Effects of prefreezing treatments on the structure of strawberries and jams

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"My religion consists of a humble admiration of the illimitable superior spirit who reveals himself in the slight details we are able to perceive with our frail and feeble mind."

- Albert Einstein

Abstract

Different chemical components and their locations in strawberry *(Fragaria x ananassa)* achenes, vascular bundles and cortical cell walls were studied by means of bright-field microscopy using different staining systems and by Fourier transform infrared (FT-IR) microscopy. The structural changes in pretreated strawberry tissues during freezing and thawing were studied by means of physical and chemical analyses as well as by bright-field and FT-IR microscopy, and by confocal laser scanning (CLS) microscopy. In addition, traditional jams made from pretreated frozen strawberries were investigated by means of physical and chemical analyses and by sensory evaluation.

Basic microstructural studies showed that the cell walls were complex and consisted of several components. Lignin was an important component of achenes and vascular tissues, whereas the cortical cell walls contained mainly pectin, cellulose and some protein. Cellulose and pectin were also detectable in the intercellular spaces of cortex, in the vascular tissue and pith. Protein was deposited inside the cortical cells, as well as in the vascular tissue and pith. The microscopical methods used were complementary.

According to microscopical studies both the pretreatments with calcium chloride ($CaCl_2$) and crystallised sucrose as well as with $CaCl_2$ and pectin methylesterase (PME) in a vacuum affected the microstructure of strawberry tissues. These pretreatments especially affected pectin, protein, lignin and structural carbohydrates in the vascular tissue and cortex compared to the untreated reference samples. The use of a vacuum appeared to make the pretreatment solutions absorb more efficiently to the cortex and pith, thus improving the stabilisation particularly of pectin and structural carbohydrates.

Firmness of thawed and particularly of jam strawberries pretreated with $CaCl_2$ and PME in a vacuum was higher than that of other pretreated or untreated berries. In all the prefreezing treatments studied, dipping of strawberries into a $CaCl_2$ solution with PME in a vacuum resulted in a significantly different sensory profile than was found in the other jams. The sensory attributes wholeness of the berries ($p<0.001$), firmness, clarity and evenness of the jam medium ($p<0.001$), softness of the berries ($p<0.001$) and faultlessness of odour and flavour ($p<0.001$) in particular were statistically significantly different among the strawberry jams. Sensory quality was perceived to decrease during 4 months of storage, even though the shapes of the sensory profiles of the studied jams did not change significantly from those evaluated after 2 weeks storage

For achieving high quality jams the pretreatment time should be short (about 5–15 min), the temperature low (below 20 °C), the vacuum level high (pressure less than 10 kPa), the $CaCl_2$ concentration moderate (about 1%) and the dosage of PME comparatively low (about 50–100 µkat/kg strawberries).

Preface

Most of the present work was carried out at VTT Biotechnology during the years 1998–2001. The research was part of the national projects 'The texture and shelf-life of berry products' and 'Enhancement of the industrial use of Finnish strawberries'; both included in the VTT Research Programme 'Minimal Processing of Foods'. The National Technology Agency (Tekes) and participating Finnish companies are gratefully acknowledged.

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List of original publications

The present thesis is based on the following publications, which will be referred to in the text by their Roman numerals. Additional unpublished data are also presented.

I   Suutarinen, J., Änäkäinen, L. and Autio, K. 1998. Comparison of light microscopy and spatially resolved fourier transform infrared (FT-IR) microscopy in the examination of cell wall components of strawberries. Lebensm.-Wiss. u. -Technol. 31, 595–601.

II   Suutarinen, J., Heiska, K., Moss, P. and Autio, K. 2000. The effects of calcium chloride and sucrose prefreezing treatments on the structure of strawberry tissues. Lebensm.-Wiss. u. -Technol. 33, 89–102.

III   Suutarinen, J., Honkapää, K., Heiniö, R.-L., Autio, K. and Mokkila, M. 2000. The effect of different prefreezing treatments on the structure of strawberries before and after jam making. Lebensm.-Wiss. u. -Technol. 33, 188–201.

IV   Suutarinen, J., Honkapää, K., Heiniö, R.-L, Autio, K., Mustranta, A., Karppinen, S., Kiutamo, T., Liukkonen-Lilja, H. and Mokkila, M. 2001. Effects of calcium chloride-based prefreezing treatments on the quality factors of strawberry jams. J. Food Sci. Accepted to be published.

V   Suutarinen, J., Honkapää, K., Heiniö, R.-L., Mustranta, A., Liukkonen-Lilja, H. and Mokkila, M. 2001. Modeling of calcium chloride and pectin methylesterase prefreezing treatments of strawberries and jams. J. Food Sci. Accepted to be published.

The author of the present thesis had the main responsibility for planning the research, FT-IR-microspectrometer study, modeling and interpretation of the results in all publications, except for the CLSM analysis in publication II, which was carried out at the Technical University of Helsinki by Patricia Moss. Interpretations of bright-field microscopy studies and sensory evaluation were made together with Karin Autio and Raija-Liisa Heiniö, respectively.

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   Publications I–V

Appendices of this publication are not included in the PDF version.
Please order the printed version to get the complete publication
(http://otatrip.hut.fi/vtt/jure/index.html)

Abbreviations

ANOVA	Analysis of variance
Ar	Argon
^{o}Brix	Soluble solids
C	Carbon
Ca	Calcium
$CaCl_2$	Calcium chloride
CLS	Confocal laser scanning
CLSM	Confocal laser scanning microscope
Cu	Copper
DE	Degree of esterification
DRIFT	Diffuse Reflectance Infrared Fourier Transform
Fe	Iron
FT-IR	Fourier transform infrared
H	Hydrogen
HM	High methoxyl
HP	High pressure
Kr	Krypton
LM	Low methoxyl
LMA	Amidated low methoxyl
LoF	Lack of fit
MCT	Mercury cadmium telluride
N	Nitrogen
N	Number of samples
O	Oxygen
p	Probability
PCA	Principal component analysis
PE	Pectinesterase
PLS	Partial least squares
PME	Pectin methylesterase
R	Correlation coefficient
RG	Rhamnogalacturonan
RSD	Residual standard deviation
Q	Prediction coefficient
s_x	Standard deviation
SS	Sum of squares
$\bar{x}$	Mean

1. Introduction

One of the major problems encountered in the processing of strawberries is their susceptibility to textural damage at almost every stage of handling. The extent of the changes that take place is influenced by the original state of the fruit (e.g. the stage of maturity), the method of picking and the degree of mechanical handling during transportation, processing and cooking (Cross 1981). Most of the production of strawberries in the world is located in the northern hemisphere (98%), although there are no genetic or climatic barriers preventing greater expansion into the southern hemisphere. In Europe the highest strawberry yields are found in Spain, where they routinely exceed $20x10^3$ kg per hectare, with Italy not far behind (Hancock 1999). In Finland the strawberry yield is approximately $3.0x10^3$ kg per hectare, which means $11.8x10^6$ kg strawberries per year (Information Centre of the Ministry of Agriculture and Forestry 2000).

In the Nordic countries, the season for fresh strawberries is short and very few industrially processed jams are made from fresh berries during the harvesting season; instead, frozen berries are commonly used. Textural changes in strawberries caused by freezing are among the leading problems faced by processors. In most cases it is changes in texture rather than in odour, flavour or colour, which make a variety unsuitable for processing (Armbruster 1967). During freezing changes in the histological structure of strawberry depend not only on the rate of freezing and the size of ice crystals but also on the structures of individual types of tissue (Derbedeneva 1971). The susceptibility to textural damage is increased at low solids content (about 10%) (Szczesniak & Smith 1969). In plant tissue, the maintenance of shape is based on turgor pressure within individual cells. The structure of the plant collapses when turgor pressure is lost. The degree of susceptibility to loss of structure depends on factors such as the rigidity of the cell wall, the strength of the middle lamella bond, and the contents of the cell (Edwards 1995).

In order to maintain the original shape of the fruit it is sometimes necessary to modify its structure by pretreatment. One way of achieving this is to use calcium (Ca), which fortifies the fruit by changing the pectin structure (Poovaiah 1986; García *et al.* 1996). Additionally, pectinesterase can be used to catalyze cleavage of the ester bonds of pectin, thus forming anionic COO^- groups with which calcium ions can form salt bridge cross links to form calcium pectate.

Examination of the cell wall of the strawberry fruit has indicated very low levels of pectinesterase (PE) (Jones 1996). However, the use of commercially available fungal PE under optimized pre-processing conditions of pH and calcium levels has resulted in an improvement in the texture of heat-processed strawberries (Grassin & Fauquembergue 1994; Coutel & Dale 1998).

1.1 Strawberry

Strawberry is the succulent carrying achenes, or fruits proper, of small plants belonging to the family *Rosaceae* in the genus *Fragaria*. Plant growth depends on the rate of cell division, cell enlargement and the shape that expanding cells adopt. A strawberry is composed of five distinct tissue zones: epidermis, hypdermis, cortex, vascular tissue and pith (Figure 1). Epidermis forms the outer layer. The outer epidermal walls possess an overlaying cuticular wax. Epidermis is a protective tissue and consists of a single layer of densely packed cells. It consists of polygonal cells and long, pointed, thick-walled hairs. Embedded in the epidermis are achenes, commonly known as seeds and recognized by their dark colour and hard texture. Each berry can have from 20 to 500 achenes, depending on cultivar and environmental conditions (Perkins-Veazie 1995). Achene development occurs prior to the final increase in receptacle enlargement. The embryo consists of two large, semielliptical cotyledons, which contain protein and fat, but no starch (Hancock 1999). Hypodermis consists of meristematic cells and no intercellular spaces. Cortex or true flesh consists of rounded cells with intercellular spaces. Vascular tissue represents conducting tissues and is composed of xylem and phloem. Vascular bundles extend from the pedicel, traversing the pith, cortex, hypodermis and epidermis to the achenes. Xylems are long hollow strands of vessel elements consisting of dead cells, the walls of which develop secondary thickenings in the forms of rings, spirals and nets. Xylems serve as water-conducting tissues, and phloems as food-conducting tissues. Pith consists of thin-walled cells which often separate during the growth of the berry, leaving large cavities (Szczesniak & Smith 1969; Reeve 1970; Jewell *et al.* 1973). During ripening the cortex grows most rapidly and the pith most slowly (Havis 1943).
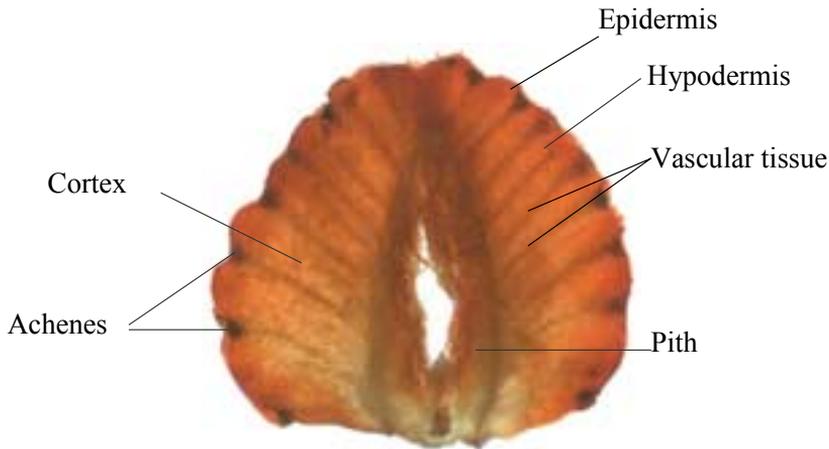
Figure 1. Cross-section of a strawberry (Fragaria x ananassa) showing the locations of different tissues.

1.2 Model of the primary cell wall

The cell wall was for a long time regarded as a non-living excretion of the living cell matter, but more evidence has been found that organic unity exists between the protoplast and the wall, especially in young cells, and that two together form a single biological unit. The cell wall grows when in contact with the protoplast. On the basis of the development and structure of plant tissues it is possible to distinguish the following principal three layers in the cell wall: the middle lamella or the intercellular layer; the primary wall; and the secondary wall. The middle lamella is the cement that holds the individual cells together to form the tissues and, accordingly, it is found between the primary cell walls of neighbouring cells. In supporting tissues it may also fill the intercellular spaces. The middle lamella consists mainly of pectic substances. The primary cell wall is the first true cell wall. The secondary wall is formed on the inner surface of the primary wall and consists of the outer layer, the central layer, and the inner layer (Fahn 1982).

A major goal in cell wall research is to understand the molecular basis of cell elongation, since it is the cell wall which places constraints on both the size and the shape of the cell (McCann *et al.* 1993). The cell wall structure of strawberries varies in the different tissues. The primary cell wall is a focus of

particular interest because it controls plant growth and morphogenesis. Secondary cell walls are virtually absent from mature fruits and the presence of any appreciable amount of secondary wall in vegetables usually makes them too tough and fibrous to be attractive as human food (Van Buren 1979). Although primary cell wall components have been chemically characterized, there is no single cohesive model of wall architecture at the molecular level (McCann & Roberts 1991). Previous models which considered the wall as one covalently linked macromolecule (Keegstra *et al.* 1973), or as a protein network coupled to a cellulose network (Lamport 1965), or as a cellulose-xyloglucan network (Fry 1989), are fragmentary in the sense that they all ignore certain major wall components (McCann & Roberts 1991).

The microscopic structure and molecular architecture of the cell wall have an important bearing on its function (Van Buren 1979). McCann & Roberts (1991) proposed a model of cell wall architecture after applying Fourier Transform Infrared (FT-IR) microspectroscopy to the sudy of plant cell walls. Figure 2 shows a type I cell wall typical to most flowering plants. A network of cellulose microfibrils is interlocked with hemicellulose. This strong framework is embedded in a dense matrix of pectic polysaccharides and reinforced by a separate network of structural proteins (Peña *et al.* 2001). In onion 5–12 nm diameter cellulose microfibrils are cross-linked by hemicellulose bridges about 20–40 nm long, which prevent the lateral association of cellulose. The primary cell wall can accommodate only about four layers of parallel-running microfibrils and no weaving is seen between layers. The middle lamella pectins are Ca^{2+} cross-linked to primary cell wall pectins forming a network, which is co-extensive with, and independent of, the cellulose/hemicellulose network, and which can be removed without affecting the structural integrity of the cellulose/hemicellulose network (McCann & Roberts 1991).
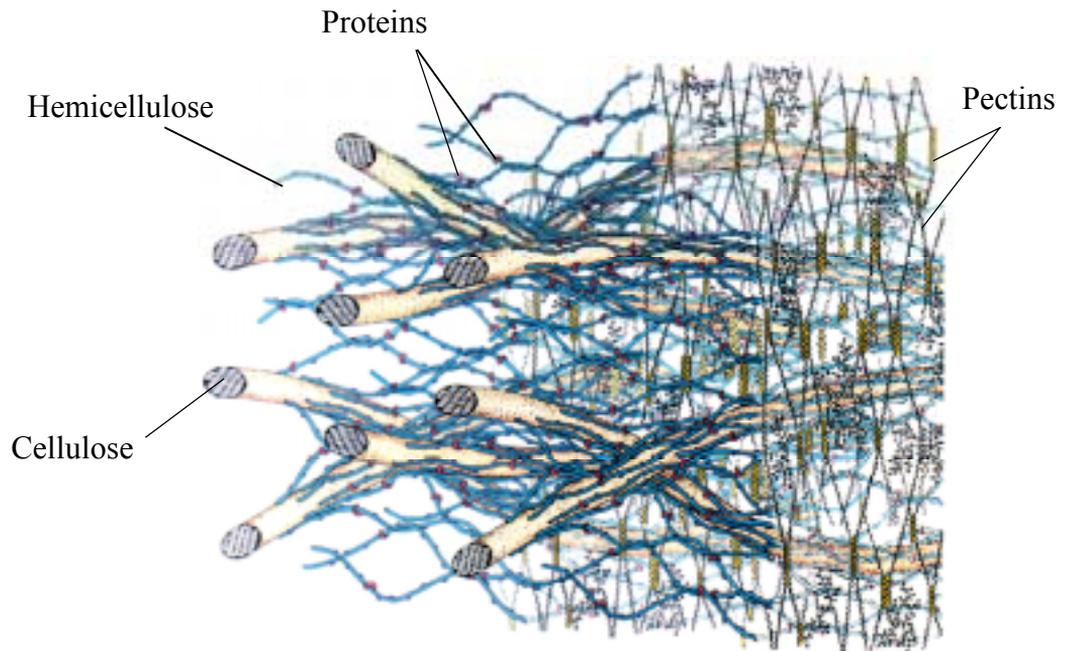
Figure 2. A three-dimensional molecular model of the wall shows the molecular interactions between cellulose, hemicellulose (blue), pectins (yellow), and wall proteins (red). Because hemicellulose has only a single face which can hydrogen bond to another glucan chain, several xyloglucans have been depicted as woven to interlace the microfibrils (Carpita & Gibeaut 1993).

1.2.1 The chemistry of textural compounds

The nutrient contents in 100 g of the strawberry fruit is as follows: 89 g water, 7.4 g carbohydrates (2.1 g sucrose, 2.4 g fructose and 2.9 g other carbohydrates), 2.4 g fibres (0.81 g pectin, 0.5 g cellulose, 0.7 g lignin and 0.4 g other fibres), 0.6 g protein, 0.47 g mineral compounds and 0.4 g fat (Rastas *et al.* 1989).

Plant cell walls are made up of cellulose microfibrils embedded in a matrix consisting largely of pectic and hemicellulosic polysaccharides and

hydroxyproline-rich glycoproteins, lignins, lower molecular weight solutes and water. The relative composition of the wall varies from the plasmalemma, separating the wall from the cytoplasm, to the middle lamella. In the primary wall there are similar percentages of pectic substances, hemicelluloses and cellulose. Water has four major functions in the walls. It is a structural component as part of the matrix gel, it can serve as a wetting agent interrupting direct hydrogen bonding between polymers, it can cooperate in stabilizing the conformation of polymers, and it serves as a solvent for the presence and transport of salts, low molecular weight organic compounds and enzymes (Van Buren 1979). Including both protein and xyloglucan in the hemicellulose fraction, cell walls contain 34% pectin, 38% hemicellulose and 26% cellulose (Keegstra *et al.* 1973). In a very simplified sense the cellulose has the function of providing rigidity and resistance to tearing, whereas pectic substances and hemicelluloses confer plasticity and the ability to stretch (Van Buren 1979).

1.2.1.1 Cellulose, hemicellulose and lignin

Cellulose is a β- (1 → 4) glucan containing 8–12 thousand glucose units per chain. It is present in the primary cell walls as linear associations of the polymer molecules called fibrils. Fibrils form a loosely interwoven network (Van Buren 1979). Analysis of the cellulose content of hydrated cell walls suggests that 60–80% of the cellulose is noncrystalline (Foster *et al.* 1996).

Hemicelluloses are non-cellulosic wall polysaccharides other than pectins. The principal chain of a particular type of hemicellulose is made up largely of one type of monosaccharide, giving rise to galactans, xylans, glucans etc. It often appears that these main or interior chains have a preponderance for another type of sugar as side chains. Thus e.g. in xyloglucan, xylose is the side chain, or in arabinogalactan arabinose is the side chain. A rather regular system such as this has been proposed for most of the hemicellulose content of the cell wall (Van Buren 1979). Only one surface of the xyloglucan backbone is able to bind to a microfibril. Alternating side chains straighten the backbone, and this conformation may facilitate the close packing of xyloglucan to cellulose. Xyloglucans occur in at least two domains in the wall. In one, they align with and bind tightly to cellulose microfibrils, and in the other, they span the distance between the microfibrils to tether microfibril neighbours (Peña *et al.* 2001).

Lignins, present in some secondary cell walls, are complex networks of aromatic compounds. They are composed mainly of the monolignols, p-coumaryl, coniferyl and sinapyl alcohols, and linked together by ester, ether or carbon-carbon bonds. The diversity of mono-lignols and their possible inter-molecular linkages give a remarkably complex structure. The aromatic network also tightly associates with cellulose and other polysaccharides in the cell wall (Peña *et al.* 2001). Lignin replaces water in the cell wall matrix and, in doing so, increases the rigidity and cohesion of the walls. Lignin is absent or in very low concentration in the cells of fruits and vegetables in their edible stages, except for the specialized vascular and structural tissue cells (Van Buren 1979).

1.2.1.2 Pectins

The principal constituent of the pectin polysaccharides is D-galacturonic acid, joined in chains by α-(1 → 4) glycosidic linkages. Inserted into the main uronide chain are rhamnose units, joined to the reducing end of the uronide by (1 → 2) linkages and the nonreducing end of the next uronide unit by (1 → 4) bonds. Rhamnose introduces a kink into the otherwise linear chain. Often arabinan, galactan, or arabinogalactan side-chains are linked (1→ 4) to the rhamnose. In the side-chains, the arabinose units have (1→ 5) linkages, whereas galactose are mutually joined mainly by (1→ 4) linkages, but (1→ 3) and (1→ 6) linkages also occur. Other sugars and acids, such as L-fucose, D-glucose, D-mannose, D-xylose, and D-glucuronic acid are sometimes found in side-chains (Van Buren 1991). Linear chains contain from 200 to 1000 galacturonic acid units (Figure 3). Pectins are described as 'block' polymers since they contain 'smooth' blocks (homogalacturonans) and 'hairy' blocks (rhamnogalacturonan) containing numerous other sugars in the side-chains as described above (Hall 1981; Darvill *et al.* 1980; Jarvis 1984). Pectinase-resistant 'hairy' blocks include rhamnogalacturonans I and II (RG I and RG II), best characterized in the walls of cultured sycamore cells (Darvill *et al.* 1980). The 'smooth' and 'hairy' blocks of apple pectin are shown in Figure 4.
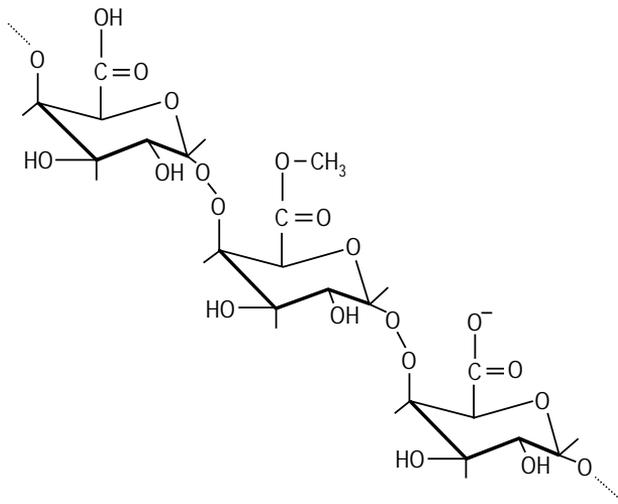
Figure 3. Structure of pectins (Oakenfull 1991).
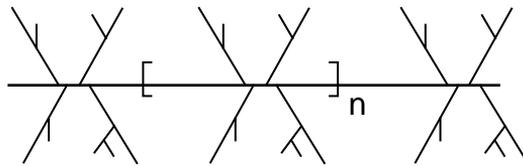
Figure 4. 'Smooth' (linear galacturonan) and 'hairy' (rhamnogalacturonan) blocks without side-chains in apple pectin (Pilnik & Voragen 1993).

