Pectic substances in the cell wall and the intercellular cohesion of potato tuber tissue during cooking

M.J.H. Keijbets

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Abstract

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The influence of ions, starch, buffer strength and pH on solubilization of pectic galacturonan from potato cell wall material during boiling was studied. The ions enhanced β-eliminative degradation of galacturonan, but calcium, copper (II) and iron (II) cations slowed down the solubilization at pH 6.1. Magnesium was ineffective. Citrate, malate and phytate anions favoured it. Maceration of potato tissue disks by pectic lyases and model cooking experiments demonstrated the ability of calcium ions to retain intercellular cohesion of potato tissue even when pectic galacturonan had been severely degraded. In a pH range of 6.1-6.5, β-eliminative pectin degradation was shown by specific periodate thiobarbituric acid staining to occur in cell wall and potato tissue boiling.

Specific gravity graded potato tubers were analysed chemically. In addition to starch, citrate, phosphorus, potassium, magnesium, pectic galacturonan and pH increased but malate, calcium and intercellular cohesion decreased with increasing sp. gr. There was a complex causal relationship between chemical composition and intercellular cohesion. Both were influenced by physiological age. Potato pectinesterase was partially characterized. Because of activation of this enzyme or leaching of ions, intercellular cohesion could be greatly enlarged in preheating experiments. Literature on structure and insolubility of pectic substances in plant cell walls and on intercellular cohesion of the cooked potato was reviewed.

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Stellingen

1. Tijdens groei en rijping van aardappelen lopen veranderingen in de chemische samenstelling parallel aan de toename van het zetmeelgehalte. Doordat bovendien laatstgenoemd verschijnsel eenvoudig is vast te stellen, is ten onrechte een causaal verband gelegd tussen zetmeel en intercellulaire cohesie na koken.

J.S. Woodman & D.S. Warren, J. Sci Fd Agric. 23: 1067-1077.
Dit proefschrift, hfdst. 7.

2. De cohesieve eigenschappen van de intercellulaire pectinegel in aardappelweefsel blijven in aanwezigheid van calcium ionen tijdens koken behouden, zelfs indien het pectine-galacturonaan in sterke mate is afgebroken.

Dit proefschrift, hfdst. 6.

3. Het verdient aanbeveling magnesium ionen niet langer onder dezelfde noemer te plaatsen als calcium ionen ten aanzien van de binding aan pectine carboxylgroepen en de onoplosbaarheid van pectine structuren in plantecelwanden.

M.A. Joslyn, 1962. Adv. Fd Res. 11: 1-107.
L.F. Molloy & E.L. Richards, 1971. J. Sci. Fd Agric. 22: 397-402.
Dit proefschrift, hfdst. 5.

4. Het verbieden van nitriet als voedingsmiddel-additief is prematuur zolang de kennis over in vivo synthese van N-nitrosaminen beperkt is, en hypocriet zolang roken van sigaretten mogelijk blijft.

K. Möhler et al., 1972. Z. Lebensm.-Unters. u. -Forsch 150: 1-11.
B.C. Challis, 1973. Nature 244: 466.
L.K. Keefer & P.P. Roller, 1974. Science 181: 1245-1247.
E. Boyland & S.A. Walker, 1974. Nature 248: 601-602.

5. Aan de aandrang vanuit de chemische en voedingsmiddelenindustrie tot fortificatie van voedingsmiddelen zoals 'snacks' dient de overheid niet toe te geven.

FAO/WHO, 1971. Joint FAO/WHO Expert Committee on Nutrition.
Eighth report. Food fortification. Protein-calorie malnutrition. Wld Hlth Org. techn. Rep. Ser. No. 477.
Voedingsraad, 1974. Voeding 35: 9-59.

6. Indien voldoende calcium ionen of andere kationen met een sterke affiniteit voor de carboxylgroepen aanwezig zijn, wordt β-eliminatie van pectine mogelijk zonder dat de carboxylgroepen veresterd zijn zoals o.a. door Albersheim et al. noodzakelijk wordt geacht.

P. Albersheim et al., 1960. Archs Biochem. Biophys. 90: 45-51.
G. Dongowski & W. Bock, 1973. Faserforsch. u. Textiltech. 24: 34-38.
M.J.H. Keijbets & W. Pilnik, 1974. Carbohyd. Res. 33: 359-362.

7. Bij de bepaling van het kiemgetal van de coli-aerogenes groep, verdient het de voorkeur glucose niet als koolhydraatbron te gebruiken.

D.A.A. Mossel et al., 1962. J. Bacteriol. 84: 381.
J.L. Cornelisse, 1974. Het isoleren van Salmonellae uit plantaardige voedermiddelen en mengvoeders. Dissertatie, Utrecht.

8. De saccharose synthase (UDPG-D-fructose-2α-glucosyltransferase, E.C. 2.4.1.13) activiteit, gemeten als saccharose splitsing, vormt een interessant en bruikbaar rijpheidscriterium voor de aardappel.

R. Pressey, 1969. Pl. Physiol. 44: 759-764.
J.R. Sowokinos, 1971 resp. 1973. Am. Potato J. 48: 37-64 resp. 50: 234-247.

9. Gezien de specificiteit van de interacties tussen pathogeen en waardplant is het weinig zinvol om macererende activiteit van niet-aardappel pathogene micro-organismen te bepalen op aardappelweefsel.

D.F. Bateman & S.V. Beer, 1965. Phytopath. 55: 204-211.
R.J.W. Byrde & A.H. Fielding, 1968. J. gen. Microbiol. 52: 287-297.
A.L.J. Cole & R.K.S. Wood, 1970. Ann. Bot. 34: 211-216.

10. In de menselijke evolutie is de teelt van voedselgewassen niet voorafgegaan aan, maar een gevolg geweest van de ontwikkeling van het koken.

A.C. Leopold & R. Ardrey, 1972. Science 176: 512-514.

11. Te vrezen valt dat bij benoeming van bisschoppen in de Nederlandse r.-k. kerkprovincie het woord 'episkopos' nogal eenzijdig geïnterpreteerd wordt als 'opzichter van Rome'.

Proefschrift van M.J.H. Keijbets
Wageningen, 6 november 1974

Ter herinnering aan mijn ouders

Pieter Jozef Hubert Keijbets
Elisabeth Maria Petronella Ploemen

Voorwoord

Bij het gereed komen van dit proefschrift gaat mijn dank uit naar velen. Niet alleen naar hen die daadwerkelijk hebben bijgedragen aan dit onderzoek, maar vooral ook naar allen die gestalte gegeven hebben aan mijn vorming en opleiding. Wat het promotieonderzoek betreft wil ik in het bijzonder noemen:

Prof. Dr. W. Pilnik, mijn leermeester in de levensmiddelenchemie en -microbiologie en promotor, onder wiens stimulerende begeleiding ik heb mogen werken en wiens diepe kennis van en enthousiasme voor de pectinestoffen meermalen nieuwe wegen van onderzoek wisten te wijzen. Mijn dank voor vele waardevolle discussies geldt ook zijn naaste medewerkers in het pectine-onderzoek.

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Marita, die niet alleen in de afgelopen jaren vele uren van wachten voor lief nam als ik door het onderzoek in beslag werd genomen, maar die bovendien het manuscript uitnemend typte.

Curriculum vitae

Martin Jozef Hubert Keijbets werd op 27 april 1944 geboren te Terwinselen (gem. Kerkrade). In 1962 behaalde hij het einddiploma gymnasium-β aan het Bisschoppelijk College te Sittard. Van 1962 tot 1970 studeerde hij aan de Landbouwhogeschool te Wageningen in de richting levensmiddelentechnologie. Het ingenieursdiploma werd met lof behaald met als vakken levensmiddelenchemie (hoofdvak, verzwaard), levensmiddelenmicrobiologie en voedingsleer. Vanaf februari 1970 bewerkte hij als promovendus dit proefschrift op het Instituut voor Bewaring en Verwerking van Landbouwprodukten (IBVL) te Wageningen. Sinds januari 1974 is hij als wetenschappelijk ambtenaar aan dit Instituut verbonden in tijdelijke dienst.

Samenvatting

De intercellulaire cohesie is een van de belangrijkste textuureigenschappen van aardappelweefsel. Al naar gelang de eigenschappen van de grondstof en de behandelingen tijdens verwerking, verandert de intercellulaire cohesie tijdens verhitten. De betekenis van de pectinestoffen, die tot de matrix behoren van de primaire celwand en middenlamel van de aardappel, voor de uiteindelijke intercellulaire cohesie van de gekookte aardappel werd aan een nader onderzoek onderworpen. Hiertoe werden maceratie-experimenten met pectine-galacturonaan depolymerases opgezet; voorts werd het oplosbaar worden van pectine-galacturonaan in modelsystemen (aardappelcelwanden, weefselschijfjes) bestudeerd. Monsters van uiteenlopend soortelijk gewicht werden uitgebreid chemisch geanalyseerd, terwijl experimenten om door vóórverhitting celwand-gebonden pectine-esterase te activeren, werden uitgevoerd.

In hoofdstuk 2 en 3 wordt een literatuuroverzicht gegeven van sommige aspecten van de intercellulaire cohesie. In hoofdstuk 2 wordt de structuur van de pectinestoffen in de matrix van de celwand en middenlamel besproken, naast die van de primaire plantecelwand zelf. Met name wordt aandacht besteed aan de onoplosbaarheid van de pectinestoffen, die verantwoordelijk wordt geacht voor de cohesie tussen de cellen. Aangenomen wordt dat de onoplosbaarheid wordt veroorzaakt door covalente verankering aan hemicellulose en glycoproteïnen in de celwand en middenlamel. Tegelijkertijd wordt benadrukt dat calcium bijdraagt aan de stapeling van galacturonaan ketenstukken in op microkristallieten gelijkende structuren.

In hoofdstuk 3 is de literatuur bijeengebracht met betrekking tot de intercellulaire cohesie van de gekookte aardappel en de samenhang hiervan met de chemische samenstelling. Intercellulaire cohesie blijkt objectief, indirect, te kunnen worden gemeten met deformatietesten, zoals meting van de samendrukbaarheid en van de penetratie, maar directe bepaling van de celseparatie aan de hand van het gewicht dat na koken achterblijft op een zeef verdient de voorkeur. Gewezen wordt op het belang van objectieve textuurmeting om invloed van verwante parameters uit te sluiten; dit geldt in het bijzonder bij het onderzoek naar de invloed van de chemische samenstelling

op de intercellulaire cohesie. Een kritisch overzicht van oudere literatuurgegevens over intercellulaire cohesie illustreert dit uitgangspunt nader. Zo kan men betwijfelen of er wel een oorzakelijk verband bestaat tussen zetmeel en intercellulaire cohesie, zoals door velen is verondersteld, omdat bij die onderzoekingen de intercellulaire cohesie meestal subjectief gemeten werd. Wel geven vroegere experimenten sterke aanwijzingen dat complexe veranderingen in de pectinestoffen de intercellulaire cohesie van de gekookte aardappel beïnvloeden.

De sleutelpositie van pectine-galacturonaan in de intercellulaire cohesie wordt aangetoond in hoofdstuk 4. Enzymen die pectine-galacturonaan depolymerizeren (laag-methoxylpectine lyase en pectine lyase) reduceerden de celcohesie sterk. Tijdens deze maceratie verliep het oplosbaar worden van pectine-galacturonaan uit weefselschijfjes van laag (1.060-1.070) en hoog (1.100-1.110) soortelijk gewicht, afkomstig uit één populatie van knollen, gelijkvormig. Derhalve werden met behulp van de gebruikte technieken geen verschillen in primaire structuur van pectinestoffen gevonden. De celseparatie was echter sneller in weefsel van hoog s.g. Een verklaring hiervoor ligt in de grotere celafmetingen in hoog s.g. weefsel, welke het verlies van intercellulaire cohesie bevorderen in vergelijking met weefsel van laag s.g. Het patroon van de enzymatische maceratie, vervolgd via de spectrofotometrische turbiditeit, bracht de belangrijke rol van calcium ionen in de celcohesie aan het licht. Calcium ionen vertraagden de celseparatie, zelfs als pectine--galacturonaan zelf sterk afgebroken was, zoals bleek uit een grote mate van oplosbaar worden bij mechanische verbreking van de weefselsamenhang.

Het oplosbaar worden van pectine-galacturonaan uit aardappelcelwanden bij koken werd bestudeerd in hoofdstuk 5. Met name werd de invloed nagegaan van de ionen in het weefsel zelf, zetmeel, pH en buffersterkte. Aangetoond werd dat pectine-galacturonaan, voor 58% veresterd, bij pH 6.1 werd afgebroken door β-eliminatie. Calcium, bivalente koper en ijzer ionen, vertraagden het oplosbaar worden van pectine-galacturonaan in vergelijking met kalium, maar magnesium deed dit niet. Toenemende concentraties van calcium en kalium ionen verhoogden de snelheid van de β-eliminatie, calcium zelfs in grotere mate dan kalium. De belangrijke rol van calcium in microkristallijne bundelingszones in de aardappelcelwandstructuur wordt geïllustreerd door de optimale onoplosbaarheid van pectine-galacturonaan bij een verhouding Ca^{2+}/COO^{-} van 1 à 2. Organische anionen zoals citraat, malaat en fytaat maakten pectine-galacturonaan oplosbaar bij koken, dankzij het binden van calcium en de versnelling van de β-eliminatieve afbraak.

Deësterificatie met sinaasappel PE verhoogde de affiniteit van de carboxylgroepen van pectine-galacturonaan voor calcium. De rol van calcium bij het onoplosbaar houden van pectine-galacturonaan werd nog bevestigd in enkele kookproeven met complexe mengsels van aardappelbestanddelen, waarvan de chemische samenstelling die van laag en hoog s.g. weefsel benaderde.

In hoofdstuk 6 worden modelstudies beschreven over de intercellulaire cohesie van gekookt aardappelweefsel. Hierbij werden dezelfde dode en gedeeltelijk uitgeloogde weefselschijfjes gebruikt als voor de enzymatische maceratie. Deze werden gekookt in een buffer van pH 6.5 en het verlies van intercellulaire cohesie werd weer turbidimetrisch gemeten. Voor het eerst wordt mededeling gedaan van het aantonen van β-eliminatieve afbraak van pectine-galacturonaan bij het koken van een plantaardig weefsel door een specifieke kleurreaktie met perjodaat-thiobarbituurzuur. Hoewel het oplosbaar worden van pectine-galacturonaan bij beide s.g. fracties weer gelijkvormig verliep, verloor het weefsel van hoog s.g. de intercellulaire cohesie het snelst. Experimenten waarin calcium en kalium ionen toegevoegd en/of verwijderd werden toonden duidelijk aan dat calcium ionen, in het weefsel aanwezig of van buitenaf toegevoegd, de pectinegel in de celwand en middenlamel stabilizeren en beschermen tegen verlies van zijn samenbindende functie, zelfs indien pectine sterk afgebroken is tijdens koken. Het versterkende effect van calcium op de intercellulaire cohesie verdween bij de verestering van de carboxylgroepen van pectine-galacturonaan en nam aanzienlijk toe bij volledige enzymatische verzeping.

Een wijd scala van chemische analyses, maar ook één bepaling van enzymactiviteit, werd in hoofdstuk 7 toegepast op soortelijk gewichtsfracties uit begrensde populaties waarin het bekende verband tussen soortelijk gewicht, ofwel zetmeelgehalte, en intercellulaire cohesie bestond. De gezamenlijke gegevens werden onderworpen aan een parametervrije trend test. Het bleek dat met stijgend s.g. niet alleen zetmeel significant toenam maar ook citraat, fosfor, kalium, magnesium en de pH. Pectine-galacturonaan, voor 50-60% veresterd, nam minder duidelijk toe. Malaat, calcium, PE activiteit en de intercellulaire cohesie namen af. De neutralizatie van niet-veresterde carboxylgroepen van pectine-galacturonaan in geïsoleerde celwanden nam ook af en bleef onder de 50% (verhouding $Ca^{2+}/COO^{-} < 0,5$). Uit deze resultaten en die van een rooitijden experiment kwam de conclusie naar voren dat het groeistadium en de mate van rijpheid bepalend zijn voor het niveau van zowel zetmeel- als niet zetmeelbestanddelen. Binnen begrensde populaties betekent dit dat de eerder genoemde relatie zetmeel-intercellulaire cohesie vervangen

wordt door een meer causaal verband tussen intercellulaire cohesie en chemische bestanddelen die met het pectine-galacturonaan reageren (hoofdstuk 5 en 6). De enorme invloed van de pH, in het bijzonder in het natuurlijke pH-gebied van de aardappel, 5,5-6,5, werd duidelijk aangetoond.

Enkele effecten van bewaring en bemesting op de chemische samenstelling en de intercellulaire cohesie van aardappelknollen werden onderzocht. Bij bewaring bij 6°C nam de intercellulaire cohesie samen met de PE activiteit af. Veranderingen in organische zuren liepen parallel met een stijging van de pH. Stikstofbemesting stimuleerde de synthese van organische zuren en de PE activiteit, terwijl de intercellulaire cohesie toenam. Fosfaatbemesting onderdrukte de citraatsynthese en dientengevolge de celseparatie bij koken. Om de veranderingen in intercellulaire cohesie tijdens verhitten van aardappelknollen te verklaren, wordt bijzondere aandacht besteed aan de 'zetmeel zwellingsdruk' hypothese van in oorsprong Atwater en de 'thermische expansiedruk' hypothese van Hoff.

Uit Bintje aardappelen, steeds gebruikt in dit werk, werd pectine--esterase geëxtraheerd. Het enzym werd gezuiverd door ammoniumsulfaatprecipitatie en gedeeltelijk gekarakteriseerd (hoofdstuk 8). Door een pH-optimum bij pH 8-8,5 en een temperatuuroptimum bij 55°C lijkt het aardappel PE op PE uit andere hogere planten. De activeringsenergie bleek 5700 cal/mol te bedragen. Met als parameters de temperatuur, de verhittingstijd en de oppervlakte van het aardappelweefsel werden vóórverhittingsexperimenten uitgevoerd. PE activering werd gemeten door veranderingen in de veresteringsgraad en trad alleen op bij 55 en 60°C, maar binnen één uur niet bij 50°C of bij 75°C waar PE reeds geïnactiveerd wordt. Bij vóórverhitting van hele, ongeschilde knollen op 55°C en langer dan één uur nam de intercellulaire cohesie sterk toe. Buiten het temperatuurgebied waar PE geactiveerd wordt, nam de intercellulaire cohesie ook toe wanneer geschilde weefselstukjes van verschillende afmetingen vóórverhit werden. De mate van toename hing af van de reeds genoemde parameters en werd veroorzaakt door het uitlogen van ionen. Het uitlogen werd kwantitatief vervolgd door het meten van de specifieke geleidbaarheid en door chemische analyse van het weefsel (kalium, calcium en citraat).

Contents

Symbols and abbreviations

A	= absorbance, cm^{-1}
AIS	= alcohol-insoluble solids
ATP	= adenosine triphosphate
COO^-	= non-esterified carboxylate anions of pectic galacturonan
DE	= degree of esterification
DEAE	= diethylaminoethyl
DMSO	= dimethylsulphoxide
DP	= degree of polymerization
E_A	= activation energy per mole; cal or J
EDTA	= ethylenediaminetetraacetate
HMP	= hexametaphosphate
HS	= high specific gravity
K_m	= Michaelis constant, mol dm^{-3}
katal	= unit of enzymic activity
LMPL	= low-methoxyl pectin lyase
LS	= low specific gravity
Milavi test	= Milner-Avigad reducing hexuronic acid test
NADP(H)	= nicotinamide-adenine-dinucleotide phosphate (reduced)
PAL	= pectate lyase
PE	= pectinesterase
PG	= polygalacturonase
pipes	= piperazine-NN'-bis-2-ethanesulfonic acid
PL	= pectin lyase
R	= gas constant per mole and per degree; 1.98 cal or 8.29 J
Relative A_{552}	= periodate-TBA $A_{552} \times 10^3$ per unit of percentage solubilized pectic galacturonan
RW	= retained weight, g (after boiling in RWCS test)
RWCS test	= retained weight cell separation test
s	= standard deviation
sp. gr.	= specific gravity = relative density d_t^t,
T	= absolute temperature, K

T_{100}	= measure of intercellular cohesion at cooking, min (RWCS test)
TBA	= thiobarbituric acid
TCS test	= turbidity cell separation test
Tris	= 2-amino-2-hydroxymethylpropane-1,3-diol
V	= maximum reaction rate at infinite substrate concentration
v_o	= initial reaction rate
ϵ	= molar extinction coefficient, $mol^{-1}dm^3cm^{-1}$
κ	= specific conductance, $mS\ cm^{-1}$
$\kappa_{corr.}$	= specific conductance corrected for alkali addition in preheating medium, $mS\ cm^{-1}$

1 Introduction

The potato tuber (*Solanum tuberosum* L.) growing as an underground stem and functioning as a storage organ rich in parenchyma tissue filled with starch, is an important food material in many European and American countries (Burton, 1966). Originally potato tubers were consumed as a staple food, but in the industrialized and prosperous western hemisphere the decreasing trend in direct potato consumption is nearly reversed by the increased consumption of industrially prepared potato products.

Both in home-cooking and industrial processing, heat treatments are applied to convert an otherwise indigestable raw product into an acceptable and even palatable endproduct. Heat treatments like ordinary boiling, baking, steaming or canning finally result in a cooked potato to which quality characteristics can be attributed. Experts of the European Association of Potato Research (EAPR) have already proposed a system for classifying cooked potato quality (Lugt & Goodijk, 1958) based on colour, flavour and texture properties.

When only texture is considered, a definition of terms seems necessary. Textural description of foods and determination of textural characteristics has not been easy for food scientists, because they lacked rational systems for nomenclature in sensorial judgments (Szczesniak, 1963) and had difficulties with instrumental measurement and characterization of texture (Kramer, 1964; Finney, 1972). Voisey (1971) defined texture of food as 'related to the physical properties sensed by the eyes before eating (except colour), the sense of touch in handling the food and the tactile receptors in the mouth during consumption'. Szczesniak (1963) attempted to classify textural characteristics of foods into more specific properties of hardness, cohesiveness, viscosity, elasticity, adhesiveness and others; Van Buren (1970) mentioned another main approach to texture by studying the relationship between chemical constituents or treatments of a food material and one or more selected texture parameters.

One of the main textural properties of potato tuber tissue is the cohesion between the cells. Intercellular cohesion is preferred to

intercellular adhesion, which was used by for instance Linehan & Hughes (1969a) and Van Buren (1970). This preference is based on definitions of Szczesniak (1963). She defined cohesiveness as the strength of the internal bonds making up the body of the product and adhesiveness as the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes in contact. Accordingly, adhesiveness is related to surface properties whereas cohesiveness is related to forces of attraction between particles and opposing disintegration. The structure and function of the intercellular layer of the cell wall in plant tissues strengthen the preference to use cohesion. The intercellular cohesion is adversely affected by heat treatments such as boiling. These result in a less coherent structure expressed in words or terms as disintegration, sloughing, breakdown, falling apart and mealiness. This loss in tissue coherence may or may not be desired, depending on the consumer's preference and the required properties of the endproduct. Linehan & Hughes (1969a) thought that intercellular cohesion was the most important textural characteristic of the cooked potato. As I agree with these authors, I shall not use terms such as falling apart (Schippers, 1962), disintegration, breakdown and sloughing when failure in intercellular cohesion is meant. These terms all indicate a partial or complete loss of intercellular cohesion, while sloughing more precisely is defined as a disintegration of tissue structure in the outer layers, mostly starting near the vascular region (Schippers, 1962).

Mealiness probably the oldest term in potato research for describing texture, is defined as the ease of mashing a cooked potato in the mouth ('mouthfeel') or with a fork or other instrument (Zaehringer & Le Tourneau, 1962). Both mealiness and (reduction in) intercellular cohesion have frequently been found to be correlated with specific gravity (dry matter, starch), so that sometimes it is thought that these texture characteristics are identical or at least interdependent.

The intercellular layer of potato and other plant tissues originates from the cell plate at cell division, develops into middle lamella and is built up essentially of pectic substances (McClendon, 1964; Shafizadeh & McGinnis, 1971; Northcote, 1972). Thus it is logical to assume that pectic substances play a substantial role in maintenance of intercellular cohesion. Cell separation or failure in intercellular cohesion might follow a degradation of pectic substances in a pH region where the galacturonan backbone of pectic substances is known to be labile during heating or another

mechanism of solubilization of middle lamella constituents (Doesburg, 1965). Until now, however, such a causal relationship between pectic substances and cooked potato intercellular cohesion has not been clearly established (Linehan & Hughes, 1969a). This must be partly because of the subjective sensorial methods of texture appraisal which do not satisfactorily separate attributes of several texture parameters. On the chemical side the large excess of starch in potato tissue obscures pectin analysis. Furthermore in the past knowledge about pectin structure and function in the native cell wall and middle lamella was rather limited.

In this study the importance of pectic substances for intercellular cohesion at cooking was determined. In Chapter 2 a literature survey was given of structure of pectic substances and cell wall. Intercellular cohesion of the cooked potato, its measurement and how it is influenced by chemical composition, were discussed in Chapter 3. The relationship between specific gravity or starch content and intercellular cohesion was used as a starting point for the experimental part. Pectin depolymerizing enzymes were applied in maceration experiments with high and low sp. gr. potato tissues, which differ widely in intercellular cohesion after cooking (Chapter 4). Differences in pectin structure of these tissues could influence maceration pattern or macerating ability of the enzymes. In Chapter 5 the mechanism of pectin solubilization during cooking was investigated in model studies with isolated potato cell walls. The high and low sp. gr. tissues used for maceration were also studied in model cooking experiments (Chapter 6). As parameters in Chapters 5 and 6 were used: ions, degree of esterification of pectic galacturonan, starch, pH, buffer concentration and boiling time. The levels of potato constituents which were found to influence pectin solubilization and intercellular cohesion were analysed in Chapter 7 in potato samples after specific gravity grading; furthermore some potato samples of experiments on lifting date and manuring were analysed. Finally the role of potato pectinesterase in intercellular cohesion was studied in preheating experiments (Chapter 8). For all experiments tubers of variety Bintje were used.

2 Pectic substances, an important structural component of cell wall and middle lamella: a survey

When dealing with intercellular cohesion, the structure of cell wall and middle lamella is important. In addition to earlier surveys in this field, it is therefore necessary to discuss the structure, occurrence and function of pectic substances within the cell wall and middle lamella complex. Kertesz (1951) compiled previous research on pectin and Joslyn (1962) reviewed the work on insolubility of native cell wall and middle lamella pectin ('protopectin'). Doesburg (1965) collected pectin research with reference to jellying phenomena and texture of fruits and vegetables. Recent surveys on pectic substances and pectin degrading enzymes have been made by Pilnik & Zwiker (1970), Pilnik & Voragen (1970), Voragen & Pilnik (1970), Rombouts & Pilnik (1972) and Fogarty & Ward (1972). Cell wall formation, composition, structure, growth and (bio)chemistry have been discussed by several authors too (Northcote, 1958, 1972; Albersheim, 1965a, b; Mollenhauer & Morré, 1966; Mühlethaler, 1967; Rogers & Perkins, 1968; Lamport, 1970; Shafizadeh & McGinnis, 1971). Attention has given to biosynthesis of cell wall components in recent reviews (Loewus, 1971; Nikaido & Hassid, 1971).

2.1 STRUCTURE OF THE PECTIC SUBSTANCES

2.1.1 Earlier research

As a result of many years of pectin research, pectic substances emerge as complex heteropolysaccharides rather than a well-defined group of homopolysaccharides with galacturonic acid or ester as a unit building block. Gradually it has become well established that pectic substances possess a rhamnogalacturonan main chain with covalently attached side chains of neutral sugars. The existence of a rhamnogalacturonan backbone structure in pectic substances from very different origins is proven by the isolation of the aldobiuronic acid 2-O-(α-D-galacturonopyranosyl)-L-rhamnose (GalpA-(1→2)-Rha) from alfalfa, lemon peel, soybean hulls and cotyledons, Amabilis fir bark, apple fruit, carnation root, sycamore callus and suspension-cultured cell

wall and mustard embryo (e.g. Rees, 1969; Aspinall, 1970a, b; Pilnik & Voragen, 1970; Rombouts, 1972). The rhamnosyl residues are not randomly distributed in the main chain, but they seem to be interspersed in a galacturonan chain as rhamnose-rich blocks (Aspinall et al., 1968a, b). The galacturonopyranosyl residues are definitely α-1,4-glycosidically linked (Jones & Reid, 1954, 1955). More information is required about linkage and composition of side chains because of their important role in the primary structure of pectic substances. However, this is difficult to obtain mainly because of the instability of the pseudo-aldobiuronic acid linkages during acid hydrolysis. The presence of neutral sugars containing side chains has become increasingly evident because it is impossible to physically separate some neutral sugars from isolated pectic substances (Aspinall, 1970a). Only by enzymatic hydrolysis, followed by methylation studies, has information been obtained about the point of attachment of side chains. Two branching points of the rhamnogalacturonan backbone have been ascertained; D-xylose and L-arabinose are linked glycosidically to C_3 of a galacturonopyranosyl residue (Bouveng, 1965; Aspinall et al., 1967b, 1968a, b) whereas both Aspinall et al. (1967a) and Talmadge et al. (1973) found evidence for attachment of side chains to C_4 of rhamnose. Besides D-galactose, L-fucose, D-glucuronic acid and 2-methylethers of xylose and fucose have been found in side chain fragments (Rees, 1969; Aspinall, 1970a, b; Rombouts, 1972). These facts and findings should not be interpreted as a simultaneous occurrence of all the mentioned sugars and linkages, because they are derived from work on pectins of different species.

2.1.2 Primary, secondary and tertiary structure

Talmadge et al. (1973) recently gave a more detailed primary structure of pectin. Their report is the first one at this level of detail and quantitation. The results can be applied to structures of other primary cell walls although their material consisted of cell walls from suspension-cultured sycamore cells (*Acer pseudoplatanus* L.) (Keegstra et al., 1973; Wilder & Albersheim, 1973). Talmadge et al. (1973) used a purified endo polygalacturonase to extract oligosaccharide fragments from cell walls. They purified and characterized these wall fractions by ion-exchange and gel permeation chromatography, and analysed the fragments using recent improvements in methylation technique.

Besides the already mentioned general features of the rhamnogalacturonan

backbone, these authors demonstrated the presence of covalently linked arabinan and galactan. The arabinan is highly branched, but the linear 4-linked galactan is attached with its reducing end to presumably C_4 of rhamnose residues, the major branch point of the main chain.

The rhamnogalacturonan molecule is built up of linear α-(1→4)-linked galacturonan parts interrupted by 2-linked rhamnosyl residues (Fig. 1). This rhamnogalacturonan also contains a tetrasaccharide structure GalpA-(1→2)--Rhap-(1→4)-GalpA-(1→2)Rha, earlier reported by Aspinall from soybean cotyledon meal, lemon peel and lucerne leaves and stem pectin (Aspinall et al., 1967b; 1968a, b). The linear galacturonan parts of sycamore cell wall pectin are determined to be 6-12 units long, whereas one of every two rhamnosyl residues is possibly branched by attachment of 4-linked galactan (Fig. 1). The Talmadge model suggests the existence of a repeating unit within the rhamnogalacturonan, an interesting detail from the point of view of biosynthesis by polymerization (Talmadge et al., 1973). The detailed sugar composition of sycamore cell wall pectic substances will be compared with that of potato pectin in Section 3.2. It is convenient, however, to remark here that the pectic substances of sycamore cell wall are rich in neutral sugars (38% galacturonic acid versus 62% neutral sugars), the most frequently occurring being arabinose, followed by galactose and rhamnose.

The Talmadge model for rhamnogalacturonan from sycamore cell walls has a zigzag shape (Fig. 1). It is likely that interspersement of 2-linked

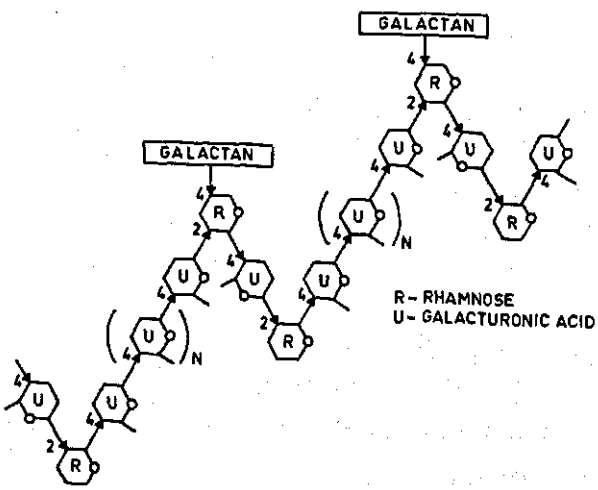

Fig. 1. Proposed structure for the rhamnogalacturonan part of pectic substances of cell wall of suspension-cultured sycamore cells (Talmadge et al., 1973). R = rhamnose; U = galacturonic acid; N = undetermined number, probably between 4 and 10.

rhamnosyl residues in an otherwise linear galacturonan chain, although a feature of primary structure, influences secondary and possibly tertiary structure (Rees, 1972a). The C1 conformation, generally ascribed to α-D-galacturonopyranosyl moieties, results in an axial-axial linked polygalacturonide with a screw axis that tends to coil (Pilnik & Voragen, 1970). On the basis of X-ray measurements of Palmer & Hartzog (1945) and Palmer et al. (1947), Rees (1969) proposed a computed conformation of a three-fold helix with a right-handed screw sense. Rees (1969) described this helix as 'a twisted, corrugated strip instead of a stretched-out, wire spring of the carrageenan type'. Sterling (1957), on the other hand, indicated a two-fold screw symmetry in oriented gels of calcium pectate. Tertiary pectin structures may result from interactions between molecules as existing in polymeric network of gels (Rees, 1969, 1972a, b). From electron microscopy Leeper (1973), in fact, found evidence for a tertiary structure of pectate molecules in elementary fibrils, which apparently contain thirteen pectate molecules in their cross-sectional area. He argued that such tertiary structures might occur in gels. As yet it has not been possible to demonstrate elementary fibrils in sol or gel state.

2.1.3 Degree of esterification and molecular weight

Two important characteristics of the pectin molecule remain to be mentioned. The galacturonopyranosyl residues of the rhamnogalacturonan main chain are esterified with methanol to various degrees, and sometimes secondary hydroxyls (C_2 - C_3) are acetylated to an appreciable extent (e.g. beet pectin; McCready, 1966). The degree of esterification, theoretically ranging from 0 to 100%, also expressed as methoxyl content (0-16.32%), varies with origin, maturity and way of extraction and purification (McCready & McComb, 1954; Gee et al., 1958; Gee et al., 1959; Raunhardt & Neukom, 1965; Potter, 1966). As a primary structure characteristic, the degree of esterification may influence secondary and tertiary structure (Leeper & Dull, 1972), but it is of considerable importance too, because degradation of pectic substances by pectin depolymerases strongly depends on this degree of esterification (Voragen et al., 1971a; Rombouts, 1972; Voragen, 1972). Polygalacturonases and low-methoxyl pectin lyases (Pilnik et al., 1973) preferentially attack low-esterified pectic substances, although not necessarily pectic acid, whereas pectin lyases show preference for less than 100% esterified substrates depending on pH, buffer and calcium (Voragen et al., 1971a; Voragen, 1972). Chemical β-eliminative (or transeliminative) breakdown of pectin is partly

governed by the degree of esterification too (Vollmert, 1950b; Albersheim, 1959; Albersheim et al., 1960b; Neukom, 1963).

Molecular weight is meaningful with relation to pectic substances in extracted state only. Number - or weight - average molecular weights have been determined for characterization of pectin preparations and are reported to range between 10 000 and 400 000 (Doesburg, 1965) according to source and extraction method. These numbers apply to the (rhamno)galacturonan part of the molecule, the side chains being removed mostly by the common acid extraction procedures. Therefore nothing is known with any precision about chain length of either rhamnogalacturonan or of side chains of pectic substances in the plant cell wall. The enzymic attack upon extracted pectin may be influenced by chain length as shown for tomato polygalacturonase (Pressey & Avants, 1971).

2.2 STRUCTURE OF CELL WALL AND MIDDLE LAMELLA WITH REFERENCE TO ANCHORAGE AND INSOLUBILITY OF PECTIC SUBSTANCES

The potato tuber tissue consists of storage parenchyma cells and hence the cell walls do not suffer from secondary thickening, not even in the vascular region (Sterling, 1966). Lignification also seems absent outside the vascular tissue. For that reason only middle lamella and primary wall will be discussed.

2.2.1 Wall formation

When a plant cell divides, vesicles derived from the so-called Golgi bodies appear during the later phases of mitosis and fuse in the equatorial plane of the cell to form the cell plate (e.g. Albersheim, 1965a; Rogers & Perkins, 1968; Shafizadeh & McGinnis, 1971). The cell plate grows from the equatorial plane perpendicular to the division axis until it reaches the side walls of the cell. In the more mature cell wall this cell plate, then called middle lamella, constitutes the common cell wall layer of the two daughter cells. At both sides of the cell plate cellulose is deposited soon, indicating that the development of the primary wall has started. At the inner side of the developing cell plate, the membranes of Golgi vesicles apparently constitute the cell membrane or plasmalemma. This means that once the plasmalemma is formed, the contents of Golgi vesicles must pass the cell membrane to reach the developing wall, a process which occurs by reverse pinocytosis, i.e.

vesicles fuse with plasmalemma and the contents are discharged without breakage of the membrane (e.g. Mollenhauer & Morré, 1966; Mühlethaler, 1967; Shafizadeh & McGinnis, 1971). It is assumed that the matrix substances, possibly including proteins (Mollenhauer & Morré, 1966) are synthesized inside the Golgi bodies in the cytoplasma. The non-matrix substances, or cellulose microfibrils seem to be synthesized outside the cytoplasm by particles at the plasmalemma surface (Mühlethaler, 1967). These particles regulate the orientation and packing of cellulose microfibrils by way of number and mode of aggregation. In the initial stages of cell wall formation (primary wall), the packing of cellulose microfibrils is fairly moderate leading to a loose and unorganized cellulose framework.

2.2.2 *Matrix substances of the cell wall*

The non-matrix *cellulose*, a polymer of β-1,4-glycosidically linked glucose is especially important for cell wall and tissue rigidity mainly obtained through formation of the secondary wall. We shall not go into the structure of this cell wall component, however, because it does not directly contribute to intercellular cohesion.

The presence of methylated polyuronides containing *pectin* in an uncompletely formed cell plate of onion root tip has been established by staining with a specific pectin stain and viewing under the electron microscope (Albersheim & Killias, 1963). Staining is done with alkaline hydroxylamine-ferrichloride (McCready & Reeve, 1955; Gee et al., 1959). With the same technique pectin is localized in the middle lamella and throughout the primary wall (Albersheim et al., 1960a; Albersheim & Killias, 1963). Dense staining was always found in the intercellular layer. In cauliflower (Saxton & Jewell, 1969) and potato tissue (Fox, 1971) similar results were obtained.

Although hemicellulose and protein form a substantial part of cell wall matrix substances, their presence cannot be revealed by histochemical methods.

The class of *hemicelluloses* comprises a group of polysaccharides, which are extracted from plant tissues with alkali after removal of starch and pectic substances (Whistler & Richards, 1970). This rather negative definition may be replaced by a more positive one, based on a functional basis formulated by Bauer et al. (1973). This definition is the result of an overall cell wall concept, emerging from the work of Albersheim and co-workers, who also established the Talmadge model for pectic substances

(Talmadge et al., 1973) (see Section 2.1.2). Bauer et al. (1973) isolated a xyloglucan polysaccharide from suspension-cultured sycamore cell walls, showing a structure of a cellulose-like β-(1→4)-glucan backbone with frequent xylosyl side chains glycosidically linked to C_6 of the glucosyl residues. It was shown that this xyloglucan is able to form strong interchain hydrogen bonds with cellulose. A similar structure was isolated from sycamore extra--cellular polysaccharides by Aspinall et al. (1969) and from cell walls of red kidney beans by Wilder & Albersheim (1973), whereas Blake & Richards (1971) noticed a strong cellulose bonding of some hemicelluloses. On this basis Bauer et al. (1973) suggested that hemicelluloses be redefined 'to include only those plant cell wall polysaccharides, which are found to bind non-covalently to cellulose'. According to them other classical hemicelluloses (xylans, gluco- and galactoglucomannans, mannans, arabinoxylans and -galactans) have structures equally suitable for hydrogen bonding to cellulose molecules.

The existence of cell wall glycoproteins has been revealed only recently. They are unusually rich in hydroxyproline and contain arabinose and galactose in their sugar part (Lamport, 1965, 1970). Lamport suggested a backbone of hydroxyproline interspersed by other amino acids and tetraarabinosides attached to the hydroxyl group of hydroxyproline. The terminal arabinose residues should be linked glycosidically to galactan side chains with an alkali-labile bond (Lamport, 1969), but according to Keegstra et al. (1973) this is ruled out since these bonds are stable to a mild alkaline treatment.

2.2.3 *Structure of the primary cell wall*

As we decided that the cell wall concept of the group of Albersheim, although worked out for sycamore primary cell wall, may be valuable for the underlying research it will be discussed here.

What is known, supplementary to the partially elucidated primary, secondary and tertiary structures of cell wall components, about the inter-connections of these cell wall building blocks? Sometimes, it has been assumed that the overall strength of the cell wall is maintained by non--covalent forces (Rees & Wight, 1969), because the cell wall might be dispersed completely by several extractions which do not break covalent bonds. This hypothesis is not supported by Knee (1970a, 1973), who could not extract pectic substances from apple fruit cell wall in appreciable amounts without degradative treatments. Knee (1973) observed that increasingly

insoluble pectic cell wall fractions contained increasing proportions of neutral sugars and even proteins, although with little hydroxyproline.

