Effects of high pressure processing on carrot tissue: a microstructure approach

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Abstract

High pressure processing (HPP) has the potential of extending the shelf life of fruits and vegetables whilst preserving nutrients and, importantly, many sensory attributes. Although there is a developing body of literature identifying the advantages of this technology for specific products under specific conditions, it is important to gain further understanding of why undesirable quality changes can also be enhanced by this process. For this reason, this work focused on the changes that HPP promotes within the microstructure of the product (carrots, *Daucus carota* L.) considering that macroscopic quality is determined at a cellular level.

This project was part of a government funded flagship programme at CSIRO Australia, where carrots were chosen as a model product of study. The effects of HPP on this commodity were studied for a range of pressures (100-600 MPa) applied for different holding times (2, 10 and 30 minutes) at ambient temperatures (20 °C). The effects were measured qualitatively and quantitatively by using several microscopy techniques, textural, physiological, biochemical and sensory analysis and through comparison with unprocessed (raw), frozen and heat processed (boiled, steamed and sous vide) carrots. The information collected provided understanding of how different pressure levels affected the physical and physiological responses of carrots based on cellular changes. It also allowed HPP to be positioned within the range of other preservation techniques and to identify relationships between quantitative and sensory quality attributes.

The key findings of the study can be divided into HPP effects below and above 200 MPa, as near this pressure a “tissue break point” was identified. Pressures below 200 MPa only slightly affected the cellular structure arrangement according to microscopy techniques, which explained small textural changes, but there was an interesting shift in the metabolic response from aerobic to anaerobic metabolism, presumably due to stress. Above 200 MPa, cell structures became less organized and more disrupted resulting in significant loss of textural characteristics such as hardness and cutting forces compared to raw carrots. This texture loss was related to cellular leakage and loss of turgidity. Considering that

texture is one of the most important quality attributes in carrots, this study searched for ways of ameliorating the impact of pressure by manipulating turgidity before and after the HPP process. One possibility was by weight loss prior to high pressure processing, but this approach did not help to overcome texture losses after HP treatments above 200 MPa, as structures were irreversibly damaged. Below 200 MPa, cells were still able to regain some turgor pressure (pressure of the cell content against the cell wall); however changes in cell permeability were evident. The addition of calcium chloride solutions in samples high pressure treated at above 200 MPa showed no quantitative texture improvements, confirming membrane damage as the principle mechanism and limited influence of biochemical reactions (pectin degradation by pectin methylesterase) affected cell walls at the conditions studied.

Sensory perception by a trained panel showed a positive response toward HPP carrots treated at 600 MPa for 2 minutes. It was interesting to observe no significant differences in many sensory attributes in comparison to raw and sous vide samples, while boiled carrots showed low acceptability due to loss of most volatiles, texture and colour attributes. Storage trials confirmed that high pressure treated samples retained higher quality after 14 days at 4°C by supporting a lower count of lactic acid bacteria and consequently having less ethanol and acetic acid production in the pack.

Overall, this research has provided a greater understanding of the application of high pressure on whole vegetable pieces by following microstructural changes. Based on this work, HPP can be considered equivalent to other 'lightly processed' technologies such as sous vide and may offer benefits as a complementary process to this or other similar preservation techniques. Future opportunities could be investigated taking advantage of the changes observed in cell permeability (< 200 MPa) for diffusion processes such as salting and candying. Health benefits arising from nutrients being more exposed and preserved after pressure treatments should be further studied by following nutrient availability and body absorption. Furthermore, studies on altering rates of compression or decompression and various pressure cycling effects could assist in optimisation of future commercial HPP applications.

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To my loving husband Andrew and my little Sam.

Thanks

The more I learn, the more I realise how little I know (Socrates).
I wish we had more time in our hands to just observe and take in
all the knowledge that is out there.

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1 Research background

The modern food industry has long provided consumers with a variety of shelf stable fruits and vegetables. These products initially required severe thermal preservation treatments to accommodate distribution and storage. Although this shelf life was valuable when household refrigerators were widely unavailable, it was obtained at the cost of considerable loss of products' attributes. Later, the advent of large scale freezing and microwave processing revolutionised the convenience foods market. Nowadays, consumers are increasing their demand for products with higher quality and freshness. Since the 1990s', the fresh-cut industry has provided vegetable and more recently, fruit products to consumers to meet these demands worldwide. These products retail at a higher cost, with shorter storage lives (typically <3 weeks) and depend on effective temperature control throughout the cold chain. As these products are marketed as ready-to-eat, without washing, they potentially pose a greater risk with respect to food safety. This was exemplified in 2006 by the presence of coliforms (*E. coli*) in some fresh produce such as spinach sold in the USA threatening the health of consumers. Therefore, the food industry has been confronted with the challenge of finding alternative technologies to meet these consumer demands for 'freshness' with low health risks.

One of the alternative technologies most readily accepted by consumers is high pressure processing (HPP) (Cardello et al., 2007). This technology offers the potential for "cold pasteurization" and has been successfully employed to produce vegetable, fruit juices and paste products such as guacamole, jams, juices and pastes, keeping those 'fresh appearance' attributes (Stewart et al., 2007 and Oey et al., 2008). There may also be an opportunity to use this technology for pre-processing in whole vegetables pieces as an alternative to pre-treatment by heat processing. The processing advantage of HPP is that it generally reduces detrimental effects on fresh product quality compared with conventional processing (Knorr, 1995). However, applying high pressures to pastes and juices is different than to whole pieces of vegetables; therefore, to be able to identify commercial opportunities for whole vegetable pieces, it is essential to investigate and understand how this technology affects the attributes of these products, in particular their tissue structures. Understanding the structural changes occurring in the product in

response to high pressure will allow identifying mechanisms involved in the process and not just obtaining a quality result given as a number.

This work was conducted at Food Science Australia (FSA) as part of the Food Futures National Research Flagship programme for CSIRO (Australian Commonwealth Scientific and Research Organization). A requirement of this programme was that carrots were selected as a model product for the study of a broad project on processing innovation. As carrots have long shelf life and are relatively low value, it is unlikely that carrots would be commercially treated by high pressures. However carrots are a good system to use to investigate the process and have been used in previous research to understand the effects as cooking, microwaving, and freezing and many other preservation processes. This is because carrots have a relatively well defined cellular structure, are highly turgid and possess different types of tissues (cells). Furthermore, carrots have a long fresh shelf life in cool storage conditions and are available all year around facilitating experimental work. In terms of their nutritional benefits, they are containing antioxidants such as carotenoids which have been shown to be degraded during heat processing. By understanding the carrot system and the effects of pressure, it would be likely to identify what may be occurring to other vegetables with similar structure and commercial opportunities.

Overall the aims of this research were firstly to identify the main quality attributes that change during HPP processing, and secondly to correlate quality attributes with microstructural modifications to allow understanding of the mechanisms that may be occurring during and after high pressure processing. The specific objectives of this work were to:

- Identify how HPP affects carrot tissues and the mechanisms involved in cellular damage. This can be assessed by following respiration rates responses and cell membrane integrity after high pressure processing at various pressure levels and time combinations.
- Correlate macro- to micro-scale changes, using microscopy and image analysis techniques to relate parameters such as shape factor and elongation to whole tissue changes (e.g. hardness). This will also involve characterising the role of endogenous vegetable enzymes on a micro-scale change in the overall result.

- To explore ways to ameliorate texture reduction by manipulating the tissue's turgidity before and after processing. This could be achieved by either reducing turgor pressure (which is known as the pressure of the cellular sap against the cell membrane) during storage or by using an osmoticum (by immersion) prior and post to treatment. This will indicate if the tissue could better withstand pressure and if the texture reductions are reversible or irreversible after pressure treatments.
- To investigate how consumers perceive HPP products and find correlations between the main quality attributes and sensorial perception in comparison with other traditional treatments such as cooked, sous vide and raw. This will allow us to identify a 'place on the processing scale' in terms of perception of HPP products.
- Finally to identify how the different treatments were able to preserve the products by measuring quality changes throughout storage of 14 days at 4°C.

Achieving these objectives will allow a more mechanistic understanding of the way HPP affects fresh vegetable pieces. This thesis will outline the most significant effects of HPP on carrot tissue properties, which will enable some prediction to be made about similar tissue's responses to pressure and consequently new applications for HPP.

2 Literature review

2.1 Introduction

In order to generate a profile of how high pressure may affect carrot tissue, it is necessary to first learn about what the main quality attributes in fresh carrots are and how storage affects these qualities, based on the fact that quality attributes start from the microstructure of the commodity. An overview of the structure and composition of cells constituting the tissue was also required. This provides better understanding of the effects of a process on the tissue and possibly ways to overcome detrimental effects due to processing.

This review also aims to present high pressure processing (HPP) as cold pasteurization already used in some countries, its advantages/disadvantages and example of products already studied. This is where, future opportunities will be identified and a window of opportunities for more research was identified.

2.2 Quality attributes of fresh fruits and vegetables during storage and processing

Fresh fruits and vegetables are unique in the sense that they are maintained as living tissues (respiring) until they are consumed (Aked, 2000). These foods are characterized by a high nutritive value, rich in antioxidants, fibre and vitamins and low in fat. For example citrus, kiwifruit and tomatoes have significant levels of vitamin C, while carrots have high levels of pro-vitamin A (Institute of Food Technologists, 1990). Consequently, many studies have investigated ways to process and extend the shelf life of these foods while maintaining their beneficial qualities. In the case of fresh consumption, slowing down senescence is the key factor to provide longer shelf life. This can be achieved by slowing respiration rates, and product deterioration (Aked, 2000). Slowing down respiration rates can be achieved by cooling or using packaging such as modified atmosphere (MA) or controlled atmosphere (CA) to control quality deterioration (Toivonen and DeEll, 2002).

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 processing is 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 (Singh, 1994; 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 foods. Nowadays, advances in biology and material sciences have given a new approach to food researchers and companies to understand foods as complex multicomponent systems (Aguilera, 2005). Considering that the majority of the elements that are involved in the physical and rheological, textural and sensorial behaviour are below 100 μm 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 or vegetables, cells normally influence chemical, physiological and biological properties of the tissue. For example during drying, tissue shrinkage is initiated at a cellular level (Konstankiewcz 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 hemicellulose. Variations in the composition will affect the structure and physiology of the tissue. Any changes in this structure 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).

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

The hierarchy of the structure in plant tissue goes from the molecules through cellular, tissue and organ levels. Each level is directly linked to a range of physical attributes. Textural changes do not only depend on one level of the 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).

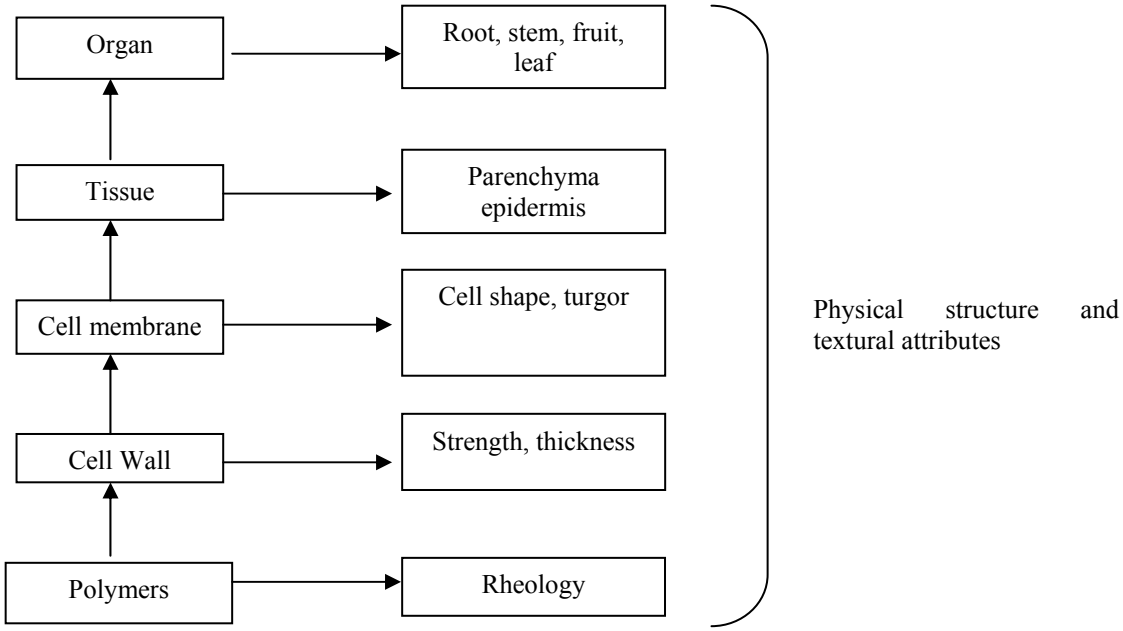

Figure 2.1: Flow diagram of structure hierarchy (adapted from Waldron et al., 2003).

As the diagram 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.

2.2.1 Respiration and packaging effects on quality during fresh fruit and vegetable storage

2.2.1.1 Respiration

Plant tissues continue to respire after harvest. Over time they senesce and eventually die (Institute of Food Technologists, 1990). Aerobic respiration results in oxygen and glucose consumption, producing carbon dioxide, water and heat (Aked, 2000). It has been observed that products with greater respiration rates (oxygen consumption and carbon dioxide release measured in a certain time) typically have shorter storage lives, as excessive respiration causes metabolic collapse, breakage of cell membranes and cellular leakage (Aked, 2000). Environmental variations significantly influence rates of respiration. Subjected to the sensitivity of the commodity to chilling injury, low uniform temperatures and air circulation are recommended to reduce respiration rate, heat liberation and condensation (Hardenburg et al., 1986). The gas composition surrounding the product will affect the physiology of the produce, taking into consideration packaging, film permeability, storage temperature and the level of wounding (Klaiber et al., 2005). There is already extensive literature on various vegetables and fruit during storage, but in the next paragraphs, research on storage and shelf life of carrot will be discussed.

Studies done on minimally processed carrots have shown that carrots can be stored as shredded packs showing gas equilibrium after 3-4 days at 4°C due to respiration. Slicing carrots exhibited increase in respiration rates compared with intact carrots as expected (Barry-Ryan and O'Beirne, 1998); this was probably due to a wound response from the tissue that had been damaged. The increase in respiration rate was dependent on the extent of wounding, the larger the number of cuts, the higher the respiration rates (number of cuts from 2 to 32 sections). Stabilization back to an initial respiration rate was observed after 9 days of storage at 10°C for all carrot sections (Figure 2.2; Surjadinata and Cisneros-Zevallos, 2003).