Pectin chains aggregate and form a polymer network, a gel. Due to their bent shape they create cavities between them, which become occupied by carboxyl and hydroxyl groups (Kratz 1993a). Homogalacturonans, which together with RG I and RG II comprise the bulk of dicotyledonous pectins, are known to form rigid insoluble gels in the presence of Ca^{2+}; cross-linking requires about 12 consecutive unesterified galacturonic acid residues, and therefore the block-wise distribution of ester groups enables local Ca^{2+} bridging (Figure 5). Pectins cement adjacent cells together, and the use of pectinolytic enzymes and chelating agents causes rapid release of single cells from many soft plant tissues (McCann & Roberts 1991).

OH OH O COO

O COO⁻ HO OH

HO Ca²⁺

OH ⁻OOC O

COO O O OH

HO
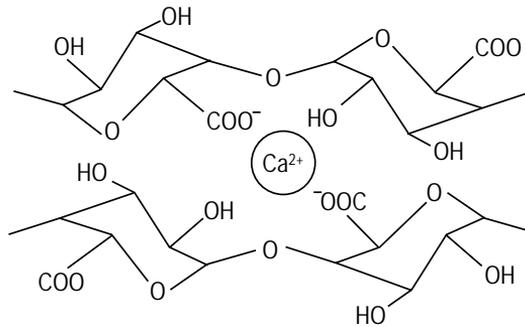
Figure 5. Chelation of calcium ions as cross-links between carboxyl groups of adjacent polyuronide chains; an 'egg-box' cavity (Axelos & Thibault 1991).

Although fruit texture changes are not completely understood, it appears that considerable softening results from degradation of the middle lamella of the walls of cortical parenchymal cells, with increased release of pectins. Hemicellulose and cellulose degradation may also contribute to softening (Perkins-Veazie 1995).

Stages of development of the berry are usually classified as small green, large green, white, pink, or red (ripe) (Perkins-Veazie 1995). The pectin content of strawberries does not change during the mature green and turning stages but decreases thereafter, during the ripening and overripening stages (Kwang *et al.* 1998). In unripe fruits, most of the pectic substances are present as insoluble proto pectin. During the processes of growth and ripening, protopectin is gradually transformed into water-soluble pectin. This transformation leads to softening of the tissue during ripening (Molyneux 1971). There is a correlation between swelling and the degree of pectin solubilisation. Swelling is associated with movement of water into voids left in the cellulose-hemicellulose network by the solubilised pectin (Redgwell *et al.* 1997).

Generally, softening of fruit is clearly a multiphase process in which both cell wall strength and cell/cell adhesion play important roles. The texture of fruit will depend on the balance between these two parameters (Hallett & Harker 1998). Thermally induced softening of onion tissues is retarded by calcium cross-linking of pectic polysaccharides involved in cell-cell adhesion, particularly

when the degree of galactan branching is developmentally reduced (Ng *et al.* 2000).

Most of the pectic substances extracted by chemical means are calcium-linked. All extracted pectin is accompanied by various levels of proteins. Xyloglucans are thought to be present in calcium-sequestered fractions because of the high levels of glucose and xylose observed in ion-exchange chromatography (Legentil *et al.* 1995). Although it is clear that pectins are released from strawberry cell walls during ripening, there is little information about why strawberry cultivars differ in firmness (Perkins-Veazie 1995).

The diversity of wall components and even the ability of oligosaccharides to elicit a variety of responses from the cell indicate that a wide range of architectural modifications must occur across a wall, between tissues, and in specialized wall domains. In onion parenchymal tissue, three distinct layers can be delimited across the cell wall: an outer zone containing methylesterified pectins, an inner zone containing unesterified pectin and a middle lamella zone which contains very densely staining rod like polymers (McCann & Roberts 1991).

1.2.1.3   Proteins

Chemical studies have shown that proteins are always present in isolated cell walls. The amount of organically bound nitrogen varies from 0.5 to 2.0% of the dry matter (Van Buren 1979). Lamport & Northcote (1960) presented the first evidence for an integral primary cell wall protein containing all the cell's hydroxyproline, and it was later proposed that hydroxyproline-rich glycoprotein must play a structural role in the wall, and that since the protein component of secondary walls is negligible, it must therefore be involved in extension. In addition to hydroxyproline-rich glycoprotein there are three other classes of structural proteins: proline-rich protein, glycine-rich protein and arabinogalactan-protein. Proteins may form a scaffold around the carbohydrate matrix and contribute to the variety of textures (Peña *et al.* 2001). According to Kwang *et al.* (1998) water- and salt-soluble protein contents in strawberry do not change throughout ripening but the cell wall protein content decreases.

1.3 Texture studies of cell walls

1.3.1 Bright-field, epifluorescence and CLS microscopy

Light microscopy is a well-developed and increasingly widely used technique for studying the microstructure and composition of food systems in relation to their physical properties and processing behaviour. The general advantage of bright-field microscopy is that it is normally carried out under ambient conditions of temperature and pressure, which tends to make specimen preparation easier. It is also possible to use a wide range of stains and contrast techniques to provide information about a specimen. Furthermore it is also relatively easy to obtain hot and cold stages, which allow temperature effects to be observed *in situ*. Two main disadvantages prevailing when using bright-field microscopy are lack of resolution and restricted depth of field, which limits 3-D presentation (Brooker 1995).

Epifluorescence microscopy has been much improved by the advent of incident light or epifluorescent instruments. Because fewer filters are required by this technique it is now possible to obtain much brighter images and consequently fluorescent stains can be used to detect constituents at rather low levels. It can also be used in conjuction with immunological techniques to indicate the specific localisation of substances (Lacey 1989).

One of the main advantages of CLS (confocal laser scanning) microscopy is the minimal degree of sample preparation that is required. It is well suited to fruits and berries containing high amounts of water. Lapsley *et al.* (1992) used a CLS microscope to differentiate apple varieties on the basis of their microstructure. Because of the optical sectioning capability of the CLS microscope, the specimen does not require prior fixing, embedding, or sectioning. Not only does this save time, but physiological processes and physical structure can also be monitored undisturbed and free of artifacts caused by complex sample preparations (Blonk & van Aalst 1993; Vodovotz *et al.* 1996; Webb & Rogomentich 1999).

The instrument uses a focused, scanning laser to illuminate a sub-surface layer of the specimen in such a way that information from this focal plane passes back through the specimen and is projected onto a pinhole (confocal aperture) in front

of a detector (Figure 6). Only the light from a defined focal plane in the specimen is able to pass through the confocal aperture, reach the detector, and produce an image, which is effectively an optical slice. By moving the specimen upwards relative to the focused laser light, large numbers of consecutive optical sections with improved lateral resolution (compared with conventional light microscopy) can be obtained with a minimum of sample preparation. The thinnest slice that can be obtained with a high numerical aperture lens is less than 1μm (Brooker 1995).
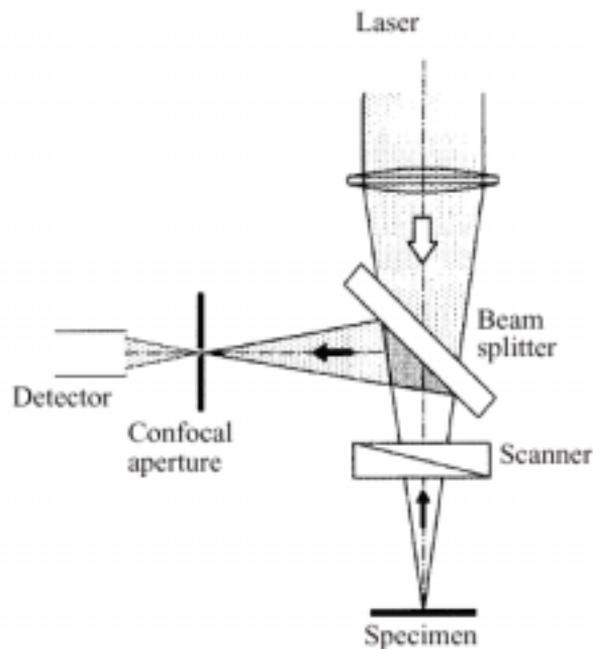
Figure 6. Diagrammatic presentation of an incident light confocal system shows the excitation beam and the reflected fluorescent light (Brooker 1995).

Most commercially available CLSM instruments are used as confocal fluorescence microscopes, although it is also common for them to have facilities for transmitted laser light and reflected light microscopy. In the fluorescence mode of operation, images of various chemical components, such as proteins, carbohydrates, lipids and ions, are produced by using the laser light to excite a selective fluorescent dye that has already been introduced or allowed to diffuse into the food system (Brooker 1995; Vodovotz *et al.* 1996). Confocal microscopes are in many cases equipped with more than one detector, which

allows simultaneous detection of more than one wavelength band, resulting in multi-component images (Blonk & van Aalst 1993).

Thus, if the laser (or lasers) in the CLS microscope produces light of two or more wavelengths, and dyes are introduced into the sample which excite at these differing wavelenghts, it is possible to obtain multiple images of the same field showing the spatial distribution of different chemical components, such as fat and protein, which can then be superimposed to show their relative distributions. The Ar/Kr laser, for instance, produces three lines at 488, 568 and 647 nm (Brooker 1995).

1.3.2 FT-IR microspectrometry

The energy of molecular vibration corresponds to the IR region of the electromagnetic spectrum. For IR radiation to be absorbed by a molecule, there must be an interaction of the radiation with an oscillating dipole moment associated with a vibrating bond. The vibration must cause a change in a dipole moment for the absorption to occur, and consequently, strong absorption occurs only for asymmetric bonds (McCann *et al.* 1992).

Fourier transform infrared (FT-IR) spectroscopy has been a used as a probe of the various chemical and conformational changes that occur during sequential polymer extraction from the cell. The results indicate a wide potential for the use of FT-IR microspectroscopy in cell wall research. The cell wall represents polymer systems in complex mixtures of both structure and composition. Wall constituents have characteristic spectral features that can be used to identify fingerprints of these polymers without, in most cases, the need for any physical separation. It is not possible to assign each IR band in the cell wall spectra because of their complexity. The variation in intensity or absence of some bands in the spectra, however, reflects compositional differences between cell walls (Kačuráková & Wilson 2001).

Figure 7 shows a typical FT-IR microscope. The FT-IR is externally very similar to an ordinary light microscope. The internal structure is identical except that all-reflecting silver mirrors replace the conventional glass lenses in order to permit the transmission of infrared light. At an appropriate time, infrared light is

directed into the microscope from the spectrometer. After reflection from many mirrors, it is focused. In this procedure, adjusting the aperture controls the part of the sample to be illuminated. After passing through the aperture, the light is eventually focused by the objective onto the sample. The light passing through the sample is then collected by the condenser and refocused through a second adjustable aperture. The infrared light then arrives at the detector and a spectrum is generated (Wilson 1995).

IR spectroscopy was not used to investigate biological systems until recently because of the severe problem of blanket absorption of water over most of the IR region. However, this has been revolutionized by Fourier transform data acquisition techniques, computer control, and new methods of sample presentation. Coupling the spectrometer to an appropriate microscope allows selection of a particular area (as small as 10 x 10 μm) in the field of view for microsampling. Spectra can be obtained from a defined region of a single cell wall or from bulk samples (Figure 8) (McCann *et al*. 1993; Wilson 1995).

Recent work with carrot has shown that biopolymers such as proteins, phenolics and pectins are oriented in carrot cell walls and that changes in their orientation occur during growth. Polarized infrared spectra of cells from the epidermis, cortex and xylem of carrot root have been acquired. The results show that different molecules are aligned in the different cell types. In the epidermis, both acidic and esterified pectin is found to be aligned, as well as a small amount of protein. Inspection of the original spectra of the epidermis shows that a considerable amount of material is aligned. In contrast, the xylem contains less aligned material, which appears to be mainly protein, with some indication of oriented phenolic material. During growth, acidic pectins are converted to methyl esterified pectins, which are then de-esterified following growth (Wilson 1995). During growth, the strength of the pectin network is reduced to facilitate expansion, with a rigid network re-formed after growth to give strength to the wall (McCann *et al.* 1993).
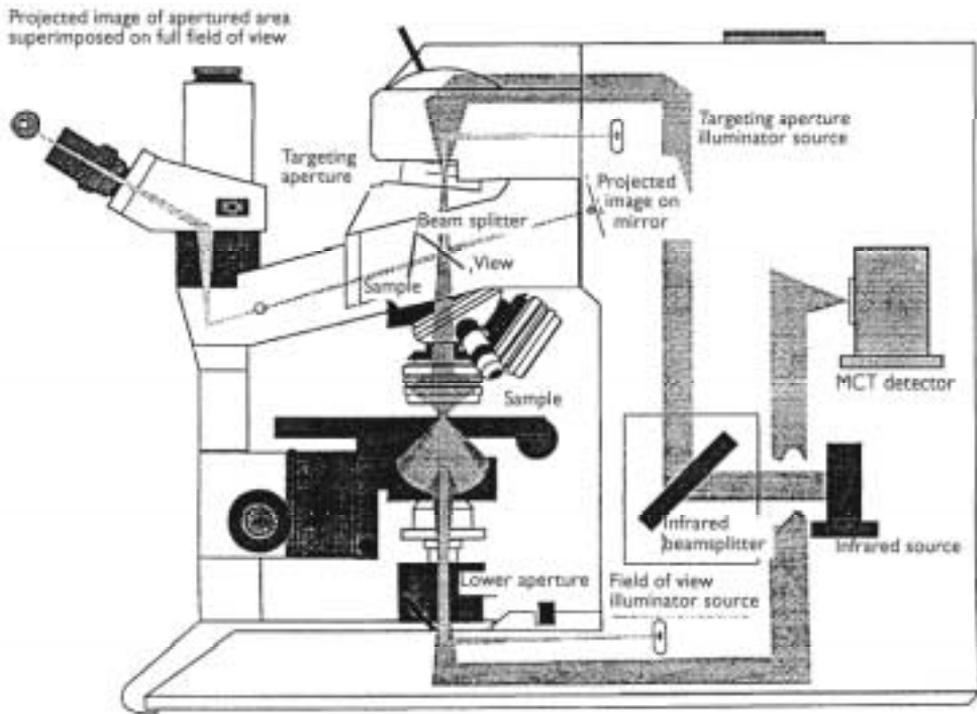
Figure 7. Cross-sectional view of an FT-IR microscope (Wilson 1995).
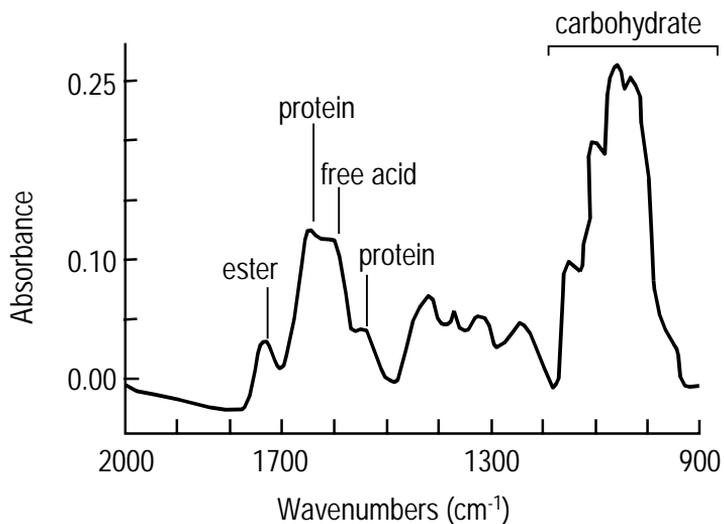
Figure 8. Spectrum of a small area (50 x 50 μm) of cell wall of tomato illustrating the main features of interest for analytical purposes (Wilson 1995).

1.4 Pretreatments

1.4.1 CaCl$_2$

Calcium has received attention because of its effects in delaying senescence and mould development and controlling physiological disorders in fruits and vegetables. Studies on leaf senescence and fruit ripening have indicated that the rate of senescence often depends on the calcium status of the tissue and that by increasing calcium levels, various parameters of senescence such as respiration, protein and chlorophyll content, and membrane fluidity are altered (Poovaiah 1986). Foliar application of calcium chloride (CaCl$_2$) to strawberry plants a few days before harvest increases fruit calcium content and influences several post-harvest senescence changes involving free sugar, organic acid, anthocyanin contents, texture and electrical conductivity (Chéour *et al.* 1990). The response to the treatment varies with the cultivar and apparently depends on the calcium content of the fruit at the time of the treatment and on the ability of the plant to accumulate and distribute calcium (Chéour *et al.* 1991).

Much of softening results from degradation of the middle lamella of the walls of cortical cells with increased release of pectins. In many fruit, such as tomato, endo-polygalacturonase begins water solubilization of pectins while exo-polygalacturonase completes hydrolysis (Perkins-Veazie 1995). Firmness changes in strawberries during senescence and in the absence of polygalacturonase have been linked to changes in ionic stability of the middle lamella. Divalent calcium ions (Ca^{2+}) normally occur between the cells, where they form crosslinks between the carboxyl groups of adjacent polyuronide chains as shown in Figure 5 (Van Buren, 1979; Main *et al.* 1986). The middle lamella is generally described by the 'egg box' model in which pectins have few ramifications and a low methoxyl rate (Figure 9). For calcium to be an effective firming agent, a low degree of methoxylation must be present. As a result of formation of calcium pectate in the cell wall, calcium may decrease softening by cell-wall macerating enzymes produced by plant pathogens. If the activity of pectin esterase is enhanced only during heating, the exogenously supplied calcium will be able to form calcium pectate, which will increase decay resistance. A major increase in calcium may actually decrease pectinesterase activity (Sams *et al.* 1993).
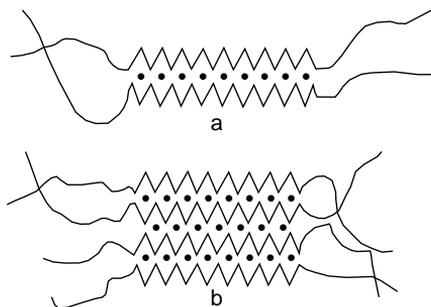
Figure 9. A schematic presentation of how calcium ions (black points) bind to polygalacturonic acids: (a) an 'egg box' dimer; (b) aggregation of dimers (Axelos & Thibault 1991).

Calcium is more effective in firming thermally processed than frozen fruit. This increased firming action is probably due to breaking of chemical bonds on the polyuronide chains during heating rendering more sites available for the formation of calcium pectate crosslinkages in the middle lamella region (Main *et al.* 1986).

Calcium tends to occur in greater concentrations in the proximal portion of the fruit than in the distal part. Calcium concentrations are highest in achenes and lowest in inner receptacle tissue (Davies & Dennis 1983; Makus & Morris 1998). Generally, the insoluble parts of many fruits are especially rich in calcium. The variation in the results of mineral analyses of whole strawberries most probably reflects differences in the relative proportion of achenes to flesh. This calcium is not completely liberated into continuous matrix of the jam during the cooking procedure and consequently only partly influences the gelation temperature of the low ester pectin. A considerable difference between total calcium and 'free' calcium in the fruit may, however, indicate a tendency of the final jam to build up additional gel strength slowly. The amount of total calcium in strawberries is about 350 ppm, of which 150 ppm should be in the free state. The bound form could play a role in the resistance of the cell walls to attack by fungal enzymes. A negative correlation (r = –0.8) is found between Ca^{2+} and the survival of fungal polygalacturonase in the liquor of sulphite-treated fruit (Davies & Dennis 1983).

Studies have been made of treating strawberries with calcium chloride (García *et al.* 1996) or calcium lactate or other calcium salts in a vacuum before freezing

and thawing (Polesello & Crivelli 1971; Morris *et al*. 1985; Main *et al*. 1986 and Berbari *et al*. 1998) in order to avoid texture rupture. The pretreatments increased the calcium contents of the fruits, enhanced their firmness independently of species and variety and also increased the soluble solids content. Pretreatments proved to be effective for reducing the drip loss and the ascorbic acid loss of thawed berries. The treatments did not affect the sensory quality of fruits.

1.4.2 PME

The ability to dictate biochemical outcomes in food with the incorporation of pectinesterase, also called pectylhydrolase and pectin methylesterase (PME), was an important development in the fruit and vegetable industry. A major product of the action of PME on pectin is methanol. The amount generated during fruit and vegetable fermentation depends on the variety, pectin content, and level of active PME in the substrate (Sajjaanantakul & Pitifer 1991) (Figure 10). PME catalyzes cleavage of the ester bonds between the methyl groups and the carboxyl groups of pectic substances, thus forming anionic COO– groups with which calcium ions can form salt bridge crosslinks. Calcium pectate is thus formed, which is assumed to anchor the pectic substances and result in an overall increase in firmness (Baker & Wicker 1996). Pretreatments could be combined with heat treatment. Heat allows demethylation of pectin by PME (Sams *et al*. 1993). PME is naturally present in many plants and micro-organisms. It can be synthesized by plants, moulds, and bacteria. Most commercial pectinases are a mixture of PME, polygalacturonase and polygalacturonate lyase, and pectin degradation therefore entails depolymerization as well as demethylation. Purified PME appears to act on polyuronides containing an unesterified carboxyl group adjacent to a methylated carboxyl group. It does not act on the ester bond of a monomer that is between two adjacent, esterified monomers. Furthermore it does not completely deesterify pectin; the action stops at a certain degree of esterification, possibly limited by the effect of small substrate polymer size. Plant PME de-esterifies pectin linearly, creating blocks of free carboxyl groups, ultimately resulting in a calcium-sensitive form of pectin. The sequential action is believed to start on the methylester groups next to free carboxyl groups. The action of microbial PE may be blockwise or random (Sajjaanantakul & Pitifer 1991).
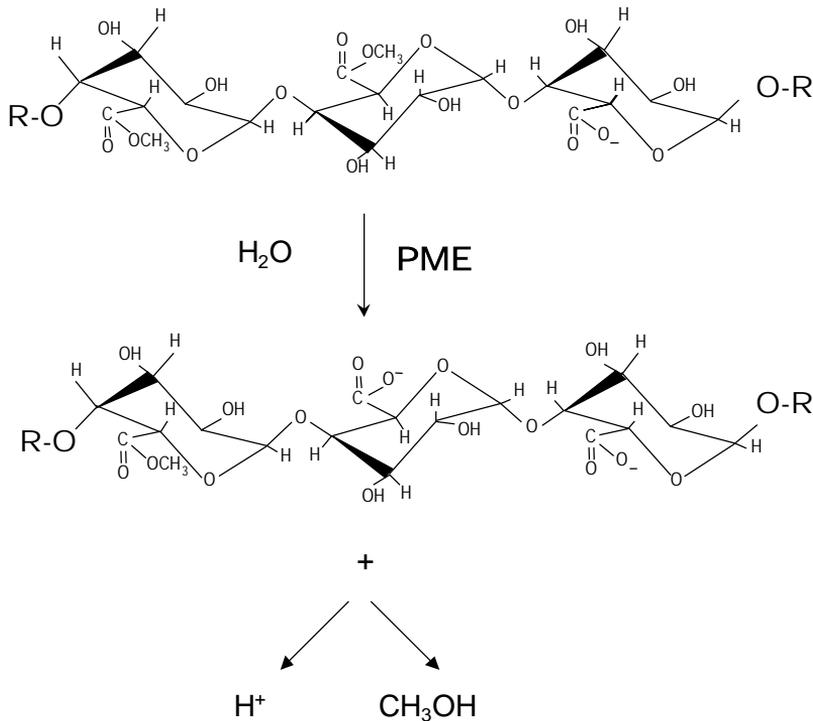
Figure 10. Pectin demethylation by PME (Sajjaanantakul & Pitifer 1991).