Contrary to Knee, the group of Albersheim made use of purified cell wall degrading enzymes, a polygalacturonase, an endoglucanase and a protease, as analytical tools, and succeeded in collecting much knowledge about interconnections of cell wall substances (Talmadge et al., 1973; Bauer et al., 1973; Keegstra et al., 1973). Finally, Keegstra et al. (1973) tried to build a molecular structure of sycamore cell wall. This primary cell wall model (Fig. 2) is based on experimental evidence and theoretical considerations. There was considerable evidence for the wall xyloglucan, which is non-covalently bonded to cellulose microfibrils and apparently covers these microfibrils with a monolayer (Bauer et al., 1973), being linked covalently to the pectic polysaccharides (Talmadge et al., 1973; Bauer et al., 1973; Keegstra et al., 1973). Most probably the reducing ends of the xyloglucan are attached to galactan side chains of the rhamnogalacturonan. Lamport (1965) put forward the hypothesis that glycoproteins ('extensin') function as cross-linking molecules by covalent bonding to pectic substances. The experimental results of Knee (1973) may confirm this, and additional evidence is presented by Keegstra et al. (1973), who found indications for a covalent linkage between galactose residues and the hydroxyl group of serine residues of the cell wall protein. From the experimental results, Keegstra et al. (1973) suggested that a highly branched arabinogalactan (3,6-linked galactosyl branching points with a single arabinosyl as predominant side chain) acts as a bridging molecule between the rhamnogalacturonan (possibly bound to the reducing ends) and the hydroxyproline-rich protein (attached to serine). The glycosidic bond between galactose and serine may be alkali-labile, thus meeting the requirements of the glycoprotein hypothesis of Lamport (1969). This alkali-lability is caused by the liability of the galactosyl-serine bond to β-elimination when the serine residue takes part in a peptide structure (Spiro, 1970).

The cell wall structure according to Keegstra et al. (1973) (Fig. 2) is characterized by the covalent interconnections between the matrix substances. The only non-covalent bonds are those between the matrix xyloglucan (= hemicellulose) and cellulose, which, however, are probably as strong as covalent ones (Bauer et al., 1973). The ultimate cell wall concept is that of *one large, interconnected macromolecule*, resembling with its proteinglycan network (Lamport, 1970), the peptido-glycan network of bacterial cell walls (Ghuysen, 1968). Keegstra et al. (1973) admitted that their model with an

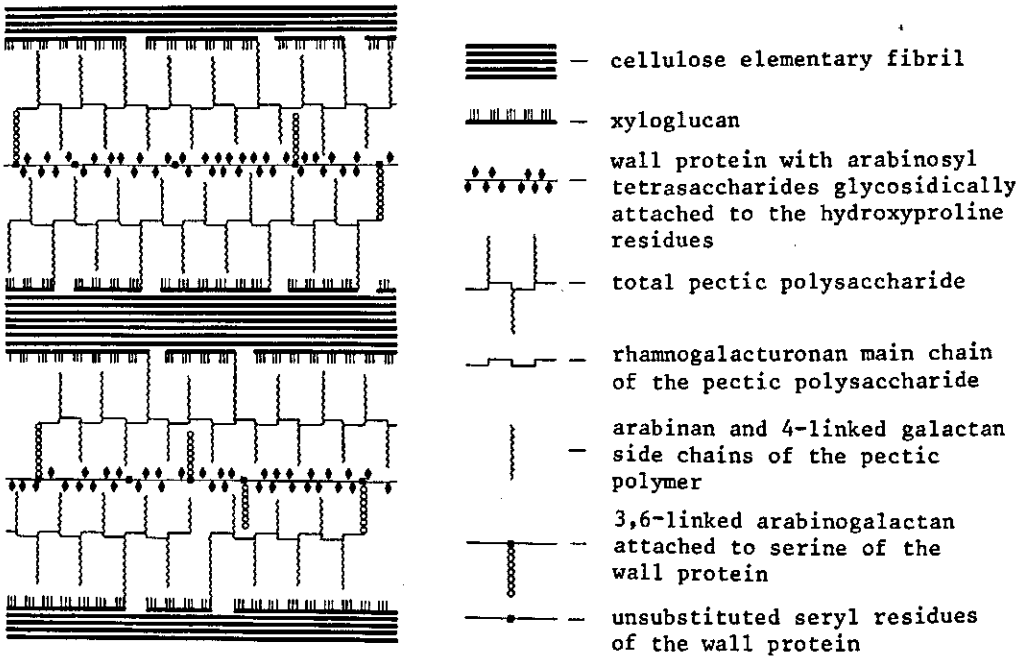

Fig. 2. Tentative structure of cell walls of suspension-cultured sycamore cells (Keegstra et al., 1973).

indirect cross-linking of cellulose microfibrils by interspersion of hydroxyproline-rich protein between the 'pectin-hemicellulose complex' by means of arabinogalactan, is not the only one possible. In another model a single pectic polysaccharide is connected to at least two cellulose fibrils through xyloglucan molecules or a single cellulose fibril is bonded to more than one pectin molecule. One can imagine that the native primary cell wall contains features of all these models.

Roelofsen (1965) stated that primary cell walls are typically one third pectic substances, one third hemicellulose and one third cellulose. The findings of Keegstra et al. (1973) essentially are in agreement with this statement. They found for sycamore suspension-cultured cells 36% pectic substances (rhamnogalacturonan, galactan, arabinan), 40% hemicellulose plus glycoprotein (xyloglucan, protein, tetraarabinosides, arabinogalactan) and 23% cellulose. So Keegstra et al. (1973) concluded that their cell wall model could be extended to other primary cell walls of higher plants, both as far as the occurrence of individual components and the quantitative composition is concerned.

2.2.4 *Structure of the middle lamella*

It is rather uncertain whether the middle lamella must be considered as an inherent part of the primary wall or as a separate entity. The layered construction of the primary wall (Keegstra et al., 1973) with alternating cellulose and matrix layers and the rapid deposition of cellulose against the cell plate before it reaches the lateral walls (Shafizadeh & McGinnis, 1971), stress, in my opinion, the unity of primary cell wall and middle lamella. Furthermore the middle lamella substances must be anchored by covalent linkages like the other matrix substances, because it is usually impossible, as indicated by Knee (1970a, 1973), to extract appreciable amounts of pectic substances without degradative agents. Exceptions may exist as appeared from a report by Kooiman (1969). However, Albersheim et al. (1960a) established a relative concentration of pectic substances (galacturonan) in the middle lamella compared with the primary cell wall. So the interstitial layer of the cell wall, cementing two daughter cells, although an inherent part of two neighbouring primary walls, keeps its own function. As a common cell wall layer of two cells, it is responsible for coherence and organization of tissue structure.

Of the matrix substances pectic substances appear to be predominant in the middle lamella. The postulated role of protein as cementing agent (Ginzburg, 1958, 1961) is rejected by Roelofsen (1965). It does not contribute to the intercellular cohesion of mature potato tissue (Linehan & Hughes, 1969c) but may have some significance in very young meristematic tissues.

2.2.5 *Insolubility of the cell wall and middle lamella pectin*

One of the most important cell wall structural components has not been mentioned yet: water (Northcote, 1972). In the living plant tissue, large amounts of water are present for all processes of living matter. The cell wall is highly hydrated too, being composed of hydrophilic material (except the secondary wall component lignin). Water influences the permeability of the wall and allows the diffusion of ions, but is of paramount importance for the existence of pectin gels (Northcote, 1972). The occurrence of native pectic substances in a gel structure is generally accepted. Albersheim (1965a) stated that 10-20% of cell wall pectin form a very firm and dense gel. Rees (1969) considered the cell wall and middle lamella as a biological gel.

Gel formation proceeds by association of polymer molecules resulting in a three-dimensional network that holds the solvent, water, ions in the interstices (sugar in pectin-sugar gels) (Rees, 1969). The nature of regions of association or junction zones differs for several types of gels, but Rees (1969, 1972a, b) presented strong arguments, theoretical and experimental ones, for the existence of so-called microcrystallites in calcium pectate, pectin-sugar and cell wall gels. He rejected hydrogen bonding, simple ionic bridging by divalent ions as calcium or chelation of single ions as prevalent causes of junction zone formation, although they may contribute (Rees, 1969).

Especially in biological cell wall gels, calcium ions are very important. The calcium-bridge hypothesis has been thought to explain the role of calcium in pectin insolubility inside the cell wall and middle lamella (Joslyn, 1962; Doesburg, 1965). Deuel et al. (1950) argued that it is unlikely that in a polyelectrolyte solution or gel, calcium or other cations are fixed by the anionic charges of the macromolecule. Only a few cross-linking points should be necessary to form a gel network, but it was found that equivalent quantities of negatively and positively charged ions were needed. The calcium--bridge hypothesis also does not explain why pectins with a degree of esterification of over 50% are not made insoluble by calcium (Deuel et al., 1950; Anyas-Weisz & Deuel, 1950). Chelation of calcium ions by pectic substances is proposed by Schweiger (1964, 1966), but Kohn concluded that calcium ions are bound to pectic carboxyls mainly by electrostatic attraction forces (Kohn & Furda, 1968; Kohn, 1968, 1971). In solutions of calcium oligogalacturonates, the activity of calcium ions decreases continuously with increasing charge of the anion (or increasing chain length) (Kohn, 1971). This is caused by accumulation of charged groups and is a normal feature of polyelectrolyte solutions where 20-80% of the counterions may be 'fixed' by localization inside the electrostatic attraction-repulsion field (Rice & Nagasawa, 1961). Such polyelectrolyte effects can contribute to formation of junction zones in pectin gel formation, when polyelectrolyte molecules are sharing a common 'atmosphere' of counterions (Rees, 1969), especially in the presence of multivalent ions as calcium. The exact nature of the strong calcium-pectin bond remains somewhat obscure. Stereochemical structure of polymerized galacturonate (Rees, 1972a) may be involved in addition to the effect of chain lengthening on calcium binding (Kohn et al., 1968a; Kohn, 1971).

What is the precise structure of the microcrystallites in pectin gels? Solms (1960) ascribed the association of large segments of homogeneous

molecules to secondary valence bonding, but Rees (1972b) developed a different theory. He suggested that junction zones in pectin gels are formed by chain stacking. Blocks of contiguous α-D-galacturonopyranosyl residues of several chains seem to be associated in a three-dimensional framework. This stacking can occur with esterified and acid uronate residues together in one lattice, at low pH to reduce ionization of free acid groups (pectin-sugar gels at low pH) or with uronic anions where calcium ions will be packed between the chains. The latter possibility will be the one of choice in cell wall and middle lamella gels. Calcium then assists the packing of galacturonan chain segments in a microcrystalline structure (Van Buren, 1970). To get enough cohesion between the molecules for stabilizing the microcrystallite, the associated blocks in the gel must have a minimum length thus accumulating the weak forces of attraction. Nothing is known about the length of these blocks, but it is assumed that rhamnose insertions and side chains diminish chain cohesion (Gould et al., 1965; Rees & Wight, 1969).

Rhamnose or rhamnose-rich blocks as emerging from the pectin model of Talmadge et al. (1973) will have a kinking effect on the linear rhamnogalacturonan chain (Rees & Wight, 1971; Rees, 1972a, b). On the other hand the rhamnose kinks may interrupt the junction zones and link different junction zones in which one molecule is involved. This results in an interconnected network that can hold water molecules within its framework (Rees, 1972a, b).

Obviously, the primary structure of pectic substances determines pectin behaviour in forming tertiary network structures such as in a gel. Thus the features of primary pectin structure (rhamnose insertion, side chains and length of uninterrupted galacturonan) are important in intercellular cohesion, if the relative pectin concentration in the intercellular cell wall layer is kept in mind. The reasons obviously are that primary structure influences pectin gel structure, and also the covalent anchorage via side chains into the whole cell wall macromolecule (Keegstra et al., 1973). This covalent bonding to hemicelluloses and proteins fully explains the reason of insolubility of native pectic substances. Until recently (Joslyn, 1962; Doesburg, 1965) a mixture of covalent bonding, mechanical enmeshing, hydrogen-bonds and calcium-bridges was thought to insolubilize the pectin inside the middle lamella and cell wall complex. These 'protopectin'-hypotheses now seem finally outdated. Abandonment of a ill-defined term like 'protopectin' might avoid much confusion. The description of middle lamella as essentially composed of low-esterified calcium pec(in)ate (Kertesz, 1951; Joslyn, 1962; Doesburg,

1965; Fogarty & Ward, 1972) and the reservation of 'protopectin' for insoluble cell wall pectin contributed to this confusion.

2.3 SUMMARY

In this chapter a short review was given of the structure of pectic substances which are of major importance in intercellular cohesion of plant tissues, because of their presence in the middle lamella. The pectic substances are heteropolysaccharides, built up from a galacturonan main chain α-1,4-glycosidically linked, interrupted by 2-linked rhamnosyl residues. Galactan-arabinan side chains are probably bound to C_4 of rhamnose. Besides the primary structure, the secondary and tertiary structures of pectin were considered. Furthermore it was pointed out that esterification of the carboxyls of galacturonan with methanol and molecular weight are important characteristics.

Further the primary cell wall and middle lamella structure was discussed, mainly with respect to insolubility and covalent binding possibilities for pectic substances. The recent structural model of Keegstra et al. for plant cell walls and the gel theories of Rees were chosen as foci for this part of the literature survey. It is thought that calcium ions assist the packing of galacturonan chain segments in microcrystalline structures, which explain the insolubility of pectic substances in plant cell walls, together with covalent anchorage via side chains to hemicelluloses and glycoproteins. The importance of pectic substances in intercellular cohesion thus becomes obvious.

3 Potato composition and intercellular cohesion of the cooked potato: a survey

3.1 ANATOMY AND COMPOSITION

The potato tuber is a modified and enlarged underground stem, growing at the distal end of a rhizome or underground stem (Reeve et al., 1969a). A wide range of constituents make up the chemical composition of the potato tuber, but starch is the main storage material and dominates the overall chemical picture. Only an approximate composition can be given, because it varies with variety, growing conditions, maturity etc.: water 70-85%, starch 10-20%, sugars 0.5-2%, protein 1-2%, ash 1-2%, cell wall substances 1-1.5% (Burton, 1966; Adler, 1971; Hoff & Castro, 1969). Anatomically, a potato tuber shows up different tissue zones which also vary in chemical composition, cell size and physiological function. The original stem character, furthermore, is reflected in a stem and bud end of the ovally shaped tuber, the youngest tissue being located at the bud end and the oldest at the stem end.

Reeve et al. (1969a) distinguished three basic meristems,which are connected with growth and differentiation of the potato tuber. The protoderm furnishes the periderm, the ground meristem cortex and pith whereas the procambium gives rise to vascular ring and perimedullary zone (Fig. 3). The tuber growth is mainly due to enlargment of the perimedullary tissue (Reeve et al., 1969a).

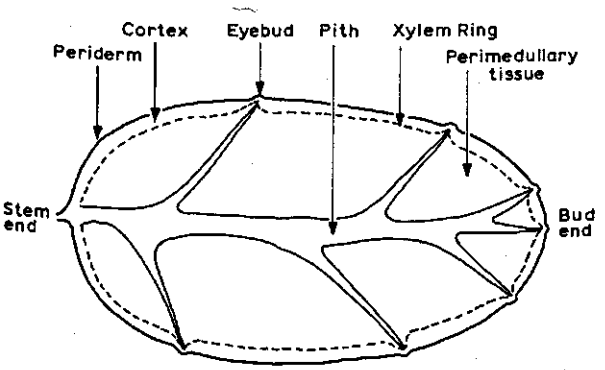

Fig. 3. Longitudinal section of mature potato tuber (Reeve et al., 1969a; 1971). Diagram showing different zones.

Table 1. Distribution of constituents over the mature potato tuber (1, 2)[1]. The arrows indicate the direction of increase of concentration.

	Outer	Inner	Reference[1]	Stem	Bud	Reference[1]
Dry matter, starch	←		1, 3	←		1, 3
N	→		1, 3	←		1, 3
K	←		8, 9	→		4, 5, 11
Ca	←		5, 10, 12			
Mg	→		5, 10, 12			
Mn, Zn, Cu	←		5			
Fe				←		4, 6
P				→		4, 5, 6, 7
Cl	→		5			
Citrate	←		8	→		4, 6, 13, 14
Malate				→		14
Phytin				→		7
pH	→		16	←		6, 15, 17

1. 1. Reeve et al., 1970.
2. Reeve et al., 1969b.
3. Bayal & Van Vliet, 1966.
4. Macklon & DeKock, 1967.
5. Johnston et al., 1968.
6. Hughes & Swain, 1962.
7. Wager, 1963.
8. Gehse, 1970.
9. Bretzloff & McMenamin, 1971.
10. Bretzloff, 1971.
11. Vecer & Bardysev, 1971.
12. Warren & Woodman, 1973.
13. Wager, 1946.
14. Heisler et al., 1964.
15. Robertson & Smith, 1931.
16. Thornton et al., 1933.
17. Iritani & Weller, 1974.

The anatomical variation within a potato tuber and the differences in physiological age contribute to the existence of gradients in chemical composition. The most important results from the literature are summarized in Table 1. Little or nothing is known about distribution of pectic substances, except the histochemically demonstrated concentration of polyuronide in the outer layers of the tuber (Warren & Woodman, 1973). It is evident that most constituents are relatively concentrated in the outer layers of the tuber like dry matter, but whereas dry matter is higher in the stem than in the bud end the chemicals are found predominantly in the bud end (Table 1).

Cell size also varies with anatomical composition. Perimedullary tissue contains the largest cells, followed by pith and cortex, and the mid regions have larger cells than bud and stem end (Reeve, et al., 1973a).

3.2 COMPOSITION OF CELL WALL AND MIDDLE LAMELLA AND OF PECTIC SUBSTANCES

There are only a few results available of analysis of the potato cell wall. Contrary to the very sophisticated and up-to-date attack on cell wall analysis of Albersheim's group, (Talmadge et al., 1973; Bauer et al., 1973; Keegstra et al., 1973) analysis of potato cell walls has previously been carried out according to the classical scheme of extractions. Le Tourneau (1956) fractionated alcohol-insoluble solids, but did not pretend to report on a quantitative basis. However, I re-interpreted these results on the basis of the present knowledge about primary cell wall structure, and calculated them on a non-starch basis (Table 2). Emiliani & Retamar (1968) and Hoff & Castro (1969) isolated potato cell walls by sieving methods, without starch gelatinization and amylase treatment (Le Tourneau, 1956). In this way any chemical degradation of pectic substances in particular is avoided. Several cell wall fractions were characterized on basis of extracting agent and subsequent analysis (Table 2).

Some differences between the three examples of potato cell wall analysis obviously exist. Le Tourneau (1956) extracted a large portion of 50%-ethanol--soluble arabinan-galactan, which is classified in the group of pectic substances (20% of dry cell wall). An araban-rich, alcohol-soluble fraction was lost by Hoff & Castro (1969) (5% of dry cell wall). The protein found by Emiliani & Retamar (1968) and Hoff & Castro (1969) is reported on a Kjeldahl basis, excluding protein-bound carbohydrate material. Nevertheless an unexplainable difference in protein content was found. The carbohydrate part of the glycoprotein will have been extracted into the pectic substances by

Table 2. A comparison of potato cell wall composition with that of cell wall of suspension-cultured sycamore cells (fractions as % of dry matter).

	Potato			Sycamore
	Le Tourneau, 1956	Emiliani-Retamar, 1968	Hoff-Castro, 1969	(Talmadge et al.,) 1973
Pectic substances[1]	60	64-66	55	36
Hemicellulose[2]	10	8-10	7	21
Cellulose	15	24-26	28	23
Glycoprotein[3]	15	1	10	19

1. Rhamnogalacturonan, araban, galactan.
2. Includes sugars like glucose, xylose and mannose.
3. Protein with tetraarabinosides and some galactan or arabinogalactan.

Emiliani & Retamar (0.05 N HCl) on account of the acid-lability of arabinosyl bonds (BeMiller, 1967). The cellulose content, determined as residue after subsequent extractions, was low in the work of Le Tourneau (1956). Emiliani & Retamar (1968) pointed out that the potato cell wall contained a low--crystalline cellulose fraction. Apart from the mutual differences between the three cell wall analyses, more striking differences emerge when these are compared with the composition of primary sycamore wall (Table 2). Potato cell walls possess more pectic substances and less hemicellulose, even when it is taken into consideration that tetraarabinosides and arabinogalactans may have been counted with potato pectic substances. Cellulose content is about the same in both types of tissue. Sycamore glycoprotein is 10% protein (cf. Hoff & Castro, 1969) and 9% tetraarabinosides.

It is interesting, furthermore, to compare the sugar composition of the two types of cell walls (Table 3). Both potato cell walls are very similar, except the already mentioned loss of arabinan by Hoff & Castro (1969). Compared with sycamore potato cell wall is rich in galacturonic acid and galactose, but arabinose is scanty. Sycamore,at the same time, is richer in rhamnose and xylose. An arabinogalactan as found in sycamore cell wall by Keegstra et al. (1973) is absent in potato cell wall, so that another rhamnogalacturonan-glycoprotein bridging molecule may be present.

Hoff & Castro (1969) provided a detailed analysis of pectic substances and hemicellulose fraction. Pectic substances are almost equally rich in galacturonan (51%) and galactan (42%) with minor quantities of rhamnose

Table 3. A comparison of sugar composition of cell wall of potato and suspension-cultured sycamore (as % of dry matter).

	Potato		Sycamore
	Emiliani-Retamar, 1968	Hoff-Castro, 1969	(Talmadge et al., 1973)
Galacturonic acid	25.2	28.7	13.4
Galactose	25.2	23.9	12.8
Arabinose	6.5	1.6	21.0
Rhamnose	1.5	1.6	3.1
Glucose[1]	29.3 (4.3)	31.3 (3.8)	26.7 (3.7)
Xylose	2.5	2.1	7.6
Mannose	0.8	0.4	0.3
Fucose	0.0	0.2	1.3

1. In parentheses non-cellulosic glucose.

(2.9%) and arabinose (2.7%). It must be concluded that the rhamnogalacturonan of potato cell wall contains a much less interrupted galacturonan main chain. Assuming that rhamnose is present in GalA-Rha-GalA-Rha blocks (Talmadge et al., 1973), the straight galacturonan parts may consist of 30-35 galacturonopyranosyl residues. It is worth mentioning that the potato hemicellulose, although a minor constituent, consists of predominantly glucose (57%) and xylose (23%), but galactose (12%) and mannose (6%) are present too (Hoff & Castro, 1969). A xyloglucan structure analogous to that of sycamore (Bauer et al., 1973) and possibly a galactoglucomannan are presumed to take part in potato cell wall structure.

Potato pectic substances are rich in galactan. A water-soluble galactan from potato tissue has been isolated by Wood & Siddiqui (1972). It is a linear β-(1→4)-linked homopolymer. The same nature is conceivable for the pectic galactans of potato cell wall and in general for other cell wall galactans (Talmadge et al., 1973; McNeil & Albersheim, 1973). Wood & Siddiqui (1972) also isolated a cell wall fraction with protein, arabinose, galactose and uronic acid. Some of the features of potato cell wall composition also appear from the incomplete cell wall analyses of Knee & Friend (1968), Friend & Knee (1969) and Knee (1970b).

3.3 OBJECTIVE MEASUREMENT OF THE INTERCELLULAR COHESION OF THE COOKED POTATO

Subjective assessment of a texture characteristic has several disadvantages (Schippers, 1962). For instance, any texture attribute scored subjectively might be influenced by other, mostly related, attributes. Consequently it is extremely difficult to study influences of chemical composition and its changes on texture.

3.3.1 Mealiness and intercellular cohesion

Mealiness of the cooked potato tuber has often been determined in the past by subjective methods, such as scoring on a hedonic scale or mashing procedures (Sweetman, 1936; Bettelheim & Sterling, 1955a; Unrau & Nylund, 1957a; Barrios et al., 1961; Zaehringer & Le Tourneau, 1962). In agreement with Linehan & Hughes (1969a), it may be taken for granted that mealiness partly depends on reduction in intercellular cohesion, especially when assessed subjectively. In the old literature this led to the confusing situation that mealiness and cell separation or loss of intercellular cohesion

were thought to be identical, although satisfactory correlations with state or degradation of the intercellular cement could not really be obtained (Sweetman, 1936; Barmore, 1937; Freeman & Ritchie, 1940; Bettelheim & Sterling, 1955b; Potter & McComb, 1957).

New light was thrown on the separation of attributes of mealiness and intercellular cohesion by Woodman & Warren (1972). They found, in a number of experiments, a strong correlation between subjectively measured cell separation (= breakdown) and mealiness or mouthfeel, but a far less evident one when these texture attributes were measured objectively. Mealiness was measured objectively by extrusion of cooked potato with a grid-extrusion cell of the Kramer Shear Press, and breakdown by collecting sediment. Extrusion force depends strongly on flow properties of the material under investigation (Bourne & Moyer, 1968), and this indicates that mealiness is not controlled by cell separation but by flow properties upon extrusion of the cooked potato. Woodman & Warren (1972) were able to confirm this conclusion by showing that total solids present correlated well with subjectively measured mealiness and extrusive force but had little effect on both subjectively and objectively estimated cell separation. Mealiness apparently depends mainly on the flow properties of cooked potato, which in turn reflect the influence of massive starch deposition in potato tissue cells. Mealiness and intercellular cohesion are definitely different texture characteristics, although some mutual influences cannot be excluded entirely.

3.3.2 *Objective indirect assessment of intercellular cohesion*

The work of Woodman & Warren (1972) again emphasizes the need of objective measurement of intercellular cohesion. Two main approaches are possible, an indirect and a direct measurement of cell cohesion. Cell cohesion is measured indirectly by a rheological procedure with the aid of deformation tests. Several kinds of deformation measurement have been practised. The penetrometer or puncture test has been used for assessment of firmess, hardness or softness of the cooked potato (Sweetman, 1936; Barmore, 1937; Thiessen, 1935, 1947; Sharma et al., 1959). Linehan & Hughes (1969b) also applied a simple puncture testing system and established a highly significant correlation between puncture and compressive measurements (Linehan & Hughes, 1969d), the latter being shown to depend on the strength of the intercellular cement by comparison with direct counting of cell separation under a microscope (Ginzburg, 1958, 1961). In simple puncture testing, however, compressive

and shear forces are involved (Bourne, 1966), whereas DeMan (1969) added flow as a possible contributer, although of minor importance in foods with a cellular structure. The additional finding of Woodman & Warren (1972) that flow interfered largely in texture measurements of cooked potato tissue with the Kramer shear-compression cell, may reduce the usefulness of puncture testing for measurement of intercellular cohesion.

Compressive strength measurement of cooked potato tissue (Linehan & Hughes, 1969c; Linehan et al., 1968) is strongly related to intercellular cohesion (Linehan & Hughes, 1969d). Whittenberger (1951a) and Finney (1972) deemed compressive strength a good measure of intercellular forces.

The reverse of compression, extension is rarely used in evaluating food texture (Kramer, 1964). Personius & Sharp (1938a) determined reduction in intercellular cohesion with an apparatus measuring tensile-strength, although Sterling & Bettelheim (1955) did not find this technique satisfactory.

Some other methods and apparatus for deformation tests were used. Doesburg (1961) measured firmess of potato and other tissues after boiling with a hardness meter, whose action combines shearing and extrusion (Bourne & Moyer, 1968) and for that reason less suited to estimate cell cohesion. Powers & Board (1973) constructed an apparatus which determines time taken to collapse during heating. Compressive forces are involved, because the sample is charged with a constant load which falls through when intercellular cohesion disappears.

3.3.3 Objective direct assessment of intercellular cohesion

It is conceivable that indirect deformation tests for assessing intercellular cohesion will be influenced by other texture attributes such as flow. Direct estimation of cell separation, for that reason, probably provides an even better measure of intercellular cohesion. Sterling & Bettelheim (1955), Ginzburg (1958, 1961) and Letham (1960) simply counted partial or full cell separation under a microscope. Letham (1960) also determined retained weight on a sieve and observed that cell aggregates passed through the sieve apertures (1,2×1,2 mm) in addition to single cells. A similar method, based on retained weight after boiling, was devised by Barmore (1938) and Pyke & Johnson (1940) and adapted by several investigators (Whittenberger & Nutting, 1950; Sterling & Bettelheim, 1955). A very useful modification of the early method of Barmore (1938) and Pyke & Johnson (1940) has been developed by Le Tourneau et al. (1962). Compared with the early

methods, small dices instead of larger cubes were used, while drying of the retained tissue was omitted. Most important, however, is that boiling is done under simultaneous mechanical agitation. This aids in separating cells when intercellular cohesion has been lowered to allow this. Mechanical agitation contributes to an increasing accuracy of the cell separation test, because some residual coherence between cells will always remain, even when the intercellular cement is degraded (Roberts & Procter, 1955; Burton, 1966). On the other hand the deformation tests may measure a reduction in intercellular cohesion even before the final stage of cell separation has occurred, while mechanical agitation in a direct cell separation procedure does a similar thing in accelerating the final cell separation. The test of Le Tourneau et al. (1962) to determine cell separation by retained weight (short RWCS test) has been further standardized (Zaehringer et al., 1963b) and modified (Zaehringer et al., 1969; Ludwig, 1972). This direct method is generally applicable and measures the change in intercellular cohesion of the cooked potato. A minor disadvantage, according to Woodman & Warren (1972) is the error due to loss of soluble material and uptake of water giving change in weight without disintegration of the tissue.

3.4 CHEMICAL COMPOSITION AND INTERCELLULAR COHESION OF THE COOKED POTATO

In Section 3.3, the importance of using a reliable, objective method of intercellular cohesion measurement, to avoid interferences by other texture parameters was pointed out. With this in mind, a critical interpretation of results from earlier investigations on cell cohesion of the cooked potato will be given.

3.4.1 Specific gravity, dry matter, starch

Starch makes up 60-80% of total solids of potato tissue (Burton, 1966; Adler, 1971). It is not surprising then that specific gravity and dry matter content are strongly dependent on starch content. After Von Scheele et al.'s initial work (1937) several mathematical relationships have been established to calculate dry matter and starch content from specific gravity because specific gravity can be determined easily by brine flotation (Burton, 1966).

Intercellular cohesion of the cooked potato is *negatively* correlated with specific gravity sometimes (Whittenberger & Nutting, 1950; Le Tourneau et al., 1962; Le Tourneau & Zaehringer, 1965; Ludwig, 1972), but other

findings report a failure in correlation, in particular when storage effects are studied or different varieties are compared (Whittenberger, 1951b; Sterling & Bettelheim, 1955; Zaehringer et al., 1969; Ludwig, 1972; Woodman & Warren, 1972; Gray, 1972). Intercellular cohesion was determined by a RWCS test, except in the work of Gray (1972).

Subjectively assessed mealiness is *positively* correlated with specific gravity (and also with dry matter and starch content) in almost all investigations (Barmore, 1937; Whittenberger & Nutting, 1950; Bettelheim & Sterling 1955; Unrau & Nylund, 1957b; Barrios et al., 1961; Zaehringer & Le Tourneau, 1962; Le Tourneau & Zaehringer, 1965). This is consistent with the findings of Woodman & Warren (1972) that mealiness depends on flow properties due to total solids and starch content of the potato tuber.

The influence of starch on cell separation during cooking has been explained by the ability of the starch to swell, leading to cell swelling, distension of cell walls and pushing apart of cells (Atwater, 1895; Whittenberger & Nutting, 1950; Whittenberger, 1951b; Sterling & Bettelheim, 1955; Reeve, 1954, 1967, 1970, 1972). Several objections can be made against this hypothesis. Cell swelling was not discovered by Bretzloff (1970) and the correlation between starch (specific gravity, dry matter) and cell cohesion was often absent. Personius & Sharp (1939b) did not observe a reduction in intercellular cohesion measured by tensile strength, when starch was gelatinized by chemicals. Hoff (1972) finally, rejected this hypothesis of starch swelling on theoretical grounds.

A *positive* correlation has been found between intercellular cohesion, measured by puncture testing, and specific gravity (Sharma et al., 1959) and starch content (Barmore, 1937; Linehan & Hughes, 1969b) too. So here intercellular cohesion is positively correlated with specific gravity or starch, while there is a negative correlation when the cohesion is measured by retained weight (RWCS test). It cannot be excluded that the interference of flow properties (Section 3.3.1) in puncture testing measurement is responsible for this phenomenon. Linehan & Hughes (1969b), however, also established a better, significant correlation between cell cohesion and level of amylose, and concluded that the cohesion-starch relationship must be a reflection of a cohesion-amylose relationship. Bretzloff (1968) reported a similar relationship between retained weight and amylose, the amylose, however, being determined as percentage of extracted starch.

The amylose effect on cell cohesion contradicts the starch swelling theory. Linehan & Hughes (1969b) proposed the alternative hypothesis that

amylose leaks through the cell walls to aid in hydrogen bonding of cells during the cooking process. In view of the pectin gel structure of the cell wall and middle lamella (Rees, 1969, 1972a, b) hydrogen bonding seems to be of secondary importance. Linehan & Hughes (1969c) themselves also measured an only small effect of amylose in re-cementation of potato cells. Furthermore, it seems unlikely that the amylose molecules are able to penetrate cell walls before most of the intercellulosic macromolecular network (Keegstra et al., 1973) has been destroyed (Bauer et al., 1973; Keegstra et al., 1973). Interference of amylose with cell cohesion, then, would occur when the intercellular cohesion had been lost because of extensive degradation of matrix substances.

The picture emerging is that starch or starch components are merely fortuitously related to intercellular cohesion of the cooked potato. Or more positively expressed, the correlation between specific gravity (or starch content) and intercellular cohesion, as found mostly in restricted populations of samples, might be a reflection of a dependence of both variables on an independent third one, for instance maturity (Woodman & Warren, 1972).

3.4.2 *Pectic substances*

Pectic substances constitute a minor component of potato tissue (0.2-0.4% on fresh weight) compared with the vast amount of starch (10-20% on fresh weight) (Thiessen, 1947; Kröner & Völksen, 1950; Burton, 1966; Adler, 1971). In the past they were often analysed and determined with methods which are now out of date, and reported both as total pectic substances (rhamnogalacturonan, araban, galactan) and as polyuronides (galacturonan) causing confusion when the results are examined nowadays. The presence of pectic substances in potato middle lamella has been ascertained by staining under the electron microscope (Fox, 1971) and by maceration (or reduction in intercellular cohesion) with purified pectolytic enzymes reported by many investigators starting with McClendon (1964) (see Section 4.1). Nevertheless a clear relationship between intercellular cohesion of the cooked potato and pectic substances has not been established.

No relationships were found between subjectively assessed *mealiness* and pectic substances of raw potato, determined by the classical schemes of extractions (Sweetman, 1936; Barmore, 1937; Freeman & Ritchie, 1940; Bettelheim & Sterling, 1955b). Potter & McComb (1957) extracted pectin in one single operation with EDTA-pectinase and determined the galacturonan

content, but they did not establish a correlation with mealiness, either.

From raw and cooked potato tissue Bettelheim & Sterling (1955b) extracted three pectin fractions: a cold water-soluble, a cold sodium hexametaphosphate (HMP)-soluble and an 85°C 0.05 M HCl-soluble. No direct relationship between any characteristic of these pectin fractions and *intercellular cohesion*, determined as sloughing by a RWCS test, was obtained. According to these authors, certain characteristics of the pectic substances (DE, calcium content and intrinsic viscosity) play a role in the development of the cooked potato texture (Sterling & Bettelheim, 1955). Exact relation coefficients, however, were only calculated with subjectively scored mealiness. Zaehringer et al. (1969) applied their modified RWCS test to look for the influence of pectic substances and other potato constituents on cell separation. The method of pectin analysis used does not seem to be very reliable and there was no clear-cut relationship between pectin and intercellular cohesion.

The method of pectin extraction utilized by Bettelheim & Sterling (1955b) is essentially the same as that performed by Sharma et al. (1959). The latter estimated intercellular cohesion as hardness-softness with a penetrometer. (puncture). Using this objective method, Sharma et al. (1959) established that within a variety the greatest hardness was correlated with the highest content of insoluble pectin (plus hemicellulose!), i.e. only soluble in a sequestering agent and HCl. Upon storage at about 17°C decrease in hardness (or intercellular cohesion) was accompanied by an increase in water-soluble pectin and a decrease in insoluble pectin. In the experiments of Linehan & Hughes (1969b) a significant statistical correlation between intercellular cohesion, assessed by puncture testing, and polyuronide, was obvious in only one of the three experiments. The overall correlation was not significant and in the one experiment where it was, amylose was at a constant low level. Pectic substances were experimentally defined as the fraction soluble in cold HMP at pH 4.6, in accordance with the opinion of Bettelheim & Sterling (1955b) and others (Joslyn, 1962; Doesburg, 1965) that this fraction soluble in a metal sequestering agent represents the structure of the intercellular cement. It has been argued that there is no foundation for this view (Section 2.2.5) on account of the present knowledge about pectin and cell wall structure.

The relationship between hardness-softness and insoluble and water-soluble pectin (Sharma et al., 1959), calls attention to some findings about pectin changes on cooking by other investigators. It is observed that cooking of potatoes caused a shift from insoluble pectin towards increasing

quantities of water-soluble pectin (Freeman & Ritchie, 1940; Bettelheim & Sterling, 1955b; Doesburg, 1961), clearly demonstrating a degradation in pectic substances. Changes in pectic substances also appear from change in staining with ruthenium red, although not highly specific for pectin (Sterling, 1970), during heating of potato tissue in water and already visible at 70°C (Roberts & Proctor, 1955) or during steaming (van Geldermalsen-de Jongh, 1963).

3.4.3 Minerals and organic acids

Quantitative aspects Interaction of calcium ions with pectic substances and pectin structures in gels, and cell wall and middle lamella was discussed in Section 2.2.5. Calcium and other metal chelating agents have been used to extract a characteristic pectin fraction in relation to cell coherence. Relationships between cations, especially calcium and magnesium, and intercellular cohesion of the cooked potato have been investigated. Naturally occurring calcium binding agents bear a close relationship to this part of cell cohesion work.

Le Tourneau & Zaehringer (1965) investigated four sets of potato samples of different origin. Apart from relationships already mentioned, they found that intercellular cohesion (RWCS test) was consistently and significantly negatively correlated with alcohol-inextractable potassium, magnesium and phosphorus, and in three of four sets with total potassium. Correlation with total calcium was *positive* although neither consistent nor significant. Zaehringer et al. (1969) estimated potassium, calcium and magnesium content of several potato cultivars. Potassium content was significantly correlated with the sum of calcium and magnesium, but these cations were not correlated with intercellular cohesion. Puncture testing, however, demonstrated a significant correlation between intercellular cohesion and calcium, magnesium or their sum in the pooled data of three experiments, but not in any separate experiment (Linehan & Hughes, 1969b).

In the opinion of Wager (1963) phytin, the hexaphosphate ester of myo-inositol, which precipitates calcium at a pH beyond 5.4 (Kaufman & Kleinberg, 1971), must influence cooked potato texture. His experimental evidence was, however, rather poor. Phytin and the calcium-chelating organic acid citrate, were included in the set of analyses carried out by Zaehringer et al. (1969). Multiple regression analysis showed that pectin content, degree of esterification of pectin, citrate, phytin, calcium and magnesium accounted for 65%

of the variability in cell separation.

Addition and removal trials By far the most valuble and promising information about the role of pectic substances, interfering cations and anions, in intercellular cohesion is available from model experiments. Mostly estimation of pectin concentration in potato tissue is even omitted, but by addition of chemicals or removal of substances changes in intercellular cohesion are introduced and interpreted with respect to the possible cementing function of pectic substances.

Potato tissue slices were kept in solutions of chemicals at a temperature of 65°C and a somewhat variable pH of 5-6. Chloride salts of divalent cations like barium, calcium, magnesium and strontium did not decrease intercellular cohesion as measured with tensile strength, while potassium and sodium did to a small extent. Calcium chloride counteracted the decrease in cell cohesion after heat or chemical treatment and underlined the role of calcium ions in improving or retaining cell cohesion (Personius & Sharp, 1939a). The firming action of calcium, measured mainly by RWCS tests, has been proven furthermore by addition to boiling liquid, immersion of the potatoes in calcium containing solutions before canning or addition to canning brine (Pyke & Johnson, 1940; Rhodes & Davies, 1945; Whittenberger & Nutting, 1950; Weckel et al., 1959; Mitchell, 1972; Ludwig, 1972). Woodman & Warren (1972) succeeded in changing cell separation by addition of calcium without a change in extrusion-measured mealiness.

In the same experiments as described for cations, Personius & Sharp (1939a) found an extensive decrease in intercellular cohesion in ammonium oxalate, sodium citrate and sodium fluoride solutions at pH 5-6, compared with water and potato juice. This effect was ascribed to removal of calcium from the intercellular cement. Having established that cell membranes become permeable at heating, Personius & Sharp (1938b, 1939a) thought that ions of the cell cytoplasm are able to diffuse into the intercellular region and to interact with the pectic structures. With the same idea in mind, Linehan & Hughes (1969c) studied changes in intercellular cohesion with compressive strength measurement using treatments of raw potato tissue. At a pH of 6.5 and 35°C, EDTA and HMP decreased intercellular cohesion to the level of the cooked potato. Heavy metal chelating agents did not do this, however, indicating that calcium, and magnesium, are important for cell cohesion. After EDTA treatment, magnesium, calcium, barium, trivalent iron and aluminium restored intercellular strength in increasing order. Sodium and potassium

were less active. During EDTA treatment of potato tissue at pH 6.5 and 35°C polyuronides were lost too. This means that reduction in intercellular cohesion must be ascribed to both calcium (and magnesium) and pectic substances. Remarkably, cations and polyuronide were lost from the tissue before reduction in intercellular cohesion really occurred. So the lost fractions may not be fully responsible for intercellular cohesion. Linehan & Hughes (1969c) ascribed the loss of polyuronide to β-eliminative degradation (Albersheim et al., 1960b).