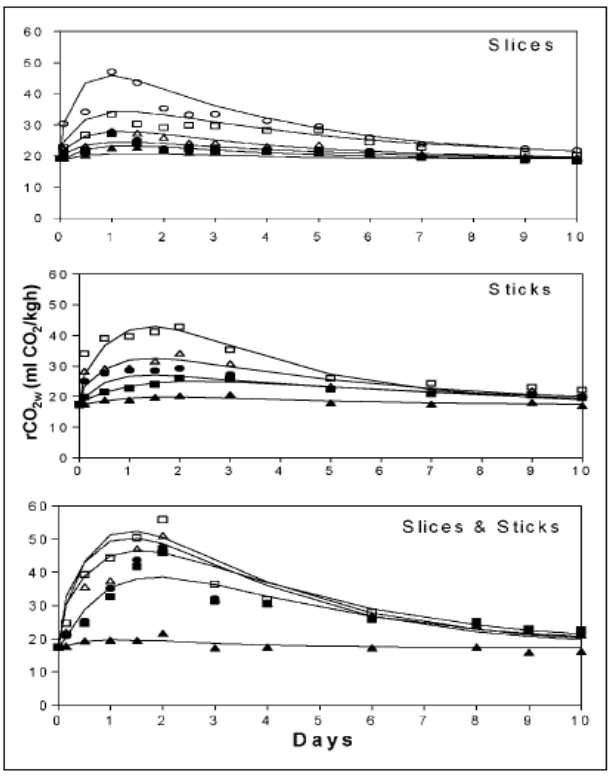

Figure 2.2: Wound-induced respiration rate (rCO_2w) of carrots compared with time for four different types of cuts. Points represent experimental data and line the fitted model. ▲: control; ■: 2 sections; ●: 4 sections; Δ: 8 sections; □: 16 sections; ○: 32 sections (Surjadinata and Cisneros-Zevallos, 2003).

Modified atmospheres can improve the quality of fresh produce during storage. The metabolism of cut carrots packed in modified atmospheres has been shown to be highly dependent on oxygen concentrations and less on carbon dioxide concentrations (Sode and Kühn, 1998). Furthermore the use of controlled atmospheres (CA) (0.5% O_2, 10% CO_2) on carrot pieces also reduced rates of respiration compared to those stored under ambient air conditions (Table 2.2). A useful indicator of product physiology is the respiratory quotient (RQ = carbon dioxide production / oxygen consumption) (Kader, 1986). The table shows how increasing the number of cuts and temperature will increase respiration rates as expected. Stick and shred samples stored in CA seemed to keep the RQ close to 1 no matter the storage temperature, while slices showed an RQ below 1, which possibly means enzymic reactions may be occurring and promoting oxidation of the tissue. Respiration rates differed according to the type of cut and storage temperature, while ethylene production was not influenced by the type of cut or by storage temperature (Izumi et al., 1996).

Table 2.2: Average rates of CO_2 production, O_2 consumption and respiration quotient (RQ) of carrot slices, sticks and shreds during CA storage (Izumi et al., 1996).

Temperature of storage	Type of cut	Treatment	CO_2 production (ml. $kg^{-1}h^{-1}$)	O_2 consumption (ml. $kg^{-1}h^{-1}$)	RQ
0°C	Slices	Air CA[a]	2.5 1.1[c]	3.9 1.7[c]	0.6 0.7
	Sticks	Air CA	7.6 3.8[c]	8.0 3.4[c]	1.0 1.1[b]
	Shreds	Air CA	5.7 3.5[c]	10.0 3.6[c]	0.6 1.0[c]
5°C	Slices	Air CA	6.5 2.4[c]	8.5 3.3[c]	0.8 0.7
	Sticks	Air CA	9.7 3.3[c]	9.9 2.5[c]	1.0 1.3[c]
	Shreds	Air CA	12.1 9.4[c]	15.2 7.4[c]	0.8 1.3[c]
10°C	Slices	Air CA	13.0 3.3[c]	31.0 6.0[c]	0.4 0.6
	Sticks	Air CA	24.3 6.2[c]	24.5 6.5[c]	1.0 1.0
	Shreds	Air CA	22.1 13.8[c]	41.0 12.7[c]	0.5 1.1[b]

[a] 0.5% O_2 + 10% CO_2, [b c] indicate significant differences ($P<0.05$ or 0.01 respectively) between paired air and CA treatments.

2.2.1.2 Microbial quality

During storage, fresh-cut packed carrots can be spoiled by growth of lactic acid bacteria producing lactic and acetic acids (Carlin et al., 1990). Non-vacuum packed sliced carrots favour the growth of *Erwinia* spp, (70% of total flora), *Pseudomonas* spp, (20%) and *Bacillus* spp (10%). This population changed after packaging and storage (Table 2.3). Vacuum packed carrots exhibited a more fermentative nature with *Leuconostoc* spp., as the predominant micro-organism, giving a slimy characteristic to the product while *Erwinia* spp were the most predominant organisms in non-vacuum packed carrots (Buick and Damoglou, 1987).

Table 2.3: Percentage distribution of bacterial generation by stored carrots at different temperatures for 8 days (Buick and Damoglou, 1987). * Lactic acid bacteria.

	Storage Temperature					
Genus	15°C Vacuum	15°C Non vacuum	10°C Vacuum	10°C Non vacuum	5°C Vacuum	*5°C* Non vacuum
Erwinia	0	90	20	90	50	80
Pseudomonas	0	10	0	10	0	10
Bacillus	0	0	0	0	10	10
*Leuconostoc**	100	0	80	0	40	0

From the above table, it is observed that the percentage of distribution of *Erwinia* was high at all temperature at non vacuum packed bags, while a significant reduction was observed in vacuum packed samples. Another observation from Table 2.3 was *Leuconostoc* growth, which occurred even at lower temperatures when vacuum packed; this is probably because carrots are still respiring, promoting fermentation and microbe growth.

2.2.2 Water loss during storage and drying

Water loss in intact vegetable products occurs by the permeation of water vapour through the cuticle and lenticels within the cell layers of the epidermis (Aked, 2000). The rate of water vapour permeation will depend on the external pressure, temperature and also tissue or epidermis damage, promoting wilting or shrivelling (Toivonen and DeEll, 2002), gloss loss and flaccidity (Paull, 1999) and crack generation (Lewicki and Pawlak, 2003). When drying, most of the water will be removed from the vacuole and not the cytoplasm (Lewicki and Pawlak, 2003). During storage, water losses decrease cell turgor affecting tissue firmness, which is the main cause for quality loss in carrots (Phan et al., 1973). Figure 2.3 shows the cross section of carrot xylem affected by different storage conditions. An increase in occurrence of gaps between cells and cell deformation is observed when storage time increases and especially at lower humidity in comparison with raw carrot tissue. Similar cellular changes were also observed by Nielsen et al., (1998) (Figure 2.3).

Pendlington and Ward (1965) studied the effects of drying on carrot tissue structure, observing that the loss of water could cause cell walls to collapse as a result of cell shrinkage. This was accompanied by the loss of plasma membrane permeability. Figure 2.4 shows the microstructure of carrot cubes. Carrot cells have a rounded shape ~ 50 μm in size and are well packed together with high cellular-cellular contact and not many air spaces. As drying of the tissue progresses, cells start to deform and finally collapse tending to form an elongated pattern (Figure 2.4C).

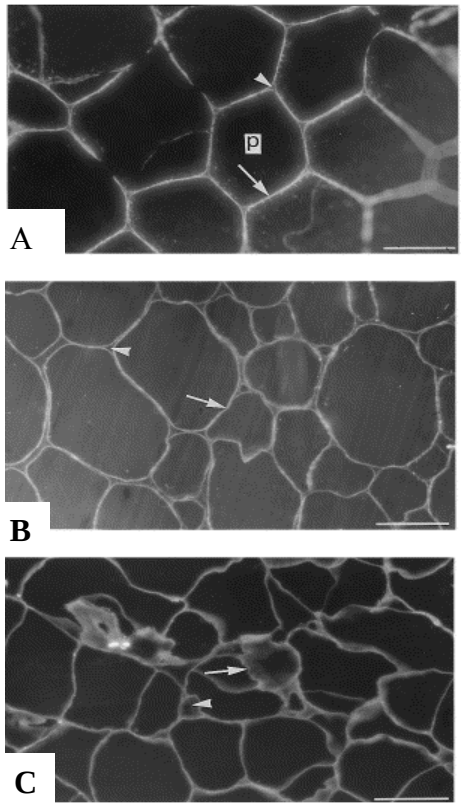

Figure 2.3: Cross sections of the xylem parenchyma region of carrot affected by different storage treatments. Figs. A, B and C are light micrographs after staining with calcofluor white fluorescent brightener (bar=50 μm). (A) fresh; (B) stored at high humidity for 19 weeks; (C) stored at low humidity for 19 weeks. Parenchyma cell (P), cell wall (arrow), intercellular space (arrow head) (Nielsen et al., 1998).

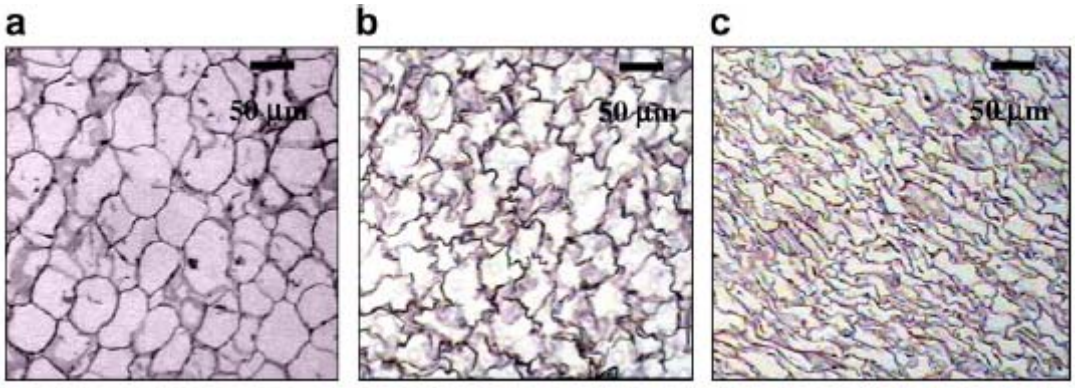

Figure 2.4: Microstructure of carrot cubes undergoing hot air drying (60°C, 0.5m/s) after 0 min (a), 150 min (b) and 300 min (c) (Kerdpiboon et al., 2007).

Potato cells, which are less regular in shape, presented larger gaps between cells. Figure 2.5 shows less cellular damage due to drying for potatoes in comparison with carrot cells. This may be due to the larger gaps between cells (at the start) allowing better accommodation and restructuring instead of cramping when drying for a prolonged time.

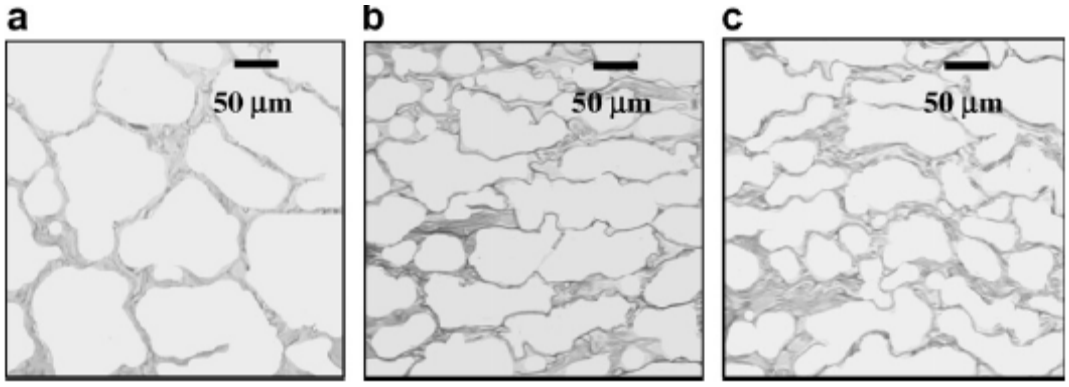

Figure 2.5: Microstructure of potato cubes undergoing hot air drying (60°C, 1.0 m/s) after 0 min (a), 150 min (b) and 300 min (c) (Kerdpiboon et al., 2007).

Dehydration can also be caused by osmotic changes. The mechanisms of osmotic dewatering consist of firstly the hypertonic solution entering into the intercellular spaces. Then the cells decreased in size due to water transfer out of the cells to try to equalise the osmotic pressure across the cell membrane (apple tissue) (Barat et al., 2001). As the solution penetrates the tissue, cells on the surface became plasmolyzed, while the inner cells still conserved their turgor. It has been found that turgor pressure gradients change, as the solution penetrates, deforming the tissue (Lewicki and Porzecka-Pawlak, 2005). Research done on turgor modification on fruits have shown impregnation of glucose in strawberries with consequent lysis of the plasma and tonoplast membranes, promoting cell walls to become disorganized. Similar responses were found in osmotic dehydration of kiwifruit, where an extensive plasmolysis of cell membranes and cell wall degradation was observed (Lewicki and Pawlak, 2003). Overall, tissue dehydration either by water loss or by osmotic dehydration will inevitably promote cellular damage or lysis. This damage will be dependent on the structural nature of the product and the given conditions.

2.2.3 Colour changes during storage

Colour is a quality attribute that relates to maturity in fruits and vegetables (Singh and Anderson, 2004). This attribute is closely linked with appearance, which is used by

consumers to evaluate the product's freshness (Fennema, 1996). In the case of carrots, pigments called carotenoids cause the characteristic orange colour. These pigments are also of high nutritional value as precursors of vitamin A and play a role in photosynthesis and photo-protection of the plant tissue. Changes in provitamin A during storage are shown in Table 2.4. Colour changes in carrots can occur due to oxidation of carotenoids as they have numerous double bonds in their molecular structure (Fennema, 1996). However, carotenoids are very stable during storage (Lavelli et al., 2006).

Table 2.4: Changes in provitamin A carotene concentrations in carrots during storage (Lee, 1986).

	Storage Days						
Carotene (μg.g^{-1} fresh weight)	5	27	50	70	100	125	155
α-Carotene	21.1	21.4	22.1	22.5	25.5	21.5	18.5
β-Carotene	54.9	58.9	61.3	62.3	62.8	63.1	58.2
β-Zeacarotene	0.7	0.8	1.3	1.1	1.2	1.6	1.6
γ-Carotene	0.8	0.9	1.9	1.9	1.3	1.5	1.3

During storage, pigments start to develop reaching a peak between 70 and 125 days. Alpha carotene content in whole carrots showed its peak at 100 days of storage (2°C and 90 % RH) (Lee, 1986). After that peak, carrots start to lose those pigments. Some can be susceptible to light, and others are lost due to oxidation (Fenemma, 1996). Table 2.4 illustrates how pigments need approximately between 100 to 125 days to develop, and then they may start to degrade in many cases.

One of the major problems related to carrot colour is discolouration or whitening of the surface. The use of minimal processing such as cutting or slicing is responsible for promoting colour changes during storage. Discolouration can affect acceptability by increasing the perceived age of the produce. Scanning electron microscopy of carrot stick surfaces indicated that the culinary knife can cause considerable tearing and shearing of the tissue resulting in white discolouration (Tatsumi et al., 1991). The reasons for this are that when cells are cut, they compress and slough against each other, leaving them more susceptible to dehydration. Studies done on the effects of slicing methods on fresh carrots have indicated that slicing promotes physical damage and

stress enhances microbial growth with a severity of blunt machine blade > sharp machine blade > razor blade. Using a machine blade caused twice as much exudation compared to a sharp blade and triple that caused by using a razor blade; this was probably due to the fact that by using a sharper blade (thinner) the cut was done cleanly passing through the cells causing less damage to adjacent tissue (Barry-Ryan and O'Beirne, 1998).

