cultivars Nicola and Irene.

sugar compositions of CWM and the accompanying starch fraction are shown in the same figures. It is only reliable to draw conclusions on basis of the composition of CWM, if for both cultivars comparable amounts of sugars were lost in the starch fractions during isolation.

Sugar compositions of CWM and starch fractions from non-cooked potato tissue of both cultivars are shown in Fig. 5.6. In CWM, all sugars were present in higher amounts for cv. Irene than for cv. Nicola. The composition of the starch fractions differed significantly only for uronic acid and arabinose. Apparently, during the isolation more arabinose disappeared with the starch fraction for cv. Nicola than for cv. Irene. However, the differences in amounts of arabinose between both cultivars in the CWM as well as in the starch fractions were very small with respect to the differences for the other sugars. Altogether, it is more reliable to conclude that non-cooked CWM *in situ* contained a comparable amount of arabinose for both cultivars. Furthermore, for cv. Irene more uronic acid disappeared with the starch fraction during isolation than for cv. Nicola. In this case, cv. Irene contained a higher amount of uronic acid in CWM and starch fraction compared with cv. Nicola.

In Fig. 5.7 the sugar compositions of CWM and starch fractions from 5 and 15 minutes cooked potato tissue are shown, respectively. After 5 minutes cooking, all sugars in

Table 5.2 DM (mol% of uronic acid content) and acetyl groups (mg/g fresh tissue) of CWM of cooked and non-cooked potato tissue and of CWM released in the cooking media for cultivars Nicola and Irene.

Fraction	Cultivar	Cooking time (minutes)	DM	Acetyl groups
CWM	Irene	0	49 ± 2	0.21 ± 0.01
		5	44 ± 1	0.28 ± 0.01
		15	44 ± 2	0.17 ± 0.01
	Nicola	0	53 ± 3	0.16 ± 0.01
		5	49 ± 1	0.22 ± 0.01
		15	45 ± 1	0.20 ± 0.01
Cooking medium	Irene	5	82 ± 5	n.d.
		15	61 ± 5	n.d.
	Nicola	5	67 ± 13	n.d.
		15	27 ± 6	n.d.

CWM were still present in higher amounts for cv. Irene than for cv. Nicola. Like in the starch fractions of non-cooked tissue, the amount of uronic acid in the starch fractions of 5 minutes cooked tissue was higher for cv. Irene than for cv. Nicola.

After 15 minutes cooking, the difference in cooking behaviour between both cultivars became clear. Pectic polysaccharides (UA + ARA + GAL) were present in higher amounts in CWM of cv. Nicola than in CWM of cv. Irene. In the starch fraction of cv. Irene, higher amounts of uronic acid and arabinose were present as compared with cv. Nicola. For cooked tissue, the pectic polysaccharides in the starch fractions were most probably solubilized in the tissue during cooking, but were at that time hindered sterically from diffusing into the cooking medium. During isolation, these pectic polysaccharides were released in the starch fractions. Therefore, cooked tissue of both cultivars could be compared on the basis of CWM, because this is the CWM that remained insoluble after cooking. Loose cells and 15 minutes cooked tissue had comparable molar compositions (data not shown).

The compositions of CWM in the cooking media of both cultivars after 5 and 15 minutes cooking are shown in Fig. 5.8. Xylose and mannose were not detected in the cooking media. After 5 minutes cooking, uronic acid and galactose appeared in higher concentrations in the cooking medium of cv. Irene. After 15 minutes cooking, the difference between both cultivars was more pronounced, because uronic acid, arabinose and galactose were present in higher concentrations in the cooking medium of cv. Irene. The glucose found in the cooking media (Fig. 5.8) most likely originated from starch and not from cellulose, which is not solubilized upon cooking. Cool storage

(24 hours at 4°C) and freeze-drying of the cooking media were favourable for transformation of the solubilized starch into resistant starch (Englyst and Cummings, 1987). Redispersion of this starch was probably not complete under the conditions used during analysis (Englyst and Cummings, 1984).

Methyl esters and acetyl groups

During cooking, the degree of methylation (DM) decreased in a comparable way for CWM from both cultivars (Table 5.2). However, the DM of the CWM dissolved in the cooking medium was significantly higher for cv. Irene after 5 and 15 minutes cooking. Non-cooked CWM of cv. Irene contained more acetyl groups than CWM of cv. Nicola. After 5 minutes cooking, this difference was still present, but prolonged cooking solubilized more acetyl groups for cv. Irene than for cv. Nicola. The numbers of acetyl groups in non-cooked and cooked tissue could not be compared due to different isolation methods.

Cell size measurement

This season, the specific cell surface area was not similar for both cultivars (Marle et al., 1994). The cell size distribution curves were similar in shape to those measured before (Marle et al., 1994), but cv. Irene had its maximum at a cell diameter of 165 µm and cv. Nicola at a cell diameter of 190 µm (curves not shown). The specific cell surface areas calculated according to Eq. [4] were $3.72 \pm 0.11 * 10^4$ m^2/m^3 for cv. Irene and $3.30 \pm 0.05 * 10^4$ m^2/m^3 for cv. Nicola.

Discussion

Non-cooked tissue of cv. Irene contained more CWM than non-cooked tissue of cv. Nicola based on fresh weight (Fig. 5.6). However, the amount of CWM per unit cell surface area gives a more reliable comparison, since this amount takes into account the differences in specific cell surface area and dry matter content between the cultivars. The calculated specific cell surface areas were comparable with those found by Hughes et al. (1975).

Table 5.3 showed that cv. Irene had significantly more CWM per unit cell surface area than cv. Nicola. This higher amount of CWM can make the cell walls of cv. Irene thicker and/or denser than those of cv. Nicola. For example, a potato cell with a diameter of 200 µm has a cell surface area of $1.3 * 10^{-7}$ m^2. From Table 5.3, it can be calculated that a cell from cv. Irene contains $2.6 * 10^{-8}$ g of CWM and a cell from cv. Nicola $2.3 * 10^{-8}$ g of CWM. Assuming that for both cultivars the cells have an equal cell wall thickness, the difference in density of their cell walls is 13%.

Table 5.3 Calculation of the amount of cell wall material per unit cell surface area for cultivars Irene and Nicola.

	cv. Irene	cv. Nicola
dry matter (%)	23.87 ± 0.05	18.01 ± 0.27
specific gravity* (10^3 g/m^3)	1097 ± 0.5	1068 ± 2.6
cell surface area (10^4 m^2/m^3 tissue)	3.72 ± 0.11	3.30 ± 0.05
cell wall material** (g/10^3 g fw)	7.0 ± 0.1	5.5 ± 0.2
cell wall material*** (10^3 g/m^3)	7.7 ± 0.2	5.9 ± 0.2
cell wall material/cell surface area (10^{-1} g/m^2)	2.1 ± 0.1	1.8 ± 0.1

* calculated according to Simmonds (1977)
** uronic acid and neutral sugars
*** cell wall material (g/m^3) = cell wall material (g/10^3 g) * specific gravity

On the other hand, assuming that for both cultivars the cells have equal cell wall densities, and that for cv. Nicola the cell wall thickness is 1 μm (Fig. 5.4), it can be calculated that the cell wall thickness for cv. Irene is 1.13 μm. The difference in thickness of the cell walls of both cultivars is 13%.

These results agree with the observations made by TEM, which suggested that cv. Irene had denser cell walls. A difference in cell wall thickness ≤ 13% is difficult to observe.

Nevertheless, the molar compositions of CWM of both cultivars were comparable (data not shown).

During cooking, middle lamellae were solubilized and cell walls became looser in structure, as shown by observations with TEM (Fig. 5.3 and 5.4) of both cultivars. After 15 minutes cooking, middle lamella breakdown seemed to be more complete for cv. Irene than for cv. Nicola. Quantification of these observations was given by the chemical characterization of the isolated CWM.

The two cultivars behaved in different ways (Fig. 5.7 and 5.8) upon cooking. For cv. Irene, significantly more uronic acid, galactose and arabinose were solubilized. Quinn and Schafer (1994) found that neutral sugars co-eluted with galacturonic acid during ion-exchange chromatography of potato pectic material, indicating that, in potato cell, walls neutral sugars are present as side chains of acid polymers. Therefore, the ratio of ARA + GAL to uronic acid was calculated to obtain information about the type of the solubilized pectic polysaccharides (Table 5.4). The ratio decreased for CWM during cooking,which suggests that relatively more unbranched than branched pectic polysaccharides were solubilized. Concurrently, the CWM solubilized in the cooking

Table 5.4 The molar ratio of ARA + GAL to uronic acid in CWM, loose cells and cooking media of cultivars Nicola and Irene. The sd is given in parentheses.

Fraction	Cultivar	Cooking time (minutes)	ARA + GAL : UA
CWM	Irene	0	2.3 (0.2) : 1.0
		5	3.3 (0.5) : 1.0
		15	4.2 (0.3) : 1.0
	Nicola	0	2.6 (0.1) : 1.0
		5	3.1 (0.3) : 1.0
		15	4.1 (0.5) : 1.0
Loose cells	Irene	15	3.9 (0.8) : 1.0
Cooking medium	Irene	5	1.5 (0.1) : 1.0
		15	0.8 (0.0) : 1.0
	Nicola	5	1.1 (0.0) : 1.0
		15	0.4 (0.0) : 1.0

medium was highly methylated (Table 5.2). Highly methylated, unbranched pectin may originate from the middle lamella (Ryden and Selvendran, 1990) but is also found throughout the whole cell wall (Carpita and Gibeaut, 1993). Certainly this type of pectin is the most easily solubilized, because the depolymerization reaction of pectin according to the ß-eliminative mechanism results in cleavage of the pectin chain next to a methyl esterified galacturonic acid residue (Keijbets, 1974). Furthermore, neutral sugar sidechains will be entangled in the pectin matrix depending on their size and conformation, thereby counteracting solubilization (Carpita and Gibeaut, 1993).

For CWM solubilized in the cooking medium, the ratio of ARA + GAL to uronic acid was higher for cv. Irene than for cv. Nicola. Rhamnose was only found in the cooking medium of cv. Irene after 15 minutes cooking. Furthermore, Table 5.2 shows that, after 15 minutes cooking cv. Irene had a higher DM than cv. Nicola. Also, the number of acetyl groups of CWM of cv. Irene was reduced between 5 and 15 minutes cooking (Table 5.2). On basis of the results given above, it can be concluded that, after 15 minutes cooking, the solubilized pectic polysaccharides were more branched, more methylated and more acetylated for cv. Irene than for cv. Nicola.

In conclusion, the composition of CWM isolated from non-cooked tissue was comparable for the mealy cv. Irene and the non-mealy cv. Nicola. However, for cv. Irene more CWM per unit cell surface area was present than for cv. Nicola. At the same time, it was found that, for both cultivars, different types of pectic polysaccharides were solubilized during cooking. Since this observation could not be

explained by a difference in composition of CWM, more research is necessary to elucidate the influence of cell walls and middle lamellae on texture development. More information concerning differences in pectin structure between both cultivars will be obtained from examination of the pectic and xyloglucan fractions of CWM from both cultivars. The results will be dealt with in a following paper.

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Chapter 6

Structural features of cell walls from potato cultivars Irene and Nicola

J.T. van Marle, K. Recourt, C. van Dijk, H.A. Schols[1] and A.G.J. Voragen[1]

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Summary

Cell wall material (CWM) isolated from non-cooked potato tissue of the cultivars Irene (mealy cooking) and Nicola (non-mealy cooking) was successively extracted with buffer, CDTA, Na_2CO_3 at 4°C and 20°C and 0.5 M KOH. The sugar composition of fractions and the remaining residues was determined. The amounts of methyl esters and acetyl groups were measured in the buffer and CDTA-fractions. Information about the structure of the pectic polysaccharides was obtained by studying the degradation of the fractions and residues with purified polygalacturonase (PG) and rhamnogalacturonase (RGase).