Removal of water-soluble substances by soaking of small thin potato dices in distilled water causes a decrease in cell separation on subsequent cooking in a RWCS test (Le Tourneau et al., 1962; Zaehringer et al., 1963a). Evidently, reduction in intercellular cohesion is reversed to a certain extent by diffusion into the water of potato constituents. Potassium ions can be removed by leaching within a short period for 70-80%, but other ions will diffuse at the same time (McDonald et al., 1960; Lorenzini, 1970; Louis & Dolan, 1970). It could be shown that intercellular cohesion after cooking was highly positively correlated with electrical conductivity of the soak water, the loss of potassium, phosphorus, magnesium, calcium, phytic acid and citric acid and loss of total solids (Davis, 1964; Davis et al., 1973). Whereas during a fixed period 20% of total solids were lost, more than 50% of present minerals were leached out. Potassium indeed diffuses to the greatest extent (70%) and calcium is most retained (35%).

A causal relationship to intercellular cohesion of the leached-out ions was confirmed in a number of experiments. Deionization of soak waters destroyed the soaking effect in RWCS test of soaked potatoes (Cunningham et al., 1967). Replacement with the aid of ion-exchangers of anions and cations of soak waters revealed and re-affirmed the important role of citrate and potassium in intercellular cohesion (Zaehringer & Cunningham, 1971). Addition of potassium chloride also reversed the effect of previous soaking, but the potassium salts of citrate and to a lesser extent malate, oxalate and phytate were more effective (Davis & Le Tourneau, 1967). Calcium chloride raised cell cohesion, magnesium chloride had no effect in the experiments of Davis & Le Tourneau (1967), but in the ion-replacement trial magnesium decreased cell separation although less than calcium (Zaehringer & Cunningham, 1971). Potato extractives when used in citrus pectin gel making, weakened the gel, while the viscosity of potato starch paste was diminished (Zaehringer & Cunningham, 1970). These results provide arguments for interference of potato constituents in weakening of the middle lamella but against the starch

swelling theory. In a similar leaching experiment, Aleshina (1966) observed the effect of soaking on intercellular cohesion and the reversion by sodium oxalate, but not by sodium chloride. Ludwig (1972) measured decreased intercellular cohesion on addition of sodium chloride to non-soaked potato dices.

From the experiments described one may conclude that addition and removal trials with potato tissue reveal the important role of pectic substances in intercellular cohesion of the cooked potato. Cations like calcium increase firmness and cellular coherence, anions like citrate, malate, oxalate and phytate exert an opposite influence. However, the disadvantageous effect of potassium on intercellular cohesion is less conceivable and probably also less specific than the forementioned ions.

3.5 INTERCELLULAR COHESION OF THE COOKED POTATO AND CELL SIZE

Because of the complex nature of interferences and interactions of chemical constituents, which probably determine intercellular cohesion of the cooked potato, a quantitative approach to this phenomenon leaves some questions unanswered. Van Buren (1970) stated that some general concepts of cohesion and interaction of molecules and surfaces may be profitably applied, whereas Linehan et al. (1968) expressed as their view that difference in cell size may account for a part of unexplained variations in intercellular cohesion (Linehan & Hughes, 1969b).

Van Buren (1970) draw attention to the treatment of cell cohesion by application of colloid theory on cell-sized particles carried out by Weiss (1968). This theory essentially considers contact processes to be regulated by a balance between potential energies of electrostatic repulsion and potential energies of attraction due to London-Van der Waals interaction. During his study Weiss (1968), calculated that the repulsive force between a glass plate and a cell decreases as the cell radius decreases. The adhesion thus increased when cell size decreased. In plant cells, however, the covalent bondings in the cell wall macromolecules and the gel character of the pectic substances (Sections 2.2.3 to 2.2.5) make the colloid theoretical considerations less useful to describe cohesion phenomena.

Linehan et al. (1968) simply thought that smaller cells would have a greater area of intercellular contact producing higher values of intercellular cohesion. They could show that intercellular cohesion, measured as compressive strength, was indeed significantly positively correlated with cell size, expressed as surface area per unit volume of tissue.

Relatively few other investigators looked for relationships between cell size and intercellular cohesion after cooking. And some of these estimated mealiness instead of intercellular cohesion or determined tuber breakdown subjectively. Thiessen (1935, 1947) compared dry-land tubers with those grown on irrigated land. The dry-land tubers had larger cells and a concurrent greater penetration on puncture testing after cooking. In dry-land tubers the greatest intercellular cohesion (or resistance to penetration) was found in the cortex where the smallest cells occurred. Gray (1972) counted the number of tubers without sign of breakdown on autoclave cooking. A significant positive correlation was established between cell surface area and freedom of breakdown, or subjectively assessed intercellular cohesion.

According to Barmore (1937) no consistent relationship between mealiness and cell size existed, although in three out of four pairs of samples, the most mealy had the largest cells. Mealiness, subjectively assessed as in the work of Barmore (1937) was significantly correlated with cell size in an investigation with four varieties (Barrios et al., 1963).

3.6 SUMMARY

Some attention was given to potato tuber anatomy, chemical composition and distribution of chemical constituents over several tuber regions. Literature data on potato cell wall and middle lamella composition were compared with those on cell walls of suspension-cultured sycamore cells, whose structure is reported in detail in literature.

In this Chapter the methods of measurement of intercellular cohesion of the cooked potato were surveyed. The importance of objective, instrumental methods was outlined for the investigation of the influence of chemical composition on texture. Compressive strength measurement and puncture testing were considered to be satisfactory, but direct determination of cell separation was preferred for measurement of intercellular cohesion (RWCS test). The literature on intercellular cohesion of the cooked potato as influenced by chemical composition was critically reviewed with reference to objective texture measurement.

Starch and starch characteristics are seen to be frequently related to intercellular cohesion, particularly in restricted populations. However, dependence of both variables on an independent third one, such as maturity is pointed out. Furthermore the influence of pectic substances, anions (organic) and cations on intercellular cohesion were discussed. Several

indications are given that changes in pectic substances during cooking, for instance solubilization and reduced binding of calcium ions, are important for intercellular cohesion. But it is concluded that no correlations with absolute pectin contents have been found.

Finally the importance of cell size or cell surface area in intercellular cohesion was briefly emphasized.

4 Enzymatic reduction of intercellular cohesion of potato tissue (maceration)

4.1 INTRODUCTION

Intercellular cohesion of potato tissue is reduced when the tissue is heated or cooked, but this phenomenon can be achieved without applying energy of heat with aid of pectin degrading enzymes, or specifically (rhamno)galacturonan attacking and depolymerizing enzymes. This kind of loss of tissue coherence, associated mainly with plant tissue modification by plant-pathogenic microorganisms (Bateman & Millar, 1966; Albersheim et al., 1969; Codner, 1971) is known as *maceration*. Maceration experiments with potato tissue have not always been carried out by incubation with definitely purified enzyme preparations, which contain only one, homogeneous, galacturonan depolymerizing enzyme protein. However there is evidence for the pectic nature of potato tissue middle lamella and the role of pectic substances in maintainance of intercellular cohesion.

Until now only endo-splitting galacturonan depolymerases, both hydrolases and lyases, have been proved to macerate potato tissue. There are reports about maceration of potato tissue by endo low-methoxyl pectin lyases (LMPL) (Dean & Wood, 1968; Hancock & Stanghellini, 1968; Hall & Wood, 1970; Mount et al., 1970; Zucker & Hankin, 1970; Mullen & Bateman, 1971; Hagar & McIntyre, 1972; Alberghina et al., 1973). Pilnik et al. (1973) preferred the term LMPL for pectate lyase (PAL) (E.C. 4.2.2.2, Poly(1,4-α-D-galacturonide)lyase) (Neukom, 1963; Koller, 1966). Endo pectin lyases (PL) (E.C. 4.2.2.10, Poly(methoxylgalacturonide)lyase) have been found effective in potato tissue maceration too (McClendon, 1964; Sherwood, 1966; Byrde & Fielding, 1968; Amado, 1970; Ishii & Yokotsuka, 1971). Whereas endo lyases tested always macerated potato tissue, this does not hold for hydrolases. Some authors reported that endo polygalacturonase (PG) (E.C. 3.2.1.15, Poly(1,4-α-D-galacturonide)glycanohydrolase) macerates potato tissue (McClendon, 1964; Sherwood, 1966; Bateman, 1968; Hall & Wood, 1970; Ishii & Yokotsuka, 1972). In other works endo PG failed to do so (Byrde & Fielding, 1968; Gremli & Neukom, 1968; Cole, 1970).

It has been pointed out that purified and characterized pectic enzymes might be valuable tools in the study of primary structure of pectic substances (Aspinall, 1970a; Rombouts, 1972). A purified endo PG (English et al., 1972) has aided the partial elucidation of sycamore cell wall structure in particular (Talmadge et al., 1973; Keegstra et al., 1973). I used endo low-methoxyl pectin lyase and pectin lyase in comparative maceration experiments of potato tissues. These tissues were derived from a restricted population, but differed widely in specific gravity (dry matter, starch content) and concurrently in intercellular cohesion of the cooked tissue. Maceration with the lyases mentioned might reveal differences in primary structure of pectic substances of the middle lamella or unequal distribution of pectic substances and its characteristics over primary cell wall and middle lamella.

4.2 MATERIALS AND METHODS

4.2.1 The potato material

Potato tubers, variety Bintje were grown under normal agricultural conditions in 1971 and 1972 on clay soils in the Noord-Oost Polder. Lots of 2-3 tons, a restricted population of identical growth history, were stored after harvest at 6°C with sprout inhibitors (isopropyl-N-phenylcarmabate and its chloro-derivative). After two (1971) or one (1972) month of storage the lots were divided in specific gravity[1] fractions by brine flotation (Burton, 1966; Smith, 1967). The storage was continued when these potato tubers had been carefully washed to remove adherent salt and were air-dry. Three (1971) or four (1972) months after the start of the storage period, tubers of low sp. gr. (1.060-1.070) and high sp. gr. (1.100-1.110) were prepared for maceration.

4.2.2 Tissue for maceration experiments

With a cork borer, cylinders of 1.6 cm diameter were taken in a transverse direction out of the mid region of the tuber. Cortex and vascular tissue were peeled off, leaving cylinders of perimedullary and pith tissue (Reeve et al., 1973a). Disks of 0.40-0.45 mm thickness were cut with a simple

1. Relative density d_t^t, is to be preferred to specific gravity, which is used here because of the normal practice in potato-processing industry.

microtome and killed by an ethanol treatment: the tissue disks were submerged in 70% ethanol immediately, and then into 96% ethanol via three changes of volume. To denature endogeneous enzymes, especially pectinesterase (PE) (E.C. 3.1.1.11, Pectin pectyl-hydrolase) the disks were boiled in 96% ethanol for 10 min (Brown, 1969). The tissue sections were returned to 70% ethanol and stored at 4°C until use. Activity of PE was destroyed, but starch granules remained intact. One may assume that this treatment does not change pectin structures dramatically. Of the 1971 tubers 3 000 disks of each sp. gr. were prepared, of the 1972 ones 1 300.

The results of pectin analyses are collected in Table 4. Due to difficulties in pectin analysis, some of the values for 1971, sp. gr. 1.100-1.110 and 1972, sp. gr. 1.060-1.070 were calculated on basis of methanol analysis of the disks and DE values of the whole tissue (Tables 22, 23). DE values of pectic galacturonan of disks and whole tissue of 1971, sp. gr. 1.060-1.070 were 54 and 52%, respectively, those of 1972, sp. gr. 1.100-1.110 55 and 56% respectively, thus quite similar.

For standard deviations see Section 7.2.2.

4.2.3 Modification of tissue disks

Tissue disks were modified in order to change the degree of esterification of pectic galacturonan, or to remove calcium ions from cell wall and middle lamella.

H disks (calcium replaced by H) were prepared by washing 400 disks four times with 250 ml 70% ethanolic 0.6 M hydrochloric acid in a beaker. Then the disks were washed with 70% ethanol until Cl^- free.

For esterification 400 H disks were washed with five volumes absolute ethanol (175 ml) and five volumes methanol (300 ml) to remove water. Esterification was adapted from the procedure for isolated pectin of Vollmert

Table 4. Pectin analyses of tissue disks.

Sp. gr.	Year	Fresh weight (mg/disk)	Pectic galacturonan		DE (%)	Non-esterified carboxyls (µmol/20 disks)
			mg/20 disks	%		
1.060-1.070	1971	94	5.80	0.31	54	14.5
1.100-1.110	1971	97	7.18	0.38	49	20.8
1.060-1.070	1972	98	6.69	0.34	52	16.8
1.100-1.110	1972	100	6.68	0.33	55	16.4

(1950a). H disks in 300 ml of methanol were cooled to -10°C in ice/acetone and 50 ml 0.4 N diazomethane in etherial solution were added (Vogel, 1967). After 30 min the residual diazomethane was removed by passing nitrogen through the solution. The esterified disks were washed thoroughly with 70% ethanol. After esterification, the pectin content appeared to be lowered. For sp. gr. 1.060-1.070 (1971) 5.18 mg pectic galacturonan was found, calculated on basis of 100% esterification, and for sp. gr. 1.100-1.110 (1971) 5.34 mg. In fact a DE of about 97% was found for solubilized pectic galacturonan during maceration.

Saponification was carried out in the maceration vessels, just before the start of maceration. To saponify 20 disks of tissue in 20 ml buffer solution, they were incubated overnight with orange PE (35 nkat). That saponification was almost complete, was seen from a DE of 4% of solubilized galacturonan.

4.2.4 Enzymes

All enzyme preparations used throughout this work were produced, isolated, purified and partly tested at the Laboratory of Food Chemistry and Microbiology of the Agricultural University of Wageningen.

Low-methoxyl pectin lyase (LMPL) was produced from the culture liquid of *Bacillus polymyxa*, which secreted this enzyme extracellularly on a medium containing pectate, as described by Uyttenboogaart (1970) and Rombouts (1972). After removal of bacterial cells, the culture liquid was concentrated by freeze-drying, purified by ammonium sulphate precipitation and dialysed against deionized water at 4°C overnight. This *Bacillus polymyxa* has been shown to secrete into the culture liquid one single pectolytic enzyme activity, pectate lyase (Nagel & Vaughn, 1961b) or low-methoxyl pectin lyase according to the new classification scheme proposed by Pilnik et al. (1973). Upon CM-Sephadex liquid chromatography, three fractions of LMPL activity have been obtained by Rombouts (1972) or four fractions when separated on CM--cellulose columns by Nagel & Wilson (1970). These LMPL-enzymes differ in pH optimum and endo mode of attack on pectic acid (Nagel & Wilson, 1970; Rombouts, 1972) and do not cleave pectic substrates in a purely random way. For one of the LMPL fractions of *Bacillus polymyxa* Rombouts (1972) established a maximum reaction rate (V) on 26% esterified pectin and maximum affinity ($1/K_m$) for a substrate with a DE of 21-31%. Lyases of *Bacillus polymyxa*

apparently do not prefer a substrate without esterified carboxyl groups (DE 0%), but exert optimum activity at 20-30% esterification and thus are properly called low-methoxyl pectin lyases.

Pectin lyase (PL) was isolated from a commercial enzyme preparation Pektolase FL 32 (Grindstedvaerket, Aarhus, Denmark) as described by Voragen (1972). At the end of the purification procedure which included calcium phosphate gel adsorption, ion-exchange and gel permeation chromatography, PE, PG and LMPL (PAL) must be absent. This PL has a rather high substrate affinity ($1/K_m$) compared with some other PLs (Voragen, 1972). The enzymic activity is influenced by the buffer medium used, citrate-phosphate and phosphate-citrate buffers giving the highest activities. In Tris-succinate buffer for a similar PL from another commercial preparation, a shift in pH optimum was found when DE decreased (Voragen et al., 1971a; Voragen, 1972). At 95% esterification of pectin substrate the optimum value of pH was 6.1, but at 74% (randomly esterified) pH optimum was 4.8. Enzymic activities on enzymically saponified substrates were much higher than on alkali saponified pectins of the same DE. The same authors reported that calcium ions (0.075-0.15 M) activated PL degradation of incompletely esterified substrates, except at pH 7.5. Calcium ions caused a shift in pH optimum of PL activity to lower or higher values dependent on degree of esterification. Therefore optimum substrate for pectin lyase is not a completely esterified pectin in all circumstances. A shift to lower esterified substrates may occur according to variation buffer in substances, pH and additional ions.

Pectinesterase (PE) was prepared from orange pulp (MacDonnel et al., 1945). Oranges were rasped to remove flavedo and pressed out. The pulp (1 kg) was homogenized in 2.5 l of buffer (42.5 g borax $Na_2B_4O_7.10H_2O$, 27.5 g boric acid and 40.0 g sodium acetate per litre) pH 8.2. After 2 h the mixture was filtered on a Büchner funnel (filter paper, Schleicher & Schull 520b). In the filtrate 1 250 g of ammonium sulphate were dissolved, and the precipitate was collected by centrifuging for 20 min at 16 000 *g*. All treatments were done at 2-4°C. The precipitate was dissolved in 50 ml deionized water, dialysed against the same overnight and freeze-dried.

4.2.5 Substrates for measurements of enzymic activities

Sodium pectate and ± 25% esterified pectin (Obipektin AG, Bischofszell, Switzerland) were used without further purification. Highly esterified pectin was prepared at the Laboratory of Food Chemistry and Microbiology (Voragen, 1972). Titrimetrically the DE was established to be 97-98% and the methylgalacturonan content 101% (Doesburg, 1965). Solutions of these pectin substrates were centrifuged at 48 000 *g* and filtered subsequently over glass filters (G_3) to get optically clear solutions according to the recommendations of Voragen (1972) for determination of lyase activities by u.v. assay.

Potato pectin was prepared from potato cell walls (Section 5.2.1), analogous to the method of Knee & Friend (1968): 2.5 g of cell wall were refluxed in 120 ml 0.1 M Tris-succinate pH 6.1 for 4 h. Four volumes of ethanol were added, the precipitate was collected by centrifugation, washed once with 70% and 100% ethanol, twice with ether and dried in vacuo. Yield was 1.15 g (air-dry). Pectin analysis with the procedures outlined in Section 4.2.9 showed a content of 91% carbohydrate material i.e. 24% galacturonan and 67% neutral sugars calculated for galactose. The DE of the pectic galacturonan was 27%. Potato pectin was used mostly after alkaline saponification in the cold.

4.2.6 Measurements of enzymic activities

According to the recommendations of the Commission on Biochemical Nomenclature (1972) enzymic activity is expressed as the amount of activity that converts one mole of substrate per second. The unit is the katal (kat).

Low-methoxyl pectin lyase activity was measured by the u.v. assay (Albersheim et al., 1960c) in a reaction mixture of 1 ml 1% (w/v) 25% esterified pectin, 1 ml 0.2 M Tris-succinate pH 8.5 containing 0.625 mM calcium chloride, 0.4 ml water and 0.1 ml enzyme solution, incubated at 30°C in a 1 cm quartz cuvette. The absorbance at 232 nm A_{232} was measured against a blank of the same components and inactivated enzyme with a Beckman DU spectrophotometer. The enzymic activity was expressed in katals by use of a molar extinction coefficient of 4 800 $mol^{-1}dm^3cm^{-1}$ (Rombouts, 1972).

Pectin lyase activity was determined also by measuring increase in A_{232}. The reaction mixture now consisted of 2 ml 0.5% (w/v) highly esterified pectin in 0.047 M Na_2HPO_4-citric acid (McIlvaine)buffer pH 6.5, 0.4 ml water and 0.1 ml enzyme solution, incubated at 30°C again. Enzymic activity was

calculated in katals with a molar extinction coefficient of 5 500 $mol^{-1}dm^3cm^{-1}$ (Rombouts, 1972).

Polygalacturonase activity was assessed by measurement of increase of reducing hexuronic acid groups (Milner & Avigad, 1967). The reaction mixture contained 2 ml 0.5% (w/v) sodium pectate dissolved in a 0.077 M McIlvaine buffer, pH 4.0, 0.4 ml water, 0.1 ml enzyme solution and was incubated at 30°C. At chosen intervals 0.1 ml of the reaction mixture was diluted to 0.5 ml and assayed immediately.

Pectinesterase assay is described in Section 7.2.2.

The activity of enzymes degrading neutral sugar parts of potato pectin and setting free reducing neutral sugars (galactose, arabinose etc.) here called arbitrarily 'galactanases' (Knee & Friend, 1968, 1970; Hoff & Castro, 1969; Cole, 1970) was determined with potato pectin as substrate. A reaction mixture of 1.15 ml 0.44% (w/v) saponified potato pectin in 0.067 M McIlvaine, pH 4.0 and 0.1 ml enzyme solution was incubated at 30°C. Total reducing sugar endgroups released were estimated with the method of Nelson-Somogyi (Somogyi, 1937; Nelson, 1944), and this result was corrected for galacturonic acid reducing endgroups determined according to Milner & Avigad (1967). The difference between the results of these two methods constitutes the quantity of neutral sugars with reducing power.

4.2.7 Maceration procedure

Potato tissue disks, after preparation stored in 70% ethanol at 4°C, were washed thoroughly with deionized water, to displace ethanol and remove loose cells or adherent starch grains and shaken in a waterbath-shaker for 5 min (Kötterman, Hänigsen, FRG). After decanting off the washwater this was repeated three times.

Twenty tissue disks were placed in the maceration vessel, a conical flask of 100 ml, and 20 ml buffer solution, 0-0.4 ml enzyme and 0.2 ml 0.02% (w/v) thiomersal (to prevent microbial growth) were added. The maceration started and proceeded for a chosen length of time at 30°C on the waterbath-shaker, at a standardized speed of 300 rev/min. Every treatment was duplicated. Enzyme solution solution was replaced by water in the blanks, when they were included. The compositions of the various maceration samples are summarized in Table 5. During the maceration experiment or when it was finished, the degree of cell separation was measured by a turbidity test. The tissue samples of 1971 were incubated with pectolytic enzyme for a fixed

Table 5. Buffers, enzymic activities and calciumchloride addition in maceration experiments.

Tissue	Enzyme		Buffer	
	LMPL nkat/0.25 ml	PL nkat/x ml	nature	pH
1971	5.17		0.02 M Tris-pipes	7.0
		4.12 (x=0.4)	0.05 M Tris-succinate	5.5[1]
		0.45 (x=0.074)	0.1 M Phosphate-citrate	5.1
1972	5.17		0.02 M Tris-pipes	7.0
	5.17		0.02 M Tris-pipes	7.0[2]

1. 0.1 M $CaCl_2$.
2. LS: 0.017; 0.08; 0.17 mM.
   HS: 0.022; 0.11; 0.22 mM.

period of time. Then enzyme action was stopped by addition of acid to lower pH (LMPL: 0.4 ml 2 M acetic acid, pH 4) (PL/Tris-succinate: 2 ml 0.5 M HCl, pH 3 and PL/McIlvaine: 0.8 ml 2 M HCl, pH 3). The macerated tissues were then prepared for further analyses. The tissue samples of 1972, however, were not used for analytical purposes apart from cell separation measurements at 3, 5, 10 and 24 h of maceration.

As just mentioned, the macerated tissue samples of 1971 were used for analyses of pectic substances. The contents of the maceration vessel were homogenized thoroughly (Bühler-homogenizer, Tübingen, FRG). The solids were removed by centrifugation or filtration over glass filter (G_3) or folded paper. The supernatant or filtrate was made up to 50 ml and analysed for solubilized pectic substances.

4.2.8 Measurement of cell separation by turbidity (TCS test)

Reduction or loss of intercellular cohesion of potato tissue results in separation of cells, which can be measured by an objective direct method such as the RWCS test described in Section 3.3.3. The small tissue samples used in maceration are less suitable for such a RWCS test in my opinion, although RWCS tests have been applied for assessment of tissue maceration (Mussell & Morré, 1969; Zucker & Hankin, 1970). For this purpose another objective and direct method was chosen. Cell separation was determined quantitatively by adaptation of a turbidity procedure (Bateman & Beer, 1965; Bateman, 1968; Melouk, 1969). In some experiments (1972 samples) a subjective counting method of the number of broken tissue disks was used too.

The procedure adapted for measurement of turbidity cell separation was as follows: a representative portion of the maceration liquid (± 3 ml), containing free cells and aggregates of some cells but avoiding larger tissue pieces, was decanted into a 1 cm glass cuvet. The cuvet, covered with parafilm, was inverted ten times, placed in the cuvet housing of a Beckman DU spectrophotometer and A_{475} was read exactly 15 seconds after inversion against a water blank. This procedure was repeated to obtain two values of A_{475} for each sample which were averaged. The A_{475} readings were converted into percentages of cell separation, ranging from 0 to 100%, with the aid of a standard curve. To establish this standard curve, twenty tissue disks were macerated overnight and complete cell separation (100%) was achieved with a magnetic bar and agitation at high speed. A_{475} at 100% cell separation was measured and of serial dilutions as well. In this way the whole range of zero to hundred percent was covered.

The turbidity cell separation test (TCS test) is a versatile method of measuring and following the course of reduction of intercellular cohesion. In fact the TCS test measures loose cells and thus is comparable with the RWCS tests used for the cooked potato tissue. However, the latter measures the retained weight or intact tissue which remains when the loose cells have been removed (Le Tourneau et al., 1962; Zaehringer et al., 1969; Ludwig, 1972). I used the TCS test for small quantities of cooked tissue (Chapter 6) as well as for macerated tissue. For larger samples of cooked tissue, the various RWCS tests are most suitable. As an objective test, the TCS test has obvious advantages over subjective appraisal of tissue maceration, like pulling apart with the fingers or pressing with needles (Brown, 1915), which was used by most workers in the past. The TCS test has the additional advantage of following maceration patterns more than giving endpoint determinations. The procedure is non-destructive, because after measurement of absorbance at 475 nm the sample can be returned to the maceration vessel. This is not possible when the RWCS tests of Mussell & Morré (1969) or Zucker & Hankin (1970) and penetration measurements according to McClendon & Somers (1960) or Sherwood (1966) are applied. The absorbance measured in a TCS test can depend on particle size (Lankveld, 1970), but according to Van Buren (1972) this is especially true at a particle radius close to the wavelength of the light used. The radius of potato tissue cells as used here (72-81 μm) (Section 4.3.3) is much greater than the wavelength of the light (475 nm) and thus variability in cell size will not influence the turbidity measurement.

The mean of cell separation values during maceration was 34% with s = 2.2%.

4.2.9 Quantitative determination of soluble pectic substances

During maceration some starch could be solubilized. As this carbohydrate material will interfere in pectin analysis, it was first removed by enzymatic hydrolysis and subsequent oxidation of glucose to gluconic acid. Gluconic acid did not react in the colorimetric tests used here to measure pectin content.

To 5 ml of the maceration extract 0.025 ml amylo-α-1,4-α-1,6-glucosidase (± 48.3 nkat) (Boehringer, Mannheim, FRG) was added. This mixture remained for 2 h at 30°C once pH was brought to 4 with diluted alkali. Then pH was further raised to 7.5 and 0.25 ml glucose-oxidase (± 291.7 nkat) (Boehringer) were admixed. The reaction sequence was assumed complete after another 4 h and then the volume was made up to 25 ml.

Pectic substances were determined with combined carbazole-sulphuric and phenol-sulphuric acid tests (Keijbets & Pilnik, 1974a). The carbazole--sulphuric acid reaction estimates galacturonic acid and was carried out according to the modification of Rouse & Atkins (1955) of the original Dische test (1947); neutral sugars were assayed with the phenol-sulphuric acid test of Dubois et al. (1956). Both colorimetric reactions are not entirely specific for galacturonic acid or neutral sugars, and for that reason a formula was used to calculate galacturonan and neutral sugar content from the absorbances measured (Keijbets & Pilnik, 1974a). The formula to be used, depends on standard curves established and may be somewhat variable. The galacturonan is calculated as anhydrogalacturonic acid, the neutral sugar part of the pectin complex as galactose, the predominant potato pectin sugar (Hoff & Castro, 1969).

Mean % solubilized pectic galacturonan in maceration experiments was 58% with s = 3.0%.

4.2.10 Analysis of β-elimination with the periodate-thiobarbituric acid test

Periodate-TBA, first described by Waravdekar & Saslaw (1957, 1959) for the determination of 2-deoxysugars, is a useful specific reagent for detection of unsaturated uronic acid residues which arise from pectic galacturonan during enzymatic or chemical β-eliminative chain splitting. At periodate oxidation β-formylpyruvic acid is formed, which is able to react with thiobarbituric acid to a violet-red chromogen with an absorption peak at about 550 nm (Weissbach & Hurwitz, 1959; Preiss & Ashwell, 1963). Rombouts (1972)

extensively described this test and thought that it might be applied for quantitative purposes although the reaction is not stoichiometric. Voragen (1972), on the other hand, denied the usefulness of the periodate-TBA test as a quantitative assay on the basis that the molar extinction coefficient ε depended on chain length for some unsaturated pectin oligomers. I investigated the dependence of ε on progressive depolymerization of purified potato pectin (extracted from cell walls with diluted hydrochloric acid) by chemical and enzymatic β-elimination. Galacturonan depolymerization was measured as an increase in reducing galacturonic acid endgroups (Milner & Avigad, 1967) (Section 4.2.12) and as an increase in unsaturated galacturonic acid endgroups (u.v. assay $A_{232-240}$ and use of ε = 5 500 $mol^{-1}dm^3cm^{-1}$). Molar extinction coefficients ε of periodate-TBA chromophore, calculated by comparison from both mentioned endgroup measurements, indeed changed with chain length of the degraded endproduct. In agreement with the results of Voragen (1972), we found that ε (periodate-TBA) increased with chain length, covering a range of 15 000 until 35 000 $mol^{-1}dm^3cm^{-1}$ for degrees of polymerization from 30 until 100 (calculated from reducing uronic acid). Hence the periodate-TBA test is useless for quantitative purposes. The u.v. measurement at 232-240 nm is not useful either because of manifold interferences in this low u.v. range by biological compounds.

However, the periodate-TBA reaction was carried out for qualitative purposes exactly as described by Rombouts (1972) who adapted the method of Weissbach & Hurwitz (1959) by application of a prolonged periodate oxidation step of 40 min (Waravdekar & Saslaw, 1959). To use the periodate-TBA data for comparison, a relative periodate-TBA A_{552} number was calculated which represents the absorbance at 552 nm per unit solubilized pectic galacturonan:

$$\frac{A_{552} \times 10^3}{\text{\% solubilized pectic galacturonan}}$$

Mean relative A_{552} number found during maceration was 0.68 with s = 0.028.

4.2.11 Total reducing sugar endgroups (Nelson-Somogyi test)

The total amount of both hexuronic and hexose endgroups were assayed with Nelson's spectrophotometric adaptation (1944) of the alkaline copper reduction method of Somogyi (1937). However, we did not make up the final volume to 25 ml, but added 4 ml of water instead. When working with potato

pectin, centrifugation appeared to be necessary in order to remove a fine flocculate which emerged after addition of arsenomolybdate reagent. The chromophore was measured at 750 nm at the absorbance maximum.

4.2.12 Reducing hexuronic endgroups (Milavi test)

Milner & Avigad (1967) developed a modified copper acetate solution which enabled them to estimate hexuronic acids sensitively and reproducibly whereas hexoses reacted poorly (aldohexoses more poorly than ketohexoses). In our experience this Milavi test must be carefully standardized to obtain satisfactory results. I added the arsenomolybdate reagent (Nelson, 1944) two min after start of cooling in ice, when sample and copper acetate solution had been boiled for ten minutes. Absorbance was measured at 750 nm, after centrifugation when needed, exactly as described for the Nelson-Somogyi reaction.

The DPs calculated from the absorbances had a mean value of 28 with $s = 2.3$.

4.2.13 Degree of esterification of soluble galacturonan

Methanol being released by alkaline saponification was determined according to the method of Wood & Siddiqui (1971), with one modification. Wood & Siddiqui reduced excess permanganate utilized for methanol oxidation prior to colour formation with 0.2 ml 0.5 M sodium arsenite in 0.12 N sulphuric acid plus 0.6 ml water. I, on the contrary, had to add 0.8 ml 0.5 M sodium metaarsenite in 0.12 N hydrochloric acid to get a sufficient reduction step. From determined methanol and galacturonan values, DE of galacturonan part of pectic substances could be calculated.

4.3 RESULTS AND DISCUSSION

4.3.1 Activities of the enzyme preparations

LMPL The low-methoxyl pectin lyase preparation from *Bacillus polymyxa* used in this work was identical with that used by Rombouts (1972) and thus free of other pectolytic activities. Pectinesterase was also absent because the *Bacillus* was cultured on a pectate medium. Potato pectin contains considerable amounts of neutral sugars, e.g. 67% for the sample we extracted from potato

cell wall or 48% for potato pectic substances according to Hoff & Castro (1969) which were largely galactose (88%), so that 'galactanase' might play a role in potato tissue maceration (Knee & Friend, 1970). I looked for 'galactanase' activity in the LMPL-preparation, and indeed at pH 4.0 some 'galactanase' activity was evident on saponified potato pectin (0.021 nkat/ml). 'Galactanase' activity, however, occurred at pH 7.5 on unsaponified potato pectin (27% esterified) in 0.2 M Tris-succinate without calcium ions at a ten times higher level (0.21 nkat/ml). This result means that 'galactanase' produced by *Bacillus polymyxa* differs from that of *Phytophtora infestans* by a pH optimum of 4 (Knee & Friend, 1970). Knee & Friend (1970) showed that their galactanase is an endo-acting enzyme, but this is unknown for the *Bacillus polymyxa* enzyme.

PL Pectin lyase, isolated and purified from the commercial preparation Pektolase FL 32, contained 'galactanase' as well (1.54 nkat/ml). The 'galactanase' from Pektolase FL 32 apparently possessed a lower pH optimum than that of *Bacillus polymyxa*, because its activity at pH 6.1 in Tris-succinate on 27% esterified potato pectin (0.12 nkat/ml) was much lower than that at pH 4 in McIlvaine buffer. No pectolytic activity was observed at pH 6.1 in Tris-succinate on 27% esterified potato pectin; of course these conditions are rather unfavourable for PL. But at pH 4 on saponified potato pectin reducing hexuronic acid groups were released. It was established that polygalacturonase (PG) was responsible (not PAL or LMPL activity, because no u.v. A_{232} was found). Voragen (1972) found no PG, PAL or LMPL and PE activity at the end of the purification operations for Pektolase FL 32 PL. The fact that some PG activity remained in the PL preparation prepared during this work, must be ascribed to experimental difficulties. I did not succeed in selective inactivation of PG by a temperature-buffer treatment and finally decided to use the PL preparation even though PG was present. The preparation used in maceration experiments with 0.05 M Tris-succinate buffer pH 5.5 + 0.1 M $CaCl_2$ (Table 5) had a PL activity of 10.30 nkat/ml, but after inactivation experiments and subsequent dilutions 6.15 nkat/ml PL activity remained for maceration in 0.1 M McIlvaine buffer, pH 5.1 (Table 5). Results of PG activity determinations on several substrates show that PG was not able to interfere in potato tissue maceration experiments (Table 6). Potato tissue used (1971) contained pectic galacturonan about 50% esterified, and no PG activity could be detected at 27% esterification when pH was raised to 5.1. On saponified potato tissue PG could be active and contribute to maceration.

Table 6. Polygalacturonase activity in Pektolase FL 32 pectin lyase preparation (6.15 nkat/ml).

Substrate	McIlvaine buffer (M)	pH	Activity (nkat/ml)
Sodium pectate	0.077	4	1.43
Saponified potato pectin	0.067	4	1.28
27% Esterified potato pectin	0.077	4	0.10
27% Esterified potato pectin	0.077	5.1	0.00

PE Pektinesterase from orange pulp is supposed to be free of galacturonan depolymerases (Mannheim & Siv, 1969). No tests for such enzymic activities thus were done.

4.3.2 General pattern of potato tissue maceration

The pattern of potato tissue maceration by LMPL was studied by measuring turbidity every hour (1971 samples). A typical example is given in Fig. 4. Three phases during enzymatic invasion of tissue can be discerned: a lag phase, a linear and a stationary phase. Bateman (1968) saw the same course of maceration with the TCS test apart from the last phase, which only appears when maceration is continued. He analysed the maceration phenomenon and established that length of lag phase and slope of linear phase are related to

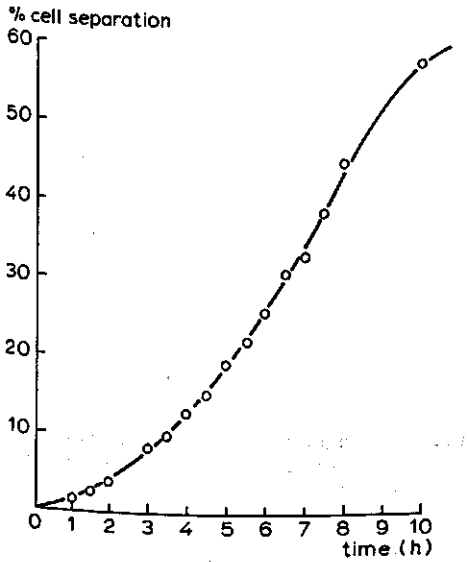

Fig. 4. General pattern of potato tissue maceration by low-methoxyl pectin lyase (1971).

log of enzyme concentration. The lower the enzyme concentration (= activity), the longer the lag phase and the smaller the slope of the linear phase. McClendon & Somers (1960) observed a lag phase only when calcium ions were added to the maceration medium possibly because of the high enzyme activity used in their experiments. The lag-linear-stationary pattern of tissue maceration is not typical for potato tissue. Zaitlin & Coltrin (1964) found this pattern with tobacco leave tissue, but only when a purified pectolytic enzyme was applied. With addition of EDTA the lag phase disappeared, probably because of calcium-binding. Potato tissue contains rather small intercellular spaces, hence diffusion of enzymes into the region of the middle lamella will be slow. Bateman (1968) thought that presence and length of lag phase depend on time of enzyme diffusion into the tissue sample and the time required to hydrolyse sufficient α-1,4-glycosidic bonds in the pectic substances of the middle lamella to permit cell separation. The results of McClendon & Somers

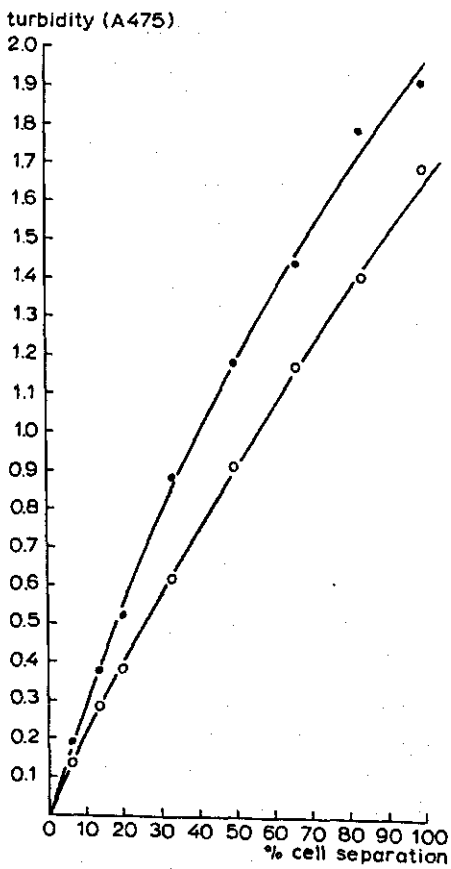

Fig. 5. Standard curves of enzymatic maceration of sp. gr. 1.100-1.110 (o) and sp. gr. 1.060-1.070 (•) (1971) for turbidity cell separation measurement (TCS test).

(1960) and Zaitlin & Coltrin (1964) suggest that calcium ions extend or even cause the existence of a lag phase in combination with enzyme activity applied. The linear phase then is the phase of rapid cell separation once enough bonds essential to intercellular cohesion are broken. A decrease in rapidity of cell release is reached in the stationary phase because of a shortage of unmacerated tissue.

For each specific gravity fraction used, a turbidity standard curve for maceration or cell separation was established. Those of the 1971 samples are reproduced in Fig. 5. At the same degree of cell separation different turbidities were measured for tissue of low and high sp. gr. The reasons for this can be found in different cell size and in the specific gravity itself. The heavier cells (sp. gr. 1.100-1.110) possibly settled down more rapidly than the lighter ones since the turbidity measurement was standardized in time.

4.3.3 Potato tissue maceration by LMPL and PL

Cell separation and galacturonan solubilization by LMPL in Tris-pipes and by PL in Tris-succinate + Ca The degree of esterification of pectic galacturonan of potato tissue disks amounted to 49-55%. Although the DE of these substrates did not seem very suitable for LMPL and PL, LMPL caused a rapid cell separation of high and low sp. gr. disks (Figs 6, 10, 11) at pH 7.0 in 0.02 M Tris-pipes buffer. The macerating capacity of PL was investigated initially in 0.05 M Tris-succinate pH 5.5, but then my attempts to initiate cell separation were unsuccessful. Addition of 0.1 M calcium chloride initiated maceration (Fig. 7), but cell separation remained at a low level compared with the activity of LMPL (Fig. 6).

In addition to % cell separation, the solubilization of tissue pectic substances was estimated. Only the results for galacturonan are given, but it was found that the value of the ratio neutral sugars (calculated for galactose) to galacturonan fluctuated around 2. Fig. 8 shows that during maceration by LMPL the solubilization of galacturonan increased almost along the same line as cell separation (Fig. 6). During maceration by PL in Tris-succinate + 0.1 M Ca, cell separation was rather limited and did not increase markedly after 6 hours of incubation. Solubilization of galacturonan followed the same pattern (Fig. 9). At 70-80% solubilization of galacturonan, LMPL caused a degree of cell separation of over 50% whereas this amounted to only 10-20% for PL (Figs 6, 7, 8, 9). Solubilized pectic substances were determined *after* homogenization of tissue. So probably all the pectic substances

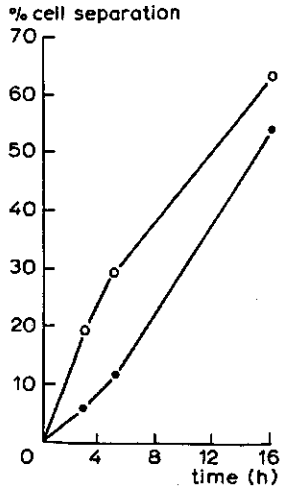

Fig. 6. Cell separation during maceration of potato tissue (1971) by LMPL in 0.02 M Tris-pipes pH 7.0. Curves marked as in Fig. 5.