The simplest form of enzyme treatments is to enhance the activity of enzymes that are naturally present in fruit or vegetable tissue (Sajjaanantakul & Pitifer 1991; Baker & Wicker 1996; Steele *et al.* 1997). The polygalacturonase and cellulase activities of strawberry fruit tissues increase markedly during ripening and the changes are reflected in a reduction in fruit firmness. Pectin esterase activity decreases during ripening but is increased in date during ripening although the degree of esterification of pectin decreases (Neal 1965; Barnes & Patchett 1976; Huber 1984; El-Zoghbi 1994).

Enhancement of PME activity occurs in the presence of either monovalent or divalent cations, but not anions. There is an optimal cation concentration for optimal activity under specific conditions. The activation is more prominent at lower pH, and is independent of enzyme and substrate concentrations. Cations increase PME activity but are not a requirement for this activity. High salt concentrations depress the activity. Differences in the effects initiated by different cations suggest that they may play different roles in PE activity. The

effect seems also to depend on the source and hence the nature of the PE itself, and on pH (Sajjaanantakul & Pitifer 1991).

Fungal PME (pH optimum 4.0–5.5) has the potential for use in the gelling of low-sugar fruit products. The generation of LM-pectin *in situ* endows PE with the ability to thicken canned foods (Sajjaanantakul & Pitifer 1991). Vacuum infiltration of peaches with a solution of PME and $CaCl_2$ for 1 h significantly increased the firmness of canned peaches. The specific activity of PME in peach halves increased more than 20-fold after infusion (Javeri *et al.* 1991).

1.4.3 Sucrose

The added sucrose acts as a dehydrating agent in order to decrease the water content of strawberries. Water is transferred out of the fruit into the syrup matrix and sucrose diffuses into the berries (Ponting *et al.* 1966). The reduction in free water causes reduced cell wall rupture and rearrangement of the cell contents during freezing. Decreasing drip losses ensure better fruit appearance, flavour, aroma and rheological behaviour in cooking. Absorption of sucrose by the pulp of the peach improves the gelatinization and firmness of the frozen fruit, due to hydrogen bonds formed between the sucrose and pectin. High concentrations of sucrose (10 and 30%) did not favour the entry of calcium from added 6% $CaCl_2$ solution into the fruit (Polesello & Maltini 1970). Sucrose addition has a significant protective effect on anthocyanin pigment content and also retarded browning and polymeric colour formation of strawberries during several years of freezer storage (Wrolstad *et al.* 1990).

1.4.4 Vacuum infusion

The gas content of some fruit tissues adversely affects the technological operations as well as the colour and flavour of the final product (Escriche *et al.* 2000). Strawberries contain 18–22 vol.% gases. Vacuum techniques have been used to increase the incorporation of sugar and firming agents in strawberries (Kolev *et al.* 1983). Fito (1994) described a rapid mass transfer phenomenon which occurs when porous structures are immersed in a liquid phase. This involves the in-flow of the external liquid through the capillary pores. Reduced

pressure is imposed in a solid-liquid system, followed by restoration of atmospheric pressure. During the vacuum step the internal gas in the fruit pores expands and partially flows out. In the atmospheric step, the residual gas is compressed and the external liquid flows into the pores as a function of the compression ratio.

Neither the duration of the time under vacuum nor at atmospheric pressure has an influence on the deformation and impregnation levels of the fruits in the examined time scales of 5–15 minutes (Salvatori *et al.* 1998). However, pressure changes can promote deformation of the fruit because of the viscoelastic properties of its solid matrix. A high pressure variation effected by rapid vacuum release can reduce the effectiveness of the process by crushing some tissues. Even when the vacuum is released gradually, tissues can be seen to compress. Ideally, vacuum release should be sufficiently slow to permit the porous tissue that is being infused to rebound to its original shape while absorbing the solution (Baker & Wicker 1996). The use of a vacuum impairs the overall appearance of the fruit somewhat, which may however not be noticeable in a jam-type product (Main *et al*. 1986).

Cell wall porosity and macromolecular size limit the effectiveness of osmotic diffusion. Treatment of soybean cells with pectin esterase enlarges the trans-wall channels without affecting cellular viability, indicating that cell wall pectins are responsible for the sieving effect (Baron-Epel *et al*. 1988). A vacuum infusion of PME is not successful in all applications. An impermeable skin or lack of interior voids may minimize the effects of infusion (Baker & Wicker 1996).

1.5 Freezing and thawing

At least three factors are involved in the firmness of fresh strawberries: the turgidity of the living cell, types and amounts of cell contents, and the nature of the cell wall and middle lamella. Detrimental changes in histological structure of strawberry during processing depend not only on the rate of freezing and the size of ice crystals but also on the structure of individual types of tissues in strawberry raw material. No changes have been found in epidermal and xylem cells during freezing but significant rupture of parenchymal cells (cortex) does

occur. Varieties with large cells suffer more damage than those with small cells (Armbruster 1967; Derbedeneva 1971; Scholey 1973).

At the freezing point the ice crystals starting to form can rupture cell walls and intercellular structure. The enzyme and substrate released by this disintegration cause a considerable increase in the rate of deterioration of flavour and colour at this stage of freezing. A slow freezing process would further favour the formation of large ice crystals, causing great physical damage to the cell walls and accordingly a soft disintegrated product upon thawing. Freezing at slow rates (–0.19^{o}C/min or –0.05^{o}C/min) causes extensive degradation of carrot cell wall, as evident from the rapid loss in tissue firmness. Severe structural damage is due to growing ice crystals together with cell separation and substantial loss of pectic material from the cell wall and middle lamella (Roy *et al.* 2001).

The rate of freezing is a more critical factor affecting the quality of frozen and thawed strawberries than the rate of thawing. Quick tunnel freezing at –80°C for 14 min followed by rapid thawing in a microwave oven (3-step thawing program) preserves the chemical, physical and sensory quality of strawberries better than other ways of freezing (at –20°C without air circulation) or thawing (at +5°C in a refrigerator). The time of harvesting and the cultivar also have a great influence on post-freezing quality of strawberries (Heiska *et al*. 1999).

According to Gormley (1970), jam made from frozen fruit is as good as that made from fresh fruit and better than that made from SO_2-treated fruit. Prior to freezing, the fresh fruit is normally washed to improve its microbiological standard and physical appearance. A rapid, continuous cold washing process is preferred in order to minimize leaching of colour and flavour (Copenhagen Pectin A/S 1993). Rapid freezing with liquid nitrogen or individual quick freezing (I.Q.F.) with an air blast give high quality dessert fruit. On thawing, these fruits have a good texture and no drip (Gormley 1970).

After thawing, high enzyme activity in the fruit is observed. To minimize undesirable enzyme reactions, it is recommended to accelerate the thawing process (thawing in a preheating tank, or even better in a continuous thawing equipment) and to pasteurize the fruit shortly after thawing (Copenhagen Pectin A/S 1993).

Before storing the product in containers, the preserved pulp must be cooled to 20°C to avoid flavour and colour deterioration due to slow cooling in the storage container (Copenhagen Pectin A/S 1993). Sucrose addition (10, 20 and 40% by weight) has a significant protective effect on the anthocyanin pigment content of frozen strawberries and it also retards browning and polymeric colour formation during freezer storage at –15°C for 3 years. Thawing accelerates these colour degradative reactions (Wrolstad *et* al. 1990). Strawberry anthocyanin pigment fortification has a protective effect on ascorbic acid (Skrede *et al*. 1992).

1.6 Jam making

The general fruit content for jam is 35 g of edible fruit per 100 g of product, and the product can be made from fruit, fruit pulp or fruit purée (or a mixture of these) (Broomfield 1996).

In jam making, high mechanical forces are used because the level of dissolved solids increases from less than 10% to almost 70% in a matter of minutes. Sugar syrup is diffused into the fruit during jam making, while at the same time osmotic effects tend to remove water and cause collapse of the fruit. Intercellular air is also replaced by syrup. In addition, the surrounding matrix is heated to the boiling point of the syrup, at which temperature the cell contents are super-heated, resulting in elevated internal pressure. Microscopical examination of fruit pieces from strawberry jams has shown that only the epidermal and vascular tissues retain their structural integrity during the boiling stage of jam production, and that the bulk of the cells rupture (Cross 1981).

Jewell *et al*. (1973) found in their microscopical studies that the vascular strands and achenes of strawberries formed the majority of the structures which maintained their structural integrity during the jam-making process, although in many cases the epidermal cells were also still intact. The remaining cells, i.e. the parenchymous and hypodermal cells, almost all exhibited severe plasmolysis, cellular collapse, and in many cases the cell walls were ruptured. It was concluded that a relationship existed between the extent to which strawberries disintegrate while being boiled to make jam and the density of achenes on the surface of berries of a given size.

1.6.1 Ingredients

1.6.1.1 Strawberries

The annual production of strawberries in world has grown steadily through the ages, with quantities doubling in the last 20 years. Total production is currently approximately 2.6×10^9 kg worldwide (Belitz & Grosch 1999; Hancock 1999) and 11.8×10^6 kg in Finland (Information Centre of the Ministry of Agriculture and Forestry 2000). Processes are rather specific with regard to yield, maturity, colour, flavour, texture and size. Colour is one of the most important aspects of the attractiveness of fruit products. The pigments in fruits are mainly flavonoids (yellow) and anthocyanins (red), which are flavonoid glycocides containing glucose, rhamnose, arabinose, galactose and xylose (Scholey 1973).

An even jam production is dependent on access to fruit all year round. Various methods of preservation of fruit are used: canning, preservation with chemicals, preservation with sugar and freezing. Preservation with sugar and freezing were discussed in Sections 1.4 and 1.5. Canned fruits are often apricots, peaches and pineapples which are aseptically packed. Fruit is sterilised by heat and then cooled and filled aseptically into sterile containers, or hot filled into semi-bulk containers that are sealed and crash cooled by immersion in cold water (Broomfield 1996). Preservation with chemicals usually means preservation in containers with a combination of SO_2 and sodium benzoate. The preservatives may diminish enzyme activity, but unless the fruit is pasteurized, a certain deterioration in flavour must be expected during storage. To minimize these reactions, a storage temperature between 0 and 5°C is recommended. As SO_2 must be disposed of by evaporation prior to the jam manufacture, colour degradation and loss of volatile flavour components must further be taken into account (Copenhagen Pectin A/S 1993).

Strawberry jam aroma is strongly dependent on the aroma of the fruit. To obtain a good aromatic quality of strawberry jam, it is therefore essential to use high quality aromatic fruits. The addition of sucrose to the fruits before heating appears to have a very limited effect on the final aroma (Lesschaeve *et al.* 1991).

Ideally, the fruits should be uniformly sized between the limits of 5 and 18 g (Scholey 1973). The stability of frozen sliced strawberries is important. High

acidity and low pH stabilize strawberry colour by inhibiting polyphenol oxidase during frozen storage and thawing. Bright colour is related to acidity, the correlation coefficient being about 0.8. Objective testing for acidity, soluble solids, pH, firmness and colour of the fresh fruit is valuable for assessing the quality to be expected in the frozen product. Most of the better cultivars do not show much difference in instrumental CDM (Color Difference Meter) 'a' (redness) value between fresh and frozen sliced products (Sistrunk & Moore 1979).

According to Skrede (1982) pH, titrable acids and sugars of fresh strawberries are of little importance for jam quality. High contents of soluble solids and ascorbic acid are related to undesirable flavour properties of jams. Furthermore, high levels of ascorbic acid adversely affect colour stability. Colour degradation in jams is parallel to flavour deterioration. The main emphasis should be placed on the characteristics of the final products rather than on those of the fresh fruits.

Colour acceptability of sensory scores after 6 months of jam storage is highly correlated with the total anthocyanin content (Spayd & Morris 1980; García-Viguera *et a*l. 1999). According to Abers & Wrolstad (1979) reactive phenolics (leucoanthocyanins, flavonols) may play a major role in the colour deterioration of strawberry preserves.

1.6.1.2 Pectin

Pectin is used in jam making in an amount of about 1% of the total amount of ingredients. Commercial pectins are normally produced either from citrus fruits (containing 25% pectin) or from apples (containing 15–18% pectin) (Pilgrim *et al*. 1991). In both cases the residue from juice pressing is utilized as the raw material for pectin production (Copenhagen Pectin A/S 1993).

Some of the galacturonic acid units in the pectin molecule are esterified and are present as the galacturonic acid methyl ester as described in Figure 11. The degree of esterification of the pectin molecule is defined as the ratio of esterified galacturonic acid units to total galacturonic acid units in the molecule. High-methoxyl (HM) pectins are pectins with a degree of esterification above 50%. Low-methoxyl (LM) pectins are pectins with a degree of esterification below

50%. Commercial LM-pectins are generally produced from plant material containing HM-pectin. The transformation (de-esterification) of HM-pectin to LM-pectin accordingly takes place under controlled conditions by treatment in either mildly acidic or alkaline conditions. If ammonia (NH_3) is used in an alkaline de-esterified process, so-called amidated low ester pectin will result (Figure 11) (Copenhagen Pesctin A/S 1993).
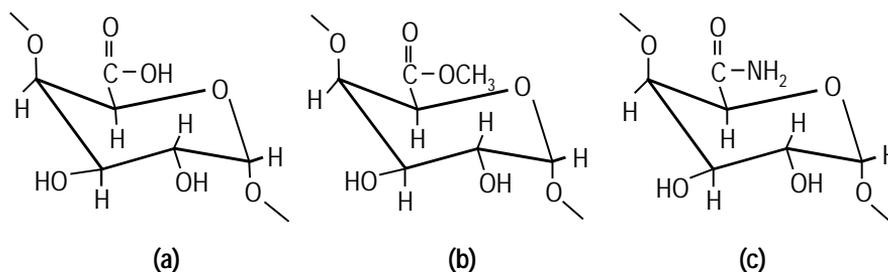
Figure 11. Functional groups: carboxyl (a); ester (b); amide (c) in low-methoxyl pectins (Axelos & Thibault 1991) .

To obtain gel formation in a system containing low ester pectins, the presence of calcium ions is crucial. LM-pectins may form gels at much lower solids concentrations than HM-pectins and greater variations in pH are tolerated without effect on gel formation. LM-pectin gels may melt when heated. Amidated low ester pectins are normally able to jellify jams at the ambient calcium level, i.e. with calcium ions originating from fruit and water. The degree of esterification and the degree of amidation largely determine the 'calcium-reactivity' of a specific LM-pectin. In practice degree of amidation and of esterification together control the relative setting temperatures of LM-pectins. Accordingly, commercial LM-pectins may be classified as rapid setting or slow setting or as more or less 'calcium-reactive'. Like HM-pectin, LM-pectin shows excellent stability at all temperatures in the pH-range 2.5–4.5 (Copenhagen Pectin A/S 1993). Mechanical reduction of pectin molecular weight significantly modifies the consistency but not the taste of the strawberry jam (Guichard *et al.* 1991).

Pectin molecules in solution adopt a helical configuration with three monosaccharide units per turn (Figure 12) and a pitch of 1.33 nm. The structure is stabilized by steric factors with a possible contribution from intramolecular hydrogen bonding (Oakenfull 1991).
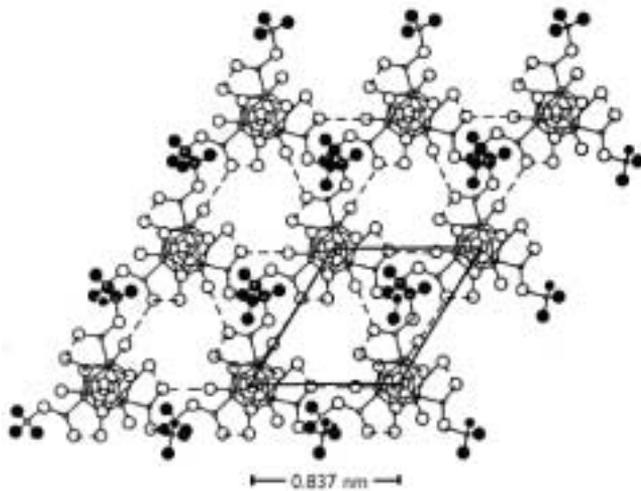
Figure 12. Polygalacturonic acid (DE = 100%) showing parallel chains packed in a hexagonal lattice. Hydrogen atoms of methyl groups are indicated by filled circles (Oakenfull 1991).

1.6.1.3 Sucrose

Generally more than 40% of total weight and 80% of total solids in a jam is sugar. In addition to its sweetening effect, the sugar has a number of functions in the jam. Sugar contributes to soluble solids, an effect which is essential for the physical, chemical and microbiological stability; it provides body and mouthfeel; improves appearance (colour and 'shine') and makes gelation of HM-pectin possible. The added sugar acts as a dehydrating agent for the pectin molecules, permitting closer contact between the chain molecules. Substituting sucrose with other sugars or polyols has an influence on the gelling characteristics of pectins and the texture of gels (Mulinari-Campos & Bileski-Candido 1995). This is due to the different water activities of the other sweeteners at similar soluble solids contents, or to substance specific differences in the stabilizing effect generated by the hydrophobic interaction (Oakenfull & Scott 1984).

The amount of sugar necessary to obtain a gel with the required firmness will depend on the pectin used and on the pH. Over 50% of sugar is needed for gel formation of HM-pectin. A higher sugar content tends to reduce spoilage (by decreasing water activity to approximately 0.8) and to increase stability in

transit; on the otherhand, an excessive amount of sugar will flocculate the pectin from solution (Molyneux 1971). The rate of osmotic dehydration does not increase significantly above about 67% sugar (Ponting *et al.* 1966).

In order to control the amount of invert sugar produced by boiling, two main factors must be considered: (1) the duration of the time during which the jam is at a high temperature; and (2) the degree of acidity or pH of the mixture. It is more satisfactory to control the formation of invert sugar by method (2), either by increasing the pH with sodium citrate or decreasing it with citric acid (Molyneux 1971).

Sucrose – beet or cane sugar – is by far the most important carbohydrate sweetener used by the jam processing industry. Various types of glucose and fructose syrups are used for health-promoting or diabetic products. It is also possible to transform the sugars in glucose syrup into the corresponding sugar alcohols (primarily sorbitol). By replacement of sucrose with other carbohydrate sweeteners (fructose, high fructose syrup, xylitol, sorbitol, lactose saccharin, cyclamate, or combinations of these) it is technologically possible to prepare jams with lower amounts of sucrose. The attainment of a suitable texture may be more difficult in xylitol and sorbitol jams than in jams with other sweeteners (Hyvönen & Törmä 1983).

1.6.1.4 Acids and preservatives

There are two reasons for adding acid: reduction of pH to a value giving satisfactory gel formation and increase of total acidity in order to enhance the fruit flavour. Acids are always added as a solution in water, usually as 50% w/v citric acid (Copenhagen Pectin A/S 1993). Acid should be added to the batch as late as possible after the boiling temperature has been reached-prior to the filling. The acid added will suppress dissociation of the free carboxyl groups and, consequently, the presence of negatively charged pectin molecules that repel each other. This facilitates closer contact between the pectin molecules and permits the formation of hydrogen bond bridges between undissociated carboxyl groups (Molyneux 1971). Without buffering, some variation in flavour and flavour intensity might be expected, since acid will migrate from the fruit pieces to the medium (Pilgrim *et al.* 1991).

A safe method of obtaining a sterile product is pasteurization of the sealed pack in a hot (90–95°C) water spray for 5–15 minutes. If preservation of the jam after the package has been opened is desired it is necessary to use chemical preservatives, commonly benzoic acid and sorbic acid either separately or together (often in the range of 0.05–0.10%). Both preservatives are only active in their undissociated form (acid form). The undissociated acid is capable of permeating the cell membrane of the microorganisms and interfering with enzyme systems in the cell to stop further growth. As both acids are sparingly soluble in water, they are always added as solutions (e.g. 20% w/v) of their neutral potassium salts immediately before addition of acid and packing, in order to avoid precipitation of the preservative (Copenhagen Pectin A/S 1993). Table 1 presents some common defects and their causes in jams.

1.6.2 Traditional jam making

Figure 13 shows a flow diagram of the basic processes in preserve manufacture. The prepared (separated from undesirable portions such as stalks, leaves, calices, etc.) fruits are weighed and charged to the boiling pans. The modern jam-boiling pan consists of a steam-jacketed hemispeherical bottomed vessel. The vessel is fitted with a low speed anchor-type stirrer fitted with wall-scraper blades. The first operation is to add the metered quantity of sugar from an overhead pipeline in the form of a syrup, invert sugar or any other syrup (Molyneux 1971). The manufacture of jams, jellies and preserves involves application of heat for the following reasons: to obtain sugar equalization (homogenous distribution of the soluble ingredients between the individual fruit pieces and the surrounding gel); to preserve product by deactivating enzymes and killing yeast and moulds present in the raw material; to concentrate the product by evaporation of water and to deaerate the product leading to better appearance (no air bubbles, deeper colour) and improved chemical stability (decreased oxidation of flavour and colour components). It is essential to carefully control processing and holding times at higher temperatures to avoid the following undesirable effects: loss of colour and flavour, sucrose inversion as well as browning reactions. Sugar should be added to the fruit at an early stage in the process to avoid extensive degradation of flavour and colour components (Copenhagen Pectin A/S 1993).

Table 1. Typical defects in jam (Pilgrim et al. 1991).

