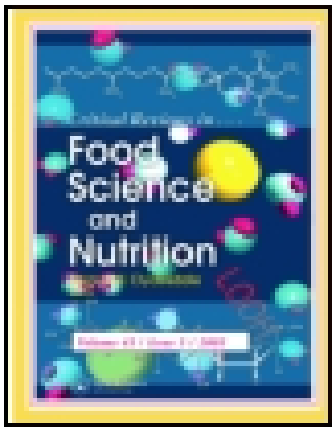

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Effect of Thermal and Nonthermal Processing on Textural Quality of Plant Tissues

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Effect of Thermal and Nonthermal Processing on Textural Quality of Plant Tissues

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Abstract

In the current fast revolving world, the consumption of processed food is increasing drastically. The population who depend on these processed foods are also cautious about the quality and safety of what they consume. This being the case, in order to satisfy the consumer it is the responsibility of the researcher and the manufacturer to check what happens to food on processing. Plant derived foods such as fruits and vegetables are sensitive produces which are to be handled cautiously through each steps involved in processing, starting from harvest to storage, processing to package, transportation to distribution, till it reaches the consumer. During processing, the plant materials, which are made up of complex structural components such as lignin, cellulose, pectin, etc. undergo changes which has its effect on the quality attributes of the final product. Texture is an important quality parameter of all the sensory properties. The relation between the structure of the plant tissue and the texture of the final product is reviewed in this paper comprehensively.

Keywords: Plant tissues: cell wall structure: parenchyma cells: texture: thermal and non-thermal processing.

1 Introduction

1.1 Vegetable and fruit processing

Since most fruits and vegetables are seasonal, they are industrially processed to guarantee a year round availability. Techniques for preserving vegetable food from natural deterioration following harvest date to prehistoric times. Among the oldest methods are drying, refrigeration and fermentation *(In Food Processing: The New Encyclopedia Britannica*, 1993). Modern preservation techniques include canning, freezing, the addition of chemicals, such as sucrose, salt, vinegar or benzoate, and to a lesser extent, drying. In addition, packaging has become increasingly important (Smittle et al., 1987). The principal causes of food spoilage are growth of microorganisms, endogenous enzyme activity, oxidation and dehydration. The form of spoilage to which food is most susceptible depends on its inherent composition and structure, and exogenous factors, such as the abundance of specific microorganisms and storage conditions. Activity and growth rates of microorganisms can be affected by temperature, moisture, oxygen concentration, pH, ionic strength, nutrients available, degree of contamination with spoilage organisms and the presence or absence of growth inhibitors. Control of one or more of these factors suffices to inhibit microbial spoilage. Heat sterilisation, or thermal processing, essentially consists of subjecting the food in a sealed can or jar to a known temperature long enough to destroy spoilage and pathogenic organisms that might be present in or on the raw food material.

The heat treatments necessary to preserve canned foods are determined by a number of factors, most importantly the acidity of the food product. Non-acid foods, such as most vegetables, require higher temperatures for adequate processing than acid foods, such as fruits.

Operations commonly included in the canning of vegetables, such as green beans, are cleaning, preparatory operations, blanching, filling and exhausting, can sealing, thermal processing, cooling, labelling and storage (Figure 1.1). Food preservation processes are still constantly being (re)designed to optimise microbial safety, nutrient retention, colour, taste, texture and other quality attributes of the end product (Sistrunk et al., 1989). Unfortunately one of the negative effects of heat sterilisation is extreme softening of the product, resulting in undesirable textural quality.

1.2 Texture

The texture of processed vegetables is an important quality attribute. Texture is defined as: “all physical characteristics sensed by the feeling of touch that are related to deformation under an applied force” (Jackman et al., 1995). Consumer perception of texture is often subconscious, but paradoxically, the pleasure experienced when eating fresh or processed plant foods is largely attributed to their texture. The importance of texture as a quality attribute is accented by the enormous real and economic losses incurred during harvesting and subsequent handling, distribution and storage that ultimately arise from textural deterioration and concomitant physico-mechanical damage.

Texture of vegetables is a sensory sensation and can best be analysed by the use of analytical sensory panels. Examples of typical textural descriptors are ‘crisp’, ‘firm’, and ‘elastic’. However when processing is investigated, the texture of the raw product as well as the product at several stages during the process has to be quantified, which is not feasible by sensory panels. Therefore, mechanical properties of food, the basic elements of texture, are often

measured instrumentally (Verlinden et al., 1996). Texture is a characteristic at the plant organ level, which is dependent on several features of plant tissues, cells, and ultimate basically on structures and interactions of molecules and ions. Plant parts used for food, either stems, leaves, roots, flowers, fruit, seeds, or tubers, contain various tissue types, which play an essential role in determining texture. At first, the relative proportion of fibrous tissues, such as collenchyma, sclerenchyma and xylem, to the more tender parenchyma tissues should be considered. Most vegetables and fruits consist mainly of parenchyma tissues.

Parenchyma cells have a diameter varying from 50 to 500 μm and are polyhedral or spherical in shape. Depending on the spatial arrangement and shapes of the constituent cells, parenchyma tissues can contain significant amounts of air space, which also influence texture. Intracellularly, the cells contain one or more vacuoles, different organelles, a cytoskeleton and inclusion bodies in which important food components, such as starch, proteins and lipids, are stored. These components also affect the texture of the processed products. For example starch is highly associated with the development of a ‘waxy’ or ‘mealy’ texture of rice and potatoes (Radhika et al., 1993). The cell is surrounded by the plasma membrane. The texture of fresh products can for a large part be attributed to the turgor pressure generated within cells by osmosis through the plasma membrane. Turgor pressure causes the cells to swell, giving rigidity and crispness to the tissue (Jackman et al., 1995). However, the contribution of the plasma membrane to texture of processed products is considered to be low, since membrane functions are very quickly lost upon heating (Greve, et al, 1994). At the cellular level, texture is a manifestation of the ease with which cells can be split open and the ease with which they can be separated (Williams, 1980). Texture of processed, low-starch vegetables and fruits is therefore

mainly determined by the primary cell wall and middle lamella, located exterior to the plasma membrane. The cell wall of a parenchyma cell is in general thin, but strong, thereby limiting expansion and generating turgor pressure (Cosgrove, 1986). The middle lamella functions as a kind of cement, sticking the cells together and generating tissue strength. For example, analyses of tomato ultrastructure during ripening showed that degradation of the middle lamellae correlated highly with softening (Jackman et al., 1995). Secondary walls can be deposited internally to the primary wall after cessation of cell growth and are important in some cereal products like wheat bran. Their presence in vegetables is associated with the development of a 'woody' or 'stringy' texture, like for example in asparagus (Waldron et al., 1990).

1.3 Structural aspects of cell wall and middle lamella constituents

Cell walls contain numerous polymeric compounds that are capable to bear applied stress (Cosgrove, 1993). This is essential for the main functions of cell walls, which are determining size, shape and strength of plant tissues. The cell wall however also has a role in resistance to pathogens (Brett, et al., 1990), and regulation of various metabolic processes (Van Cutsem, et al., 1994). The main cell wall constituents are water, pectin, hemicellulose and cellulose, but also protein and lignin can be present. The middle lamella is merely composed of pectin, and cannot always be discerned from the primary cell wall. Its main function is to cement the cells together.

Older concepts of the cell wall as a static, inert structure (Darvill, et al., 1980) have given way to new, more dynamic models (Carpita, et al., 1993). These newer models differ predominantly from the earliest models in that the individual polymers are not all covalently linked to each other. At present, the cell wall is envisaged as three structurally independent, but

interacting domains (figure 1.3). The first domain constitutes the cellulose-hemicellulose network, which is embedded in a second domain, a matrix of pectic substances. The third domain consists of structural proteins, oriented radially within the cell wall matrix. Cell wall polysaccharides are built up of about a dozen main sugars, which can be linked in an enormous variety of ways. The types of linkages, as well as the sugars themselves, dictate the properties of polysaccharides and thus their possible functions in the plant cell wall and effect on texture (Fry, 1988). Properties commonly used to characterise the different cell wall polysaccharides are sugar composition, molecular mass distribution, shape, charge, extractability, and binding with other polymers.

1.3.1 Water

The primary cell wall is a highly hydrated structure, with water being one of its most variable features. Water is believed to have four major functions in the cell wall (Northcote, 1972)

- a structural component of the matrix gel
- a wetting agent, hindering direct hydrogen bonding between polymers
- a stabiliser of polymer conformation
- a solvent or transport medium for salts, enzymes and low molecular mass organic compounds.

1.3.2 Pectic substances

Pectic substances are very abundant in fruit and vegetable cell walls. Since they are considered to be very important in determining the texture of processed vegetables, they are

discussed here in more detail than the other cell wall constituents. Pectin is not one single polymer, but comprises rather a group of so-called 'pectic substances'. Pectins are defined as hetero-polysaccharides rich in D-galacturonic acid, including side chains of such polysaccharides (Fry, 1988). The term 'protopectin' is sometimes used to describe *native*, water unextractable, pectic polysaccharides within the cell wall, in recognition of the fact that the methods used to extract them are degradative. Pectins from middle lamella and cell walls are not distinguishable after extraction. There is some evidence that pectins from the middle lamella are less branched than those from the cell wall (Selvendran, 1991). There are contradictionary data about the degree of methylation of middle lamella pectin, being either low or high (Liners et al., 1992). In general, three types of pectic components have been studied: homogalacturonan (HG or 'smooth regions'), and rhamnogalacturonan type I and II (RGI or 'hairy regions' and RGII). Though the usage of this nomenclature came into practice recently, the group of Albersheim used the nomenclature HG, RGI and RGII since the eighties, based on their work with cell walls from suspension cultured sycamore cells. (Albersheim et al., 1996).

1.3.2.1 Polymer structure:

Pectins are 'block' polymers (Jarvis, 1984). They contain linear homogalacturonan blocks ('smooth regions') and elsewhere in the same molecule, branched blocks of rhamnogalacturonan ('hairy regions') with neutral side chains (De Vries, et al., 1982) (Figure 1.4).

Homogalacturonan consists of a backbone of mainly α-(1, 4) galacturonic acid which can be interspersed with some α-(1, 2) rhamnose residues. The spacing of the rhamnose residues may

be regular, but this is not generally established (Powell, et al., 1982). The galacturonic acid residues can be substituted with methyl and acetyl esters on respectively the carboxylic acids on C-6 and the secondary alcohols on C-2 and C-3 (Komalavilas, et al., 1989). It has been suggested that the rhamnose residues act as molecular 'punctuation marks', with the intervening homogalacturonan blocks being in some cases fully methylesterified and in others not esterified at all (Mort, et al. 1993). The proportion of acetyl groups varies greatly with the plant origin, being for example very high (> 30 %) in sugar beet pectins. The molecular distribution of acetyl groups along the rhamnogalacturonan chain is unknown. Rhamnogalacturonan consists of a backbone of α - (1,2) rhamnosyl and α -(1,4) galacturonic acid units. The ratio between rhamnose and galacturonic acid is not necessary 1:1, as was proposed in the early RGI and RGII models of Albersheim and co-workers (Colquhoun, 1990) but varies between 0.05 and 1 (Schols, 1995). Side chains consisting essentially of arabinans, galactans, arabinogalactans and single xylose residues are attached to the backbone. The main side for attachment is the O-4 of the rhamnosyl unit. The proportion of rhamnosyl units with attached side chains varies between 20 to 80 %, depending on the source of the polysaccharide (Albersheim, et al. 1996). The amounts and length of the side chains varies with the cell type and physiological state. Talbott and Ray (1992) proposed that these neutral polysaccharides also occur as free polymers. It cannot be excluded however, that these free neutral pectic polymers in their experiments originate from side chains split off from the pectic backbone during the cell wall preparation procedure. Feruloyl groups can be ester linked to the non-reducing arabinose and/or galactose termini of the pectic side chains in the 'hairy regions' of for example sugar beet and spinach pectins (Fry, 1993). Rhamnogalacturonan II is a small and complex pectic polymer (Puvanesarajah, et al.

1991). The structure is extremely complex with as much as twelve different glycosyl residues, including rare sugars such as apiose, 2-*O*-methyl- L-fucose, aceric acid and common monosaccharide's involved in unusual glycosidic linkages such as a fully substituted rhamnose. The main structure seems to be highly conserved during evolution and the complexity of the molecule suggests a role as signal molecule, rather than a structural function.

1.3.2.2 Cross-linking of pectins in the cell wall

The types of cross-links in primary cell walls as indicated in Figure 1.5 have been reviewed by Fry (1993). Some pectin can be solubilised with water, which indicates that they are not cross-linked at all. Nonesterified pectic homogalacturonan blocks of sufficient length are able to associate intermolecular in the presence of calcium ions (Figure 1.6) (Garnier, et al. 1994). This type of association is called 'egg-box' (Grant, et al. 1973). Liners and co-workers have proposed, using a monoclonal antibody against calcium induced supramolecular conformation of PGA that an uninterrupted sequence of at least nine galacturonic acid residues is necessary on each of the two chains to dimerise with five calcium ions (Liners, et al. 1992). Subsequent induced aggregation of these homogalacturonan dimers into tetramers; hexamers etc. can occur (Morris, et al. 1982). However, at this moment there is some confusion about the existence and importance of 'egg-box' structures *in vivo* (Liners and Cutsem, 1992). Acidic pectins could also be ionically linked to basic proteins such as extension (Fry, 1986). In addition to ionic binding zones in the 'smooth regions', pectins may be covalently cross-linked to other pectins or hemicelluloses by the formation of for example ferulic acid dimers (Ralph, et al.,

1994). There is also some evidence for the existence of other ester linkages between uronic acid residues and neutral sugars from for example cellulose (Fry, 1986).

1.3.2.3 Structure-function relationships of pectins

As structural polysaccharides, pectins contribute to the strength and flexibility of cell walls from nonlignified plant organs and ripening fruits (Jarvis, 1984). McCann and co-workers proposed that pectic compounds, including the neutral side chains, mainly determine the porosity of the cell wall (McCann, et al., 1991). By limiting the porosity below a certain level, pectins inhibit the free diffusion of enzymes through the wall and thereby enzyme-substrate interactions and hence cell wall autolysis. The cementing function of pectic substances is thought to depend mainly on their non methylesterified homogalacturonan regions, which can form 'egg-box' structures with calcium, as is described above. Nevertheless, in suspension cultured carrot cells, the pectin of tightly attached young callus was found to be composed of high methylesterified galacturonic acid, while that of loosely attached old callus was composed of non-methyl-esterified galacturonic acid (Liners, 1992).This suggest that the formation of so-called 'egg-box' structures is not associated with intercellular cohesion *in vivo*.