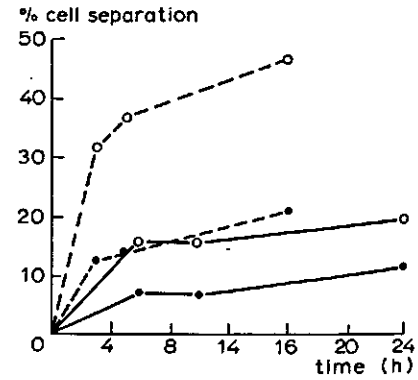

Fig. 7. Cell separation during maceration of potato tissue (1971) by PL in 0.05 M Tris-succinate pH 5.5 + 0.1 M $CaCl_2$ (——) and 0.1 M McIlvaine pH 5.1 (---). Curves marked as in Fig. 5.

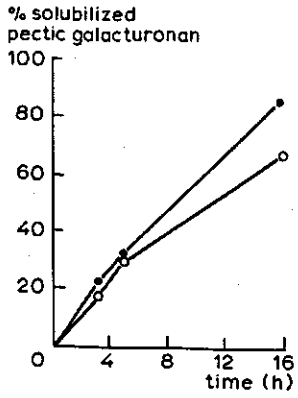

Fig. 8. Solubilization of pectic galacturonan during maceration of potato tissue (1971) by LMPL in 0.02 M Tris-pipes pH 7.0. Curves marked as in Fig. 5.

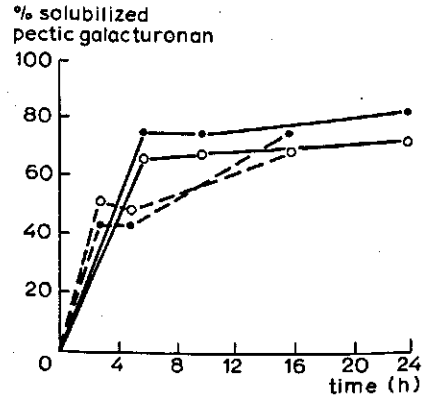

Fig. 9. Solubilization of pectic galacturonan during maceration of potato tissue (1971) by PL in 0.05 M Tris-succinate pH 5.5 + 0.1 M $CaCl_2$ (——) and 0.1 M McIlvaine pH 5.1 (---). Curves marked as in Fig. 5.

degraded or detached from covalent bonding to such an extent that they were soluble in aqueous medium, became soluble only when the tissue was destroyed artificially by mechanical forces of homogenization. Before tissue destruction, these potentially soluble pectic substances kept their ability to cement cells by means of calcium association as shown in the maceration experiments with PL.

Cell separation and galacturonan solubilization by PL in phosphate-citrate buffer The experiments with PL were repeated in 0.1 M phosphate-citrate pH 5.1 (McIlvaine) without calcium ions, because these ions were suspected to retard and reduce cell separation during maceration in Tris-succinate + 0.1 M Ca. On the other hand these ions had been found to initiate cell separation, probably by activating pectin lyase working on a rather low esterified substrate (Voragen, 1972). A concentration of 0.02 M McIlvaine was not sufficient to obtain cell separation. In blank samples, 0.1 M McIlvaine did not cause cell separation which proved that the buffer itself was not responsible for maceration (for instance by binding calcium ions). In 0.1 M McIlvaine buffer pH 5.1 about 10% of the PL units, (Table 5) used in the previous experiments with 0.05 M Tris-succinate pH 5.5 + Ca, caused a separation of cells which greatly exceeded that in the calcium-containing buffer (Fig. 7); even the same amount of galacturonan was solubilized (Fig. 9). The importance of calcium ions in intercellular cohesion of potato tissue is indicated strongly by these results of enzymic tissue degradation.

Galacturonan depolymerization The depolymerization of solubilized galacturonan was estimated tentatively by measuring reducing hexuronic acid groups (Milavi) and unsaturated uronic acid groups (periodate-TBA). As pointed out by Voragen et al. (1971b) chemical endgroup determinations are useful and reliable on a comparative basis merely to measure enzyme activity. But these methods are less suited to determine molecular weights because breakage of glycosidic linkages may occur during performance of the methods. The Milavi test, although carried out in an acid environment, also has these disadvantages. The results of Milavi test, expressed as degree of polymerization (DP), and of periodate-TBA test, expressed as relative A_{552}, thus are for comparative purposes only. In Table 7 the results on the depolymerization of pectic galacturonan by LMPL and PL are collected. During prolonged incubation with enzymes the DPs of solubilized pectic galacturonan decreased, although to a limited extent. The strongest decrease was found for LMPL, which also caused the greatest increase of solubilization (cf. Figs 7, 9). The changes in

Table 7. Depolymerization of pectic galacturonan during maceration of potato tissue (1971).

Enzyme	Buffer	Time of incubation (h)	Sp. gr. 1.060-1.070		Sp. gr. 1.100-1.110	
			DP (Milavi)	Relative A_{552}	DP (Milavi)	Relative A_{552}
LMPL	Tris-pipes	5	40		30	
		16	27	0.48	18	0.35
PL	Tris-succinate + Ca	6	15	0.89	20	1.01
		10	16	0.91	17	0.97
		24	11	0.78	14	1.01
PL	Phosphate-citrate	3	39	0.38	44	0.48
		5	38	0.41	44	0.51
		16	35	0.38	32	0.59

relative A_{552} were irregular because of the dependence of ε on DP (Section 4.2.10). The lowest DPs were connected with PL in Tris-succinate + Ca and the high activity applied (Table 5). A high activity of LMPL was used too, but the pH of maceration (7.0) was rather unfavourable for this enzyme which has an optimum pH of 8.5-9.3 (Uyttenboogaart, 1970). The optimum pH for PL of 4.8-6.1 agreed better with the maceration pH used (5.1-5.5) (Voragen, 1972).

Removal and addition of Ca, and cell separation by LMPL The role of calcium ions in the tissue coherence was further investigated in experiments with LMPL. Tissue disks of both sp. gr. classes were treated with ethanolic hydrochloric acid to remove calcium ions from cell wall and middle lamella. This treatment indeed resulted in considerable loss of ions. Potassium ions were removed for 79-92% and calcium ions for 52-58% (see Section 6.3.1, Table 14 for more specified figures). The residual calcium and potassium ions were possibly starch-bound and thus more difficult to wash out. The H tissue disks were macerated faster than the untreated ones (Figs 10, 11). The differences for sp. gr. 1.060-1.070 (Fig. 10) were smaller than for sp. gr. 1.100-1.110 when cell separation was assessed objectively by the TCS test.

Subjective judgment of tissue maceration afforded additional evidence for a difference in maceration rate (Table 8). After 3 and 5 hours of incubation, large differences existed in the number of broken disks which were not reflected in the turbidity measurement. This is probably because breakage of a tissue disk does not automatically mean release of free cells which cause turbidity. However, after 10 hours of incubation the number of broken disks was similar (Table 8) whereas percentages of cell separation of 36 to 54% were measured (Figs 10, 11). Removal of calcium ions apparently diminished

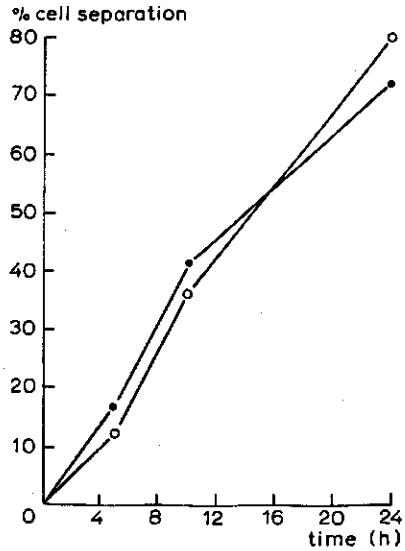

Fig. 10. Cell separation during maceration of potato tissue (1972) by LMPL in 0.02 M Tris--pipes pH 7.0: sp. gr. 1.060-1.070, unmodified disks (o) and H-disks (•).

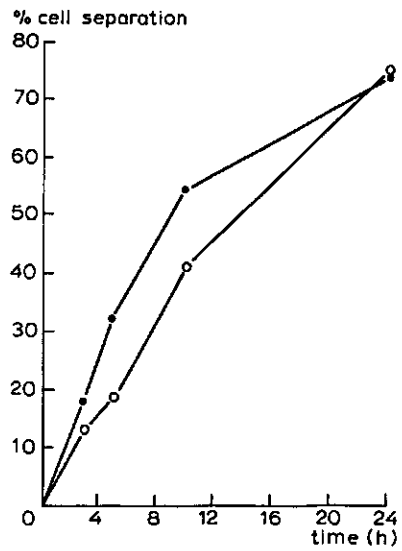

Fig. 11. Cell separation during maceration of potato tissue (1972) by LMPL in 0.02 M Tris--pipes pH 7.0: sp. gr. 1.100-1.110. Curves marked as in Fig. 10.

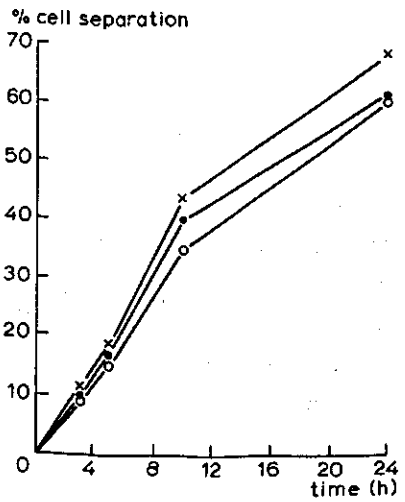

Fig. 12. Cell separation during maceration of potato tissue (1972) by LMPL: sp. gr. 1.060-1.070, 4.5% (o) - 22.8% (•) - 44.8% (x) of non-esterified pectic galacturonan carboxylic acid groups neutralized by Ca^{2+} added in 0.02 M Tris-pipes pH 7.0 buffer (H disks).

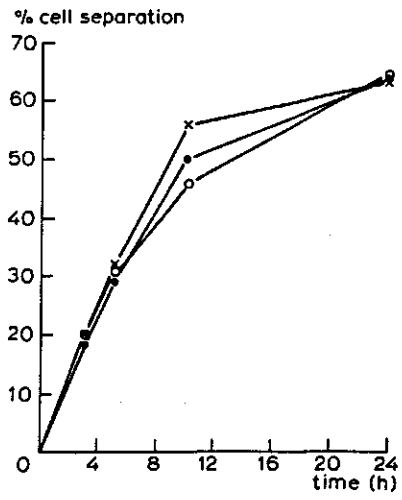

Fig. 13. Cell separation during maceration of potato tissue (1972) by LMPL: sp. gr. 1.100-1.110, 4.1% (o) - 20.7% (•) - 40.9% (x) of non-esterified pectic galacturonan carboxylic acid groups neutralized by Ca^{2+} added in 0.02 M Tris-pipes pH 7.0 buffer (H disks).

Table 8. Subjective judgment of tissue maceration during incubation with LMPL by counting of number of broken disks (1972 tissue). H: tissue disks washed out with ethanolic HCl.

Sp. gr.	Time of incubation (h)			
	3	5	10	24
1.060-1.070	0	1	18	20
1.060-1.070 - H	0	9	18	20
1.100-1.110	0	6	19	20
1.100-1.110 - H	6	15	20	20

the tissue coherence. Tris-pipes, the buffer used in these experiments, does not bind calcium ions (see Section 5.2.5) so that interference of the buffer ions is excluded.

This calcium trial was continued by re-addition of calcium ions in the maceration buffer to account for 4.5, 22.8 and 44.8% respectively of non-esterified pectic galacturonan carboxylate equivalents of sp. gr. 1.060-1.070 or 4.1, 20.7 and 40.9% of sp. gr. 1.100-1.110. Progressive neutralization of non-esterified galacturonan carboxylic acid groups, however, did not result in increased intercellular cohesion as expected but the opposite effect was encountered (Figs 12, 13 cf. Figs 10, 11). The explanation of this unanticipated calcium effect probably lies in the calcium dependent activity of *Bacillus polymyxa* LMPL (Nagel & Vaughn, 1961a; Rombouts, 1972), which is optimally active in 0.25 mM calcium ion solution. The highest amount of calcium ions supplemented was equivalent to a 0.17-0.22 mM concentration. Hancock & Stanghellini (1968) found a similar calcium effect with potao tissue and a pectate lyase of *Hypomyces (Fusarium) solani* f. sp. *cucurbitae* at 0.1 mM calcium addition, but at 1 mM level calcium already retarded maceration. The interaction of calcium dependent enzyme activity for LMPL and PL and calcium dependent galacturonan solubility or cell cohesion obviously causes difficulties in the interpretation of maceration phenomena.

Comparison of high and low specific gravity Potato tissues of low and high specific gravity differ widely in intercellular cohesion after cooking. Do the maceration experiments with the pectin depolymerizing lyases reveal differences in primary structure of their pectic substances? One of the theoretical possibilities is a different pattern of esterification between middle lamella and primary wall pectic galacturonan for the high and low

Table 9. Average maceration rate of low and high sp. gr. potato tissue (1971).

Enzyme	Time of incubation (h)	% Cell separation per hour	
		sp. gr. 1.060-1.070	sp. gr. 1.100-1.110
LMPL	3	2.0	6.3
	5	2.4	5.8
	16	3.2	4.0
PL (+Ca)	6	1.2	2.7
	10	0.7	1.6
	24	0.5	0.8
PL (-Ca)	3	4.3	10.7
	5	2.8	5.4
	16	1.3	2.9

sp. gr. fraction. I found, however, DEs ranging from 49-62% for solubilized pectic galacturonan for both sp. gr. fractions during the maceration. This is equal to or somewhat higher than the values in the untreated tissue. No differences between sp. gr. fractions were found. The patterns of solubilization of galacturonan (Figs 8, 9, 15, 17) ressembled each other strongly, while the results of reducing endgroup and unsaturated bond determinations (Table 7) did the same. Thus the enzymatic degradation patterns of pectic galacturonan of both sp. gr. classes were rather similar, so that differences in primary structure of these two extremes were not found. It is evident, however, from all results that the intercellular cohesion of the high sp. gr. class tissue (1.100-1.110) was reduced at a faster rate than that of the low sp. gr. tissue (1.060-1.070) (Figs 6, 7, 10, 11, 12, 13) (Table 9). Differences in cell size may account for this according to Linehan et al. (1968) and Van Buren (1970). With a linear measuring method as described by Reeve et al. (1971) I established that the 1971 tissue contained larger cells in the high sp. gr. disks ($\bar{d}$ = 162 µm; s = 20) than in those of low sp. gr. ($\bar{d}$ = 144 µm; s = 18). Another contributing factor might be a difference in cell wall and middle lamella calcium level.

4.3.4 The effect of DE of tissue galacturonan on maceration by LMPL and PL

Saponification of potato tissue by orange PE is specific. This is not true for the chemical esterification by diazomethane, which may depolymerize pectin (Neukom & Deuel, 1958; Heim & Neukom, 1962; Smit & Bryant, 1969). In fact some pectic galacturonan was lost during this treatment as seen from

the analyses (Section 4.2.3). Furthermore diazomethane-treated tissue disks were rather fragile and lost coherence in maceration blanks at long incubation times due to the continuous shaking force. These blank values of cell separation were subtracted only when tissue disks were broken, which was necessary in the LMPL trials where long incubation periods were taken (16 h).

LMPL LMPL preferred to attack the unmodified tissue, because % cell separation of saponified tissue (Fig. 14) as well as the amount of solubilized galacturonan (Fig. 15) were lower. The depolymerization, on the other hand, seemed markedly increased after saponification because a DP of 5 (Milavi) and a relative A_{552} of 1.95 were measured for sp. gr. 1.060-1.070 (cf. Table 7). Enhanced binding of calcium to non-esterified pectin carboxylic acid groups at a low DE may be responsible for this effect of retarded cell separation and pectin solubilization. When galacturonan is low-esterified, calcium ions are able to form insoluble calcium pectate. LMPL, after correction for blank, did not macerate esterified tissue.

PL PL progressively macerated potato tissue as the DE increased, although there was one exception (Fig. 16). The patterns of cell separation and galacturonan solubilization differed strikingly (Figs 16, 17). This is evident from the experiments in 0.05 M Tris-succinate pH 5.5 + 0.1 M $CaCl_2$, particularly. Whereas cell separation increased strongly from unmodified to esterified tissue, this could not be ascribed to enhanced solubilization of pectin. It must be assumed that this was a calcium effect again. Esterified pectic galacturonan contains very few ionizable carboxylic acid groups and this prohibits considerable calcium bonding (Deuel et al., 1950; Anyasz-Weisz & Deuel, 1950). Also the effect of buffer disappeared at a high DE at the same time (Fig. 16). Saponified potato tissue retained its structural integrity when incubated with PL, although the PG activity might have caused cell separation. But after homogenization only up to 17% of pectic galacturonan was soluble (Fig. 17).

Galactanase During maceration by PL of saponified tissues the ratio neutral sugars (calculated for galactose) to galacturonan was raised from about 2 up to 8-11, possibly by 'galactanase' activity in the PL preparation. Knee & Friend (1970) described some maceration experiments with an endo galactanase and concluded that this enzyme had some influence on tissue coherence (subjective assessment according to Brown (1915)). I isolated 'galactanase' from

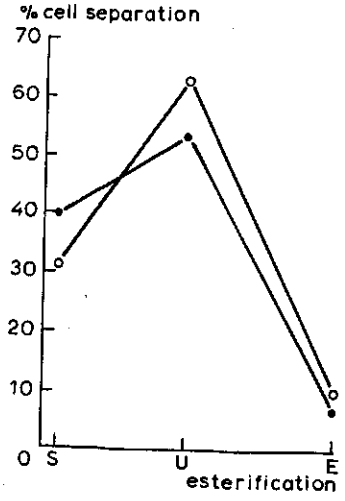

Fig. 14. Cell separation during maceration by LMPL of modified potato tissue in comparison with unmodified tissue (1971).
S = saponified tissue
E = esterified tissue
U = unmodified tissue
Curves marked as in Fig. 5.

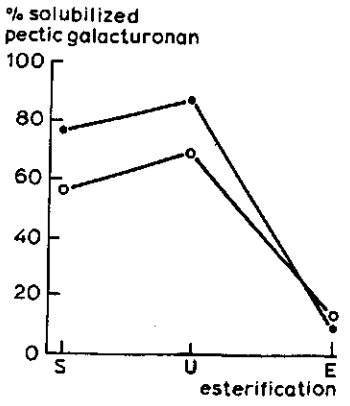

Fig. 15. Solubilization of pectic galacturonan during maceration by LMPL of modified tissue in comparison with unmodified tissue (1971). Curves marked as in Fig. 5; U, S, E as in Fig. 14.

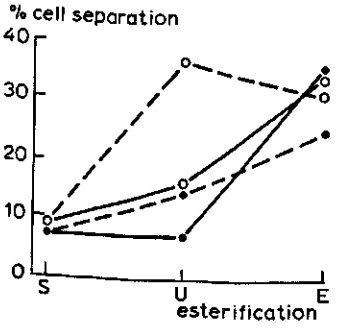

Fig. 16. Cell separation during maceration by PL of modified potato tissue in comparison with unmodified tissue (1971). Curves marked as in Fig. 5, 7; U, S, E as in Fig. 14.

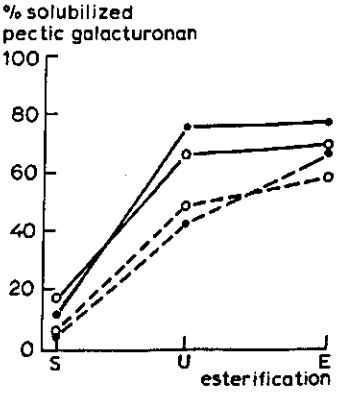

Fig. 17. Solubilization of pectic galacturonan during maceration by PL of modified tissue in comparison with unmodified tissue (1971). Curves marked as in Fig. 5, 7; U, S, E as in Fig. 14.

Phytophtora infestans too, but found no effect of this enzyme on intercellular cohesion measured by turbidity. Breakage of tissue disks was not noticed either. Solubilization of neutral sugars did not occur. The 'galactanase' activity present in the PL preparation, although the endo or exo character is obscure, solubilized neutral sugars from saponified tissue probably because of polygalacturonase activity on this substrate, but this did not contribute to cell separation (Fig. 16).

4.4 CONCLUSIONS AND SUMMARY

Reduction of intercellular cohesion or maceration of potato tissue by two pectic galacturonan depolymerases was investigated. The tissues of extreme specific gravity fractions from a restricted population of potatoes were compared. Procedures and methods of analysis have been described. The degree of loss of intercellular cohesion or cell separation could be conveniently measured by determination of turbidity. The pattern of tissue maceration was studied. Maceration experiments were carried out (1) with unmodified potato tissue disks, (2) after removal of calcium from cell wall and middle lamella and (3) after DE of pectic galacturonan had been changed.

Attack of unmodified tissue by low-methoxyl pectin lyase and pectin lyase revealed identical patterns of solubilization of pectic galacturonan (and neutral sugars) and of depolymerization (reducing hexuronic acid and unsaturated acid endgroups) for both low (1.060-1.070) and high (1.100-1.110) sp. gr. tissue. Differences in primary structure of pectic substances could not be observed with the techniques used. Cell separation, however, in the high sp. gr. tissue exceeded that in the low sp. gr. samples. It is suggested that the greater cell size or smaller surface area of cells of high sp. gr. tissue caused this difference. The maceration of potato tissue, established already by many investigators, emphasizes again the inevitability of pectic galacturonan in cell cohesion.

The important influence of calcium ions in intercellular cohesion emerged from several experimental results. Calcium ions retarded strongly cell separation when 100 mM of these ions were present during maceration by pectin lyase in Tris-succinate buffer. Calcium removal from the tissue and partial neutralization of non-esterified pectic galacturonan carboxylic acid groups confirmed the retarding effect of calcium on cell separation during maceration by low-methoxyl pectin lyase. At the same time it became clear that the use of lyases in tissue maceration is somewhat confusing when the

role of calcium has to be investigated. This must be ascribed to the enzyme--activating influence of calcium ions. Although calcium ions retarded cell separation, even when pectic galacturonan was degraded severely, solubilization of galacturonan was not limited upon tissue destruction by chemical means.

It was established that pectin lyase preferentially macerates highly esterified potato tissue (97% esterified) and does not attack saponified tissue (4% esterified). These findings are in agreement with the properties of pectin lyase described in literature. Low-methoxyl pectin lyase, on the other hand, did not macerate highly esterified tissue but surprisingly its macerating action on saponified tissue was inferior to that on unmodified tissue (49-55% esterified).

5 Behaviour of pectic substances in the potato cell wall and middle lamella during boiling

5.1 INTRODUCTION

When potatoes are cooked, a shift from insoluble pectic substances into water-soluble pectin has been observed (Freeman & Ritchie, 1940; Bettelheim & Sterling, 1955b; Doesburg, 1961). Other authors measured during cooking a loss of intercellular cohesion objectively (Section 3.3.2 and 3.3.3). Although calcium ions retard cell separation during enzymatic maceration, increase in cell separation and in solubilization of pectic galacturonan parallel each other mostly (Section 4.3.3). A cause and effect relationship between reduction of intercellular cohesion and solubilization of pectic substances is suggested by these data.

The natural hydrogen ion concentration or pH of potato tissue ranges between 5.5 and 6.5 (Burton, 1966). It is well-known now that chemical degradation of pectic galacturonan in this pH region during heating or boiling is due to the β-eliminative (or transeliminative) mechanism (Fig. 18) (Albersheim et al., 1960b; Neukom, 1963; Doesburg, 1965). The reaction rate of β-eliminative degradation at boiling temperature depends on pH (hydroxyl ion concentration, Fig. 18) (Albersheim, 1959; Doesburg & Grevers, 1960; Schmidt, 1965; BeMiller & Kumari, 1972) and the presence of a methylester at C_6, next to which chain cleavage occurs (Albersheim et al., 1960b). Keijbets & Pilnik (1974b) found that nature and quantity of ions in the pectin surrounding medium influence β-elimination. Both negatively and positively charged ions enhance pectin breakdown.

It is assumed, that during heating of potato tissue above 60°C the plasmalemma becomes permeable (Bartolome & Hoff, 1972; Hoff, 1972, 1973) as indicated by a sharp increase in electrical conductivity (Personius & Sharp, 1938b), probably caused by diffusion of intercellular solutes into the cell wall. In potato tissue the predominant ions are potassium and citrate (Davis, 1964; Burton, 1966; Adler, 1971; Davis et al., 1973). These ions, and others, might penetrate into the cell wall and middle lamella and effect solubilization of pectic substances.

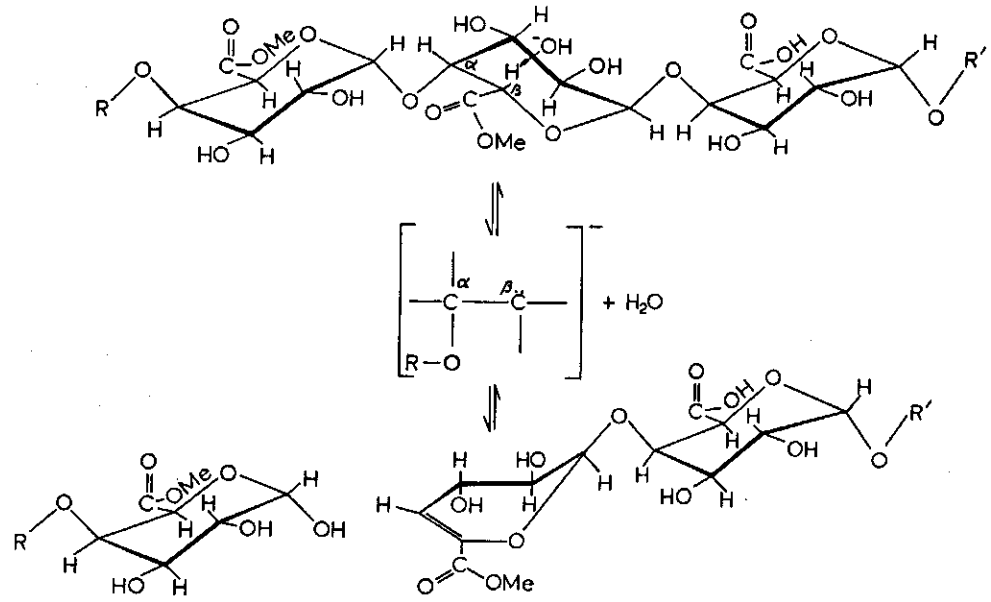

Fig. 18. Depolymerization of a partially esterified pectic galacturonan chain by chemical β-elimination according to the ElcB mechanism.

To study the solubilization of pectic substances and pectic galacturonan especially, and the interaction with several potato constituents like ions and starch, potato cell walls were isolated. The cell wall/middle lamella complex is an ideal model for solubilization studies, because the normal potato constituents are removed and can be added when wanted in a controlled way.

5.2 MATERIALS AND METHODS

5.2.1 Isolation of potato cell walls

Two kg of potatoes (Bintje, 1971, sp. gr. 1.080-1.090, stored 4.5 months, see Section 4.2.1) were peeled, chopped and disintegrated with 2 l of ethanol in an Ultra-turrax homogenizer (Janke & Kunkel, Staufen in Br., FRG). Free starch grains were washed out with excessive deionized water over cheese cloth (Knee & Friend, 1968). To destroy further unbroken cells the solids were homogenized with water at high speed in a Bühler homogenizer with water-cooling (50 000 rev/min). The washing and blending procedures were repeated until all cells were broken and all starch grains had disappeared (microscope).

A few small thick-walled cells with small starch grains remained intact. The cell wall preparation was freeze-dried, ground and stored in an exsiccator (yield 24.8 g) at room temperature.

5.2.2 H cell wall

Calcium and other cell wall ions were removed by treating 10 g of dry potato cel wall with four volumes of 250 ml 0.6 N ethanolic hydrochloric acid; the cell wall was washed free from chloride with 70% ethanol, 96% ethanol and ether. Finally this preparation, containing free pectic galacturonan carboxylic acid groups (H cell wall), was dried in vacuo at room temperature overnight.

Pectin analysis was carried out with the Cu^{2+} ion-exchange method of Keijbets & Pilnik (1974a). The H cell wall contained 16.0% of esterified galacturonan. The galacturonan was esterified for 58%, which means that 36.5 µmol of free carboxylic acid groups were present in 100 mg air-dry H cell wall.

5.2.3 Saponified H cell wall

Partial saponification with orange PE (Section 4.2.4) occurred by incubation of 50 mg of H cell wall with 70 nkat of enzyme activity for 2 and 5 hours in 0.02 M Tris-pipes, pH 6.1. These samples were boiled then according to the standard procedure outlined below. The DE was lowered to 42% (2 h) and 33% (5 h) respectively.

A larger bath of PE-saponified cell wall was prepared by incubation of 1 g H cell wall with 70 nkat orange PE, 25 ml water, 2.5 ml 4% (w/v) NaCl and 1.5 ml 0.02% (w/v) thiomersal overnight while the pH was maintained at 7.5 with a pH-stat (Radiometer Automatic Titration Assembly TTT 11b, Radiometer A/S, Copenhagen, Denmark). The cell walls were prepared for use by repetition of the ethanolic hydrochloric acid washing procedure etc. Pectin analysis resulted in a content of 14.7% esterified galacturonan, DE of 2% and 81.8 µmol of free carboxylic acid groups per 100 mg.

Another 1 g H cell wall was saponified in the cold (4°C) for 1 h with 50 ml 0.1 N NaOH. The saponified cell wall was converted to H cell wall. Pectin analysis resulted in a content again of 14.7% esterified galacturonan, DE of 1.3% and 82.6 µmol per 100 mg.

5.2.4 *Starch*

Potato starch was purchased from British Drug Houses (BDH Chemicals Ltd, Poole, England). H starch, containing free phosphoric acid groups, was prepared by a washing procedure with diluted aqueous hydrochloric acid (Winkler, 1960). It was dried overnight at 30°C in a ventilated incubator. Dry matter was determined by drying at 130°C for 2 h (Richter et al., 1968). Calcium starch was prepared by titrating H starch in aqueous suspension with saturated calcium hydroxide to pH 7. Not-ionically bound calcium was washed out with deionized water. The primary Ca starch (phosphate groups only half neutralized (Winkler, 1960)) was dried again at 30°C overnight in the ventilated incubator.

The phosphate content of starch samples was determined by Cu^{2+} ion-exchange as used for pectic galacturonan (Keijbets & Pilnik, 1974a). This method gave identical results as potentiometric titration (H starch) (Winkler, 1960). It has the additional advantage of being applicable without previous conversion to H starch because calcium and other ions bound to starch-phosphate are replaced by copper ions. This is possibly due to the high selectivity of phosphate groups for copper ions (Haug & Smidsrød, 1970).

Calcium content of starches was determined after exchange with hydrogen ions (0.5 N HCl) and analysis of the exchanged liquid with a colorimetric calcium determination according to Milligan & Lindstrom (1972). One modification was applied, because the use of sodium sulphide solution to establish an alkaline pH was unsuccessful. A 0.2 M sodium hydroxide solution was found suitable. The sodium hydroxide used here contains only very small amounts of calcium (5 µg per g) (Merck AG, Darmstadt, FRG), and such a low-calcium alkali was not available to Milligan & Lindstrom (1972).

The results of phosphate and calcium analysis were: BDH starch (unmodified), 5.04 µeq P and 1.2 µeq Ca per 100 mg or 24% of phosphate neutralized by calcium. H starch contained 5.14 µeq P per 100 mg. Ca starch 5.14 µeq P and 2.68 µeq Ca per 100 mg or 52% of phosphate neutralized by calcium.

5.2.5 *Chemicals*

Pipes or piperazine-N,N'-bis-2-ethanesulphonic acid (BDH) is a buffer with pK_a at 20°C = 6.8. This acid does not bind calcium, magnesium or copper ions (BDH, 1973). Tris-pipes buffer is a useful buffer in the pH region of 6-7, which does not interfere in the study of interaction of metal ions with pectin solubilization.

All salts, alkalis and acids used are commercially available, except phytic acid which was prepared from sodium phytate (Sigma Chemical Company, St. Louis, USA) by ion-exchange on Amberlite IR-120 (H^+) according to Kaufman & Kleinberg (1970).

Stock solutions of 0.3 M Tris-pipes pH 6.1, 1.0105 N citric acid, 0.9426 N phytic acid, 0.9955 N malic acid were prepared. The acid solutions were checked by titration as also the alkali solutions of 0.0994 N potassium hydroxide and 0.0402 N calcium hydroxide. Furthermore 1 N KCl, $CaCl_2$ and $MgCl_2$ solutions and 0.01 N $CuSO_4$ and $FeSO_4$ solutions were used.

5.2.6 Boiling mixtures and boiling procedure

The boiling mixtures consisted of 50 mg H cell wall or saponified H cell wall, which were weighed into a 50 ml conical flask. The ions under investigation were added with 10 ml 0.02 M Tris-pipes buffer pH 6.1, except in a few experiments where they were supplemented in up to 0.4 ml volume. During preparation of the diluted buffer solution (0.02 M) the required amounts of standard salt, acid or alkali were added to the buffer. In the experiments with organic anions (citrate, malate, phytate), the non-esterified pectic galacturonan carboxylic acid groups were neutralized with the appropriate amount of calcium or potassium hydroxide. As excess cations and anions were added as alkali and acid, the pH became slightly more than 6.1, so that some additional hydrochloric acid was necessary. Thus the diluted buffer concentration alone was unable to maintain the pH exactly, but no higher buffer molarity was used to minimize the influence of buffer ions themselves on pectin solubilization. The influence of buffer concentration and pH of buffer was studied too. Hydrogen ion concentration of Tris-pipes buffer was adjusted with 0.02 M Tris. Potato starch was added to the boiling mixtures by weighing (air-dry substances).

The boiling proceeded under refluxing on a hot plate shaker for 30 min. In one series of experiments the boiling time was used as parameter. At the end of the boiling period, the mixture was cooled down immediately and filtered over folded paper (Schleicher & Schull, 595). The filtrate was used for analyses.

The experiments generally were not duplicated, but their reproducibility had been found to be satisfactory. For seven repeated experiments, the mean % solubilized pectic galacturonan was 46% with s = 1.6% and the mean relative A_{552} number was 0.47 with s = 0.026.

5.2.7 Boiling mixtures which simulate the composition of low and high sp. gr. potato tissue

The composition of these boiling mixtures was derived from analyses of potato tissue of Bintje potatoes of 1972 (Section 7.3.3). In the diluted Tris-pipes buffer ions were added in the proportions as they occurred in the potato tissues relative to cell wall content. The final composition in the boiling mixture simulating tissue of low sp. gr. (1.060-1.070) (LS), expressed as ratio ion/COO^- (= non-esterified carboxylate of galacturonan) was: Ca 2.2; Mg 3.0; K 10.1; citrate 10.3; malate 3.0; phytate 0.7; in 0.02 M Tris-pipes pH 5.89 (pH was lowered as a result of additions!). The composition for tissue of high sp. gr. (1.100-1.110) (HS) amounted to: Ca 1.8; Mg 4.4; K 11.5; citrate 14.0; malate 2.1; phytate 1.0; whereas pH was adjusted with potassium hydroxide (K 2.7) to 6.19. Only magnesium was added as salt (chloride), while phytate accounted for one fourth of P analysed (Nowotny & Samotus, 1965). More phosphate was omitted. The HS experiment was repeated with a buffer solution at pH 5.89 (additional K 1.7).

In a similar experiment H cell wall was replaced by cell walls isolated from low and high sp. gr. fractions described in Section 7.2.1. The LS cell wall contained 18.9 µeq of non-esterified galacturonan carboxylic acid groups and 8.0 µeq of Ca per 50 mg, the HS cell wall 16.7 µeq of non-esterified carboxylic acid groups and 5.5 µeq of Ca. The same buffer mixtures just mentioned were used, apart from correction for calcium already present in the cell wall.

Furthermore an experiment was done without any additional calcium ion and the HS sample was boiled at pH 5.89 again.

5.2.8 Pectin analyses after boiling

In the filtrate, soluble pectic substances were determined as described in Section 4.2.9. Unsaturated uronic acid groups were also determined (Section 4.2.10), but with a 1 ml sample, appropriate quantities of reagents and final measurement of A_{552} in a 5 cm cuvet. For calculation of the relative A_{552} number, the absorbance measured was divided by 5. Determination of reducing hexuronic acid groups was omitted, because pipes interfered in this analysis. When starch was present in the boiling mixture, it had to be removed partly by freezing overnight and thawing, followed by centrifugation (Sorvall RC-B, swing-out rotor, 16 000 *g*). The soluble part was further removed enzymically as described in Section 4.2.9 but somewhat greater enzyme

activities were used. Periodate-TBA analysis then was omitted, because the glucose-oxidase enzyme contained pectic lyase activity.

5.3 RESULTS AND DISCUSSION

5.3.1 Cations

K, Ca, Cu, Fe When all non-esterified carboxylic acid groups of pectic galacturonan were neutralized by potassium or calcium ions, and the boiling time was varied, calcium ions were seen to retard the solubilization of pectic galacturonan (Fig. 19). The difference amounted to 20-25% of total pectic galacturonan. As boiling proceeded, the rate of galacturonan solubilization slowed down. The pattern of solubilization was rather similar for both cations, apart from the difference in level.

That cell wall pectic galacturonan was indeed depolymerized by ß-elimination, could be seen from the concurrent increase in periodate-TBA absorbances (Fig. 20), although differences in pattern were evident (Figs 19, 20). A periodate-TBA absorbance spectrum recorded for the potassium-neutralized cell wall sample boiled for 60 min, when 69% of pectic galacturonan had been solubilized (Fig. 19), showed the presence of unsaturated uronosyl residues with an absorbance maximum at 550-552 nm (Fig. 21).

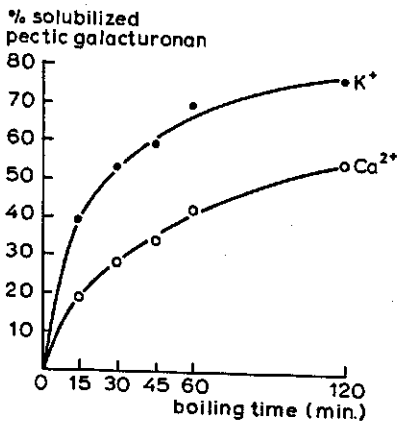

Fig. 19. Solubilization of pectic galacturonan from potato cell walls during boiling with calcium and potassium ions (equiv. K^+ = equiv. Ca^{2+} = equiv. COO^-).

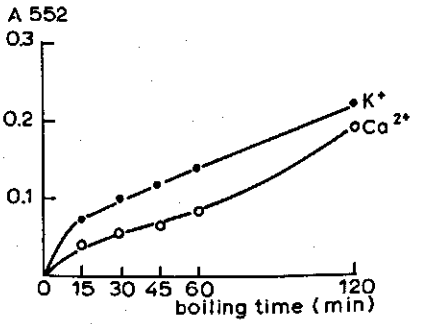

Fig. 20. Periodate-TBA absorbances during boiling of potato cell walls as in Fig. 19.

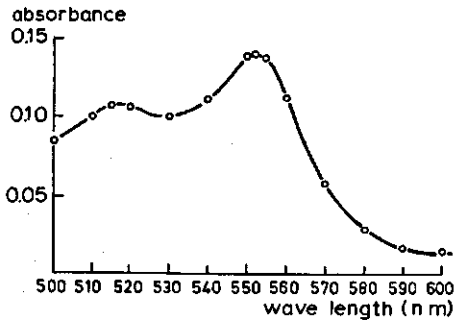

Fig. 21. Periodate-TBA absorbance spectrum of solubilized pectic galacturonan (K cell wall, 60 min; cf. Fig. 19).

Preliminary experiments showed that the second, lower absorbance maximum at about 517 nm, must be ascribed to the relatively high neutral sugar content in the sample (ratio neutral sugars (calculated for galactose)/galacturonan = 1.9). In the periodate-TBA spectra reproduced by Rombouts (1972), which represent reaction mixtures of purified pectic acid (77% galacturonan) and enzyme preparations, a shoulder at about 515 nm was merely found, but the neutral sugar content of the pectate preparation was rather low. When the neutral sugar content of my samples was higher than about ratio 2 and few unsaturated bonds were present, the peak at 517 nm exceeded that at 552 nm. The violet-red colour then turned to red-brown.

In the absence of potassium (and chloride) ions during boiling, the same pattern for the solubilization of pectic galacturonan was obtained as with potassium ions (Fig. 19). Apparently the addition of this amount of potassium ions is without influence. This is congruent with the finding that addition of ten to twenty times as much potassium ions (expressed as ratio K^+/COO^-) did not essentially change the solubilization of pectic galacturonan (Fig. 22). Only at ratio K^+/COO^- 50-100, did more galacturonan come into solution at a fixed boiling period of 30 min.

Changing amounts of other cations were studied as well. At a ratio cation/COO^- < 1 divalent calcium, copper and iron ions inhibited the solubilization of pectic galacturonan progressively (Fig. 22). Iron and calcium were equally effective, but copper ions exerted a stronger insolubilizing action, which is in agreement with the high affinity (Haug & Smidsrød, 1970) and precipitation strength (Wunsch, 1952; Tibensky et al.,

1963) of copper for pectin solutions.

The results were very interesting when there were more calcium ions than non-esterified pectic carboxylate ions (ratio $Ca^{2+}/COO^{-} > 1$). The higher the calcium level became, the more pectic galacturonan was solubilized. From the results of Fig. 22 it can be derived that the increase of calcium solubilization exceeded that of potassium. An explanation of this unexpected calcium effect will be found in periodate-TBA values and some theoretical considerations.