Defects	Causes
Too-rigid gel	a) Too high soluble solids content
	b) Too high pectin content
	c) Too high calcium content (LM-pectin)
Too-soft gel	a) Too high pH
	b) Too low soluble solids content
	c) Too low pectin content
	d) Too low calcium content (LM-pectin)
	e) Aged pectin (rapid-set converted to slow-set; HM converted to LM)
	f) Degraded pectin (heat + acid + time)
Excessive Foaming	a) Traces of protein
	b) Saturation with air
Haze formation	a) Impure ingredients (dust, fibre, etc.)
	b) Undispersed pectin (too large mesh size)
	c) Unclarified juice
Discoloration	a) Caramelization (non-uniform heating)
	b) Metal ions (Fe, Cu contamination)
Crystallization	a) Surface evaporation of water
	b) Too high glucose content
	c) Too high soluble solids content
	d) High tartrate content (grape juice)
Flaccid fruit tissue	a) Over-ripe fruit
	b) Excessive precooking
	c) Heat labile fruit
	d) Freeze labile fruit
	e) Oversized fruit (berries)
Pregelation	a) Non-uniform distribution of acid and calcium
	b) Cold additions of calcium or acid solution
	c) Temperature and concentration gradients
Syneresis	a) Unsuitable pectin choice
	b) Too high soluble solids content
Microbial contamination	a) Microscopic leaks (negative container pressure)

| Prepared fruit | Prepared pectin | Sucrose source | Glucose syrup | Minor ingredients |

Mixing vessel

Boiling process

Holding vessel

Filling process

Hold & despatch
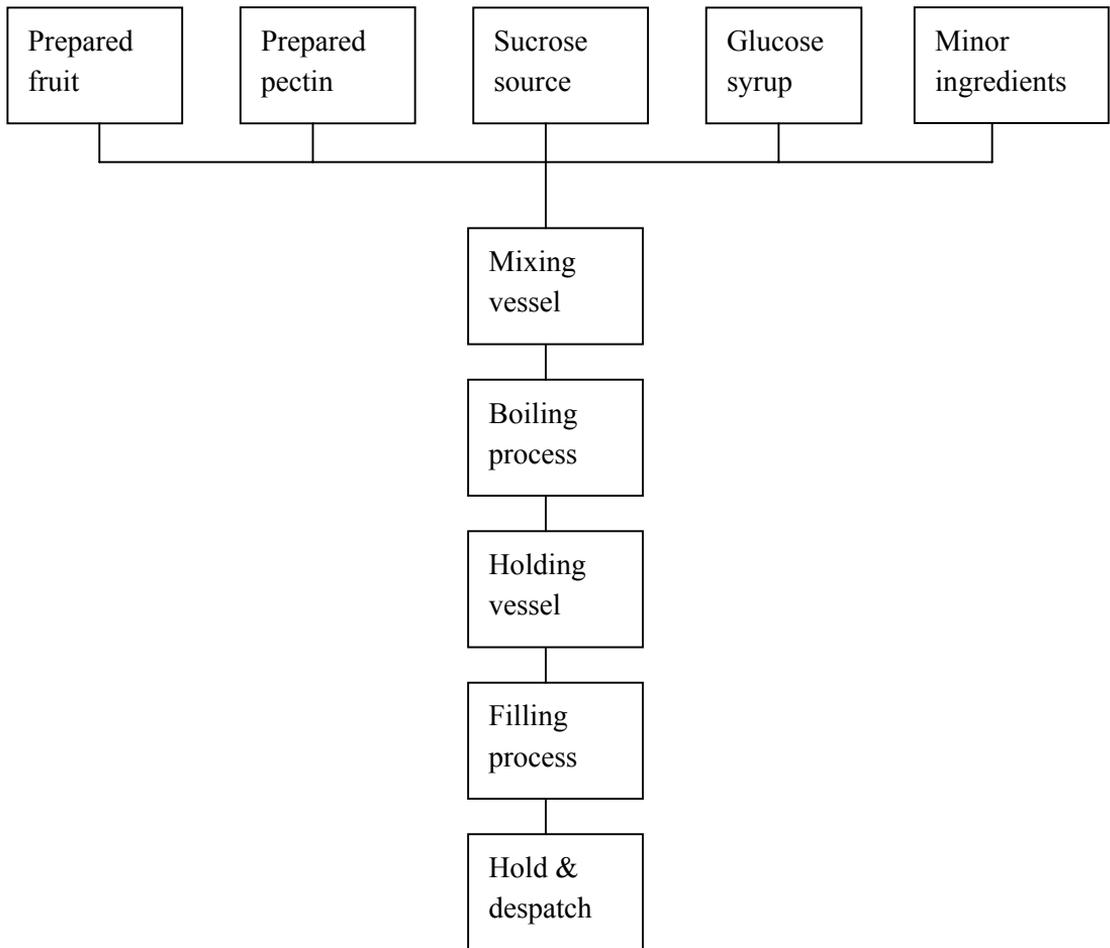
Figure 13. Flow diagram of the basic processes in preserve manufacture (Broomfield 1996).

The concentration of soluble solids is checked by a refractometer. Assuming that all the control tests (for example temperature, pH and soluble solids) are satisfactory, the hot pasteurised jam is blown to an insulated overhead tank fitted with a slow-moving spiral ribbon stirrer. From this tank, the hot jam is run to the charging tanks on the can-filling machines. The jam should fill the container while still hot (Molyneux 1971; Broomfield 1996).

Setting time is the time between the moment that all ingredients necessary for forming the gel are present in the heated solution in the correct proportions, and

the moment that the gel develops into a coherent mass. The setting time is determined by the cooling rate, i.e. the faster the cooling, the shorter the setting time. Also, the faster the cooling, the lower the setting temperature. The setting time is to a limited degree directly related, and the setting temperature is inversely related to pH; below a critical pH range (2.5–3.1), gel strength is independent of pH, but declines rapidly at higher pH. Setting time is inversely related to sugar concentration and pectin grade. Pectin grade is the ability of pectin to gel a quantity of sugar (Pilgrim *et al.* 1991).

1.6.2.1 Open pans

Traditional jam processing in open (atmospheric) pans may be considered as a simple upscaling of the domestic preservation process. The pans are hemispherical, with a large lip extension to assist in the prevention of boilover. The hemispherical part of the pan is steam jacketed, with high-pressure steam providing the heat source. Extra heating may be provided by the use of internal steam coils. The capacity of the pans may range from a few kilograms up to 100 kg or more (Broomfield 1996). Efficient evaporation takes place at temperatures above $100^{o}C$ and the product is often treated at this high temperature for extended periods of time. As a result the product is characterized by a considerable loss of flavour and colour, a high amount of invert sugar, and often extensive caramelizing and browning reactions. On the other hand the open pan process is unsurpassed with respect to terminating enzyme activity, ensuring complete sugar equalization and completely sterilizing the jam (Copenhagen Pectin A/S 1993). A reduced boiling time (4 min at atmospheric pressure) retains more natural fruit colour in the jam but does not affect ascorbic acid levels in strawberry jam. After 6 months of storage most of the aroma and flavour benefits of reduced boiling time have been lost (O' Beirne *et al.* 1987).

1.6.2.2 Vacuum pans

Preserves may be boiled under vacuum using either batch or continuous methods as well as by a combination of atmospheric pressure and vacuum. The capacity of batch vacuum cookers also varies from a few kilograms up to several tonnes (Broomfield 1996). The advantage of vacuum boiling is that the whole process

takes place at lower temperature (normally 60–75°C). The result is a lower fuel cost, better preservation of the natural colour and flavour of the fruit, and a reduction of the inversion and caramelization of sugar (Copenhagen Pectin A/S 1993). The pre-heated fruit/sugar mix is fed from the pre-heater by negative pressure into the kettle and the volume is reduced by boiling under vacuum with constant stirring. The vessel has a domed cover with a steam outlet to a barometric condenser and a jet ejector to maintain a low vacuum on the pan. To prevent foaming, edible oils and fats may be added during cooking (Kratz 1993a).

The low pressure applied during the process may further help to deaerate the fruit, leading to a shiny product with a deeper colour and less browning. For optimum aroma and colour preservation, the holding time in the process must be kept to a minimum. The whole cooking operation from the beginning of heating until the batch is transferred to the filler or holding tank should not exceed 25 minutes (Copenhagen Pectin A/S 1993).

The ratio between volatiles remaining in jam and lost during cooking varies according to cooking pressure, design of the cooker and structure of the volatiles. Condensation of vapours during cooking and incorporation of the condensate in pectin solutions could result in a more highly flavoured product (Lesschaeve *et al.* 1991).

1.6.2.3 Continuous cooking

By using scraped surface heat exchangers, continuous manufacture of jam in a closed system is possible. Using continuous jam manufacture, the processing time at high temperatures can be minimized. As a result, natural flavour and colour of the fruit is optimally preserved and energy expenditure is also lower, but the system may sometimes present problems with respect to obtaining complete sugar equalization between larger fruit particles or berries with a hard skin and the continuous phase of the product (Copenhagen Pectin 1993). Various plant layouts are used (Anonym 1977; Anonym 1979; Anonym 1985), but most systems comprise the following jam processing units: premixing tanks, preheating section, cooking or evaporation section, sterilization section and buffer tank for the filling line (Copenhagen Pectin A/S 1993).

One example of the automated process lines of a low soluble solids product is at the BOB Industries plant in Kumla, Sweden. In this process fruit, sugar and pectin are automatically metered into specially designed horizontal mixing tanks, features of which include an agitator designed to operate very gently so that there is thorough mixing of ingredients without breaking down the nature of the fruit itself. Two mixing tanks are filled in alternate sequences, the process being controlled from a central control panel. There is some preheating in the mixing tanks and the product is fed via an Ibex pump to three vertical scraped surface Contherm heat exchangers mounted in series, in which the product is continiously heated by steam to 95°C for a short period. It is then kept in holding tubes for 3 min to allow even penetration of the sugar and ingredients before being moved for cooling by water to the required filling temperature in a Contherm heat exchanger. Finally, it is piped through a buffer tank for filling into jars or plastic containers. pH adjustment is made automatically just before filling (Anonym 1979).

1.6.2.4 Filling and cooling

From the holding reservoir the jam is filled into an appropriate container. The container size will vary from 20 g portion packs to 20 tonne bulk tankers for large bakery users (Broomfield 1996).

The setting temperature is usually lower than the filling temperature which prevents pregelling. When organizing the filling operation it must be borne in mind that any holding of the product at temperatures above 40°C leads to quality reduction. The higher the temperature the faster the deterioration processes: loss of fruit flavour components; loss of fruit colours (anthocyanin); inversion of sucrose and browning reactions; loss of gelling power through pectin degradation. When using high filling temperatures (smaller jars), production and filling capacity must be kept equal in order to to keep the holding time prior to filling at a minimum. Smaller jars should not be finally stacked on pallets or packed in boxes until the product temperature is below 40°C. Jams to be filled into large containers can consequently not be made with rapid-set pectin, and medium- or slow-set pectins are normally used (Copenhagen Pectin A/S 1993). After filling and capping, the jars pass through a tunnel cooler and are sprinkled with cold water which decreases their temperature to 40–50°C. The rapid

reduction in temperature prevents caramelization and colour changes (Kratz 1993b). Table 2 shows some advantages and disadvantages of the presented jam making methods.

Table 2. Advantages and disadvantages of different jam making methods (Stolt 1997).

Method	Advantages	Disadvantages
Open pans	• Almost sterile product • Enzyme inactivation • Caramelization gives special flavour • Berries absorb sugar well • Partial inversion of sucrose	• High temperatures • Small batches • Major changes in colour and flavour • Broken berries • Pectin degradation
Vacuum pans	• Low temperatures • Large batches • Small variations in colour and flavour • Deaeration easy • Berries absorb sugar well • Pectin degradation	• Difficult to control • Broken berries • No caramelization • No inversion of sucrose • Micro-organisms are not totally inactivated • Enzymes are not totally inactivated
Continuous cooking	• Short heating time • Small changes in colour and flavour	• Berries have insufficient time to absorb enough sucrose • Broken berries

1.7 Aims of the present study

The aim of this study was to promote the industrial use of domestic strawberries by developing methods to control the quality of frozen Finnish strawberries and strawberry jams. The specific aim was to develop a prefreezing method in order to improve the texture of jam strawberries. To achieve this the chosen steps of the study were:

a) to study the microstucture of strawberries in order to obtain knowledge about the chemical composition of strawberry tissues and the proportional amounts of chemical compounds in different tissues.

b) to study how $CaCl_2$, sucrose and PME prefreezing treatments either at normal air pressure or in a vacuum affect the microstructure of the different strawberry tissues and their cell wall components during freezing and thawing.

c) to study how the above prefreezing treatments affect the physical, chemical and sensory quality of the pretreated strawberries and traditional jams made from them.

d) to model the best prefreezing treatment of the studied methods in order to obtain knowledge about the most effective factors and their favourable ranges for achieving high quality strawberry jam.

2. Materials and Methods

A detailed description of the experimental procedures is presented in the original publications I–V.

2.1 Strawberries

Fresh Finnish strawberries (*Fragaria x ananassa*) cv. Senga Sengana (I) and Jonsok (II, IV & V); fresh Spanish strawberries (*Fragaria x ananassa*) cv. Oso Grande (I) and Camarosa (III) and fresh Egyptian strawberries (*Fragaria x ananassa*) cv. unknown (II) were used for the studies.

2.2 CaCl$_2$, sugar and enzyme

Calcium chloride (CaCl$_2$ x 2 H$_2$O, Riedel-de Haën AG, Seelze, Germany and tap water, 90 mmol/L) solutions were used for the experiments (II–V). Sucrose was either used as water-sucrose solutions, as invert sugar (Neste 65, Suomen Sokeri, Kantvik, Finland) or as dry, crystallized sucrose (crystal size approximately 0.53 mm) (Suomen Sokeri Oy, Kantvik, Finland) (II–IV). Pectin methylesterase (PME) preparations (EC 3.1.1.11) used in the experiments were either from orange peel (P 5400, Sigma, Deisenhofen, Germany) (III) or from *Aspergillus oryzae* (NovoShape, Novo Nordisk Ferment Ltd., Dittingen, Switzerland) (IV–V).

2.3 Pretreatments

CaCl$_2$ concentrations of pretreatment solutions were the following: 1, 5.5 or 10 g/L water (II–IV); 1.1, 10.5 or 20 g/L water (V). Dosage of crystallized sucrose was 150 g/kg strawberries (II–III). Sucrose solutions were the following: 350 and 700 g/1L water (II–III). In the tests of CaCl$_2$ and invert sugar (Neste 65) the ratio of CaCl$_2$ and invert sugar was 1:9. The final sucrose concentration was 59.7% (IV). PME dosages were: 4 200 nkat/kg (III), 200 000 nkat/kg (IV) and 50 000 nkat/kg, 275 000 nkat/kg or 500 000 nkat/kg fresh strawberries (V).

CaCl$_2$-based pretreatments were carried out at 25, 37.5 or 50°C at normal air pressure (101.3 kPa) for 0.25, 7.63 or 15 min (II) or at 37°C at normal air pressure for 15 min or in a vacuum at pressure of 16.67 kPa (a vacuum chamber W.C. Heraeus GmbH, Hanau, Germany) for 10 min (III–IV) or at 10, 25 or 40°C at pressures of 6.67, 23.3 or 40 kPa for 5, 10 or 15 min (V).

2.4 Jam making

Frozen strawberries, crystallized sucrose and water were mixed together. The mixture was heated until it boiled. The mixture was allowed to boil for 10 min, after which citric acid (Riedel-de Haën AG, Seelze, Germany), pectin (Grindsted TM Pectin LA 410, Danisco Ingredients, Brabrand, Denmark) and preservative solutions (Atamon, Tørsleff, Denmark or Sigma-Aldrich Chemie, Germany) (IV–V) were added. The degree of esterification of the amidated LM pectin LA 410 was about 29% and degree of amidation about 20%. Calcium-reactivity of the pectin was high. The jam was allowed to cool at room temperature for 60 min before filling 0.5 L glass jars. After filling the jars were closed and stored at 5°C for 1 week (III) or 2 weeks and 4 months (IV) or at 22°C for 1 month with daily illumination (V) before analysis.

2.5 Microscopical studies

2.5.1 Bright-field, epifluorescence and CLS microscopy

The fresh (I) or frozen (II–IV) strawberries were freeze-dried (Dr Morand freeze-drier, Germany), cut into smaller pieces, mixed gently in an infiltration solution, polymerized with a Historesin Embedding Kit (Jung, Germany) and cut into 4 μm sections with a Microm HM355 microtome (Microm Laborgeräte GmbH, Germany). Sections were stained with specific staining solutions (Szczesniak & Smith 1969).

For the fluorescence microscopic examinations, the sections were stained with Calcofluor White M2R or Alizarin red S (IV) and measured with excitation wavelenghts from 400 to 410 nm (I) or from 420 to 480 nm, respectively

(Kiernan 1990). Samples were examined and photographed with an Olympus BX-50 microscope (Olympus, Japan).

Confocal laser scanning microscopy (Leica CLSM) was used to examine cryo-sections of fresh and frozen strawberries. The CLSM was used in fluorescence mode with an excitation wavelength of 488 nm (II). Samples were stained with acridine orange. Series of optical sections were collected and then added together to make an extended focus image of the sample area (II).

An additional confocal laser scanning microscopy (BIO RAD Confocal Scanning System Radiance Plus and Nikon Eclipse E800 microscope) study was used to examine sections of fresh strawberries. The CLSM was used in fluorescence mode with an excitation wavelength of 488 nm. Two wavelength regions were detected: green (wavelengths from 500 to 560 nm) and red (wavelengths from 570 to 650 nm). An approximately a 1 mm thick axial specimen of a strawberry was cut with a blade and stained with acridine orange for 10 min and washed with water. Series of optical sections were collected and then added together to make an extended focus image of the sample area.

The images were viewed with a Soft Imaging System AnalySIS 3.0 software (Soft Imaging System GmbH, Germany) and printed.

2.5.2   FT-IR studies

All FT-IR spectra were recorded using a Bruker FTS 66 (Spectroscopin AB, Germany) microspectrometer. The spectrometer was equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. Pre-dried nitrogen gas was injected into the FT-IR spectrometer system.

2.5.2.1   DRIFT measurements

Transmission spectra of four commercial compounds (pectin, lignin, protein and cellulose) were obtained by using the DRIFT (Bruker Diffuse Reflection Accessory) technique from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1} ; 200 scans were co-added. Each sample was measured in an open 14 mm diameter sample

cup. A reference spectrum was recorded using a pure plane mirror from 4000 to 600 cm^{-1}; 250 scans were co-added (I).

2.5.2.2 FT-IR microspectrometric studies

Fresh (I) and frozen (II–III) strawberries were used for FT-IR microspectrometric studies. The fresh berries were frozen at –40°C in a cryostat. When studying frozen berries they first had to be thawed at room temperature before frozen at –40°C in a cryostat (Leitz, Germany), because it was not otherwise possible to cut thin sections from the frozen berries. Sections of 7 μm were then cut in a cryostat (Leitz, Germany). Aluminium foil was used to surround the specimens. The aperture (36 x) was adjusted to frame the desired portion for scanning and to exclude unwanted tissue. The transmission mode was used and 50 scans were accumulated to produce a spectrum over the range of 4000 to 700 cm^{-1}at a resolution of 4 cm^{-1}. A reference was scanned using the aluminium foil free of any tissue and 100 scans were accumulated. After logarithmic transformation and baseline correction the absorbance spectra were stored.

2.6 Physical analyses

2.6.1 Drip loss and soluble solids

Drip loss was calculated by averaging the percentages of the original weights of the strawberries of three replicates after putting the berries onto a plastic screen and allowing to drip for 30 s (II). The content of soluble solids in the jam matrix was determined by collecting duplicate ^{o}Brix readings with a refractometer (Opton 74016, West Germany) (III–V).

2.6.2 Firmness

After 2 weeks (III) or 2 months (II, IV–V), freezer storage strawberries were thawed and equilibrated to 17°C for firmness measurements. The compression force of strawberries was measured with a Texture Analyser (model TA-XT2,

Stable Micro Systems, U.K.) with a 25 kg load cell (II) or a Texture Analyser (model TA-HDi, Stable Micro Systems, U.K.) with a 250 kg load cell (III–V) using an Ottawa Cell (A/OTC) with a Holed Extrusion Plate (A/HOL). The result was taken as an average of maximum force of three to four replicates (II–III) or as an average area of the deformation curve of five to six replicates (IV–V). After cooking, the jams were stored at 5°C for 1 week (III) or 2 weeks (IV) or at 22°C for 1 month (V) and equilibrated to 20°C. For firmness measurements strawberries were separated from the jam medium with a spoon onto a plate. The compression force of strawberries or of the medium was measured using an A/BE/45 Back Extrusion Rig (Stable Micro Systems, U.K.). The area of the deformation curve of three to six replicates was recorded as the result (III–V).

2.6.3 Jam colour

Instrumental jam colour of strawberries and jam medium was measured with a Minolta Chroma Meter CR-200 (Minolta Camera Co., Ltd., Osaka, Japan) in the reflection mode after 2 weeks (IV–V) or 4 months (V) of jam storage.

2.7   Chemical analyses

2.7.1 pH

The pH of the jam medium was measured with an Orion Digital Ionanalyser 501 (Orion Research Inc., Cambridge, MA, USA and electrode Orion 8155SC, Orion Research Inc., Boston, MA, USA) (III–V).

2.7.2 Calcium

After 1 week (III) or 2 months (IV–V) freezer storage frozen strawberries as well as jam berries and medium after 1 week (III) or 1 month (IV–V) storage were used for calcium analysis. The homogenized sample was dissolved in dilute nitric acid after dry ashing at 550°C. Calcium was then analysed after dry washing by atomic absorption spectrometry (AAS) using the flame technique (method VTT-4289-91, accreditated by the Finnish Accreditation Service).

2.7.3 Sugar, titrable acidity and vitamin C

For the total sugar content determination fresh or frozen (2 months) strawberries were weighed with water, mixed and clarified. An aliquot was treated with HCl at 70°C for 10 min to invert the disaccharides to reducing sugars. After cooling, two drops of 1% phenolphthalein were added and the contents were neutralized to a pink colour using 30% NaOH solution. Invert sugars were then determined by titrimetry according to the Luff-Schoorl method (EC 1979) (IV).

Titrable acidity was determined for the fresh and for the frozen (2 months) strawberries as well as for the jams both 2 weeks and 4 months storage after homogenisation according to AOAC Method 942.15 (1995) (IV).

HPLC analysis of vitamin C was carried out with fresh and frozen (2 months) strawberries as well as with jam berries and medium after both 2 weeks and 4 months storage. Strawberries were homogenized and vitamin C was directly analysed as dehydroascorbic acid according to Speek *et al.* (1984). HPLC analysis was carried out using a Waters 6000A solvent delivery system equipped with a Waters 712 autosampler and Waters 474 fluorescence detector (Waters Corporation, Milford, MA, USA). The system was controlled and data was treated using a Millennium 2.15 Workstation (IV).

2.8 Sensory evaluation

The sensory quality of the strawberry jams was evaluated by a trained panel with proven skills (n = 10–13) using descriptive analysis (Lawless & Heymann 1998). Sensory evaluation was carried out after 1 week (III), 2 weeks or 4 months storage (IV) at 5°C or after 1 month storage at 22°C with daily illumination (V).