Studies of colour during storage have shown that slices of vacuum packed carrot remained at acceptable colour and appearance after storage at 4°C for 8 days (Buick and Damoglou, 1987). Similar results were also observed by Rocha et al., (2007), who found that vacuum packed grated carrots stored at 2°C had a maximum storage life of 8 days. In contrast, non-vacuum packed carrots presented high levels of dryness when stored for 2, 4 and 5 days at 15, 10 and 4°C respectively (Buick and Damoglou, 1987). The storage temperature is a critical variable as when carrots were stored at 10 and 15°C, vacuum was lost and carrots were unusable after 4 and 2 days, respectively.

Changes in colour during storage are also possible when the accumulation of lignified material intensifies giving the white blush or discolouration in carrots (Cisneros-Zevallos et al., 1995; Toivonen and Brummell, 2008). Previous research done on quality losses in fresh carrot sticks stored at 4°C have used the white index as a colour sensitive parameter perceived by consumers (Lavelli et al., 2006). It is suggested that carrot dehydration (water loss) rates can be slowed with packaging films (~1 $g.m^{-2}$ day^{-1}; 25°C, 75% RH) preventing whitening (Klaiber et al., 2005). Peeling methods can be an important variable in the shelf life of carrots. It has been found that coarse abrasion can significantly increase weight loss (Barry-Ryan and O'Beirne, 2000).

Edible coatings (cellulose based polymers) could reduce some of the white surface incidence and improve appearance (Howard and Dewi, 1996; Li and Barth, 1998). Studies done by Toivonen and Brummell, (2008) indicated that white discolouration may also be reduced by a change of pH and enzyme activity involved in the lignification process. Meanwhile calcium dipping of shredded carrots could improve firmness and reduce microbial load but had no effects on whitening, which seems to be mainly a result of water loss (Izumi and Watada, 1994).

2.2.4 Organoleptic changes during storage

Organoleptic changes during storage may be caused by natural senescence, environmental conditions or microbial contamination. Fresh whole fruits and vegetables will respond differently to minimal processing, which exposes larger areas of damaged tissue allowing microbes to act and biochemical reactions to take place, thereby changing the organoleptic characteristics of the product. Carrots can vary in sweetness and bitterness as well as in their intrinsic woody and green flavours. The sugar content at harvest depends on the growing location and conditions (Suojala, 2000). Sucrose is the main sugar in carrots and its concentrations tend to be higher in colder years than warmer years (Suojala, 2000). Storage trials indicate that during the first few months' storage, hexose concentration tends to increase while sucrose concentration decreases (Phan et al., 1973).

The perceived organoleptic quality of carrots in general is related to chemical quality attributes such as sugar concentrations, dry matter (or moisture) content, titratable acidity and soluble solids content (Alabran and Mabrouk, 1973).

Volatile compounds in carrots, such as mono-sesquiterpenes, are also affected by growing, handling and storage conditions (Varming et al., 2004). During storage, terpene concentration can reduce significantly and lipid oxidation occur affecting flavours (Varming et al., 2004). Lafuente et al., (1996) showed that ethylene-induced formation of isocoumarin was related to ethylene-enhanced respiration in both whole and cut carrots. In cut carrots, stress may promote the generation of bitter compounds (isocoumarin) affecting the flavours of the product (Varming et al., 2004). Seljåsen et al., (2001) found higher isocoumarin and lower sugar levels in carrots that had been stressed after harvest. Washing could also promote stress in carrots; however studies indicated that washing by hand or machinery did not significantly affect the carrot flavour (Seljåsen et al., 2004).

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 intercellular 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 submitted 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 compounds lost by leakage.

2.2.5 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 lignification. (b) cell-to-cell adhesion, which includes the middle lamellae 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, stems 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, hemicellulose and pectins (Abbot and Harker, 2004). Cellulose provides rigidity and resistance to cell walls whilst hemicellulose gives plasticity and the ability to stretch (Van Buren, 1979). Pectins are responsible for the adhesive or cementing forces between cells (van Dijk 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; this is normally expressed in terms of degree 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 can be classified having low and high methoxyl groups; the high methoxyl group is represented by DE values between 50 and 80%, while low methoxyl groups have DE values below 50% (Baker *et al*, 20005). 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).

Gómez Galindo et al., (2004c) 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 (Gómez Galindo et al., 2004d).

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 2.6 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.

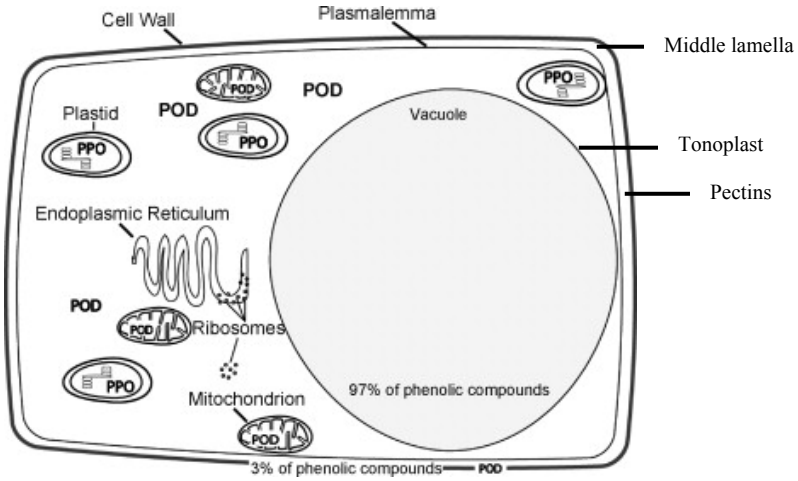

Figure 2.6: The internal and external localization of phenolic compounds and phenolic oxidizing enzymes (polyphenol oxidase (PPO) and peroxidase (POD) in a typical plant cell. This model was constructed from previous published work (Toivonen, 2004).

When relating texture to structure it is also known that 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-hemicellulose bonding).
- Adhesive forces between cells given by pectin.

Carrots structure develops from a secondary growth of the cambium, which creates the xylem (inner part or core) and phloem (outer part or cortex) (Figure 2.7). The phloem part is mainly formed by parenchyma cells (with thinner cell walls), while the xylem is mainly formed by lignified cells, which are characterized by having stronger and thicker cell walls (Davis and Gordon, 1980).

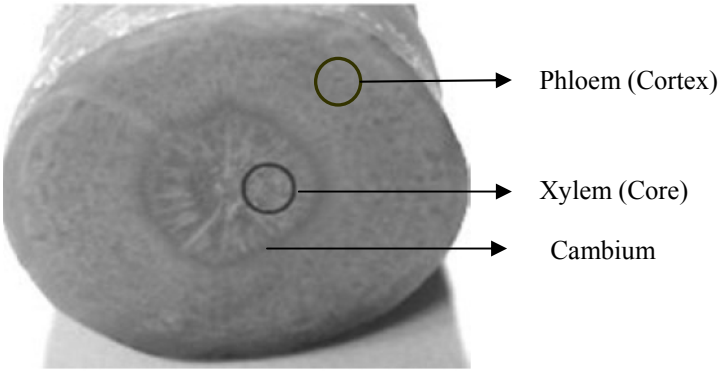

Figure 2.7: Cross sectional carrot structure (modified from Zdunek and Umeda, 2005a).

The presence of lignin in the xylem region can reduce structural damage, while carrot phloem displays a greater tendency to cell wall fracture (Davis and Gordon, 1980). This is further exemplified by the 2-fold increase of the puncture force required to penetrate the xylem in comparison to the phloem tissue at both day 0 and after 60 days of pickling (Llorca et al., 2001). The lignified cells in the xylem also helped to retain its integrity and rigidity upon heating, as it is composed mainly of non-living material (Davis and Gordon, 1980). Lignin, (which is a complex biopolymer containing phenolics) gives a woody texture when it accumulates in the cell wall during storage (Institute of Food Technologists, 1990). This will have an effect on the final texture perception of the product.

Figure 2.8 shows a comparison between potato and carrot tissue distribution and cellular pattern. It is observed that potatoes have a more regular distribution of cells, while carrots showed large variation of smaller and larger cells forming a less regular pattern. In terms of cellular shape, potatoes present a more square and rounded shape cells, while carrots are more rectangular shape. The scattered vascular bundles observed (round cells in the centre) in carrot tissue are lignified cells known as xylem vessels; this provides strength and gives the brittle characteristic.

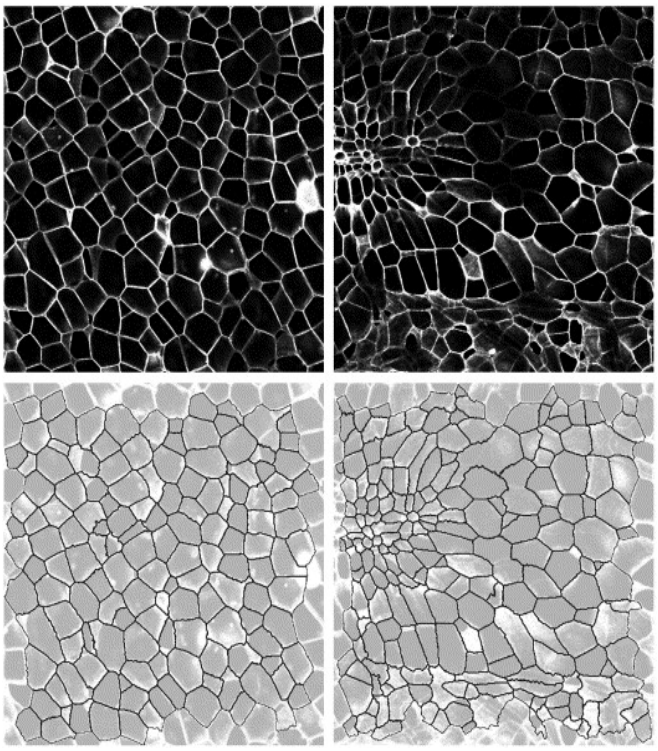

Figure 2.8: Microscopy images of intact potato tissue (left images) and intact carrot tissue (right images) together with the result of image analysis is shown as the dark lines on source images in background (Zdunek and Umeda, 2005b).

2.2.5.1 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 Dijk 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-cell adhesion, they may break and liberate their inner contents. In fresh produce, cell adhesion can depend on the strength of the middle lamella, plasmodesmata connections and cell-to-cell contact (Harker, 1997). Goldberg et al., (1996) explains that pectins are the only polymers present in a tricellular junction joining the cells (cell-to-cell contact). Figure 2.9 shows how the turgor-induced stress is distributed at tricellular junctions. Forces are classified into two components: Ft is the first component of stress on the place of each cell to cell contact, while Fc is the second component that describes the radial forces that separate the cells at the corners.

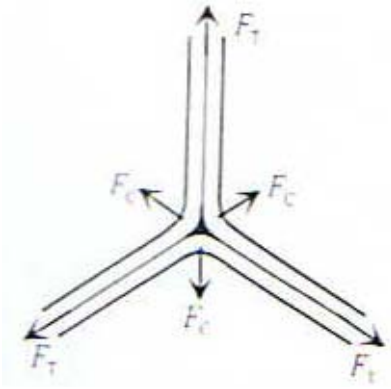

Figure 2.9: Distribution of turgor-induced stress at a tricellular junction. Ft is the stress component between each cell wall and Fc is the force that separates the cells in the corners (radial forces) (Golberg et al., 1996).

In a fresh tissue, the mechanisms 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 walls 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-enzymic 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 not 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 the main factor for firmness loss (Greve et al., 1994b).

Moving from cell wall changes to cell membrane, it is found that cellular membranes are formed by proteins associated with a bilayer of lipid (Fan et al., 2005). 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 homeostasis and compartmentalization (Fan et al., 2005). External environmental factors such as temperature abuse, dehydration or toxic contamination can alter these membranes affecting respiration, leakage and cell injury (Fan et al., 2005). Leakage due to membrane disruption can lead to turgor losses. Greve et al., (1994b) studied the effect of plasmolyzing carrot tissue by immersion in mannitol (1M) before cooking so it could better 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 that cell walls and cell membranes have their own mechanisms 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 compressed, 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 between the cell wall 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 will 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.

2.2.5.2 Enzymes involved in carrot 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 many 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 2.10) 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 of free carboxyl groups that will further interact with bivalent ions (Ca^{2+}) (Figure 2.11; Van Buren, 1979; 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 combining high pressures with heat as a process.

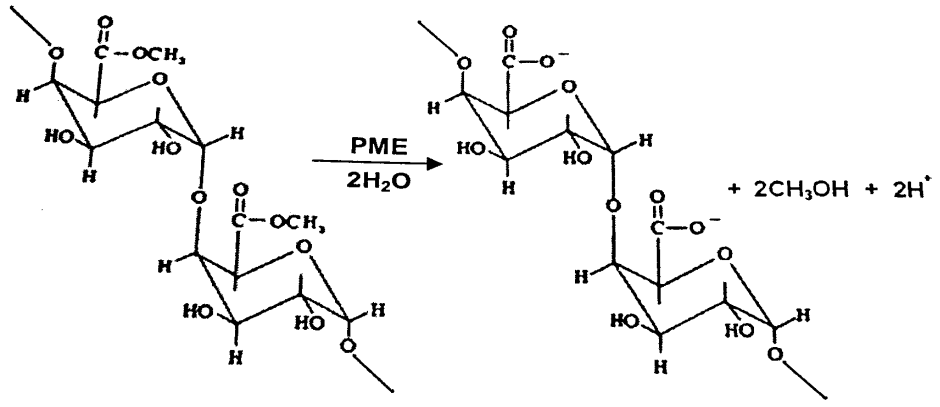

Figure 2.10: Pectin hydrolysis reaction by pectin methylesterase (Ly Nguyen, 2004).