Moreover, the ratio of arabinose to galactose was highly correlated (R = 0.94) with the formation of cell clusters and intercellular attachment which, in contrast to the general idea, suggests an important role for these neutral side chains in intercellular adhesion (Kikuchi, et al. 1996). Very few specific data on the contribution of pectic side chains on textural properties are available (Hwang, et al., 1993). Neutral side-chains of pectins are usually considered to hinder gel formation. In fact, they themselves may be capable of weak non-covalent interactions, such

as double helices of β-(1,4)-galactan and partly crystalline aggregates of linear α-(1,5)-arabinans (Churms, et al., 1983). However, there is no experimental evidence that binding of this type contributes to the strength of plant cell walls. A hint for the importance of side chains in relation to intrinsic texture is presented by Batisse and co-workers (1996). Pectic side chains of crisp cherry fruits were found to be longer and more cross-linked in comparison with pectin from soft fruits. For potatoes however, it was reported that either a greater amount or longer pectic side chains resulted in less firm potatoes and less sensibility to prewarming (Quinn & Schafer, 1994). A decrease in branching and concomitant higher solubility is described for pectin from many fruits during storage, ripening and concurrent softening. This is the result of either overall degradation of existing pectins, such as described for tomato (Seymour, et al., 1990), guava (El-Buluk et al., 1995), mango (Muda et al., 1995), peach (Fishman, et al., 1993), nectarine (Lurie, et al. 1994) and kiwi (Redgwell, et al. 1992), or a changed metabolic turnover, as was shown for apples (MacLachan, et al. 1994). In the case of pectin degradation, the softening seemed to be related both with a lower degree of branching and polymerisation. In the case of turnover however, the newly synthesised pectins also had a low degree of branching, but a high degree of polymerisation. This was also accompanied by a higher DM, which was altogether proposed to result in less calcium complexed intercellular cohesion and hence tissue softening (Jarvis, 1984).

1.3.3 Cellulose

Cellulose is a structural component of all higher plant cell walls. The structure consists of linear chains with β-(1, 4) glucosyl residues. The chains are associated into microfibrils with crystalline and amorphous regions, which make the cellulose structure more diverse than the single building

units suggest. The degree of crystallinity depends on the origin of the cellulose. The role of cellulose is generally viewed upon as a skeleton function. The microfibrils are laid down in different parallel layers or lamellae, which must be free to move with respect to each other during elongation (McCann & Roberts 1991).

1.3.4 Hemicelluloses

Hemicelluloses are defined as non-cellulosic cell wall polysaccharides other than pectins which can be extracted by aqueous alkali (McCann & Roberts 1991). Hemicelluloses presumably play a role in microfibril spacing, important for wall assembly. The main function of the resulting cellulose-hemicellulose network is to provide shape and strength to the cell wall (McCann & Roberts 1995). In the cell walls of dicotyledonous plants xyloglucans and acidic arabinoxylans are the main hemicellulosic polymers found, but also (gluco- or galacto) mannans can be present (Carpita, et al. 1993). Xyloglucans are structurally related to cellulose in that they possess a β-(1, 4) glucosyl backbone. They differ from cellulose in that approximately 75 % of the glucosyl backbone is substituted at C-6 with a α-xylose residue. Arabinose, galactose and fucose can be attached to the xylose (Kiefer, et al. 1990). O'Neill and Selvendran (1985) proposed a block type structure for the xyloglucan of *Phaseolus coccineus* (Figure 1.7).

Xylans consist of a β-(1,4) xylose backbone with single arabinose units at the O-3 and single glucuronic acid residues at the O-2 of the xylosyl units (Carpita & Gibeaut, 1993). Xyloglucans and unsubstituted xylans can be linked to cellulose through hydrogen bounding (Valent & Albersheim, 1974). In addition there is evidence for covalent linkage of arabinoxylans of grasses to other cell wall polymers through diferuloylesters (Ralph, et al. 1994).

1.3.5 Cell wall proteins

Cell walls contain a variety of different proteins, most of which are glycosylated (Brett & Waldron, 1990). These include structural proteins as well as enzymes. The most well studied structural proteins are the hydroxyproline rich proteins, such as extensin. Extensins are present in the primary cell walls of dicots in varying quantities, making up 1 - 10 % (w/w) of the wall. They are particularly abundant in the cell walls of cultured cells and also in the seed coat of soybeans (Fry, 1988). Extensin is thought to play a role in the skeletal construction of the cell wall, the organisation of the cellulose microfibrils, the restriction of growth and the exclusion of invading pathogens (Carpita, et al. 1993). Related proteins are the arabinogalactan proteins (AGP's), which are slimy, water soluble proteins found in the apoplastic fluid of many tissues. The biological role of AGP's is unclear: suggestions for their function include lubrication and cell/cell recognition (Fry, 1988). A structural protein which received attention only very recently is expansin, a structural protein involved in the expansion of cells probably by breaking the hydrogen-bonds of hemicelluloses and cellulose thereby promoting expansion (Cosgrove, 1997). The enzymes present in the wall include peroxidase, cellulase, polygalacturonase, pectin methylesterase, and several exo- and endoglycosidases.

1.3.6 Lignin

Certain differentiated cell types contain lignin, a phenolic polymer that is laid down after cell elongation has ceased (Brett & Waldron, 1990). The precursors of lignin include the three aromatic alcohols, coumaryl, coniferyl and sinapyl alcohol. These precursors are linked by a

wide variety of bounds in the final polymer. Lignin initially is laid down in the middle lamellae and primary walls of certain cells with secondary walls, being mainly vessel elements, tracheids, fibres and sclereids. It later also accumulates in the secondary walls of these cells. Lignin is a filler material, replacing the water and permanently preventing further growth. Its most probable biological function is provision of physical strength (Fry, 1988).

1.4 Thermal processing for food preservation

Modern western consumers increasingly demand minimally processed, 'additive' free foods that are nutritionally sound, convenient, 'fresh -like' and yet have sufficient shelf-life to be compatible with modern weekly shopping habits. These demands have presented significant technical challenges for the food industry. Ambient stable foods are those which have a significant shelf-life without a requirement for chilling. Conventional processes for achieving ambient shelf stability include drying, aseptic processing and canning. The shelf-life of such foods varies quite widely depending upon the nature of the product. Taking canned products as an example, the shelf-life of canned fruit and vegetables can vary between around 9 months and 3 years (Holdsworth 1983). Handling, container construction and storage conditions can all influence the achievable shelf-life. Conventional thermal preservation technologies for ambient stable 'canned' products have an excellent safety record (when applied correctly), are generally high throughput and can provide convenient, reasonable quality foods at a low cost (Mintel 2007).

1.5 Emerging methods for food preservation

A number of alternative ‘emerging’ preservation processes have attracted research interest because such technologies may provide products having improved quality over those made using conventional thermal preservation and which address some of the limitations described previously. Many of these technologies remain very much in the research arena, some are on the brink of commercialisation and some (such as high pressure pasteurisation) are already in use, albeit on a relatively limited scale.

1.5.1 Power ultrasound

Power ultrasound (typically in the range of 20 kHz to 100 kHz) has numerous non-preservation applications such as cutting, cleaning, emulsifying, de-gassing and foam breaking (Patist & Bates 2008). It is not thought to be currently in use for microbiological inactivation in food production but there is evidence at laboratory scale demonstrating antimicrobial effects. For the inactivation of microorganisms, it is usually suggested that ultrasound is combined with moderate heating and, in some cases, slightly elevated pressure; processes known respectively as thermosonication and manothermosonication (Bermúdez-Aguirre & Barbosa-Cánovas, 2008).

1.5.1.1 Application of ultrasound**:**

Ultrasound can be used for: 1) extraction of phenolic compounds from vacuolar structures by disrupting plant tissue; 2) extraction of Betacyanin (red pigments, e.g., from beets) and Betaxanthin (yellow pigments); 3) extraction of lipids and proteins from plant seeds, such as soybean (e.g., flour); 4) improvement of oil extraction from oil seeds; 5) cell membrane

permeabilization of fruits, such as grapes, plums, and mango; 6) processing of fruit juices, (e.g., orange, grapefruit, mango, grape, and plum), purees, sauces (e.g. tomato, asparagus, bell pepper, and mushroom), dairy products; and 7) improve stability of dispersions, such as orange juice, i.e. reduce settling.

Mechanism of action: Extraction enhancement by ultrasound has been attributed to the propagation of ultrasound pressure waves, and resulting cavitation phenomena. High shear forces cause increased mass transfer of extractants (Jian-Bing et al., 2006). The implosion of cavitation bubbles generates macro-turbulence, high-velocity inter-particle collisions and perturbation in micro-porous particles of the biomass which accelerates the eddy diffusion and internal diffusion. Moreover, the cavitation near the liquid– solid interface sends a fast moving stream of liquid through the cavity at the surface. Cavitation on the product surface causes impingement by micro-jets that result in surface peeling, erosion and particle breakdown. This effect provides exposure of new surfaces further increasing mass transfer. This phenomenon was confirmed by performed scanning electron micrography on peppermint plant leaves and trichomes. After these were ultrasonically treated for menthol extraction, microscopy results indicated that there were two mechanisms involved in extraction: (a) the diffusion of product through the cuticle of peppermint glandular trichomes and (b) the exudation of the product from broken and damaged trichomes (Shotipruk, Kaufman, & Wang, 2001).

If the substrate is dry then ultrasound may be used to facilitate swelling and hydration and cause enlargement of the pores of the cell wall (Vinatoru, 2001). Diffusion through the plant cell walls, disruption and washing out of the cell contents were also attributed to improved

extraction performance. The corresponding reduction in the size of the vegetal material particles by ultrasound disintegration will increase the number of cells directly exposed to extraction by solvent and ultrasonic cavitation. Intensive ultra-sonication can also serve the purpose of reducing the particle size in tomato juice (Food Science Australia unpublished data). As large amplitude ultrasound waves pass through a mass media, cavitational bubble collapse can occur in close vicinity or at the surface of the plant membranes causing microfractures. The occurrence of microfracture by ultrasound was demonstrated in soybean flakes (Haizhou et al., 2004). Cavitation collapse can occur on the plant surfaces, resulting in a micro-jet directed into the solid surface (Figure 1.8). Cavitation at cell surfaces has the ability to punch holes through cell wall as recently demonstrated with studies of bacterial cell sonication (Ugarte-Romero, Feng, Martin, Cadwallader, & Robinson, 2006). Preferentially micro-jetting will occur onto hydrophilic particle surfaces (Arora, Claus-Dieter, & Knud, 2004). Variation in the extraction yield from different plant varieties may result from structure, rheology (hardness of the seed structure) or the compositional differences resulting in varying degrees of susceptibility to ultrasound shock waves and likelihood that cavitation bubble will contact with the plant surface causing micro. Factors such as plant tissue turgor and the mobility of particles such as starch granules within the cell cytoplasm can be expected to influence ultrasound energy dispersion and extraction effectiveness (Zhang, Niu, Eckhoff, & Feng, 2005).

1.5.2 High Intensity Pulsed Light

Pulsed light is a surface preservation method in which a material is subjected to very short pulses (of the order of milliseconds) of broad-spectrum white light including wavelengths

in the ultraviolet and near infrared range. The product is typically exposed to 1-20 pulses having an energy density in the range of 0.01 to 50 J.cm^{-2} at the surface (IFT 2000). Pulsed light suffers from some of the same limitations affecting continuous ultraviolet light treatment, namely that areas on the treated surface that are exposed to the light might not be effectively decontaminated. This problem can be alleviated by treatment chamber geometry and by the feed methods used to present the sample to the light source. On smooth surfaces such as stainless steel, vegetative bacterial cells can be reduced from 10^6 cfu/cm^2 to the limits of detection after only 1 pulse of 300-microsecond duration (Shaw *et al.* 2009). The level of microbial inactivation that can be achieved on food surfaces is however limited due to surface roughness effects. Trials at Campden BRI have determined that a 1-3 log reduction in total viable counts is typically achievable on food surfaces such as meat, cheese and fruit (Shaw *et al.* 2009) and similar results have been reported by other groups (Ozer & Demirci 2006).

1.5.2.1 Production of pulse light:

Pulse light is produced effeiciently by amplifying power by storing the electromagnetic energy in a capacitor for fractions of second followed by releasing it in the form of light (Dunn et al, 1995). One more Xenon lamp units, a high voltage connection and a power unit make the necessary part of the pulse light producing units. High voltage connection permits transfer of high current electric pulse. The wavelength range of pulse light is 1000-11000Å comprising UV rays (1000-4000Å), visible light (4000-7000 Å) and infra red (7000-11000 Å). For effective inactivation of microorganism in food preservation, the pulse light of 120 flashes per sec with energy range of 0.01-50 joule cm^{-2} should be used (Barbosa-Canovas, 1998).

1.5.2.2 Mode of action:

The pulse light is more effective in inactivation of microorganisms than UV rays due to its high penetration, emission capacity and peak power distribution due to the several times amplification of energy during their production(FDA, 2000; XDemirci, 2002; McDonald et al, 2000). The rise in temperature in the Pulsed light is less when compared to UV rays sue to short duration of pulses and cooling effect in between pulses (Krishnamurthy et al., 2004). The composition of the emitted spectrum largely determine the effectiveness of pulse light and the microbial inhibition by pulsed light is due to the broad spectrum UV content and the energy density applied Marquenie et al.(2003a,b), Oms-Oliu et al.(2010). Rowan et al. (1989) reported the reduction of food pathogens were higher by using high UV spectrum than low UV spectrum in Pulse light. Oms-Oliu et al (2010) suggested the use of UV with short pulse and high width instead of traditional UV.

Wang et al. (2005) explained the mechanism of microbial inactivation by pulse light due to photochemical effect that results in denaturation of DNA and pyramid formation in dimmers in bacteria, virus and other pathogens. The microbial cell is disintegrated due to overheating of its constituents (Wekhof, 2003) and membrane disruption due to steam productionin the cell (Fine and Grevais, 2004). The inactivation of microbes is also due to prevention of replication of cells (Rowan et al, 1999). The additional microbial inactivation due to photothermal and photophysical effects caused by pulse light has also been described (Wuytack et al, 2003; Krishnamurthy et al, 2007). When exposed to pulse light, the rapid increase in temperature is seen after achieving a minimum threshold of energy (Wekhof, 2003). Under the exposure to

longer wavelength, the photochemical effects of Pulsed light are reversed by the photoreaction (McDonald et al, 2000). Thus sufficient precautionary measure such as wrapping etc should be taken to avoid it (MacGregor et al, 1998; Rowan et al, 1999; Anderson et al, 2000).