The assessors were familiarised with the sensory descriptors and the attribute intensities used prior to the evaluations. Attribute intensities were rated on continuous, unstructured, graphical intensity scales. The scales were verbally anchored at each end, and the left side of the scale corresponded to the lowest or opposite intensity (value 0) and the right side to the highest intensity (value 10). The sensory attributes evaluated were redness of colour, wholeness of berries,

clarity of medium, evenness of medium, firmness of medium, softness of berries, faultlessness of odour and flavour (III–V) as well as sweetness, sourness and balance of odour and flavour (III).

In all experiments the samples were presented to the panelists coded and in random order, and water and crackers were provided for cleansing the palate between the jam samples. The samples were analysed on two sequential days in two sensory replicates, both divided into two sessions in which the untreated reference samples was presented to the assessors.

2.9 Experimental design

In the model each of the studied response variables (Y_k) occured as a linear term with the following form:

$$Y_k = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \varepsilon \tag{1}$$

where β_0 was constant, β_1 and β_2 were regression coefficients, X_1 and X_2 were factors and ε was noise. The interaction terms were determined by addition (V). The resulting model was the following:

$$Y_k = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + ... + \varepsilon \tag{2}$$

Regression coefficients for each linear model were calculated using partial least squares (PLS) regression with 95% confidence levels.

In the first calcium chloride test series, a full factorial screening design (2^n) with three centre points containing 11 tests was used. The three-level factors were $CaCl_2$ concentration, pretreatment time and pretreatment temperature. In the case of sucrose pretreatment a factorial screening design (2^{n-1}) containing 4 tests was used. The two-level factors were the pretreatment method (dipping/sprinkling), pretreatment concentration and pretreatment time (II). A fractional factorial screening interaction was used in the second calcium chloride test series. The resolution of the design was V+, which meant that the main effects were unconfounded with two-factor interactions and two-factor interactions were unconfounded with each other. The design contained 16 tests with four centre

points (V) (Cochran & Cox 1957, Martens & Næs 1989). The experimental design was created and analysed using Modde for Windows 4.0 software (Umetri Ab, Umeå, Sweden).

2.10 Statistical analysis

Analysis of variance (ANOVA) and Tukey's HSD test ($p < 0.05$) were executed with a Statgraphics Plus (Ver. 7.1, Manugistics, Inc., Rockwille, MD, USA) and SPSS software (SPSS Ver. 8.0, SPSS Inc., 1997). ANOVA was used to test statistical differences in attributes between samples ($p < 0.05$). When the difference in the analysis of variance was statistically significant, pairwise comparisons of the attributes between the samples were analysed by Tukey's test (II–V). Additionally, a principal component analysis (PCA) was performed for sensory results using an Unscrambler software package (Unscrambler Ver. 7.5, CAMO ASA, Trondheim, Norway) (IV) and a linear regression method was performed to analyse the interrelations of the response variables with a Statgraphics Plus (V).

3. Results and discussion

3.1 Microstructure

3.1.1 Bright-field, epifluorescence and CLS microscopy (Publications I–IV)

3.1.1.1 Fresh berries (I–II)

The locations of pectin, lignin, protein and cellulose in strawberry cell walls of cortical and vascular tissues (Figures 14 and 15) and in achenes (Figure 16) were studied (I). Thionin stains cellulose violet, light green and iodine stain proteins green or yellow, rhutenium red stains pectin pink and phloroglucinol stains lignin light pink.

Cellulose could be detected in the cortical cell walls and intercellular spaces. Protein was deposited inside the cells where the cytoplasm appears as a thin layer between the large vacuole and the cell wall. Some protein was found in the cell wall. Pectin was localized both in the primary cell wall and in the middle lamella. Lignin was not detected. The results support the cell wall theory of McCann & Roberts (1991) that cellulose fibres in plant cell walls are dispersed in a gel composed of pectin and hemicellulose.

Vascular tissue (Figure 15) contained long fibres composed of cellulose, protein, pectin as well as lignin spirals. McCann *et al.* (1993) obtained similar results by studying the vascular tissues of carrot with FT-IR microspectrometry.

The main component of achenes (Figure 16) appeared to be lignin, which was surrounded by protein and pectin. No cellulose was observed in achenes. Lignin may have dominated the cell wall structure of achene so that no cellulose was detectable despite the fact that cellulose is the important component of plant cell walls.

Figure 17 shows the CLSM image (Bio Rad Radiance Plus Confocal Scanning System and Nikon Eclipse E 800 microscope) of lignin spirals in vascular tissue of fresh strawberry. Lignin spirals were very close and coherent. The CLSM image of achene is shown in Figure 18. The lignified cell walls around the

embryo were very dense and coherent. In the CLSM study samples were stained with acridine orange which appeared to stain lignin much more intensely than other cell wall components. According to CLSM studies in the cortex tissue of fresh, unpretreated strawberries the cell content was rather homogeneously distributed inside the cell walls. Intercellular spaces could also be detected. The cell walls around the vascular tissue were undamaged and the coherent lignin spirals were clearly shown (II).
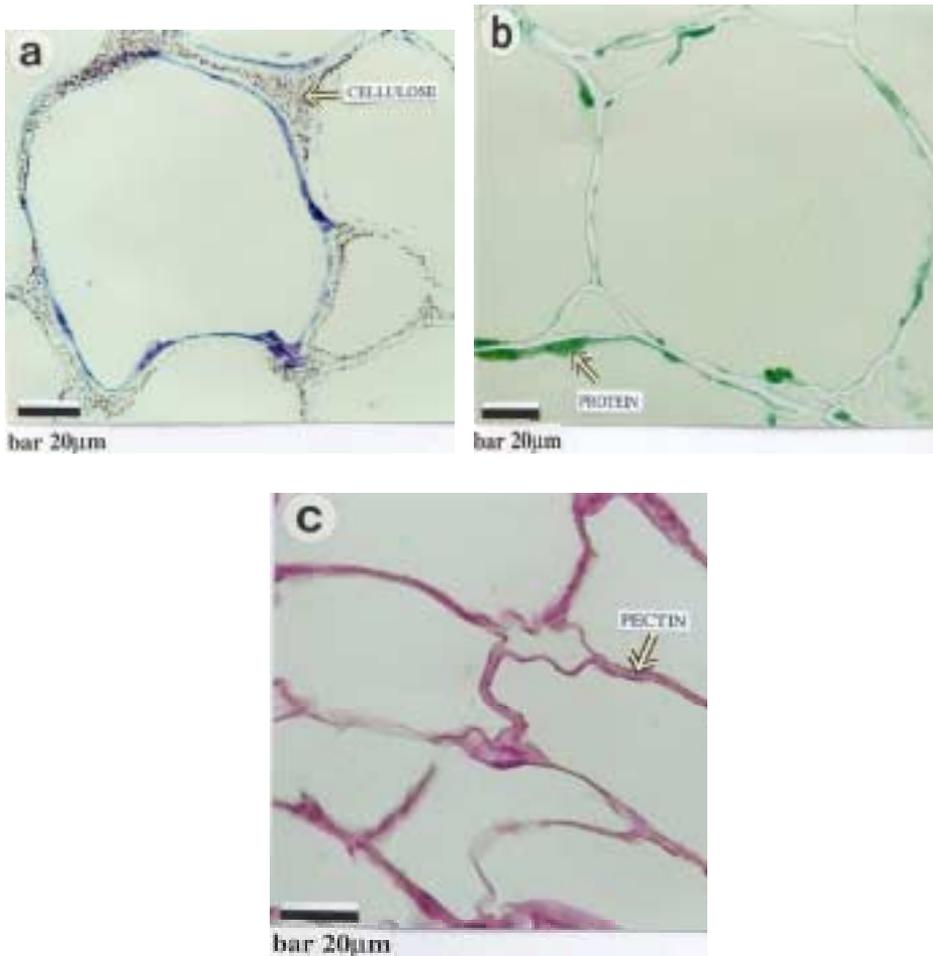
Figure 14. Micrographs of cortical tissue. (a) cellulose (marked with an arrow) appears violet ('sticks'), (b) protein (marked with an arrow) appears green, (c) pectin (marked with an arrow) appears red.
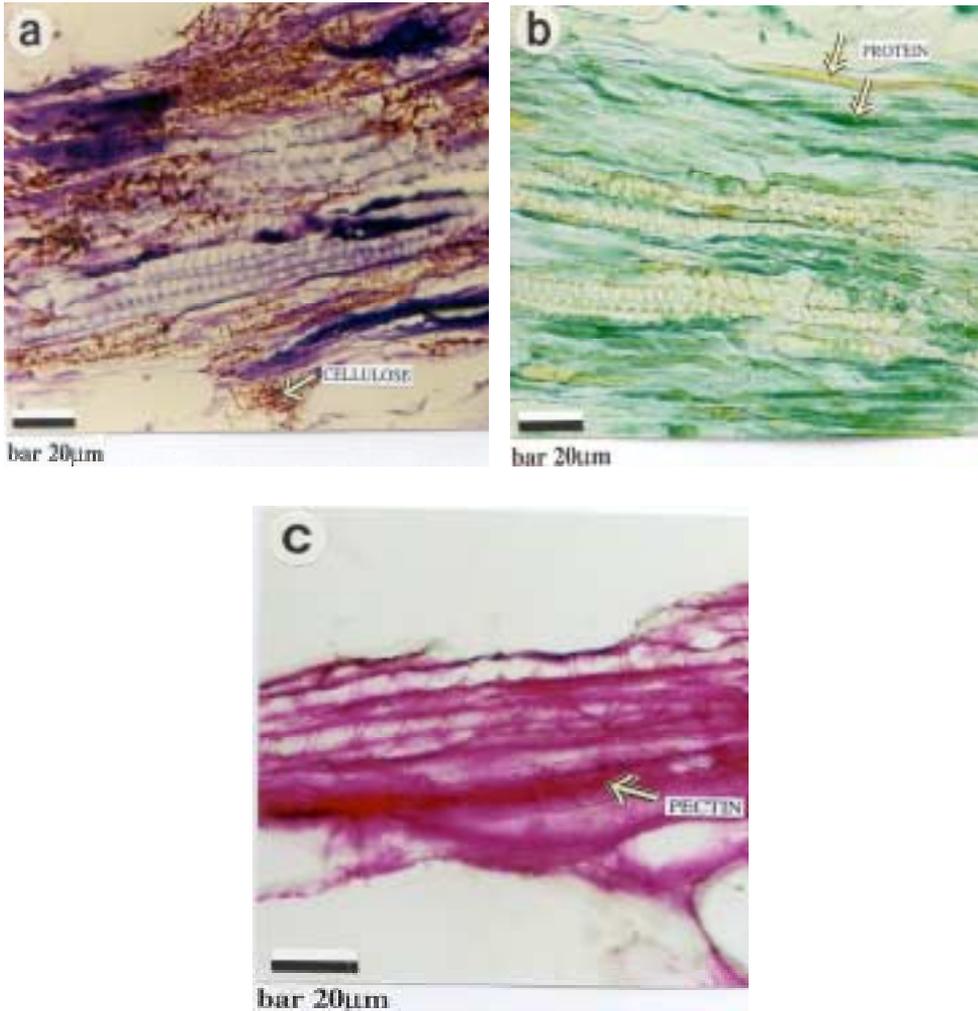
*Figure 15. Micrographs of vascular tissue. (a) cellulose (marked with an arrow) appears violet ('sticks'), (b) protein (marked with an arrow) appears yellow or green, (c) pectin (marked withan arrow) appears red.*
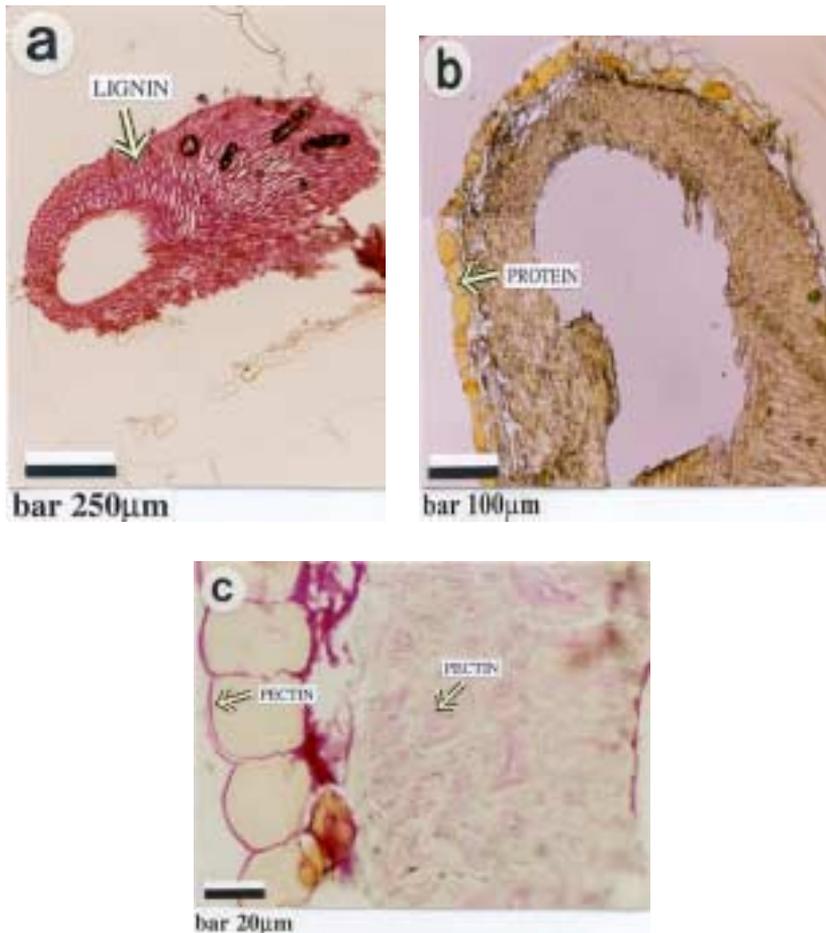
Figure 16. Micrographs of achene. (a) lignin (marked with an arrow) appears light pink, (b) protein (marked with an arrow) appears yellow, (c) pectin (marked with an arrow) appears red.

Figure 17. CLSM image of vascular tissue of fresh strawberry (41 sections taken at 0.5 μm steps). Bar is 50 μm.
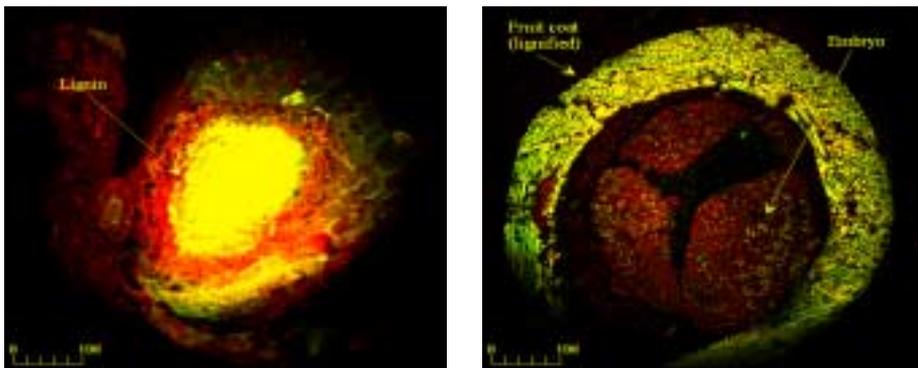

Figure 18. CLSM images of achene of fresh strawberry (81 sections taken at 1.25 μm steps). Bar is 100 μm.

3.1.1.2 Pretreated frozen berries (II–IV)

Microscopical studies of the unpretreated and pretreated ($CaCl_2$, sucrose, PME, $CaCl_2$+PME in a vacuum) frozen berries indicated the following: In the untreated reference sample pectin in the middle lamella of the cortical cells as well as in the vascular tissue was poorly stained compared to the pretreated samples. Moreover, the cell walls appeared to be broken and pectin occured outside the middle lamella. In $CaCl_2$-treated strawberries cell walls were more dense and pectin was more strongly stained and quite homogeneously spread in the middle lamella than in the reference or sucrose-treated samples. In some parts the middle lamella had swollen and was filled with pectin (II–IV). In the PME-treated strawberries pectin appeared outside the middle lamella due to the broken cell walls partly caused by cellulase and protease side activities of the PME preparation used (III). Micrographs of the strawberry cortical and vascular tissues of the untreated reference and the $CaCl_2$- and PME-treated strawberries in a vacuum after pectin staining (IV), are shown in Figure 19.

Protein was poorly stained in the unpretreated reference sample and appeared to have spread into the middle lamella due to the breaking of cell walls and plasma membranes by ice crystals (III). Additionally, the same effect could be seen with protein in the sucrose-treated (II) as well as in the PME-treated strawberries (III). Protein was more strongly stained in the cortical and vascular tissues of the strawberries treated with $CaCl_2$ or $CaCl_2$ and PME in a vacuum than in the unpretreated reference, or in sucrose- or PME-treated strawberries. Micrographs of the strawberry vascular tissues of the untreated reference and the $CaCl_2$- and PME-treated strawberries in a vacuum after protein staining (III), are shown in Figure 20.

The vascular tissues of the PME-treated strawberries were broken (III). The cell walls of the cortical tissues of the $CaCl_2$- and PME-treated strawberries especially were less broken and protein appeared to remain as it was in fresh strawberry (III). In the vascular tissue of the untreated reference strawberries lignin spirals appeared to be severely broken. The lignin was less changed in the sucrose-treated sample, which was in agreement with the CLSM results (II). The lignin-rich, spiral-type structures appeared to be quite intact and coherent in the vascular tissues of $CaCl_2$- and PME-treated strawberries in a vacuum (II–IV).

(a) Reference, pectin

(a) Calcium + PME, in a vacuum, pectin

(b) Reference, pectin

(b) Calcium + PME, in a vacuum, pectin
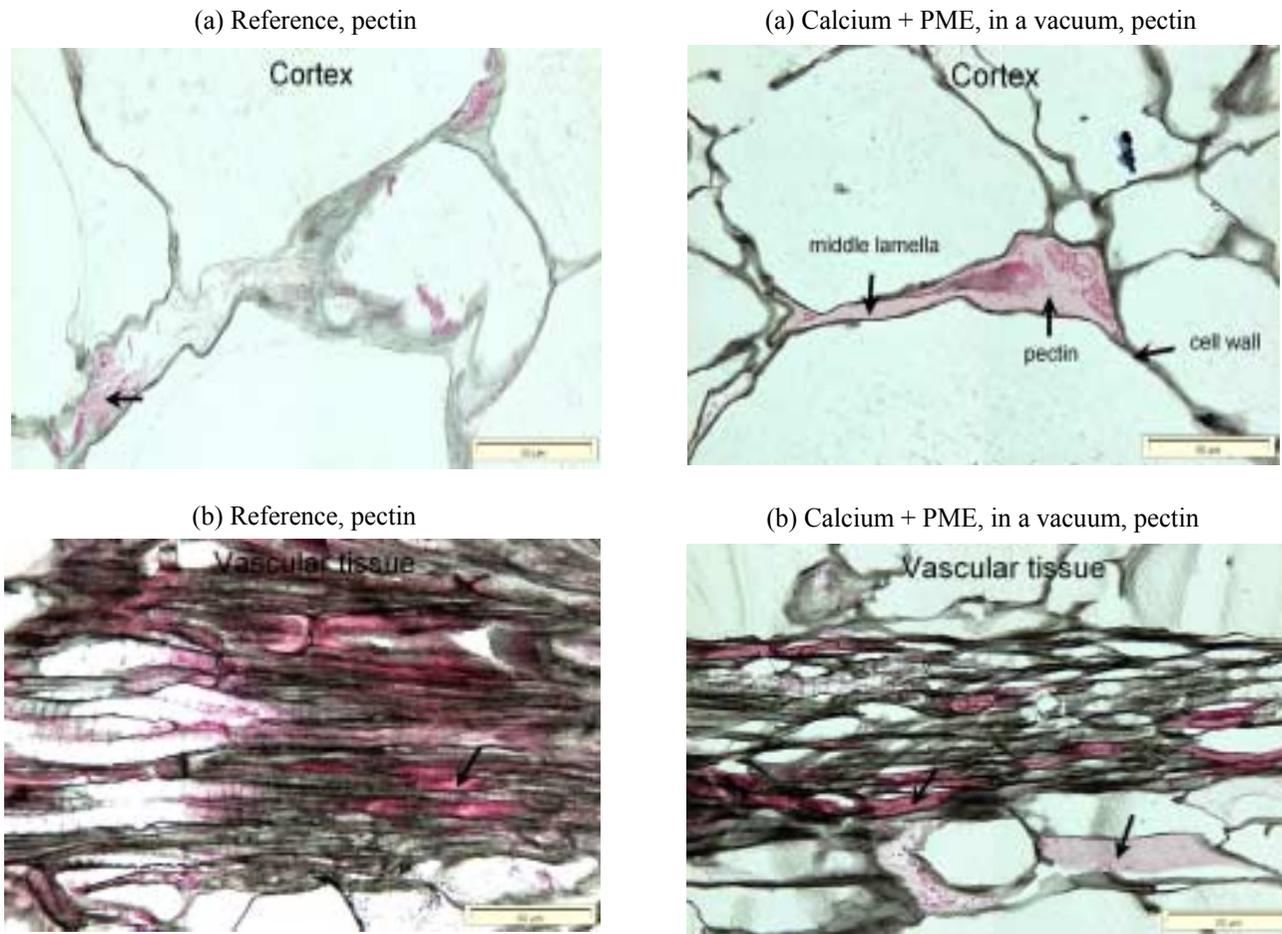
Figure 19. Micrographs of strawberry (a) cortical and (b) vascular tissues. Pectin (marked with an arrow) appears pink. Bar is 50 μm (Suutarinen et al. 2000).

(a) Reference, protein
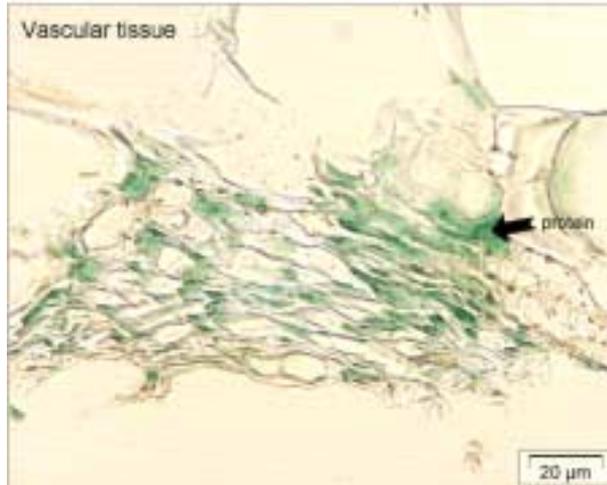
(b) CaCl$_2$ + PME, in a vacuum, protein
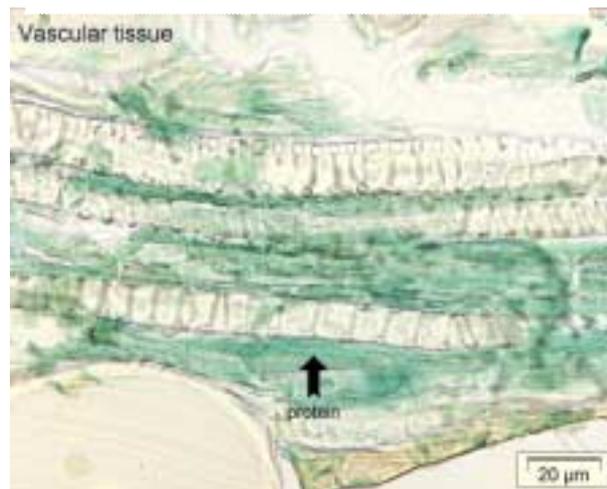
Figure 20. Micrographs of (a) untreated reference and (b) CaCl$_2$- and PME-treated (in a vacuum) strawberry vascular tissues. Protein (marked with an arrow) appears yellow or green. Bar is 20 μm.