Comparing CDTA, both Na_2CO_3 and KOH-fractions with each other revealed that with more severe extraction conditions the ratio of ARA+GAL to uronic acid increased and more PG-resistant material was present. The pectic polysaccharides extracted by buffer, CDTA and cold Na_2CO_3 were more branched for cv. Irene than for cv. Nicola, comparing sugar composition and the ratio of ARA+GAL to uronic acid. Differences in the PG-degradation patterns of the CDTA-fractions from both cultivars indicated the presence of longer and/or more homogalacturonan regions for cv. Nicola than for cv. Irene. From the remaining CWM of cv. Nicola more branched pectic polysaccharides were extracted with Na_2CO_3 at 20°C than from CWM of cv. Irene. The yield of the 0.5 M KOH-fraction was higher for cv. Nicola in comparison with cv. Irene. Finally, 24% more residue was left for cv. Irene than for cv. Nicola. It is proposed that the pectin matrix in the primary cell wall of cv. Irene had a less porous structure than the matrix in the cell wall of cv. Nicola.

[1] Agricultural University, Department of Food Science, Wageningen, The Netherlands

Introduction

Structure and composition of potato cell walls, and in particular the pectic polysaccharides, have been studied for many years (Ishii, 1981; Jarvis et al., 1981; Ryden and Selvendran, 1990).

Pectin is the main component of the potato cell wall (54%) (Jarvis et al., 1981; Ryden and Selvendran, 1990). Three types of pectic polysaccharides are present in all primary plant cell walls, namely homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II (Albersheim et al., 1997). Pectin determines cell wall porosity and thickness (Bacic et al., 1988; Carpita and Gibeaut, 1993) and it plays an important role in maintaining tissue integrity. Upon processing of potatoes, pectic material is degraded and partly solubilized into the cooking media (Hughes et al., 1975a; Hughes et al., 1975b; Marle et al., 1994). This degradation greatly influences intercellular adhesion and the structure of the remaining cell walls, which are both important texture parameters (Marle et al., 1992). However, little is known about differences in structure and composition of pectic polysaccharides with respect to texture development during processing. Recently, Quinn and Schafer (1994) characterized the pectic substances of two potato cultivars with different sensitivities to prewarming. They reported differences in yield and composition of the pectic material extractable by sodiumhydroxide. Our previous study revealed differences in total amount of cell wall material and amount and composition of solubilized pectic material for the potato cultivars Irene (mealy cooking) and Nicola (non-mealy cooking) (Marle et al, 1996).

Using either purified enzymes (Ishii, 1981) or chemical extractants (Jarvis et al., 1981; Ryden and Selvendran, 1990) several fractions of pectic polysaccharides were isolated originating from different parts of the potato cell walls, being different in composition and structure. For instance, pectic polysaccharides from the middle lamellae were more easily solubilized than those present in the primary cell walls (Ishii, 1981; Ryden and Selvendran, 1990). Furthermore, the pectic polysaccharides solubilized with more severe extractants had a higher ratio of neutral sugars to galacturonic acid indicating the presence of more hairy regions (Ryden and Selvendran, 1991).

More knowledge about the relative abundance of homogalacturonan and/or branched rhamnogalacturonan regions in the various fractions can be obtained by enzymatic degradation of those fractions with purified endopolygalacturonase (endoPG) and rhamnogalacturonase (RGase) in combination with high performance size-exclusion chromatography (HPSEC) and examination of the structure and composition of the oligomers formed (Kravtchenko, 1992; Schols, 1995).

In this study, the structure of the pectic polysaccharides present in cell walls of the cultivars Irene and Nicola is examined. Cell wall material was fractionated using

chemical extractants. The composition of the fractions was analyzed and the structure of the pectic polysaccharides was studied by degradation with purified endoPG and RGase.

Materials and methods

Potatoes

The isolation procedure and the characterization of cell wall material (CWM) from the potato cultivars Nicola and Irene, non-mealy and mealy cooking respectively, were described in a previous paper (Marle et al., 1996). For this study, the CWM isolated from non-cooked tissue was used.

Fractionation of CWM

For the sequential extraction of CWM the method described by Selvendran et al. (1985) was used, which was slightly adapted.

A suspension (100 g; 2% w/w CWM in 70% ethanol) was centrifuged (15 minutes; 12,000 x g). Ammonium acetate buffer (150 ml 0.05 M), pH 4.7, was added to the residue and the suspension was stirred for 16 h at 20°C and centrifuged. Subsequently, the residue was washed with 50 ml of the same buffer and 50 ml demineralized water respectively. Supernatant and washings were combined and this "buffer"-fraction was dialysed. The residue was suspended in 150 ml 0.05 M CDTA, pH 6.5, and stirred for 16 h at 20°C. After centrifugation, the residue was washed twice with 50 ml CDTA and once with 50 ml demineralized water. Supernatant and washings formed the CDTA-fraction. This fraction was dialysed three times against NH_4OAc buffer, pH 4.7 (48 h) and demineralized water respectively (Mort et al., 1991). The residue was suspended in 150 ml cold (4°C) 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$ and stirred 16 h at 4°C. After centrifugation, the supernatant was filtered through a GF/C-glass fibre filter (Whatman) and the filtrate was acidified to pH 5 with HAc and dialysed (Na_2CO_3 (4°C)-fraction). The residue was suspended in 150 ml 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$ and stirred for 3 h at 20°C and centrifuged. The residue was washed twice with 50 ml Na_2CO_3 and once with demineralized water. Supernatant and washings were combined, filtered through a GF/C-glass fibre filter and subsequently the filtrate was acidified to pH 5 and dialysed (Na_2CO_3 (20°)-fraction). Potassium hydroxide (100 ml 0.5 M) containing 10 mM $NaBH_4$ was added to the residue, stirred for 16 h at 20°C and centrifuged. The residue was washed with 50 ml KOH. Supernatant and washing were combined and filtered through a GF/C-glass fibre filter and the filtrate was acidified to pH 5 and dialysed (KOH-fraction). The residue was suspended in 75 ml demineralized water, acidified to pH 5 and was

dialysed (residue). After dialysis all fractions and the residue were freeze-dried and milled with a ball-mill. For each cultivar the fractionation was performed in duplicate.

Chromatography

High-performance size-exclusion chromatography (HPSEC) was carried out as described by Schols et al. (1990). High-performance anion-exchange chromatography (HPAEC) was performed on a HPLC (Pharmacia LKB low pressure mixer, Waters 625 LC pump and autosampler 2157) equipped with a Carbopack PA1 column (250 x 4 mm, Dionex) as described by Schols et al. (1994). For analysis of the RGase-digests the method described by Schols et al. (1994) was slightly adapted. After sample injection a linear gradient was started from 150 mM NaOAc in 100 mM NaOH to 250 mM NaOAc in 100 mM NaOH within 40 minutes. For analysis of the PG-digests the method described by Kravtchenko et al. (1993) was used, which was slightly adapted. After sample injection two successive linear gradients were used, 400 mM NaOAc to 670 mM NaOAc in 100 mM NaOH within 35 minutes and 670 NaOAc to 1 M NaOAc in 100 mM NaOH within 5 minutes. The column was washed with 1 M NaOAc in 150 mM NaOH for 5 minutes and equilibrated with 400 mM NaOAc in 100 mM NaOH for 15 minutes.

Saponification

Buffer and CDTA-fractions (25 mg) were stirred with 2 ml 0.1 N NaOH (16 h; 4°C) to remove the methyl esters and acetyl groups. The solutions were neutralized with 2 ml 0.1 N HAc and 0.05 N NaOAc buffer, pH 5.0, was used to make the final concentration 3 mg/ml.

Enzymic hydrolysis

The different fractions and residues (3 mg/ml 0.05 M NaOAc pH 5.0) and the saponified samples were incubated with either PG (10 nkat/ml) or RGase for 16 h at 40°C as described by Schols et al. (1995). PG was purified from *Kluveromyces fragilis* and RGase was purified from *Aspergillus aculeatus*. Both enzymes were provided by the Department of Food Science, Agricultural University, Wageningen (Schols et al., 1995). After incubation, the enzymes were inactivated (10 minutes; 100°C). The digests were centrifuged (12,000 x g for 10 minutes) and the supernatants were analyzed by HPSEC and HPAEC.

Analytical methods

Starch content

Starch content was determined enzymatically using the Boehringer test kit (Mannheim GmbH) on two replicates (if enough material was available). For sample preparation, amounts of sample and reagents were adapted to obtain a final solution with a starch concentration between 15-200 µg/ml in a total volume of 1 ml. This solution (0.10 ml) was used for the starch assay, which was adapted by using half the amounts of reagents resulting in a final volume of 1.16 ml in the cuvette.

Neutral sugar and uronic acid content

All fractions were hydrolysed with 1 M H_2SO_4 at 100°C. Only the residues were pretreated with 72% H_2SO_4. After neutralization with $BaCO_3$ the hydrolysates were analyzed for neutral sugars by HPLC (Pharmacia LKB Low pressure mixer, Waters 625 LC pump and autosampler 2157) equipped with a Carbopack PA1 column (250 x 4 mm, Dionex). The eluents, consisting of Milli Q water and 150 mM NaOH, were sparged and pressurized with helium. Prior to injection, the system was equilibrated with 30 mM NaOH for 13 minutes at a flow rate of 1.0 ml/min at ambient temperature. At 0.1 minute after injection, the eluent was switched from 30 mM NaOH to Milli Q water. After each run, the column was regenerated with 150 mM NaOH for 10 minutes. Sugars were detected using a pulsed amperometric detector (Dionex) fitted with a gold working electrode as described by Stolle-Smits et al. (1995). The data system used was a Millenium 2.0 Chromatography Manager (Waters Corporation). Each sample was analyzed in duplicate.

Non-starch glucose content was determined as the difference between glucose content as measured with the HPLC and with the Boehringer test kit.

Uronic acid content was determined in the filtrate (0.2 ml) using the m-hydroxydiphenyl method of Ahmed and Labavitch (1977). Determinations were made on three replicates.

Number of methyl esters and acetyl groups

Methyl esters and acetyl groups were determined by HPLC according to Voragen et al. (1986).

Results

Yield and composition of fractions

The yields (mg/g CWM) of buffer, CDTA and both Na_2CO_3-fractions were comparable for the cultivars Irene and Nicola (Table 6.1). However, on basis of fresh weight the

Table 6.1 Yield and sugar composition of CWM, fractions and residues for the cultivars Irene and Nicola (mg/g CWM). The SD is given in parentheses.