1.5.3 Irradiation

Food irradiation is described as "a processing technique that exposes food to electron beams, X-rays or gamma rays, and produces a similar effect to pasteurisation, cooking or other forms of heat treatment, but with less effect on look and texture". X-rays and radioactivity were discovered at the end of the 19th century and in the early years of the twentieth century, the possible application of these concepts to the preservation of food was already being investigated (Brennan *et al.* 1990). Irradiation of strawberries, for example, was investigated in Sweden in 1916; a paper on the elimination of *Trichinella spiralis* in pork with X-rays was published in 1921; and a patent for food preservation using ionising radiation was issued in France in 1930 (Brennan *et al.* 1990).

Three types of ionising radiation can be used for food irradiation – gamma rays, X-rays and electron beam radiation. Both gamma rays and X-rays are photons, whereas an electron beam is made up of β-particles. In all cases, the energy of the radiation source is limited or selected such that when the food is irradiated, electrons can be ejected from their orbits around molecular nuclei, but there is no interaction with the nuclei themselves, which would induce radioactivity (Grandison 2006; Woolston 2006).

High energy electrons generated by an electron accelerator can also be used to irradiate food. However, application of this method is limited by the penetration depth relative to gamma

or X-ray radiation. Electron beam irradiation has a maximum penetration depth of around 4 cm (Grandison 2006). This effectively limits the maximum sample thickness that can be treated is around 8 cm if irradiated from two sides. Electron beam irradiation therefore tends to be used on products that can be arranged in thin layers during the process.

The 'wholesomeness' of irradiation in terms of its effects on food toxicity, microbiological safety and nutritional status has perhaps been more widely investigated than any other novel preservation method, having been extensively studied for over 50 years (Farkas 2006). The FAO/IAEA/WHO expert committee on food irradiation concluded that "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard…" and "introduces no special nutritional or microbiological hazards" (Brennan *et al.*1990; Farkas 2006; Leadley *et al.* 2002).

Irradiation has been so comprehensively studied (Farkas 2006) that it can almost be considered as a 'conventional' non-thermal preservation method. Consumer confidence would appear to be the main barrier to irradiation being considered as a credible preservation method in the UK.

1.5.4 Pulsed Electric Field processing (PEF)

Pulsed electric field processing is a technique in which a food is pumped between paired electrodes and exposed to a pulsed high voltage field (typically 20-80 kV/cm for antimicrobial purposes). Treatment times are of the order of less than 1 second for pasteurisation applications. This process reduces levels of microorganisms whilst minimising undesirable changes in the sensory properties of the food. Pasteurisation using PEF is ideally suited to pumpable

homogenous products. Pasteurisation of foods having large particulates is not feasible because of the physical restrictions relating to the gap between electrodes.

Products having a high salt content are also unsuitable for PEF treatment because their higher electrical conductivity reduces the resistance of the chamber and more energy is therefore required to reach the required electrical field strength for pasteurisation (IFT 2000). Examples of potential pasteurisation applications include the non-thermal treatment of various products such as fruit juices, liquid dairy products, beers and wines, egg products and tomato products (Floury *et al.* 2006).

A PEF-treatment of biological tissue can not only be used to induce tissue disintegration to enhance mass transfer processes, but can also be used in such a way that the structural properties can be changed significantly. During pressing of apple a tissue softening and loss of compressibility showed to have a detrimental effect or at least to require an adjustment of separation technique used, but it is obvious that a change of textural properties can also be utilized during production and processing of various biological products. Exemplarily the impact on textural properties of potato tissue has been investigated. A force displacement curve of potato samples after a PEF-treatment in comparison to untreated samples is shown in Figure 1.9. Tissue softening of apple, potato and carrot tissue has also been described by Fincan and Dejmek (2003) and Lebovka *et al.* (2004), a reduction of elasticity modulus was reported. Approx. 50 % steady state cutting force reduction and improvement of cut quality have been described for sugar beet (Kraus 2003), which is in good agreement to own results.

The schematic depiction of mechanism of membrane permeabilization by electro-compressive forces induced by an external electrical field is showed in the figure 1.10. Increasing treatment intensity will lead to formation of large, irreversible membrane pores.

The tissue softening effect of PEF, based on cell membrane electropermeabilization and loss of turgor can be utilized to reduce the energy required for cutting of plant material. With a continuous, short time and low energy (~10 kJ/kg) PEF-treatment of potato tissue, a reduction of grinding energy similar to that of thermal or enzymatic treatment can be achieved at lower energy input processing times. In addition a PEF pretreatment therefore appears to be applicable to influence cutting behavior. Loss of turgor pressure and elastic modulus will change cutting properties of the tissue. Dependent on process requirements and parameters a cell membrane permeabilization and loss of turgor can be utilized for softening and tissue modification and replace or enhance conventional processing techniques.

1.5.5 Ohmic heating

Ohmic heating, where heat is generated by the passage of alternating electrical current (AC) through a body such as food, has been in use since the nineteenth century. Other names for this technology include resistance heating, direct resistance heating, Joule heating and Electroheating^{TM} (Christian & Leadley 2006). Ohmic technology relies on the electrical resistance of the food to generate heat. Therefore if the electrical resistances of all components of the product are constant then the product heats uniformly. Particulate products can therefore be heated uniformly, overcoming the limitations of heat transfer in conventional HTST systems such as plate, tubular or scraped surface heat exchangers. Products such as fruit juices and

concentrates, shelf-stable milk, puddings, soups and liquid egg products can be heated rapidly, uniformly, and with reduced impact on the organoleptic properties of the product (Christian & Leadley 2006). Ohmic heating is not a new principle, several processes were patented for the use of electrical currents to heat pumpable liquids in the 19th Century and it was used for milk pasteurisation in the early 20th Century (Ruan *et al.* 2004).

Although rapid volumetric heating offers quality benefits over conventional canning it is important to note that ohmic heating is still a thermal process and in this respect there are limits to what can be achieved in terms of preserving 'fresh-like' quality. The process is also difficult to operate without closely matching the physical properties of the different phases within the food.

1.5.6 Microwave and radio-frequency heating

Microwave and radio frequency heating are well-established thermal processing technologies that have found application in many process sectors. Commercial installations are common in the plastics, textiles, paper and board and wood (Decareau & Peterson 1986) and applications in the food industry are now increasingly common (Sumnu *et al.* 2005). The use of Radio Frequency processing for food thawing was commercialised in the 1960's and its use for food preservation was suggested in the early 1970's (Marra *et al.* 2009). Radio frequency has found wide-spread use as a post-baking drying process for cookies and snack foods, an application that began in the 1980s (Marra *et al.* 2009). Microwave processing has many applications where pasteurisation or sterilisation is not necessarily the key objective. These include cooking, thawing, freeze drying, tempering, rendering, frying, blanching and drying

(Sumnu *et al.* 2005). Food applications for preservation using microwave or radio-frequency heating are not however in widespread use.

In microwave and RF heating, as in ohmic heating, product heating occurs volumetrically. As a result, particulate heating is not dominated by conduction heating. This makes both techniques potentially attractive as rapid methods for thermal pasteurisation or sterilisation. Microwave and radio frequency heating refers to the use of electromagnetic waves at particular frequencies used in order to generate heat in a food material. The frequency bands allocated for Industrial, Scientific and Medical (ISM) use are listed in Table 1.1. Of these frequencies, 896/915 MHz is generally used for microwave processing applications and 2450 MHz is used for domestic microwave ovens and some industrial process applications. Radio frequency heating utilises each of the frequencies outlined (although 40.68MHz is rarely used) depending on the application.

The major advantage of microwave/RF heating is, as for ohmic heating, the rapid and volumetric temperature rise that can be achieved in foods. This result from two primary heating mechanisms, a dielectric effect and an ionic heating effect (IFT 2000). Dielectric heating occurs when the water molecule, the principal constituent in most food materials, oscillates at the very high frequency of the microwave/RF field; such oscillations produce rapid heating. Ionic heating occurs when the oscillatory motion of ions in the food, under the influence of the microwave/RF fields, produces a heating effect. In food processing, the advantages of the rapid heating effects are amplified by the fact that both microwave and RF frequencies can penetrate the food to a depth of several cm (microwave) or tens of cm (RF), promoting a volumetric heating effect

throughout the food. This can offers significant advantages over conventional thermal processing as high-temperature-short-time processes can minimise thermal damage to the food product but as has been noted for ohmic heating, microwave and RF are still in essence thermal processes with a limited capability to preserve 'fresh-like' quality.

1.5.7 High pressure processing (HPP)

High pressure processing is a non-thermal pasteurisation process in which a food is subjected to pressures in the region of 150 MPa to around 700 MPa (1500 to 7000 bar) and held at pressure for a time, generally under 5 minutes. The extremely high pressures used for HPP can inactivate vegetative microorganisms, yeasts, moulds and certain enzymes (Simpson & Gilmour 1997) and a wealth of data are available to demonstrate its efficacy for this purpose in a wide range of food products. The extent of microbiological inactivation is affected by many factors including; the intrinsic properties of the food such as its pH, water activity, fat content, protein content, mineral and sugar content (Black *et al.* 2007a.; Rendueles *et al.* 2011); life cycle effects (organisms tend to be more sensitive to HPP in the exponential phase of growth) and the pressure, temperature and time combinations that are applied (Patterson *et al.* 2007; Rendueles *et al.* 2011).

Because the process often does not involve significant heating, the sensory and nutritional quality of products can be remarkably similar to their unprocessed counterparts. The nonthermal nature of high-pressure pasteurisation makes it an excellent candidate process for preserving the 'fresh-like' characteristics of foods. Unfortunately, bacterial spores are very resistant to commercially achievable pressures. An additional challenge presented by HPP

pasteurisation is that the response of enzymes to HPP is complex and substrate dependent. For example, HPP resistance of Pectin Methyl Esterase (PME) has variously been reported to be everything from "extremely resistant to moderately labile" (Katsaros *et al.* 2010) largely depending on the source of the enzyme. Many studies have explored the effects of HPP on PME inactivation in foods such as tomato products, citrus juices and various other fruits and vegetables (Boulekou *et al,.* 2010; Broeck *et al.,* 2000).

2 Quality attributes of fresh fruits and vegetables during processing

Convenient ready-to-use vegetables with fresh-like quality are in demand not only by consumers but also by food service industries (Ahvenainen, 1996). Minimal processing technologies have been developed to provide fresh-cut products with similar characteristics to fresh but in a ready-to-eat format. To achieve this, the commodity generally undergoes several steps such as: trimming, peeling, cutting, washing and (commonly) disinfection. However, these steps promote wounding responses, which accelerate deterioration and limit shelf life (Aked, 2000). As expected all these processing methods are employed at the cost of some fresh quality loss. In the case of fresh-cut products, cell disruption may influence the formation of off-flavours and browning, which occur due to reactions between cell constituents catalysed by phenolase released from the tissue (Fennema, 1996). Controlling this type of reaction requires a deeper understanding of the tissue structure and components to optimize the final quality.

In the past food engineers neglected the effects of microstructure on the properties of food. Nowadays, advances in biology and material sciences have given a new approach to food researchers and companies to understand foods as complex multi component systems.

Considering that the majority of the elements that are involved in the physical and rheological, textural and sensorial behaviour are below 100μm in size, the use of microscopy is required to understand structures such as plant cells, cell walls and starch granules (Aguilera, 2005).

In the case of fruit and vegetables, cells normally influence chemical, physiological and biological properties of the tissue. For example during drying, tissue shrinkage is initiated at a cellular level (Konstankiewez et al., 2002) affecting the tissue composition, shape and metabolism. Similar conclusions were found with the role of pectin in cell walls of processed fruits and vegetables in a broad review by Sila et al. (2009). The plant primary cell wall is mainly composed of pectins, cellulose and hemicelluloses. Variations in the composition will affect its functionality and the quality attributes of the product such as organoleptic perception, physiological responses to the environment and final quality.

During senescence, storage and processing, fruits and vegetables will present changes in colour, flavour, texture and nutritional value. The following table summarizes the major quality attributes of foods during processing and storage. (Table 2.1)

The hierarchy of the structure in plant tissue goes from the molecules through cellular, tissue organ levels. Each level is directly linked to a range of physical attributes. Textural changes do not only depend on one level of hierarchy but a combination of them (Van Dijk and Tijskens, 2000). In the case of carrots, texture will depend on the physical structure, cell composition and turgor pressure (Figure 2.1).

As the diagram, Figure 2.1, shows, cell wall and cell membrane changes are responsible for loss of tissue strength or turgor pressure during cellular deformation, since cell walls support

the structure in combination with middle lamellae. The relationship or interaction between these components based on their natural structure will define the final quality of a product.

2.1 Organoleptic perception of texture

The organoleptic perception of texture can be influenced by two main factors that occur while tissue is in the mouth: firmness and juiciness. For example a tissue may feel more firm and less juicy if it has a larger cell-to-cell contact and low amount of intracellular air spaces (Toivonen and Brummell, 2008). Overall, perception of texture will therefore relate to the structure of the tissue. This will differ from product to product and will encounter changes when subjected to any type of processing. Therefore, it is possible that any damage or changes localised at the cell or tissue level will potentially affect the organoleptic perception of the final product. For this reason it is important to evaluate what structural changes occur in the tissue before and after processing. In the case of heat- processed carrots, new compounds may be formed and other compound lost by leakage.

2.2 Relating structure to texture

Quality changes in fruits and vegetables, in particular texture changes are normally measured using an indirect technique or by instrument. Mebatsion et al. (2006) mentioned that most quality changes observations in horticultural products are expressed in terms of macro results that occur as a consequence of a number of mechanisms or reactions at the micro level and that are normally treated as a black box. Unfortunately this is a common practice but not the best approach to provide any information about the product as such. For this reason it is important to identify the main components that relate to microstructure of the tissue to

understand textural changes. In the case of carrots, it is possible to identify four main components that will have an influence on textural changes: (a) type of cells, cell wall strength (thickness of cells) and composition such as lignifications. (b) cell to cell adhesion, which includes the middle lamella and calcium binding. (c) turgor pressure, inside the cell and finally (d) the cellular arrangement, which includes the organization of cells as groups and their size and shape. All these components may also interact with each other, creating a sequence of responses that will result in a macro texture change of the product. For example, if the cell wall integrity is being jeopardized by external forces such as pressure or heat, then this will affect the strength of the network holding the structure together and cellular contents. Weakening of the structure will put stress on cellular joining points, leading to collapse if not strong enough. In addition, weakening of cell-to-cell wall cementing (middle lamella) would lead to cell separation and a softer texture with a mealy feel. Turgor pressure could also change as a result of cell membrane damage properties promoting leakage and softening of the tissue (Sajnin et al., 1999).