The increase of periodate-TBA absorbance (A_{552}) in the presence of calcium ions clearly surpassed the increase when potassium ions were added (Fig. 23). At a ratio cation/COO^{-} = 10 the A_{552} value for Ca even surpassed that for K absolutely. This strong increase was found again in the relative periodate-TBA numbers or the absorbance at 552 nm per unit solubilized pectic galacturonan (Section 4.2.10). When the ratio cation/COO^{-} rose from 1 to 100, this number rose for Ca from 0.47 to 1.21 and for K from 0.41 to 0.76 only. An increase in A_{552} might be due to an increase in molar extinction coefficient ε, i.e. an increase in DP (Section 4.2.10), but if A_{552} per unit solubilized pectic galacturonan increased concurrently with percentage solubilized galacturonan itself, this would mean that more galacturonan had been solubilized as larger molecules. This assumption is rather untenable. So it can be concluded that increasing amounts of calcium ions (above ratio cation/COO^{-} = 2) favoured β-eliminative degradation of pectic galacturonan. The enhanced depolymerization of galacturonan ultimately caused the increased solubilization of galacturonan, which was unexpected because of the demonstrated effect of calcium to retard solubilization (Figs 19 and 22 when ratio cation/$COO^{-} < 1$).

The ability of calcium ions to keep pectic galacturonan insoluble even when progressively depolymerized during boiling, is demonstrated convincingly as follows: at a level of 50% solubilized pectic galacturonan (Fig. 22), this galacturonan was depolymerized extensively when calcium ions were present (Fig. 23) and only for a minor part with potassium ions (TBA-A_{552} for Ca three times that for K). Keijbets & Pilnik (1974b) have already presented evidence that with calcium ions β-elimination cleavage of pectin molecules during boiling occurs easier than with potassium ions. They also established that increasing amounts of calcium (and potassium) ions increase β-elimination of pectin solutions.

The resultant of the galacturonan insolubilizing effect of calcium and the counteracting stimulation of β-elimination led to a minimum net yield of

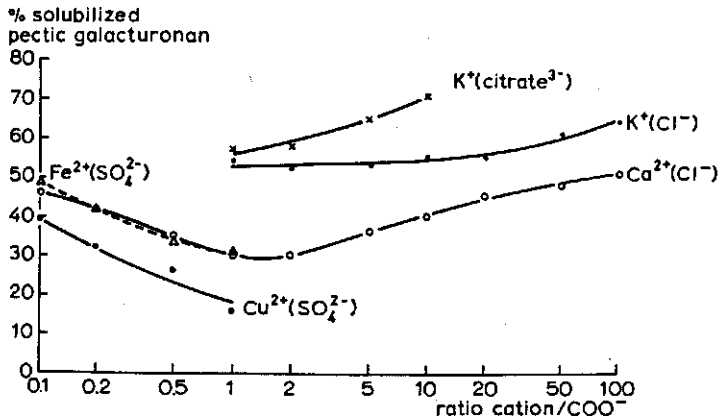

Fig. 22. Effect of cation neutralization of non-esterified carboxylic acid groups of pectic galacturonan on its solubilization during boiling of potato cell walls.

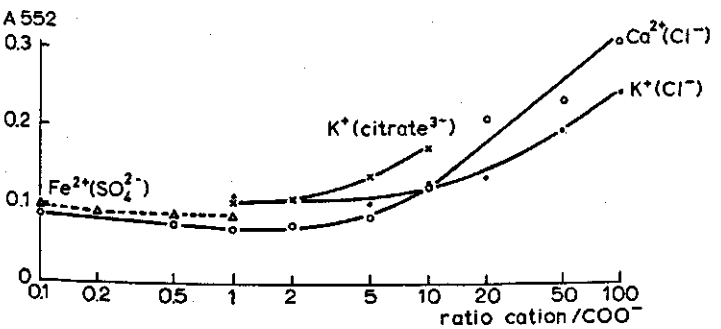

Fig. 23. Periodate-TBA absorbances during boiling of potato cell walls as in Fig. 22.

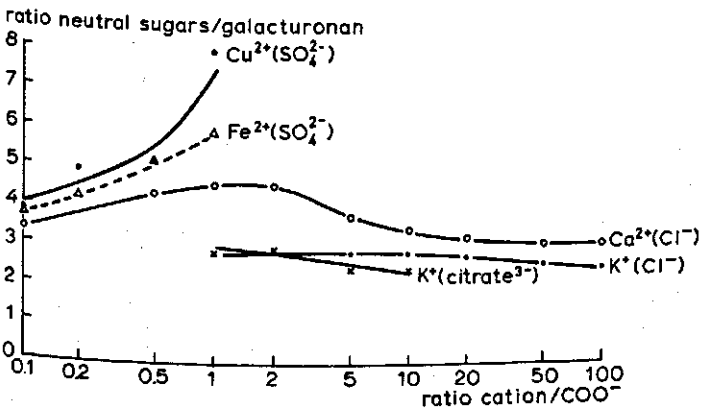

Fig. 24. Ratio neutral sugars (calculated for galactose) to galacturonan solubilized during boiling of potato cell walls as in Fig. 22.

soluble galacturonan during boiling of potato cell wall (Fig. 22) at a ratio Ca^{2+}/COO^{-} = 1-2. At least all pectic galacturonan carboxylic acid groups must be neutralized by calcium ions to obtain insolubility during boiling. This finding is another argument against the 'calcium-bridge' theory (Joslyn, 1962) and in favour of the 'microcrystallite' hypothesis for gel behaviour (Rees, 1969, 1972a, b), because optimum stability of the pectin structure inside the cell wall and middle lamella complex of potato requires more than a few calcium galacturonan carboxylate bonds or cross-links.

Mg Magnesium ions are often thought to aid in the insolubilization of native pectic substances ('protopectin') by polyvalent ion bonding (Joslyn, 1962; Doesburg, 1965). Their function might be similar to that of calcium and other polyvalent cations (iron, copper). However, magnesium ions exerted no insolubilizing influence during boiling on the pectic galacturonan of potato cell wall compared with potassium ions (Table 10), although they seemed to favour β-elimination. The latter result is derived from relative periodate-TBA numbers, which increased for magnesium from 0.33 to 0.56 and only from 0.41 to 0.46 for potassium when ratio cation/COO^{-} increased from 1 to 10. Stimulation of β-elimination of pectic galacturonan confirmed previous results of

Table 10. Influence of magnesium on the solubilization of pectic galacturonan from potato cell wall during boiling with some anions.

Ratio		Anion[1]	Additional Cl^{-}/COO^{-}	Solubilized galacturonan (%)	Relative A_{552}
Mg^{2+}/COO^{-}	K^{+}/Mg^{2+}[2]				
1		Cl^{-}		54	0.33
10		Cl^{-}		52	0.56
	1	Cl^{-}		55	0.34
	10	Cl^{-}		58	0.36
	1	$citrate^{3-}$	1.0	59	0.36
	10	$citrate^{3-}$	2.4	71	0.41
	1	$malate^{2-}$	1.0	57	0.39
	10	$malate^{2-}$	1.5	66	0.45
	1	$phytate^{12-}$	1.4	58	0.36
	10	$phytate^{12-}$	4.8	65	0.42

1. Equiv. anion as Cl^{-} = equiv. Mg^{2+} when no K^{+} is present.
   Equiv. anion as Cl^{-} = equiv. K^{+} + Mg^{2+} when K^{+} is present.
   Equiv. anion as organic anion = equiv. K^{+}.
2. Equiv. Mg^{2+} = equiv. COO^{-}.

Keijbets & Pilnik (1974b). The lack of a magnesium insolubilizing effect during boiling is in agreement with findings of Molloy & Richards (1971b) that magnesium was not bound significantly to a grass pectin, consisting of 87% galacturonan which was 12% esterified (calculated from methoxyl content). Haug & Smidsrød (1970) established a higher selectivity towards calcium than magnesium for pectate. This selectivity has been directed towards preferential bonding of the larger ions and confirms the view of Joslyn (1962) that the smaller ion radius of magnesium compared to calcium is responsible for decreased affinity.

Solubilization of neutral sugars Neutral sugars estimated as galactose, one of the predominant potato pectic sugars (Hoff & Castro, 1969) came into solution upon boiling of potato cell wall simultaneously with galacturonan. Unless solubilization of pectic galacturonan was retarded by Ca^{2+}, Fe^{2+} or Cu^{2+} ions, the ratio (solubilized) neutral sugars/galacturonan ranged from 2-3 (Figs 22, 24). When galacturonan was solubilized more slowly, solubilization of neutral sugars was not retarded to the same extent if at all (fig. 24). This result suggests that a part of the neutral sugar complex is no inherent part of pectic substances. However, the potato pectin prepared by boiling at pH 6.1 for 4 hours from the cell wall preparation used here (Section 4.2.5) and making up 46% of that cell wall, could not be separated into subfractions on DEAE-cellulose columns according to Knee (1970a). Therefore, I think that all neutral sugars released on boiling are substantial parts of potato cell wall pectic substances or are linked to these by interconnections as emerge from the cell wall structural investigations of Keegstra et al. (1973). Barrett & Northcote (1965) were able to separate a β-eliminatively degraded apple pectinic acid into two fractions: one rich in and the other poor in neutral sugars. The former, according to Aspinall (1970a), is possibly derived from rhamnose-rich regions in the rhamnogalacturonan main chain. This is in agreement with the recent suggestion of Talmadge et al. (1973) that neutral sugar side chains will be attached to rhamnose of the main chain. During the boiling of potato cell wall, the divalent cations probably insolubilize galacturonan-rich parts of the pectic main chain, while rhamnose-rich regions bearing neutral sugar side chains because of β-eliminative chain cleavage, are less retarded and go into solution.

5.3.2 Anions

The experiments to investigate some anion effects on solubilization of cell wall galacturonan, were designed in such a way that as many calcium ions were present to account theoretically for the non-esterified pectic galacturonan carboxylate ions. Potassium salts of chloride, citrate, phytate and malate were added in one to 10-fold excess (ratio anion/COO^- = 1-10). The results are summarized in Figs 25 and 26. Chloride ions, like potassium ions (Fig. 22), were unable to solubilize pectic galacturonan markedly (Fig. 25). According to Kohn & Furda (1967), a growing excess of potassium ions over calcium decreased the stability of calcium pectinate, possibly by ion exchange. I found that removal of 50% of calcium ions (decrease of ratio Ca^{2+}/COO^- from 1 to 0.5) resulted in only a small increase in solubilization of pectic galacturonan from 30 to 35% (Fig. 22), but the level of 35% was not reached at 10-fold chloride (and potassium) excess (Fig. 25).

Organic anions, on the other hand, effectively enhanced solubilization of pectic galacturonan (Fig. 25), probably because of the calcium-binding ability of these anions. Organic acids, especially α-hydroxy carboxylic acids, chelate metal cations (Martell & Calvin, 1962) and phytic acid precipitates calcium ions at a pH above 5.4 (Møllgaard, 1946; Kaufman & Kleinberg, 1971).

However, at a 10-fold excess of citrate ions (+ K^+) over calcium, the level of galacturonan solubilization surpassed the limit of 50-55% that was expected at removal of all calcium ions from their binding sites to pectic substances. The solubilization of galacturonan increased linearly for citrate and malate, suggesting a further increase at increasing ratio anion/COO^-. So it could be supposed that the organic anions, besides chelating calcium, facilitated β-eliminative degradation of pectic galacturonan as found for pectin solutions by Keijbets & Pilnik (1974b) where citrate was most effective. I confirmed the role of citrate ions as β-elimination stimulator in an experiment without calcium ions. The chelating action could not interfere here. Citrate ions enhanced solubilization of pectic galacturonan from 50 to 71% at an increase of ratio anion/COO^- from 0 to 10 (Fig. 22). Concurrently a fairly large increase in periodate-TBA absorbance was observed (Fig. 23) whereas the relative periodate-TBA number increased from 0.37 to 0.49 (cf. to 0.41-0.46 for chloride). The patterns of periodate-TBA absorbances for the organic anions ressembled those of pectic galacturonan solubilization strongly (Fig. 26 cf. Fig. 25). These results gave no additional indication of the influence of anions on β-elimination!

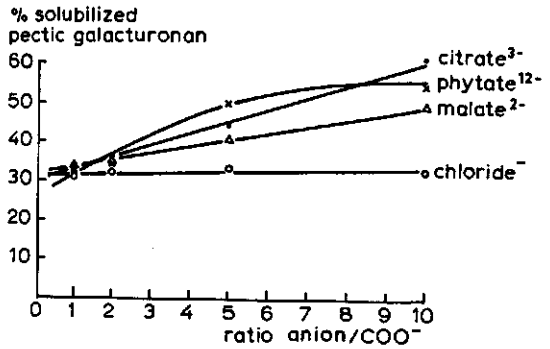

Fig. 25. Influence of anions on solubilization of pectic galacturonan at complete neutralization of non-esterified carboxylic acid groups by calcium during boiling of potato cell walls (equiv. anion = equiv. K^+; equiv. COO^- = equiv. Ca^{2+}

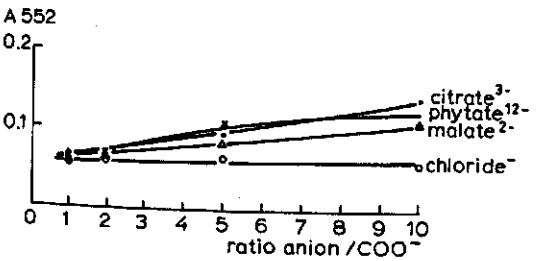

Fig. 26. Periodate-TBA absorbances during boiling of potato cell walls as in Fig. 25.

The pattern of galacturonan solubilization by phytate seems somewhat anomalous (Fig. 25). At ratio anion/COO^- = 5 its solubilizing ability was higher than for citrate but at ratio = 10 the positions were reversed. Beyond ratio = 5 the solubilizing ability of phytate did not increase as for citrate and malate. This behaviour might be caused by the changing ratio of calcium to phytate and the increasing amount of chloride ions necessary to maintain pH (Section 5.2.6). These ions are possibly disadvantageous to calcium precipitation or binding (Mattson, 1946; Møllgaard, 1946; Crean & Haisman, 1963; Kaufman & Kleinberg, 1971). According to Crean & Haisman (1963), the composition of insoluble salts of phytic acid changes between the penta-calcium and the pentamagnesium phytate. Tricalcium phytate is already soluble (Mattson, 1946). At a ratio phytate/COO^- = 5 while equiv. Ca^{2+} = equiv. COO^- no insoluble calcium phytate will be precipitated. However, the solubility of calcium (and magnesium) phytates decreases with increase in temperature (Mattson, 1946; Crean & Haisman, 1963).

The calcium-binding capacities of organic acids versus pectic galacturonan have been studied in the past. Deuel et al. (1957) established that the calcium selectivity of a cross-linked pectic acid ion-exchanger is

decreased strongly by citrate. Citrate ions were shown to be able to dissolve calcium pectinate precipitates. Oxalate anions dissolved Ca pectinate gels (Speiser et al., 1947) and caused swelling of dried pectin films containing calcium (Doesburg, 1957, 1961). Molloy & Richards (1971a, b) studied the binding of calcium and magnesium ions by cell wall fractions and organic acids of a grass species by an ion-exchange equilibrium procedure. Citric and malic acid were very active in complexing both alkaline earth metals. A shift of pH to the alkaline region (5 to 7) increased the complex formation for citrate in particular, but not for oxalic acid. The cell wall pectic fraction (87% galacturonan, which was 12% esterified) was less active in calcium binding than citric and malic acid, but equally effective compared with malonic and oxalic acid. When pectic galacturonan is esterified to a greater extent than in the potato cell wall used (DE = 58%), the selectivity for calcium ions in the calcium-potassium ion-exchange will decrease (Kohn & Furda, 1967). Compared with the organic anions, the binding activity of pectic galacturonan for calcium will be lowered then. The results of the cell wall boiling experiments, which are a resultant of complexing calcium and influencing β-elimination reaction rate, nevertheless show that organic anions exert calcium binding activity when applied at rather large excess. The effect of a decrease in esterification of pectic galacturonan will be discussed in Section 5.3.3.

In some experiments the influence of organic anions and chloride anion was investigated, while calcium was replaced by magnesium. The failure to be complexed by pectic galacturonan is clearly illustrated in Table 10. As found for potassium citrate in Fig. 22, citrate was most effective in solubilization of galacturonan, followed by malate and phytate. This difference must be partly ascribed to β-elimination (Table 10).

5.3.3 Decrease of degree of esterification

Kohn et al. (1968b) suggested that the bond strength of calcium ions with the carboxyl groups of pectic substances also depended on the distribution of non-esterified groups. The stability of calcium pectinate thus was greater for pectin which was partially deesterified by PE than for pectin of the same DE deesterified by alkali. Nothing was known, however, of the distribution of charged groups in the 58% esterified H cell wall. Upon deesterification of H cell wall the selectivity and the binding ability of pectic galacturonan for calcium ions, as found by Kohn & Furda (1967), indeed was seen to increase

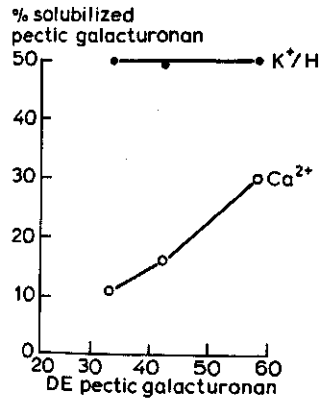

Fig. 27. Influence of partial saponification by orange PE and interaction with cations on solubilization of pectic galacturonan from potato cell walls during boiling (equiv. Ca^{2+} = equiv. K^+ = equiv. COO^- before saponification) (H: no cation addition).

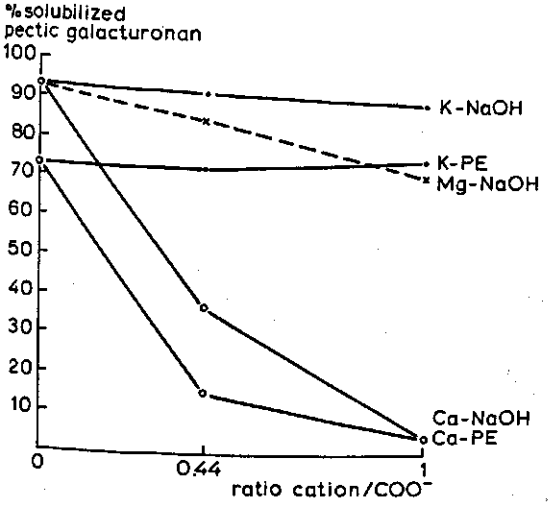

Fig. 28. Effect of partial and complete cation neutralization of non-esterified carboxylic acid groups of PE and alkali saponified potato cell walls on solubilization of pectic galacturonan during boiling (equiv. COO^-: non-esterified carboxylic acid groups after saponification).

(Figs 27, 28).

Partial saponification by orange PE decreased the quantity of pectic galacturonan dissolved by boiling in the presence of calcium, although the ratio Ca^{2+}/COO^- progressively decreased too (Fig. 27). Surprisingly, at complete deesterification by PE the level of solubilization was raised. This must be because of the successive treatments like second ethanolic HCl washing etc. which possibly result in pectin solubilization without boiling (before PE saponification already 25% was soluble in cold water). Partial and full neutralization of non-esterified carboxylate ions with calcium were again seen to insolubilize the pectic galacturonan (Fig. 28). It was known already that low-esterified pectin forms water-insoluble calcium precipitates (Deuel et al., 1950; Anyas-Weisz & Deuel, 1950). Pectic galacturonan will be depolymerized,

but as long as no very small oligomers arise, calcium containing precipitates remain insoluble. The limit DP for insolubility, however, is not known.

The same results were found when alkali saponified potato cell wall was used. The original level of solubilization then was almost 100%, due again to treatments and alkali itself in particular. The cell wall pectic acid (1.3% esterified) complexed magnesium ions to a small but definite extent, another proof of increased selectivity for alkaline earth metals at a low degree of esterification (Fig. 28).

It was expected that decrease of DE itself would decrease the rate of β-elimination (Vollmert, 1950b; Albersheim et al., 1960b). This was not confirmed, however, by my results because no decrease of solubilization of pectic galacturonan was observed (Fig. 27). Only upon addition of calcium ions was a decrease of solubilization noticed. The fact that only moderate increases in percentage of solubilized galacturonan at boiling were encountered upon addition of organic anions, if at all, again points to the increased selectivity towards calcium of deesterified cell wall pectic galacturonan (Table 11). Citrate ions, for instance, did not increase the solubility of pectic galacturonan at partial carboxylate neutralization (44%) and did increase for only 11% at complete neutralization (100%) by calcium (Table 11). However, 58% esterified potato cell wall lost 30% of galacturonan at 100% calcium-neutralization and another 30% over the same range of

Table 11. Influence of organic anions on solubilization of pectic galacturonan from PE saponified potato cell wall during boiling.

Ratio Ca^{2+}/COO^-	Ratio K^+/Ca^{2+}	Anion[1]	Additional Cl^-/COO^-	Solubilized galacturonan (%)
0.44	0	Cl^-		15
0.44	5	$citrate^{3-}$	0.8	12
0.44	10	$citrate^{3-}$	1.3	14
0.44	5	$phytate^{12-}$	1.4	18
0.44	10	$phytate^{12-}$	2.3	23
0.44	5	$malate^{2-}$	0.7	8
0.44	10	$malate^{2-}$	0.9	5
1	0	Cl^-		4
1	5	$citrate^{3-}$	1.9	4
1	10	$citrate^{3-}$	2.4	15
1	5	$phytate^{12-}$	2.9	4
1	10	$phytate^{12-}$	4.7	12

1. Equiv. anion as Cl^- = equiv. Ca^{2+}.
   Equiv. anion as organic anion = equiv. K^+.

citrate addition (ratio K^+/Ca^{2+} 0-10).

5.3.4 Starch

Potato starch is characterized by covalently bonded phosphate groups. Orthophosphoric acid is esterified to carbon 6 in some glucose residues of the amylopectin fraction mainly (Nowotny & Samotus, 1965; Richter et al., 1968). Two acid groups are ionizable and can bind cations. The first acid group is neutralized at pH 5-6, the second at 8.5-9 (Winkler, 1960). This means that potato starch is half-neutralized in normal conditions. According to Richter et al. (1968), calcium and magnesium ions form the bulk of potato starch phosphate bound cations, although potassium is certainly present. It is assumed that 25% calcium bonding occurs at most in native potato starch.

The quantities of starch added to potato cell wall were calculated on basis of rough proportions of pectic galacturonan and starch in low and high sp. gr. potatoes (1.060-1.070 and 1.100-1.110). H starch, containing no cations, did not change the known level of about 50% pectic galacturonan solubilization (Fig. 22) without calcium ions (Fig. 29). Addition of enough calcium to this system to neutralize all non-esterified carboxylic acid groups limited the solubilization to the previously described 30% level. The level of solubilization which was reached when adding Ca and BDH starch (52 and 24% of phosphate neutralized, respectively) was lower than 50%. Obviously calcium ions were transported from starch phosphate to carboxylic acid groups of galacturonan, by which they were stronger complexed. This is possibly

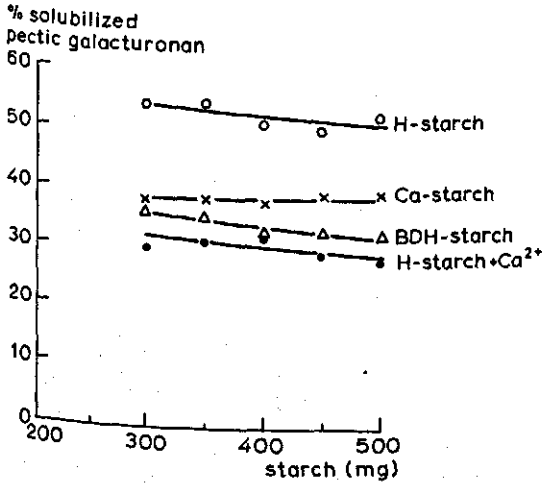

Fig. 29. Influence of starch on the solubilization of pectic galacturonan during boiling of potato cell walls (H starch + Ca^{2+}: equiv. Ca^{2+} = equiv. COO^-).

understandable because only 25.2 µeq P were present compared with 18.2 µeq of carboxylic acid groups (ratio P/COO^- = 1.4) in the system used. In spite of the higher calcium level in Ca starch than in BDH starch, more pectic galacturonan was solubilized in the presence of the former. This could be due to a difference in gelling behaviour. BDH starch formed a rather stiff gel on boiling but Ca (and H) starch remained more fluid.

5.3.5 *Buffer concentration and pH*

At increasing Tris-pipes pH 6.1 buffer concentration, the solubilization of pectic galacturonan was enhanced (Fig. 30). From 0.02 until 0.1 mol/l a sharp increase was noticed, but from there on a sudden fall in increase became evident. The use of a diluted buffer throughout the cell wall boiling experiments indeed minimized the influence of buffer concentration. The increased solubilization must be ascribed to increase of β-eliminative depolymerization, which shows the unspecific effect of ionic strength on this mechanism (Fig. 30).

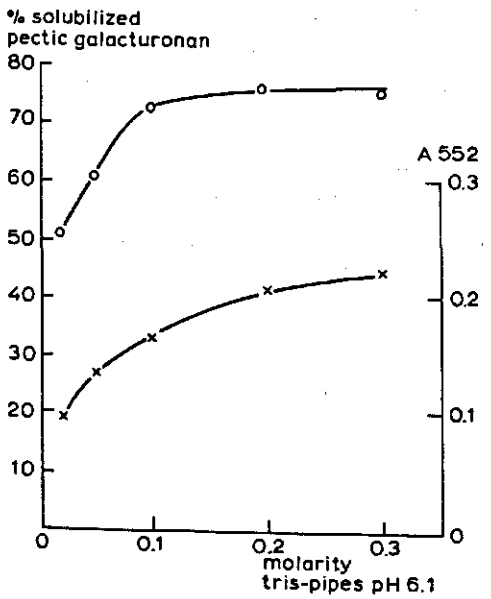

Fig. 30. Effect of buffer concentration on solubilization of pectic galacturonan (o) and periodate-TBA absorbances (x) during boiling of potato cell walls.

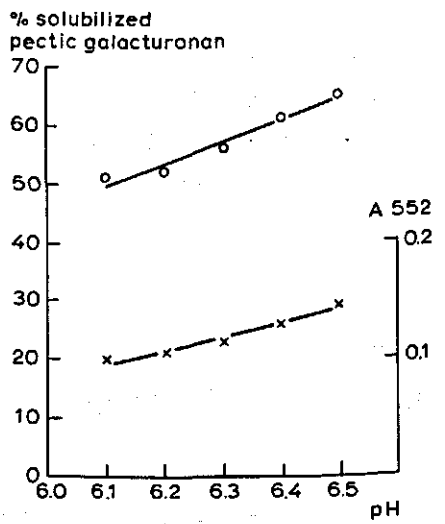

Fig. 31. Effect of buffer pH on solubilization of pectic galacturonan (o) and periodate-TBA absorbances (x) during boiling of potato cell walls.

It was known that β-elimination was increased with higher hydroxyl ion concentration, because these ions initiate the reaction (Fig. 18). Concurrently the solubilization of pectic galacturonan increased (Fig. 31).

5.3.6 Boiling of potato cell wall in mixtures simulating the composition of low (LS) and high (HS) sp. gr. potato tissue

Analyses, as presented in Section 7.3.3, revealed that a difference in pH existed between potatoes of low and high sp. gr. of a restricted population. This difference in pH deeply influenced the level of solubilized pectic galacturonan from potato cell wall as seen from Fig. 31. Without further additions in the boiling medium, 0.3 pH unit caused a difference of about 10-12% solubilization of galacturonan (Fig. 31). Addition of potato ions led to a much greater difference of 24% (= 68-44) (Table 12) to 28% (= 71-43) - 31% (= 58-27) (Table 13). When H cell wall was boiled at pH 5.89 for both the LS and HS samples, the difference of 24% was reduced to 7% (Table 12). It is conceivable that here the pH had a rather strong influence.

The LS and HS cell wall isolates contained calcium ions which accounted for 42 and 33% of non-esterified carboxylic acid groups of pectic galacturonan. Boiling in 0.02 M Tris-pipes pH 6.1 without extra ions resulted in

Table 12. Solubilization of pectic galacturonan in a low (LS) and high (HS) sp. gr. potato model during boiling: H cell wall.

Sp. gr.	pH	Solubilized galacturonan (%)	Relative A_{552}
LS	5.89	44	0.55
HS	6.19	68	0.76
HS	5.89	51	0.60

Table 13. Solubilization of pectic galacturonan in a low (LS) and high (HS) sp. gr. potato model during boiling: LS and HS cell walls.

Sp. gr.	pH	Additional Ca	Solubilized galacturonan (%)	Relative A_{552}
LS	5.89	+	27	1.01
HS	6.19	+	58	0.85
LS	5.89	-	43	0.96
HS	6.19	-	71	0.74
HS	5.89	-	58	0.70

solubilization of 22 and 27% galacturonan for LS and HS, respectively (Section 7.3.5). Boiling with or without additional calcium ions in the boiling medium raised the level of solubilization and increased the difference partly due to the pH difference (Table 13). Even without this pH difference, a rather important difference in solubilization remained. Remarkably, in Table 13 the relative periodate-TBA numbers were lower the more pectic galacturonan was solubilized. This effect probably was a result of the dependence of periodate-TBA ε on the degree of polymerization, indicating a lower starting DP for the HS sample than for the LS one (see also Section 7.3.5).

5.4 CONCLUSIONS AND SUMMARY

Isolated potato cell walls, washed free of ions, were boiled at pH 6.1 in Tris-pipes buffer with anions, cations and starch. Boiling time, degree of esterification of pectic galacturonan, pH and buffer concentration were varied. It was shown by specific periodate-thiobarbituric acid staining that pectic galacturonan was degraded β-eliminatively.

Calcium ions and divalent iron and copper as well, slowed down the solubilization of pectic galacturonan at boiling. Copper ions exerted a strong affinity for pectic galacturonan in particular. Evidence was obtained that calcium ions, when present in excess proportional to non-esterified pectic galacturonan carboxylate ions (ratio $Ca^{2+}/COO^{-} > 2$) no longer retarded solubilization, but on the contrary enhanced it. It could be demonstrated that increased β-eliminative breakdown of galacturonan is responsible. Potassium ions at the same time did not retard galacturonan solubilization, but facilitated it by the same mechanism. Increasing amounts of buffer ions also enhanced the solubilization of galacturonan, convincingly indicating the unspecific nature of this effect. It must be emphasized, however, that the calcium ions had a pronounced galacturonan insolubilizing influence, even when galacturonan was progressively degraded.

Optimum insolubility during boiling was encountered at Ca^{2+}/COO^{-} ratio 1-2. This requirement of complete neutralization of non-esterified carboxylic acid groups of galacturonan is an argument in favour of the existence of microcrystalline junction zones in cell wall and middle lamella gels, whose strength partly depends on the degree of completeness of neutralization of mutual repulsive forces in the pectic structure.

Citrate, phytate and malate anions when present in increasing amounts, progressively solubilized pectic galacturonan, completely neutralized by

calcium, from potato cell wall. Citrate was most effective. It appeared that both withdrawal of calcium ions and acceleration of β-elimination contributed.

Decrease of esterification of pectic galacturonan shifted the calcium binding ability in the direction of pectic galacturonan compared with the organic anions. Magnesium ions made completely deesterified galacturonan slightly insoluble, but were far less effective than calcium ions. Magnesium was not able to stimulate galacturonan insolubility when esterified for 58%, as calcium did. Its role in insolubility of native plant tissue pectin ('protopectin') is rather doubtful.

Potato starch, containing bound phosphate groups, seems to be of minor importance in calcium binding compared with cell wall galacturonan when present in relative proportions as in potato tissue. Calcium ions were shown to be easily withdrawn from potato starch phosphate and to aid in insolubilization of pectic galacturonan.

The experiments carried out in this chapter indicate the importance of potato constituents, which are present inside the cell wall boundary and in the cytoplasm, during solubilization of pectic galacturonan from the cell wall and middle lamella. Some experiments, therefore, were conducted to simulate the composition of low and high sp. gr. tissue during boiling and to confirm some of the findings. The importance of pH and differences in concentrations of anions and cations, which may affect solubilization of pectic galacturonan and hence intercellular cohesion, were affirmed. Cell wall calcium ions seemed to be rather important again in fixing the pectin structure.

6 Influence of chemical constituents on intercellular cohesion of potato tissue in a model cooking study

6.1 INTRODUCTION

Numerous attempts have been made to establish relationships between intercellular cohesion of the cooked potato and chemical composition of the raw material (see Section 3.4). Use of inadequate methods of measurement of intercellular cohesion, problems in analysis of pectic substances and complexity of the processes during cooking, which determine the texture appearance after cooking, have probably prevented the establishment of simple relationships between chemical factors and texture parameters. For this reason I carried out model experiments to obtain information about the influence of chemical factors on intercellular cohesion. Addition and removal trials were the subject of investigations by Personius & Sharp (1939a), Linehan & Hughes (1969c) and the group of Zaehringer and Le Tourneau (Davis & Le Tourneau, 1967; Zaehringer & Cunningham, 1971; Davis et al., 1973 etc.). During these investigations, however, solubilization of pectic substances was not accounted for.

The model cooking study, described below, was carried out with dead, leached potato tissue of the two extreme sp. gr. fractions also used for maceration studies. Upon addition of ions and change of degree of esterification of pectic galacturonan, cell separation as an objective measure of change in intercellular cohesion was studied together with solubilization and degradation of pectic substances.

6.2 MATERIALS AND METHODS

6.2.1 General

For source and preparation of potato tissue, modification of tissue disks, the turbidity cell separation test and chemical analyses see Section 4.2. Potassium and calcium analyses of tissue disks are described in Section 7.2.2. Periodate-TBA reaction proceeded starting with 1 ml of sample

(Section 5.2.8). The results of the turbidity cell separation (TCS) test expressed in absorbances at 475 nm were converted into percentages of cell separation with a standard curve. These standard curves were established anew for cooked tissue cells. The number of broken tissue disks were counted as well.

6.2.2 Boiling procedure

Potato tissue disks were taken from storage in 70% ethanol at 4°C and prepared for maceration (see Section 4.2.7). Twenty disks were boiled in 20 ml 0.02 M Tris-pipes pH 6.5 buffer in a conical flask of 100 ml. Calcium and potassium ions (chloride as anion) were added in a small volume of less than 1 ml, but always to ethanolic hydrochloric acid washed tissue disks, apart from one experiment with calcium ions and 1972 tissue. Citrate ions were included in an experiment with 1972 tissue (potassium as cation) with untreated tissue disks too. In this citrate series, citrate ions were replaced by chloride at the highest level applied. Boiling proceeded in duplicate with refluxing for the time chosen on a hot plate. After boiling, the conical flasks were transferred to the Köttermann waterbath-shaker for 15 min at 300 rev/min. In this way loosened cells were separated from the tissue disks as in the maceration experiments. Turbidity was then assessed.

The boiled tissues were prepared for chemical analyses by homogenization in the Bühler-apparatus. The solids were centrifuged off at 16 000 *g* (Sorvall RC-B, swing-out rotor). The supernatant was decanted and made up to 50 ml. Solubilized starch was removed in a two-step procedure. At first the supernatant was liberated from starch partly by freezing-thawing (Section 5.2.8) and the other part was removed enzymically (described in Section 4.2.9). The starch-free boiling extract was analysed for pectic galacturonan and neutral sugars (Section 4.2.9). Determinations of unsaturated uronosyl residues and of methanol for DE of galacturonan were carried out after only partial starch removal by freezing-thawing (Sections 4.2.10 and 4.2.13). The periodate-TBA analysis proceeded with 1 ml sample (Section 5.2.8). For percentage cell separation a mean of 22% was found with s = 2.6%, while a mean of 13 broken disks was counted with s = 2.0. The mean percentage solubilized pectic galacturonan was 54% with s = 2.0%. The mean relative periodate-TBA number was 0.19, s = 0.020.

6.3 RESULTS AND DISCUSSION

6.3.1 Some characteristics of the tissue (Ca, K, TCS test)

The tissue used in the model cooking study differs considerably from living, untreated potato tuber material. The native pectinesterase and other endogeneous enzymes are killed (Section 4.2.2) leaving a leached, dead tissue. The plasmalemma will be freely permeable for many potato constituents, but starch granules are not gelatinized and the cell wall and middle lamella structure is presumed to be essentially unchanged. The ethanol inactivation treatment promoted the leaching of ions in particular as demonstrated for the 1972 tissue in Table 14. Additional potassium and calcium ions were lost upon ethanolic HCl washings (tissue from 1972 and 1971). This treatment is supposed to remove calcium and other ions from the cell wall and middle lamella. The removal of calcium ions by the washings is facilitated more than that of potassium ions. Davis (1964) and Davis et al. (1973) established that compared with potassium and total solids, calcium ions were retarded on leaching in aqueous medium.

The ethanol-soluble ions in the tissue might contribute to reduction of intercellular cohesion during cooking. Cunningham et al. (1967) found that an ethanolic extract of potato tissue indeed reduced intercellular cohesion. The effect of ethanolic washing is conveniently shown as follows. Two hours

Table 14. Removal of potassium and calcium from potato tissue disks by washings with ethanol and ethanolic HCl. Loss calculated relative to ethanol-washed (1971) or fresh (1972) disks; in parentheses loss calculated relative to ethanol-washed disks (1972).

Sp. gr.	Year	Treatment	mg/100 disks		% Loss	
			K	Ca	K	Ca
1.060-1.070	1971	ethanol	12.26	0.48		
		eth.-HCl	0.18	0.14	99	72
1.100-1.110	1971	ethanol	17.13	0.31		
		eth.-HCl	0.93	0.16	95	45
1.060-1.070	1972	fresh	33.95	0.94		
		ethanol	9.92	0.42	71	55
		eth.-HCl	2.09	0.20	94(79)	78(52)
1.100-1.110	1972	fresh	37.10	0.55		
		ethanol	15.69	0.42	58	24
		eth.-HCl	1.21	0.17	97(92)	68(58)

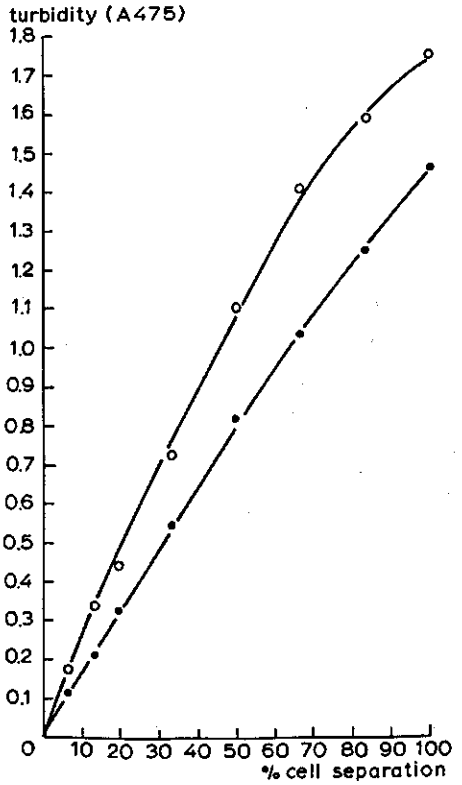

Fig. 32. Standard curves for the turbidity cell separation test of cooked potato tissue (1971) of sp. gr. 1.060-1.070 (•) and sp. gr. 1.100-1.110 (o).

of boiling of 1971 disks, were not sufficient to reach 50% cell separation for either of the sp. gr. fractions, whereas 50% of the living potato tissue of the same origin was separated into cells after 6-19 min (RWCS test).

Standard curves for turbidity measurement of cooked potato tissue are given for the 1971 tissue (Fig. 32). Compared to the standard curves for enzymic maceration (Fig. 5) the position of curves for the sp. gr. fractions is reversed. This may be because the higher the specific gravity or starch content the greater the uptake of water at starch swelling and gelatinization during cooking of these thin tissue disks.

The TCS test possessed one disadvantage for measurement of cell separation of cooked potato tissue. Because some gelatinized starch molecules were dissolved, boiling liquids became turbid before breakage and disintegration of tissue disks and release of cells. This turbidity was not really related to cell separation or reduction of intercellular cohesion. A correction in these cases was applied by subtraction of the lowest measured turbidity (A_{475}) of a sample showing no sign of disintegration (number of broken cells = 0-1)

within the experimental series from other absorbances of similar samples.

6.3.2 Mechanism of pectin degradation during boiling

The periodate-TBA test of the boiling liquid at first resulted in a brownish-red instead of the well-known violet-red colour. The absorbance spectrum clearly showed a large neutral sugar peak at 515-520 nm (Fig. 33) (see also Section 5.3.1, Fig. 21). After starch removal by freezing-thawing and DEAE-cellulose gradient elution according to Knee (1970a), the periodate--TBA spectrum was recorded again showing convincingly the reduction of the neutral sugar peak to a shoulder (Fig. 33). Attempts to demonstrate the unsaturated uronosyl bond in the ultraviolet (232-240 nm) were less successful, but here problems arose of interferences and of choice of a suitable blank to measure against.

The periodate-TBA absorbance spectrum of boiled potato tissue liquid (Fig. 33) strongly indicates that during cooking native pectic galacturonan was degraded along the line of the β-elimination. Van Buren (1970) pointed out that the extent to which β-elimination takes place in foods during processing is completely unexplored. This report is the first about β-elimination in heated plant tissue, shown by the periodate-TBA spectrum (Fig. 33). Some authors felt that β-elimination must play a role in degradation of pectin when plant tissues are heated in a pH range above 4-4.5. Both Doesburg (1961) and Goto et al. (1969) compared the behaviour of dried-pectin films or pectin solutions on boiling with that of plant tissues (firmness) and concluded without direct evidence that degradation of pectic substances in the plant tissues should be ascribed to β-elimination.