	CWM	Fraction						
		Buffer	CDTA	Na_2CO_3 (4°)	Na_2CO_3 (20°)	KOH	Residue	Total
Cv. Irene (0.95 g CWM/100 g fresh weight)								
Yield	1000	64 (11)	54 (7)	95 (5)	107 (6)	222 (2)	497 (9)	1039 (40)
UA	167 (10.5)	5.5 (0.7)	19.4 (4.3)	41.6 (2.7)	26.6 (1.5)	28.3 (1.8)	66.9 (1.0)	188 (12.1)
ARA	39.9 (0.8)	2.1 (0.3)	1.3 (0.4)	3.4 (0.1)	5.5 (0.4)	9.6 (0.5)	16.4 (0.6)	38.4 (2.2)
GAL	312 (2.7)	19.2 (2.3)	15.4 (4.9)	30.3 (0.3)	48.8 (2.7)	93.8 (4.5)	89.4 (2.8)	297 (17.5)
RHA	9.1 (0.1)	0.3 (0.0)	0.3 (0.1)	0.7 (0.1)	0.7 (0.1)	1.3 (0.1)	0.8 (0.2)	4.0 (0.5)
MAN	14.8 (0.0)	0.2 (0.0)	0.1 (0.0)	0.1 (0.1)	0.1 (0.0)	0.0 (0.0)	6.7 (0.6)	7.1 (0.7)
XYL	9.1 (0.8)	0.1 (0.0)	0.1 (0.0)	0.2 (0.0)	0.2 (0.0)	1.6 (0.1)	13.3 (0.4)	15.5 (0.6)
GLU*	243 (3.2)	2.3 (1.3)	0.8 (0.0)	1.1 (0.4)	0.9 (0.2)	29.9 (0.9)	146 (11.6)	181.2 (14.5)
NSP**	795 (18)	30 (5)	37 (10)	77 (3)	83 (5)	165 (6)	340 (17)	731 (44)
Starch	51 (17)	11 (7)	1.7 (0.6)	0.5 (0.2)	0.5 (0.3)	11 (2)	0.6 (0.0)	25 (10)
OMe	14.4 (0.6)	0.44(0.03)	0.34(0.00)					
OAc	24.4 (0.6)	0.82(0.11)	0.71(0.12)					

* Non-starch glucose

**NSP = non-starch polysaccharides

	CWM	Fraction						
		Buffer	CDTA	Na_2CO_3 (4°)	Na_2CO_3 (20°)	KOH	Residue	Total
Cv. Nicola (0.79 g CWM/100 g fresh weight)								
Yield	1000	60 (5)	55 (6)	84 (8)	114 (2)	299 (26)	379 (19)	991 (66)
UA	157 (5.7)	5.2 (0.6)	22.2 (2.9)	37.3 (5.3)	27.1 (1.1)	37.5 (6.1)	44.3 (1.9)	174 (18.0)
ARA	41.3 (0.1)	1.7 (0.1)	1.1 (0.1)	3.0 (0.4)	6.0 (0.2)	14.4 (2.2)	11.5 (0.3)	37.7 (3.4)
GAL	316 (5.3)	12.6 (1.4)	10.0 (0.8)	25.5 (3.7)	56.4 (2.4)	133 (15.1)	54.5 (6.5)	292 (29.8)
RHA	8.5 (0.0)	0.2 (0.0)	0.3 (0.0)	0.6 (0.1)	0.8 (0.1)	2.0 (0.1)	0.8 (0.1)	4.9 (0.4)
MAN	14.2 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	5.5 (0.6)	5.8 (0.7)
XYL	7.1 (0.4)	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)	0.2 (0.0)	1.5 (0.2)	11.6 (1.2)	13.6 (1.5)
GLU*	235 (15.8)	2.3 (1.1)	1.5 (0.1)	1.5 (0.0)	1.4 (0.0)	48.4 (2.7)	132 (10.7)	187 (14.6)
NSP**	779 (27)	22 (1)	35 (4)	68 (10)	92 (4)	237 (21)	260 (4)	715 (43)
Starch	72 (6)	19 (0.4)	4.0 (0.1)	0.8 (0.1)	0.8 (0.0)	10 (3)	1.0 (0.2)	36 (4)
OMe	14.7 (0.8)	0.38(0.12)	0.51(0.07)					
OAc	22.7 (0.2)	0.68(0.09)	0.38(0.01)					

* Non-starch glucose

**NSP = non-starch polysaccharides

yield of these fractions was higher for cv. Irene than for cv. Nicola, since cv. Irene contained 0.95 g CWM per 100 g fresh weight and cv. Nicola 0.79 g CWM per 100 g fresh weight (Marle et al., 1996). Furthermore, cv. Nicola had a higher yield of the KOH-fraction, resulting in more residue for cv. Irene, both on basis of CWM and fresh weight (Table 6.1). Comparing fractions and residues of both cultivars on basis of total non-starch polysaccharides (NSP) gave similar results as the comparison made on basis of yield, except for the buffer-fraction. The total NSP content of the buffer-fraction was slightly higher for cv. Irene than for cv. Nicola.The sugar composition of CWM and the respective fractions and residues is also shown in Table 6.1. The isolated CWM contained 3.4 to 7.8% starch, despite several washing and sieving (mesh: 90 μm) steps (Marle et al., 1996). Observations with a light microscope revealed that large starch granules (dia > 90 μm) were left in the CWM together with starch granules in some small cells, which were not broken during ball milling. During fractionation, a large amount of the residual starch present in the CWM was found in the buffer-fractions (11% and 19% of the fractions for cv. Irene and cv. Nicola respectively). Furthermore, the CDTA and KOH-fractions also contained ca. 5% starch. The presence of starch in these first two fractions was not expected, but in particular decanting of the buffer solution after centrifugation was difficult due to a quite instable residue.

The total sugar content of the buffer-fractions accounted for 47% and 37% of the isolated material for cv. Irene and cv. Nicola respectively (Table 6.1). This low yield is mainly due to the large amount of starch present. For the CDTA, Na_2CO_3, KOH-fractions and the residues, the total sugar content accounted for 70% to 80% of the isolated material (Table 6.1). Ryden and Selvendran (1990) found a total sugar content of ca. 60% for the CDTA-fractions. In our CDTA-fractions of the cultivars Irene and Nicola, CDTA was probably removed to a larger extent due to dialysis against buffer (instead of demineralized water) as was also reported by Mort et al. (1991).

The sugar composition of the CWM from the two cultivars was discussed previously (Marle et al., 1996). The amounts of arabinose, rhamnose, mannose and xylose were slightly different in the CWM of the two cultivars. However, the molar composition of the CWM was comparable for both cultivars. The composition of the pectic polysaccharides in the buffer, CDTA and cold Na_2CO_3-fractions were quite comparable for both cultivars, with the exceptions that for cv. Irene more rhamnose was found in the buffer-fraction and more galactose in the buffer and cold Na_2CO_3-fractions compared with cv. Nicola.

Sequential extraction of the CWM with buffer, CDTA, Na_2CO_3 (at 4°C and 20°C) and 0.5 M KOH yielded different fractions of pectic polysaccharides and still a considerable fraction of pectic material (ca. 30% uronic acid, ca. 35% arabinose, ca. 25%

Table 6.2 The molar ratio of arabinose + galactose to uronic acid in CWM, fractions and residues of the cultivars Irene and Nicola. The sd is given in parentheses.

cultivar	sample	molar ratio ARA + GAL : UA
Irene	CWM	2.3 (0.2) : 1.0
	buffer	4.3 (0.3) : 1.0
	CDTA	0.9 (0.2) : 1.0
	Na_2CO_3 (4°C)	0.9 (0.1) : 1.0
	Na_2CO_3 (20°C)	2.3 (0.0) : 1.0
	KOH	4.1 (0.3) : 1.0
Nicola	CWM	2.6 (0.1) : 1.0
	buffer	3.1 (0.2) : 1.0
	CDTA	0.6 (0.0) : 1.0
	Na_2CO_3 (4°C)	0.8 (0.1) : 1.0
	Na_2CO_3 (20°C)	2.6 (0.1) : 1.0
	KOH	4.4 (0.4) : 1.0

galactose) remained in the residues. Galactose was the most abundant neutral sugar in the CWM, the fractions (except the CDTA and cold Na_2CO_3-fractions) and the residues. The following differences between fractions and residues of both cultivars were observed. At room temperature, Na_2CO_3 extracted more galactose and glucose for cv. Nicola than for cv. Irene. With KOH more rhamnose, arabinose, galactose, uronic acid and glucose were extracted for cv. Nicola than for cv. Irene. Finally, in the residue of cv. Irene more arabinose, galactose, uronic acid and xylose were found than in the residue of cv. Nicola.

The molar ratio of ARA+GAL to uronic acid can give information about the branching of the pectic polysaccharides, assuming that all neutral sugars are present as sidechains. Ryden and Selvendran (1990) fractionated their pectic extracts by anion-exchange chromatography. They reported that uronic acid was the main constituent of all obtained fractions, except for one small neutral fraction (rich in arabinose and galactose) obtained from the CDTA-extract. However, this small fraction accounted for only 1.7% of the total potato cell wall material. Therefore, the molar ratio of ARA+GAL to uronic acid was calculated for the two cultivars (Table 6.2). A higher ratio implicates the presence of more and/or longer neutral sugar sidechains. The consecutive fractions from the CDTA-fraction up to and including the KOH-fraction showed an increase in the molar ratio from ca. 1 to ca. 4.5. Buffer extracted pectic

polysaccharides with a ratio between 3 and 4.5. Comparing both cultivars, the ratio was higher in the buffer and CDTA-fractions of cv. Irene and in the CWM and Na_2CO_3 (20°C)-fraction of cv. Nicola.

The molar ratio of uronic acid to rhamnose can be used as an indication for the number of sidechains. Although it should be kept in mind that this ratio may be not very reliable, since only part of the rhamnose residues are substituted (Bacic et al., 1988; Carpita and Gibeaut, 1993) and furthermore the rhamnose content of the cell wall is relatively low. For cv. Nicola a higher ratio was found in the buffer fraction (21 for cv. Nicola and 18 for cv. Irene) and for cv. Irene the ratio was higher in the Na_2CO_3 (20°C)-fraction (38 for cv. Irene and 30 for cv. Nicola).

For both cultivars, buffer and CDTA extracted about 6% of the methyl esters and acetyl groups, while 16% of the uronic acid present in the CWM was extracted in these fractions. For both cultivars a similar degree of methylation (DM = mol methyl esters per 100 mol uronic acid) was established for the CWM, buffer-fractions and CDTA-fractions. Furthermore, the DM of the buffer-fractions (43%) was comparable with the DM of the initial CWM (51%), while the DM was lower for the CDTA-fractions (about 12%). This low DM of the CDTA-fractions was expected, since CDTA was used with the aim to extract calcium-bound pectate. The degree of acetylation (DA = mol acetyl groups per 100 mol uronic acid) was comparable for the CWM of cv. Irene and cv. Nicola (ca. 44%) and for the buffer-fractions of cv. Irene and cv. Nicola (ca. 42%). The CDTA-fractions had a lower DA and moreover there was a difference between cv. Irene (11%) and cv. Nicola (5%).

Enzymic degradation of fractions

To obtain additional information about the relative abundance of homogalacturonan and branched rhamnogalacturonan regions in the pectic polysaccharides, the fractions and residues of both cultivars were degraded by PG and RGase. The buffer and CDTA-fractions were saponified to remove the methyl ester and acetyl groups, which block degradation by PG and RGase. Fractions and residues were not completely soluble in the buffer solution used for the enzymic degradation studies. The HPSEC-elution patterns of the saponified buffer and CDTA-fractions and the Na_2CO_3 and KOH-fractions and residues before and after enzymic degradation are shown in Fig. 6.1.