Vegetables normally come from roots stem and leaves that may have relatively stronger cells than fruits (Toivonen and Brummell, 2008). One common factor is that most edible tissues are composed of parenchyma cells. The mechanical properties of these cell walls will be determined by the mixture of structural polysaccharides such as cellulose, hemicelluloses and pectins (Abbot and Harker, 2004). Cellulose provides rigidity and resistance to cell walls whilst hemicelluloses give plasticity and the ability to stretch (Van Buren, 1979). Pectins are responsible for the adhesive or cementing forces between cells (Van Djik and Tijskens, 2000). Pectins are hetero polysaccharides consisting of a linear chain of D-galacturonic acid residues linked by α-(1-4) glycosidic bonds (Baker et al. 2005). The galacturonic acid residues can have

various degrees of esterification (DE) or methoxyl content (Baker et al, 2005). An unaltered intercellular pectic will have 83% of DE, but this can vary with processing and extraction methods. Commercial pectins may range for example between 20 and 70%DE. Pectins are classified having low and high methoxyl groups: the high methoxyl group is represented by DE values between 50 and 80% while low methoxyl groups is represented by DE values below 50% (Baker et al, 2005). Low methoxyl pectins can form gels in the presence of divalent cations such as calcium, which may be the case for most vegetable products (Baker et al., 2005).

Gomez Galindo et al., (2004c, d) found that pectins in carrot cell walls can be crosslinked during the first 12 weeks of storage, thereby improving carrot firmness. This occurs probably by linking with internal available calcium. But, there are other compounds that may help to keep the texture, such as the existence of proteins called extensins. These proteins can strengthen the cell wall through cross- linkages between themselves, forming an independent network that will eventually change the architecture of the cell wall. Extensins are essential for cell wall assembly and growth by cell extension in carrots (Gomez Galindo et al., 2004c, d).

Figure 2.6 shows a plant cell structure. It is observed that the vacuole is surrounded by a membrane called the tonoplast. The permeability of the tonoplast will be an important variable in the response toward drying and osmotic traffic into and out of the cell (regulating permeability of solutes). Figure 2.2 also shows the location of the main enzymes present in plant cells responsible for many of the quality changes due to processing and storage. By identifying the location of the enzymes and compounds it is easier to understand how easily substrates and

enzymes can be exposed when the system is disrupted. By losing compartmentation, enzymes and substrates can mix, enhancing reactions and consequently quality changes.

When relating texture to structure it is also known that the cell separation or debonding may result in the weakening of middle lamellae. This will give a mealy or dry texture due to the cells being left unbroken and therefore not releasing intracellular contents when chewed. Several physical forces relate to the structure and will have an effect on the final texture such as:

- Turgor pressure inside the cells affecting tissue tension.
- Specific compounds within the cell giving strength (e.g starch)
- Cohesive forces within cells giving strength (e.g cellulose- hemicelluloses bonding)
- Adhesive forces between cells given by pectin.

2.3 Physical changes

The cellular structure of fruits and vegetables can be characterized by many factors as mentioned earlier such as: cell wall thickness, cell size, cell shape, cell adhesion and cell organization (Van Djik and Tijskens, 2000). The rigidity of the cells is affected by the elasticity of the cell walls, osmotic potential of the cell contents and water availability (turgor pressure). The mode of failure under an applied load will also depend on the structure of the raw material, for example the strength of the middle lamella (Waldron et al. 1997). Another physical change in vegetables is the loss of water or loss of turgidity as mentioned previously. Water loss in vegetables can be controlled by selecting the appropriate packaging. However in minimally

processed vegetables there is a reduction in membrane integrity and leakage of cellular contents into the apoplastic space that results in turgor loss (Toivonen and Brummell, 2008).

In terms of physical changes, when an external force is applied, the cell-cell adhesion in the tissue will be affected. Tissues that have low cell-cell adhesion will break easily between cells keeping the cell contents mainly intact; this may be perceived as mealiness (Van Dijk and Tijskens, 2000). Where cells have higher cell adhesion, they may break and liberate the inner contents. In fresh produce, cell adhesion can depend on the strength of middle lamella, plasmodesmata connections and cell-to-cell contact (Harker, 1997). Goldberg et al. (1996) explains that pectin is the only polymers present in a tricellular junction joining the cells (cell-to-cell contact). Figure 2.3 shows how the turgor induced stress is distributed at tricellular junctions. Forces are classified into two components. F_t is the first component of stress on the place of each cell to cell contact, while F_c is the second component that describes the radial forces that separate the cells at the corners.

In a fresh tissue, the mechanism of actions of the components indicate that when stress is applied by turgor pressure, cells start to stretch in the plane of each cell wall and a further radial force can tend to pull the tricellular junctions apart delaminating the wall at the corners. If air spaces are to grow between the tricellular gaps, then the cells would become more rounded increasing pressure between cells (Goldberg et al.,1996). However, during processing such as heat, cell debonding or separation is explained by the pectin degradation via β-elimination (non-enzymatic reactions) and less by the physical state (Greve et al., 1994a). The impact of heating carrots and their cell wall components have indicated that differences in carbohydrate

composition (cell wall) did ot explain texture retention; however there was a correlation found between the size of the pectic polymers and texture retention (Greve et al., 1994a). Furthermore, cell wall changes are apparent after several minutes when temperatures reach 100˚C and firmness is lost. It is thought that many cellular changes may occur in early heating stages but may not necessarily be detected (below the threshold). During heat processing for example, membrane disruption may be main factor for firmness loss (Greve et al., 1994 a,b).

Moving from cell wall changes to cell membrane, it is found that cellular membranes are formed by proteins associated with bilayer of lipid. Their function is to regulate the inflow and outflow of molecules, ions and water of the cell. These membranes are considered important for the control of homoestasis and compartmentalization (Fan et al., 2005). Leakage due to membrane disruption can lead to turgor losses. Greve et al., (1994a,b) studied the effect of plasmolyzing carrot tissue by immersion in mannitol (iM) before cooking so it could withstand turgor losses. Their findings indicated that tissue with less turgor before cooking would retain firmness in comparison to untreated tissue (turgid tissue) which showed no turgor pressure left after cooking.

From the above information, it seems cell walls and cell membranes have their own mechanism of failure when subjected to processing such as cooking. These components will affect the final quality of the product including texture colour and sensory attributes (flavours and odours) by exposure of cellular components. Experiments done on cell wall stress due to turgor pressure have indicated that the rate of liquid leaving the cells when the cells are compresses, depends on the hydraulic conductivity (which describes the ease with which water

can move through pore spaces or fractures), cell surface area and turgor pressure across the cell wall (Pitt and Chen, 1983). Stiffer cells will show more stress with compression, whereas softer cell walls will not change much as the force is shared beteen the cell and the cell turgor pressure, consequently with less chances to fail or rupture (Pitt and Davis 1984).

In the case of application of compression, it is expected to reach a certain point where cells could only resist without rupturing and excess of leakage; however above this pressure which is possibly be tissue dependent, cells would probably fail to hold their shape together. The cell wall elasticity plays an important role when cells are losing water. As water is lost, their volume is reduced until their turgor is lost; this is however product dependent as well. Water loss in carrots during storage will probably promote a more flaccid type of texture, indicating parenchyma cells becoming flexible.

2.4 Enzymes involved in textural changes during processing

Texture in plant tissue can be modified by enzyme activity. Texture is not determined by the enzyme activity at a given time, but rather by the cumulative action of any enzymes over a period of time (Hendrickx et al., 1998). Tissue softening during heating may be due to pectin degradation; this occurs when pectin methylesterase (PME) partially demethylates the pectins producing methanol and galacturonic acid (Ly Nguyen, 2004) (Figure 2.4). The less methylated pectins are then depolymerised by polygalacturonase (PG), resulting in shorter pectin chains and texture losses as a consequence. However, PME can act in two different ways: (1) randomly on pectin chains, promoting the action of polygalacturonase (PG) or (2) it can act linearly, forming blocks o free carboxyl groups that will further ineract with bivalent ions(Ca^{2+}) (Figure 2.5: Vu et

al, 2004). Sila et al., (2004) found significant hardness losses of carrots after heat treatments (95-110˚C). This texture loss was able to be reduced by calcium pre-treatments before heat treatments or by combing high pressures with heat as a process.

2.5 Quality changes after conventional thermal processes

Heat is the most common technique for preserving foods; however it can promote various chemical and biochemical reactions that can be used to control vegetative pathogens and inactive enzymes involved in quality deterioration. Severe heat treatments (e.g. canning) can destroy compounds such as vitamins and pigments and cause significant changes in texture of most foods. Research has been done on the effect of heat on pigments measured by colour of carrot juice have shown a change from an orange colour to a less desirable yellow colour (Chen et al., 1995). Colour deterioration occurs as a result of isomerisation of caotenoids (cis/trans), affecting the provitamin A activity (Fennema, 1996). Another reaction that occurs during heating is nonenzymatic browning, where sugar molecules form unsaturated rings such as furans, which can result in colour changes. Simon and Lindsay (1983) have shown that during cooking (e.g canning) carotenoid concentration diminished 20%, while 70% of terpenoids were lost compared with the initial values in fresh-cooked carrots.

Tissue softening during thermal processing is due to turgor loss, membrane degradation and cell wall separation (Lillford, 2000); while rigidity is directly affected by cell turgor pressure (Ramana and Taylor, 1994). Heating can also promote the splitting of glycosidic bonds (covalent bond) of pectins by β- elimination leading to increased pectin solubilisation and consequent

texture loss, Theil and Donald (1998) suggest stabilizing pectins using binding compounds such as Ca^{2+}.

Literature on the material properties influenced by tissue porosity indicates that tissue with strong intercellular adhesion and low porosity such as carrots, would fail by cell rupture and release of fluid when compressed (Ormerod et al., 2004). The same author supports previous findings indicating that thermal degradation of pectins in the cell wall and middle lamella will weaken bonds, allowing cell separation to take place (Figure 2.6). Texture changes in heat processed carrot discs have been shown to follow a biphasic pattern, with a rapid phase during the first 1 to 6 minutes of cooking and a slower phase thereafter.

De Belie et al., (2000) explained that when temperatures reached 50˚C in the cell membranes, the membranes begin to degrade resulting in rapid turgor loss whereas pectins are only affected after 6 minutes (Greve et al., 1994). Ng and Waldron (1997) found cell separation in carrots cooked at 100˚C for 20 minutes. A precooking stage could reduce the loss of texture by enhancing the cell- cell adhesion and the degree of methyl esterification of the cell wall pectic polymer. Pre-heating with calcium has been shown to reduce softening by forming calcium complexes with pectins. Meanwhile, osmotic treatments (e.g., using mannitol solutions) can reduce turgidity, helping to better withstand firmness loss during heating (Greve et al., 1994b).

In addition to the effects of heat on microbial survival, effects on sensory properties, nutritional values and other quality factors must also be considered. The high temperatures and times needed to achieve a shelf-stable product will cause reactions affecting the quality. The effects are both of a qualitative and quantitative nature. Changes in appearance, taste and texture

are examples of qualitative losses (Awuah et al., 2007). Nutritional degradation, on the other hand, is an example of a quantifiable loss that will occur.

Data on the effects of elevated temperatures on chemical reactions are valuable for determining the quality changes resulting from a thermal process. Food scientists use a parameter called z-value for describing the temperature dependence of chemical reactions (Brennan, 2006). It is defined as the temperature change that results in a tenfold change in the decimal reduction time, which is the time required for a 90 % reduction of the substance under study. The lower the z-value, the more temperature sensitive is the reaction. The z-value of most chemical reactions associated with quality losses in food products is at least two times higher than the z-values for microbial inactivation, which generally have z-values around 10 °C (Toledo, 2007). The z-values of some quality degrading chemical reactions are shown in Table 2.2. As can be understood from these figures, properties such as flavor, color, texture and nutritional values are generally more sensitive to long times than to high temperatures. For this reason, it is common practice in the food industry to increase the temperature to decrease the time, thereby minimizing the quality degrading reactions without lowering the microbial inactivation (Toledo, 2007).

2.6 Quality changes after mild-thermal and non thermal processing

Minimal processing of fruits and vegetables includes steps such as washing, peeling, trimming, cutting and disinfecting to provide fresh, ready-to-eat products that will subsequently be packed and chilled (Orsat et al., 2001). Several methods are available to maximise the quality of minimally processed. Produce such as selecting high quality raw materials, optimal choice of

peeling and cutting methods, cleaning, washing, browning inhibition, the use of edible coatings (Ahvenainen, 1996).

In carrots, undesirable changes are associated with high CO_2 and low O_2 concentrations. As a consequence, loss of firmness and development of off flavours will be expected. This is because of the proliferation of lactic acid bacteria producing ethanol and lactic acid (Orsat et al., 2001).

Sous vide (mild heat under vacuum) is a cook- chilled technology which has also been used to extend the shelf life of prepared foods (Rodgers, 2004). As sous vide is a relatively mild treatment (90°C – 10 minutes or equivalent), most food quality attributes such as flavours are preserved (Werlein, 1998). Sous vide is an interesting processing alternative, which has produced carrots with better texture (shear force) and color (more orange) in comparison to conventionally processed or cooked carrots (Werlein, 1998). Other applications are heat-shock and calcium lactate treatments. These in combination could help to maintain turgor and texture in carrots (Rico et al., 2007).

Non thermal minimal processing technologies have been evaluated to prolong shelf life of foods including irradiation, pulsed electric fields, ultrasound and high pressure processing. Apart from minimal processing there are other mild preservation processes such as pickling, which is a popular traditional technique for preservation of vegetables. Previous work done on carrot pickling showed 50% reduction in puncture force after a day, this was due to cell plasmolysis (Llorca et al. 2001).

Pulsed electric fields are used to modify biological materials in a short period of time. The way it works is by applying very short electric pulses in a range of 0.1-1kVcm^{-1} field intensity, this causing reversible permeabilization in plant cells by pore developments in cell membranes (Knorr et al., 2011). On its application, membranes can become permeable or damaged (Lebovka et al., 2004). The literature indicates that the advantages of PEF are less damage to plant ingredients in comparison with heat treatments (Lebovka et al., 2004). However, the irreversible damage caused to cells is used in advantage to improve mass transfer during drying and extraction resulting in processes with higher yields and less energy consumption. (Toepfl et al., 2006).

High pressure processing is another technology that can be used without the application of external heat (apart from adiabatic heating generated when pressure rises). Fruits and vegetables such as oranges, apples, peaches, citrus juices, carrots, tomatoes, strawberries and raspberries have been shown to maintain sugars, vitamin C and carotenoids (Butz et al., 2003) with no apparent damage to sensorial attributes (Hendrickx et al., 1998).