The cell walls in the cortex of the CaCl$_2$-treated strawberries appeared to have brighter blue staining (staining with calcofluor white M2R) than the sucrose-treated or the unpretreated strawberries (II) (Figure 21). The location of calcium (staining with alizarin red S) in the primary cell wall and middle lamella of cortex of the strawberries treated with CaCl$_2$ and PME in a vacuum could be

demonstrated as more orange spots than in the unpretreated reference sample (IV).

Accordingly to the CLSM study there was some shrinkage of the cytoplasm in the cortical cells of $CaCl_2$-treated strawberries due to osmotic water loss, although the cell walls themselves appeared to be relatively undamaged (II).

Although the results indicated that CLS microscopy can be used to study strawberry microstructure, further work should be carried out to find a fluorochrome in the 450–500 nm range which would be carbohydrate-specific, especially for pectins, to better identify the components of the middle lamella.

(a) Reference
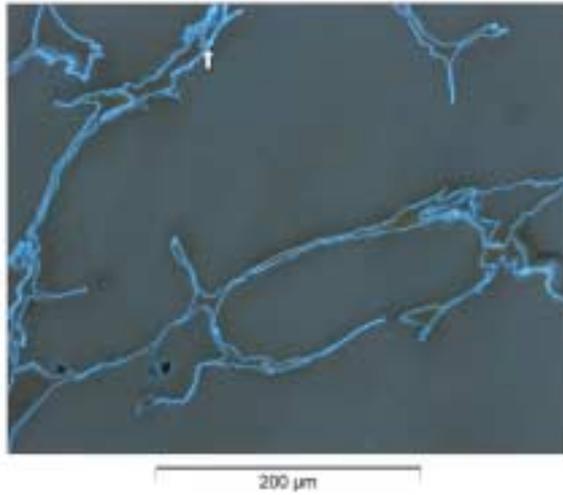
(b) CaCl$_2$
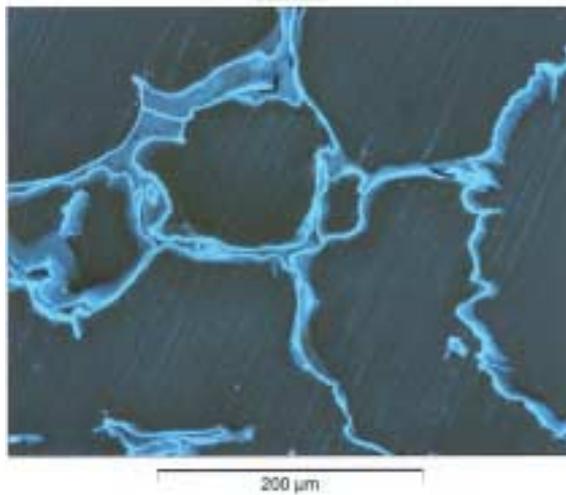
Figure 21. Micrographs of (a) untreated reference and (b) CaCl$_2$-treated strawberry cell walls of cortical tissues. Bar is 200 μm.

3.1.2 FT-IR studies

3.1.2.1 DRIFT measurements (I)

Since the different cell walls in strawberry gave complicated spectra, the spectra of the pure commercial compounds pectin, lignin, protein and cellulose were measured. Figure 22 shows the spectra obtained from pectin, lignin, protein and cellulose over the 4000–700 cm^{-1} region. In each spectrum the following bands were observed: the OH and the CH stretching vibration bands approximately in the 3500–3300 cm^{-1} and in the 2956–2900 cm^{-1} regions, respectively. In each spectrum the carbohydrate band in the 1200–1000 cm^{-1} region was also present.

In the 1800–1600 cm^{-1} region of the pectin spectrum strong CO stretching vibration bands were observed suggesting that both esterified and acified groups at approximately 1759 cm^{-1} and 1641 cm^{-1} were present. In the lignin spectrum the typical CC stretching vibration bands were observed at approximately 1601 and 1516 cm^{-1} and an HOH deformation band at approximately 1653 cm^{-1}. In the protein spectrum, the NH stretching vibration band was present at 3300 cm^{-1} and the amide I and amide II bands due to the strong CO and CN stretching vibration bands appeared at approximately 1689 and 1552 cm^{-1}, respectively. In the case of cellulose, CH and CO stretching vibration bands were observed in the 3500–3300 and 2956–2900 cm^{-1} regions respectively, and the HOH deformation band was observed at approximately 1653 cm^{-1}.
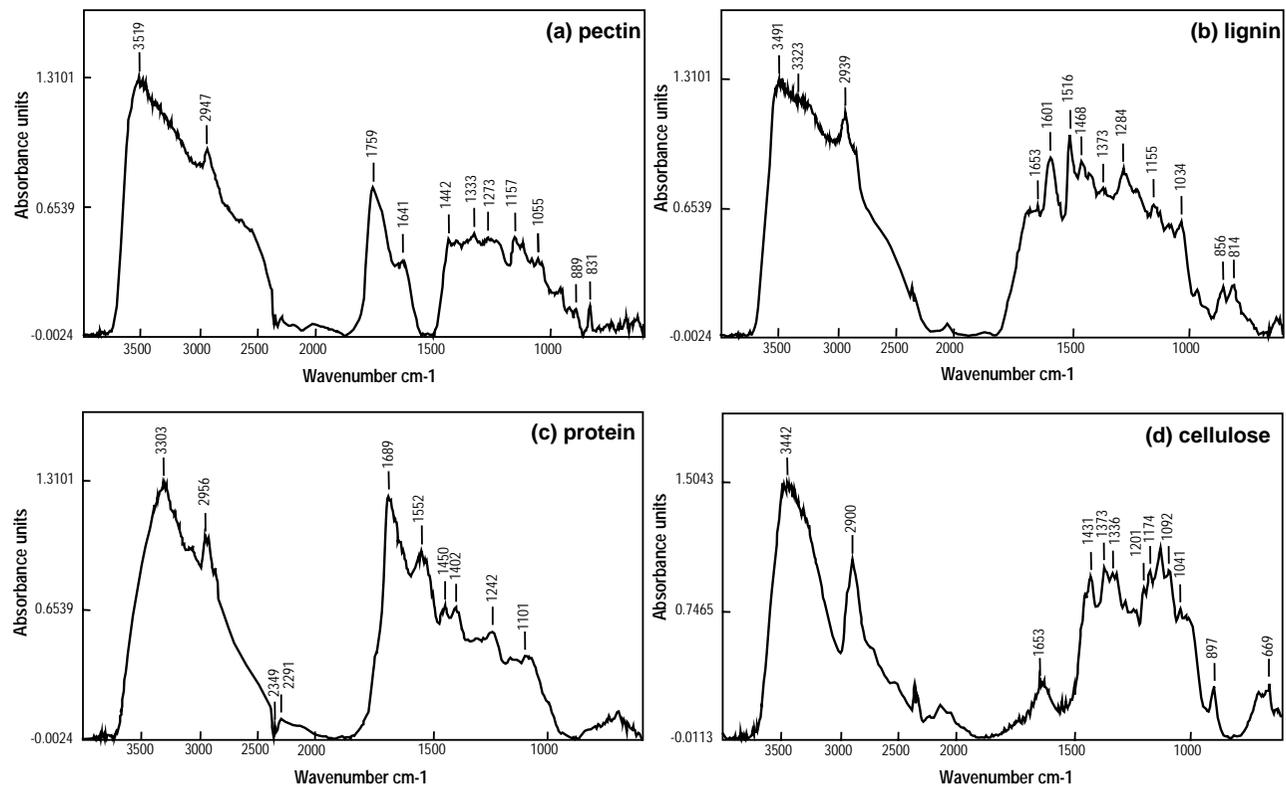
Figure 22. Spectra of (a) pectin, (b) lignin, (c) protein, and (d) cellulose.

3.1.2.2 Fresh berries (I)

The spectra of strawberry cortical and vascular tissues and achene were measured from 4000 to 700 cm^{-1}. Due to the complex nature of the spectra, scanning across one tissue to another was carried out in order to study the changes in peak sizes, which were indicative of changes in the contents of the different chemical components.

Generally, achene, vascular tissue and pith contained more cell wall material than cortex. The high lignin content in the achene was evident from the 1601 and 1516 cm^{-1} peaks. Small amide I and amide II peaks at approximately 1680 cm^{-1} and 1552 cm^{-1} representing protein were seen. Furthermore in achene spectra there was a strong carbonyl band at 1740 cm^{-1} representing pectin.

The lignin, pectin, protein, cellulose and carbohydrate peaks remained strong, transferring from the outer layer of the achene to the edge of the vascular tissue. This was in agreement with bright-field microscopy results. After the edge of the achene and vascular tissue the pectin, protein, cellulose and carbohydrate peaks (cellulose at 1460–1330 cm^{-1} and carbohydrates at 1200–1000 cm^{-1}) first decreased and then remained constant until the edge of the vascular tissue and the outer edge of the pith. From the edge of the vascular tissue to the centre of the pith the amounts of protein, cellulose and pectin remained constant, whereas the carbohydrate content appeared to vary to some extent, being high in both the edge of the vascular tissue and in the centre of the pith. In the epidermis and hypodermis a rather strong carbonyl peak at 1726 cm^{-1} could be seen, representing pectin.

The carbohydrate content (1200–1000 cm^{-1}) appeared to be dominant in all the spectra of epidermis, hypodermis, cortex, vascular tissue and pith. Cellulose was also present in the epidermis and hypodermis as shown by the peaks in the range 1460 to 1330 cm^{-1}. The pectin content appeared to decrease when transferring from the epidermis and hypodermis to the cortex, but remained constant thereafter. Small protein peaks could be seen in the cortical tissue. In the vascular tissue the contents of pectin, protein, cellulose and carbohydrates at first clearly increased and then remained relatively constant. The pectin content decreased as the point of observation moved from the epidermis and hypodermis

to the cortex and the pectin, protein, cellulose and carbohydrate contents decreased as the point of observation moved from the pith and vascular tissue to the cortex.

The region from 1700 to 1500 cm^{-1} was rather difficult to analyse because in this region peaks typical for acidified pectin, protein and lignin appear. For that reason lignin was not clearly detected from the vascular tissue even if lignin spirals were quite evidently present in the vascular tissue in the light microscopy and CLSM studies.

3.1.2.3 Pretreated frozen berries (II–III)

Figure 23 shows typical spectra of the tissues of unpretreated reference strawberries and of the tissues treated with CaCl$_2$+PME in a vacuum. Spectra are shown in the fingerprint region approximately between 2000 and 900 cm^{-1}.

An overview of series of spectra of unpretreated reference, CaCl$_2$- and sucrose-treated strawberry tissues showed that peak absorbances of spectra of the CaCl$_2$-treated strawberries were higher and their areas larger in vascular tissue and cortex compared to the sucrose-treated and unpretreated reference strawberries (II).

In each of the achene spectra a strong CO stretching vibration band was present at approximately 1740 cm^{-1} due to an ester stretching vibration. This is characteristic of pectin. At approximately 1680 and 1550 cm^{-1}, small amide I and amide II bands were present in the spectra of the calcium- and sucrose-treated strawberries. At approximately 1605 and 1510 cm^{-1}, peaks typical to lignin were present in the achene spectrum of the pretreated and unpretreated reference. Carbohydrate bands (1200–1000 cm^{-1}) appeared to be weaker in the achene of the pretreated strawberries, which was probably due to the overall destruction of the structural compounds in the achenes of these strawberries. In the vascular tissue of the CaCl$_2$-treated strawberries there were stronger (higher and larger) pectin peaks at 1720 cm^{-1} than in the sucrose-treated strawberries and much stronger pectin peaks than in the untreated reference spectra. Furthermore the amide I and amide II peaks (at about 1650 and 1550 cm^{-1}) of protein appeared to be stronger in the vascular tissue of CaCl$_2$-treated than in the
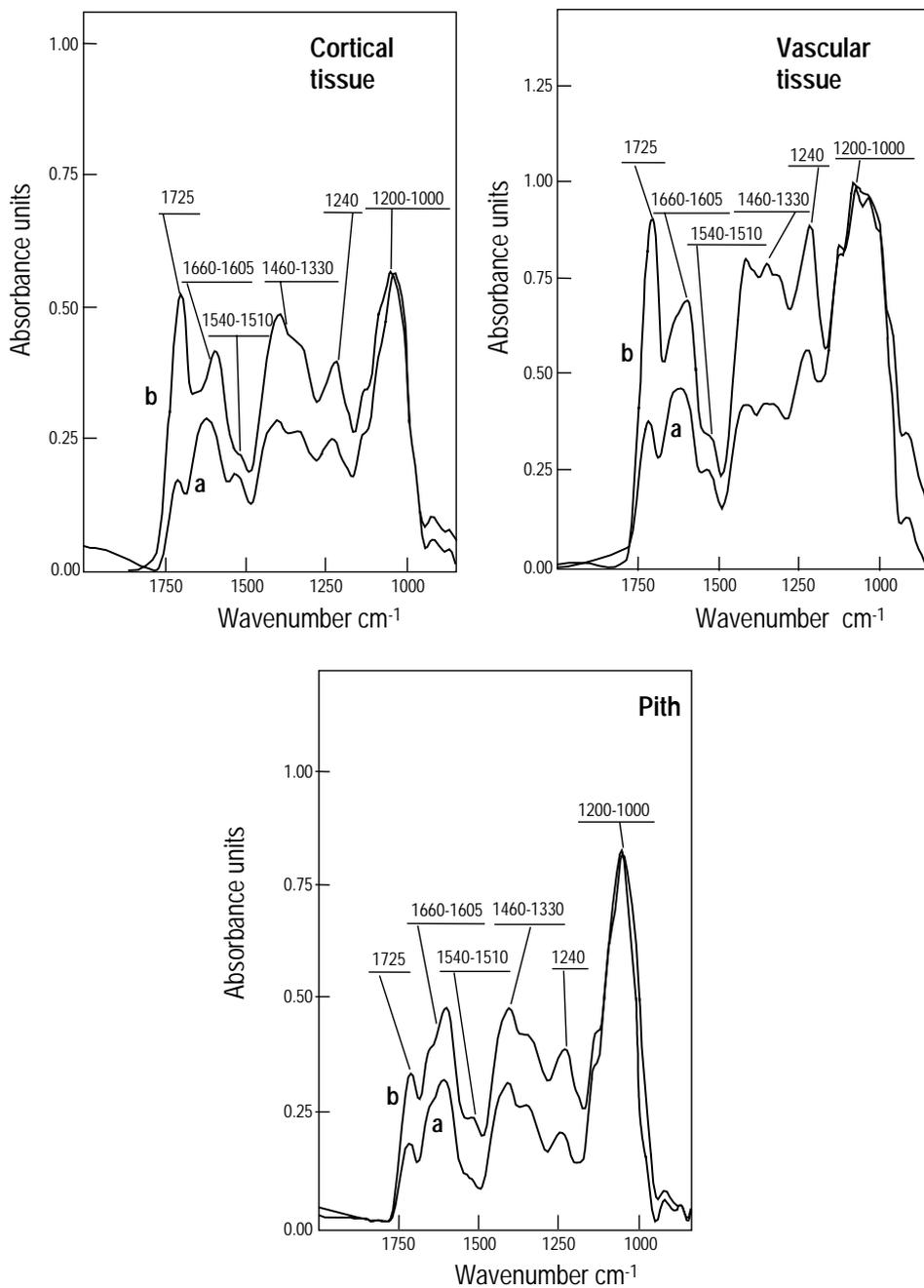
Figure 23. Spectra of the tissues of (a) unpretreated reference strawberries and of the tissues treated with (b) CaCl$_2$+PME in a vacuum in the fingerprint region.

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sucrose-treated or in the unpretreated reference. Cellulose (1460–1330 cm^{-1}) and carbohydrate (1240–1000 cm^{-1}) bands appeared to be higher and larger in the vascular tissue of the CaCl$_2$-treated than in the sucrose-treated or reference strawberries. No differences were found in the spectra of the pith between the pretreated or unpretreated reference strawberries. Furthermore, no differences were observed in the spectra of epidermis and hypodermis between the samples.

In all the spectra of the cortical tissue of the studied pretreated and unpretreated strawberries there were rather strong carbonyl bands at approximately 1725 and 1640 cm^{-1}, representing esterified and acidified groups of pectin. This is in agreement with the findings of McCann & Roberts (1991) for onion cortical tissue. At approximately 1550 cm^{-1} there was a strong peak of amide II. Cellulose bands were present in the 1435–1360 cm^{-1} and carbohydrate bands in the 1240–1000 cm^{-1} region. In the cortical tissue of the CaCl$_2$- and sucrose-treated strawberries the pectin and protein peaks were slightly higher and their areas larger than in the reference (II).

There were no significant changes in lignin, pectin, protein, cellulose or carbohydrate bands of the achene, epidermis or hypodermis between the samples of the CaCl$_2$+PME-treated strawberries in a vacuum and other pretreated or unpretreated reference strawberries. By contrasts, in the vascular tissue and pith the pectin, protein, cellulose and carbohydrate peaks of the CaCl$_2$+PME-treated strawberries in a vacuum were higher and their areas larger than in the other pretreated or unpretreated strawberries.

In the cortical tissue of the CaCl$_2$+PME-treated strawberries in a vacuum the pectin, protein and cellulose peaks were higher and their areas larger than in the cortical tissues of the other pretreated or the unpretreated reference. Vacuum clearly strengthened the absorption of the pretreatment solution of CaCl$_2$+PME into the cortical tissue and pith.

Because of the partial destruction of the compounds during or after the pretreatments, the analyses of achenes, epidermis and hypodermis were rather difficult. In the spectra of the vascular tissue of the reference and pretreated strawberries the typical lignin peaks could not be detected even if the lignin was quite evidently present in the vascular tissue, which could also be seen in CLSM images (II). Generally, the overlapping effects of acidified pectin, amide I and

lignin peaks in the 1700–1500 cm^{-1} region, together with their small natural amounts in strawberry, decreased the spectral resolution. Therefore, it was not possible to recognize these compounds from this area of the spectra very clearly (III).

3.2 Physical quality

3.2.1 Drip loss and soluble solids (Publications II–V)

It was not possible to model drip loss of the pretreated (CaCl$_2$ or sucrose at normal air pressure), thawed strawberries (II). One reason for this was too few replicates of drip loss measurements. Standard deviations of the measurements were also rather high and the range of the drip loss rather narrow. These, together with the fact which was later (V) observed that dipping time and temperature did not much affect for the pretreatment models, perhaps explain why it was impossible to obtain a suitable model for drip loss (II). Linear regression correlations between drip loss and firmness of CaCl$_2$- or sucrose-treated thawed strawberries were R = –0.46 (p<0.01) and R = 0.53 (p<0.01), respectively. According to Skrede (1980) there was no correlation between the firmness of fresh and thawed strawberries. Furthermore, drip loss of thawed berries and firmness of fresh berries and jams gave a negative correlation.

All soluble solids contents were rather, low between 47 and 50 or between 48 and 56 ^{o}Brix (III–IV), or between 41 and 49 ^{o}Brix (V). The amidated LM pectin (LA 104) used for the study was intended for jams, with a ^{o}Brix value around 45. In these investigations it was more important to follow the effect of calcium and PME on the strawberries than to optimize the jam making. Therefore, the amounts of soluble solids were not adjusted to a specific level, but the jams were cooked using similar total amounts of each ingredient.

3.2.2 Firmness (Publications II–IV)

It was not possible to model firmness of CaCl$_2$- or sucrose-treated thawed strawberries (II). Reason for this were the rather high standard deviations

between the replicates of measurements as well as the rather narrow range of firmness values of the pretreated samples.

$CaCl_2$+PME prefreezing treatment in a vacuum had statistically the most significant influence on the firmness of frozen, thawed strawberries when measuring the area of the deformation curve (IV).

Jams made from frozen strawberries treated with $CaCl_2$+PME in a vacuum (III–IV) or $CaCl_2$ and crystallized sucrose (III) or $CaCl_2$+PME and sucrose solutions in a vacuum (IV) had significantly increased firmness values, approximately twice or threefold higher than the untreated reference samples. Jams made from the untreated reference sample had a significantly harder medium than other jams, except for jams made from strawberries treated with crystallized sucrose (III).

3.2.3 Jam colour (Publication IV)

All the instrumental colour coordinate values (*L, *a and *b) of the studied jams made from frozen berries treated with $CaCl_2$-based solutions decreased during 2 weeks or 4 months storage at 5 ^{o}C in darkness. Jams made from berries that had been dipped only in $CaCl_2$ solution in a vacuum or $CaCl_2$ and PME solution in a vacuum or in $CaCl_2$, PME and sucrose solution in a vacuum generally resulted in darker strawberries and lighter, redder media in the jam than the untreated reference berries.

According to García-Viguera *et al. (*1999), simulated daylight conditions caused insignificant colour and anthocyanin losses during storage, compared with jams stored in total darkness. However, no direct relationship was found between anthocyanin loss during processing and storage, and resulting jam colour. Storage of fruit for one year at –20°C prior to jam preparation resulted in anthocyanin losses of 77%, whereas fruit stored for 6 months had less than 20% anthocyanin loss.

3.3 Chemical quality

3.3.1 pH (Publications III–V)

The pH values of the jams were about 3.1, 3.4 (III–IV) or 3.7 (V). These pH values were very convenient for the LMA-pectin used. LMA-pectin forms gel at 30–70°C in the pH-range 1–5 (Hoefler 1991).

3.3.2 Calcium (Publications III–IV)

$CaCl_2$-based prefreezing treatments clearly increased the calcium content of strawberries relative to the control. The amount of calcium in the strawberries that had been dipped in the $CaCl_2$ solution in a vacuum particularly increased relative to the control berries. The amounts of calcium in strawberries that had been pretreated in $CaCl_2$- or $CaCl_2$- and PME-solutions in a vacuum were about three times higher than in the reference berries.

The same effect could be seen in jam strawberries and in the media. Jams which were made from pretreated frozen berries that already contained a high level of calcium also had higher (twice or threefold) amounts of calcium in their media. By contrast, the differences in calcium contents between untreated reference and other pretreated jam berries except the $CaCl_2$-treated berries in a vacuum were not as high as expected. This might be due to the effects of density differences between sucrose syrup, PME and the $CaCl_2$ solution and the abilities of strawberries to absorb calcium into their tissues (Sacchetti *et al.* 2001).