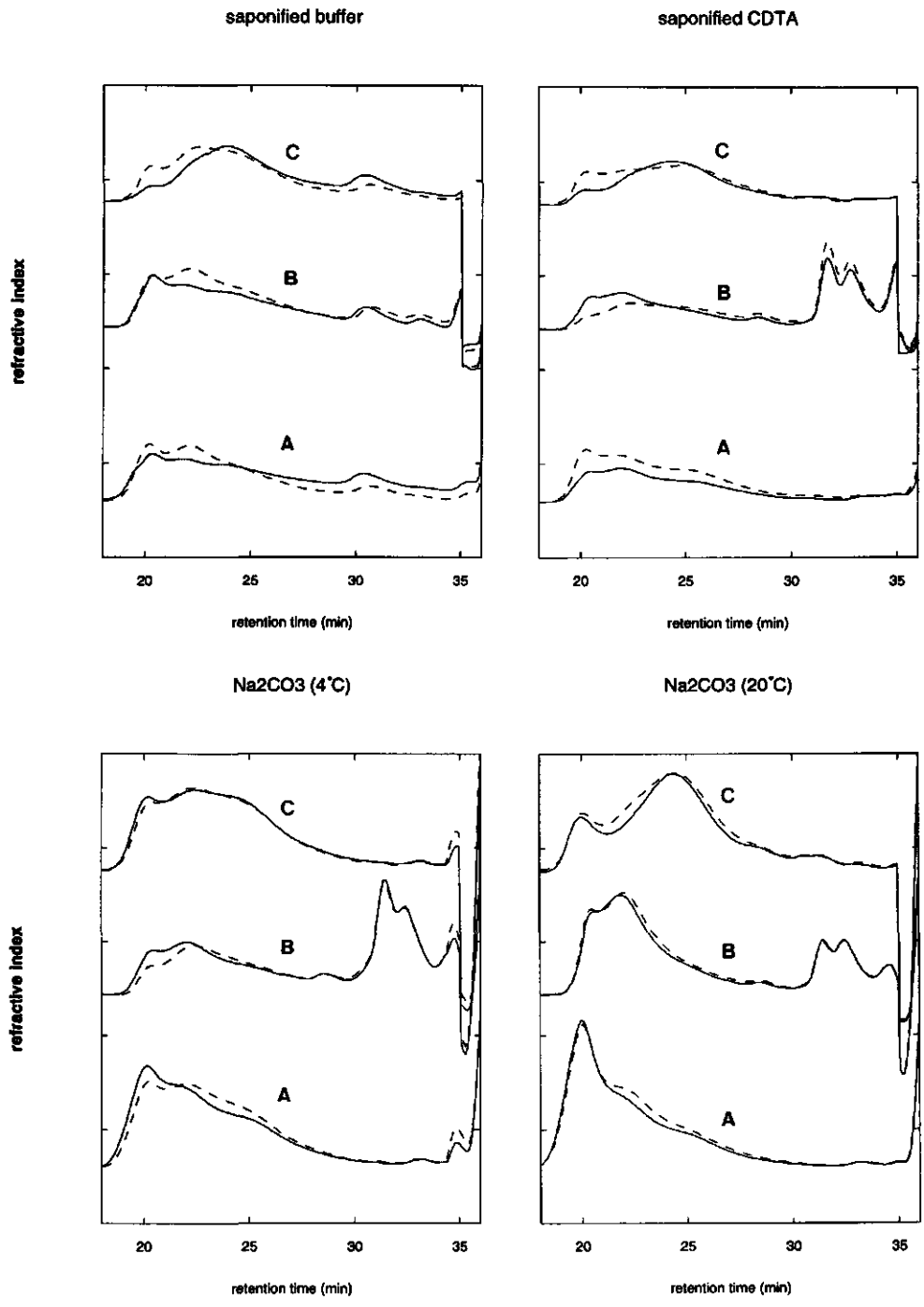
For the buffer and to lesser extent for the CDTA-fractions broad molecular weight-distributions were found. This is probably due to the presence of relatively low molecular weight starch in these fractions, which also give a refractive index. Low molecular weight starch may be a result of the combination of mechanically damaged starch granules (due to ball-milling of fractions and residues) and subsequent heating to 100°C used to inactivate enzymes after enzymic hydrolysis. The HPSEC-elution

patterns of the CDTA-fractions showed for cv. Nicola in particular more material with retention times between 20 and 26 minutes than was found for cv. Irene. This difference was not (only) due to the difference in starch content between the CDTA-fractions of both cultivars. Undigested non-saponified buffer and CDTA-fractions gave comparable HPSEC-elution patterns as the saponified fractions.

The HPSEC-elution patterns of the Na_2CO_3 (4°C), Na_2CO_3 (20°C), KOH-fractions and residues showed the presence of high amounts of high molecular weight material with retention times between 18 and 21 minutes. In these fractions starch was not interfering, since it was almost absent. Differences between the cultivars were found in the KOH-fraction, where material of cv. Nicola was more (34%) soluble and in the residues, where material of cv. Irene was more (23%) soluble as measured from the RI signal from the respective HPSEC-elution patterns.

After incubation of the various fractions and residues with PG, material with retention times between 21 and 28 minutes, oligo- and monomers, DP 1-4, (HPSEC: retention times between 30 and 36 minutes; confirmed with HPAEC, results not shown) were formed at the expense of high molecular weight material. Comparing both Na_2CO_3-fractions and the KOH-fraction with each other revealed that less oligomers were released resulting in relative more material with retention times between 21 and 28 minutes in the consecutive fractions. In the NaOH-fraction and residue of apple cell walls also molecular weight material with a retention time of 26 minutes was formed after incubation with PG (Schols et al., 1995). Remarkable differences between cultivars were found in the following fractions. After treatment of the saponified CDTA-fractions with PG, for cv. Irene more material with retention times between 20 and 26 minutes was present than for cv. Nicola. This observation contrasted with the elution patterns of the undigested CDTA-fractions. PG degraded relative more material for cv. Nicola than for cv. Irene. The solubility of material in the residues increased after incubation with PG and comparable amounts were solubilized for both cultivars.

The respective fractions and residues were also incubated with RGase. Non-saponified buffer and CDTA-fractions showed comparable HPSEC-elution patterns before and after incubation with RGase (Fig. 6.1). A similar observation was found for the cold buffer-fraction from apple cell wall material (Schols et al., 1995). Saponification of buffer- and CDTA-fractions and subsequent incubation with RGase resulted for both cultivars in the formation of material with retention times between 22 and 26 minutes at the expense of initial higher molecular weight material (Fig. 6.1). No oligomers could be detected. Incubation of the Na_2CO_3, KOH-fractions and residues with RGase resulted in the formation of material, with an increasing average retention (from 22 to 25 minutes) for the consecutive fractions. For the Na_2CO_3 (20°C), KOH-fractions and residues, the elution-patterns obtained by HPAEC showed the presence of typical


saponified buffer
saponified CDTA
refractive index
C
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20
25
30
35
retention time (min)
Na2CO3 (4°C)
Na2CO3 (20°C)

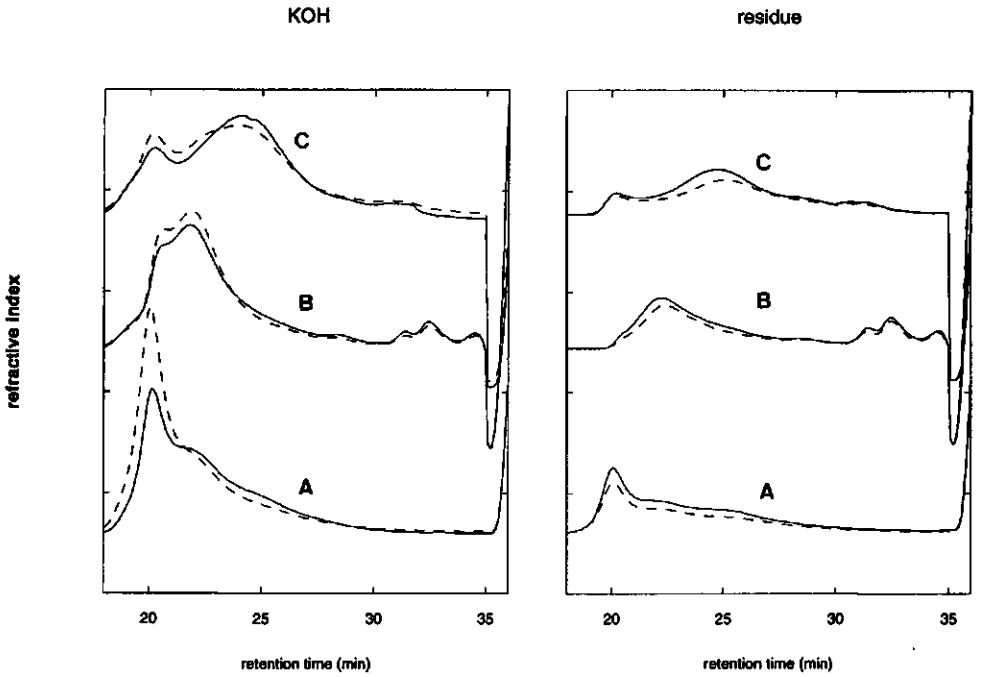

Fig. 6.1 HPSEC-elution patterns of fractions and residues from the cultivars Irene (solid line) and Nicola (dashed line). (A) before enzymic degradation and after treatment with (B) PG and (C) RGase.

RGase oligomers. These patterns were comparable with those obtained by Schols et al. (1994). Schols et al. also identified the structures of the RGase oligomers. On basis of these results, it was concluded that for both potato cultivars mainly the hexamer and octamer (of alternating galacturonic acid and rhamnose residues) were found, which contained two rhamnose residues substituted with a galactose side-chain.

Discussion

Previously, it was reported that upon cooking different types and amounts of pectic material were solubilized for potatoes of the cultivars Irene and Nicola (Marle et al., 1994; Marle et al., 1996). These results can be explained by differences in structure of the non-cooked cell wall material for these cultivars. To validate this hypothesis, non-cooked cell wall material from both cultivars was fractionated by sequential extraction with various solvents to obtain information about the composition, structure

and amounts of the different types of pectin present in the cell wall.
The pectic material extracted by buffer, CDTA and cold Na_2CO_3 originated from the middle lamellae (Ishii, 1981; Jarvis et al., 1981; Ryden and Selvendran, 1990; Selvendran et al, 1985). This material constituted 20% of the CWM for the cultivars Irene and Nicola. Ryden and Selvendran (1990) solubilized 36% of their CWM after extraction with CDTA and cold Na_2CO_3, while Jarvis et al. (1981) reported that 55% of the CWM was extracted with hot oxalate-citrate buffer and cold Na_2CO_3.
The branched pectin extracted with Na_2CO_3 at 20°C probably originated from the primary cell wall (Ryden and Selvendran, 1990) and contained more neutral sugars than galacturonic acid (Table 6.2). Using 0.5 M KOH, an additional amount of more branched pectic material and a small part of the xyloglucans were extracted. Vincken (1996) reported that stronger alkali (1 M and 4 M KOH) extracted most of the xyloglucan from blanched potato tissue. After sequential extraction including 0.5 M KOH, for cv. Irene 70% of the galactose and 64% of the uronic acid and for cv. Nicola 81% of the galactose and 75% of the uronic acid present in CWM were solubilized. Ryden and Selvendran (1990) extracted 40% of the galactose and 60% of the uronic acid present in the CWM after the extraction with 0.5 M KOH, but Jarvis et al. (1981) found the main part of galactose (about 90%) and uronic acid (about 95%) in the oxalate-citrate buffer and cold Na_2CO_3-fractions. As appeared from the above results the extractability of potato pectic polysaccharides is strongly cultivar and/or method dependent.
In the residues of both cultivars considerable amounts of pectic material were found, which are not extracted probably due to alkali-resistant cross-linking and/or entanglement with other cell wall material (Ryden and Selvendran, 1990; Schols et al., 1995).
Galactose is the most abundant neutral sugar in potato cell walls (Jarvis, 1981) and also in the respective fractions and residue (Table 6.1). The relative high amount of galactose (and also arabinose) and the allied high ratio of ARA+GAL to uronic acid found in the buffer-fractions was unexpected, since during the isolation procedure no elevated temperatures were used, which could cause degradation of pectic material (Selvendran and O'Neill, 1987; Ryden and Selvendran, 1990). Maybe this was due to interference of contaminating suspended material from the residue after centrifugation.
The ratio of neutral sugars to uronic acid increased with more severe extraction conditions, comparing the compositions of both Na_2CO_3-fractions and the KOH-fraction with each other (Table 6.2). This increase in ratio is correlated with the presence of more PG-resistant material (Fig. 6.1). These observations implied the presence of more branched rhamnogalacturonan regions in the consecutive Na_2CO_3 and KOH-fractions. The pectic polysaccharides in these fractions are slightly more methylated