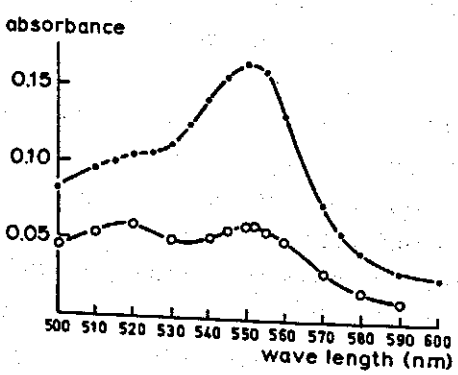

Fig. 33. Periodate-TBA absorbance spectra of cooked potato tissue (1971, sp. gr. 1.100-1.110) liquid, before (o) and after (•) starch removal.

Although in my experiments β-elimination was found during cooking in a buffer, one can readily assume that the same mechanism will function when living, unchanged potato tissue is cooked with its pH of 5.5-6.5 (Burton, 1966) (Section 7.3.3). The conclusion seems warranted that pectin degradation in the pH region above 4-4.5 during heating of solutions, tissues or even by vibration milling in the air-dry state (Dongowski & Bock, 1973) occurs by β-elimination.

6.3.3 Boiling of potato tissue disks without additions

Continued boiling of potato tissue of two sp. gr. fractions resulted in patterns which are similar for cell separation (Fig. 34) and almost identical for solubilization of pectic galacturonan (Fig. 35). After 60 min of boiling, maximum levels for both parameters were reached. A causal relationship between cell separation and solubilization of pectic galacturonan is suggested as for enzymic maceration (Section 4.3.3). It is evident as well that low sp. gr. (1.060-1.070) retained a larger intercellular cohesion of the cooked tissue than high sp. gr. (1.100-1.110) (Fig. 34). The contribution of cell size to differences in cell separation has been outlined before (Section 4.3.3).

Above pH 5.5 chemical β-elimination is generally accompanied by deesterification (Albersheim et al., 1960b; Doesburg & Grevers, 1960;

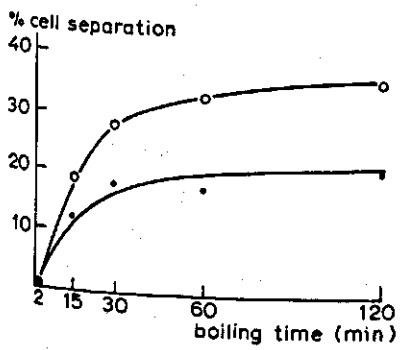

Fig. 34. Cell separation during cooking of potato tissue (1971). Curves marked as in Fig. 32.

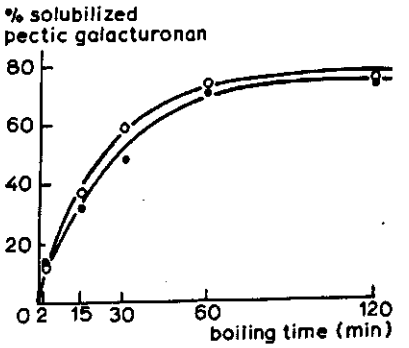

Fig. 35. Solubilization of pectic galacturonan during cooking of potato tissue (1971). Curves marked as in Fig. 32.

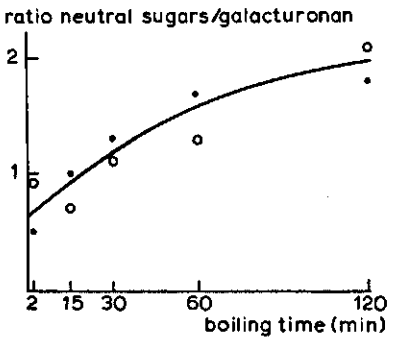

Fig. 36. Ratio neutral sugars (calculated for galactose) to galacturonan during cooking of potato tissue. Curve marked as in Fig. 32.

Slavickova, 1961; BeMiller & Kumari, 1972). I also found some deesterification at pH 6.5, but this was of minor importance after two hours of boiling. The lowest DE measured was 42%. During even shorter boiling periods deesterification should be neglected.

The ratio neutral sugars/galacturonan, both solubilized during boiling, increased with boiling time (Fig. 36). At first less neutral sugars (calculated for galactose) than pectic galacturonan were lost on homogenization of the cooked disks, but finally a surplus of neutral sugars was observed. Knee (1973) established that increasingly insoluble pectic fractions of apple tissues contained increasing proportions of neutral sugars. My finding is consistent with that of Knee.

6.3.4 Addition of cations

When potato tissue disks were washed with ethanolic HCl, nearly all residual potassium and calcium were removed (Table 14). The intercellular cohesion of cooked, ethanolic HCl treated disks was significantly decreased, although solubilization of pectic galacturonan was not essentially changed

Table 15. Effect of ethanolic HCl washing on cooking of potato tissue. Boiling time 30 min (1971 tissue).

Sp. gr.	Eth.-HCl	Cell Separation (%)	Solubilized galacturonan (%)
1.060-1.070	-	18	55
	+	33	61
1.100-1.110	-	28	59
	+	42	59

(Table 15). This effect must be due to removal of calcium from cell wall and middle lamella, because simple leaching out should increase intercellular cohesion.

To the ethanolic HCl pretreated tissue disks, cations (anion chloride) in increasing proportions (cation/COO^-) were added. Potassium ions did not significantly affect cell separation or solubilization of pectic galacturonan (Table 16), apart from a slow increase in both parameters with increasing ratio (% cell separation at K^+/COO^- 3.1 and 6.2, sp. gr. 1.100-1.110, were unexplainably low!). Breakdown of pectic galacturonan seemed fairly equal too and increased slowly as well (Table 16). The differences between the extreme sp. gr. fractions had been disappeared to a great extent. The relative periodate-TBA values remained at a low level, compared to enzymatic degradation in maceration of potato tissue (cf Table 7). The absence of an obvious potassium effect seems congruent with its role as established during boiling of the potato cell walls (Section 5.3.1).

Calcium ions largely inhibited cell separation in the low sp. gr. tissue, even in the presence of excess potassium ions, and decreased cell separation appreciably in the high sp. gr. tissue (Table 16). This intercellular cohesion promoting effect of calcium is very well-known (Section 3.4.3). The most remarkable finding, however, was that calcium ions reduced cell separation although solubilization of pectic galacturonan and degradation were scarcely affected. This contradictory result can be explained if it is kept in mind that solubilization was determined *after* tissue destruction by mechanical homogenization. The same problem was encountered in tissue maceration by pectin lyase in the calcium containing Tris-succinate buffer (Section 4.3.3). Both with or without calcium ions, less pectic galacturonan was measured as being soluble before than after homogenization of tissue disks (Table 17). When (almost) all tissue disks were broken during cooking (Table 17; sp. gr. 1.100-1.110) and when no calcium was added, the differences in solubilization were smallest. It is very remarkable that tissue coherence of sp. gr. 1.060-1.070 was maintained in the presence of calcium although almost 50% of pectic galacturonan was soluble without tissue destruction (Table 17). The tissue disks, however, were very fragile after cooking.

It was shown in the previous Chapter (Section 5.3.1) that calcium ions retarded the solubilization of pectic galacturonan from potato cell walls, but also stimulated β-eliminative depolymerization in accordance with Keijbets & Pilnik (1974b). During boiling of potato tissue with calcium ions, pectic galacturonan was strongly degraded so that it could be solubilized on

Table 16. Effect of cations on cooking of potato tissue (1971, ethanolic HCl washed tissue). Boiling time 30 min.

Sp. gr.	Ratio			Cell separation (%)	Number of broken disks	Solubilized galacturonan (%)	Relative A_{552}	Ratio neutral sugars/ galacturonan
	Ca^{2+}/COO^-	K^+/COO^-	K^+/Ca^{2+}[1]					
1.060-1.070	0			33	19	61	0.12	1.3
	0.6			2	1	68	0.12	1.4
	1.2			4	1	64	0.10	1.5
	3.1			4	2	71	0.12	1.7
	6.2			5	2	73	0.15	1.8
		0.6		36	20	59	0.14	1.8
		1.2		38	20	59	0.13	1.9
		3.1		37	19	61	0.14	1.7
		6.2		41	19	64	0.16	1.8
			1	1	1	59	0.12	1.8
			2	0	0	61		1.9
			5	4	2	61	0.17	2.0
			10	13	5	64	0.17	2.0
1.100-1.110	0			42	20	59	0.17	2.3
	0.6			22	17	55	0.13	1.8
	1.2			21	15	59	0.13	1.8
	3.1			24	16	66	0.15	2.1
	6.2			21	16	68	0.17	2.2
		0.6		40	20	56	0.15	2.4
		1.2		42	20	57	0.17	2.5
		3.1		32	20	58	0.16	2.6
		6.2		30	20	62	0.20	2.7
			1	19	12	51	0.14	2.0
			2	21	14	51	0.15	2.2
			5	24	17	54	0.17	2.2
			10	20	18	54	0.17	2.3

1. Equiv. Ca^{2+} = 0.6 equiv. COO^-

Table 17. Influence of mechanical homogenization (HO) of tissue on solubilization of pectic galacturonan from cooked potato tissue (1972). Boiling time 45 min. Ratio Ca^{2+}/COO^- = 0.5.

Sp. gr.	Ca^{2+}	Cell separation (%)	Number of broken disks	Solubilized galacturonan (%)		Ratio neutral sugars/ galacturonan	
				-HO	+HO	-HO	+HO
1.060-1.070	-	19	13	56	66	0.7	1.6
	+	0	0	49	65	0.7	1.7
1.100-1.110	-	34	20	73	77	1.0	1.3
	+	26	19	67	75	1.0	1.2

homogenization (Table 16). However, calcium ions aided in stabilizing the pectin gel structure inside the cell wall and middle lamella. So the pectin gel apparently retained its ability to function as cohesive material in the intercellular layer even when the pectic substances were depolymerized and solubilized partially.

In the cell wall boiling experiments of Chapter 5, addition of calcium and divalent iron and copper ions caused an increase of the ratio neutral sugars/galacturonan of the solubilized pectic substances, at least when the solubilization of pectic galacturonan was slowed down (Fig. 24). During boiling of potato tissue disks, on the contrary, a slight decrease of this ratio was observed when calcium was added (Table 16) compared with potassium, or this effect was even absent (Table 17). Determination of the ratio neutral sugars/galacturonan after tissue destruction also explains this result. Without tissue destruction by mechanical homogenization the ratio was seen to be lower than with homogenization (Table 17). The solubilization of neutral sugars then was retarded in accordance with the findings of Knee (1973).

6.3.5 *Addition of anions*

Only a few results are available. Citrate and chloride anions (potassium as cation) were added to tissue disks, which were not pretreated with

Table 18. Influence of anions on cooking of potato tissue (1972). Boiling time 45 min.

Sp. gr.	Ratio		Cell separation (%)	Number of broken disks	Solubilized galacturonan (%)	Relative A_{552}
	$citrate^{3-}/COO^-$	Cl^-/COO^-				
1.060-1.070	0		17	13	65	0.14
	0.3		16	8	68	0.16
	1.7		20	13	67	0.14
	3.4		11	11	69	0.15
	6.7		16	15	74	0.20
		6.7	14	9	66	0.16
1.100-1.110	0		27	20	68	0.18
	0.5		23	20	72	0.20
	2.5		33	20	77	0.24
	5.1		31	20	80	0.23
	10.1		26	20	85	0.25
		10.1	25	20	78	0.19

ethanolic HCl. Citrate ions did not change cell separation, but the results were rather variable (Table 18). It can be concluded that citrate addition in increasing amounts favoured solubilization of pectic galacturonan, and concurrently the rate of β-elimination. At ratio anion/COO^- 6.7 or 10.1, for low and high sp. gr. respectively, chloride ions were less active in solubilization and degradation of pectic galacturonan (Table 18). This result is in full agreement with the effect of citrate ions on depolymerization of pectic galacturonan as observed by Keijbets & Pilnik (1974b) for pectin solutions and in Section 5.3.2 for boiling of potato cell wall. One may speculate why no citrate effect on intercellular cohesion was present, especially for sp. gr. 1.060-1.070. Davis & Le Tourneau (1967) and Zaehringer & Cunningham (1971), on the other hand, established a pronounced citrate effect in reduction of intercellular cohesion of previously soaked potato tissue.

6.3.6 Esterified tissue

Esterification of potato tissue resulted in rather fragile tissue disks (Section 4.3.4). Nevertheless the level of cell separation was lower than with unmodified and ethanolic HCl pretreated tissue (Figs 37, 38). Whether the esterification conditions themselves affect the ease of cell separation is not clear. The solubilization of pectic galacturonan seemed facilitated as would be expected because of the highly esterified nature of galacturonan

Table 19. Cooking of highly esterified potato tissue with or without additional cations. Boiling time 30 min (1971).

Sp. gr.	Addition	Cell separation (%)	Number of broken disks	Solubilized galacturonan (%)	Relative A_{552}
1.060-1.070	unboiled blank[1]	2	0	15	0.16
	no	13	11	86	0.26
	K^{+2}	16	15	85	0.30
	Ca^{2+2}	15	14	82	0.29
1.100-1.110	unboiled blank[1]	3	0	16	
	no	23	20	87	0.32
	K^{+2}	28	20	92	0.28
	Ca^{2+2}	23	20	83	0.33

1. Averaged blank of no addition, Ca^{2+} and K^+.
2. Equiv. Ca^{2+} = equiv. K^+ = 0.6 equiv. COO^- before esterification.

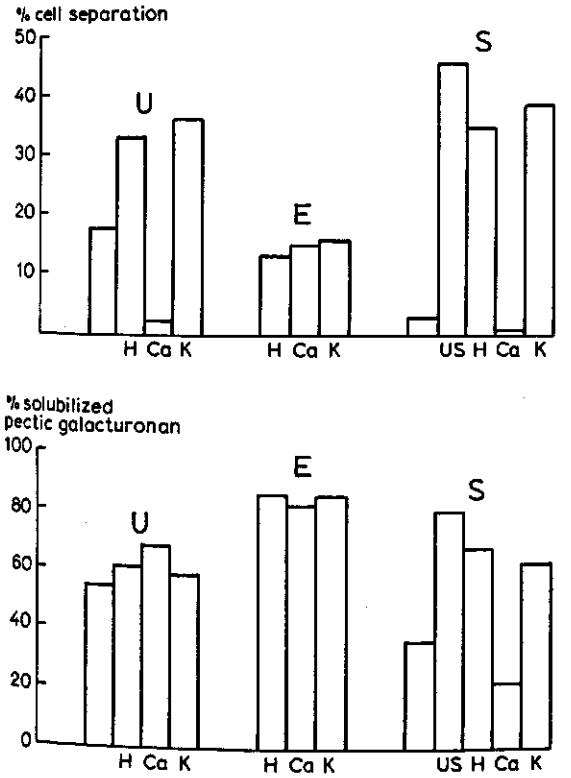

Fig. 37. Effect of degree of esterification of potato tissue galacturonan, removal of endogeneous ions and addition of cations on cell separation and solubilization of pectic galacturonan of cooked potato tissue (1971, sp. gr. 1.060-1.070). U = unmodified; E = esterified; S = saponified; H = ethanolic HCl washed; Ca, K = cations added to H disks; US = unsaponified, ethanolic HCl washed. Boiling time U and E = 30 min, S = 120 min.

(Section 4.2.3, over 90% DE) (Table 19) (Figs 37, 38) and the concurrent enhanced liability to β-elimination (Albersheim et al., 1960b). This is reflected in fairly high periodate-TBA numbers (Table 19) (compare with Table 16), although β-elimination due to diazomethane esterification treatment can not be excluded completely (see unbloiled blank sp. gr. 1.060-1.070,

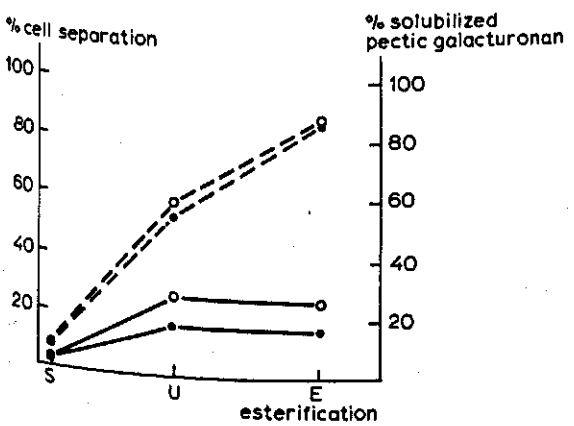

Fig. 38. Influence of degree of esterification of potato tissue galacturonan on cell separation (——) and solubilization of pectic galacturonan (---) of potato tissue (1971) cooked for 30 min. E, U, S see Fig. 37; curves marked as in Fig. 32.

Table 19).

Although a few carboxyl groups of pectic galacturonan will have remained non-esterified and calcium ions were removed prior to esterification, the cell separation level was distinctly higher for the high sp. gr. tissue. The influence of cell size emerges here again. Calcium ions lost their capacity to increase intercellular cohesion of the cooked potato tissue. The lack of enough non-esterified galacturonan carboxylate ions prohibited interaction with calcium ions, but, possibly due to covalent bonding of the pectin complex, the pectin cell wall gel in the esterified state (Rees, 1972b) retained its cementing ability.

6.3.7 Saponified tissue

Comparison of the results of continued boiling of saponified tissue (Table 20, Fig. 39) with the same of unsaponified tissue (Figs 34, 35) (blank Table 20) revealed that cell separation and solubilization of pectic galacturonan were reduced after saponification by orange PE. This effect of retarded solubilization could not be detected during the cell wall boiling experiments (Fig. 27; Section 5.3.3) unless calcium ions were added. However, when potato tissue is cooked, the calcium ions of the cell wall and middle lamella as well as the retarded reaction rate of β-elimination (Albersheim et al., 1960b) play a role in this reduction phenomenon. This is conveniently demonstrated by data in Tables 20 and 21 of tissue samples boiled for 120 min. Removal of calcium from cell wall and middle lamella by ethanolic HCl washing resulted in increased cell separation and galacturonan solubilization of saponified, cooked tissue. Once saponification of ethanolic HCl pretreated

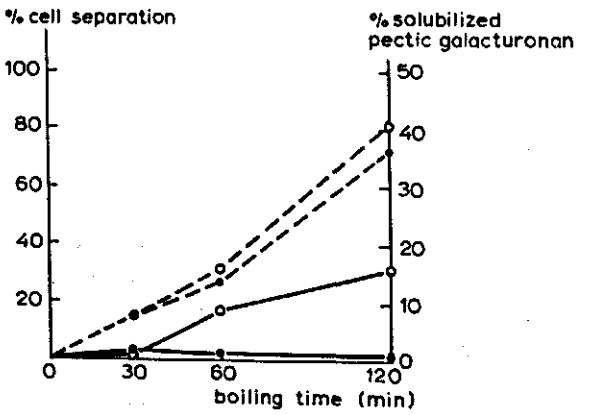

Fig. 39. Cell separation (——) and solubilization of pectic galacturonan (---) during cooking of saponified potato tissue (1971). Curves marked as in Fig. 32.

Table 20. Cooking of PE saponified potato tissue (1971) for different lengths of time.

Sp. gr.	Boiling time (min)	Cell separation (%)	Number of broken disks	Solubilized galacturonan (%)	Relative A_{552}	Ratio neutral sugars/ galacturonan
1.060-1.070	30	3	0	8		3.0
	60	2	0	14		2.0
	60(BL)[1]	26	13	73	0.18	1.6
	120	3	1	36		1.5
1.100-1.110	30	2	0	8		3.3
	60	18	4	16		1.8
	60(BL)[1]	40	20	64	0.23	2.0
	120	31	18	41		2.2

1. Unsaponified blank, incubated overnight at 30°C as saponified samples.

Table 21. Influence of cations on cooking of PE saponified potato tissue (1971; ethanolic HCl washed). Boiling time 120 min.

Sp. gr.	Addition	Cell separation (%)	Number of broken disks	Solubilized galacturonan (%)	Relative A_{552}	Ratio neutral sugars/ galacturonan
1.060-1.070	BL[1]	46	20	79	0.21	1.8
	no	35	20	67	0.08	1.5
	K^{+2}	39	20	62	0.12	1.6
	Ca^{2+2}	1	0	22	0.21	3.0
1.100-1.110	BL[1]	67	20	74	0.31	2.5
	no	45	20	66	0.15	1.8
	K^{+2}	51	20	54	0.20	2.3
	Ca^{2+2}	1	0	12	0.71	6.0

1. See note 1 of table 20.
2. Equiv. Ca^{2+} = equiv. K^{+} = 0.6 equiv. COO^{-} before saponification.

tissue was omitted, an additional increase in both parameters was found. Moreover, the relative periodate-TBA number was considerably increased (Table 21). It is evident that slowing down of the rate of β-elimination was responsible for the latter effect.

Re-addition of calcium ions to ethanolic HCl pretreated tissue, surpressed cell separation completely (Table 21) in tissues of both sp. gr. contrary to the situation without saponification (Table 16). In agreement with the results

of cell wall boiling trials after saponification (Figs 27, 28) pectic galacturonan was insolubilized by calcium ions as is seen from Tables 20 and 21 and Fig. 37, although only about 30% of non-esterified carboxylic acid groups were neutralized by calcium after saponification. This can be explained by the findings of Anyas-Weisz & Deuel (1950) and Deuel et al. (1950) that galacturonan gives insoluble precipitates with calcium when the DE is under 50%. A high value of the ratios neutral sugars/galacturonan (6.0), especially in tissue of high sp. gr., interfered in periodate-TBA measurement, resulting in considerable increase in the relative A_{552} number (Table 21).

The influence of degree of esterification of pectic galacturonan on cell separation and solubilization of pectic galacturonan during cooking of potato tissue is summarized in Fig. 38. To a certain extent these results are comparable with those of attack of pectin lyase on the same potato tissue disks in McIlvaine buffer (Figs 16, 17), because both enzymic and chemical β-elimination occur next to an esterified galacturonan carboxyl group.

It is evident that enzymic saponification of potato tissue pectic galacturonan may prevent undesirable loss of intercellular cohesion during potato processing and cooking (Tables 20, 21; Figs 37, 38, 39). This will be discussed further in Chapter 8.

6.4 CONCLUSIONS AND SUMMARY

In this chapter a model cooking study of potato tissue of two extreme sp. gr. fractions is described. Dead, partially leached tissue was cooked in Tris-pipes buffer at pH 6.5. Boiling time, ions and degree of esterification of pectic galacturonan were the variables included. Apart from intercellular cohesion, solubilization and degradation of pectic galacturonan were estimated to study the cooking phenomenon.

By specific staining of unsaturated uronosyl bonds with periodate--thiobarbituric acid, particularly when dissolved neutral sugars (starch) were removed, it was established that degradation of pectic galacturonan during cooking in buffer at pH 6.5 proceeded according to the β-elimination mechanism. Thus for the first time, even if in a model study, direct proof was found for occurrence of β-elimination when plant tissue material is heated at a pH beyond 4-4.5.

As in enzymic maceration (Chapter 4) differences in cell size were thought to explain partly the higher degree of intercellular cohesion of the tissue of low specific gravity.

Removal of ions (calcium) from cell wall and middle lamella enhanced cell separation significantly. Potassium ions were rather ineffective but re-added calcium ions strikingly lowered the level of cell separation. It appeared that calcium ions keep potato tissue firm on cooking, although the cell wall and middle lamella galacturonan was degraded to such an extent that on mechanical homogenization progressive dissolution was accomplished. Without mechanical destruction of residual tissue coherence solubilization of pectic galacturonan was retarded. Calcium ions, externally applied or internally present, thus stabilized the pectic cell wall gel so that it kept its cohesive function in the interstitial layer of cell walls even when severely degraded.

During cooking of potato tissue disks, citrate ions did not affect cell separation when present in increasing amounts but solubilization and β-elimination of pectic galacturonan were raised to a higher level, especially when compared with chloride.

Esterification of potato tissue dissipated the pronounced calcium effect on promotion of intercellular cohesion, demonstrating convincingly the requirement of non-esterified carboxylate galacturonan anions to interact with calcium. Upon saponification two effects converged with respect to intercellular cohesion and solubilization of pectic galacturonan of the cooked tissue. The rate of β-eliminative breakdown of galacturonan was diminished because of removal of esterified carboxyl groups and the binding affinity of calcium for pectic galacturonan was increased. These combined effects led to both a large reduction of cell separation and solubilization of pectic galacturonan of PE saponified cooked potato tissue. Re-addition of calcium ions to previously ethanolic HCl washed, saponified potato tissue prevented tissue degradation even after prolonged boiling.

7 Relationship between intercellular cohesion of the cooked potato and chemical composition

7.1 INTRODUCTION

In Chapters 5 and 6 it has been established which chemical factors influence the solubilization of pectic galacturonan during model experiments on boiling of cell wall and tissue. A distinct relationship between solubilization of pectic galacturonan and loss of intercellular cohesion was found.

Within restricted populations of potato tubers, but also outside these, several correlations have been found between chemical constituents and intercellular cohesion (Section 3.4). The most obvious relationship, which outside restricted populations is often absent, is that between specific gravity (total solids, starch) and intercellular cohesion. This experimental result has been theoretically based on the starch swelling pressure theory, originally mentioned by Atwater (1895) and used by, for instance, Reeve (1954, 1967, 1970, 1972). When potato tissue is heated and cooked, starch granules gelatinize between 58 and 70°C (Reeve, 1954, 1967). Then the starch might swell and exert pressure on the cell walls rounding off the cells. Simultaneously pectic substances of the middle lamella are degraded leading to cell separation, so that intercellular cohesion is distinctly reduced. Indeed, a negative correlation between specific gravity and intercellular cohesion, measured by RWCS test, was found (Section 3.4.1).

During cooking and the previous warming-up phase in particular, the permeable properties of the plasmalemma change at approximately 60°C (Personius & Sharp, 1938b), allowing diffusion of cell solutes into the cell wall (Bartolome & Hoff, 1972). Anions and cations then can interfere in the solubilization of pectic galacturonan. The relationship of specific gravity or starch, which can be calculated from specific gravity (Von Scheele et al., 1937; Burton, 1966; Nissen, 1967), with intercellular cohesion, has a weak theoretical basis. It could be replaced by a more causal relationship with the complicated chemical composition of the potato tuber.

For that reason chemical composition of restricted (specific gravity fractions from one lot of tubers) and less restricted potato populations was

studied.

7.2 MATERIALS AND METHODS

7.2.1 The potato material

In addition to the specific gravity fractions graded from populations of 1971 and 1972 (Section 4.2.1), those of 1970 were used for two experiments after two months of storage at 6°C. Pectinesterase activity, intercellular cohesion and dry matter content were determined with fresh material, directly after brine flotation. Other analyses were carried out on freeze-dried potato powder from unpeeled tubers, for which purpose a representative portion was dried. After freeze-drying the potato material was ground in a hammer mill. Freeze-drying proceeded after storing at 6°C the 1971 sp. gr. fractions for 2.5 months, and after several storage periods at 6°C starting at brine flotation with the 1972 ones.

Of the 1970 potatoes, sp. gr. 1.060-1.070 and 1.100-1.110 tubers were cut in 1.2 mm thick slices (as for RWCS test). These slices were dissected along the vascular ring to obtain cortex and non-cortex, perimedulla plus pith, tissue. The intercellular cohesion of these tissues was determined in the RWCS test. Tubers of sp. gr. 1.080-1.090 and 1.100-1.110 of the 1970 stock were boiled in the RWCS test in citrate solutions, with hydrochloric acid and sodium hydroxide adjusted to pH 2.7 to 12.

Further potato material was obtained from a trial on lifting date. Tubers (variety Bintje) were lifted from June until September 1971. The potato tubers were prepared for analyses as described, but they were freeze--dried immediately after harvest.

In a field manuring trial (1972) there were eight treatments with fertilizers. Nitrogen, phosphorus and potassium fertilizers were applied as single and combined treatments on clay soil. Nitrogen (360 kg/ha) was given as sodium nitrate, phosphorus (400 kg/ha) as monocalcium phosphate and potassium (600 kg/ha) as potassium sulphate. After lifting the potatoes were graded for size. The 35-50 mm sized tubers were freeze-dried immediately after harvest and used for analyses. The levels of fertilizer application are very high and do not represent normal agricultural practice.

7.2.2 *Characterization of the material*

The intercellular cohesion of the cooked potato tissue was assayed with the RWCS test, an objective direct procedure, which measures cell separation after cooking (Section 3.3.3). The original method of Le Tourneau et al. (1962), standardized by Zaehringer et al. (1963b), was modified by replacement of the standard boiling time by a series of boiling times (Zaehringer et al., 1969; Ludwig, 1972). The retained weights, obtained in duplicate after boiling of 100 g of diced potato tissue (10×10×1.2 mm) under agitation, were plotted against the boiling time, resulting in a cooking curve. A wide range of intercellular cohesion can thus be covered. Intercellular cohesion is expressed as the time required to reach a pre-established level of cell separation and not as percentage of cell separation proper (as in the TCS test, Section 4.2.8). This pre-established level was 100 g of retained weight, which was shown to be highly significantly correlated with the 50 g level (= 50% cell separation) (Zaehringer et al., 1969). The 100 g level was chosen to save time.

The RWCS test was carried out essentially according to Zaehringer et al. (1969) and intercellular cohesion is presented as T_{100} in min.

From 36 duplicated determinations of retained weight a mean value of 99 g with s = 2.4 g was obtained.

Dry matter About 1.5 kg of potato tubers were chopped in a cutter. A portion of 500 g of tissue was spread on a metal plate and pre-dried at 70°C overnight. The material was finely ground then in a hammer mill and samples for finish-drying at 105°C were taken with a sample divider (Retsch, Ham, FRG). The dry matter content of freeze-dried potato powder was determined by drying at 105°C until constant weight. For the sp. gr. fractions 1971 dry matter was not determined but calculated from the sp. gr. converted into weight in water and tables presented by Nissen (1967).

Pectinesterase A representative sample of 100 g of diced potato, taken during preparation for RWCS test (from about 2.5-3 kg) was homogenized with 200 ml of 1 M NaCl (Ultra-turrax). The pH of the homogenate was adjusted to 8 and maintained for six hours at room temperature by continuous or periodical addition of dilute sodium hydroxide. The solubilized PE was obtained by suction on a Buchner funnel (Schleicher & Schüll, 589/1 or 604) and the filtrate made up to 500 ml after washing (Unilever, 1966).

Most plant PEs have an optimum pH of 7.5 in common. The activity of PE is often measured at 30°C (Kertesz, 1951; MacDonnel et al., 1945; Somogyi & Romani, 1964; Vas et al., 1967; Leuprecht & Schaller, 1968). Other optimum conditions for PE activity measurements were thoroughly investigated by Vas et al. (1967) and Leuprecht & Schaller (1968). My procedure was adapted from Vas et al. (1967). The carboxylic acid groups of the pectin substrate liberated by potato PE were continuously titrated at pH 7.5 with a Radiometer Titrator TTT 11b connected with a PHM 28 pH meter and an autoburette ABU 11; the alkali consumption was recorded with a Titrigraph SBR 2. In a cylindrical titration glass 50 g 1% (w/v) apple pectin, approximately 65% esterified (Obipektin), was kept at 30°C with the aid of a circulating waterbath, 5 ml 4% (w/v) NaCl were added and some anti-foam. To reduce uptake of CO_2, nitrogen was bubbled through. When the pH of the mixture was adjusted to about 6 with 0.5 N NaOH, 10 ml of the PE extract already equilibrated at 30°C were added. The titrator was started to raise the pH to the pre-set level of 7.5 and the consumption of 0.1 or 0.05 N NaOH was recorded for 30 min. The PE activity was calculated from the slope of the alkali consumption and expressed as nmol of carboxyl groups liberated per second per g fresh weight of potato tissue (nkat/g). The activity was recorded in triplicate.

The activity of orange PE (Section 4.2.4) was determined using 14 ml 4% NaCl and 1 ml enzyme extract.

The mean PE activity measured was 32.4 nkat/g with s = 0.45.

For all other analyses representative samples were taken with the sample divider from the freeze-dried potato powder. All analyses were done in duplicate apart from cell wall isolation, pH measurement and solubilization of pectic galacturonan from cell wall by boiling.

Cell wall and middle lamella complex Cell wall and middle lamella material was isolated quantitatively from 20 g of freeze-dried powder as in Section 5.2.1 starting with the washing procedure. During some isolations the starch, washed out, was collected and dried until air-dry at room temperature.

pH of potato tissue was measured by suspending 10 g potato powder in 100 ml of deionized water and stirring for 5 min. The pH was recorded.

Pectic galacturonan and DE

a. Potato tissue: the procedures of Keijbets & Pilnik (1974a) were essentially

followed. Pectic galacturonan was extracted by enzymic degradation with Ultrazym-100 (Dr. Schubert AG, Basel, Switzerland). The degraded solubilized pectic galacturonan was analysed as described in Section 4.2.9. Methanol, released by alkaline saponification of tissue, was estimated colorimetrically (Section 4.2.13) after distillation (Keijbets & Pilnik, 1974a). The mean content of pectic galacturonan was 0.35 g/100 g, s = 0.0064 g/100 g. Mean DE was 53% with s = 1.9%, while the non-esterified carboxyl groups (COO^-) had a mean value of 0.91 meq/100 g with s = 0.038 meq/100 g (Chapters 7 and 8).
b. Cell wall material: the Cu^{2+} ion-exchange procedure of Keijbets & Pilnik (1974a) was applied to 50 mg. The cell walls had a mean content of pectic galacturonan of 0.163 g/g dry weight, s = 0.0017 g/g. The mean DE was 55% with s = 0.3%
c. Soluble pectic galacturonan: 2 g potato powder were extracted four times with 50 ml of deionized water or 50 ml of 0.05 M EDTA - 0.1 M Na_2HPO_4 (pH 6.9) on a glass filter (G_3). The combined filtrates were made up to 500 ml. Pectic galacturonan was determined (Section 4.2.9) after proper dilution.

Starch was solubilized with DMSO-HCl from 100 mg of potato powder and estimated enzymically. The solubilized starch was hydrolysed by an amylo-glucosidase, and glucose was determined after phosphorylation with hexokinase in the presence of ATP. The NADP-dependent oxidation with glucose-6-phosphate dehydrogenase was measured at 366 nm (Bergmeijer et al., 1970; Boehringer, 1972).

A mean value of 14.3 g/100 g was measured, s = 0.20 g/100 g.

Citrate and malate were extracted from 1 g potato powder for 3 hours with 0.075 M trichloroacetic acid under moderate shaking (waterbath-shaker) at room temperature. The extraction was continued overnight at 4°C. The slurry was filtered over folded paper (Schleicher & Schüll, 595 or 604) and the volume of the filtrate adjusted to 100 ml (adopted from Hughes et al., 1962). For assay of the acids enzymic methods were used. Citrate was converted into oxalacetate and acetate by citrate lyase. The consumption of NADPH during reduction of oxalacetate and its decarboxylation product pyruvate in the presence of malate dehydrogenase and lactate dehydrogenase was measured at 366 nm (Möllering & Gruber, 1966; Boehringer, 1972).

Malate was oxidized in the presence of excess NADP to oxalacetate by malate dehydrogenase in alkaline medium containing hydrazine. The change in absorbance at 366 nm was measured (Hohorst, 1970; Boehringer, 1972).

The mean citrate content found was 4.99 meq/100 g, s = 0.072 meq/100 g. Malate had a mean value of 1.05 meq/100 g, s = 0.046 meq/100 g (Chapters 7 and 8).

N, P, K, Ca and Mg analyses in potato tissue were carried out at the Bedrijfslaboratorium voor Grond- en Gewasonderzoek (Laboratory for Soil and Crop Testing) (Oosterbeek, the Netherlands) with potato powder. Nitrogen was determined according to Kjeldahl with a selenium catalyst during destruction.

For phosphorus the material was wet-ashed in a mixture of concentrated sulphuric acid and nitric acid and assayed according to Murphy & Riley (1962).

The cations were determined after dry ashing at 440°C and extracting the ash with 2 M hydrochloric acid. Potassium and calcium were determined by flame photometry, magnesium by atomic absorption spectrophotometry. The temperature of dry ashing was fairly low, compared with the 550°C used by other authors (Krauss & Marschner, 1971; Bretzloff & McMenamin, 1971). This lower temperature, however, seems to facilitate the extraction of calcium.

For N a mean value of 0.306 g/100 g was found with s = 0.0031 g/100 g. P had a mean value of 1.47 meq/100 g, s = 0.042 meq/100 g. The mean K content of potato tissue was 4.15 meq/100 g with s = 0.052 meq/100 g. For Ca the standard deviation was very small and for Mg a mean of 1.27 meq/100 g, s = 0.030 meq/100 g was calculated (Chapters 7 and 8).

Calcium bound to cell wall pectic galacturonan The calcium ions of 50 mg material were exchanged for hydrogen ions with three changes of 5 ml of 0.5 M HCl on a glass filter (G_3). The extract was neutralized with 15 ml of 0.5 M sodium hydroxide and analysed with the method of Milligan & Lindstrom (1972) as mentioned in Section 5.2.4 (calcium in starch).

The mean value of ratio Ca^{2+}/COO^- calculated was 0.32 with s = 0.021.

Solubilization of pectic galacturonan during boiling was tested as follows: 50 mg of cell wall material was handled as described in Section 5.2.6. The boiling medium was 0.02 M Tris-pipes pH 6.1. The analyses applied are described in Section 5.2.8.

7.3 RESULTS AND DISCUSSION

7.3.1 Analysis of pectin in potato tissue

Some of the problems encountered in pectin analysis of potato tissue have been outlined by Keijbets & Pilnik (1974a). An extraction method of pectic substances with pectolytic enzymes as described originally by McCready & McComb (1952) was preferred; non-extraction methods give erroneous results when applied to potato tissue because of absorption of alkali with a titrymetric method (Gee et al., 1958; Jaswal, 1969; Warren & Woodman, 1973) and possible phosphate interference with an ion-exchange method (Raunhardt & Neukom, 1964; Keijbets & Pilnik, 1974a). Pectin contents of potato tubers reported in literature range from 0.2-1% on fresh weight (e.g. Kröner & Völksen, 1950) but even higher values have been found (Sweetman, 1936; Sharma et al., 1959). It is not clear whether these pectin contents were defined as pectic galacturonan or as pectic substances, including side chain material. This rather confusing situation may be partly caused by the use of the calcium pectate precipitation method of Carré & Haynes (1922) which determined not only covalently bound side chain material but also physically adsorbed non-pectic material (Doesburg, 1965).

The advantage of the non-extraction methods is the simultaneous assay of DE of pectic galacturonan, which is an important pectin characteristic (β-elimination; calcium binding). As it is impossible to use these methods methanol was determined after saponification and distillation.

With the combined pectolytic extraction/methanol analysis I found contents of pectic galacturonan on fresh weight of potato material ranging from 0.27-0.45% (Tables 22, 23, 26, 32; Fig. 44d) and DEs from 48-60%. Potter & McComb (1957) and Krause & Bock (1973) have found similar, low values for pectin content (calculated for anhydrogalacturonic acid) with an enzymic extraction method. From cell wall isolation and analysis by Emiliani & Retamar (1968), Hoff & Castro (1969) and Eipeson & Paulus (1973) similar results can also be obtained for pectin content of potato tissue. Only a few, correctly determined values for DE are known in literature. Hoff & Castro (1969) found a DE of 40% for cell wall pectin while Krause & Bock (1973) established a DE of 60% using methanol analysis.

7.3.2 Effect of pH on intercellular cohesion

Boiling of two sp. gr. fractions (1970) at a pH range of 2.7-12 resulted in distinct maxima of intercellular cohesion (as T_{100}) in the RWCS test at pH 4.7 (Fig. 40). Doesburg (1961) found a similar curve for several edible plant tissues, potato tissue included, though he measured intercellular cohesion with a hardness meter (see Section 3.3.2). Doesburg, moreover, found soluble pectin to be lowest when hardness was highest. Beyond pH 4.7 (Fig. 40) intercellular cohesion decreased sharply, emphasizing the role of β-eliminative degradation of pectic galacturonan (Albersheim, 1959; Doesburg & Grevers, 1960; Doesburg, 1965) (see also Section 6.3.2). At the acidic side of the T_{100} peak, detachment of the pectic complex from the intercellulosic cell wall network by degradation of side chains and calcium exchange for hydrogen ions possibly explains loss of intercellular cohesion (Doesburg, 1961; Goto et al., 1969).

Even in the pH range of potato tissue (5.5-6.5), the influence of hydrogen ion concentration on intercellular cohesion was very obvious (Fig. 40).

7.3.3 Chemical and other characteristics of several specific gravity fractions

The distribution of potato tubers of restricted populations (1971 and 1972) over specific gravity classes is presented in Fig. 41. Most tubers were found in the classes 1.080-1.090 and 1.090-1.100, although less preponderant for the 1972 distribution.

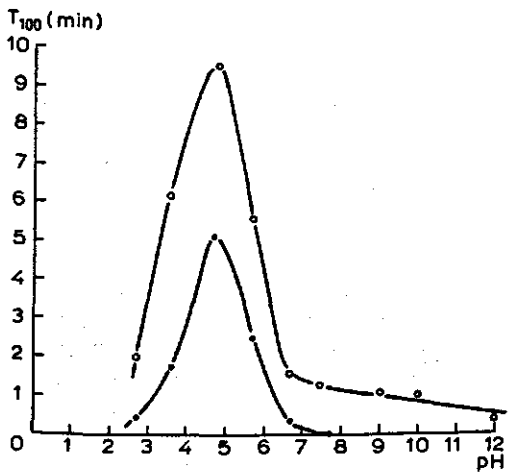

Fig. 40. Influence of pH of boiling medium on intercellular cohesion (T_{100}) of sp. gr. fractions 1.080-1.090 (o) and 1.100-1.110 (•) in the RWCS test (1970 tissue).