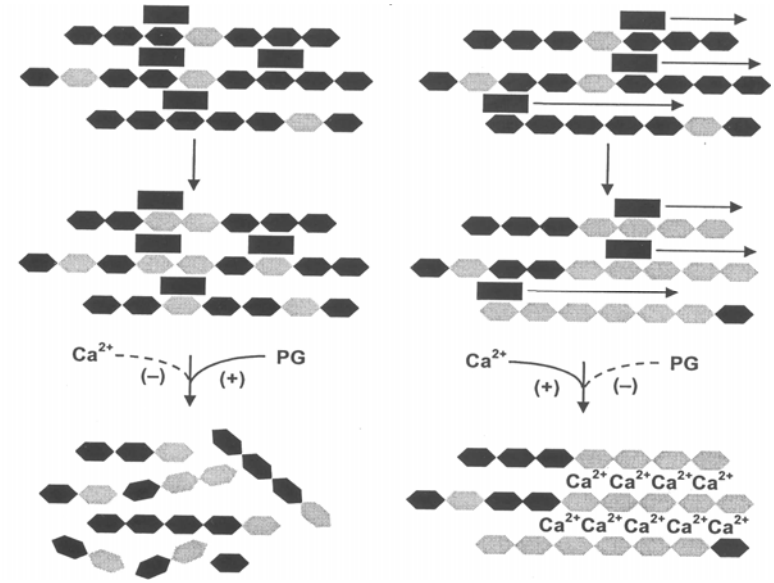

Figure 2.11: Schematic modes of action of PME (black rectangle). Left: random action; right: linear action. Esterified galacturonic acids are black hexagons and de-esterified galacturonic acids in grey hexagons (Micheli, 2001 cited by Ly Nguyen, 2004).

2.3 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 are often detrimental to food quality. Mild heat treatments can be used to control vegetative pathogens and inactivate 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 carotenoids (cis/trans), affecting the provitamin A activity (Fennema, 1996). Another reaction that occurs during heating is nonenzymic browning, where sugar molecules form unsaturated rings such as furans, which can result in colour changes (Fennema, 1996).

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.

A way of reducing the detrimental effects of high temperature on quality is to apply high temperature for a short time. Such treatments produce the equivalent lethality but fewer detrimental changes to quality (Fennema, 1996). Furthermore, milder heat treatments such as steaming (for 3 seconds) before packing can extend the shelf life of carrots by controlling plant pathogens (Afek et al., 1999). Blanching can also be used to improve colour in carrots (giving a brighter orange colour) by inactivating lipoxygenase (Werlein, 1998).

Tissue softening during thermal processing is due to turgor loss, membrane degradation and cell wall separation (Greve et al., 1994b; Kidmose and Martens, 1999; 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 solubility and consequent softening (Keijbets and Pilnik, 1974; Van Buren, 1979). To prevent solubilisation and consequent texture loss, Thiel and Donald (1998) suggest stabilizing pectins using binding compounds such as $Ca^{2+.}$

During heat processing, membrane structures can be altered irreversibly, causing release of compounds for further reaction (Fennema, 1996). Depending on the temperature and duration of heat application, water blanching can cause cells to collapse and break. Studies of carrot tissue using light microscopy have demonstrated separation of cell walls in the middle lamella region after severe heating. However less cell degradation was found after steam blanching (Jewell, 1979). 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 2.12).

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 (Greve et al., 1994a).

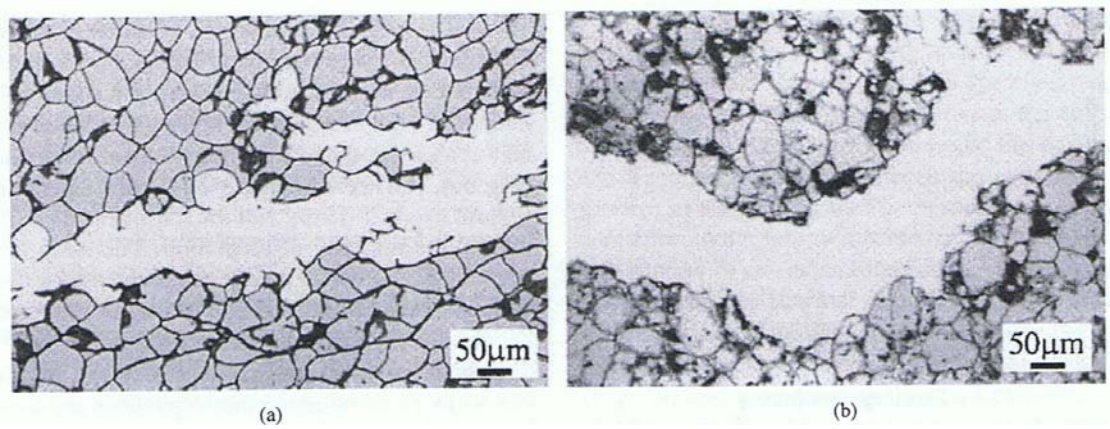

Figure 2.12: Carrot compressed at 500 mm/min to 25% strain: (a) Raw carrot; brittle fracture through cells and (b) Carrot heated for 30 min, fracture between cells (Ormerod et al., 2004).

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., 1994a). 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 (Van Buren, 1979). Meanwhile, osmotic treatments (e.g., using mannitol solutions) can reduce turgidity, helping to better withstand firmness loss during heating (Greve et al., 1994b).

There is indeed a space for further research in the area of high pressure processing by investigating whether texture changes are similar to different from those found during cooking.

2.4 Quality changes after mild-thermal and non-thermal processing

Minimal processing (MP) 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 modified atmosphere packaging and /or active packaging and 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). An example of a minimally processed product is presented by shredded carrots packaged under a modified atmosphere, where a mix of 5% CO_2 and 95% N_2 provided the best gas combination for preventing deterioration at 4 and 10 °C (Kakiomenou et al., 1996).

S*ous 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 s*ous 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 colour (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 MP technologies have been evaluated to prolong shelf life of foods including irradiation, pulsed electric fields, ultrasound and high pressure processing. Apart from minimal processing (MP) 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 1 day, this was due to cell plasmolysis (Llorca et al., 2001).

Pulsed electric fields (PEF) 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 (Angersbach et al., 2000; Zimmermann et al., 1974 and 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 are 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 (HPP) 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., 2002; Butz et al., 2003) with no apparent damage to sensorial attributes (Hendrickx et al., 1998). Table 2.5 shows some examples of current applications of HPP.

Table 2.5: Applications of HPP (Ohlsson, 2002a).

Product	**Manufacturer**	**Process conditions**
Jams, fruit dressing, fruit sauce topping, yoghurt, jelly	Meidi-ya Company, Japan	400 MPa, 10-30 minute, 20°C
Grapefruit juice	Pokka Corp., Japan	120-400 MPa, 2-20 minute, 20°C
Mandarin juice	Wakayama Food Ind., Japan	300-400 MPa, 2-3 minute, 20°C
Non-frozen tropical fruits	Nishin Oil Mills, Japan	50-200 MPa (freeze –18°C)
Avocado	Avomex, USA	700 MPa, 600-800 L/h
Orange juice	UltiFruit, France	500 MPa, 5-10 cycles 1 minute hold

High pressure processing is the most commercially advanced and accepted of these technologies (Cardello, 2003). Understanding the actual effects of HPP on the product starting from the micro level such as cells should be always considered. The following part of this literature review is directed at fresh produce during storage and processing, including HPP and its potential role as a minimal preservation technology.

Many studies have been done on the applications of HPP to fruits, pastes, jams and juices indicating retention of colour and flavours (Ludikhuyke et al., 2002). However there is little information on the application of HPP to vegetable pieces and how HPP affects their quality attributes based on their structure and sensory perception.

2.5 High pressure processing: background and principle

2.5.1 Introduction and technological aspects

High pressure processing in foods is not a new technique as it has been practised since 1899 for milk preservation. However in the early eighties research it started again in USA, Japan and Europe with some preserved products on the shelves as a result (Earnshaw, 1996). Conceptually, this technology is known as a non-thermal treatment with the ability to reduce and/or inactivate the microbial flora and maintain food constituents undamaged without altering the sensorial quality (taste, flavour and colour) (Hendrickx et al., 1998).

Based on Pascal's principle of hydraulics, the system uses an incompressible fluid such as water or oil to transmit a force from one location to another. In other words, the increase in pressure at any point in a confined fluid will be equal at every other point within the container (Figure 2.13).

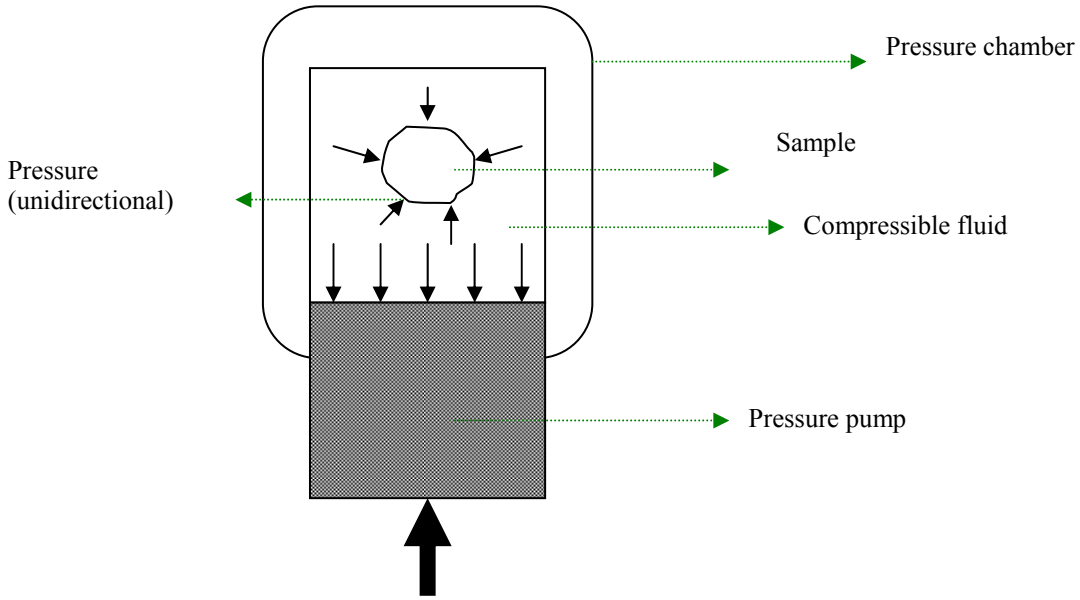

Figure 2.13: Principle of high hydrostatic pressure processing. The product will be compressed by a uniform pressure in all directions and return to its original state when the pressure is released (Olsson, 1995).

The fundamental effect of high pressure transmission is based on Le Chatelier's principle, which explains that when a constraint is placed on a system, the system will respond to minimise the effect of that constraint (Johnston, 1995). This means that the application of high pressure will impose a volume constraint and enhance reactions with

negative volume changes (ΔV) or negative activation volumes (ΔV*); this marks the distinction between high pressure and heat where free energy (ΔG) or activation free energy (ΔG*) are the more familiar terms (Johnston, 1995).

During pressurization, a 4% decrease in water volume is reached when applying 100 MPa, while a 15% volume reduction occurs at 600 MPa (at 22°C) (Cheftel, 1992). During the pressure cycle (compression, holding time and decompression), compression heat causes temperature to rises in the pressure transmitting fluid and in the product. Adiabatic heating occurs during compression, increasing water temperatures 2 to 3°C per 100 MPa. The temperature then decreases by the same amount during pressure release (Cheftel, 1992). The temperature increase is dependent on the combination of pressure and temperature and also on the foods thermodynamic properties such as heat capacity (C_p), thermal expansion coefficient (α) and specific volume (v).

Figure 2.14 shows how tomato paste and water specific volumes' decrease when pressure increases at various temperatures. Measurements of specific volumes were taken every 50 MPa steps during the high pressure cycle. As the trends show in Figure 2.14, volume reductions of water were higher than the observed in tomato paste; this is because tomato paste has a lower moisture content (71%) is therefore less and compressible than water.

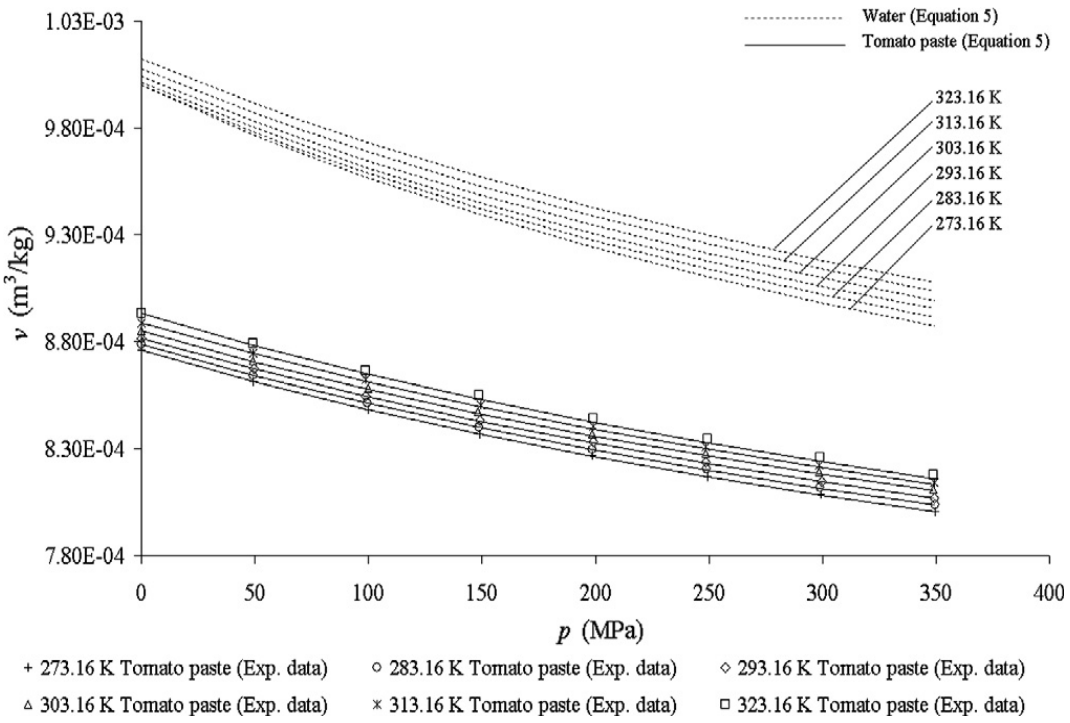

Figure 2.14: Specific volume of tomato paste and water as a function of pressure and temperature (from: Aparicio et al., 2011).

Following the specific volume reductions of water at various pressures and ambient temperature (293.16 K) from Figure 2.14, a specific volume of 9.8E-04 is observed at atmospheric pressure. Based on the theory that a 15% reduction can be achieved at pressures of 600 MPa (Cheftel, 1992), it would be expected to find a specific volume of around 8.33E-04 (theoretical value at this pressure). By extrapolating the compressibility data in Figure 2.14 for 600 MPa using a simple polynomial curve, a specific volume of 8.36E-04 (fitted value R^2 of 0.99) at 600 MPa was achieved.

This agrees well with the theoretical of 8.33E-04 given above. Based on the fact that specific volume reductions is found as pressure increase, it would be expected that cells would also be exposed to such as compression forces, resulting in changes in conformation or damage. Even though volume reductions of food products may be less than 15% as found in water, it may still be considerable to promote structure changes and consequently quality changes. It would therefore be interesting to investigate how whole vegetable tissue would sustain its volume after the application of high pressures and correlate those cellular volume changes to quality parameters such as texture or juiciness.