and acetylated than in the initial CWM, because relative more uronic acid than methyl esters and acetyl groups was extracted with buffer and CDTA from the CWM (Table 6.1). Furthermore, it was observed that incubation with RGase led to the formation of material with an increasing retention time (due to the presence of shorter homogalacturonan regions between branched rhamnogalacturonan regions) and release of more RGase oligomers for the consecutive fractions, which is in agreement with the above results. Similar observations were made for fractions of apple cell wall material (Schols et al., 1995). During incubation with RGase only small amounts of oligomers were formed. Also incubation of CDTA-insoluble apple cell wall material (Renard et al., 1993) and fractions of cell wall material from apple (Schols et al., 1995) with RG released low amounts of oligomers compared with RGase-incubation of (modified) hairy regions from apple (Schols et al., 1990; Schols et al., 1995). The presence of relative high amounts of the mentioned hexamer and octamer after treatment with RGase was also reported by Schols et al. (1995) for the hairy regions isolated from the alkali-fraction and residue of apple cell walls.

The following differences between the cultivars Irene and Nicola were found with respect to the pectic polysaccharides extracted by buffer, CDTA and cold Na_2CO_3. Comparing the two cultivars, the ratio of ARA+GAL to uronic acid was higher for cv. Irene, while the ratio of uronic acid to rhamnose was higher for cv. Nicola (Table 6.2). Furthermore, the CDTA-fraction had a higher DA for cv. Irene compared with cv. Nicola (Table 6.1). These results indicate that the pectic polysaccharides in the middle lamellae of cv. Irene contain more and/or longer sidechains than those of cv. Nicola. Such a difference in structure was also observed comparing the HPSEC-elution patterns of the saponified CDTA-fractions for both cultivars. The undigested, saponified CDTA-fraction of cv. Nicola contained more material with retention times between 20 and 26 minutes than this fraction of cv. Irene. This material was relatively more degraded after incubation with PG for cv. Nicola than for cv. Irene (Fig. 6.1). Since the CDTA-fractions had a low DM (ca. 12%), saponification and subsequent incubation with PG gave similar results. These observations demonstrate the presence of more and/or longer homogalacturonan regions for cv. Nicola. This is an indication that for cv. Nicola more (stable) calcium-pectate complexes may be present in the middle lamellae than for cv. Irene.

Comparison of the sugar composition of the Na_2CO_3 (20°C), KOH-fractions and residues of both cultivars revealed differences between pectic polysaccharides and the xyloglucan-cellulose-network in the primary cell walls of both cultivars.

The branched pectic polysaccharides extracted by Na_2CO_3 at 20°C form a relative similar weight fraction of the cell walls of both cultivars. However, a higher ratio of ARA+GAL to uronic acid for cv. Nicola (Table 6.2) and a higher ratio of uronic acid to

rhamnose for cv. Irene (Table 6.1) are an indication for the presence of more and/or longer sidechains in the pectic polysaccharides for cv. Nicola than for cv. Irene.
The yield of the KOH-fraction was 7.7% higher for cv. Nicola than for cv. Irene due to the extraction of relative more pectic polysaccharides and glucans (Table 6.1). An indication for a difference in structure between the pectic polysaccharides extracted by KOH for both cultivars is the presence of more soluble material in the KOH-fraction of cv. Nicola than in this fraction of cv. Irene (Fig. 6.1).
For cv. Irene 50% of the CWM remained in the residue, which is about 24% more than for cv. Nicola (Table 6.1). This difference is mainly due to the presence of more uronic acid, arabinose and galactose in the residue of cv. Irene compared to the residue of cv. Nicola. This may be an indication that for cv. Irene the neutral sugar sidechains are more strongly and/or to a larger extent bound to or entangled in the xyloglucan or cellulose-material than for cv. Nicola.
The differences found between the pectic polysaccharides of both cultivars may influence the solubilization of pectic polysaccharides during cooking of potatoes. In our previous paper (Marle et al., 1996) observations made by transmission electron microscopy (TEM) showed that at least part of this cell wall material solubilized during cooking originated from the middle lamellae. The solubilized material was relatively more branched and higher methylated and acetylated for cv. Irene than for cv. Nicola (Marle et al., 1996). These results agree with the data obtained by the fractionation study. The pectic polysaccharides extracted by buffer, CDTA and cold Na_2CO_3 contained more branched rhamnogalacturonan regions for cv. Irene than cv. Nicola.
Since non-cooked cells of cv. Irene had significant more CWM per unit cell surface area than the cells of cv. Nicola (Marle et al., 1996) and comparable amounts of pectic polysaccharides were extracted from the CWM with buffer, CDTA and cold Na_2CO_3 for both cultivars, the cell walls remaining after these extractions still contained more CWM per unit cell surface area for cv. Irene than for cv. Nicola. Therefore the remaining cell walls of cv. Irene will be thicker and/or more compact compared with the cell walls of cv. Nicola. For cv. Nicola, from the remaining cell walls more branched pectic polysaccharides (higher ratio of ARA+GAL to uronic acid) were extracted with Na_2CO_3 (20°C) (Table 6.2) and relative higher amounts of pectic material were extracted with KOH (Table 6.1). This is an additional indication that the primary cell walls of cv. Nicola have a less compact structure than the ones of cv. Irene. In the model proposed by Carpita and Gibeaut (1993), the cellulose-xyloglucan network is embedded in a pectin matrix, which defines, amongst other functions, the porosity of the cell wall. It can be proposed that for cv. Irene in the cell walls remaining after extraction with buffer, CDTA and cold Na_2CO_3, the cellulose-xyloglucan construction is embedded in a less porous pectin matrix than for cv. Nicola. A similar postulation

can be made comparing the structure of the cell walls remaining after cooking for both cultivars. These results agreed with the observations made by cryo-scanning electron microscopy showing cooked cells with more intact cell walls for cv. Irene than for cv. Nicola (Marle et al., 1992).

In conclusion, during cooking more branched pectic polysaccharides were solubilized for cv. Irene than for cv. Nicola, which reflects the difference in middle lamella composition between both cultivars. Middle lamellae of cv. Nicola contained probably more (stable) calcium-pectate complexes than those of cv. Irene. However, the difference in construction of the remaining cell walls for cv. Irene and cv. Nicola seemed to be also important in explaining differences in texture development. Fractionation of CWM from both cultivars gave different types of pectic polysaccharides. Although, relative small differences between the structures of pectic polysaccharides in the cell walls and middle lamellae were found for both cultivars, when using the ratio of ARA+GAL to uronic acid as an indication for the abundance and/or length of neutral sugar sidechains, these differences were confirmed by the enzymic degradation studies.

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Chapter 7

General discussion

Potato production, processing and consumption are all important daily pursuits in the Netherlands. Processing, involving heat treatment, is necessary to make potatoes suitable for consumption. An important quality aspect of the heated product is texture, which exhibits a great deal of diversity. This has led to numerous investigations into describing cooked potato texture based on a sensory perception and into explaining the development of different texture types using histological, (bio-)chemical and physical approaches.

The different approaches were usually subjects of different studies using different cultivars and different processing conditions, which makes comparison or combination of the obtained data very difficult. Furthermore, it is complicated (or simplistic) to relate measured characteristics of the potato (both sensory and instrumental), like mealiness, firmness, cell separation, to certain potato constituents, since statistical correlations do not implicate causal relations. Based on knowledge concerning the ultrastructure, structure and composition of potato tissue and the impact of processing hereupon, hypotheses can be made concerning the development of different texture types during processing. Subsequent investigations will lead to causal relations.

Potato parenchyma tissue is built up of potato cells, which are surrounded by cell walls and filled with starch granules (or gelatinized starch after heating). Both starch and cell walls are thought to play a role in determining potato texture (Jarvis and Duncan, 1992; Keijbets ,1974; Shomer, 1995). Cell walls are also known to be important in determining texture of fruits (apple, guava, kiwi, papaya) (Dawson and Melton, 1991; Marcelin et al., 1993; O'Beirne et al., 1981; Westerlund et al., 1991) and vegetables (carrots, olives, tomatoes, snap beans) (Ben-Shalom et al., 1992; Jiménez et al., 1994; Plat et al., 1988; Shomer et al., 1991; Stolle-Smits et al., 1995). Whereas starch is known to play a role during processing of legume seeds (Liu, 1995).

The aim of the study described in this thesis was (i) to obtain the most relevant sensory texture descriptors, which reflect the diversity in cooked potato texture, (ii) to visualize the change in ultrastructure of potato tissue during cooking to reveal the relative importance of cell walls, middle lamellae and starch with respect to texture development, (iii) to measure the degradation of middle lamellae during cooking for potatoes with a different texture type, (iv) to determine if and/or how ions and composition and structure of potato cell walls contribute to texture development.

Sensory evaluation of potato texture

Ten potato cultivars, which represented a large part of the diversity in texture types of cooked potatoes (in the Netherlands) were sensory evaluated (Chapter 2). Most of

the difference in texture was explained by the difference between mealy and non-mealy (waxy) cooking potatoes. The origin of these differences was subsequently studied using the potato cultivars Irene and Nicola.
Additionally, the sensory evaluation revealed that potato cultivars with an intermediate dry matter content (between 20.5% and 24%) and with moderately mealy and waxy characteristics may differ significantly in firmness. The origin of this difference in firmness was not further investigated, but it can be supposed that cell wall strength and intercellular contacts are the most important factors.The information obtained from the sensory evaluation was not available at the moment of the cryo-SEM study described in Chapter 3. Therefore, in this chapter the contrast mealy <-> firm was used expressing the main difference in texture according to Parlevliet et al. (1991). However, the sensory evaluation revealed that the contrast mealy <-> non-mealy should have been used. Furthermore, the choice of the cultivars for the cryo-SEM study was also based on the classification system of the EAPR (Parlevliet et al., 1991) and cultivars Nicola and Eersteling were chosen as representatives for firm potato texture. Sensory evaluation revealed that cv. Nicola had a waxy texture and that cv. Eersteling had a moderately waxy and mealy, not firm texture. Cryo-SEM showed that fracture planes of potatoes from these two texture types were characterized by the presence of ruptured cell walls and large intercellular contacts.