3.1 Effects of thermal processing on cell wall chemistry

Vegetables become softer and lose their desired crispness and firmness during processing mainly due to a loss of cell wall and middle lamella structure and a variety of changes in the cell wall matrix components (Van Buren, 1979). Cellulose is a rather inert material. The only change in cellulose during processing is an increase in the degree of hydration (Sterling & Shimazu 1961). No effect of heating on hemicelluloses has been reported in literature. Pectins are more chemically reactive than other cell wall polymers and can undergo a variety of chemical

modifications under conditions similar to those associated with food processing. These modifications are influenced by a number of factors, primarily pH and the concentration and types of salts that are present. Enhanced softening at low pH (pH < 3) has been ascribed to hydrolytic cleavage of glycosidic bonds of neutral sugar components of the cell wall. The enhanced softening during heating at neutral and alkaline pH's has likewise been associated with polymer cleavage, in this case through the ß-eliminative degradation of polyuronides (Van Buren, et al., 1990) (Figure 1.8). A higher methylester content results in a larger degradation (Sajjaanantakul, et al., 1989). In addition Loh and Breene (1982) reported thermal protopectin degradation of another kind for potatoes, which seemed more important than ß-elimination. Huang and Bourne (1983) studied the kinetics of thermal softening of vegetables and postulated that the rate of softening reflects two simultaneous first order mechanisms. Mechanism 1 was proposed to be due to pectic changes in the middle lamella region and accounted for 85 - 97 % of the original tissue firmness. Its contribution to firmness decreased practically to zero during processing. Mechanism 2, whose nature has not been identified yet, was suggested to be responsible for the residual firmness of vegetables after prolonged heating. The rate constants of both mechanisms could be influenced by preheating (Bourne, 1987).

Various ions have been shown to enhance the rate of ß-elimination in native plant tissues (Van Buren, et al., 1990 or in a model system of purified pectin (Sajjaanantakul, et al., 1993). The enhancement effect in a model system with purified carrot pectin increased in the order Zn^{+} > Ca^{++}> Cd^{++}~ Sr^{++}> Mg^{++}~ Na^{+} ~ K^{+} > $NH4^{+}$. The accelerating effect of ions was more pronounced with low-methoxyl pectins than with high methoxyl pectins. The mechanism behind this effect has not been identified yet, but was not a simple function of the charges, ionic radii or

hydration radii of the cations. Calcium has two opposite effects on texture. At one hand it may firm the tissue through complexes with pectic substances, and at the other hand it may soften the tissue by enhancing ß-eliminative degradation. The net result of calcium addition has invariably been to firm the tissue (Javeri, et al. 1991). In addition to the effect of salts during the heating process, there is also an effect when salts are added after processing, to the cooked material. Van Buren (1993) reported that the firming effect was most prominent with Ca++ and Mg++, while monovalent ions had a softening effect. Another method exploited to reduce tissue softening during thermal processing is preheating of the samples at moderate (50 - 80 °C) temperatures (Quinn et al, 1994). Bartolome and Hoff (1972) suggested that the cell membranes are destroyed, thus allowing salts, such as K+, to activate endogenous pectin methyl esterase (PME). This enzyme can subsequently partially demethylate the pectic compounds. This PME firming effect was hypothesised to involve two separate phenomena: (1) the decrease in susceptibility of the pectin to ß-eliminative degradation during subsequent cooking or sterilisation and (2) increase the formation of pectate-calcium complexes (Ng, et al. 1997).

3.2 Effect of ultrasound treatment on plant tissues:

The effects of ultrasound on plant cells and tissues can be mechanical or thermal in nature (Wu & Wu 2007; Ahn et al. 2003). When plants absorb ultrasonic waves, energy associated with the wave is converted into heat, causing a “thermal” effect. Ultrasound can also cause acoustic cavitation in plant cells. Documented biological effects of sonication on plant cells includes chromosomal anomalies, disruption or collapse of gas vesicles and subsequent loss of buoyancy, damage to or destruction of cellular organelles, cell death, changes in cellular osmotic potential, inhibition of

photosynthesis and cell division, destruction of cell membranes, and formation of free radicals (Wu & Wu 2007; Zhang et al. 2006; Hao et al. 2004; Ahn et al. 2003; Lee et al. 2002; Nakano et al. 2001; Soar 1985). These effects have been reported after short exposures to ultrasonic waves, from seconds (Lee et al. 2002) two minutes (Zhang et al. 2006; Hao et al. 2004; Soar 1985). Ahn et al. (2003) reported that algal cell densities and chlorophyll *a* concentrations of *Microcystis aeruginosa* were significantly decreased after 3 days of ultrasonication (20 kHz applied twice daily for 2-minute exposures).

3.3 Effect of combination treatment of Osmotic dehydration and ultrasound on tissues:

Osmotic dehydration causes changes in the structure of plant tissue. During Osmotic dehydration treatment there is a contemporaneous counter-diffusion of water from the tissue into the osmotic solution, while the sucrose moves into the intercellular and extracellular spaces of the tissue (Deng and Zhao, 2008; Fernandes et al., 2009 and Malgorzata Nowacka et al, 2013 Lebovka et al. 2001; Angersbach et al. 2002). These transport phenomena can lead to the damage of the structure at microscopic level. The changes between untreated and osmodehydrated freeze dried tissue are shown in Figure 3.2 a and b. Untreated material was characterized by a round shape cells (Fig. 3.2a). However, the cells in the osmodehydrated tissue were irregularly shaped, distorted and showed numerous breakdown of cell walls (Fig. 3.2b). Also Fernandes et al., (2008) and Panarese et al., (2012) observed the alteration of OD treated cellular tissue, in particular the increase of intercellular spaces creation probably due to the solubilizing of chelator-soluble pectin of the middle lamella.

It could be observed in Figure 3.2b, that ultrasound application caused alteration of microscopic structure and the distribution of the cells changed, shifting to the larger field surface suggesting that cells were damaged and combined with each other leading to a larger area. Moreover the microscopic channel creation could be appreciated in figure 3.2b. Those channels and also the breaking down of the cell walls could be responsible for increasing of mass transfer rate during OD because of reducing the resistance for water diffusion through the cells.

Ultrasound pre-treatment performed for 30 minutes promoted clear changes of the kiwifruit tissue microstructure, leading to the increase of the average cross-section area. Moreover microscopic channels creation in the cell structure was observed, which could be responsible for the increase on water diffusivity from the tissue into the osmotic medium. However the increase of mass transfer rate was observed only for short term osmotic treatment (till 30 min).

3.4 High pressure effects on quality attributes of fruits and vegetables

From literature it was found that the effects of high pressure on enzymes for example varied according to the product's characteristics. Unfortunately this may lead to different optimal conditions for different products showed undesirable organoleptic changes at 300MPa. Meanwhile, others reported that high pressures (up to 350MPa) can be applied to plant systems without major effects on texture and structure (Knorr, 1995).

Experiments done on lettuces treated at 300 to 400MPa at 20˚C displayed increased browning, probably as a result of PPO activation or exposure to substances; while tomatoes lost their skin but maintained a firm flesh (Arryo et al., 1997). Bacterial count reduction was

achieved in onions following treatment with 300MPa for 140 mins at 40˚C, but browning was seen at 100MPa (Butz et al., 1994). Some texture recovery was achieved in carrots after treatment at low pressures (100MPa) (Basak and Ramasamy, 1998). Maintaining firmness after pressure treatment could be due to cell disruption allowing enzymes to mix with pectins, creating cross-linkages with divalent ions, thereby increasing cell structure compactness (Ludikhuye et al., 2002). In general, textural changes after low pressure application are characterized by an initial loss of firmness, also called instantaneous pressure softening (IPS), and followed by a gradual change during the pressure hold time (Basak and Ramasamy, 1998). This gradual change has been described as firmness recovery, which is one study on carrots reached 100% at low pressures for long processing times (100MPa for 30 mins) (Basak and Ramasamy, 1998).

Michel and Autio (2001) discussed the loss of firmness in various fruits and vegetables, concluding that many samples could regain their firmness after the first 100MPa, while others did not regain their firmness. In the case of pears, pineapples and oranges, it was shown that their first firmness loss at 100MPa could be recovered during holding time of 30 and 60 min, while carrots did not show recovery. The mechanisms involved in firmness recovery or losses after HPP are not clear. Structural dependence and turgidity seem to be important; however more work needs to be done on for example cell shape deformation analysis such as pectin methyl esterase. It is thought that microscopy tools would also provide evidence of the mechanism involved in textural changes and provide a better understanding of the high pressure effects on whole produce pieces.

The figure 3.3 shows the possible mechanism, from the structural level, which occurs in carrot tissue when subjected to high pressures.

3.5 Effect of High pressure homogenization on fruits and vegetables:

Next to thermal processing, high-pressure homogenization (HPH) is another unit operation that can possibly affect cell wall polysaccharides thereby affecting the textural characteristics of processed plant-based food products (Sandy Van Buggenhout et al, 2013). This was clearly demonstrated in two recent studies of Christiaens at al. (2012a/b) in which the consistency of differently prepared carrot purées (untreated, low temperature blanched and high temperature blanched) and of (untreated) broccoli purée was significantly affected by applying a HPH step during the production of the purées. The consistency of all the purées was significantly reduced by applying an HPH treatment at 100 bar. Also the degree of separation between the serum phase and the pulp phase was significantly reduced for all purées. The decrease in consistency compared to the corresponding blended samples could be explained by the reduction in particle size provoked by HPH. On the other hand, pectin solubility was affected by HPH. For the carrot purées, HPH induced a remarkable shift towards more water-soluble pectin (for example 30.3% to 56.6% for the untreated carrot purée) and less chelator-soluble pectin (for example 42.6% to 23.7% for the untreated carrot purée) and sodium carbonate-soluble pectin (for example 19.9% to 12.7% for the untreated carrot purée). This shift towards a higher relative amount of water-soluble pectin was, compared to the corresponding blended samples, more pronounced for the untreated carrot purée compared to the low temperature and high temperature blanched purées. A similar increase in water-soluble pectin occurred when carrots were high

temperature blanched. For example for the untreated carrot purée, the water-soluble fraction shifted to 48.4% while the chelator-soluble and sodium carbonate-soluble fraction respectively shifted to 32.5% and 12.3%. HPH also had a significant effect on the MM distribution of the water-soluble pectin fraction: a shift towards smaller MM was observed. Similar changes, but to a larger extent, were observed for carrot purées that were high temperature blanched indicating that HPH caused some pectin depolymerization. HPH also had an effect on the pectin solubility in broccoli purée. A clear shift from the sodium carbonate-soluble pectin (30.7% to 23.6%) to the chelator-soluble pectin (25.4% to 35.5%) upon HPH was observed, a similar shift as could be found upon high temperature blanching of the broccoli purée (sodium carbonate-soluble pectin fraction from 30.7% to 17.6% and chelator-souble pectin fraction from 25.4% to 39.7%) . In case of broccoli purée, no change in the MM of pectin upon HPH was found. These results clearly show that HPH induces a higher level of tissue and cell wall disruption compared to conventional blending. Thereby, HPH seems to provoke a certain degree of pectin solubilisation and depolymerisation, similar to the effect that is induced by thermal treatments, thereby affecting the rheological properties of plant-based food systems like purées.

3.6 Effect of Pulsed light (Pulsed UV light) treatment on fruits and vegetables

It has been reported that UV–C affects several physiological processes in plant tissues and, what it is more important, damages microbial DNA (Kuo et al., 1997; Lucht et al., 1998). Lado and Yousef (2002) reported that UV–C radiation from 0.5 to 20 kJ/m2 inhibited microbial growth by inducing the formation of pyrimidine dimers which alter the DNA helix and block

microbial cell replication. Therefore, cells which are unable to repair radiation-damaged DNA die and sub-lethally injured cells are often subject to mutations. A number of *in vitro* studies have demonstrated the efficiency of UV–C radiation on microbial inhibition (Gardner and Sharma, 2000).

Liu et al. (2011a) studied gene expression of tomato fruit in response to postharvest UV-C irradiation (4 kJ/m2), during 24 h after the treatment. They concluded that UV-C irradiation can induce the expression of a number of defence response genes, and suppress the expression of major genes involved in cell wall disassembly, lipid metabolism and photosynthesis. These gene changes underline the biochemical and physiological changes induced by UV-C such as increased defence ability, delayed softening, better maintenance of nutritional and sensory qualities and extension of shelf-life in tomato fruit.

UV-treatments of tomato fruits reduce the gloss of the tomato surface because those treatments affect the morphology of fruit surface wax (Charles et al., 2008a). UV-treatment may have induced biochemical modifications of the surface wax layers. The overall impact of these changes has two contrasting effects. On the one hand, changes in the physical and biochemical modifications that occur in the epidermal cell in response to UV-treatment may be conducive to an improved ability of the plant tissue to resist pathogen attack. On the other hand, altered wax layers can affect light reflectance characteristics of the fruit surface, and may also contribute to increased water loss from cuticular transpiration, both leading to changes in the appearance of the fruit.

UV-C light exposure, if applied at mild intensity, was demonstrated to be an effective nonvisible technology for food surface decontamination. Manzocco et al. (2011) studied the effect of UV-C light treatments at 1.2, 6.0, 12.0 and 24.0 kJ/m2 relative to germicidal efficiency and changes in fresh-like appearance of fresh-cut apple. Independently of UV-C light intensity, all treatments showed the same germicidal effect with 1–2 log reduction in total viable counts. Treatments at an intensity exceeding 1.2kJ/m2 had detrimental effects over the cells of the surface apple tissue.

3.7 Electric Field Effects in Plant Tissues

Electric fields (PEF or AC) produce a current through the biological tissue and may result in damage of membranes and volumetric ohmic heating. As a result, a number of different phenomena, such as intracellular liquid release, diffusion of solutes, and membrane resealing processes, develop inside the cellular structure after their treatment. Specific effects like electro-osmotic flow and electrolysis phenomena can be also important.

3.7.1 Origin of Electropermeabilization

The unique property of PEF application is related to selective damage of the biological membranes. An external electric field *E* induces a transmembrane potential *um* on a membrane. When the transmembrane potential exceeds some threshold value (typically about 0.2–1.0 V), electric field cause a temporary loss of semipermeability by the cell membranes (electropermeabilization) or their damage. The exact mechanism of permeabilization is not precisely understood yet, but it is accepted that electroporation consists of different stages including (Teissi´e et al. 1999; Teissie et al. 2005; Krassowska and Filev 2007):

(1) charging and polarization of the membranes (charging time of ≈1 μs);

(2) temporal destabilization and creation of pores (reported as occurring on time scales of 10ns (Tarek 2005));

(3) expansion of pore radii and aggregation of different pores (in a time range of 100 μs);

(4) resealing of pores and memory effects (lasting from seconds to hours).

Proposed theories account for pore formation (electroporation), and for electromechanical, electrohydrodynamical, viscous-elastic, electrothermal, and electroosmotic instabilities (Ho and Mittal 1996; Weaver and Chizmadzhev 1996; Chen et al. 2006). Sufficiently strong PEF exposure (high electric fields and long time of treatment) leads to formation of large pores, deformation of membranes, and cell lysis (Pliquett et al. 2007). The other possibilities of cell lysis may be explained by chemical imbalances resulting from the enhanced transmembrane transport (Dimitrov and Sowers 1990) and Joule overheating of the membrane surface (Lebovka et al. 2000b). Reversibility of electroporation is closely related to the pulse protocol, i.e. electric field strength, shape of pulses, pulse duration, and intervals between pulses (Canatella et al. 2001). The PEF application can results in transient or stable electroporation.