2.5.2 High pressure effects on biological structures

The physicochemical changes in biological entities submitted to pressure vary according to their structure. Intercellular air spaces can increase damage associated with the application of pressure, as compression results in cell breakage and disruption, loss of compartmentalization and liberation of cellular compounds. This is probably due to the simple hydrostatic pressure force exerted on the cells. Therefore, it seems sensible to consider filling those air spaces with water to prevent damage (Ludikhuyze and Hendrickx, 2001); except that gas spaces are important for gas diffusion and gas exchange. Experiments done on gas filled intercellular spaces in cucumber have shown that the spaces are not completely interconnected and in some parts are disrupted by HPP (Kuroki et al., 2004). The findings suggested that some air spaces got filled with water during storage, subsequently affecting gas diffusion (Kuroki et al., 2004). In the case of application of HPP to whole pieces of vegetables, it is expected that some of these air spaces will be filled with water either from the pressure medium or water within the tissue that is mobilized during compression and decompression, and may affect the gas diffusivity of the produce. There is little information on the effects of HPP on physiological responses such as respiration rates of whole pieces of vegetables, so it would be interesting to investigate to what extent air spaces can become filled with water during the pressure cycle and how respiration rates are affected as an indicator of cell membrane damage.

Literature also indicates that the application of high pressure (>100 MPa) to biological membranes could promote membrane disruption affecting the ATPase activity (Kato et al., 1999b; Kato et al., 2002). However the mechanisms remain poorly understood at the fundamental level of shape and cell interactions (Smith et al., 2000; Norton and Sun, 2008). It is possible to apply 100 MPa to kill microorganism by structural damage due to physiological imbalance, limiting their growth (Shimada et al., 1993). It is possible therefore to find similar mechanisms in plant cells after high pressure applications, especially regarding on cell wall modifications as cell walls are the major components in plant tissue structures. Dorenburg and Knorr, (1993) found that most plant cells can sustain pressures up to 100 MPa without damage. Pressure levels between 100 – 200 MPa can induce the formation of a gel phase in cell membranes from crystalline liquid

while, over 220 MPa, membrane destruction due to protein unfolding and membrane separation can occur in animal cells (Table 2.6) (Kato et al., 2002).

Table 2.6: Changes in membranes due to high pressure in animal cells (Kato et al., 2002).

Pressure Level (MPa)	Effect
≤100 MPa	Decrease in fluidity of lipid bilayer and reversible conformational changes of transmembrane proteins leading to membrane disorder.
100-200	Reversible phase transition of lipid bilayer, protein subunit dissociation, formation of transmembrane tunnels.
≥200	Irreversible protein unfolding, fragmentation of lipid layer, destruction of membrane structure.

Volume change during high pressure processing will also affect biological structures. According to Cheftel (1995) various morphological changes like gas vacuole compression, separation of cell membranes from cell walls, cell lengthening, and modification of the cytoskeleton and intracellular organelles can promote microbiological inactivation. Studies on the application of HPP to yeast cells have shown changes in volume. These volume changes are due to cell compression promoting mass transfer across membranes and physicochemical balance (Perrier-Cornet et al., 1995).

Mechanisms of bacteria inactivation have shown that HPP can interrupt cell reproduction and functions by membrane damage and cellular effects on the fluid transport of nutrients (intake and waste). Cell fluid leakage during held pressure was evident after membrane damage (Torres and Velazquez, 2005). Furthermore, microbial enzymes can be denatured due to structural damage or activated given the appropriate conditions (Figure 2.15). This will depend on the microbe type, morphology and resistance to pressure.

Enzyme kinetics can also be affected during pressurization where the binding of enzyme-substrate is changed due to deformation of active sites.

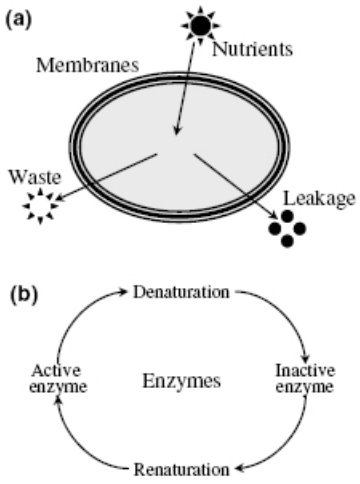

Figure 2.15: Hydrostatic pressure effects on selected microbial functions (a) cellular membranes; (b) microbial enzymes (Torres and Velazquez, 2005).

In addition high pressures can affect the chemistry of molecules by increasing ionization when water and acid molecules are pressurized causing changes in the biochemistry of living cells and consequently death of microorganisms (Earnshaw, 1996). Studies done on yeast cell mass transfer related to HPP have shown that there is an irreversible mass transfer across membranes at conditions of 250 MPa/15 min, due to water loss (Perrier-Cornet et al., 1995). In the same study the authors identified three phases during a HPP cycle. In the first phase there was a volume decrease as pressure increased compared to a bulk cell at ambient pressure. The volume decrease was equivalent to 12%. The second phase happened during holding time where the cell volume still decreased even though pressure was maintain constant, causing water and internal solutes to exit the cell. Finally the third phase showed membrane decompressions during pressure unload, with no total cell volume recovery (Perrier-Cornet et al., 1995).

As mentioned before, changes in cell permeability have been observed in yeast cells, and this was also observed in both spinach and cauliflower cells when processed at 400 MPa for 30 minutes at 5°C (Préstamo and Arroyo, 1998). Water under pressure could also promote ionic dissociation reducing pH and promoting protein denaturation and micro-organism inactivation (Cheftel, 1992). Cell morphology changes are reversible when low pressures are applied but irreversible (showing completely deformed cytoplasmic organelles) at 400 MPa (Alemán et al., 1994). Structural damage can be

demonstrated by leakage of the internal substances as observed in yeast cells (Shimada et al., 1993). This leakage increased as pressure increased especially above 200 MPa.

A model was recently developed to describe the effect of hydraulic pressure on yeast cells, simulating cell damage and disruption at pressures of 415 to 460 MPa; the damage was thought to be due to inactivation mechanisms such as the release of membrane proteins and cell wall disruptions (Hartmann et al., 2006). Based on the previous findings, it is not yet clear if similar conditions would have an effect on plant tissues considering their distinctive structure of rigid cell walls and high turgor. Earnshaw (1996) explains that vegetable and fruits vary in response to the application of HPP, depending on their structural resistance. It is expected that plant structures containing air vacuoles will be more affected in their volume during compressions. If the product is in direct contact with the fluid of the unit, then these vacuoles may be filled with liquid, changing their structural integrity and density (Earnshaw, 1996).

Consequently high pressures could modify the tissue structure promoting release of intracellular contents and therefore further reactions will occur by the mixing of substrates and enzymes. However, under what conditions this may happen and how tissue would respond to a whole range of pressure and time combinations needs to be investigated.

There is not much literature available on high pressure processing affecting flavours of juice and paste products. But a separate question can be proposed as to how HPP affects flavours of whole commodity pieces where compartments may or may not be destroyed or disrupted releasing flavour components from the cells? Furthermore, if texture is one of the most important quality attributes in whole vegetable pieces, the question is, if the tissue is damaged by pressure, how is texture affected and how does it compare with other treatments? What are the mechanisms involved in the process of textural change? Is there a role for enzymes such as PME when applying high pressures at ambient temperatures? Do cell walls and membranes appear to be affected at similar pressure to those reported for yeast cells? Does leakage affect the final texture? Can cell leakage be controlled? Finally, how do panellists perceive the resulting sensory changes? All these questions based on previous findings need to be addressed for future applications of high pressures in foods.

2.5.3 High pressure effects on proteins

A protein structure can be defined at four levels: the primary structure is characterised by the amino acid sequence joined by covalent peptide bonds, which determines the specific function of the protein in the metabolism (Fennema, 1996). The secondary structure is defined by the alpha helices, beta sheets and random coils formed by polypeptide chains from intra- or intermolecular hydrogen bonds (Fennema, 1996). The tertiary structure is the three dimensional configuration of the secondary structures generally formed with non-covalent bonds between amino acid side chains. Finally, the quaternary structure is the spatial arrangement of the tertiary subunits, also held together by non-covalent bonds (Hendrickx et al., 1998). Protein functionality can be modified by the surrounding environment and external factors.

At low pressures, covalent bonds are not affected and therefore nor is the primary structure of the protein. At high pressures, hydrogen bonds can be broken and therefore secondary structures can be affected (Cheftel, 1995). Pressure levels of 200 MPa or higher caused significant changes to tertiary structures, while quaternary structures are affected by pressures of 150 MPa or higher (Hendrickx et al., 1998). Similarly Cheftel (1995) after applying pressures between 100 and 200 MPa concluded that in this region there could be partial unfolding or protein denaturation, aggregation or gelation, depending on the protein concentration.

2.5.4 High pressure effects on enzymes

Enzymes are proteins with three-dimensional structures and active sites. Small changes in the active site can cause loss of their activity or functionality (Fennema, 1996). It has been found that high pressures can either activate or inactivate enzymes (Hendrickx et al., 1998). A pressure activation effect can be described by decompartmentalization of intact tissues, resulting in the leakage of enzyme and substrate and increasing their contact (Hendrickx et al., 1998). The result of this contact can induce catalysis or reaction, depending on the pressure levels, types of enzymes, pH, medium composition and temperatures (Hendrickx et al., 1998). For some enzymes a maximum pressure level at which they became inactive has been observed (Hendrickx et al., 1998). To achieve greater enzyme inactivation, pressure should be applied in numerous cycles (if equipment is available) (Hendrickx et al., 1998). An example of a HPP cycling process

is given in Figure 2.16, where the time and temperature were experimentally collected from the centre, between centre and border of the high pressure unit, and predicted using a numerical model for heat transfer in an enzymatic model system (Denys et al., 2000).

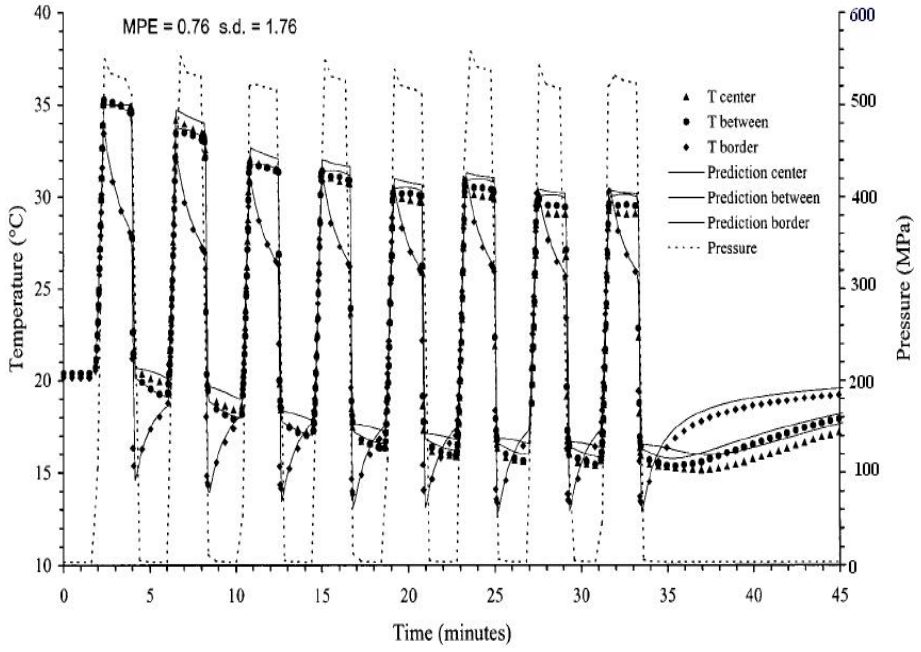

Figure 2.16: Experimental and simulated time-temperature profile for HPP cycling process of an enzymatic model system (Denys et al., 2000).

In plant cell systems, enzymes such as pectin methylesterase, polygalacturonase, polyphenoloxidase, lipoxygenase and peroxidase can play an important role in quality, affecting texture, colour, flavour and odours, respectively. The following section will discuss the effects of high pressure on these enzymes for various applications.

2.5.4.1 High pressure effects on pectin methylesterase and polygalacturonase

Pectin methyl esterase (PME) and polygalacturonase (PG) are enzymes that modify pectin chains thereby affecting the product texture, as described in section 2.1.6.3. Examples of the impact of these enzymes in food systems include tomato paste consistency, cloud destabilization in juices and gelation of juice concentrates (Hendrickx et al., 1998).

Studies done on high pressure inactivation of PME in apple juice showed substantial inactivation depending on the pressure level and holding time used. PME activity was

reduced almost ten-fold in apple juice after treatments at 400 MPa for 25 minutes (Rihai and Ramaswamy, 2003). Ogawa et al., (1990) achieved pectinesterase inactivation at very high pressures (1000 MPa) at 20°C or at lower pressures combined with higher temperatures (600 MPa for 10 minutes at 57°C) in mandarin juice; while up to 90% irreversible inactivation of PME in orange juice was obtained at 600 MPa for 10 minutes (Irwe and Olsson, 1994). PME activation was also observed in freshly squeezed orange juice after treatment at 250-400 MPa for 15 minutes at room temperature (Cano et al., 1997).

Studies done on the effect of using HPP on PG in tomato purée showed an increase in purée viscosity as the pressure level increased in comparison with non-HPP treated samples. The viscosity increased two to three-fold with pressure increase from 700 to 900 MPa, showing PG inactivation as pressure increased (Krebbers et al., 2003). Tomato PG was shown to be pressure sensitive following first order inactivation kinetics between 300-600 MPa at 50 °C (Fachin et al., 2002). Studies on purified tomato PG also showed a decrease in enzyme activity with an increase in pressure at a constant temperature (0.1-500 MPa) (Verlent et al., 2004). From these results, it seems possible to achieve a product with the desired viscosity combining PME and PG activities at given temperature/pressure conditions, according to their level of sensitivity.

2.5.4.2 High pressure effects on polyphenoloxidase

Polyphenoloxidase (PPO) is the enzyme responsible for enzymatic browning (Fennema, 1996). It is found in the cytosol of plant cells and when phenolic compounds are released from the cells, they can be exposed to the enzyme resulting in oxidation and brown complex formation (Michel and Autio, 2001). Different commodities have different PPO pressure sensitivity. For example apricot requires 100 MPa to achieve inactivation; strawberry 400 MPa; grapes 600 MPa; avocados 800-900 MPa and apples 100 to 700 MPa depending on the pH (Hendrickx et al., 1998). At low pressures (100 MPa), products like apple, onion, pear and strawberry showed an increase in PPO activity (Hendrickx et al., 1998). Pressures of 100 MPa induced browning in onions due to PPO activity, and at higher pressure (700 MPa) the effect was even stronger (Butz et al., 1994). This can be explained by the effects of decompartmentation of PPO in the cell. As HPP is applied to a tissue, there will be an increase in membrane

permeability and consequently browning by exposure of enzymes to substrates unless HPP inactivates PPO, which may require very high pressures. Browning reactions are also frequently found in potatoes and mushrooms, where PPO is usually inactivated by the use of heat. However high pressures (800-900 MPa) have been shown to be capable of reducing the PPO activity (Hendrickx et al., 1998). Very high pressures (900 MPa) were required to produce a 1 log reduction of PPO activity in avocado (Weemaes et al., 1998). High pressures (600 MPa, 25°C, 15 minutes) did not inactivate PPO in guava purée, resulting in colour changes during storage (Yen and Lin, 1996). Limited (29%) PPO inactivation was found after treatment at 800 MPa for 15 minutes (18-20°C) in red raspberry and strawberries; (Garcia-Palazon et al., 2004). Studies on isolated PPO under pressure have indicated that pressures up to 500 MPa can increase the maximum activity by 42%, whilst above this pressure lower activity was observed (Butz et al., 1994). Overall, this suggests that the application of HPP on PPO cannot be predicted and must be determined for each product under study.