Potato cell wall characteristics in relation to texture development

Microscopic observations
Fracturing of non-cooked potato tissue resulted in cell cleavage (Chapter 3). However after cooking, fracture occurred alongside cells resulting in cell separation. Cell separation is a result of solubilization of middle lamella material, which is clearly visualized by observation with TEM (Chapter 5). With cryo-SEM more cell separation was observed in cooked tissue of mealy cultivars than in cooked tissue of non-mealy cultivars (Chapter 3). This was in agreement with the higher amount of cell sloughing for the mealy cv. Irene than for the non-mealy cv. Nicola as determined in Chapter 4. In Chapter 3, it was observed that for the non-mealy cultivars, the cell walls in cooked tissue were ruptured after fracturing. Since observations with TEM (Chapter 5) showed that cooked cells are still surrounded by cell walls which were not ruptured, it is concluded that the cell walls in cooked tissue of non-mealy cultivars ruptured during fracturing.

The cellulose-xyloglucan network in potato cell walls
About 13% more cell wall material (per unit cell surface area) was isolated from potato

tissue of cv. Irene than from tissue of cv. Nicola (Chapter 5). Chemical fractionation of the CWM from both cultivars revealed that 80% of the CWM was not removed after extractions with buffer, CDTA and cold Na_2CO_3 for cv. Irene and for cv. Nicola. The remaining cell walls contained almost similar amounts of cellulose and xyloglucans for both cultivars (Chapter 6). Combining the observations from both chapters revealed that cv. Irene contained at least 13% more cellulose and xyloglucan per unit cell surface area compared with cv. Nicola, resulting in a more dense and/or thicker cellulose-xyloglucan network.

The cellulose-xyloglucan-network is the major load-bearing system in cell walls (Iraki et al., 1989; Shedletsky et al., 1992). The above results indicate that for cv. Irene this network has higher load-bearing capacities than for cv. Nicola, either due to a more compact structure or due to a more extended volume. Similar results were found by comparing the physical and chemical structure of unadapted tobacco cells and tobacco cells adapted to osmotic stress (Iraki et al., 1989) and by comparing cell wall structures of unadapted tomato and tobacco cells and adapted cells, grown in a medium with a cellulose-synthesis inhibitor (Shedletsky et al., 1992). The adapted cells had less cell wall material compared to normal cells and the cell wall material of adapted cells had relative lower amounts of cellulose compared to the cellulose content of unadapted cells (total cellulose content was reduced to 10-25%). The tensile strength of the cell walls from adapted cells was lower (about 30%) than for the unadapted cells, measured as described by Carpita (1985).

The pectic polysaccharide network in potato cell walls

The pectic polysaccharides extracted with buffer, CDTA and cold Na_2CO_3 probably originated from the middle lamellae (Ishii, 1981; Jarvis et al., 1981; Selvendran et al., 1985). These fractions were present in comparable relative amounts for both cultivars. However, on basis of fresh tissue weight, cv. Irene contained more pectic polysaccharides originating from the middle lamellae than cv. Nicola. Furthermore, these pectic polysaccharides were more branched for cv. Irene than for cv. Nicola (Chapter 6).

The remaining pectic polysaccharides are thought to be part of the primary wall and control cell wall thickness and porosity (Chapter 1). In the first place, more pectic polysaccharides were present per unit cell surface area for cv. Irene than for cv. Nicola based on fresh weight. Furthermore, for cv. Irene these polysaccharides were more tightly connected with the cellulose-xyloglucan-network than for cv. Nicola, since more branched pectic polysaccharides were extracted with Na_2CO_3 at room temperature for cv. Nicola and since the yield of the KOH-fraction was higher for cv. Nicola as compared with cv. Irene (Chapter 6). Therefore, these results implicate that non-

Table 7.1 Amount of pectic material (measured as uronic acid) in non-cooked potato tissue of the cultivars Irene and Nicola and the amounts of this pectic material (given as % of total uronic acid present in non-cooked tissue) in the different fractions after 15 minutes cooking, as determined in Chapter 4 and 5.

	Chapter 4		Chapter 5			
Cultivar	Non-cooked	Cooked	Non-cooked	Cooked		
	Tissue (mg/g f.w.)	Cooking medium (%)	Tissue (mg/g f.w.)	CWM (%)	Starch fraction (%)	Cooking medium (%)
Irene	3.2	35	3.2	21	55	10
Nicola	2.9	18	2.5	32	57	3

cooked cell walls of cv. Irene contain a thicker and more dense/less porous pectic polysaccharide network.

Heating of potato tissue led to solubilization of pectic material due to β-elimination and decreased stability of the calcium-pectate-gel (Chapter 1). In Chapter 4 and 5 the release of pectic material into the cooking media was determined. The results of both chapters are compared with respect to the solubilization of galacturonic acid after 15 minutes cooking (Table 7.1).

It was checked and found that this difference in pectin release into the cooking media was not due to the fact that in Chapter 4 the uronic acid content was directly determined in the cooking media, while in Chapter 5 it was determined in the AIS of the freeze-dried cooking media.

The difference in uronic acid solubilization can be explained by the fact that during the experiments described in Chapter 4 the potato disks were cooked in small amounts and in Chapter 5 in large amounts. The amount of friction between the disks during boiling was probably smaller in the large scale experiment resulting in a smaller release of pectic material in the cooking medium. Indications for this postulation were found in Chapter 5, comparing the amount of pectic polysaccharides in CWM and starch-fractions of 15 minutes cooked tissue. Table 7.1 shows that more than 50% of the uronic acid present in fresh tissue is lost with the removal of starch during isolation of CWM from 15 minutes cooked potato tissue and recovered in the starch-fraction. Therefore, it was postulated in Chapter 5 that this pectic material was solubilized in the tissue during cooking, but was at that moment sterically hindered to diffuse into the cooking medium. The amount of friction may result in more cell sloughing and thus

less sterical hindrance. The pectic material recovered in the starch fraction also had another structure (ARA+GAL to uronic acid ratio of 1.4) than the pectic polysaccharides in the CWM of 15 minutes cooked tissue (ratio 2.5).
Another result which is in agreement with the previous postulation is the fact that during isolation of CWM from the fraction "loose cells" isolated from the cooking media of 15 minutes cooked tissue of cv. Irene, relatively less galacturonic acid was lost in the starch fraction compared with the isolation of 15 minutes cooked tissue of cv. Irene.
In conclusion, the amount of friction exerted between potato cells and the amount of intercellular adhesion eventually determine the amount of cell sloughing and pectin release, which are cultivar dependent. The amount of intercellular adhesion depends on the pectic polysaccharides in the middle lamellae. Beside small differences in structure, the main difference is the presence of a higher absolute amount of these pectic polysaccharides for cv. Irene than for cv. Nicola. Maybe a more compact and/or thicker middle lamella can be more completely solubilized due to less interconnections with the primary cell walls (in which these middle lamella pectic polysaccharides are anchored (Chapter 1)). The observation that for cv. Nicola less neutral sugar side-chains are solubilized during cooking than for cv Irene agrees with this postulation.

Influence of ions on potato cell walls during cooking

Ionic strength

The effect of ion transfer on the solubilization of pectic polysaccharides in potato cell walls during cooking is complex. The ionic strength (I) influences the stability of the calcium-pectate-gel (Garnier et al., 1993; Garnier et al., 1994; Kohn and Furda, 1967). The difference in ionic strength between cv. Irene and cv. Nicola can be estimated (I_{est}) using the amounts of potassium and citrate, the most abundant cation and mineral acid respectively, which were determined in Chapter 4. For cv. Irene I_{est} = 0.24 M and for cv. Nicola I_{est} = 0.16 M. According to Kohn and Furda (1967) the stability constant of calcium-pectate of several origins (sugar beet, apple, citrus, sunflower) is lower at higher ionic strength. The ionic strength in potato fluid is higher than used in the experimental conditions used by Kohn and Furda (1967). However, during cooking a large part of the ions is transferred to the cooking medium (Chapter 4) and the actual ionic strength in cooked potato cell walls will be lower than calculated. Since the initial ionic strength is higher for cv. Irene and the transfer rates of potassium and citrate are comparable or lower for cv. Irene than for cv. Nicola, the ionic strength in cell walls of cooked potato tissue is most probably higher for cv. Irene.
The difference in ionic strength between the cultivars Irene and Nicola may lead to a

Table 7.2 Molar ratios in potato tissue of the cultivars Irene and Nicola.

Ratio	cv. Irene	cv. Nicola
K^+/COO^-	12	11
Ca^{2+}/COO^-	0.27 (1.1*)	0.27 (1.0*)
$citrate^{2-}/COO^-$	3.7	2.8
$Ca^{2+}/phosphate^{-}$ **	0.006	0.006

* ratio calculated using the amount of uronic acid extracted with CDTA.
** calculated using a phosphor content of 0.08% for starch (on dry matter basis) (Swinkels, 1985).

different stability of the calcium-pectate-gels in the respective cell walls. The actual effect is difficult to estimate and needs more attention in future research.

Specific interaction of ions with calcium-pectate

Although the actual concentrations of specific ions in the cell walls during cooking are also not known, for some ions the effects of the differences in concentration between both cultivars will be estimated. For the cultivars Irene and Nicola the amounts of uronic acid, potassium, calcium and citrate were determined (Chapter 4). The DM was found to be about 50% for both cultivars (Chapter 5). Combining these results, the following ion/COO^- ratios were calculated (Table 7.2).

When present in these ratios potassium had no effect, citrate enhanced and calcium retarded the solubilization of pectic polysaccharides from isolated cell walls (Keijbets, 1974). The effect of the Ca^{2+}/COO^- ratio on pectic polysaccharide solubilization was comparable for both cultivars. The difference in $citrate^{2-}/COO^-$ ratio could result in the solubilization of 4% more galacturonic acid for cv. Irene than for cv. Nicola, neglecting the fact that for both cultivars not enough calcium was present to neutralize all COO^- groups (as was the case in the experimental conditions used by Keijbets (1974)).

Furthermore, the number of binding places in potato tissue (pectate, phosphate, citrate) far exceeds the available calcium ions. To estimate the role of calcium in potato cell walls, the distribution of calcium over cell walls, starch and cytoplasm should be known.

In the studies described in this thesis potato tissue disks are used instead of isolated potato cell walls. The structure of potato tissue (including cell walls and gelatinized starch) is than another important factor influencing the transfer of ions and solubilization of pectic polysaccharides, which should be taken into account.

Cooking disks of potato tissue revealed that the transfer of ions into the cooking medium was much faster than the release of pectic material (Chapter 4). Therefore

the ratio of ion/COO^- in cooked potato tissue is lower than calculated above.
Comparing the cultivars Irene and Nicola with respect to ion transfer and ability of pectic polysaccharides to solubilize, the following differences were found.
The transfer rates of citrate and potassium were higher or similar for cv. Nicola as compared to cv. Irene (Chapter 4). For cv. Irene higher transfer rates were expected, since the surface area increased more during cooking as a result of more sloughing compared with cv. Nicola. This deviant behaviour may be due to the higher dry matter content for cv. Irene compared to cv. Nicola. Andersson (1994) also found that the effective diffusivity of glucose was lower for potatoes with a higher dry matter content due to the hindering effects of potato polymers. Although the main potato polymers are starch polymers, also cell wall polymers can affect the diffusion coefficients of ions (Grignon and Sentenac, 1991). The fact that cv. Irene has more dense and/or thicker cell walls than cv. Nicola (Chapter 5 and 6) probably also contribute to the lower transfer rates than expected for cv. Irene.
Selvendran et al. (1990) postulated that retarded citric acid leakage led to enhanced middle lamella breakdown in mealy cultivars. Although a smaller difference in citrate leakage between cv. Irene and cv. Nicola was found than reported for the mealy and non-mealy cultivar by Selvendran et al. (1990), it can be assumed that both the higher citrate/COO^- ratio and the lower transfer rate of citrate for cv. Irene compared to cv. Nicola enabled more solubilization of the calcium-pectate-gel present in the cell walls of cv. Irene.
The pectic polysaccharides which form calcium-pectate complexes were extracted by CDTA. For both cultivars, this fraction constituted 5.5% of the CWM, but it was (slightly) more branched for cv. Irene than for cv. Nicola. This might indicate that more (stable) calcium-pectate complexes are found in the middle lamellae of cv. Nicola than in those of cv. Irene.