3.7.2 Stability of Membranes and Cells

In electroporation theory, the lifetime τ_m of a membrane can be estimated as (Weaver and Chizmadzhev 1996):

$$\tau_m = \tau_\infty \exp W/kT\,(1 + (u_m/u_o)^2), \qquad (1)$$

where W is the membrane damage activation energy, τ_∞ is a parameter, k=1.381.10−23 J/K is the Boltzmann constant, T is the absolute temperature, and u_o is a parameter characterizing the electroporation response of the membrane. For lipid membranes, the following estimations of parameters were obtained experimentally: W≈270 kJ/mol, u0 ≈0.17 V, and $\tau\infty$ ≈ 3.7.10−7s (Lebedeva 1987); however, these values depend on the structure and composition of membranes in plant cells. For example, for membranes in sugar beet cells the values of W ≈ 166 kJ/mol and τ_∞ ≈10−23 s were obtained experimentally (Lebovka et al. 2007a). The mean lifetime of a spherical cell τ_c may be estimated by averaging of τ_m^{-1}over the cell surface. (Lebovka et al. 2002; Lebovka and Vorobiev 2004)

$$\tau_c^{-1} = \int_0^\pi \tau_m^{-1} d\cos\theta/2 \qquad (2)$$

where τ_m is determined by Equations (1).

The lifetime τ c of a spheroid depends also on its orientation in the external field (Lebovka and Vorobiev 2007). For example, the lifetime of a prolate spheroid is minimum for its orientation along the external field E (θ =0◦) and maximum for its perpendicular (θ =90◦) orientation.

This result is an accordance with maximum of the transmembrane potential and electropermeabilization for cells oriented by their longest axes in parallel to the external electric field, which was reported for different ellipsoidal microorganisms (Valic et al. 2003; Toepfl et al. 2007; Agarwal et al. 2007).

3.7.3 Electrically Induced Damage in the Cellular Tissue*s*

In cell suspensions and in biological tissues, electroporation is a complex function of cell orientation and distribution of cell sizes and may be influenced by aggregation of cells, their arrangement, local cell density and solute concentration, and distribution of local electric field (Canatella et al,. 2004; Pucihar et al., 2007; Pavlin et al., 2007). Moreover, an external field can affect orientation (Lebovka and Vorobiev, 2007) and aggregation of cells (Toepfl, 2006) in suspensions. Redistribution of the local fields inside a biological tissue is possible also during the PEF treatment (Lebovka et al., 2000a; Lebovka et al., 2001).

3.7.4 Estimation of the Damage Degree

The damage degree *P* can be defined as the ratio of the damaged cells and the total number of cells. The direct estimation of the damage degree can be done through microscopic observation of the PEF-treated tissue (Fincan and Dejmek, 2002), but this procedure is not simple and it is ambiguous. It is possible to estimate the damage degree from diffusion coefficient measurements in the PEF-treated biological materials (Jemai and Vorobiev, 2001; Lebovka et al., 2007b)

$$P \approx (D - Di)/(Dd - Di) \qquad (3)$$

where *D* is the measured apparent diffusion coefficient and the subscripts *i* and *d* refer to the values for intact and totally destroyed material, respectively. The apparent diffusion coefficient can be determined from solute extraction or convective drying experiments. Unfortunately, diffusion techniques are indirect and invasive for biological objects, and they may

impact the structure of the tissue. Moreover, validity of Equation (1) approximation is still controversial (Vorobiev et al., 2005; Lebovka et al., 2007b).

A conventional method of damage degree *P* estimation is based on electrical conductivity measurements. The local electrical conductivity is elevated near the damaged cells, and averaged electrical conductivity increases as the damage degree grows. The conductivity disintegration index *Z* can be defined as (Rogov and Gorbatov 1974):

$$Z = (\sigma - \sigma i)/(\sigma d - \sigma i), \qquad (4)$$

where σ is the electrical conductivity value measured at low frequency (1–5 kHz) and indexes *i* and *d* refer to the conductivities of intact and totally destroyed cellular system, respectively. This equation gives *Z*=0 for the intact tissue and *Z*=1 for the totally disintegrated material.This method is useful for tissues and colloidal biosuspensions (Lebovka et al. 2000a; Vorobiev and Lebovka 2006; El Zakhem et al. 2006a, 2006b). But it requires determination of σd from supplementary measurements for maximally damaged material after freeze-thawing or strong PEF-treatment with high strength electric field and long duration of PEF-treatment (Lebovka et al. 2007a).

Another method is based on electrical conductivity measurements at low (≈1 kHz) and high (3–50 MHz) frequencies (Angersbach et al., 2002):

$$Z = (k\,\sigma^{0} - \sigma_i^{o})/(\sigma_i^{\infty} - \sigma_i^{o}),$$

where $k = \sigma_i^{\infty}/\sigma^{\infty}$ and the indexes *o* and ∞ refer to the low and high conductivity limits, respectively.

Unfortunately, there exists no exact relation between disintegration index Z and damage degree P, though it may be reasonably approximated by empirical Archie's equation (Archie 1942):

$$Z \approx P^{m}, \qquad (5)$$

where exponent m falls within the range of 1.8–2.5 for biological tissues, such as apple, carrot and potato (Lebovka et al. 2002).

Evolution of Damage and Transient Effects

Examples of the time dependence of the conductivity disintegration index Z are presented schematically in Fig. 3.4. It is useful to introduce the characteristic damage time τ defined as a time needed for attaining a half of the maximal damage ($Z \approx 1/2$) (Bazhal et al. 2003).

The damage evolution in tissue can be approximated by the following transition function (Bazhal et al., 2003):

$$Z = \left[1 + (\tau/t)^{K}\right]^{-1}, \qquad (6)$$

where k is an empirical exponent. For damage caused by PEF-treatment, time t corresponds to the total time of PEF-treatment: $t=t_{PEF}=nt_p$, where n is the number of pulses and t_p is the pulse duration. It follows from Equation (6) that $Z=1/2$ at $t=\tau$ so, the definition of the characteristic damage time τ is evident. The thermally induced damage requires a long time and is accelerated by the temperature T increase. The PEF-induced damage depends on the treatment protocol and

its rate grows with increase of the electric field strength E and temperature T (Lebovka et al., 2005).

At moderate electric fields (E <300 V/cm) and room temperature, disintegration index Z may reach plateau at long PEF-treatment. It was experimentally observed that the saturation level Z_s increased with increase of both E (Lebovka et al. 2001) and T (Lebovka et al. 2007a). For example, the maximal disintegration index Z_s was of the order of 0.75 at E=100 V/cm for sugar beet tissue (Lebovka et al. 2007a). The saturation behavior possibly reflects existence of a complex structure and wide spread of the cell geometries and sizes. At higher fields, E >500 V/cm, the saturation behavior was not observed for tissues with relatively homogeneous structures (potatoes, apples, etc.), and it was possible to attain the maximal disintegration($Zs \approx 1$) for these materials. But inhomogeneous materials, such as the red beetroot tissues, for example, can display a step-like behavior of the conductivity disintegration index Z even at higher fields. These steps evidently reflect existence of different domains in the red beetroot tissues and the presence of the cell survivability distribution (Shynkaryk et al. 2008; Shynkaryk 2007).

If PEF stops at the saturation level (Fig. 3.4), the scenario of the further evolution can be different. At small level of disintegration, the cells can partially reseal (Knorr et al. 2001). But higher level of disintegration usually results in further increase of Z after a relatively long time (Lebovka et al. 2001; Angersbach et al. 2002) and acceleration of the thermally induced damage as shown in Fig. 3.4. The nature of the PEF-induced transient effects is not fully understood yet and requires more thorough study in the future.

3.7.5 Changes induced by PEF treatment**:**

Significant structural changes occur in cell walls and plant tissues as a result of the interaction between electromagnetic fields and cellular material during pulsed electric field treatment. These changes have important influence on juice extraction and tissue pressing process. Some of the significant changes induced by the PEF treatment, which influences its application in food processing are:

1. Reduces permeability

In this study it was shown that the effects of PEFs on plant tissues are similar to those observed in response to other stress conditions. Electropulsation, in the range of 100–500 V/cm (one 1 ms rectangular pulse), slows the diffusion of the dye FM1-43 through the cell wall of potato tissue, suggesting a significant decrease in cell wall permeability at nanometer scale. Oxidative cross-linking is likely to reduce the pore dimensions, restricting the movement of particles through the apoplast. It may appear surprising that the diffusion of a small molecule such as FM1-43 (molecular weight 451 Da) was retarded by the putative cross-linking (Federico Gomez Galindo, et al., 2008).

2. Increases diffusivity

   PEF treatment noticeably accelerated the diffusion process, which required one hour with mild heating at 40°C, allowing to disintegrate efficiently the biological cells and noticeably decreasing the diffusion temperature (up to 40–50°C) without any loss of solution purity (Lebovka et al., 2007a).

3. Enhances drying

PEF utilization as a pretreatment of apples enhances the drying process. Application of PEF in the investigated range of parameters increased the cell disintegration index and, as a consequence, modified the kinetics of the operation. It reduced the time necessary for air drying apple tissue by 12% and increased the effective moisture coefficient by 20% compared to the intact tissue. Drying time reduction can result in a reduction of energy consumption. (Artur Wiktor, et al, 2013)

4. Induces shrinkage on drying:

Treating the apple samples with PEF significantly (at the 5% level) affected bulk density, skeletal density, porosity, pore size and volume shrinkage (M.I Bazhal et al., 2002). Control samples had higher volume shrinkage than treated samples. Treating apple slices with PEF resulted in a 22% less volume-shrinkage than control samples. Volume shrinkage is related to structural collapse during drying (Karathanos and others 1996, Zogsas and others 1994). There was apparently a more severe collapse of structure in the control apple samples than in the PEF treated samples. This can be attributed to weakening of the cell walls in pulsed tissue as a result of integrity violation due to induced pores.

5. Alteration in bulk and skeletal density

The mean bulk and skeletal densities obtained in this study for the control samples were in the range of the data reported in literature. PEF treatment of samples resulted in lower bulk densities due to the resulting less shrinkage during drying of the treated materials. The higher mean skeletal density obtained with PEF treated samples are

attributed to the increased porosity resulting from electroplasmolysis. With increased pores and with no moisture occupying the pores, skeletal density of the tissue was largely influenced by the densities of the various constituents of tissue such as carbohydrate with density in the range of 1.5 to 1.6 kg/m3 (French 1984).

6. Pore formation

Dry apple samples can contain such pores as internal cell and intercellular spaces, holes in the cell walls and cell membranes. Considering that the difference between pore size distribution for control and pulsed samples is constituently revealed in the range of 0.1 to 5mm as shown in figure 3.5, it can be concluded that treating the apple tissue with electric field resulted in generation of more pores of sizes in order of cell wall thickness. Electroplasmolysis affects therefore not only cell membranes but also cell wall integrity.

The current investigation showed that PEF treatment changes apple tissue porosity from 63.9% to 68.3%. Sizes of the induced pores were mostly smaller than the average sizes of pores in the untreated samples. Sizes of the PEF induced pores were less than the cell wall thickness.

7. Loss of turgor pressure

The turgor pressure apparently reduced due to the electric field induced pores in cell walls thus explaining the decrease in compression modulus. Therefore, bioyield stress decreased with intensification of electroplasmolysis. Apple tissues become more compressible after PEF treatment even at low tissue destruction degree.

4 Conclusion:

Plant cells are enclosed with cell wall which is composed of constituents such as pectin, lignin, homogalacturonan, etc. These constituents and their network form the basal structure of the plant tissue. From the review, it is understood that the parenchyma cells undergo the characteristic changes, which has its ultimate impact on the texture, which is the most vital quality of the final product. The processing methods, be it thermal or non thermal, has its own effects on the cell structure to some extent, which determines the textural quality. The characteristics change in the texture depends on the principle mechanism of the treatment. For example, in pulsed electric field treatment, the electric field pulses used results in electroporation of the cell wall/ membrane. Whereas, when it is subjected to thermal treatment, tissue softening occurs due to turgour loss; membrane degradation and cell wall separation. Overview of this paper indicates that the study on the final textural quality of the product, which undergoes the characteristics changes, requires a detailed biochemical analysis of cell wall or a microstrcutural study.

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Table 1.1. Electromagnetic frequency allocations for ISM purposes (Mullin, 1995)

Nature	Frequency
Microwave	896MHz(UK) or 915 MHz(US)
	2450 MHz
	5800 MHz
	22125 MHz
Radio frequency	13.56 MHz
	27.12 MHz
	40.68 MHz

Table 2.1: Quality attributes and undesirable changes during storage and processing (Singh and Anderson, 2004)

Quality Attributes	Undesirable Changes
Colour	Off-colours (darkening, bleaching, browning)
Flavour	Off flavours (hydrolytic, oxidative rancidity, caramelization)
Texture	Loss of solubility, water holding capacity, toughening or softening
Nutritive value	Vitamins, minerals, proteins or lipid degradation.