3.3.3 Sugar, titrable acidity and vitamin C (Publication IV)

Sucrose pretreatments together with $CaCl_2$ or PME (at normal air pressure or in a vacuum) increased the total sucrose content in frozen berries by about 25–45%, being higher when berries were treated with $CaCl_2$ and invert sugar (1:9) in a vacuum. The organic acid contents appeared to remain similar during frozen storage and only slightly decreased during jam storage, and thus were independent of the studied pretreatment itself. Vitamin C contents of the studied pretreated frozen berries did not change during 2 months frozen storage compared to the untreated fresh reference berries. Similar results have been

reported by Hong & Ueda (1993). According to their results sugar, acid and vitamin C contents of strawberries did not change markedly during frozen storage, whereas the anthocyanin content was reduced to less than half after 6 months of storage at -20, -40 or -80^oC.

After jam cooking, the vitamin C content decreased dramatically to about 25% from the original values of all the studied jams and appeared to be independent of the studied pretreatment. After 4 months storage jam berries and medium of the jam made from berries treated with $CaCl_2$, PME and sucrose in a vacuum had a slightly higher C vitamin content (1–2 mg/100g) than other jams.

3.4 Sensory quality (Publications III–IV)

The jam made from berries treated with $CaCl_2$ and crystallized sucrose was found to be redder in colour than the reference jam. The medium of the jam from the untreated reference strawberries was firmer and more turbid than those of the jams made from strawberries treated with $CaCl_2$ and PME in a vacuum. Jam made from strawberries treated with crystallized sucrose had a medium as firm as that of the reference (III). There appeared to be a negative correlation between clarity and firmness of the medium.

The following attributes were statistically different among the strawberry jams: redness of the colour ($p<0.05$) (III), wholeness of the berries ($p<0.001$), firmness, clarity and evenness (IV) of the jam medium ($p<0.001$), softness of the berries ($p<0.001$) (IV) and faultlessness of odour and flavour ($p<0.001$) (IV).

After 2 weeks storage none of the jams was evaluated to have more even medium than the untreated reference jam (IV). The jams which were made from strawberries kept in $CaCl_2$, PME and sucrose solution during freezing (test 6) or from frozen strawberries which were put into a similar pretreatment solution to that used in test 6 just before jam making (test 7) and kept there until the end of jam making were evaluated to have significantly more uneven medium. After 4 months storage at 5^oC none of the jams were evaluated to be significantly redder or browner in colour compared to the untreated reference ($p<0.001$) (IV). Furthermore, none of the jams were evaluated to contain more whole berries or to have more even medium than the reference ($p<0.001$). After 4 months storage

jam berries treated with CaCl$_2$ and PME in a vacuum were still evaluated as significantly harder than the untreated reference. Additionally, differences in clarity and firmness of medium between jams made from untreated reference berries and berries treated with CaCl$_2$ and PME in a vacuum were no longer recorded. After 2 weeks and 4 months storage all the jams except test jams 6 and 7 were still evaluated to be faultless in odour and flavour.

According to principal component analysis (PCA), after 2 weeks storage the first factor (66%) separated the jams mainly on the basis of the medium attributes and the second factor (29%) on the basis of berry texture. After 4 months storage the first factor (68%) still separated the jams on the basis of the medium attributes and the second factor (19%) on the basis of strawberry texture (Figure 24). Total explanation of the variance of the attributes with the first and second factors was high (95%) and only slightly decreased (by 8%) after 4 months storage.
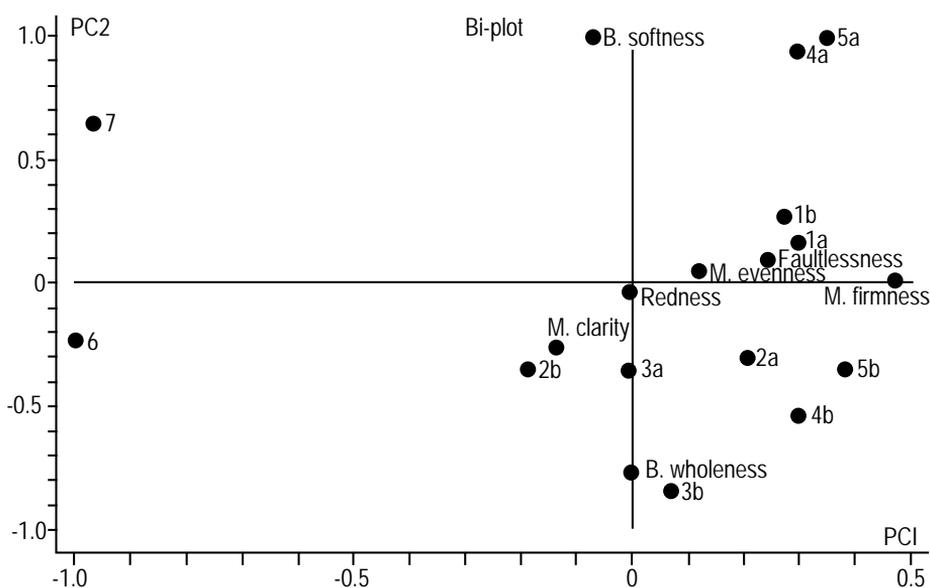
Figure 24. PCA score and loading biplot of the sensory data of the strawberry jams after 4 months storage. The numbers indicate the tests, and the abbreviations of the sensory attributes are: Redness = redness of colour, B. wholeness = wholeness of berries, M. clarity = clarity of medium, M. evenness = evenness of medium, B. softness = softness of berries, M. firmness = firmness of medium and Faultlessness = faultlessness of odour and flavour.

Figure 25 shows graphically the significant difference in the sensory results after 2 weeks storage of the jams made from frozen CaCl$_2$- and PME-pretreated strawberries in a vacuum and from frozen unpretreated reference berries.
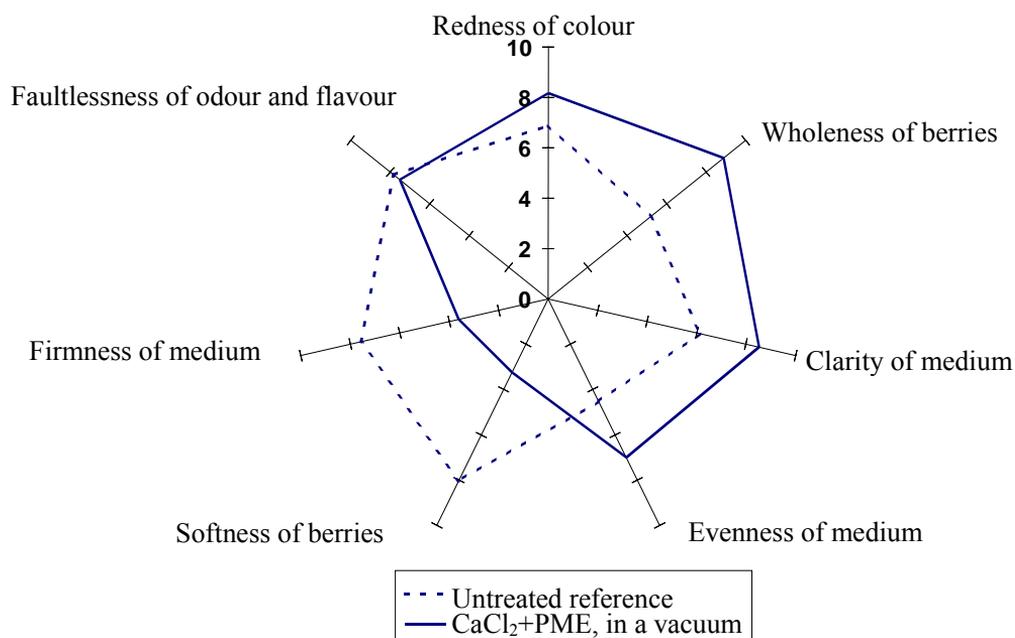
Figure 25. Sensory profiles of the strawberry jams after 2 weeks storage.

3.5 Modeling (Publication V)

The pretreatments at the studied levels did not affect °Brix- or pH-values. The factor with the greatest effect on calcium in frozen or jam berries and medium was naturally calcium pretreatment. Futhermore, in frozen strawberries increasing temperature and in jam berries increasing pretreatmet time favoured increasing calcium content.

García *et al.* (1995) found in their investigation that the temperature of post-harvest heat treatment reduced significantly strawberry decay during shelf life. Heating at 45°C (other temperatures used were 25, 35 and 55°C) for 15 min produced the lowest values for post-harvest losses, weight loss, and titrable

acidity, the highest values of fruit firmness and soluble solids content, and the best values for appearance. However, these fruits lost initial calyx colour and calyx and fruit brightness most rapidly.

Heating at 45°C for 15 min enhanced the penetration of the calcium from 1% $CaCl_2$ dips into the fruits. In strawberries, both post-harvest treatments, temperature and calcium applied by dips may act synergistically to maintain or even enhance the initial firmness values of the fruits during shelf life. The combination of $CaCl_2$ dips and heat treatments probably allows the formation of salt bridge crosslinks with the pectin molecules of the cell wall after their heat-induced demethylation (García *et al.* 1996).

When studying the effects of PE, holding time and holding temperature on strawberry firmness, a temperature increase from 40 to 50°C was found to have the greatest effect on fruit or vegetable firmness improvement. The enzyme amount and holding time had little effect on the process (Coutel & Dale 1998).

The calcium content of jam medium increased when the vacuum level decreased (pressure increased). According to linear regression results of the responses, increasing the $CaCl_2$ concentration in frozen berries increased calcium in jam berries (R = 0.67, p<0.01) and medium (R = 0.86, p<0.001) as well as instrumental firmness in jam berries (R = 0.89, p<0.001) and jam medium (R = 0.87, p<0.001), but decreased the colour coordinates lightness (R = –0.62, p<0.01), redness (R = –0.81, p<0.001) and yellowness (R = –0.74, p<0.001) as well as the clarity (R = –0.73,p<0.001) and evenness (R = –0.80, p<0.001) of the jam medium. Increasing calcium in frozen berries also increased the sensory firmness of jam medium (R = 0.71, p<0.001).

Instrumental firmness of jam berries increased when $CaCl_2$, temperature and vacuum level increased (pressure decreased). The pretreatment time appeared to have no effect on the firmness of the jam berries or of the medium. This is supported by the results of Hoover & Miller (1975) and Salvatori *et al.* (1998). $CaCl_2$ alone (in the range from 0.1 to1.0%) affected the firmness of jam medium. Above this concentration, increasing vacuum level also increased the firmness of jam medium. Comparison of the mean values of firmness of jam strawberries and medium by linear regression showed that jams with firmer berries also had firmer medium (R = 0.87, p<0.001).

It was not possible to model instrumental redness or yellowness of jam berries. This meant that the pretreatments did not affect these parameters in the studied range. Generally, the lightness of jam berries and of the medium increased when temperature and the amount of $CaCl_2$ decreased. PME favoured increasing lightness of the jam medium. According to García-Viguera *et al.* (1999) a jam boiling time of longer than 15 min has a detrimental effect on jam colour. The jam boiling time in our study was about 10 min. Use of the linear regression method showed that there were high positive correlations between redness and lightness of jam berries and media as well as between redness and yellowness of jam berries and jam media, respectively (R = 0.74 and R = 0.99, p<0.001). Redness of the jam medium correlated positively with its clarity (R = 0.73, p<0.001) and evenness (R = 0.84, p<0.001) but negatively with its sensory firmness (R = –0.82, p<0.001). Lightness and yellowness had similar effects on the above variables.

$CaCl_2$ was the most significant factor for sensory attributes of evenness and firmness of medium. Redness of colour could not be modelled. The use of a vacuum was shown to have a significant effect on the wholeness of berries, PME and temperature affected the clarity of the medium and, temperature had an influence on the faultlessness of odour and flavour. The pretreatment time alone did not affect the sensory attributes in the given ranges.

Instrumental firmness of jam berries correlated positively with the wholeness of berries (R = 0.64, p<0.05). The instrumental firmness of jam medium correlated positively with its sensory firmness (R = 0.83, p<0.001), whereas the evenness of jam medium correlated highly negatively with its sensory firmness (R = –0.94, p<0.001). The evenness of jam medium had a positive correlation with the clarity (R = 0.76, p<0.001) of medium and the sensory softness of jam berries (R = 0.80, p<0.001). The yellowness of the jam medium had a positive correlation with the evenness of the medium (R = 0.81, p<0.001). The jams in which the instrumental firmness of jam berries and medium increased, had decreased clarity and evenness of the medium.

4. Feasibility of the study

The studied prefreezing treatment incorporating $CaCl_2$ and PME in a vacuum is particularly interesting for the jam making industry because fresh berries are not available for jam making all year round. Furthermore, harvesting conditions and the size and chemical structure of berries selected for jam making vary. For industrial jam making it is of primary importance to ensure consistent conditions of jam making and the integrity of berries. The studied pretreatment also provides the option of using several different strawberry varieties in industrial production, whereas the domestic jam industry nowadays solely employs one variety, cv. Senga Sengana. Thus the dependence on a single variety is eliminated. A further advantage of the studied pretreatment is that jams made of these berries contain a significant amount calcium ions. This is advantageous for many people requiring dietary calcium, e.g. people allergic to milk and milk products. Further, it appears that the vacuum reduces the content of air in the strawberries, so that there is less air which may cause frothing in jam making.

In addition to strawberries, the studied pretreatment could also be applied to other soft berries such as raspberries and cloudberries. The pretreatment is suitable before freezing or for frozen berries before jam making (Int. Patent Appl. WO patent 01/30178). The pretreatment is suitable for exported berries, which can be treated before freezing and transport to foreign countries.

The vacuum equipment includes a pump, an air-pressure chamber and pipes. Costs of the pretreatment equipment and chemicals will be reasonable. A pump which can be used for decrease in pressure of a 200 L chamber to 7 kPa with a suction effect of 100 m^3/h would cost about 1300–2000 EUR (Busch Vakuumteknik Oy, Finland). Equipment can be designed for batch or continuous processes. Chemicals including $CaCl_2$ (1%) and PME enzyme (50 µkat/kg strawberries) would cost about 45 cent per 100 kg strawberries. The cost of food-grade $CaCl_2$ is about 3 EUR per kg (Telko Oy, Finland) and that of PME (50 µkat/kg strawberries) (Novo Shape) is about 54 EUR per L (S.O. Strömberg Ky, Finland). The amount of pretreatment solution is needed 120 L for 100 kg strawberries. The solution must be changed every 2 h, which means that the total amount of solution per 8 h day will be 500 L. One pretreatment will take about 15 min. About 30 batches will be treated per day, which means about 3000 kg strawberries per day. About 8.5 L extra pretreatment solution must be added into

the chamber after each treatment to compensate for solution absorbed into the berries and for losses during pouring. From this it follows that chemical costs for the treatment of 3000 kg strawberries would be about 27 EUR per day.

The growing consumer demand for high quality, minimally processed, additive-free and microbiologically safe foods has created a need for new food processing methods. High pressure (HP) technology has proved to be a potential new method allowing the production of foods which fulfil consumer expectations. The preservation of porous fruits and vegetables by HP is often problematic, since they contain significant air voids within the tissue. On being subjected to pressure any gases contained in a porous material will be almost completely compressed, which can cause an irreversible collapse of the tissue. The oxygen dissolved in the product also causes trouble in preserving quality. Various oxidative reactions (which can be both non-enzymatic and enzymatic, depending on how well the enzymes have been deactivated), can take place in the product during storage. Reactions affecting product quality include for example discoloration, formation of off-flavours and decomposition of vitamin C. The studied pretreatment of $CaCl_2$ and PME solution in a vacuum can be used for strawberries and similar berries having a sensitive and soft texture before subjecting the berries to HP treatment (Int. Patent Appl. WO patent 01/58286). In this case, the berries do not need to be drained at all, or are only partially drained after the pretreatment. Sugar, salt or other agents including gelling and/or thickening agents, optionally in liquid medium, are then added to the pretreatment medium. These agents may also be added before the actual pretreatment. The berries are then packaged in a flexible container and subjected to a pressure of 400 to 700 MPa at 0–40 $^\circ$C for 1–60 min. The results of the firmness measurements of HP- treated strawberry slices soaked in a liquid containing $CaCl_2$ and PME indicated that the firmness results that was about nine times higher than that of berries which were not pretreated at all.

For industrial applications, any high pressure equipment may be employed for generating the required high isostatic pressure. Typically, the equipment comprises low alloy steel vessels having a cylindrical configuration. The pressure is generated through direct or indirect compression of the pressure medium. Direct compression is obtained by using a piston-type press. Indirect compression can be attained by using an HP intensifier pump (Mertens 1993). HP technology is a very expensive technology which is a significant disadvantage delaying its

adoption in industry. The investment in a 600 MPa HP food processor is of the order of 0.6 to 3 million EUR. Processing 6000 L/h at 600 MPa without a hold time and with a fill factor in the press of 50% would cost around 5 cent per L. The estimate cost is based on a five year depreciation and 15% interest rate and includes operating and maintenance costs (Olsson 1995).

5. Conclusions

Different chemical components and their locations in strawberry *(Fragaria x ananassa)* achenes, vascular bundles and cortical cell walls were studied by means of bright-field microscopy using different staining systems and by Fourier transform infrared (FT-IR) microscopy. The structural changes in pretreated strawberry tissues during freezing and thawing were studied by means of physical and chemical analyses as well as by bright-field and FT-IR microscopy, and by confocal laser scanning (CLS) microscopy. In addition, traditional jams made from pretreated frozen strawberries were investigated by means of physical and chemical analyse and by sensory evaluation.

Basic microstructural studies showed that the cell walls were complex and consisted of several components. Lignin was an important component of achenes and vascular tissues, whereas the cortical cell walls contained mainly cellulose, pectin and some protein. Cellulose and pectin were also detectable in the intercellular spaces of cortex, in the vascular tissue and pith. Protein was deposited inside the cortical cells, as well as in the vascular tissue and pith. The pectin content decreased as the point of observation moved from the hypodermis to the cortex, and the pectin, protein and carbohydrate contents also decreased as the point of observation moved from the vascular to the cortical tissue. The microscopical methods used were complementary.

Microscopical studies showed that $CaCl_2$ and PME treatment improved tissue integrity. Pectin, protein and structural carbohydrates of strawberries pretreated with $CaCl_2$-based solutions remained more stable after freezing and thawing and were less damaged. The $CaCl_2$ and PME treatment in a vacuum appeared to stabilize first the structure of the vascular tissue and then the cortex and pith. Use of a vacuum enhanced the diffusion of the pretreatment solution into the cortex and pith.

Neither of the pretreatments appeared to affect epidermis or hypodermis. On the other hand epidermis and hypodermis comprised only a few cellular layers, which were compressed after thawing and refreezing and thus were difficult to recognize. Generally, overlapping effects in the spectral region $1700-1500$ cm^{-1} followed by smoothing decreased the resolution of spectra, and lignin, amide II and acidified pectin were to some degree undistinguishable.

The textural properties of the jams made from strawberries treated with $CaCl_2$-based solutions were influenced significantly. The treatments were found to preserve the shape of the strawberries. Treatments with $CaCl_2$-solution followed by sprinkled crystallized sucrose retained the red colour of the strawberries. The results of instrumental texture and sensory evaluation were similar.

The $CaCl_2$ and PME solution in a vacuum, together with a quick freezing method with liquid nitrogen, stabilized the original structure of the strawberries during freezing, jam making and storage.

For achieving high quality and palatable jams from pretreated strawberries, the use of high $CaCl_2$ concentrations and high pretreatment temperatures should be avoided. Optimally, the pretreatment time should be short (about 5–15 min), the temperature low (less than 20°C), the vacuum level high (pressure less than 10 kPa), the $CaCl_2$ concentration moderate (about 1%) and the dosage of PME comparatively low (about 50–100 μkat/kg strawberries).

References

Abers, J.E. & Wrolstad, R.E. 1979. Causative factors of color deterioration in strawberry preserves during processing and storage. J. Food Sci., Vol. 44, pp. 75–81.

Anonym, 1977. Modernisation of Hartley's new jam line. Food Trade Rev., Vol. 47, pp. 656–657.

Anonym, 1979. Jam for Sweden's largest food organisation. Food Proc. Ind., Vol. 48, pp. 48–51.

Anonym, 1985. Chivers Hartley's new fruit handling and processing plant. Food Trade Rev., Vol. 55, pp. 219–224.

AOAC. 1995. Official Methods of Analysis of AOAC. Method 942.15 and 975.03. 16th ed. Association of Official Analytical Chemists and AOAC International, Arlington, Virginia 22201-3301, USA.

Armbruster, G. 1967. Cellular and textural changes in three varieties of strawberries as a result of pre-freezing treatments. Amer. Soc. Hortic. Sci., Vol. 91, pp. 876–880.

Axelos, M.A.V. & Thibault, J.-F. 1991. The chemistry of low-methoxyl pectin gelation. In: R. Walter (ed.). The Chemistry and Technology of Pectin. New York: Academic Press. Pp. 109–118.

Baker, R.A. & Wicker, L. 1996. Current and potential applications of enzyme infusion in the food industry. Trends in Food Sci. & Techn., Vol. 7, pp. 279–284.

Barnes, M.F. & Patchett, B.J. 1976. Cell wall degrading enzymes and the softening of senescent strawberry fruit. J. Food Sci., Vol. 41, pp. 1392–1395.

Baron-Epel, O., Gharyal, P.K. & Schindler, M. 1988. Pectins as mediators of wall porosity in soybean cells. Planta, Vol. 175, pp. 389–395.

Belitz, H.-D. & Grosch, W. 1999. Food Chemistry. 2. Ed. Heidelberg: Springer. P. 752.

Berbari, S.A.G, Nogueira, J.N. & Campos, S.D.S. 1998. Effect of different pre-freezing treatments on the quality of frozen strawberry variety Chandler. Ciénc. Tecnol. Aliment., Vol. 18, pp. 82–86.

Blonk, J.C.G. & van Aalst, H. 1993. Confocal scanning light microscopy in food research. Food Research Int., Vol. 26, pp. 297–311.

Brooker, B.E. 1995. Imaging food systems by confocal laser scanning micro-scopy. In: E. Dickinson (ed.). New Physio-Chemical Techniques for The Characterization of Complex Food Systems. Pp. 54–68.

Broomfield, R.W. 1996. The manufacture of preserves, flavourings and dried fruits. In: D. Arthey & P. R. Ashurst (eds.). Fruit Processing. London: Chapman & Hall. Pp. 165–195.

Carpita, N.C. & Gibeaut, D.M. 1993. Structural models of the primary walls of flowering plants. Plant J., Vol. 3, pp. 1–30.

Chéour, F., Willemot, C., Arul, J., Desjardins, Y., Makhlouf, P.M., Charest, P.M. & Gosselin, A. 1990. Foliar application of calcium chloride delays postharvest ripening of strawberry. J. Amer. Soc. Hortic. Sci., Vol. 115, pp. 789–792.