Starch in relation to texture development

The cultivars Irene and Nicola contain 15% f.w. and 12% f.w. starch respectively (Chapter 4). This difference in starch content may influence texture development. The role of starch was not studied in this thesis, but some estimations can be made.
The concentration of the starch gel in the cells will be 20% and 15% for cv. Irene and cv. Nicola respectively. This difference in starch concentration may result in a different degree of swelling of the starch granules for both cultivars (Keetels, 1995; Ring, 1985; Steeneken, 1987). When studying starch gels inside potato cells, it must be kept in mind that the amount of available water is limited and that the degree of swelling is limited to the cell size.

For instance, a potato cell with a diameter of 200 μm has a volume of $4.2*10^{-12}$ m^3. Since the specific gravity of potato tissue is $1097*10^3$ g/m^3 for cv. Irene and $1068*10^3$ g/m^3 for cv. Nicola (Chapter 5), it can be calculated that for cv. Irene the weight of one cell is $4.6*10^{-6}$ g and for cv. Nicola $4.5*10^{-6}$ g. One cell contains $0.69*10^{-6}$ g starch for cv. Irene and $0.54*10^{-6}$ g starch for cv. Nicola (Chapter 4). According to Keetels (1995) 1 g potato starch swelled to a volume of 140 ml ($\approx 0.14*10^{-3}$ m^3) when dispersed in distilled water and heated to 80°C. Using this swelling capacity the starch inside a cell of cv. Irene can swell to a volume of $9.7*10^{-11}$ m^3 (23 times cell volume) and for cv. Nicola to a volume of $7.6*10^{-11}$ m^3 (18 times cell volume).
Taking into account the conditions inside potato tissue, the presence of ions will reduce the degree of swelling. For instance, the presence of 0.1 M NaCl reduced the starch swelling capacity of potato starch about 4.5 times (Keetels, 1995). Furthermore, the amount of water inside cells is limited and during cooking potato cells gain (cv. Irene) or loose (cv. Nicola) weight (Chapter 3) for which mainly water is responsible. However, according to the calculations made, a difference in starch swelling behaviour between both cultivars is possible. The influence of conditions inside potato tissue need more attention to verify the postulation that a difference in starch swelling pressure contributes to the development of different texture types (Jarvis et al., 1992). Another important feature of the starch gel inside potato cells is its rheological behaviour. The Young modulus for 15% and 20% potato starch gels heated at 90°C differed a factor 3 (Keetels, 1995), implicating that a more concentrated gel behaved more stiff. A comparable difference in gel behaviour may be present between starch gels in potato cells of the cultivars Irene and Nicola (as was postulated in Chapter 3), *although the influence of conditions inside tissue should be taken into account.*

Potato texture development

The strength of non-cooked potato tissue is determined by the strength of the primary cell walls (surrounding individual cells) and the strength of the middle lamellae (keeping adjacent cells together) respectively. Fracturing of non-cooked tissue takes place through cell walls (the line of least resistance) demonstrating the strength of intercellular contacts.
Cooking of potato tissue causes solubilization of the middle lamellae, weakening of the cell wall and gelatinization of starch. After cooking fracturing of potato tissue takes place alongside cell walls. Thus, the strength of the middle lamellae is relatively more reduced than the strength of the primary cell walls.
The strength of cooked tissue depends both on the remaining intercellular contacts in

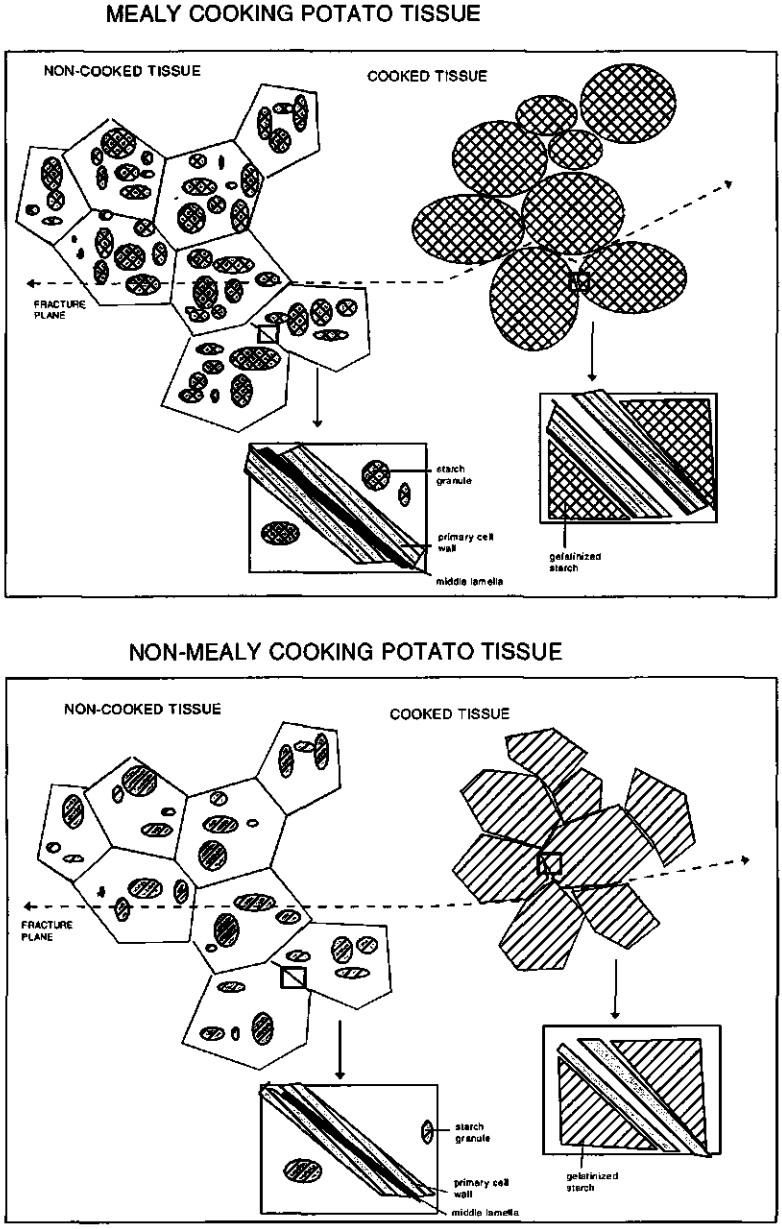

Fig. 7.1 Schematic representation of mealy and non-mealy cooking potato tissue.

the middle lamellae and on the rigidity of the cells. The rigidity of the cooked cells is dependent on the strength of the remaining primary cell wall and the rigidity of the gelatinized starch inside the potato cells. The features of the two extreme types of texture (mealy and non-mealy respectively) can be represented as follows. A solid cell wall and a rigid starch gel render rigid cells. These cells take up a more or less round shape (energetically most favourable), probably resulting in degradation of more intercellular contacts. The cells become more or less separate unities and are not easily damaged upon handling. A weak cell wall and/or a weak starch gel give few support to the cells. The cells keep lying against each other and are easily damaged upon handling. The features of non-cooked and cooked potato tissue with mealy and non-mealy characteristics are schematically represented in Fig. 7.1.

The strength of the middle lamella is determined by pectic polysaccharides and among other things dependent on the amount and stability of calcium-pectate complexes. The strength of the cell wall is determined by the cellulose-xyloglucan-network. Ions affect the solubilization of pectic polysaccharides and the rigidity of the gelatinized starch.

Future research should be focused on the (physical measurement of) forces and strengths that are present in potato tissue or develop during processing and, in particular, on differences between these forces within tissues with different texture. Forces and strengths of interest are the strength of the middle lamellae, the strength of the cell walls, the swelling pressure of starch during gelatinization and the rigidity of cooked potato cells. Combination of the results from (bio-)chemical studies and physical studies is essential to confirm the postulations made in this thesis.

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Summary

Texture of cooked potatoes is an important quality aspect. Much diversity in texture is observed between potato cultivars and also within a cultivar between potatoes with different dry matter content. The aim of the research described in this thesis was to gain insight in the development of different texture types between cultivars during cooking.

An overview of the literature concerning potato texture is given in Chapter 1. Furthermore, attention is paid to structure and composition of potato tissue with emphasis on the role of cell wall composition and structure, with special attention for pectic polysaccharides, and on the role of starch and ions.

In Chapter 2, research into the sensory diversity in texture types is described. Ten table potato cultivars were sensory evaluated by a trained panel. The texture descriptors used concerned both appearance and mouthfeel. This study reveals (i) the relative contribution of the used texture descriptors in discriminating between different cultivars and (ii) correlations between the used descriptors. Most of the diversity in texture types could be explained by differences between mealy and non-mealy characteristics. Furthermore, cultivars which have similar mealy/non-mealy characteristics could be discriminated on basis of firmness of potato tissue. Subsequent investigations were focused on the origins of the difference between mealiness and non-mealiness (waxiness).

Observations with cryo-scanning electron microscopy (cryo-SEM) showed that fracture planes of mealy and non-mealy cooked potato tissue have different structures (Chapter 3). In both cases fracturing occurs alongside cells. However, more intercellular contact and more ruptured cell walls are observed for non-mealy tissue than for mealy tissue. Therefore, structure and composition of cell walls and middle lamellae were subject of further investigations using the cultivars Irene and Nicola, which have a mealy and a non-mealy texture respectively.

In Chapter 4, the degradation of the middle lamellae was determined by recording solubilization of pectic material (measured as galacturonic acid) and cell sloughing during cooking (0 to 30 minutes) of potato tissue disks from both cultivars. Relatively more release of pectic material and more cell sloughing are observed in the cooking medium of the mealy cooking cv. Irene compared with the non-mealy cooking cv. Nicola. Both cultivars have similar specific cell surface areas and therefore the difference in pectin release is not due to a difference in cell size distribution. Since a

given percentage release of pectic material results in more cell sloughing for cv. Irene than for cv. Nicola, it was postulated that either differences in structure and composition of the pectic polysaccharides in the cell wall or middle lamella and/or differences in the ionic conditions in cell wall and middle lamellae may result in a cultivar dependent degradation of pectic material.

Firstly, the ion composition in potato tissue was studied during cooking (0 to 30 minutes) by recording the transfer of potassium (most abundant cation in potato), citrate (most abundant anion in potato) and calcium (complex formation with pectate and citrate) from potato tissue disks into the cooking medium for both cultivars. 80% of the calcium present in potato tissue could not be transferred, probably due to complexation with other potato constituents (for instance starch and pectate). However, more than 70% of the potassium and citrate present in potato tissue is released into the cooking media. Using k_s*A as a measure for the transfer rate, it was observed that citrate and potassium are transferred in higher or similar amounts for cv. Irene than for cv. Nicola. Since the amount of sloughing is higher (resulting in more cell surface area) for cv. Irene than for cv. Nicola, this result was unexpected. Especially more retarded citrate leakage for cv. Irene than for cv. Nicola may lead to solubilization of more calcium-pectate, which is present in the middle lamellae. However, also differences in structure and composition of the pectic material from both cultivars should be taken into account and were subsequently studied.