2.5.4.3 High pressure effects on lipoxygenase and peroxidase

Lipoxygenase (LOX) is the enzyme often responsible for the development of off-flavours and colour degradation in vegetables. Currently, blanching is an effective pre-treatment often employed to inactivate this enzyme. Studies of the pressure and temperature combinations required to achieve LOX inactivation have been performed on green bean extract, indicating that inactivation occurs by heating from 55 – 70°C in combination with 200-500 MPa (Indrawati et al., 1999a). First order inactivation kinetics were observed in LOX from green bean juice at low and high temperature-pressure combinations (50-650 MPa / -10 – 55°C) (Indrawati et al., 1999b), opening an opportunity to improve quality retention using a single step (high pressure-freezing) instead of blanching then freezing (Indrawati et al., 2000).

Peroxidase (POD) is another enzyme related to colour and off-flavours in vegetables; pressures below 396 MPa increased peroxidise stability, however at 600 MPa and 45°C, 91% inactivation was observed in carrots (Soysal et al., 2004). Later studies observed that lower pressures and temperatures (350 MPa at 20°C) decreased to 16% the residual activity of POD in carrots within the first 30 minutes, and further down to 9% after 60 minutes (Çağdaş et al., 2006). These findings confirm a time dependency on enzyme inactivation as mentioned in section 2.5.2 where during holding time, a volume decrease

may still occur allowing the release of cell contents from compartments. In the case of enzymes, it can also be possible to find active sites being damaged due to pressure with consequent changes in enzyme activity.

2.5.5 High pressure effects on nutrients and pigments

Most fruits and vegetables have been shown to retain quality attributes such as colour as well after pressure treatments as observed after blanching. This is because most covalent bonds are not affected by pressure, so pigments are preserved (Basak and Ramaswamy, 1998). Modelling of water soluble vitamins showed high retention (100 % and 102 %) of vitamin B1 and B6, respectively when treated at 600 MPa for 30 minutes at 20°C (Sancho et al., 1999). Overall, vitamin C levels in a multivitamin model appears to be more susceptible to degradation during high pressure processing, with 87.83 % retention at 200 MPa and 88.58 % at 600 MPa (Sancho et al., 1999). Levels of vitamins were claimed to be fully maintained in stabilized orange juice after high pressure treatments during 2 months of storage at 3°C (Donsi et al., 1996). Furthermore, vitamin C and provitamin A were highly preserved in orange juice processed at 350 MPa for 2.5 minutes (30°C) (Sánchez-Moreno et al., 2003). Similar findings were observed with respect to the antioxidative capacity, vitamin C, sugar and carotene content during high pressure treatments (500-800 MPa, 5 minutes) in orange, orange-lemon and carrot juice (Fernández García et al., 2001). Similarly, no changes in the total concentration of lycopene or β-carotene in tomatoes were observed after 60 minutes of high pressure treatment at 600 MPa (Butz et al., 2002).

Changes in the functional properties of broccoli showed that chlorophyll a and b hardly changed after extremely long treatments at 600 MPa at 75°C (Butz et al., 2002). Consequently, no changes in broccoli colour were observed after processing at 800 MPa for 8 hours at 40°C, which was in contrast to the combination of heat (>50°C) and pressure, where pigmentation was significantly reduced (Van Loey et al., 1998).

High pressures (500 MPa for 10 minutes) were able to produce a microbiologically safe apple-broccoli juice (chilled 5°C for 30 days) and the juice also retained more sulforaphane (an anti-cancer substance) in comparison with frozen pre-treatments (Houška et al., 2006). Studies of anthocyanins in raspberries indicated good stability when processing at 200 and 800 MPa at 20°C and with storage at 4°C for up to 9 days

(Suthanthangjai et al., 2005). In general, increasing pressure between 0.1 and 500 MPa increases the rate of pigment degradation, but not as much as the increase associated with increasing temperature by 10°C when over 70°C (Van Loey et al., 1998). However, the stability of these compounds throughout storage would also need to be studied.

2.6 High Pressure effects during storage

Quality attributes such as colour, flavour and texture can be lost or retained after applying high pressures. Storage experiments done on HPP products have indicated that concentrations of antioxidants decreased, probably because pressure does not completely inactivate enzymes or otherwise inhibit the pathways of deterioration (Butz et al., 2003). A clear example is given by most green vegetables, which displayed browning during storage as a result of polyphenoloxidase activity (Michel and Autio, 2001). However, pressure treated green beans presented a uniform color change in comparison to raw beans when stored for one month at 6°C (Krebbers et al., 2002a).

2.7 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. Studies done by Arroyo *et al*., (1997) applying HPP to vegetables products showed undesirable organoleptic changes at 300 MPa. Meanwhile, others reported that high pressures (up to 350 MPa) can be applied to plant systems without major effects on texture and structure (Knorr, 1995).

Experiments done on lettuces treated at 300 to 400 MPa at 20°C displayed increased browning, probably as a result of PPO activation or exposure to substrates; while tomatoes lost their skin but maintained a firm flesh (Arroyo et al., 1997). Bacterial count reduction was achieved in onions following treatment with 300 MPa for 140 minutes at 40°C, but browning was seen at 100 MPa (Butz et al., 1994). Some texture recovery was achieved in carrots after treatment at low pressures (100 MPa) (Basak and Ramaswamy, 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 (Ludikhuyze 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 Ramaswamy, 1998). This gradual change has been described as firmness recovery, which in one study on carrots reached 100% at low pressures for long processing times (100 MPa for 30 minutes) (Basak and Ramaswamy, 1998).

Michel and Autio (2001) discuss the loss of firmness in various fruits and vegetables, concluding that many samples could regain their firmness after the first 100 MPa, while others did not regain their firmness. In the case of pears, pineapples and oranges, it was shown that their first firmness loss at 100 MPa 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, and leakage or loss of turgidity in conjunction with enzyme analysis such as pectinmethylesterase. 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.

2.7.1 High pressure effects on carrots

Carrots are vegetables that have high turgidity and carotenoids responsible for their orange colour. The application of HPP as a pre-treatment for diced carrots resulted in colour and texture retention before drying and freezing in contrast to heat treatments which showed affected colour (pigment degradation) and texture (Eshtiaghi et al., 1994). Most vitamins and antioxidants were preserved after processing carrots with 600 MPa for 10-40 minutes at 25°C (Butz et al., 2002).

Texture in carrots can be related to PME activity. Studies done on HPP and heat in carrots have shown a decrease in PME activity as temperature increased. Carrot pieces treated at 100 MPa at 20°C displayed a recovery of texture after sustained pressure for 60 minutes, while at above 200 MPa more than 50% of the carrot firmness had been lost within the first 10 minutes and no further changes were observed with longer durations (Basak and Ramaswamy, 1998). Pressurization did not cause significant firmness loss in

carrots; however an increase in rupture strain was found to be 20% for raw and 100 MPa, while 30% of rupture strain was observed at 200 MPa (Kato et al., 1997).

Studies of the effects of low pressure and moderate heat on carrots indicated that samples subjected to 100 MPa before heating at 75-85°C for 3 to 4 hours were firmer than samples without prior pressure treatments (Islam et al., 2003). At the above conditions, there was no evidence of an effect on colour change, as the main cause of carotene degradation is oxidation stimulated by oxygen and light and not by HPP (Islam et al., 2003). It seems that the application of low pressures in combination with ambient temperatures (18°C-20°C) would allow the carrot tissue to recover after a certain time. In addition pressures caused insignificant firmness losses below 100 MPa. But what is happening to the tissue during these mild pressure treatments needs to be further investigated. Are cell membranes still intact and therefore less turgor losses are found? What is happening to the cell walls at high pressures and when does PME play an important role in the textural changes? It would be interesting to be able to correlate those changes happening to the cells in terms of actual texture measurements such as hardness to widen our understanding of pressure effects on whole pieces of tissue.

2.8 Applications of pressure without external heat

The use of high pressure at ambient temperatures can be considered a cold pasteurization process. During compression, adiabatic heating occurs up to the point the maximum pressure is reached followed by cooling to the medium temperature. Figure 2.17 shows there was an increase in temperature of 20 °C (15 to 35°C) during compression to 600 MPa, followed by a reduction in temperature to 15°C after 8 minutes at 600 MPa.

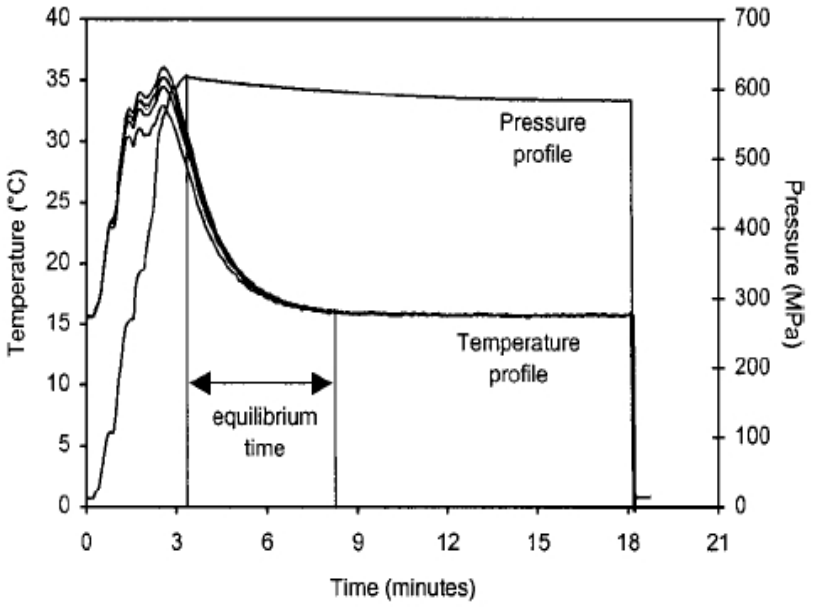

Figure 2.17: Pressure and temperature profiles of the pressure medium in a multi vessel high-pressure machine at 600 MPa and 15°C (Indrawati et al., 2000).

There have been many examples of product development without heating using HPP. Guava purée HPP treated without external heat (600 MPa at 25°C) for 15 minutes retained similar quality attributes to the fresh purée and maintained acceptability during 40 days of storage at 4°C (Yen and Lin, 1996). Shelf life extension was achieved in pressure treated orange juice (350 MPa for 1 minute, without external heat). The product could be stored at 8°C for at least two months without changes in microbiological counts or chemical components such as vitamins, sugars and organic acids (Donsí et al., 1996). Up to 12 weeks storage at 4°C was also achieved in Navel orange juice processed with high pressure (600 MPa for 60 seconds at 20°C) without changes in ascorbic acid or β-carotene concentrations (Bull et al., 2004).

In the case of whole vegetables, low pressures (100 MPa) at ambient temperatures resulted in gradual texture recovery for pears, orange, apple and pineapple during treatment (Basak and Ramaswamy, 1998). The texture of carrots and green peppers deteriorated with treatments above 200 MPa (Basak and Ramaswamy, 1998). But there is little information explaining those textural changes per se and the effects of enzymes in those changes at lower pressure conditions.

Microbial responses, such as spore inactivation using high hydrostatic pressure, are dependent on the pressure and time combination. Research done on *Bacillus* and *Clostridium* showed a requirement of approximately 810 MP to achieve inactivation at 20°C, with inactivation increasing as a function of the process holding time (Sale et al., 1970). Experiments conducted with vegetables showed that at 100 and 200 MPa (for 10 minutes at 20°C) the microbial population was not reduced significantly, whilst at 300 MPa considerable decreases were observed. Population reductions of one log cycle were obtained in both tomatoes and lettuce when treated at 300 MPa at 20°C (Arroyo et al., 1997). Every product may show different results, because microbial inactivation depends on the conditions applied as well as the product's composition, pH and water activity. As a consequence, each product will require a particular set of conditions to achieve a desired degree of inactivation. It would be possible to speculate that tissue structure and cell components may play an important role on reaction responses to high pressures. Therefore it would be possible to group products into composition categories to be able to better predict their responses to pressures.

2.9 Applications of high pressure combined with heat

Sterilization can be achieved when heat is combined with pressure (and the associated adiabatic heating) over a short time (Master et al., 2004). Figure 2.18 shows an example of the temperature/time profile during a combined heat and pressure sterilization process for spinach. The figure shows the differences in temperature intensity between retorting and HP-sterilization. Differences are also expected in the final product quality, especially in compounds sensitive to heat. Another application of this combined treatment was explored by Krebbers et al., (2003), who used 700 MPa for 30 seconds at 90°C on meatballs in tomato purée inoculated with 4.5 log units of *B. stearothermophilus* spores, achieving a temperature of 121°C for 30 seconds. The consistency of the product appeared to depend directly on the level of pressure applied through the effect of pressure on the enzymic reactions, specifically inactivation of pectinmethylesterase and polygalacturonase. Krebbers et al., (2003) subjected tomato purée to a traditional heat process as well as a combined heat and pressure process. The traditional process resulted in a product with greater syneresis (water release), which continued to increase through 2 months of storage in comparison with heat-HP processed products.

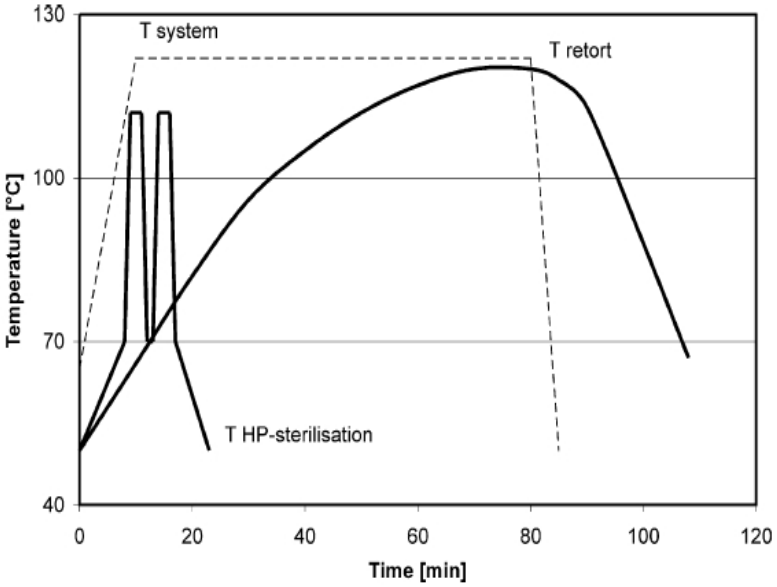

Figure 2.18: Temperature in the centre of a 500 mL can of spinach during conventional heat sterilisation (T retort), compared to the temperature of the retort unit (T system) and temperature during high pressure processing sterilization (T HP-sterilization) (Matser et al., 2004).