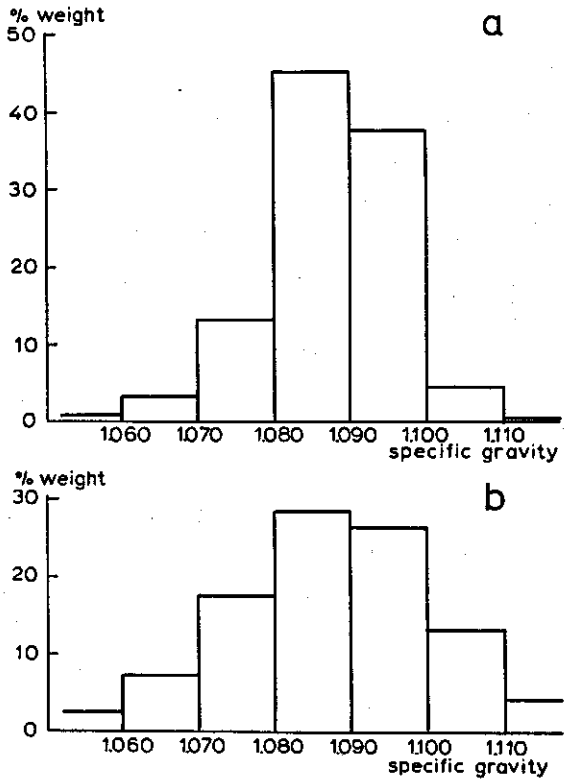

Fig. 41. Distribution of potato tubers from restricted populations over sp. gr. fractions (a = 1971; b = 1972).

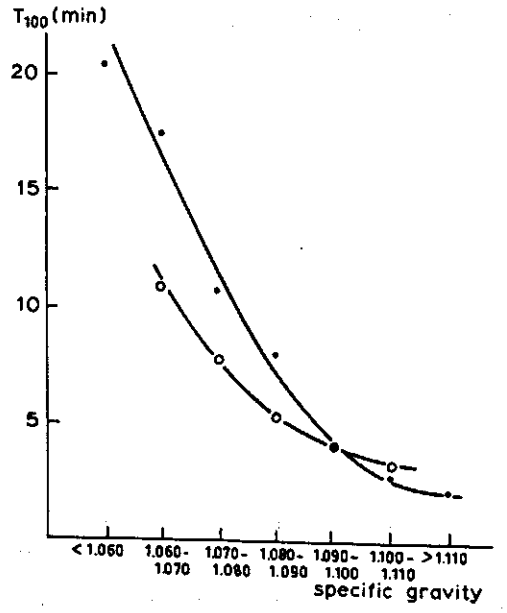

Fig. 42. Relation between specific gravity and intercellular cohesion (T_{100}) in the RWCS test in the 1971 (o) and 1972 (•) restricted populations.

The influence of specific gravity on intercellular cohesion of the cooked tissue (RWCS test) is shown in Fig. 42. The curves for two years differ, but the negative effect of increasing sp. gr. on texture confirms results obtained by Whittenberger & Nutting (1950), Le Tourneau et al. (1962), Zaehringer et al. (1963b) and Ludwig (1972).

Only those constituents were analysed that proved to influence solubilization of pectic galacturonan from cell wall material (Chapter 5) and cell separation of cooked tissue disks (Chapter 6). The results of this survey are collected in Tables 22 and 23, for the populations of 1971 and 1972 respectively. Not all analyses of the potatoes of 1971 were carried out after the same storage period, but it was shown in Section 7.3.4 that no essential changes in trends occurred even after prolonged storage at 6°C. For statistical analysis these results were pooled, and Kendall's correlation coefficient tests (Kendall, 1962) (parameter free) were carried out (Table 24). The sign of the trend coefficient Q indicates an ascending relationship between increasing sp. gr. and characteristic (+) or a descending one (-). The higher this coefficient (maximum possible here is 3.97), the better the trend is. The trend coefficients are graphically presented in Fig. 43.

In addition to starch, citrate, phosphorus, potassium and magnesium increased significantly with specific gravity; so did the pH value. Pectic galacturonan and nitrogen also increased with sp. gr., but the trend is not as obvious. The increase of degree of esterification is fairly weak, and as a result a trend of the non-esterified carboxyl groups of pectic galacturonan is absent (Table 24). Besides intercellular cohesion (T_{100}), the chemical constituents malate and calcium were the only ones to decrease to a certain extent, while the activity of the enzyme PE decreased with increasing specific gravity as well (Tables 22, 23, 24; Fig. 43).

Several authors described how dry matter and starch content increase during growth of the potato tuber (Nowotny & Samotus, 1965; Burton, 1966; Hughes & Evans, 1969; Münster, 1971; Grisson & Besson, 1973 and others) (see also Fig. 44a). When growth becomes stationary and the increase of starch content slows down, the tuber becomes mature. Analyses of chemical composition of specific gravity fractions showed that starch is not the only component that increases quantitatively during growth and maturation, if the sp. gr. tubers with more dry matter are considered to be more mature. The starch/dry matter ratio of Table 23, however, also showed that increase of starch with sp. gr. exceeded the increase of non-starch constituents.

Table 22. Physical, chemical and enzymic characteristics of specific gravity fractions from one restricted population (1971). All analyses calculated on fresh weight.

Characteristic	Unit	Specific gravity fraction				
		1.060-1.070	1.070-1.080	1.080-1.090	1.090-1.100	1.100-1.110
T_{100}[1]	min	10.8	7.7	5.2	4.2	3.3
pH		5.82	5.93	5.99	6.03	6.18
PE[1]	nkat/g	35.7	34.3	29.3	23.8	19.7
Starch	g/100 g	10.2	11.8	13.4	15.0	16.4
Galacturonan	g/100 g	0.37	0.37	0.38	0.41	0.40
DE	%	52	54	50	48	49
N	g/100 g	0.259	0.310	0.327	0.271	0.263
COO^-	meq/100 g	0.97	0.93	1.04	1.17	1.12
Citrate	meq/100 g	5.18	5.34	5.60	5.67	6.09
Malate	meq/100 g	1.13	1.01	1.19	0.82	0.66
P[2]	meq/100 g	1.43	1.65	1.91	1.70	1.89
K	meq/100 g	4.77	4.87	5.05	5.28	5.52
Ca	meq/100 g	0.97	0.72	0.80	0.77	0.79
Mg	meq/100 g	0.76	0.86	0.95	0.93	1.02

1. Determined directly after brine flotation; other analyses after 2.5 months of storage (Section 7.2.1).
2. P: equivalent weight = 15.5 because most P groups have 2 free acid groups.

An increase of starch content during tuber growth and maturation has been seen to be associated with changes in starch characteristics. Geddes et al. (1965) showed that granule size and amylose content increased, whilst the temperature of gelatinization of starch decreased. Due to the difference in retrogradation properties of the amylose and amylopectin fractions, a change in amylose/amylopectin ratio influenced the texture properties of precooked potato products such as granules and flakes (Potter, 1954; Reeve, 1954, 1967, 1972). The finding that potato tubers of high sp. gr. contained larger starch granules than tubers of low sp. gr. affirmed the maturity specific gravity relationship (Sharma & Thompson, 1955; Unrau & Nylund, 1957a; Barrios et al., 1963). The phosphorus content of starch from maturing tubers remained fairly constant (Geddes et al., 1965), but Samotus & Schwimmer (1962) established that increase of tuber phosphorus during growth was due to incorporation into starch in the early stage and into phytic acid later. The phosphorus content of potato starch seems to be a varietal characteristic (Samotus, 1965).

The increase of pectic galacturonan with sp. gr. as found here was rather unexpected, although Dastur & Agnihotri (1934) already found that the

Table 23. Physical, chemical and enzymic characteristics of specific gravity fractions from one restricted population (1972). All analyses calculated on fresh weight.

Characteristic	Unit	Specific gravity fraction						
		<1.060	1.060-1.070	1.070-1.080	1.080-1.090	1.090-1.100	1.100-1.110	>1.110
T_{100}	min	20.5	17.4	10.8	8.0	4.2	2.8	2.2
pH		5.78	5.85	5.85	5.92	6.04	6.10	6.21
PE	nkat/g	44.8	42.7	33.8	33.3	30.0	23.8	23.2
Dry matter	g/100 g	14.5	16.7	18.8	20.6	23.4	25.5	27.5
Starch	g/100 g	8.1	10.3	12.8	14.1	15.9	17.2	19.1
Starch/ dry matter	%	56.1	61.7	67.7	68.2	68.0	67.5	69.2
Galacturonan	g/100 g	0.38	0.36	0.35	0.36	0.38	0.38	0.42
DE	%	50	49	51	54	56	55	54
N	g/100 g	0.255	0.297	0.301	0.324	0.325	0.306	0.322
COO^-	meq/100 g	1.04	1.00	0.94	0.90	0.91	0.93	1.05
Citrate	meq/100 g	4.53	5.37	5.68	6.03	6.40	7.21	7.56
Malate	meq/100 g	1.49	1.40	0.94	0.81	0.82	0.98	0.92
P[1]	meq/100 g	1.22	1.36	1.38	1.39	1.61	1.87	1.98
K	meq/100 g	4.60	4.76	4.91	4.94	5.40	5.40	5.71
Ca	meq/100 g	1.21	1.04	0.94	0.78	0.77	0.85	0.84
Mg	meq/100 g	1.23	1.43	1.51	1.66	1.88	2.05	2.21

1. See note 2 of Table 22.

Table 24. Kendall's correlation coefficient tests of physical, chemical and enzymic characteristics of specific gravity fractions. Pooled data of Tables 22 and 23.

Characteristic	Trend coefficient Q	Probability P
T_{100}	- 3.97	<0.01
pH	+ 3.87	<0.01
PE	- 3.97	<0.01
Starch	+ 3.97	<0.01
Galacturonan	+ 2.15	0.03
DE	+ 0.77	0.44
N	+ 1.66	0.10
COO^-	+ 0.38	0.70
Citrate	+ 3.97	<0.01
Malate	- 1.92	0.05
P	+ 3.71	<0.01
K	+ 3.97	<0.01
Ca	- 1.92	0.05
Mg	+ 3.20	<0.01

total pectin content of potato tubers increased during growth. Bretzloff (1970) suggested that cell wall synthesis was retarded when starch synthesis was accelerated because of a common metabolic pool for carbohydrate synthesis. I did not find this for pectic galacturonan (Tables 22, 23, 24; Fig. 43). The clear-cut trend of PE, which might change DE, and the absence of a DE trend, rules out a causal relationship between these characteristics of potato tissue.

Increase of citrate, phosphorus, potassium and magnesium with increasing sp. gr. have been found also by Davis (1964) and Davis et al. (1973). Besides, these authors reported in a high sp. gr. fraction compared with a low sp. gr. fraction more ash, calcium and phytic acid phosphorus, but less nitrogen. The calcium and nitrogen trend coefficients, presented in Table 24 and Fig. 43, deviate from their results, but the calcium and nitrogen trends were fairly weak. Le Tourneau & Zaehringer (1965) also found an increase of potassium with sp. gr.. Ng et al. (1957) showed that high total solids of several varieties were related with high calcium. An increase of ash content with sp. gr. and total solids was seen by Le Tourneau (1963) as well. That nitrogen trends are probably rather inconsistent can be derived from the work of Fitzpatrick et al. (1964), who found that nitrogen was almost constant in specific gravity graded tubers. Further evidence for increasing citrate levels,

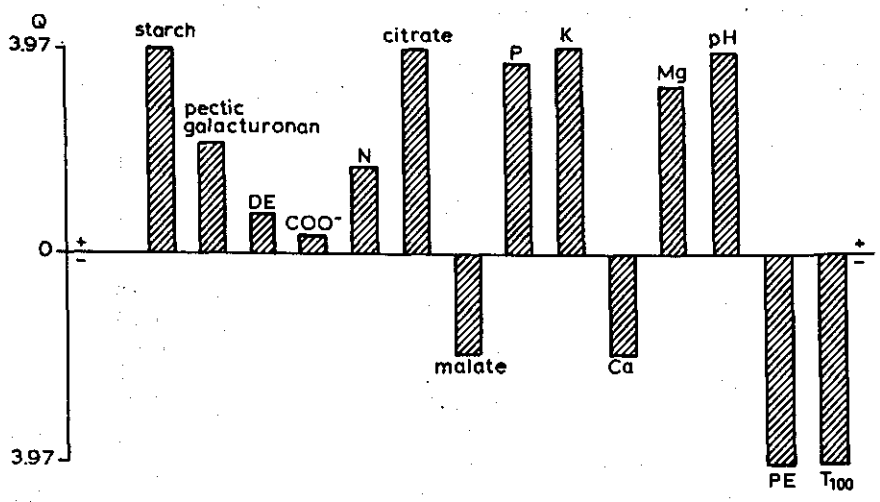

Fig. 43. Trend coefficients Q of physical, chemical and enzymic characteristics of specific gravity fractions. Data derived from Table 24.

when sp. gr. increased, was presented by Schwartz et al. (1961, 1966), but the trends in malate investigated by these workers were somewhat variable because decreases and increases were observed. I found opposite trends for citrate and malate (Tables 22, 23, 24; Fig. 43) and Schwartz et al. (1961) mentioned the possible interconvertability of these acids, which are closely related metabolically (citric acid cycle). The most predominant organic acid in potato tubers is citrate (Burton, 1966; Adler, 1971), followed by malate and oxalate. For that reason, citrate and malate were analysed, whereas determinations of other organic acids were omitted.

Some other potato characteristics also vary with maturity or with specific gravity. Schulze (1931) said that cell size and starch granule size increased when tubers matured. A significant positive correlation between sp. gr. and cell size was observed by Barrios et al. (1963), while Gray (1972) also measured increasing cell size at increasing sp. gr. within restricted populations. During growth of the potato tuber, increasing cell size and increasing cell wall thickness were noticed by Reeve et al. (1973a, 1973b, respectively). An increase of cell size during maturation of the potato tuber was found furthermore by Hughes & Faulks (1972). I measured only a few cell sizes of pith tissue (tissue disks, Section 4.3.3) and the results indeed suggested an increase of cell size with sp. gr.

That pH increased when sp. gr. increased is an important finding (Tables 22, 23, 24; Fig. 43), when the effect of pH on intercellular cohesion of the cooked tissue is considered (Fig. 40). This increase in pH occurred together with an increase of citrate and potassium, the ions which mostly make up the potato's buffer system.

The results mentioned hitherto strongly suggest that sp. gr. of potato tubers, derived from one restricted population, is a function of physiological age and ultimately maturity. The intercellular cohesion of the cooked potato tissue then also varies as a function of physiological age, but is not causally related to specific gravity (or starch!). When the potato tuber grows and becomes more mature, constituents like pectic galacturonan, citrate, phosphorus (phytin-P?), potassium and magnesium increase, while calcium and malate more or less decrease. Furthermore pH becomes higher and cell size certainly will increase. These changes altogether must result in enhanced solubilization of pectic galacturonan from the cell wall and middle lamella complex, if it is supposed that, due to changing permeability of plasmalemma during heating, all ions are able to react with pectic galacturonan. The neutralization of non-esterified pectic carboxylic acid groups by calcium seems affected

unfavourably as resultant of all trends of chemical constituents with sp. gr. β-Eliminative degradation of pectic galacturonan wil be enhanced by the same trends in the same direction. Thus a complex causal relationship between chemical composition and intercellular cohesion will replace the non-causal starch intercellular cohesion one. This relationship could only emerge because of the dependence of both chemical composition and starch on physiological age as an independent third variable (Woodman & Warren, 1972).

Within one tuber compositional gradients exist as mentioned in Table 1 from the outside to the centre and from stem to bud end. Starch and most non-starch substances are concentrated in the cortex tissue. Tissue outside the vascular region (= cortex) had less intercellular cohesion in the RWCS test than tissue inside the vascular region (Table 25) though the differences for sp. gr. 1.100-1.110 were not meaningful. This result agreed with those of Reeve (1954) and Burton (1966). Cunningham et al. (1967) showed that extracts of bud ends caused more cell separation than those of stem ends. Indeed in bud ends compared with stem ends, potato constituents apart from dry matter are relatively concentrated. It is known that stem ends of tubers are more readily prone to after-cooking blackening partly because of lower citrate content. These differences in intercellular cohesion in different tuber regions confirm that chemical constituents other than starch influence intercellular cohesion.

7.3.4 Influence of storage at 6°C

During storage several potato constituents and characteristics were analysed for the sp. gr. fractions of 1972. Only the results after six months are compiled in Table 26 and they should be compared with those of Table 23. The dry matter contents were assumed to be unchanged, an assumption which is

Table 25. Intercellular cohesion of different tissue zones (1970).
CT = cortex tissue; PMT = pith and perimedulla tissue; WT = whole tissue.

Sp. gr.	Tissue zone T_{100} (min)		
	CT	PMT	WT
1.060-1.070	8.2	12.2	11.0
1.100-1.110	3.5	3.8	3.4

Table 26. Physical, chemical and enzymic characteristics of specific gravity fractions from one restricted population (1972) after 6 months of storage at 6°C. All calculations based on fresh weight.

Characteristic	Unit	Specific gravity fraction						
		<1.060	1.060-1.070	1.070-1.080	1.080-1.090	1.090-1.100	1.100-1.110	>1.110
T_{100}	min		12.2	9.2	6.6	4.1	3.3	2.4
pH		6.07	6.10	6.05	6.21	6.33	6.50	6.52
PE	nkat/g	45.0	32.2	27.3	22.3	20.5	17.7	15.2
Starch	g/100 g	7.4	9.9	12.2	13.5	15.7	17.7	19.4
Galacturonan	g/100 g	0.39	0.36	0.37	0.38	0.41	0.45	0.45
DE	%	51	54	58	55	54	51	51
COO^-	meq/100 g	1.04	0.90	0.84	0.93	1.03	1.20	1.20
Citrate	meq/100 g	3.84	4.84	5.46	5.75	6.23	6.31	6.70
Malate	meq/100 g	2.15	1.69	1.40	1.01	1.21	1.04	0.88

not entirely true, because some water is usually lost upon storage. Changes in starch were of minor importance. The intercellular cohesion was reduced apart from the low, fairly constant values of the high sp. gr. fractions (sp. gr. $\geq$ 1.090). The trend in pectic galacturonan remained fairly equal upon storage. The contents of pectic galacturonan increased. We also determined water-soluble and EDTA-soluble pectic galacturonan for sp. gr. fractions 1.060-1.070 and 1.100-1.110. Only relatively small differences existed between water-soluble and EDTA-soluble pectic galacturonan contents of both sp. gr. fractions and during storage (Table 27). Slightly more pectic galacturonan was shown to be soluble in EDTA-phosphate than in water. It was remarkable that such a large part of pectic galacturonan became soluble even in water.

Citrate was lowered during storage and malate increased, but the trends were conserved (Table 26). From literature data it appeared that changes in

Table 27. Water and EDTA soluble pectic galacturonan of two sp. gr. fractions (1972) as percentage of pectic galacturonan content.

Sp. gr.	Storage period (months)	Soluble pectic galacturonan (%)	
		in water	in EDTA
1.060-1.070	0	22	27
	6	23	28
1.100-1.110	0	29	32
	6	28	36

these acids partly depend on temperature. Schwartz et al. (1961, 1966), using 5°C, found initial changes in organic acids, which were reversed during continued storage. At 10°C, Rumpf (1972) established a regular decrease in citrate and an increase in malate, confirming my results at 6°C and the probable interconvertability of both acids. These changes in organic acids were observed by Swiniarski (1973) as well. Potatoes, lifted in August, increased in citrate and decreased in malate, but the storage conditions are not known. Oxalate also decreased. During storage the changes in organic anions, some of which were measured here, resulted in averaged increases of pH of 0.3 unit (Table 26). It is assumed that the change of pH influenced the change of intercellular cohesion upon storage in particular. The activity of PE declined during storage.

7.3.5 Analysis of cell walls of sp. gr. fractions

The results of cell wall isolations and analyses are collected in Tables 28 and 29. Kendall's correlation coefficient tests again were done with pooled data (Table 30).

The most remarkable data of Tables 28, 29 and 30 certainly are the high losses of pectic galacturonan during cell wall isolation. Even a trend in loss of pectic galacturonan with sp. gr. is significant (Table 30). Because of this trend in loss of pectic galacturonan, it is likely that the significant trend in cell wall content with sp. gr. can be an artefact especially as the variations in cell wall content were small (Tables 28, 29). Williams (1963) also found an inverse relationship between total solids and cell wall material, but Eipeson & Paulus (1973) saw a small increase of cell wall content with increasing dry matter content for two varieties.

Although the loss of pectic galacturonan was partly caused by the disappearance of water-soluble substances as pointed out in Table 27, it was shown after completing this work, that very small fragments of cell wall slip through the cheese-cloth apertures during washing. These small fragments are released during the dry milling action upon the freeze-dried potato tissue. It could be shown too that only negligible quantities of cell wall material were lost when fresh tissue was used as starting material.

Contrary to the trend coefficient of pectic galacturonan in potato tissue, that for pectic galacturonan in cell wall material decreased with increasing sp. gr. with small variations between samples again. The neutralization by calcium of non-esterified carboxylic acid groups of pectic

Table 28. Content and some characteristics of cell wall isolated from specific gravity fractions from one restricted population (1971). fr. w. = fresh weight; dr. w. = dry weight.

Characteristic	Unit	Specific gravity fraction				
		1.060-1.070	1.070-1.080	1.080-1.090	1.090-1.100	1.100-1.110
Cell wall	g dr. w./100 g fr. w.	1.19	1.10	1.05	1.02	1.00
Galacturonan (cell wall)	g/g dr. w.	0.161	0.166	0.163	0.157	0.161
Galacturonan (tissue)[1]	g/100 g fr. w.	0.215	0.183	0.171	0.159	0.161
Loss galacturonan	%	42	51	55	61	60
DE	%	53	57	56	59	58
Ca^{2+}/COO^-		0.33	0.29	0.30	0.32	0.27
Solubilized galacturonan (boiling)	%	21.7	26.6	25.7	31.8	29.6
Relative A_{552} (boiling)		1.09	0.88	0.90	0.69	0.61

1. Calculated from cell wall and galacturonan (cell wall) contents.

Table 29. Content and some characteristics of cell wall isolated from specific gravity fractions from one restricted population (1972). fr. w. = fresh weight; dr. w. = dry weight.

Characteristic	Unit	Specific gravity fraction						
		<1.060	1.060-1.070	1.070-1.080	1.080-1.090	1.090-1.100	1.100-1.110	>1.110
Cell wall	g dr. w./100 g fr. w.	1.31	1.30	1.34	1.33	1.28	1.27	1.28
Galacturonan (cell wall)	g/g dr. w.	0.177	0.174	0.173	0.162	0.161	0.159	0.162
Galacturonan (tissue)[1]	g/100 g fr. w.	0.232	0.226	0.232	0.215	0.206	0.202	0.207
Loss galacturonan	%	39	37	34	40	46	47	51
DE	%	53	55	56	54	56	56	56
Ca^{2+}/COO^-		0.48	0.42	0.37	0.33	0.31	0.33	0.29
Solubilized galacturonan (boiling)	%	20.2	21.6	22.0	23.4	24.4	26.7	26.3
Relative A_{552} (boiling)		0.92	1.04	0.89	0.78	0.65	0.60	0.67

1. See note 1 of Table 28.

Table 30. Kendall's correlation coefficient tests of some characteristics of isolated cell walls from specific gravity fractions. Pooled data of Tables 28 and 29.

Characteristic	Trend coefficient Q	Probability P
Cell wall	- 2.58	<0.01
Galacturonan (cell wall)	- 2.73	<0.01
Loss galacturonan	+ 2.94	<0.01
DE	+ 2.35	0.02
Ca^{2+}/COO^-	- 2.94	<0.01
Solubilized galacturonan (boiling)	+ 3.20	<0.01
Relative A_{552} (boiling)	- 2.94	<0.01

galacturonan decreased when sp. gr. increased, favouring simultaneously the solubilization of pectic galacturonan during boiling of cell wall (Section 5.3.1) (Tables 28, 29, 30) and providing an argument once more for a faste loss of intercellular cohesion. The trend coefficient Q for the relative periodate-TBA number was almost as large as that for solubilized pectic galacturonan, but with opposite sign (Table 30). Because of the dependence of periodate-TBA ε on molecular weight (Section 4.2.10), it is suggested that the higher the sp. gr. the lower the DP of pectic galacturonan of the cell wall samples. This, in turn, could contribute, together with variations in calcium neutralization of non-esterified carboxyls, to the increasing water and EDTA solubility of pectic galacturonan in sp. gr. 1.100-1.110 compared with sp. gr. 1.060-1.070 (Table 27).

Similar results for cell wall content of potato tissue were reported by Emiliani & Retamar (1968), Hoff & Castro (1969) and Eipeson & Paulus (1973). These authors found higher contents of pectic galacturonan (25-28%) than those presented here (Tables 28 and 29). They probably omitted to correct for neutral sugar interference in carbazole-sulphuric acid reaction (Keijbets & Pilnik, 1974a) and this might have raised the pectic galacturonan level. The calcium contents of cell wall material of Eipeson & Paulus (1973) were higher than in my analyses as well.

A preliminary attempt was made to look for distribution of phosphorus and calcium over cell wall, starch and rest of tuber tissue. It was seen that phosphorus became progressively incorporated into starch in a high sp. gr. fraction (Table 31), due - of course - to synthesis of starch itself. The distribution of calcium was calculated, with the assumption that the ratio Ca^{2+}/COO^- (of pectic galacturonan) as found in Tables 28 and 29, could be

Table 31. Distribution of calcium and phosphorus over starch, cell wall and residual parts of potato tissue of two specific gravity fractions (1972). Calculations of P and Ca in meq per 100 g fr. w. and % (in parentheses).

Sp. gr.	P		Ca		
	starch	rest	cell wall	starch	rest
1.060-1.070	0.87 (63)	0.49 (37)	0.42 (40)	0.10 (10)	0.52 (50)
1.100-1.110	1.59 (85)	0.28 (15)	0.31 (36)	0.11 (13)	0.43 (51)

applied for all COO^- groups of pectic galacturonan as calculated in Tables 22 and 23. The neutralization by calcium of starch-phosphate groups was even much lower than that of cell wall carboxyls (up to 40%), namely 11% for sp. gr. 1.060-1.070 and 7% for sp. gr. 1.100-1.110. About 50% of tissue calcium were not bound to cell wall or starch. Bartolome & Hoff (1972), on the other hand, reported about 80% of tissue calcium to be localized in cell wall and starch, but the potato tubers I used contained 2-4 times as much calcium as those of Bartolome & Hoff.

7.3.6 Influence of lifting date

By lifting potato tubers from early June until the end of September, increasing levels of maturity were available. The results of analyses are shown in Figs 44a-j. The patterns of dry matter and starch (a), pH (c), nitrogen (g), citrate and potassium (h), phosphorus (i), calcium and magnesium (j) showed a striking qualitative similarity. No simple, ascending curves were observed. After an inital strong increase of starch and several non--starch constituents, a sudden drop occurred in the curves for the lifting period 16/7-30/7, probably due to a wet climatic period after a dry one. At the end of the growing season another drop appeared (27/8-10/9). Phosphorus (i), magnesium (j) and pH (c) remained constant in the period 16/7-30/7. The intercellular cohesion after cooking (b), PE (d), pectic galacturonan (e), its non-esterified carboxylic acid groups (f) and malate (i) followed quite a different pattern, which was approximately opposite to those first mentioned. The four latter characteristics, indeed, increased in the period 16/7-30/7, but the intercellular cohesion continued to decrease, although the non-starch constituents which affect solubilization of pectic galacturonan decreased as well. The explanation might be found in the strong decrease in calcium (decrease by over 50%) and absence of decrease of pH, combined with a large

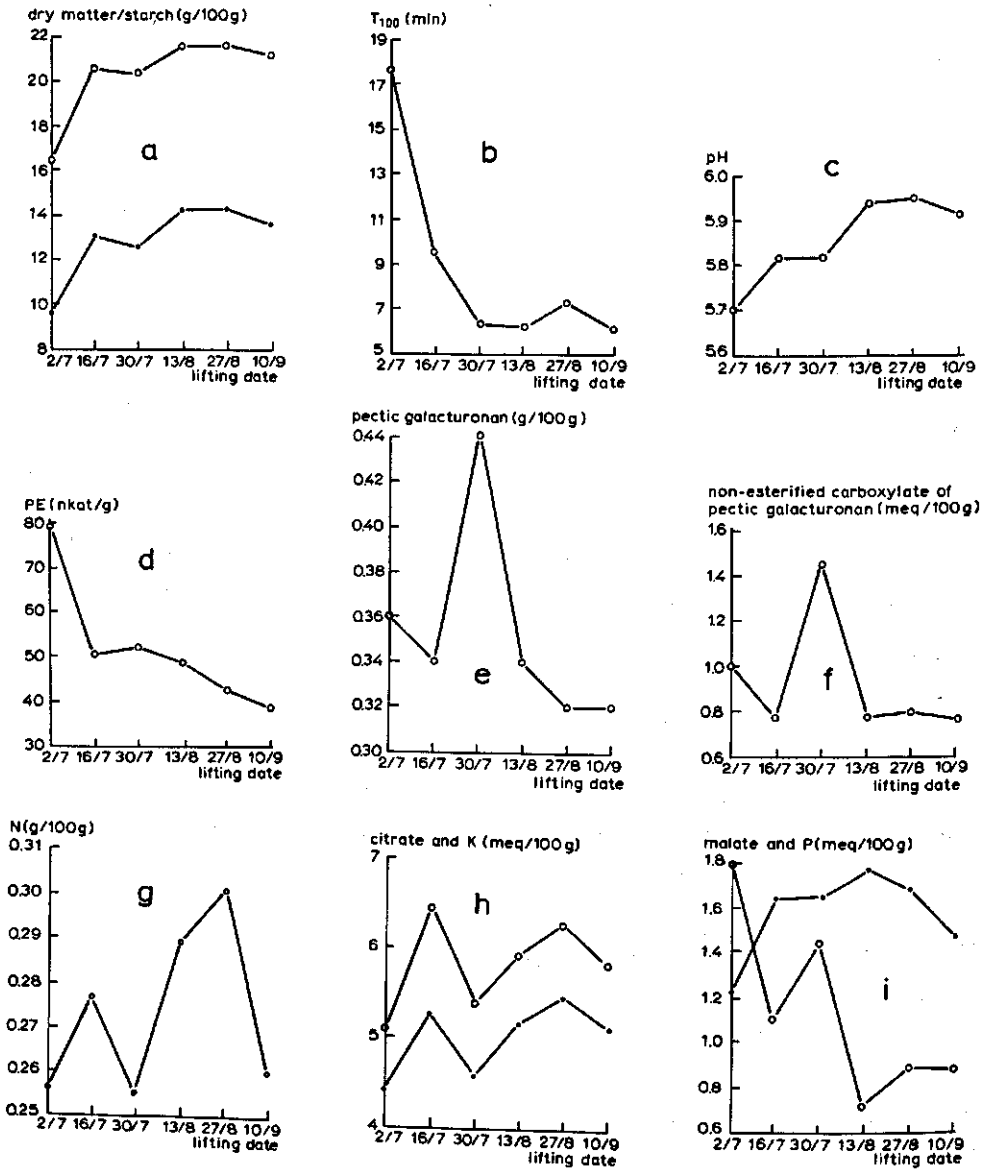

Fig. 44. Some characteristics of potato tubers, as determined at subsequent lifting dates.
a. Dry matter (o) and starch (•)
b. Intercellular cohesion as T_{100}
c. pH
d. PE activity
e. Pectic galacturonan
f. Non-esterified carboxylic acid groups of pectic galacturonan
g. Nitrogen
h. Citrate (o) and potassium (•)
i. Malate (o) and phosphorus (•)
j. Calcium (o) and magnesium (•)

increase of the non-esterified carboxyls of pectic galacturonan (f). The influence of cell size was not known, although generally cell size will increase during maturation of the potato tuber (Schulze, 1931; Hughes & Faulks, 1972; Reeve et al., 1973a).

The patterns of pectic galacturonan and its non-esterified carboxylic acid groups (Figs 44e, f) were rather surprising. At lifting date 30/7 the pectic galacturonan content of the potato tubers was very high, but it diminished at increasing maturity. The overall decrease of pectic galacturonan with increasing maturity seems to contradict the increasing trend found for sp. gr. fractions (Table 24), but this trend was rather weak. It is supposed that in the period 16/7-30/7 cell wall synthesis was accelerated in a rapid growth phase which then resulted in a temporary dilution of potato constituents. The activity of the cell wall bound PE (Jansen et al., 1960; Nakagawa et al., 1971) also was higher at 30/7. The large changes in non-esterified carboxyls are essentially due to changes in pectic galacturonan because the DE was fairly constant. Their relation to those of the calcium level might strongly affect the pectin gel in the cell wall and middle lamella.

The results obtained in this trial are thought to support the hypothesis of concurrent increase of several non-starch constituents of the potato tuber with starch as a function of physiological age or growth phase. Both calcium and nitrogen now followed the general pattern, although calcium content was lower at 10/9 than at 2/7 and nitrogen sharply decreased at the end of the growing period.

7.3.7 Influence of manuring on some chemical and other characteristics

The material used in these experiments was not especially grown for this study, but it was still interesting to look for influences of fertilizers on intercellular cohesion. The levels of manuring were extremely high. Although phosphate fertilization resulted in higher levels of phosphorus in the tuber, the effect of nitrogen fertilization was even more obvious. The nitrogen manured tubers contained more total nitrogen, while dry matter and starch were lower than in the samples without nitrogen fertilization (applied as sodium nitrate) (Table 32). These findings were in full agreement with earlier ones (Burton, 1966; MacLean et al., 1966; Smith, 1967).

Maturity effect According to Timm et al. (1963) the growing period of the

Table 32. Physical, chemical and enzymic characteristics of potato tubers derived from a NPK manuring trial. All calculations based on fresh weight.

Characteristic	Unit	Blank	P	K	PK	N	NP	NK	NPK
T_{100}	min	5.9	7.9	6.4	7.7	9.8	11.1	10.0	10.7
pH		5.93	6.02	5.95	6.00	5.92	5.93	5.96	5.91
PE	nkat/g	37.5	36.8	29.0	35.3	41.7	49.0	43.7	53.2
Dry matter	g/100 g	21.7	22.8	22.9	23.1	19.1	21.8	19.5	20.0
Starch	g/100 g	14.6	16.4	16.3	16.3	13.5	14.4	12.9	13.0
Starch/ dry matter	%	67.4	71.9	71.1	70.7	70.6	65.9	66.0	65.0
Galacturonan	g/100 g	0.33	0.30	0.31	0.30	0.29	0.30	0.28	0.27
DE	%	54	60	59	57	58	57	54	59
N	g/100 g	0.271	0.274	0.268	0.277	0.315	0.401	0.384	0.362
COO^-	meq/100 g	0.83	0.65	0.69	0.70	0.66	0.70	0.70	0.60
Citrate	meq/100 g	5.25	4.31	4.67	4.76	5.51	3.76	5.70	5.77
Malate	meq/100 g	0.85	0.75	0.69	0.69	1.06	0.79	0.88	1.03
P^1	meq/100 g	1.13	1.38	1.09	1.37	0.99	1.54	0.95	1.54
K	meq/100 g	3.28	3.71	3.65	3.64	3.17	3.10	3.32	4.02
Ca	meq/100 g	0.85	0.87	0.80	0.88	0.79	0.82	0.66	0.79

1. See note 2 of Table 22.

nitrogen fertilized tubers will be extended and tuber maturation delayed. Cells are smaller (Reeve et al., 1971) as a result of nitrogen application, confirming the existence of delayed maturity (Reeve et al., 1973a) (nitrogen was applied as ammonium sulphate). The activity of PE was favourably affected by nitrogen application. Pectic galacturonan was lower in the nitrogen treatments, as were dry matter and starch.

The maturity retarding influence of nitrogen manuring was reflected in enhanced intercellular cohesion of the N samples (Table 32). Similar results were found by Ludwig (1972). Schippers (1961), who measured cell cohesion subjectively, suggested the same effect of nitrogen fertilization as well as Zuk & Gupalo (1970) and Zuk (1970), whose method of assessment of intercellular cohesion was not given.

Citrate Zuk (1970) and Zuk & Gupalo (1970) found a decrease of citrate when the level of nitrogen fertilization (ammonium nitrate) was raised, which would causally affect intercellular cohesion. In my experiments, on the contrary, the tubers fertilized with nitrogen contained more citrate and malate than those without nitrogen except the NP sample (Table 32), in particular when potassium was applied as well. There was no decrease of intercellular cohesion as expected when citrate (and malate) levels increased.

However, the manuring samples do not represent one restricted population. The effect of nitrogen fertilization on maturity, and presumably cell size which was not measured, might dominate the effect of citrate on intercellular cohesion. Nitrogen application delayed tuber maturity, but probably favoured the tuber metabolism enhancing synthesis of enzyme proteins (PE, Table 32) and of organic acids. The fertilizer treatments resulted in several restricted populations, which is conveniently illustrated by the absence of opposite patterns of citrate and malate.

Fertilization with phosphate, in the absence and presence of nitrogen, suppressed citrate metabolism, which was related to a high level of intercellular cohesion for the P and NP samples in their N- and N+ classes (Table 32). The same results were found in a duplicate manuring trial on a more sandy soil type (results not presented) for all influences mentioned up to now. Zuk & Gupalo (1970) already established such a phosphate suppression of citrate and cell separation. These authors, working with variable NPK-ratios, found a good negative relationship between citrate content of potato tubers and intercellular cohesion, although they did not mention this.

Several authors reported effects of fertilizers on citrate content of potato tubers. Chloride replacement of sulphate (potassium as cation) led to a decrease of citrate (Swain et al., 1963; Vertregt, 1968; Zuk & Gupalo, 1970), which might aid in retaining intercellular cohesion at cooking. The reason evidently is that chloride takes the place of citrate anions in the ionic balance. The availability of potassium by soil and fertilizer also affects the amount of citrate synthesized, because the citrate ions apparently counteract potassium in the ionic balance. Several authors thus found positive correlations between citrate and potassium (Hughes & Evans, 1967, 1969; Zuk & Gupalo, 1970; Swiniarski, 1973). Ammonium ions and urea (sources of nitrogen) reduced the uptake of potassium and depressed the amount of citrate synthesized (Swain et al., 1963; Hughes & Evans, 1963), possibly explaining the decreasing effect of ammonium nitrate on citrate level as found by Zuk (1970) and Zuk & Gupalo (1970). Cultivation methods and a proper choice of fertilizers, could influence intercellular cohesion, but an adverse effect of after-cooking blackening should be avoided when citrate content becomes low (Hughes & Evans, 1967, 1969).

The level of citrate appears to be a varietal characteristic (Hughes & Evans, 1967; Rumpf, 1972; as well as other reports, viz. Zaehringer et al., 1969; Zuk, 1970). Schwartz et al. (1968), though recognizing the importance of variety, stressed the influence of location, but Hughes & Evans (1967)

showed that varietal differences (soil and climate) were preserved on different locations.

Cell wall composition From three fertilizer treatments, P, N and NP, cell wall material was isolated and analysed (Table 33). Compared to the data of Tables 28 and 29, the cell wall contents here were fairly low and the losses of pectic galacturonan even higher. The DE of cell wall galacturonan also was somewhat lower than that of whole tissue (Table 32), but Cu^{2+} ion exchange and combined pectolytic extraction/methanol determination gave essentially the same results. The cell wall analyses did not demonstrate significant differences in any characteristic apart from the relative periodate-TBA numbers, which suggest that P > NP > N possessed higher polymerized pectic galacturonan molecules.

Table 33. Content and some characteristics of cell wall isolated from three samples of a NPK manuring trial. fr. w. = fresh weight; dr. w. = dry weight.

Characteristic	Unit	P	N	NP
Cell wall	g dr. w./100 g fr. w.	0.62	0.72	0.76
Galacturonan (cell wall)	g/g dr. w.	0.147	0.169	0.160
Galacturonan (tissue)[1]	g/100 g fr. w.	0.091	0.122	0.122
Loss galacturonan	%	70	58	59
DE	%	50	51	48
Ca^{2+}/COO^-		0.29	0.27	0.27
Solubilized galacturonan (boiling)	%	22.1	22.4	20.7
Relative A_{552} (boiling)		1.24	0.88	1.02

1. See note 1 of Table 28.

Table 34. Distribution of calcium and phosphorus over starch, cell wall and residual parts of potato tissue of some samples of a NPK manuring trial. Calculations of P and Ca in meq per 100 g fr. w. and % (in parentheses).

Manuring	P		Ca		
	starch	rest	cell wall	starch	rest
P	1.29 (93)	0.09 (7)	0.19 (22)	0.08 (9)	0.60 (69)
N	1.04 (100)	-0.05 (0)	0.18 (23)	0.06 (7)	0.55 (70)
NP	1.28 (83)	0.26 (16)	0.18 (22)	0.07 (9)	0.57 (69)

Localization of P and Ca Phosphorus was found to be concentrated in the starch fraction, in particular for the N tubers (Table 34). Calcium distribution was calculated again ignoring the pectic galacturonan losses during cell wall isolation. Then even about 70% of total tissue calcium was not bound to starch and cell wall material.

7.4 A HYPOTHESIS FOR CHANGES IN INTERCELLULAR COHESION DURING COOKING

An important role has been ascribed to starch in causing cell separation when potato tissue is heated in the 'starch swelling pressure' theory (Section 3.4.1; introduction to this chapter). Hoff (1972, 1973) considered the existence of a 'starch swelling pressure' to have no theoretical foundation. Upon heating there would be no net mass transport to the interior potato cells as they form a closed system (it is questionable whether this reasoning will be valid for small, thin disks as used in the RWCS test, which indeed can absorb up to 20% water during cooking). In a closed system upon heating from room to boiling temperature shear stresses will be generated due to a volumetric expansion of 4% (Hoff, 1972, 1973). The stresses depend on module of elasticity and tensile strength of the wall and the initial turgor pressure. As long as the limit of elasticity of middle lamella or primary wall is not exceeded, no cell separation or cell wall rupture will be observed. In potato cooking cell separation is the common experience (Sterling, 1955), but rupture of cells and extrusion of starch has been observed (Reeve, 1954).