Table2.2: z- values for quality degrading chemical reactions. (From Toledo (2007) with minor alterations)

Texture	**Z(°C)**	**Overall sensory quality**	**Z(°C)**
Apples	21-28	Beets	19
Black beans	35	Broccoli	44
Navy beans	37	Carrots	17
Soy beans	42	Corn kernels	32
Beets	40	Green beans	29
Brussel sprouts	21	Peas	32
Carrots	18	Potatoes	26
Potatoes	11	Squash	26
Enzymes	**Z(°C)**	**Colour loss**	**Z(°C)**
Peroxidase	28	Green pigment	30
Catalase	8	Red pigment	31
Lipoxygenase	9	Browning	32
Polyphenol oxidase	8		
Pectin esterase	16		

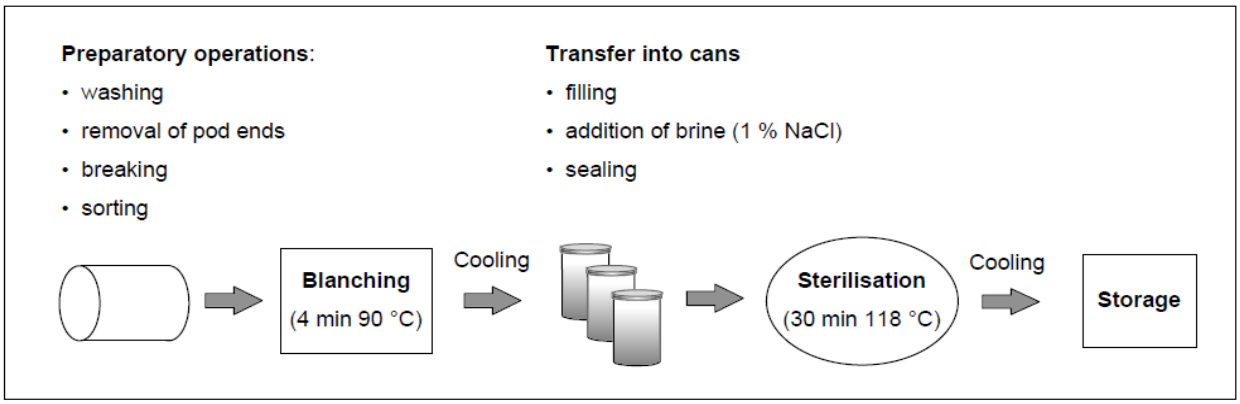

Figure1.1 Overview of operations in the standard canning process of green beans

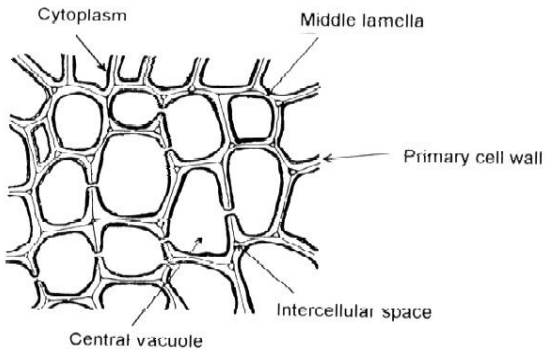

Figure 1.2 Schematic overview of typical parenchyma tissue (Greve, et al, 1994 a, b).

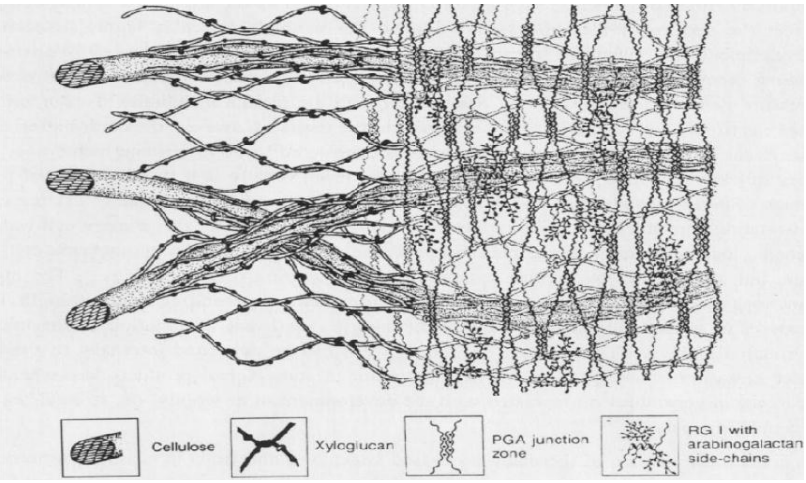

Figure 1.3**:** A schematic representation of the primary cell wall of most flowering plants except grasses (adapted from Carpita, et al., 1993).

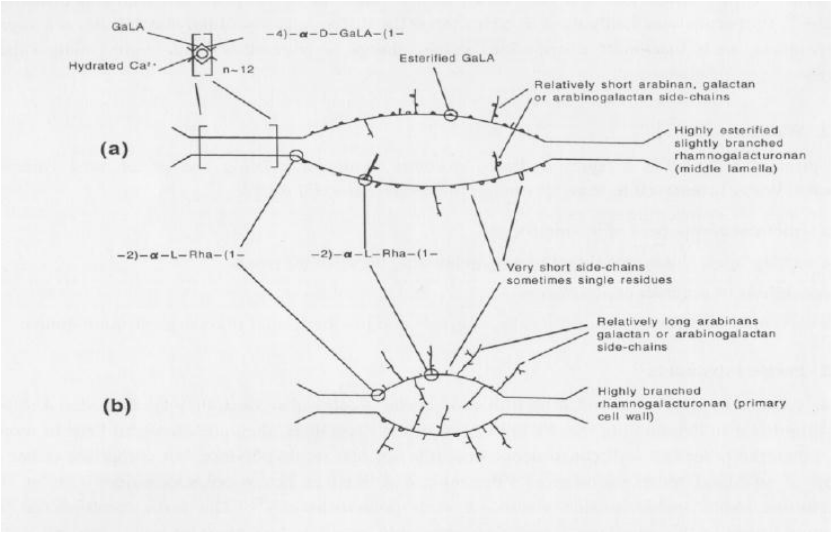

Figure 1.4 Overview of structure of pectic polysaccharides in (a) the middle lamella and (b) cell wall (from Brett 1990).

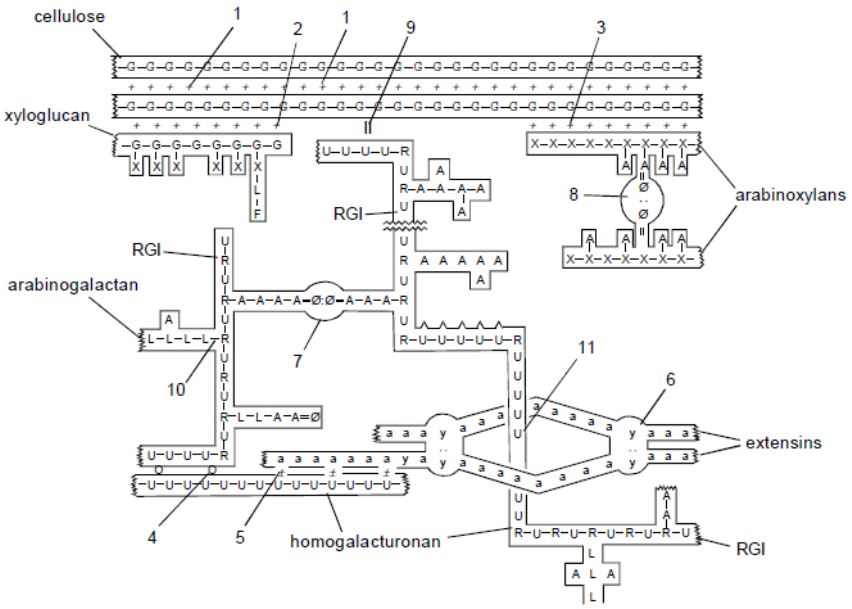

Figure 1.5 Overview of the types of crosslinks in the primary wall (Fry 1993). 1. cellulose-cellulose, 2. xyloglucan-cellulose, 3. xylan-cellulose, 4. HG-HG 5. extensin-pectin, 6. extensin-extensin, 7. pectin-pectin, 8. arabinoxylan-arabinoxylan, 9. pectin-cellulose, 10. arabinogalactan-RGI, 11. pectin in extensin, (+)H-bonds, (o) Calcium bridges, (±) other ionic bonds, (:) coupled phenolics, (=) ester bonds, (-)glycosidic bonds, A = arabinose, F = fucose, G = glucose, L = galactose, R = rhamnose, U = galacturonic acid, Û = galacturonic acid methylester, a = amino acid other than tyrosine, y = tyrosine, y:y = isodityrosine, Ø = ferulic acid, Ø:Ø = diferulic acid.

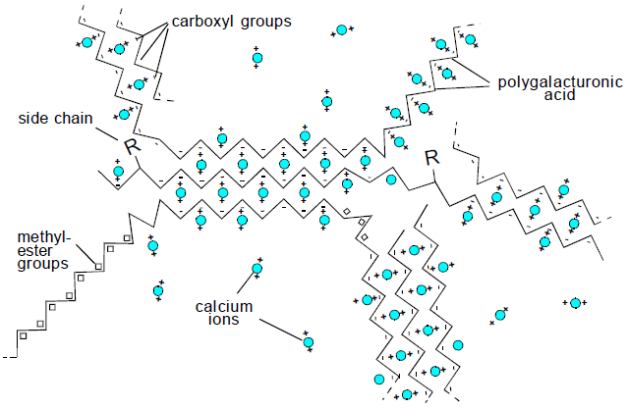

Figure 1.6 Egg box model of (Morris et al., 1982) showing cross-linking between unsubstituted homogalacturonan and the factors that are likely to influence such cross-linking within the cell wall (Brett & Waldron 1990).

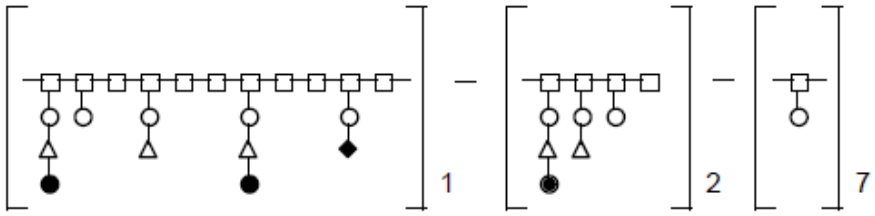

Figure 1.7: Tentative structure for the xyloglucan from cell walls of Phaseolus coccineus (modified from O'Neill & Selvendran 1985). □ , β(1,4)Glc; ○ , α(1,6)Xyl; △ , β(1,2)Gal; ● , α(1,2)Fuc; ◆ , (1.2) Ara.

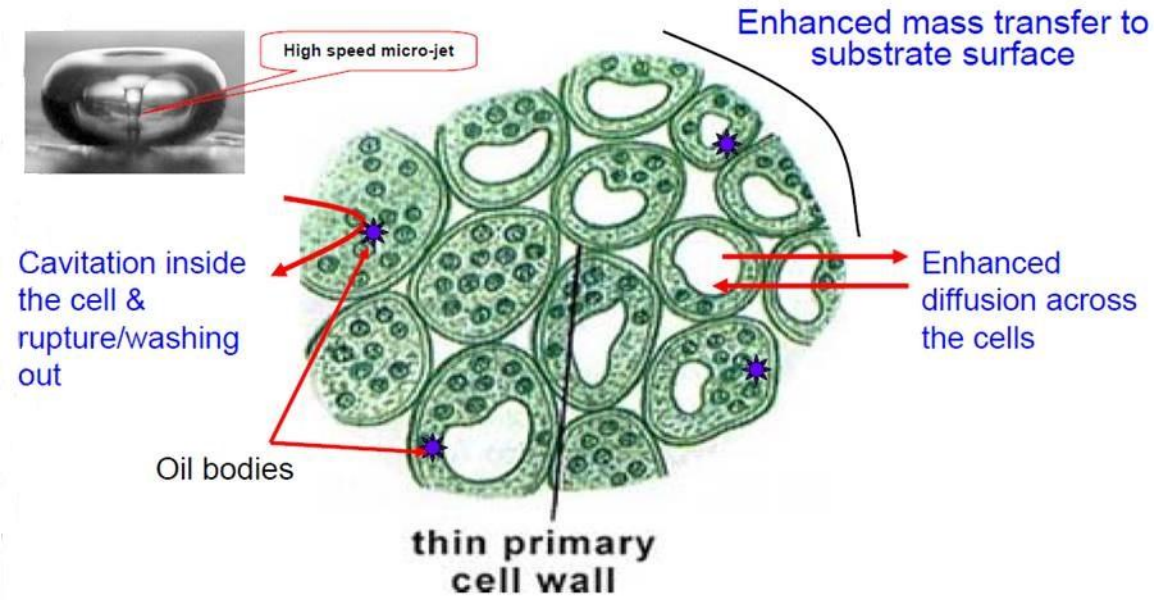

Figure 1.8: Pictorial representation of action of ultrasound treatment

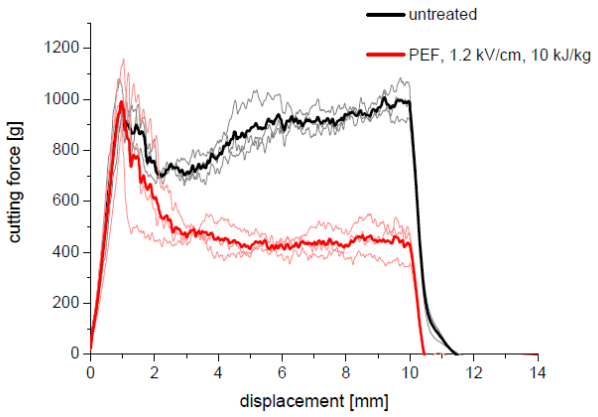

Figure 1.9: Impact of a PEF-treatment on textural properties of potato tissue, three samples for untreated (black) and PEF-treated (red) samples and average (bold lines) are shown.

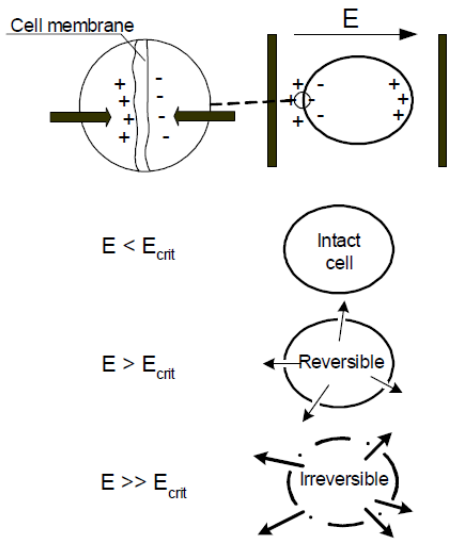

Figure 1.10: Schematic depiction of mechanism of membrane permeabilization of cell in PEF treatment.

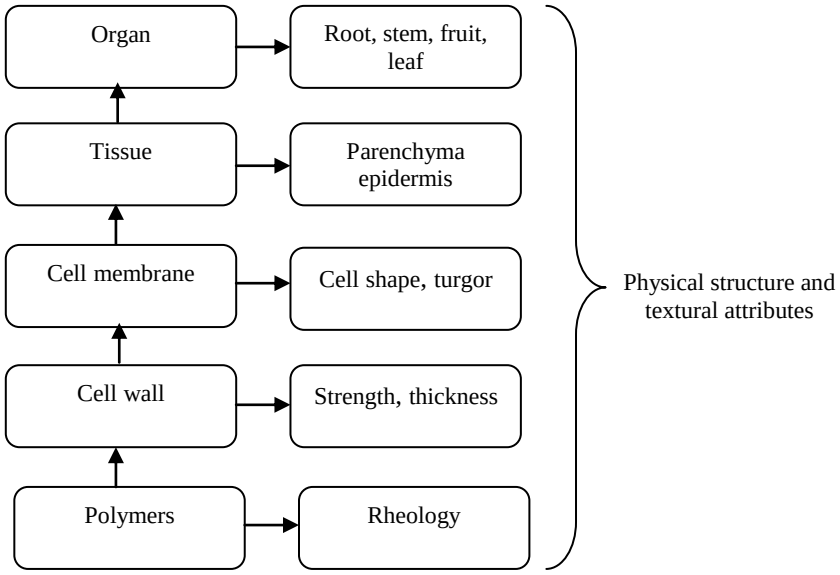

Figure 2.1: Flow diagram of structure hierarchy (adapted from Waldron et al. 2003).