Chéour, F., Willemot, C., Arul, J., Makhlouf, J. & Desjardins, Y. 1991. Postharvest response of two strawberry cultivars to foliar application of $CaCl_2$. Hort Sci., Vol. 26, pp. 1186–1188.

Cochran, W.G. & Cox, G.M. 1957. Experimental Designs. John Wiley & Sons Ltd. 611 p.

Copenhagen Pectin A/S. 1993. Handbook for The Fruit Processing Industry. Denmark.

Coutel, Y.A.G. & Dale, R.H.S., inventors; Gist-Brocades Co., assignee. 1998 Nov 26. A method for fruit processing. WO patent 98/52423.

Cross, D.E. 1981. Changes during processing – preserves. In: P.W. Goodendough & R.K. Atkin (eds.). Proceedings of 7th Long Ashton Symposium. Quality in Stored and Processed Vegetables and Fruit. 1979 April 8–12. University of Bristol. London: Academic Press. Pp. 383–387.

Darvill, A., McNeill, M., Albersheim, P. & Delmer, D.P. 1980. The primary cell walls of flowering plants. In: N.E. Tolbert (ed.). The Plant Cell. New York: Academic Press. Pp. 91–162.

Davies, A.M.C. & Dennis, C. 1983. Distribution of potassium, calcium and magnesium in strawberry fruits in relation to breakdown of the sulphited fruit. Food Chem., Vol. 12, pp. 219–228.

Derbedeneva, Z.A. 1971. Histological changes in plant tissue during freezing and thawing of strawberries. Kholodil'naya Tekhnika, Vol. 48, pp. 36–39.

EC. 1979. Determination of reducing sugars expressed as invert sugar or dextrose equivalent. Off. J. Eur. Comm. No L 239, 22.9. Pp. 41–43.

Edwards, M. 1995. Change in cell structure. In: S.T. Beckett (ed.). Physicochemical Aspects of Food Processing. London: Blackie Academic & Professional. Pp. 212–233.

El-Zoghbi, M. 1994. Biochemical changes in some tropical fruits during ripening. Food Chem., Vol. 49, pp. 33–37.

Escriche, I., Chiralt, A., Moreno, J. & Serra, J.A. 2000. Influence on blanching-osmotic dehydration treatments on volatile fraction of strawberries. J. Food. Sci., Vol. 65, pp. 1107–1111.

Fahn, A. 1982. Plant Anatomy. 3 Ed. Oxford: Pergamon Press. 544 p.

Fito, P. 1994. Modelling of vacuum osmotic dehydration of foods. J. Food Eng., Vol. 22, pp. 313–318.

Foster, T.J., Ablett, S., McCann, M.C. & Gidley, M.J. 1996. Mobility-resolved-1-3C- NMR spectroscopy of primary plant cell walls. Biopolymers, Vol. 39, pp. 51–66.

Fry, S.C. 1989. The structure and functions of xyloglucan. J. Exp. Bot., Vol. 40, pp. 1–11.

García, J.M., Aguilera, C. & Albi, M.A. 1995. Postharvest heat treatment on Spanish strawberry (*Fragaria x ananassa* cv. Tudla). J. Agric. Food Chem., Vol. 43, pp. 1489–1492.

García, J. M., Herrera, S. & Morilla, A. 1996. Effects of postharvest dips in calcium chloride on strawberry. J. Agric. Food Chem., Vol. 44, pp. 30–33.

García-Viguera, C., Zafrilla, P. & Tomás-Barberán, F. A. 1999. Influence of processing and storage conditions in strawberry jam color. Ciên. Tecnol. Aliment. Int., Vol. 5, pp. 487–492.

Gormley, T.R. 1970. Freezing strawberries for jam. Farm and Food Research, Vol. 1, pp. 106–107.

Grassin, C.M.T. & Fauquembergue, P.C.L., inventors; Gist-Brocades Co., assignee. 1994 June 9. Use of pectinesterase in the treatment of fruit and vegetables. WO patent 94/12055.

Guichard, E., Issanchou, S., Descourvieres, A. & Etievant, P. 1991. Pectin concetration, molecular weight and degree of esterification: influence on volatile composition and sensory characteristics of strawberry jam. J. Food Sci., Vol. 56, pp. 1621–1627.

Hall, M.A. 1981. Cell wall structure in relation to texture. In: P.W. Goodendough & R.K., Atkin (eds.). Proceedings of 7th Long Ashton Symposium. Quality in Stored and Processed Vegetables and Fruit. 1979 April 8–12. University of Bristol. London: Academic Press. Pp. 53–64.

Hallett, I.C. & Harker, F.R. 1998. Microscopic investigations of fruit texture. Acta Horticulturae, Vol. 464, pp. 411–416.

Hancock, J.F. 1999. Strawberries. Oxon: Cab Interantional. 237 p.

Havis, A.L. 1943. A developmental analysis of the strawberry fruit. Amer. J. Bot., Vol. 30, pp. 311–314.

Heiska, K., Heiniö, R.-L., Suutarinen, J., Karppinen, S., Kiutamo, T. & Mokkila, M. 1999. Enhancement of the industrial use of Finnish strawberries. Progress Report Dnro 8/2/99 (in Finnish). Pp. 14–22.

Hoefler, A.C. 1991. Other pectin food products. In: R. Walter (ed.). The Chemistry and Technology of Pectin. New York: Academic Press. Pp. 51–66.

Hong, D. & Ueda, Y. 1993. Effects of freezing methods and storage temperature on flavor stability and ester contents of frozen strawberries. J. Japan Soc. Hortic. Sci., Vol. 62, pp. 633–639.

Hoover, M.W. & Miller, N.C. 1975. Factors influencing impregnation of apple slices and development of a continuous process. J. Food Sci., Vol. 40, pp. 698–700.

Huber, D.J. 1984. Strawberry fruit softening: The potential roles of polyuronides and hemicelluloses. J. Food Sci., Vol. 49, pp. 1310–1315.

Hyvönen, L. & Törmä, R. 1983. Examination of sugars, sugar alcohols, and artificial sweeteners as substitutes for sucrose in strawberry jam. Product development. J. Food Sci., Vol. 48, pp. 183–185, 192.

Information Centre of the Ministry of Agriculture and Forestry: Horticultural Enterprise Register. 2000. Production of berries and fruit grown outdoor. Helsinki, Finland.

Int. Patent Appl. WO patent 01/30178. Suutarinen, J., Honkapää, K. & Mokkila, M., inventors; Technical Research Centre of Finland, assignee. 2001 October 27. A process for preparing jam.

Int. Patent Appl. WO patent 01/58286. Stolt, M., Suutarinen, M. & Autio, K., inventors; Technical Research Centre of Finland, assignee. 2001 February 12. Process for preserving foodstuff.

Jarvis, M.C. 1984. Structure and properties of pectin gels in plant cell walls. Plant Cell Env., Vol. 7, pp. 153–164.

Javeri, H., Toledo, R. & Wicker, L. 1991. Vacuum infusion of citrus pectin-methylesterase and calcium effects on firmness of peaches. J. Food Sci., Vol. 56, pp. 739–742.

Jewell, G.G., Rantsios, A. & Scholey, J. 1973. Factors influencing the break-down of fruit in strawberry jam. J. Text. Stud., Vol. 4, pp. 363–370.

Jones, S.A. 1996. Optimisation of texture in heat processed fruits. Flair-Flow seminar, VTT Biotechnology and Food Research, Espoo, Finland, 24th of May.

Kačuráková, M. & Wilson, R.H. 2001. Developments in mid-infrared FT-IR spectroscopy of selected carbohydrates. Carbohydrate Polymers, Vol. 44, pp. 291–303.

Keegstra, K., Talmadge, K.W., Bauer, W.D. & Albersheim, P. 1973. The structure of plant cell walls. Plant Physiol., Vol. 51, pp. 188–196.

Kiernan, J.A. 1990. Histological and Histochemical Methods. Theory and Practise. 2nd ed. New York: Pergamon Press. Pp. 222–223.

Kolev, D., Kafedzhiev, I. & Atanasov, I. 1983. Technology of vacuum degassing of apples. Konservna Promishlenost, Vol. 12, pp. 26–27. (in Bulgarian).

Kratz, E. 1993a. Jams, jellies, marmelades Part I: Basic information. Food Marketing & Tech., Vol. 7, pp. 5–13.

Kratz, E. 1993b. Jams, jellies, marmelades Part II: The phenomenon of syneresis and methods of manufacturing, Food Marketing & Tech., Vol. 7, pp. 17–21.

Kwang, H.L., Kwang, S.K., Mi, H.K., Seung, R.S. & Kyung, Y.Y. 1998. Studies on the softening of strawberry during circulation and storage. I. Changes of cell wall components, protein and enzymes during ripening. J. Korean Soc. Food Sci. Nutr., Vol. 27, pp. 29–34.

Lacey, A.J. 1989. The principles and aims of light microscopy. In: A.J. Lacey (ed.). Light Microscopy in Biology: A Practical Approach. Oxford: Oxford University Press. Pp. 1–24.

Lamport, D.T.A. & Northcote, D.H. 1960. Hydroxyproline in the cell walls of higher plants. Nature, Vol. 188, pp. 665–666.

Lamport, D.T.A. 1965. The protein component of primary cell walls. In: R.D. Preston (ed.). Advances in Botanical Research. New York: Academic Press. Pp. 152–218.

Lapsley, K.G., Escher, F.E. & Hoehn, E. 1992. The cellular structure of selected apple varieties. Food Struc., Vol. 11, pp. 339–349.

Lawless, H.T. & Heymann, H. 1989. Sensory Evaluation of Food: Principles and Practices. New York: Chapman & Hall. Pp. 341–378.

Legentil, A., Guichard, I., Piffaut, B. & Haluk, J.P. 1995. Characterization of strawberry pectin extracted by chemicals means. Lebensm.-Wiss. und -Technol. Vol. 28, pp. 569–576.

Lesschaeve, I., Langlois, D. & Etiévant, P. 1991. Volatile compounds in strawberry jam: influence of cooking on volatiles. J. Food Sci., Vol. 56, pp. 1393–1398.

Main, G.L., Morris, J.R. & Wehunt, E.J. 1986. Effect of preprocessing treatments on the firmness and quality characteristics of whole and sliced strawberries after freezing and thermal processing. J. Food Sci., Vol. 51, pp. 391–394.

Makus, D.J. & Morris, J.R. 1998. Preharvest calcium applications have little effect on mineral distribution in ripe strawberry fruit. Hort. Sci., Vol. 33, pp. 64–66.

Martens, H. & Næs, T. 1989. Multivariate Calibration. New York: John Wiley & Sons Ltd. 419 p.

McCann, M.C. & Roberts, K. 1991. Architecture of the primary cell wall. In: C.W. Lloyd (ed.). The Cytoskeletal Basis of Plant Growth and Form. London: Academic Press. Pp. 109–129.

McCann, M.C., Hammouri, M., Wilson, R., Belton, P. & Roberts, K. 1992. Fourier transform infrared microspectroscopy is a new way to look at plant cell walls. Plant Physiol., Vol. 100, pp. 1940–1947.

McCann, M.C., Stacey, N.J., Wilson, R. & Roberts, K. 1993. Orientation of macromolecules in the walls of elongating carrot cells. J. Cell Sci., Vol. 106, pp. 1347–1356.

Mertens, M. 1993. Developments in high pressure food processing (Part 1). Internation Food Manufacturing ZFL, Vol. 44, pp. 100–104.

Method VTT-4289-91 (in-house method). 1991. Foodstuffs. The determination of metal contents after dry ashing by atomic absorbtion spectrometry. VTT Biotechnology.

Molyneux, F. 1971. Manufacture of jams, sauces and pickles. Process Biochem., Vol. 6, pp. 17–18.

Morris, J.R., Sistrunk, W.A., Sims, C.A. & Main, G.L. 1985. Effects of cultivar, postharvest storage, preprocessing dip treatments and style of pack on the processing quality of strawberries. J. Amer. Hort. Sci., Vol. 110, pp. 172–177.

Mulinari-Campos, A. & Bileski-Candido, L.M. 1995. Formulation and physico-chemical and rheological evaluation of low soluble solids jams with different sweeterners. Ciênc. Tecnol. de Aliment. Vol. 15, pp. 268–278. (in Portuguese).

Neal, G.E. 1965. Changes occurring in the cell walls of strawberries during ripening. J. Sci. Food Agric., Vol. 16, pp. 604–611.

Ng, A., Parker, M.L., Parr, A.J., Saunders, P.K., Smith, A.C. & Waldron, K.W. 2000. Physicochemical characteristics of onion (allium cepa l.) tissues. J. Agric. Food Chem., Vol. 48, pp. 5612–5617.

Oakenfull, D.G. & Scott, A. 1984. Hydrophobic interaction in the gelation of high methoxyl pectins. Food Sci., Vol. 49, pp. 1093–1098.

Oakenfull, D.G. 1991. The chemistry of high-methoxyl pectins. In: R. Walter (ed.). The Chemistry and Technology of Pectin. New York: Academic Press. Pp. 87–108.

O'Beirne, D., Egan, S. & Healy, N. 1987. Some effects of reduced boiling time on the quality of fruit preserves. Lebensm.- Wiss. u.-Technol., Vol. 20, pp. 241–244.

Olsson, S. 1995. Production equipment for commercial use. In: D.A. Ledward, D.E. Johnston, R.G. Earnshaw & A.P.M. Hasting (eds.). High Pressure Processing of Foods. Nottingham: University Press. Pp. 167–180.

Peña, M.J., Vergara, C. & Carpita, N.C. 2001. The structure and architectures of plant cell walls define dietary fibre composition and the textures of foods. In: B.V. McCleary & L. Prosky (eds.). Advanced Dietary Fibre Technology. London: Blackwell Science. Pp. 42–60.

Perkins-Veazie, P. 1995. Growth and ripening of strawberry fruit. In: J. Janick (ed.). Horticultural Reviews. London: John Wiley & Sons. Pp. 267–297.

Pilgrim, G.W., Walter, R.H. & Oakenfull, D.G. 1991. Jam jellies and preserves. In: R.H. Walter (ed.). The Chemistry and Technology of Pectin. California: Academic Press. Pp. 23–50.

Pilnik, W. & Voragen, A.G.J. 1993. Pectic enzymes in fruit and vegetable juice manufacture. In: T. Nagodawithna & G. Reed (eds.). Enzymes in Food Processing. New York: Academic Press. Pp. 363–399.

Polesello, A. & Maltini, E. 1970. Studies on uptake of calcium salts during pretreatment of peaches for freezing. Ind. Agr. (in Italian), Vol. 8, pp. 199–205.

Polesello, A. & Crivelli, G. 1971. Addition of calcium salts to fruits as pretreatment for quick freezing. Ind. Agr. (in Italian), Vol. 9, pp. 413–422.

Ponappa, T., Scheerens, J.C. & Miller, A.R. 1993. Vacuum infiltration of polyamines increases firmness of strawberry slices under various storage conditions. J. Food Sci., Vol. 58, pp. 361–364.

Ponting, J.D., Watters, G.G., Forrey, R.R., Jackson, R. & Stanley, W.L. 1966. Osmotic dehydration of fruits. Food Tech., Vol. 20, pp. 1365–1368.

Poovaiah, B.W. 1986. Role of calcium in prolonging storage life of fruits and vegetables. Food Tech., Vol. 40, pp. 86–89.

Rastas, M., Seppänen, R., Knuts, L.-R-, Karvetti, R.-L. & Varo, P. (eds.). 1989. Nutrient Composition of Foods. Publications of The Social Insurance Institution, Finland. Hämeenlinna: Karisto Ltd. 187 p.

Redgwell, R.J., Mac Rae, E., Hallett, I., Fischer, M., Perry, J. & Harker, R. 1997. In vivo and in vitro swelling of cell walls during fruit ripening. Planta, Vol. 203, pp. 162–173.

Reeve, R.G. 1970. Relationships of histological structure to texture of fresh and processed fruits and vegetables. J. Text. Studies, Vol. 1, pp. 247–284.

Roy, S.S., Taylor, T.A. & Kramer, H.L. 2001. Textural and ultrastructural changes in carrot tissue as affected by blanching and freezing. J. Food Sci., Vol. 66, pp. 176–180.

Sacchetti, G., Gianotti A. & Dalla Rosa, M. 2001. Sucrose-salt combined effects on mass transfer kinetics and product acceptability. Study on apple osmotic treatments. J. Food Eng., Vol. 49, pp.163–173.

Sajjaanantakul, T. & Pitifer, L.A. 1991. Pectinesterase. In: R. Walter (ed.). The Chemistry and Technology of Pectin. New York: Academic Press. Pp. 135–164.

Salvatori, D., Andrés, A., Chiralt, A. & Fito, P. 1998. The response of some properties of fruits to vacuum impregnation. J. Food Proc. Eng., Vol. 21, pp. 59–73.

Scholey, J. 1973. Suitability of fruit for jam manufacture. Food Manufacture, Vol. 48, pp. 19–20.

Sams, C.E., Conway, W.S., Abbott, J.A., Lewis, R.J. & Ben-Shalom, N. 1993. Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. J. Amer. Soc. Hortic. Sci., Vol. 118, pp. 623–627.

Sistrunk, W.A. & Moore, J.N. 1979. Evaluating strawberry selections and cultivars for processing. Arkansas Farm Research, Vol. 28, p. 7.

Skrede, G. 1980. Strawberry varieties for industrial jam production. J. Sci. Food Agric., Vol. 31, pp. 670–678.

Skrede, G. 1982. Quality characterisation of strawberries for industrial jam production. J. Sci. Food Agric., Vol. 33, pp. 48–54.

Skrede, G., Wrolstad, R.E., Lea, P. & Enersen, G. 1992. Color stability of strawberry and blackcurrant syrups. J. Food Sci., Vol. 57, pp. 172–177.

Spayd, S.E. & Morris, J.R. 1980. Prediction of sensory color of strawberry jam. Arkansas Farm Research, Vol. 29, p. 12.

Speek, A.J., Schrijver, J. & Schreurs, W.H.P. 1984. Fluorometric determination of total vitamin C and total isovitamin C in foodstuffs and beverages by high-performance liquid chromatography with precolumn derivatization. J. Agric. Food Chem., Vol. 32, pp. 352–355.

Steele, N.M., McCann, M.C. & Roberts, K. 1997. Pectin modification in cell walls of ripening tomatoes in distinct domains. Plant Physiol., Vol. 114, pp. 373–381.

Stolt, M. 1997. Factors that affect the structure of strawberry jam. Master's Thesis for the Helsinki University of Technology. Otaniemi, Espoo, Finland.

Suutarinen, J., Heiska, K., Autio, K. & Mokkila, M. 2000. The effect of $CaCl_2$ and PME prefreezing treatment in a vacuum on the structure of strawberries (abstract). In: T. Hietaranta, M.-M.Linna, (eds.). 4th International Strawberry Symposium Book of Abstracts; 2000 July 9–14; Tampere, Finland: Kaarinan Tasopaino Oy Ltd. P. 228.

Szczesniak, A.S. & Smith, B.J. 1969. Observations on strawberry texture a three-pronged approach. J. Text. Stud., Vol. 1, pp. 65–89.

Van Buren, J.P. 1979. The chemistry of texture in fruits and vegetables. J. Text. Stud., Vol. 10, pp. 1–23.

Van Buren, J.P. 1991. Function of pectin in plant tissue structure and firmness. In: R. Walter (ed.). The Chemistry and Technology of Pectin. New York: Academic Press. Pp. 1–22.

Vodovotz, Y., Vittadini, E., Coupland, J., McClements, D.J. & Chinachoti, P. 1996. Bridging the gap: use of confocal microscopy in food research. Food Technol., Vol. 50, pp. 74–82.

Webb, R.H. & Rogomentich, F. 1999. Confocal microscope with large field and working distance. Appl. Optics, Vol. 38, pp. 4870–4875.

Wilson, R.H. 1995. Recent developments in infrared spectroscopy and microscopy. New Physico-Chemical Techniques. In: E. Dickinson (ed.). New Physico-Chemical Techniques for the Characterization of Complex Food Systems. Glasgow: Blackie Academic & Professional. Pp. 177–195.

Wrolstad, R.E., Skrede, G., Lea, P. & Enersen, G. 1990. Influence of sugar on anthocyanin stability in frozen strawberries. J. Food Sci., Vol. 55, pp. 1064–1065.

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Title

Effects of prefreezing treatments on the structure of strawberries and jams

Abstract

Different chemical components and their locations in strawberry (*Fragaria x ananassa*) achenes, vascular bundles and cortical cell walls were studied by means of bright-field microscopy using different staining systems and by Fourier transform infrared (FT-IR) microscopy. The structural changes in pretreated strawberry tissues during freezing and thawing were studied by means of physical and chemical analyses as well as by bright-field and FT-IR microscopy, and by confocal laser scanning (CLS) microscopy. In addition, traditional jams made from pretreated frozen strawberries were investigated by means of physical and chemical analyses and by sensory evaluation.

Basic microstructural studies showed that all the cell walls were complex and consisted of several components. Lignin was an important component of achenes and vascular tissues, whereas the cortical cell walls contained mainly cellulose, pectin and some protein. Cellulose and pectin were also detectable in the intercellular spaces of cortex, in the vascular tissue and pith. Protein was deposited inside the cortical cells, as well as in the vascular tissue and pith. The microscopical methods used gave comparable results.

According to microscopical studies both the pretreatments with calcium chloride ($CaCl_2$) and crystallised sucrose as well with $CaCl_2$ and pectin methyesterase (PME) in a vacuum affected the microstructure of strawberry tissues. These pretreatments especially affected pectin, protein, lignin and structural carbohydrates in the vascular tissue and cortex compared to the untreated reference samples. The use of a vacuum appeared to make the pretreatment solutions absorb more efficiently to the cortex and pith, thus improving the stabilisation particularly of pectin and structural carbohydrates.

Firmness of thawed and particularly of jam strawberries pretreated with $CaCl_2$ and PME in a vacuum was higher than that of other pretreated or untreated berries. In all the prefreezing treatments studied, dipping of strawberries into a $CaCl_2$ solution with PME in a vacuum resulted in a significantly different sensory profile than was found in the other jams. The sensory attributes wholeness of the berries ($p<0.001$), firmness, clarity and evenness of the jam medium ($p<0.001$), softness of the berries ($p<0.001$) and faultlessness of odour and flavour ($p<0.001$) in particular were statistically significantly different among the strawberry jams. Sensory quality was perceived to decrease during 4 months of storage, even though the shapes of the sensory profiles of the studied jams did not change significantly from those evaluated after 2 weeks storage.

For achieving high quality jams the pretreatment time should be short (about 5–15 min), the temperature low (below 20 °C), the vacuum level high (pressure less than 10 kPa), the $CaCl_2$ concentration moderate (about 1%) and the dosage of PME comparatively low (about 50–100 μkat/kg strawberries).