According to the view of Hoff (1972, 1973) the *primary factor* for maintance of intercellular cohesion is the strength of the middle lamella and cell wall structure, with which I agree. Furthermore, in my opinion, the strength of middle lamella and cell wall, apart from primary up to tertiary or quaternary structure, depends mainly on the stability of the pectin gel during heating. This is especially true for the intercellular layer which is concerned in intercellular cohesion. Solubilization of the pectin gel due to depolymerization of the (rhamno)galacturonan main chains, breaking of covalent bonds with the cellulose-hemicellulose framework (Keegstra et al., 1973) and removal of calcium from its key-position in the microcrystalline junction zones (Rees, 1969, 1972a, b), will ultimately destroy the resistance to internal shear stresses or externally exerted pressure (puncture test, compression tests, RWCS tests, shaking of tissue). Once the problem of intercellular cohesion of the cooked potato tissue is translated from strength of

middle lamella into stability of the intercellular pectin gel, the chemical constituents of the tuber (anions, cations, ionic strength, pH) which interact with the pectin gel during heating (above approximately 60°C) and the permeability of the plasmalemma belong to the definition of primary factors responsible for intercellular cohesion (Fig. 45) (Chapters 5 and 6). Bartolome & Hoff (1972) postulated that the ionic strength of the vacuolar solution affects the texture of the cooked potato by desorbing PE, which then would change the DE of pectic galacturonan. This mechanism, however, will be important in preheating treatments only because PE is inactivated above 65-70°C (Bartolome & Hoff, 1972). Ionic strength, however, is important in β-elimination (Keijbets & Pilnik, 1974b) (Chapter 5) of pectic galacturonan and influences solubilization in that way. The permeability of the plasmalemma determines the level of interactions between cell wall pectic galacturonan and vacuolar components.

Other factors influencing intercellular cohesion and mentioned by Hoff (1972, 1973) must be considered as *secondary factors*. These are cell size, cell wall thickness, mechanical properties of the cell wall and turgor. Physiological age and storage time are strongly related to chemical composition (this Chapter) and determine the level of the primary factors and of starch and its properties (granule size, amylose/amylopectin ratio, retrogradation, amylose diffusion). Cell size, cell surface area or intercellular contact area (Section 3.5), although a secondary factor will still be of major importance for cell cohesion. Hoff (1972, 1973) pointed out that the internal pressure would be proportional in any direction to the original diameter of the cell. Maximum shear stresses would be associated with the contact area of small and large cells in the vascular region, where the most severe separation of cells takes place (Reeve, 1954; Burton, 1966; Hoff, 1972, 1973). Hughes & Faulks (1972) established that 92% of the variation in intercellular cohesion (texture) in an experiment on time of harvest could be explained by the amount of pectic substances released during cooking and cell size. A highly significant negative correlation between texture and released pectic substances expressed per cm^2 of cell wall during cooking was observed. Little is known about module of elasticity and tensile strength of the potato cell wall. Anisimova (1968) established a direct correlation between elasticity of the raw potato and reduction of intercellular cohesion after cooking. Huff (1967) found the tensile strength of the tissue to be largest in the centre of the tuber with a decrease in the direction of the periderm; this was found by Personius & Sharp (1938a) to a certain extent,

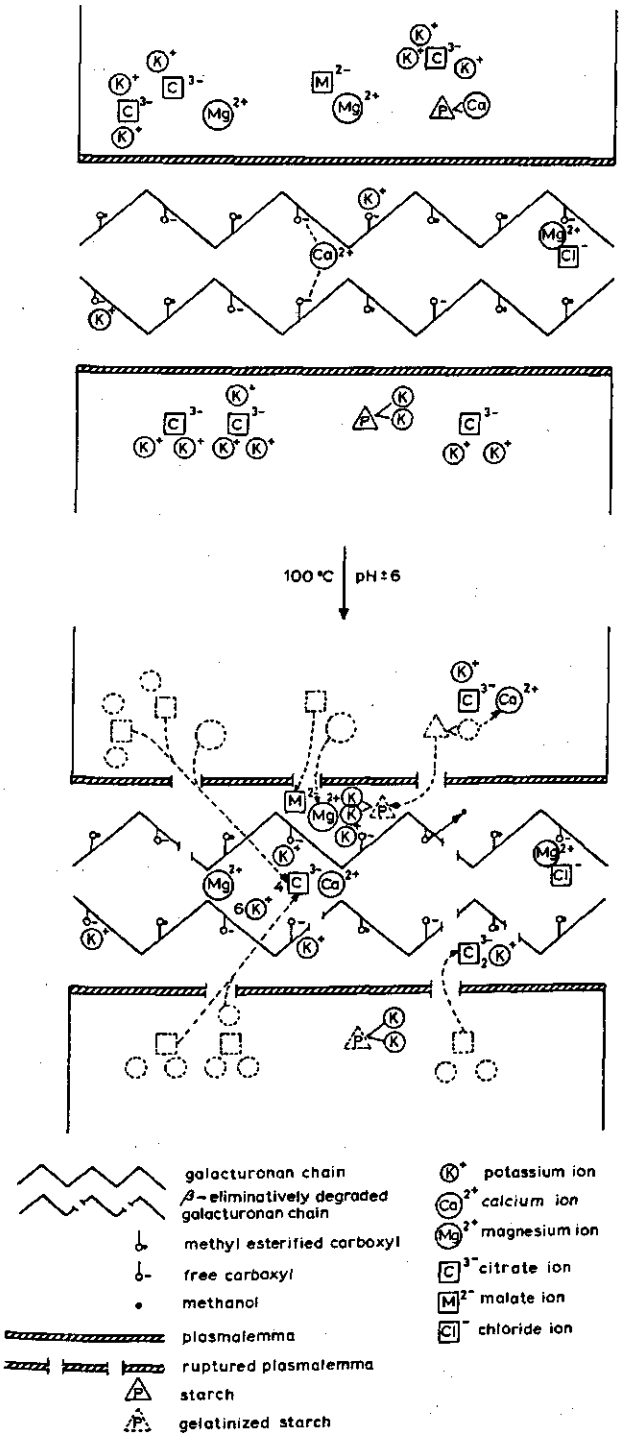

Fig. 45. Proposed action of potato constituents in the intercellular layer during cooking.

but they could not deduce a relationship between cell cohesion and tensile strength of individual potato tubers. Intercellular cohesion, on the other hand, is mostly reduced in the outer tuber region (Table 25; Reeve, 1954; Burton, 1966).

7.5 CONCLUSIONS AND SUMMARY

The influence of the chemical composition of the potato tuber on intercellular cohesion after cooking was investigated using an objective texture measurement procedure, the RWCS test. The starch intercellular cohesion relationship, which appeared frequently within restricted populations of tubers and which was explained by the 'starch swelling pressure' hypothesis, was studied in more detail.

Both starch and non-starch constituents, as citrate, phosphorus, potassium, magnesium and pH increased with increasing specific gravity. The increase of pectic galacturonan was less significant while the degree of esterification of pectic galacturonan was not affected. Malate and calcium decreased, although less significant, together with PE activity and intercellular cohesion. The neutralization by calcium of non-esterified galacturonan carboxylic acid groups, as shown by cell wall isolation and analysis, also decreased and did not exceed 50% (ratio $Ca^{2+}/COO^{-} < 0.5$). Because of the already established interactions of potato constituents with pectic galacturonan during heating, resulting in solubilization and possibly cell separation, the idea emerged to replace the hypothetical starch intercellular cohesion relationship by a concept of a causal relationship between chemical composition and intercellular cohesion. Within restricted populations both starch and non-starch levels depend on the stage of growth and maturity. In the pH region of 5.5-6.5 in the potato tissue, intercellular cohesion was strongly affected by small changes in pH. Only about 50% of tuber calcium was found to be incorporated in cell wall material and starch, which was unexpectedly low. That growth and maturity determine chemical composition of the potato tuber was further illustrated by the results of a lifting date trial.

During storage of specific gravity fractions at 6°C it was seen that intercellular cohesion was reduced or remained constant. Citrate decreased and malate increased. As a possible net effect pH increased considerably. PE activity diminished.

Some interesting effects of high doses of fertilizers in a field trial

were noticed. Nitrogen (as nitrate) increased the levels of organic acids and PE activity, but due to delayed maturity and a supposed effect of smaller cell size, intercellular cohesion was better in the nitrogen-treated tubers. Phosphate suppressed both citrate synthesis and cell separation. No essential differences in isolated cell wall material could be detected.

Some comment is given to an alternative hypothesis of Hoff, who replaced 'starch swelling pressure' by a less speculative 'thermal expansion pressure'. The stability of the intercellular cell wall layer was thought to be the primary factor responsible for tissue coherence of the cooked potato. The chemical constituents, which affect (in)solubility and gel structure of the middle lamella pectic substances when the permeable properties of the plasmalemma change at heating, were included in this primary factor. All other factors, such as cell size, cell wall thickness, turgor and mechanical features of the cell wall were considered as secondary factors, some of which, cell size in particular, will be of major importance.

8 Preheating, activation of pectinesterase and intercellular cohesion

8.1 INTRODUCTION

Some results of the model cooking experiments with tissue disks (Section 6.3.7) indicated that deesterification by pectinesterase (PE) of cell wall and middle lamella pectic galacturonan - which was esterified up to about 55% - resulted in retardation of cell separation on subsequent cooking. Endogeneous calcium ions or externally applied calcium strengthened the effect of pectin saponification markedly.

It has been demonstrated by several authors that, due to activation of PE, the firmness of plant tissues could be improved by a preheating or blanching procedure before processing (Doesburg, 1965; Van Buren, 1973). Such firming treatments were reported for cauliflower (Hoogzand & Doesburg, 1961), snap beans (Van Buren et al., 1960a, b, 1962; Sistrunk & Cain, 1960; Kaczmarzyk et al., 1963; Van Buren, 1968), tomatoes (Hsu et al., 1965), apple slices (Wiley & Lee, 1970) and sour cherries (Van Buren, 1973). A preheating procedure to activate potato PE, to be applied before freezing immature tubers (Unilever, 1966), was described without proof of enhanced firmness. Bartolome & Hoff (1972) showed that potato PE was activated during heating at 60-70°C, resulting in a smaller loss of intercellular cohesion on cooking. At the same time, I also was carrying out preheating experiments to establish suitable conditions for PE activation and firming of potato tissue, after having established the presence of pectinesterase in the tissue (Section 7.3.3).

8.2 MATERIALS AND METHODS

8.2.1 The potato material

Potato PE, utilized for partial characterization, was extracted from tubers of sp. gr. fractions of the 1969 harvest (variety Bintje). For the preheating experiments sp. gr. fraction 1.090-1.100 of harvests 1970, 1971

and 1972 was taken (see Sections 4.2.1 and 7.2.1), stored at 6°C.

8.2.2 Partial characterization of potato PE

PE was extracted from 1-2 kg of tubers as before (Section 7.2.2). The enzyme was salted out at 80% ammonium sulphate saturation, after previous 40% and 70% saturation steps, and collected by centrifugation (6 000 *g*). The precipitate was dissolved in 40 ml 0.01 M Tris-HCl pH 7.8 containing 0.1 M NaCl. The dependence of PE activity on pH and temperature was determined. To account for chemical deesterification, blanks were included with heat--denatured enzyme solution. In the reaction mixture 0.5-1 ml of enzyme was added. For the activity measurement see Section 7.2.2.

8.2.3 Procedures of preheating

Experiment I (1970 tubers, stored for 8 months) Tubers were sliced and diced to different sizes. Slices of variable thickness were used, in the other experiments as well, but the 10×10×1.2 mm dices as standardized in the RWCS test were also used (Section 7.2.2). 2 000 g Tissue were treated in 6 l of deionized water for 1 h at 30 and 46°C and usually at pH 7.5. The pH was adjusted with 1 N sodium hydroxide with the aid of the automatic Radiometer titration assembly. After one hour the pH was re-adjusted to 6 with concentrated hydrochloric acid. During preheating the specific conductance was measured periodically with a Philips conductance measuring bridge GM 4249. The intercellular cohesion of the preheated tissue was assessed as usual (RWCS test). In one treatment 0.05 M calcium chloride was added to the medium, while in two others the tissue was infiltrated with water by vacuum application. All treatments were done once.

Experiment II (1971 tubers, stored for 4 months) In the same procedure as used above without pH change, 2 200 g tissue were treated in duplicate at three temperatures: 30°, 50° and 75°C. In addition some analyses were performed: pectic galacturonan, DE of pectic galacturonan, citrate, K, Ca and pH (Section 7.2.2). These analyses were carried out after freeze-drying of the preheated tissue.

Experiment III (1972 tubers, stored for 2 months) 100 g Diced tissue (10×10×1.2 mm) (three replicates) were treated in 300 ml of deionized water

at 30°-45°-60°-80°C for 0.5-1-2 hours. Immediately afterwards 900 ml of boiling water were added and the RWCS test was carried out with one standard boiling time. As standard time the pre-established T_{50} of the untreated tissue was used, e.g. 8 min. The retained weight was determined in duplicate. The third replicate was used for methanol analysis after storage in ethanol and conversion to AIS. The methanol, still bound to pectic galacturonan, was liberated by saponification, collected by distillation (Keijbets & Pilnik, 1974a) and assayed as described before (Section 4.2.13).

The twelve treatments had a mean RW of 87 g with s = 5.2 g. The determination of intercellular cohesion thus was subjected to fairly large variation when one standard boiling time was used (compare with T_{100} determinations in Section 7.2.2).

Experiment IV (1972 tubers, stored for 4 months) 3 kg Unpeeled tubers were heated at 55°C for 1-2-3-6-18 hours in a waterbath. The RWCS test and the pectin analyses were carried out as described in Experiment III.

The mean methanol value of the Experiments III and IV was 1.28 µg/g fresh weight with s = 0.040 µg/g.

8.3 RESULTS AND DISCUSSION

8.3.1 Partial characterization of potato PE

When measuring PE activity, there should be no chemical deesterification of the pectin substrate, because the validity of the results is questionable under such conditions (Kertesz, 1951). For that reason Vas et al. (1967) thought it advisable to determine PE activity at pH 7.5 when assaying PE of higher plants. The 'at random' chemical deesterification can influence PE activity since the number and position of carboxyl groups are changed, possibly creating new places for attack by PE, which occurs linearly along the chain of the molecule (Solms & Deuel, 1955; Kohn et al., 1968a; Lee et al., 1970). I found that chemical deesterification of 65% esterified apple pectin at 30°C started at a pH above 7.5-8 (Fig. 46) and at pH 7.5 at 40°C (Fig. 47). Potato PE activity therefore was measured at pH 7.5 and at 30°C.

pH A broad pH optimum for potato PE was found at pH 8-8.5 (Fig. 46). This PE resembles other higher plant PEs, which are most active in the pH region around 7.5. The relationship between pH and activity is also known to be

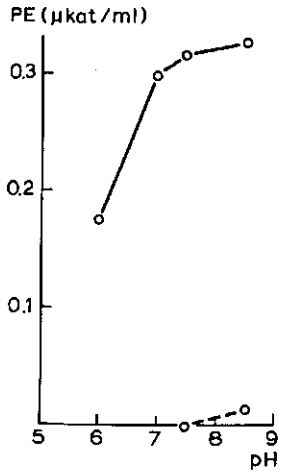

Fig. 46. Activity of potato PE at 30°C as influenced by pH (——) and corrected for chemical deesterification (---).

influenced by the salt concentration in the assay mixture as shown for PE of alfalfa (Lineweaver & Ballou, 1945), orange (MacDonnel et al., 1945), snap bean (Van Buren et al., 1962) and tomato (Nakagawa et al., 1970). Furthermore pH optima at approximately 7.5 have been found for PE of southern peas (*Vigna sinensis*) (Collins, 1970), strawberries (Leuprecht & Schaller, 1968) and apples (Lee & Wiley, 1970). Hultin & Levine (1963) determined optima at pH 9-9.5 for two banana PE fractions, pointing out at the same time the existence of several fractions of PE with different properties.

Temperature Optimum activity of potato PE was established at 55°C, but a small chemical saponification was already observed at 40°C (Fig. 47). The recording of activity curves for 30 min also suggested that thermal inactivation begins at 55°C because of deviations of linearity (see also Fig. 48). Bartolome & Hoff (1972) found initial inactivation at 50°C. A temperature optimum of 55°C was also established for strawberry PE (Leuprecht & Schaller, 1968) and apple PE (Vas et al., 1967; Lee & Wiley, 1970). Collins (1970) measured a broad peak at 50-60°C for southern peas and Vas et al. (1967) found PE from orange albedo to be most active at 65°C. Potato PE activity was greatly diminished at 70°C (Fig. 47). From the Arrhenius plot in Fig. 48 (straight line through points of 30-40-50°C only) the activation energy was calculated:

$$E_A = - 2.303\ R \frac{\Delta \log v_o}{\Delta T^{-1}}$$

$$E_A = - 2.303 \times 1.98 \times \frac{2.760 - 2.635}{(3.10 - 3.20)10^{-3}} = 5\ 700\ \text{cal/mol}$$

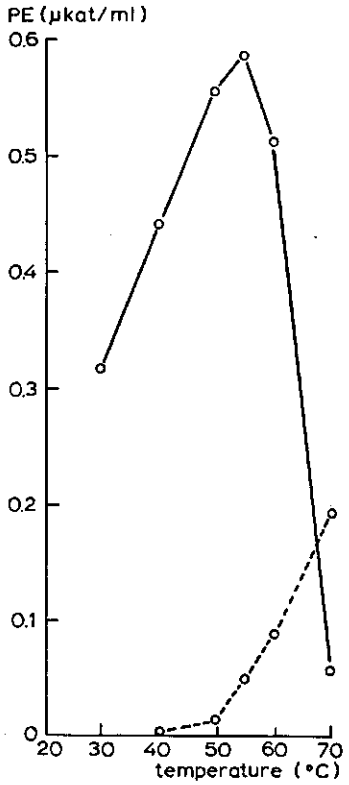

Fig. 47. Activity of potato PE at pH 7.5 as influenced by temperature (——) and corrected for chemical deesterification (---).

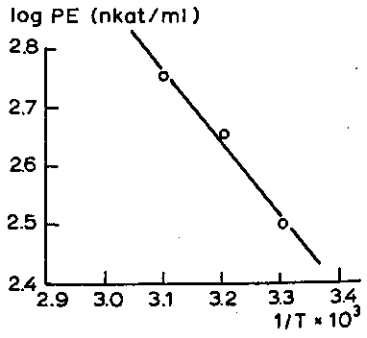

Fig. 48. Arrhenius plot for potato PE.

For a proper estimation of the activation energy, according to Voragen (1972) the substrate concentration must be high enough throughout the temperature range used to saturate the enzyme. The use of maximum rate V therefore is recommended. However, I did not determine V for potato PE at the temperatures used, but applied the initial velocity in the Arrhenius plot at a substrate concentration of 0.77% of 65% esterified apple pectin. Vas et al. (1967) established for orange albedo PE that the activity only slightly depended on pectin (75% esterified) concentration in the range of 0.25-2%. Nakagawa et al. (1970) found a K_m value for tomato PE at 30°C of 0.24% for citrus pectin. Therefore it is rather uncertain whether I determined the activation energy E_A at substrate saturation, which was possibly in the range of 2-5% pectin

concentration. However, from a practical point of view a substrate concentration of over 2% is impossible (viscosity of pectin solutions!). The activation energy was somewhat higher than 5 400 for strawberry PE (Leuprecht & Schaller, 1968), almost equal to 5 800 for apple PE (Lee & Wiley, 1970) but much lower than the 8 000 - 9 000 for tomato PE in the 27-31°C region (Nakagawa et al., 1970). At higher temperatures this tomato PE was already activated at ± 3 000 cal/mol.

8.3.2 Four preheating experiments

Preheating experiment I (Table 35) No pectin analyses were available. The work of Bartolome & Hoff (1972), however, makes it rather unlikely that potato PE could have been active in situ at pH 6.6 and 30°C. Nevertheless the intercellular cohesion was appreciably raised from 4.3 to 11.2 min (T_{100}). Removal of water-soluble substances from the tissue (Le Tourneau et al., 1962; Zaehringer et al., 1963b), resulting in increased specific conductance κ of the preheating medium (Davis, 1964; Davis et al., 1973), are now known to be related to reversion of cell separation of the cooked potato tissue (Cunningham et al., 1967; Davis & Le Tourneau, 1967; Zaehringer & Cunningham, 1970, 1971). When, however, the pH was raised to 7.5, activation of PE was possible. κ corrected for alkali addition was seen to increase to a lesser extent (less diffusion of ions?), but intercellular cohesion was even higher (10×10×1.2 mm pieces) (Table 35). Results of Cu^{2+} ion-exchange (Keijbets & Pilnik, 1974a) indicated that saponification of pectic galacturonan indeed occurred. At pH 7.5, potato PE could have been solubilized from its binding to the cell wall as found before for orange PE (MacDonnel et al., 1945) and tomato PE (Nakagawa et al., 1971), while simultaneously the activity increased (Fig. 46). Calcium ions leaching out and passing the cell wall and middle lamella region have the opportunity to react with non-esterified pectic galacturonan carboxylic acid groups (for leaching of Ca see Experiment II (Table 36) and Davis (1964) and Davis et al. (1973)).

External application of calcium ions resulted in a vast increase of intercellular cohesion (42.3 min). At calcium addition κ, corrected for alkali consumption, decreased instead of increasing. The drained weight after preheating increased unlike to the previous samples which lost weight (Table 35). It is assumed that calcium ions are taken up by the tissue in preference to sodium. Then the addition of sodium ions probably does not equal the calcium uptake. The calcium ions will diffuse into the free space (intercellular

Table 35. Influence of pH, temperature and contact area during preheating on intercellular cohesion (experiment I; 1970). T_{100} is corrected for weight change during preheating.

Potato tissue size (mm)	pH	Temp. (°C)	κ (mS/cm)	NaOH added (meq)	$\kappa_{corr.}$ (mS/cm)	Weight change (%)	T_{100} (min)
Blank							4.3
10×10×1.2	6.6	30	2.51	0	2.51	- 8.6	11.2
10×10×1.2	7.5	30	3.01	30.3	1.79	- 9.5	20.2
10×10×1.2[1]	7.5	30	9.36	33.0	8.03	+10.5	42.3
1.2	7.5	30	2.04	18.1	1.30	+24.0	17.4
10	7.5	30	0.43	10.8	0	+12.5	6.6
10[2]	7.5	30	0.44	7.5	0.18	+18.1	7.9
10	7.5	46	1.86	19.5	0.91	+ 2.7	6.5
10[3]	7.5	46	1.53	12.5	0.90	+ 8.9	10.5

1. Preheating in 0.05 M $CaCl_2$ (κ = 9.36 mS/cm).
2. Vacuum infiltration in 2 l water for 30 min. For pretreatment 4 l water were added.
3. Vacuum infiltration in 2 l water for 30 min and pretreatment in fresh water (6 l).

space, cell wall) and be taken up into the cytoplasm causing an osmotic flow of water into the tissue.

The effect of slicing of tissue (or magnitude of surface area) is evident when results of κ and intercellular cohesion are inspected (Table 35). Preheating of 10 mm thick slices at 30°C reduced increase of κ to (almost) zero after correction for alkali, but intercellular cohesion after cooking was also low. Previous vacuum infiltration of potato tissue with preheating medium appeared to be effective, especially at subsequent preheating at 46°C where the infiltration water was removed before preheating. Vacuum infiltration will fill the intercellular spaces with water and probably favour the diffusion of potato ions. It was furthermore expected that upon vacuum infiltration, it might be easier to establish a correct pH value of 7.5 in the cell wall boundary to activate PE.

Preheating experiment II (Table 36) The pH was not raised during preheating. At *30°C* an increase of intercellular cohesion of 10×10×1.2 mm pieces was not associated with a decrease of DE of pectic galacturonan. Once more the change in texture appeared to be related to loss of ions, reflected in κ and analyses of citrate (59% loss), K (49% loss) and Ca (46% loss). The pH decreased markedly for the 10×10×1.2 mm pieces, but only to a small extent

Table 36. Influence of temperature and contact area during preheating on intercellular cohesion (experiment II; 1971). All analyses calculated on fresh weight without correction for weight change.

Potato tissue size (mm)	Temp. (°C)	κ (mS/cm)	T_{100} (min)	Galacturonan (g/100 g)	DE (%)	meq/100 g			pH
						citrate	K	Ca	
Blank			3.6	0.36	49	5.23	4.66	0.39	6.21
10×10×1.2	30	2.43	7.5	0.30	54	2.14	2.39	0.21	6.03
10	30	0.37	3.8	0.34	50	4.12	4.42	0.33	6.18
50	30	0.15	4.0	0.36	48	4.55	4.57	0.38	6.18
10×10×1.2	50	4.94	28.4	0.35	50	0.71	1.40	0.20	6.16
10	50	2.20	6.8	0.35	46	2.94	3.57	0.28	6.19
50	50	1.31	4.8	0.36	48	4.14	4.38	0.35	6.21
10×10×1.2[1]	75	5.46	11.4	0.23	52	1.45	1.61	0.15	6.20

1. Preheating time only 15 min; T_{100} corrected for weight increase.

for bigger pieces. When 10 and 50 mm thick slices were preheated at 30°C intercellular cohesion remained unchanged and indeed loss of ions and increase of κ were sharply reduced. Again the loss of citrate was most evident (21 and 13% respectively). Variability in pectic galacturonan (Table 36) was caused by weight changes, which were not corrected for.

Preheating at *50°C* resulted in much higher values of κ due to the effect of temperature itself and the acceleration of diffusion. The 10×10×1.2 mm tissue pieces lost 86% of citrate, 70% of K but only 49% of Ca, while DE of pectic galacturonan was not lowered. Leaching out of ionic constituents therefore effectively increased intercellular cohesion. A large contact area between tissue and surrounding water, of course, favours leaching. PE activity was not found and indeed according to Bartolome & Hoff (1972) the preheating temperature of 50°C was too low to desorb and activate PE. Moreover, the preheating period of one hour is short because of the rate of heat penetration particularly in thicker potato slices. Bartolome & Hoff showed that preheating must preferentially occur at 60-70°C. They found that most methanol was formed by potato PE at 60°C although thermal inactivation started at 50°C. These investigators isolated cell wall material from pretreated tissue and found that simultaneously with methanol formation the methoxyl content of cell walls decreased and migration of calcium and magnesium (the latter will not contribute to insolubility of pectic galacturonan markedly; Section 5.3.1) into the cell wall increased. Bartolome & Hoff (1972), on the other hand, did not account for the influence of leaching of cell

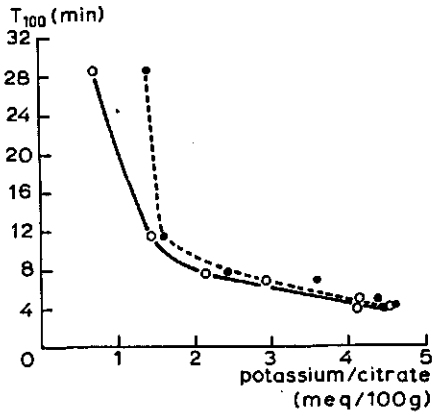

Fig. 49. Relationship between citrate (——) and potassium (---) contents of preheated tissue and intercellular cohesion (T_{100}) (preheating experiment II).

solutes in their preheating experiments, although this effect would have affected their results.

At *75°C*, PE in potato tissue will rapidly be inactivated (Fig. 45; Bartolome & Hoff, 1972). My experiments showed that after only 15 min of preheating κ was rather high and concurrently 72% of citrate, 65% of K and 62% of Ca were lost. The similarity of leaching patterns of citrate and K at all temperatures (Fig. 49) confirms the strong relation between these ions (Section 7.3.7). When starch has been gelatinized the loss of calcium was facilitated. Preheating at 75°C resulted in increased intercellular cohesion, due also to loss of ionic constituents. Precook heatings at 70-75°C are known to be applied in potato processing, in particular for dehydrated mashed products (Potter et al., 1957; Eskew, 1967; Adler, 1971) to obtain a product without undesirable cell rupture and stickiness caused by exuded gelatinized starch. A chilling treatment, however, after the heating period is essential to enhance retrogradation of starch molecules. In these preheating processes change of pectic substances by PE will be absent. If an increase of intercellular cohesion is desired a LTLT-preheating treatment (low temperature long time) at about 60°C will be most successful (Hoogzand & Doesburg, 1961).

Preheating experiment III (Table 37) The effect of leaching on intercellular cohesion during preheating could be reversed by carrying out the pretreatment in a part of the boiling water of the RWCS test as suggested by results of Zaehringer et al. (1963b). Nevertheless, an increasing RW (or increasing intercellular cohesion) was observed at *30°C* and even more obviously at *45°C*. The amount of methanol, esterified to pectic galacturonan, however, was not

Table 37. Influence of temperature and length of preheating period on intercellular cohesion (experiment III; 1972). fr. w. = fresh weight.

Temperature (°C)	Time (h)	RW (g)	Ester-bound methanol (µg/g fr. w.)
Blank		50	1.51
30	0.5	77	1.42
	1	77	1.50
	2	103	1.47
45	0.5	100	1.42
	1	120	1.32
	2	118	1.51
60	0.5	116	1.14
	1	116	1.10
	2	113	1.00
80	0.5	89	1.21
	1	38	1.28
	2	26	1.23

markedly reduced in these attempts (Table 37). At *60°C* preheating temperature, ester-bound methanol started to decrease. Intercellular cohesion remained fairly constant at 110-120 g RW, which represents a maximum level encountered in many RWCS cooking curves (not given here) (see for instance Zaehringer et al., 1969). Preheating at *80°C* for more than 30 min resulted in faster separation of cells at cooking. The low contents of ester-bound methanol at 80°C must be due at least partially to loss of degraded pectic galacturonan as demonstrated during preheating at 75°C in Experiment II (Table 36). The low pectic galacturonan content there resulted from both degradation and water uptake which was not corrected for.

Preheating experiment IV (Fig. 50) In a recent patent (Schoch & Sloan, 1972) the idea was put forward to change texture by preheating whole, even unpeeled tubers. A temperature between 50° and 60°C just below the gelatinization temperature of starch, applied with hot air or water for 3-24 hours, would produce 'a firm potato tissue which resists physical breakdown during further processing'. This result would be obtained by association of starch molecules in the granules without gelatinization. This idea was used in my work, because unpeeled tubers could be prevented from losing soluble substances as experienced in the preceding experiments.

At *55°C* the intercellular cohesion of whole, unpeeled tubers was much greater after an initial lag period of one hour. After 3-6 hours increase in cell cohesion at cooking gradually slowed down. The ester-bound methanol

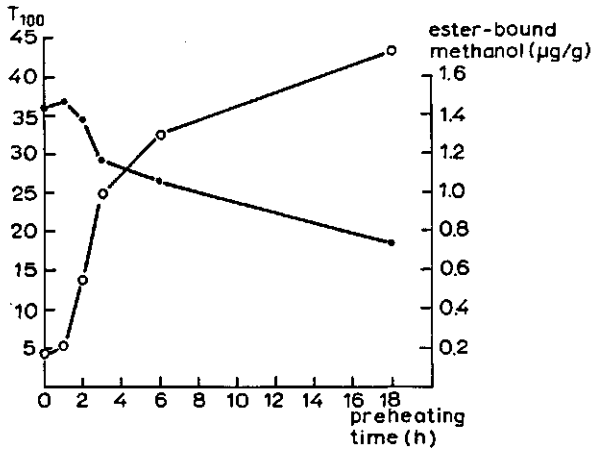

Fig. 50. Changes in ester-bound methanol (•) and intercellular cohesion (T_{100}) (o) during pre-heating at 55°C of whole, unpeeled tubers (pre-heating experiment IV).

content also decreased sharply after a lag period of 1-2 hours and was halved after 18 hours. These results proved that at 55°C and prolonged incubation PE was activated to change DE. Diffusion of calcium ions through the destroyed plasmalemma subsequently led to neutralization of non-esterified carboxylic acid groups of pectic galacturonan and stabilization of the intercellular pectin gel structure against breakdown and loss of tissue coherence during cooking. The explanation for these firming phenomena as given by Schoch & Sloan (1972) seems to be deduced from the starch retrogradation theory of Potter (1954) and Reeve (1954, 1972) and must be rejected in favour of the PE activation hypothesis of Bartolome & Hoff (1972). It is uncertain whether only diffusion of vacuolar calcium ions into the cell wall boundary, without PE activity on pectic galacturonan, could produce more intercellular cohesion.

Off-odours may become a major disadvantage for potato quality during prolonged preheating (Bartolome, 1971). Discoloration, on the other hand, can be prevented by addition of sodium bisulphite (Schoch & Sloan, 1972).

8.4 CONCLUSIONS AND SUMMARY

Potato PE was partially characterized after extraction and a ammonium sulphate precipitation. The relationships between activity and pH or temperature were found to be comparable to those of PE enzymes from other higher plants and particularly vegetables. An exact pH optimum at 30°C was not established, but the potato enzyme appeared to be most active at pH 8-8.5,

and not at the usual pH of activity assessment, which is 7.5. The temperature optimum for activity was 55°C. At 40°C and pH 7.5 and at pH 8.5 and 30°C chemical deesterification was initiated on 65% esterified apple pectin. The activation energy for potato PE was calculated to be 5 700 cal/mol.

Preheating experiments with potato tissue were carried out at variable conditions of time, temperature and tissue surface area. Without PE activation, as indicated by determination of DE of pectic galacturonan or ester-bound methanol, considerable firming effects or reduction of loss of intercellular cohesion during cooking could be obtained at 30°, 45° and 50°C, even at 75°C. Diffusion out of the tissue of ionic constituents as citrate, potassium and calcium, reflected in specific conductance measurements, was thought to stabilize the potato tissue against thermal breakdown. This leaching phenomenon or the rate of diffusion of soluble constituents was favoured by increase of surface (or contact)area, rising temperature and duration of pretreatment.

More obvious activation of PE, resulting in a decrease of ester-bound methanol, was only achieved by preheating at 60°C, using potato dice of 10×10×1.2 mm. However, due to use of measurement of retained weight instead of T_{100} in the RWCS test, the effect of PE activation was not quantitatively distinguishable from the effect of preheating at 45°C (no PE activation!) on intercellular cohesion of the tissue. Moreover, interference of ion-diffusion phenomena could not be excluded but after preheating of whole, unpeeled tubers at 55°C. Loss of ions in the surrounding water then was impossible and PE activation and increase of intercellular cohesion at cooking occurred together. A preheating period of more than one hour until six hours caused large increases of cell cohesion.

In accordance with the findings of Bartolome & Hoff it was assumed that at 55-70°C the permeable properties of the plasmalemma change to release ions into the cell wall region, which desorb and activate PE. The DE of pectic galacturonan is decreased and migration of calcium ions (magnesium ions are less important) leads to firming of the cell wall and middle lamella pectin gel. The reaction of calcium ions with non-esterified pectic carboxylic acid groups especially will be of most importance.

Summary

Intercellular cohesion is one of the most outstanding textural characteristics of potato tuber tissue. During heating the intercellular cohesion changes, depending on the properties of the raw material and the processing conditions. The role of the pectic substances, which form part of the matrix substances in the primary potato cell wall and middle lamella, in the ultimate intercellular cohesion of the cooked potato was studied further. For that purpose maceration experiments with pectic galacturonan depolymerizing enzymes were designed, and solubilization of pectic galacturonan was studied in model systems (potato cell walls, tissue disks). Specific gravity fractions were chemically analysed and preheating experiments were done to activate pectinesterase bound to cell walls.

In Chapters 2 and 3 a literature review of some aspects of intercellular cohesion was given. In Chapter 2, therefore, the structure of pectic substances of the cell wall and middle lamella matrix and the structure of the primary plant cell wall was discussed. Special attention was paid to the insolubility of the pectic substances, which is thought to be responsible for cohesion between cells. The insolubility was thought to be caused by covalent anchorage to hemicellulose and glycoprotein substances in the cell wall and middle lamella, while the role of calcium in assisting the packing of galacturonan chain segments in microcrystalline structures was also emphasized.

In Chapter 3 intercellular cohesion of the cooked potato as influenced by chemical composition was surveyed. Reference was made to the overall composition of the potato tuber, the individual constituents and the detailed cell wall composition. It was shown that intercellular cohesion can be measured objectively by deformation tests such as compressive strength and puncture testing, but probably better by direct determination of cell separation based on retained weight on a sieve after cooking. The importance of objective texture measurement to exclude influences of related texture parameters was pointed out, particularly when the influence of chemical composition on intercellular cohesion of the cooked potato was investigated.

This was illustrated in a critical review of older literature data on intercellular cohesion. The well-known starch intercellular cohesion relationship thus becomes doubtful. The causal explanation mostly was based on subjective measurements of intercellular cohesion. On the other hand, many experimentally obtained results indicate that complex changes in pectic substances influence intercellular cohesion of the cooked potato.

The key role of pectic galacturonan in intercellular cohesion of potato tissue was shown in Chapter 4. Pectic galacturonan depolymerizing enzymes, low-methoxyl pectin lyase and pectin lyase, strongly reduced the cell cohesion. During this maceration, the patterns of solubilization of pectic galacturonan from tissue disks of low (1.060-1.070) and high (1.100-1.110) specific gravity from one population of tubers were similar and therefore, with the techniques used, no differences in primary structure of pectic substances were found. However, cell separation in the high sp. gr. tissue was faster. This can be explained by the greater cell size in high sp. gr. tissue which enhanced loss of intercellular cohesion compared with the low sp. gr. tissue. Enzymic maceration measured by a spectrophotrometric turbidity procedure, revealed the important role of calcium ions in cell cohesion. Calcium ions retarded cell separation even when pectic galacturonan was strongly degraded as was shown by strong solubilization at mechanical distortion of the tissue.

The solubilization of pectic galacturonan from potato cell walls at boiling as influenced by potato cations, anions, starch, pH and buffer strength, is studied in Chapter 5. Pectic galacturonan, which was esterified for 58%, was seen to be degraded by β-elimination at pH 6.1. Calcium, divalent copper and iron but not magnesium ions slowed down the solubilization of pectic galacturonan compared with potassium. Increasing concentrations of calcium and potassium ions increased the rate of β-elimination reaction, calcium even more than potassium. The important role of calcium in microcrystallite junction zones in the potato cell wall structure is illustrated by the optimum insolubility of pectic galacturonan at ratio $Ca^{2+}/COO^{-} = 1\text{-}2$. Organic anions, citrate, phytate and malate, solubilized pectic galacturonan at boiling, due to calcium binding and acceleration of rate of β-elimination. The affinity of pectic galacturonan carboxylic acid groups for calcium could be increased by deesterification with orange PE. Some boiling experiments were carried out with complex mixtures of potato constituents to simulate the chemical composition of low and high sp. gr. tissue. The pectic galacturonan insolubilizing role of calcium was confirmed here.

In Chapter 6 model studies on intercellular cohesion of the cooked

potato tissue are described using the same dead, partially leached tissue disks as for enzymic maceration. Intercellular cohesion or cell separation was measured again turbidimetrically, after cooking in a buffer at pH 6.5. β-Eliminative degradation of pectic galacturonan during cooking of a plant tissue was demonstrated by specific periodate thiobarbituric acid staining and reported for the first time. Although patterns of solubilization of pectic galacturonan were similar again, the high sp. gr. tissue lost intercellular cohesion at a faster rate. Addition and removal trials with calcium and potassium ions clearly showed that calcium ions, internally present or externally applied, stabilize the pectin gel in cell wall and middle lamella against loss of its cohesive function even when severely degraded during cooking. The intercellular cohesion strengthening effect of calcium disappeared when the carboxyls of pectic galacturonan were esterified and increased considerably at complete enzymic deesterification.

Specific gravity fractions from restricted populations, with the well-known relationship between sp. gr. (starch) and intercellular cohesion, were analysed for a range of chemical constituents and one enzymic activity in Chapter 7. A parameter free trend test of pooled data showed that with increasing sp. gr., starch as well as citrate, phosphorus, potassium, magnesium and pH significantly increased. Pectic galacturonan, esterified to 50-60%, increased to a lesser extent. Malate, calcium, PE activity and intercellular cohesion decreased. The neutralization of non-esterified carboxylic acid groups of pectic galacturonan in isolated cell walls also decreased and did not exceed 50% (ratio $Ca^{2+}/COO^{-} < 0.5$). From these results and those of a lifting date trial it is concluded that the stage of growth and maturity determines the level of both starch and non-starch constituents. Within restricted populations the already mentioned starch intercellular cohesion relationship is replaced by a more causally explainable one between intercellular cohesion and chemical constituents which react with pectic galacturonan (Chapters 5 and 6). The enormeous influence of pH on intercellular cohesion, particularly in the natural potato pH range of 5.5-6.5, was clearly demonstrated. Effects of storage and fertilizers on chemical composition and intercellular cohesion of potato tubers were investigated to some extent. During storage at 6°C intercellular cohesion decreased together with PE activity. Changes in organic acids and a rise of pH also concurred. Nitrogen fertilizer stimulated synthesis of organic acids, raised PE activity and affected intercellular cohesion favourably. Phosphate fertilizer surpressed citrate synthesis and consequently cell separation at cooking. Some hypotheses

for explanation of changes in intercellular cohesion during heating of potato tubers are discussed with special reference to the 'starch swelling pressure' hypothesis of originally Atwater and the 'thermal expansion pressure' hypothesis of Hoff.

Pectinesterase, extracted from Bintje potato tubers, the variety used throughout this work, was purified by ammonium sulphate precipitation. Its partial characterization was described in Chapter 8. The optimum pH was established to be pH 8-8.5 and the temperature optimum for activity was found at 55°C. Potato PE thus ressembles other higher plant PEs. The activation energy was calculated to be 5 700 cal/mole. Preheating of potato tissue was carried out with temperature, heating time and surface area as parameters. Activation of PE, detected by measuring changes in degree of esterification of pectic galacturonan, only occurred at 55°and 60°C but not at 50°C for one hour or at 75°C where inactivation occurs. Preheating of whole, unpeeled tubers at 55 C for more than one hour caused large increases of intercellular cohesion. During preheating of peeled tissue pieces of different sizes at temperatures outside the PE activation range, intercellular cohesion also increased, depending on the parameters mentioned already caused by leaching of ionic constituents. This leaching phenomenon was quantitatively studied by specific conductance measurements and chemical analyses (potassium, calcium, citrate) of the tissue.

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