Matser et al., (2004) found that sterilization of a fresh basil product could be achieved by combining heat and pressure resulting in retention of more essential oils than conventional sterilization processes. Figure 2.19 presents another example of the benefits of sterilization using a combination of heat and pressure in comparison with other traditional treatments, in this case on the firmness of green beans where firmness could be maintained at almost 75% of firmness of the raw samples by PHPP while frozen and retorted samples lost almost all their firmness.

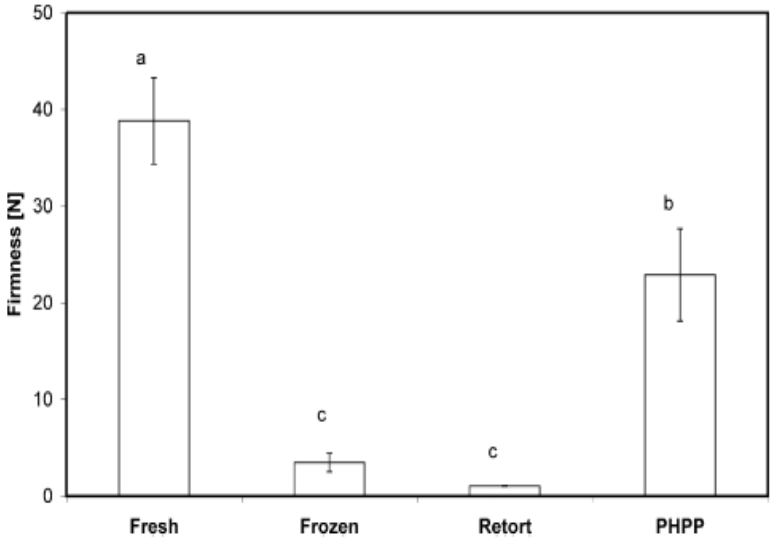

Figure 2.19: The effects of pulse high-pressure sterilization (PHPP) (two pulses, 75°C, 1000 MPa) and conventional treatments on the firmness of green beans (Krebbers et al., 2002b) (Different letters are equivalent to significant differences ($P< 0.05$).

Overall, the application of pressure in combination with heat appears to have significant potential for quality improvement in comparison to traditional processes; however, further studies of the sensory properties, shelf life and stability of the products are required before commercialisation can occur (Guerrero-Beltrán et al., 2005).

2.10 Conclusions and research opportunities

The scientific literature highlights the relevance of microstructure with respect to quality attributes especially texture and associated changes occurring during any type of processing. In the case of whole vegetable pieces, cellular and tissue changes including by those caused by enzyme reactions will directly have an impact on the products' texture and acceptability. There is already a robust amount of scientific information on the effects of high pressure on fruit and vegetable products (such as pastes and juices), in the area of enzyme activation and inactivation and quality parameter changes as a result and safety. However, there is less information on explaining why those changes may happen and the importance of tissue integrity and physical structure in whole vegetable pieces. Information about the structure will provide the basis for protection against further biochemical and quality changes that may occur during processing. Research done on physiological responses such as respiration rates after high pressure treatment has not yet been reported. This unexplored area is essential to understand tissue damage and the optimizing of processing, packaging and storage shelf life of

products. On the basis that different tissue structures may respond differently to a process due to cell composition and the proportion of air spaces inside the cells, there is also a need to research the microstructural changes and mechanisms to provide understanding of macro quality changes such as textural changes (i.e. hardness or stiffness losses) before and after the application of high pressures.

Research on carrot texture and the application of high pressures have indicated significant firmness losses above 200 MPa, while texture recovery has been obtained at 100 MPa after 60 minutes. However, there is less information on the effects of HPP on different tissues based on their cellular composition, and answers to why firmness may be lost at some specific pressure and not another. Key biochemical effects, such as PME activity, need also to be evaluated using pressure - time combinations at ambient temperatures to confirm if the enzyme's activity has been modified by high pressures. If pressure is applied without the use of external heat, then would it degrade pectins (as found in thermal processes)? Questions can also be asked about the effects of pressure on the cellular structure and whether or not anything can be done to ameliorate those effects on the tissue. Can consumers perceive changes in those quality attributes after HPP? Can high pressures modify the volatile composition of whole vegetable pieces? and how do they compare to other processing treatments throughout product shelf life?

This work aims to study mechanisms that may be involved in the use of high pressures on carrot tissue, using a microstructural approach. Starting from the basis that changes do occur at a micro level, more clear insights into what the process effects are will be found. Finally, this approach would better identify optimum conditions for processing and future commercial opportunities for HPP vegetable products.

3 Carrot tissue damage on processing: an introductory study (*)

3.1 Introduction

As discussed in the literature review, high hydrostatic pressure is a non-thermal technology that may offer an opportunity for the food industry to satisfy consumers' demands for 'fresher' fruits and vegetables, enabling effective preservation in comparison to traditional processes. Physical properties of fruits and vegetables relate directly to cell morphology and turgor pressure as well as to the amount of intercellular fluid or air between cells (Pitt, 1982). Carrots are formed by a core central area; cambium and the cortex area formed by phloem and parenchyma tissue. The core consists of central pith surrounded by xylem, which is composed of vascular tissue with many lignified cells with thicker cell walls and is responsible for the transport of water along the roots (Jewell, 1979). Cambium cells separate the xylem and phloem cells. The cortex is the region outside the cambium, comprising the phloem and parenchyma tissue; parenchyma cells are round in shape with thin walls. These cells are found in most edible plants and are involved in synthesis and storage of food materials (Jewell, 1979). Based on these differences in cell composition, it is expected that different preservation techniques such as heating, freezing and the applications of high pressures will affect carrot cells differently.

Considering cell modification, Earnshaw (1996) explains that fruits and vegetables will be affected by high pressures if the product is in direct contact with the liquid medium during processing, as the air gaps could be filled with that liquid helping to maintain structural integrity. In the case of carrots, literature indicates that the cells are highly turgid with a compact cell structure and small air spaces between cells (Kerdpiboon et al., 2007). Many studies have been done on the application of high pressure as a pre-thermal treatment of carrots, showing better preservation than pure heating by reducing cell separation. This is attributed to pectins being stabilized and the enhancement of pectin methylesterase activity to crosslink with calcium ions at pressures above 200 MPa, 60°C for 15 minutes (Sila et al., 2007).

(*) Material from this chapter is included in the paper: Trejo Araya X. I, Smale N.J., Stewart, C., Mawson, A. J., and Tanner, D.J. (2005). Effects of High Pressure Processing on Carrot Tissue. Acta Horticulturae, (687): 379-381.

It is expected that the use of high pressures would cause cell deformation, elongation or even breakage depending on the level applied. Based on the composition, it is also possible to cause larger conformational changes within the parenchyma than in lignified cells. To confirm morphological changes, the objectives of this part of the study were to observe tissue structural changes using light microscopy across both core and cortex tissues. Observations of the effects of high pressure applications were made using a range of pressures (100, 200, 300, 400, 500 and 600 MPa) and holding time durations of 1, 2, 5 and 10 minutes at 600 MPa. Changes were compared to raw, steam blanched (2 minutes) and frozen (-18°C, 24 h) carrot tissues. Quantification of tissue damage was evaluated using dye techniques and relative electrolyte leakage. The results of this investigation provide an initial understanding of the tissue responses and structural changes in comparison to other treatments.

3.2 Materials and methods

3.2.1 Plant material

Fresh pre-packed carrots (*Caucus carota* L., var. 'Stephano') were purchased from the Sydney local market and stored at 1°C. The following day, carrots were washed; end parts were removed leaving the middle to be cut transversely into 1 cm high slices using a razor blade. Samples were then vacuum packed into Cryovac® FS 7100 series film bags and processed at various pressures (100, 200, 300, 400, 500 and 600 MPa) for 2 minutes and 600 MPa for various durations (1, 2, 5 and 10 minutes). The rest of the samples were left unprocessed or were steam blanched (100 °C, 2 minutes) or frozen using a blast freezing at -18°C for 24 h for comparison.

High pressure processing was performed at ambient temperatures (18-20°C), using a 2L high pressure vessel (Flow International Corporation, USA), with a compression time of less than 10 seconds and a decompression time of 5 seconds.

3.2.2 Microscopy

For microscopy purposes, processed samples were finely cut into triangular pieces to be able to identify differences between tissues such as core and cortex (Figure 3.1). Samples were then fixed in 10% w/v neutral buffered formalin for 72 hours at room temperature, embedded in paraffin, sectioned at 5 μm thickness and stained with Safranine O (lignified cell walls, cuticle and nuclei), followed by staining with Fast

Green FCF (cellulosic cell wall, plastids) (Xia and Steeves, 1999). For tissue damage observations, carrots were processed and then cut into disks (1 cm height, 1.25-1.5 cm radius), and stained with Trypan blue, which is a solution that can be taken up only by non-viable cells (damaged cells), as previously used by Noor Azian et al., (2004) and Gómez Galindo et al., (2004b), respectively. Stained samples were immediately rinsed with distilled water to remove the excess dye and left to dry before observation. Stained disks provided a visual indication of how the tissue responded to different treatments.

An overall quantification of area and perimeter of non-viable cells stained by Trypan blue was obtained by using the program Image J. This was done to be able to compare numerically differences between tissue damage before and after different treatments. Image J is a free public domain Java programme that can analyse image formats by calculating areas of similar pixels statistically; it can be easily download from any internet source (http://rsbweb.nih.gov/ij/) (National Institutes of Health, Bethesda, MD) (Rasband 1997 – 2008). Images were transformed into a binary format before quantification. The number of pixels fulfilling the specified threshold criteria was converted into mm².

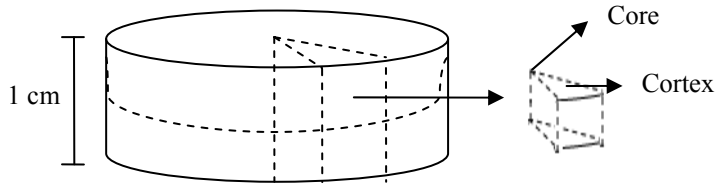

Figure 3.1: Carrot slice sectioned into triangular samples including the core and cortex tissue for microscopy preparation.

3.2.3 Relative electrolyte leakage

Processed disk samples were weighed (~ 7.5 - 9.0 g) and put into containers with distilled water (80 mL) to measure electrolyte leakage (in triplicate) after 24 h at 20°C using a conductivity meter (WP-81, TPS Australian manufacturer) with a k=1.0 sensor (20 µS/cm - 200 mS/cm), accuracy ± 5% and calibrated with a KCl standard solution of 2.76 mS/cm. Conductivity measurements were then standardized using the sample weight (considering all samples are similar surface area); a similar procedure was performed by Gómez Galindo et al., (2004a). Figure 3.2 presents a flow diagram of the methodology and treatments applied in this study.

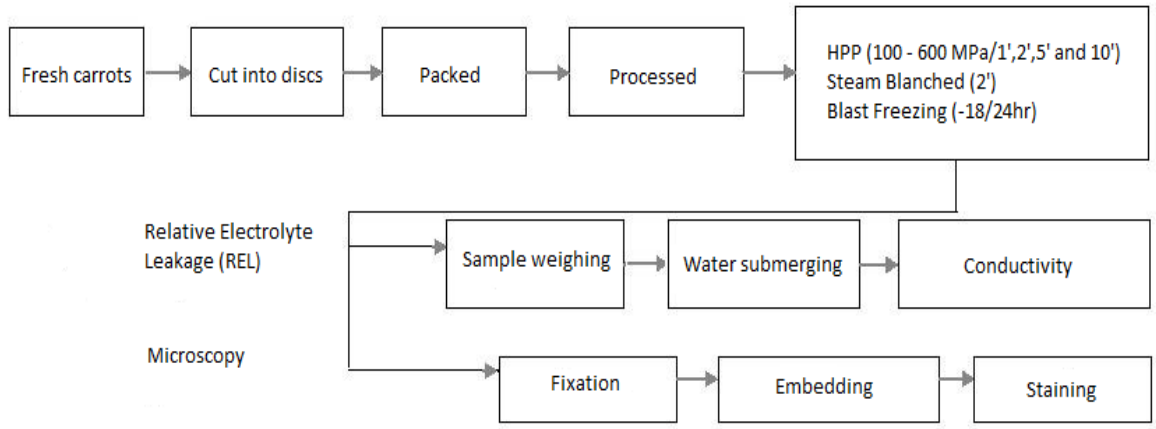

Figure 3.2: Flow diagram of methodology and treatments applied to carrot tissue.

3.2.4 Statistical analysis

Significant differences among treatments were analysed using analysis of variance (ANOVA) and Tukey's test for pair wise comparisons with a confidence interval of 95% (MINITAB version 13.31). Analyses included the interaction effects of treatments and processing duration (pressure application) for relative electrolyte leakage measurements.

3.3 Results and discussions

3.3.1 Light microscopy image analysis

Image analysis was performed using light microscopy for samples cut from the core and cortex tissue of carrot disks (Figure 3.3).

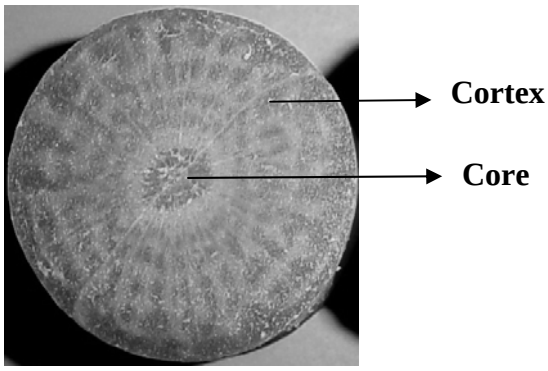

Figure 3.3: Types of tissue in raw fresh carrots analysed by light microscopy.

The core tissue is characterized by the presence of lignified cells (stained red) and larger parenchyma cells (stained blue).

Figure 3.4 shows little damage to core cells after pressure treatment at 600 MPa for 2 minutes in comparison to the raw samples. As the pressure process duration increased to 10 minutes, the cells became elongated (less round and more rectangular in shape). At 600 MPa and 10 minutes processing, cells were more damaged by pressure tending to elongate as if they were under shearing forces. Steamed samples showed some deformation and broken areas. Furthermore, frozen core tissue presented broken areas and the cells had "sharper" or less rounded shapes.