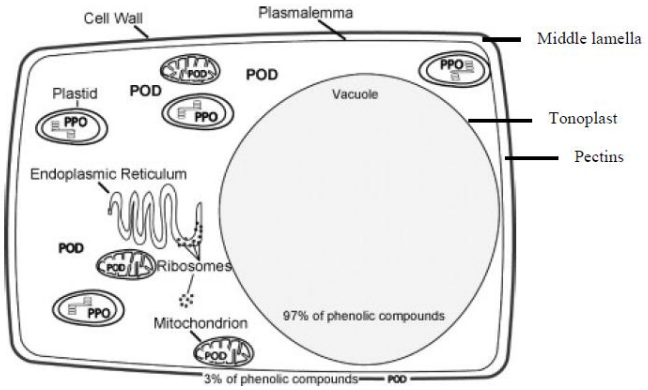

Figure 2.2: The internal and external localization of phenolic compounds and phenolic oxidizing enzymes (polyphenol oxidase (PPO) and peroxidise (POD) in a typical plant cell. This model was constructed from previous published work (Toivonen, 2004).

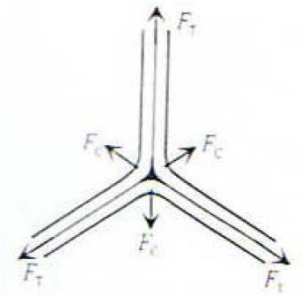

Figure 2.3 Distribution of turgor- induced stress at a tricellular junction. F_t is the stress component between each cell wall and F_c is the force that separates the cells in the corners (Golberg et al. 1996).

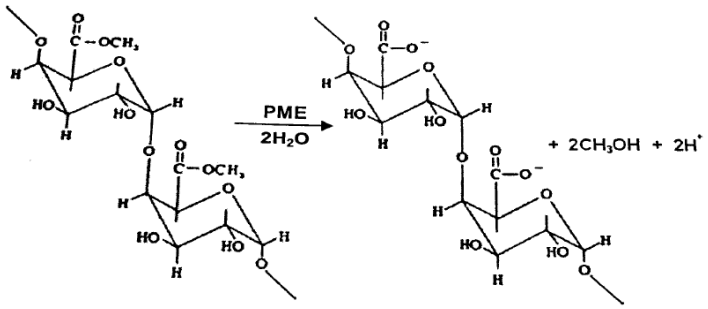

Figure 2.4: Pectin hydrolysis reaction by pectin methylestaerase (Ly Nguyen, 2004).

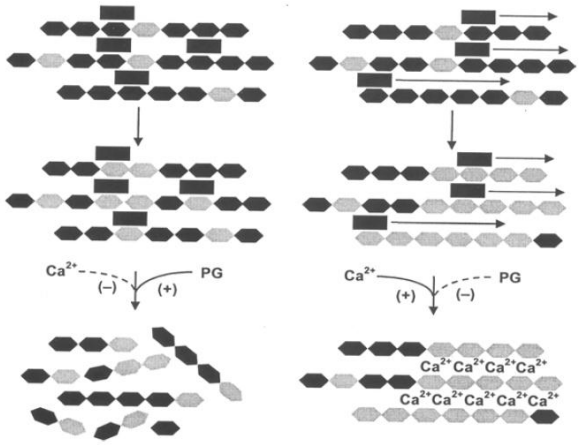

Figure 2.5: Schematic modes of action of PME (black rectangle). Left: random action; right: linear action. Esterified galacturonic acids bleak hexagons and de-esterified galaturonic acids in grey hexagons (Vu et al, 2004).

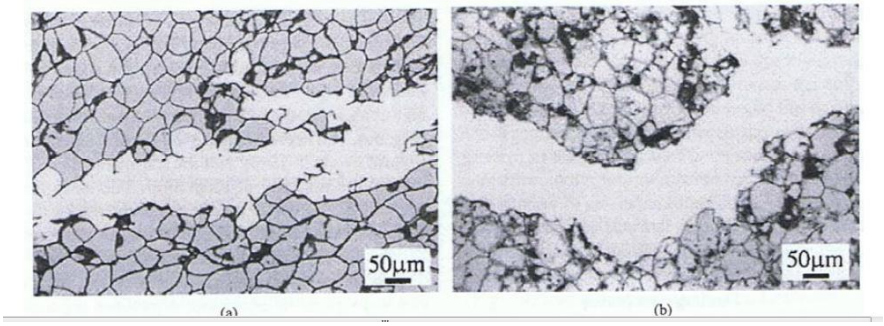

Figure 2.6: Carrot compressed at 500mm min to 25% strain. (a) Raw carrot: brittle fracture through cells and (b) Carrot heated for 30 min, fracture between cells (Ormerod et al., 2004).

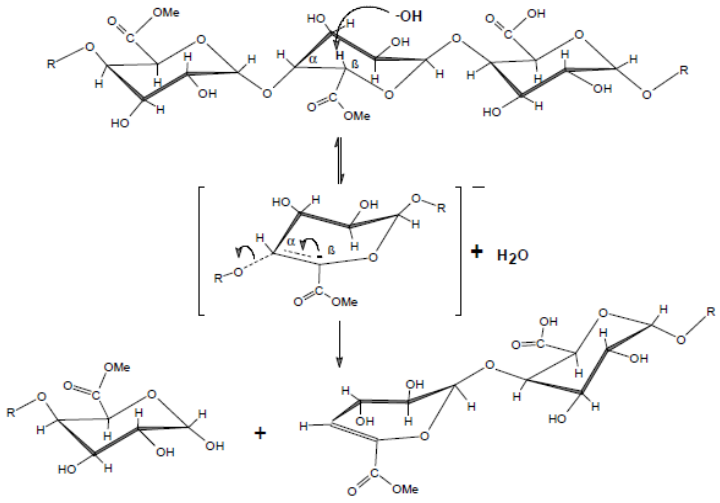

Figure 3.1: Depolymerisation of a partially esterified pectic galacturonan chain by β-Elimination.

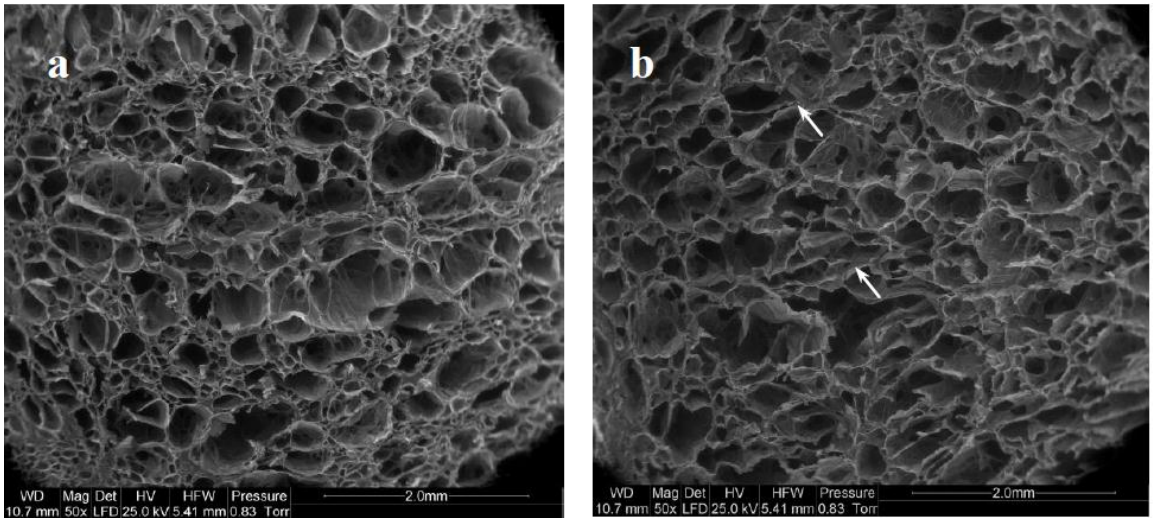

Figure 3.2 shows the microstructure of ultrasound treated sample prior (3.2a) and after (3.2b) osmotic dehydration process.

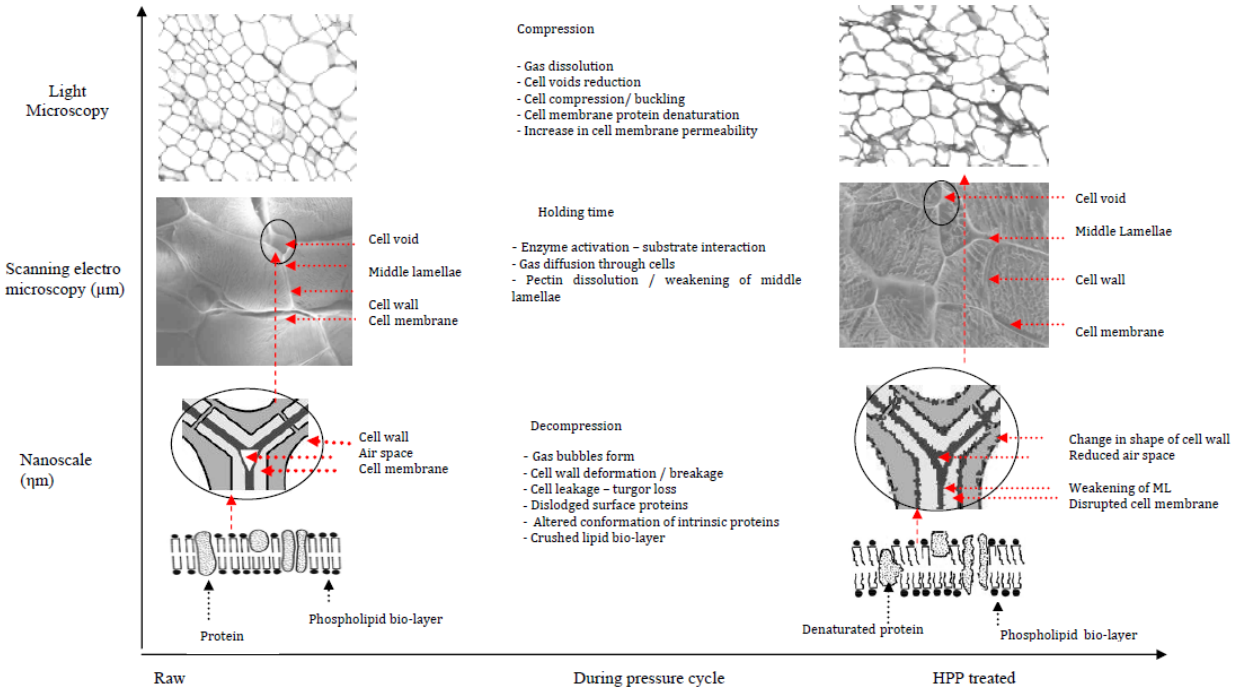

Figure 3.3: Effect of high pressure processing on carrot tissues.

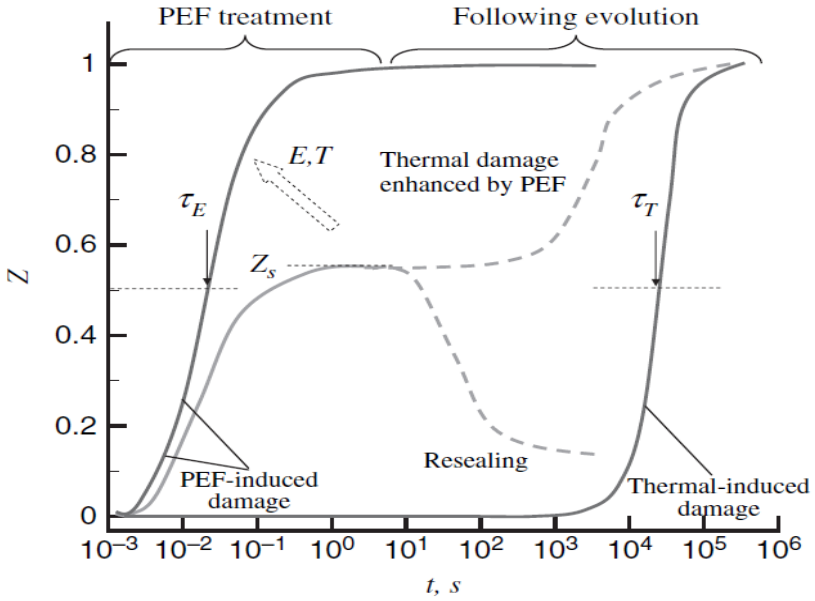

Fig. 3.4 Evolution of the conductivity disintegration index Z under the PEF and thermal treatment. Here, τ_E and τ_T are the electric and thermal characteristic damage times, respectively, Z_s is the level of disintegration index saturation

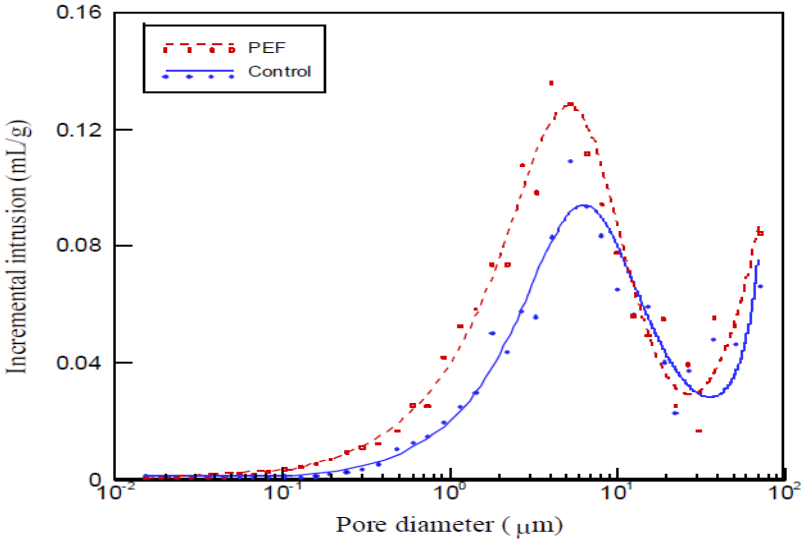

Figure 3.5: Distribution of pores in untreated and PEF treated apple samples with conductive disintegration index in the range of 0.9-1 (n= 60 pulses, E= 1000V/cm, t_1= 300μs, f = 1 Hz). Points represent averaged data from three determinations. Lines were obtained with least square polynominal fiting of experimental data.