Cell wall material (CWM) was isolated from non-cooked and cooked potato tissue and accompanying cooking media for both cultivars (Chapter 5). The mealy cooking cv. Irene contains more CWM per unit cell surface area than the non-mealy cooking cv. Nicola. These results confirm observations of potato cell walls made by transmission electron microscopy (TEM). The molar sugar composition of CWM of non-cooked tissue is comparable for both cultivars. However, different types of pectic polysaccharides are solubilized between 5 and 15 minutes cooking. The solubilized pectic material is relatively more branched and higher methylated and acetylated for cv. Irene than for cv. Nicola. The released amount of pectin is not consistent with the results found in Chapter 4, probably due to the different methods used during cooking. Subsequent research was focused on differences in structure of cell walls from both cultivars, which could explain the observed differences in solubilized pectic polysaccharides.

CWM of non-cooked potato tissue was successively extracted with buffer, CDTA, Na_2CO_3 at 4° and 20°C and 1 M KOH (Chapter 6). Beside the sugar composition also the structure of the polysaccharides in the fractions and residues was determined. Information about the presence of smooth and hairy regions in pectic polysaccharides was obtained using degradation with purified polygalacturonase (PG) and

rhamnogalacturonase (RGase) in combination with high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC). The pectic polysaccharides originating from the middle lamellae (extracted by buffer, CDTA and cold Na_2CO_3) form 20% of the CWM for both cultivars. Both sugar composition and pectin fragments obtained after incubation with PG and RGase revealed that this material is more branched for cv. Irene than for cv. Nicola. This may be an indication for the presence of more (stable) calcium-pectate complexes. Pectic polysaccharides in the primary cell wall were extracted with Na_2CO_3 at room temperature and 1 M KOH. Since for cv. Nicola the Na_2CO_3-fraction contains more branched pectic polysaccharides and a higher yield of the KOH-fraction is obtained compared with cv. Irene, it is proposed that the pectin matrix of the primary cell wall of cv. Irene had thicker and/or a more dense/less porous structure than the matrix in the cell wall of cv. Nicola.

In Chapter 7, the results from the preceding chapters are compared and combined. The roles of the xyloglucan-network and of the pectin matrix are discussed with respect to the texture development of cv. Irene and cv. Nicola. The influence of ions upon the solubilization of pectic polysaccharides is discussed. The role of starch was not studied, but on basis of results from literature some estimations are made with respect to starch swelling pressure and the rigidity of the starch gel inside cells. Finally, the development of different types of potato texture is described in broad outline.

Samenvatting

Textuur is een belangrijk kwaliteitsaspect van gekookte consumptie-aardappelen. Verschillen in textuur worden gevonden tussen aardappelen van verschillende rassen en binnen een ras tussen aardappelen met een uiteenlopend droge stof gehalte. Het doel van het onderzoek dat beschreven is in dit proefschrift, was het verkrijgen van meer inzicht in het ontstaan van verschillen in textuur tussen rassen tijdens koken.
Een overzicht van de literatuur over textuur van aardappelen wordt gegeven in Hoofdstuk 1. Verder wordt de structuur en de samenstelling van aardappelweefsel beschreven met nadruk op de rol van de samenstelling en structuur van celwanden, met speciale aandacht voor pectine, en op de rol van zetmeel en ionen.
In Hoofdstuk 2 wordt onderzoek naar de sensorische verschillen in textuur beschreven. Tien consumptie-aardappelrassen werden gekeurd door een getraind sensorisch panel. De descriptoren die werden gebruikt, hadden betrekking op zowel het uiterlijk als het mondgevoel van het gekookte aardappelweefsel. Dit onderzoek geeft aan (i) welke relatieve bijdrage de gebruikte textuur descriptoren leveren aan het onderscheid tussen de verschillende rassen en (ii) welke correlaties aanwezig zijn tussen de descriptoren. De grootste variatie in textuur wordt verklaard door verschillen tussen melige en niet-melige eigenschappen. Rassen die vergelijkbare melige/niet-melige eigenschappen hebben, kunnen onderscheiden worden op basis van de stevigheid van het aardappelweefsel. In het verdere onderzoek werd met name gekeken naar de oorzaken van het verschil tussen meligheid en niet-meligheid.
Opnamen met een cryo-scanning elektronen microscoop (cryo-SEM) lieten zien dat breukvlakken van melig en niet-melig aardappelweefsel een verschillende structuur hebben (Hoofdstuk 3). In beide typen weefsel vindt breuk plaats langs het celoppervlak. Echter niet-melig weefsel vertoont meer intercellulaire contacten en meer beschadigde celwanden dan melig weefsel. Naar aanleiding hiervan werden de structuur en de samenstelling van celwanden en middenlamellen verder onderzocht voor de rassen Irene en Nicola, die respectievelijk een melige en niet-melige textuur hebben.
In Hoofdstuk 4 werd de afbraak van de middenlamel bepaald door tijdens het koken (0 tot 30 minuten) van aardappelschijfjes van beide rassen enerzijds het oplossen van pectine te meten en anderzijds het loslaten van cellen te meten. Relatief meer opgelost pectine en meer losse cellen worden gevonden in het kookwater van het melig kokende ras Irene in vergelijking met het niet-melig kokende ras Nicola. Het verschil in opgelost pectine kan niet verklaard worden door verschil in

celgrootteverdeling, omdat beide rassen een vergelijkbaar specifiek celoppervlak hebben. Echter omdat een gegeven percentage opgelost pectine meer losse cellen tot gevolg heeft voor het ras Irene dan voor het ras Nicola, werd verondersteld dat *of* verschillen in structuur en samenstelling van het pectine in celwand of middenlamel *en/of* verschillen in ionensamenstelling in celwand en middenlamel de pectine-afbraak rasafhankelijk maken.

Allereerst werd de ionensamenstelling van aardappelweefsel tijdens koken (0 tot 30 minuten) bestudeerd door transport van kalium (meest voorkomende cation in aardappel), citraat (meest voorkomende anion in aardappel) en calcium (vorming van complexen met pectaat en citraat) uit aardappelweefsel in het kookwater te meten. 80% van het calium dat aanwezig is in niet-gekookt weefsel wordt niet getransporteerd, mogelijk als gevolg van complexvorming (bijvoorbeeld met zetmeel en pectaat). Echter meer dan 70% van de hoeveelheid kalium en citraat aanwezig in niet-gekookt weefsel lost op in het kookwater. Door k_s*A te gebruiken als maat voor de transportsnelheid bleek dat een grotere hoeveelheid citraat en een vergelijkbare hoeveelheid kalium in oplossing gaan voor het ras Irene dan voor het ras Nicola. Dit was een onverwacht resultaat omdat tijdens koken meer cellen loslaten voor het ras Irene (resulterend in een groter celoppervlak) dan voor het ras Nicola. Met name het feit dat citraat minder snel in oplossing gaat voor het ras Irene dan voor het ras Nicola kan ertoe leiden dat meer calcium-pectaat, dat aanwezig is in de middenlamel, in oplossing gaat. Vervolgens werd onderzocht of ook verschillen in structuur en samenstelling van pectine van beide rassen een rol spelen.

Uit niet-gekookt en gekookt aardappelweefsel en kookwater werd celwandmateriaal (CWM) geïsoleerd voor de rassen Irene en Nicola (Hoofdstuk 5). Het melig kokende ras Irene heeft meer CWM per eenheid celoppervlak dan het niet-melig kokende ras Nicola. Deze resultaten bevestigen opnamen gemaakt met een transmissie elektronen microscoop (TEM). De molaire suikersamenstelling van CWM afkomstig van niet-gekookt weefsel is vergelijkbaar voor beide rassen. Toch lossen verschillende soorten pectine op tussen 5 en 15 minuten koken. Het opgelost pectine is relatief meer vertakt en bevat meer methylesters en acetylgroepen voor het ras Irene dan voor het ras Nicola. De opgeloste hoeveelheid pectine is niet vergelijkbaar met de resultaten, die in Hoofdstuk 4 gevonden zijn. Dit is waarschijnlijk een gevolg van het verschil in kookmethode. Tijdens verder onderzoek werd gekeken of dit een gevolg was van verschillen in structuur van de celwanden van beide rassen.

CWM afkomstig van niet-gekookt aardappelweefsel werd achtereenvolgens geëxtraheerd met buffer, CDTA, Na_2CO_3 bij 4°C en 20°C en 1 M KOH (Hoofdstuk 6). Naast de suikersamenstelling werd ook de structuur van de polysacchariden in de fracties en residuen onderzocht. Informatie over "smooth" en "hairy regions" in pectine

werd verkregen door afbraak met gezuiverd polygalacturonase (PG) en rhamnogalacturonase (RGase) in combinatie met HPSEC ("high-performance size-exclusion chromatography") en HPAEC ("high-performance anion-exchange chromatography"). Voor beide rassen wordt 20% van het CWM gevormd door pectine afkomstig uit de middenlamel (geëxtraheerd met buffer, CDTA en Na_2CO_3 bij 4°C). Zowel de suikersamenstelling als de pectinefragmenten verkregen na incubatie met PG en RGase wijzen erop dat dit pectine meer vertakt is voor het ras Irene dan voor het ras Nicola. Mogelijk duidt dit op de aanwezigheid van meer (stabiele) calium-pectaat complexen. Na_2CO_3 bij 20°C en 1 M KOH extraheren pectine uit de primaire celwand. De Na_2CO_3-fractie van het ras Nicola bevat meer vertakt pectine en de opbrengst van de KOH-fractie is hoger voor het ras Nicola in vergelijking met het ras Irene. Deze resultaten leidden tot de hypothese dat de pectine matrix in de celwand van het ras Irene dikker is en/of een meer dichte/minder poreuze structuur heeft dan de matrix in de celwand van het ras Nicola.
In Hoofdstuk 7 worden de resultaten van de voorgaande hoofdstukken vergeleken en gecombineerd. De rol van het xyloglucaannetwerk en de pectine matrix met betrekking tot de textuur van de rassen Irene en Nicola wordt besproken, evenals de invloed van ionen op de oplosbaarheid van pectine. De rol van zetmeel is niet onderzocht, maar op basis van gegevens uit de literatuur wordt een schatting gemaakt van de invloed van een zogenaamde "starch swelling pressure" en de stevigheid van een zetmeel gel in aardappelcellen. Tot slot wordt in grote lijnen het ontstaan van verschillende typen textuur geschetst.

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Nawoord

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Wageningen,
1 december 1996

Netty

Curriculum vitae

Jacoba Trinette (Netty) van Marle werd op 20 januari 1965 geboren te Rijnsburg. In 1983 behaalde zij haar Atheneum-B diploma aan het Christelijk Lyceum te Alphen aan den Rijn. In hetzelfde jaar begon zij op Laboratoriumschool Rijnland te Leiderdorp. Na het behalen van de propedeuse HLO startte zij in september 1984 met de studie Levensmiddelentechnologie (orientatie Levensmiddelenleer) aan de toenmalige Landbouwhogeschool te Wageningen. Zij deed afstudeervakken bij de sectie Levensmiddelenchemie en bij de sectie Zuivel en Levensmiddelennatuurkunde. Tijdens haar stageperiode werkte zij aan de "Université Nationale du Bénin" (West-Afrika). In november 1989 studeerde zij af aan de Landbouwuniversiteit Wageningen. Van 1 februari 1990 tot 1 mei 1994 werkte zij als wetenschappelijk onderzoekster op het Agrotechnologisch Instituut ATO-DLO te Wageningen. Het onderzoek is beschreven in dit proefschrift.