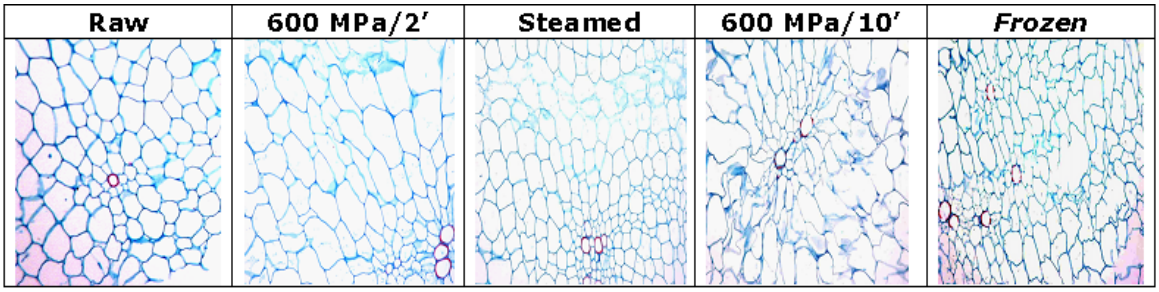

Figure 3.4: Light microscopy images for different treatments on core carrot tissue.

Images of cortex tissue indicated that after treatments at 100 MPa, cells began to lose their compacted order with an increase in intercellular space (Figure 3.5). It is important to consider in this case that the cortex tissue is mainly composed of parenchyma cells, which have less structural strength than lignified cells. An abrupt change was found between 100 and 200 MPa (where cells showed disruption) providing evidence of a "tissue damage break point" between 100 – 200 MPa. No further visual damage was observed in cells with increased pressure. However after processing for ten minutes at 600 MPa, some cell enlargement and deformation was evident. Frozen samples presented areas of broken tissue and release of cell contents. Changes in the cell wall and collapse in some areas of steam blanched carrot cortex were also observed.

Overall, frozen carrot tissue presented the most severe damage and a noticeable drip loss after thawing. This is likely due to the formation of ice crystals causing cell buckling and folding. Some cell lysis, tissue disruption and losses of the internal cell contents were observed throughout the cortex of frozen samples (Figure 3.5).

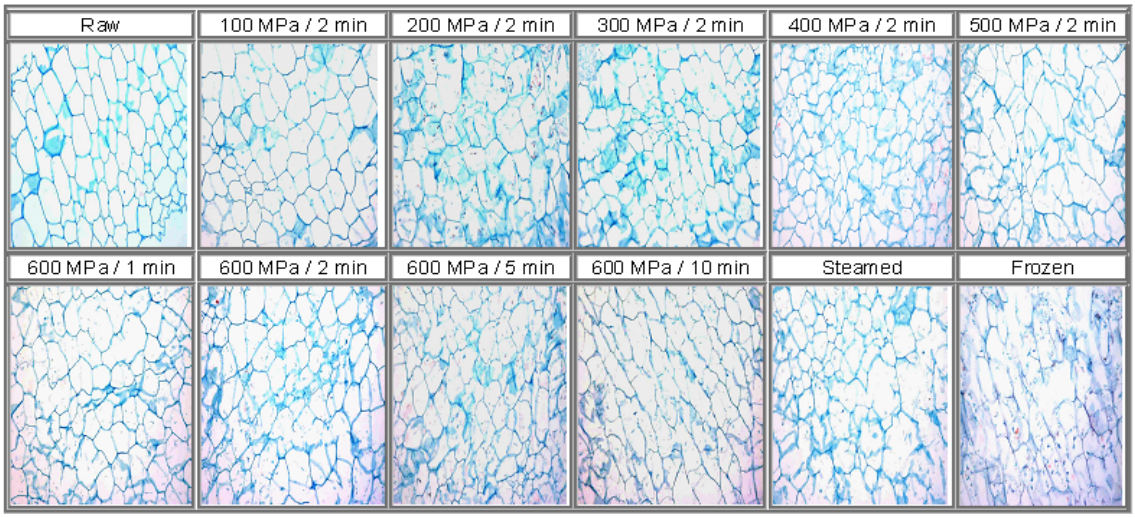

Figure 3.5: Light microscopy images for different treatments of cortex carrot tissue.

Tissue damage was also evaluated using Trypan blue staining solution, which is only taken up by non-viable (damaged) cells (Figure 3.6). An area from the core tissue was selected to evaluate the extent of tissue damage for the various treatments. The images showed a predictable damage in the raw carrot tissue due to the cutting process using a blade when preparing the samples. This inevitable damage will probably be constant in all samples due to preparation. The images indicated that damage intensity increased in the order of pressure treatment < raw < steamed < frozen treatment, respectively. Visually, raw tissue showed diffuse small areas of Trypan blue concentrations.

These areas expanded in steamed carrot tissue with dramatic large damaged areas in frozen tissue. However, high pressure processed carrots, showed reduced damaged areas.

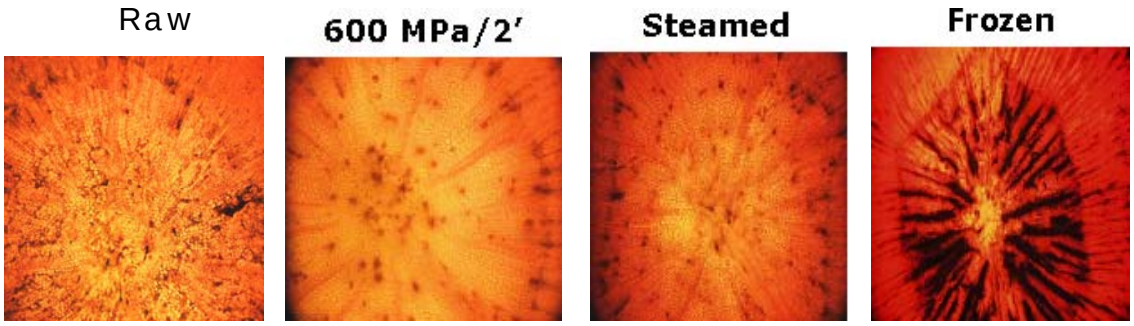

Figure 3.6: Treatment effect on carrot xylem using the dye Trypan blue as an indicator of cellular damage.

The dark sections in frozen samples followed the radial trend of cells, which are probably rows of parenchyma cells. In this experiment Trypan blue could help to identify damaged tissue for all samples. However, quantifying damage helped to differentiate the extent of damage between raw, HPP and steamed carrots (Table 3.1).

Table 3.1: Fractional area of damaged (non-viable) carrot tissue stained by using Trypan blue.

Carrot Samples	Area Fraction
Raw	31
Frozen	58.6
HPP 600 MPa	24.5
Steamed	42.4

Table 3.1 shows the different area fractions for each treatment, confirming the trend observed in Figure 3.6, where HPP carrot tissue showed less damage than raw, steamed and frozen samples. The area quantification showed that 31% of damage may be a result of sample preparation. This damage was reduced after high pressure processing to 24.5%. It seems hard to believe that HPP could present less tissue damage than raw carrots, but it could be possible that by applying high pressure (isostatic and instantaneously), cells are forced together into a new reorganized form, weakening the cell cementing material (middle lamella). If this is the case, then HPP may be enhancing cell debonding by promoting cell separation instead of breaking the cells in front of the cutting blade, reducing dye penetration by increasing gaps between cells. Alternatively, if leakage occurs, then the dye may have leaked out of the cells during rinsing because more disrupted membranes.

Cutting through cells will lead to cellular breakage. This assumption is further investigated in section 5 to confirm such a response, because if cell debonding is happening at a cellular level, then many physical qualities attributes such as texture, juiciness and the actual sensory perception of the carrot tissue may be affected.

Cellular damage was also quantified by using relative electrolyte leakage (REL). The proportion of leakage in treated samples was compared to raw samples, for all treatments. Figure 3.7 shows a “tissue break point” was found between 100 - 200 MPa, where leakage increased significantly ($P<0.05$) from 1.6 to 8.7 times respectively in comparison to the control (raw samples). There was less significant difference in REL

(8.7 – 11.1 respectively) between pressures of 200 and 600 MPa. Variation in processing time (1, 2, 5 and 10 min) showed no significant effect at 600 MPa, in contrast to structural changes observed previously. This could be because images are observed at a cellular level and disruption or increase in permeability is related to cell membranes. What could be happening is that cell membranes have reached some level of breakage or perturbation independent of the processing time applied.

Steamed samples presented similar damage (as measured by REL) to samples treated at 300 and 500 MPa. However major damage was found in frozen tissue, which had REL 14 times higher than the raw sample (Figure 3.7). This result was expected as cells were shown in previous images to be torn due to ice crystal formation.

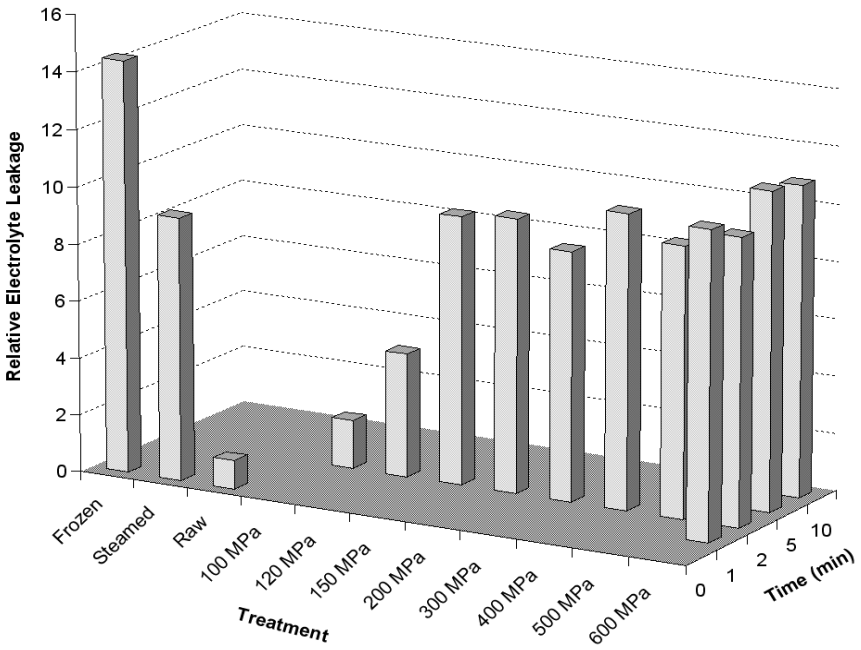

Figure 3.7: Relative electrolyte leakage (treatment/raw) in carrot tissue after different processing and time conditions (n=3).

3.4 Conclusions

This current introductory study focused on identifying the different carrot tissue structures and changes after the application of traditional and high pressure treatments. By using microscopy, it was possible to identify cell elongation, loss of roundness and breakage in many areas, especially when freezing was applied. High pressure processing caused less tissue damage than steaming and freezing treatments. During the

application of a range of pressures a "tissue break point" was found between 100 MPa and 200 MPa for 2 minutes of pressure processing. There was no significant increase in damage above 200 MPa. Furthermore, processing time did not show significant differences at 600 MPa when measuring REL, but some cellular shape changes were observed in the cells due to processing time such as an increase in elongation. In this study, Trypan blue facilitated visual differentiation between treatments, showing increasing cell damage in the order HPP < raw < steamed < frozen tissue. Relative electrolyte leakage was a very useful and simple method to estimate and compare tissue damage between treatments by quantifying cell content leakage when membranes became more permeable or broken.

From these observations and based on the findings from the introductory chapter, it was decided to further research the following areas: A non-destructive way to measure tissue damage at a cellular membrane level would be to follow physiological patterns such as respiration rates. This would provide an indication of the metabolic and stress responses caused by high pressures. If structural changes were observed such as cell elongation, then how would this affect a macro quality parameter such as texture (e.g. hardness)? Quantification of structural changes at different pressures and times could be correlated to macro quality measures. From the observations of tissue damage by using Trypan blue, it would be interesting to investigate if high pressure promotes cell debonding during cutting, and if so, how this affects the juiciness of the tissue.

From this introductory chapter, it was concluded that studying microstructural changes would be an essential step to further investigate quality changes affected by high pressures. It would be valuable to study textural changes before as well as after high pressures for both types of tissues found in carrots as significantly different changes were observed in the core and cortex cells. It was also important to keep in mind that textural changes might also occur due to enzymatic reactions such as the action of pectinmethylesterase hydrolysing pectins. Therefore, it is decided to investigate this enzyme activity before and after pressure treatment for both types of carrot tissues to confirm their role in those changes if that is the case. In this way, a more complete set of information integrating microstructure, macro quality attributes and biochemical changes would be provided.

4 Identifying carrot tissue damage after high pressure processing by following respiration rates patterns

4.1 Introduction

Chapter 3 showed that cell shape modifications such as elongation occurred during high pressure processing, using light microscopy. Tissue damage was also observed when using Trypan blue (take up by non-viable cells) as a selective dye. Due to membrane damage and cellular changes on HPP, products will inevitably suffer undesirable biochemical, physiological and physical changes as a response to wounding or stress. This chapter focuses on the physiological responses (measured as respiration rates) of carrot tissue before and after high pressure treatments.

Literature indicates that a mild physical stress on fruits and vegetables can cause changes in the metabolism such changes as in respiration rate, increasing ethylene production, phenolic metabolism and wound healing (Surjadinata and Cisnero-Zevallos, 2003). However, a physical stress such as cutting done on shredded cabbage, cauliflower florets and carrot sticks does not necessarily affect many quality attributes such as in controlled or modified atmospheres (Kaji et al., 1993; Voisine et al., 1993; Izumi et al., 1996). Controlled atmosphere conditions (5% CO_2, 95% N_2 and 10°C) could delay spoilage of shredded carrots in terms of texture, colour and odour parameters in comparison with air stored samples at up to 28 days of storage (Izumi et al., 1996). Studies done on carrot shreds and sticks (as minimal processed products) showed an increase in respiration rates during storage at 10°C, which was tentatively explained by wound healing of cut cells (Izumi et al., 1996). Some changes in respiration rate can last for a few hours or even days (Hardenburg et al., 1986). Yahraus et al., (1995) explained that stressed plant cells may undergo an oxidative burst in response to a mechanical disturbance.

There are reports that the application of low pressures (100-200 MPa) may increase membrane permeability and cause membrane disorders affecting the ATPase activity in animal cells (Kato et al., 2002), as well as gel formation in cell membranes and lead to membrane destruction due to protein unfolding (Kato et al., 2002). There is scarce information on how fruits and vegetables would respond to pressure applications in

terms of physiological metabolism. If at low pressures membranes can be damaged as found by Kato et al., (1999b), then changes in respiration rates are expected to happen. The question is what physiological responses carrot tissue would manifest when submitted to different high pressure/time conditions? Is high pressure a treatment severe enough to destroy all physiological mechanisms of the product? Or is the product able to survive and continue respiring as found in minimally processed carrots? The aim of this section of the research was to follow respiration rates after HPP at a range of pressures and time combinations to classify HPP as either a mild or a severe process and compare it with other traditional technologies (e.g. thermal). The study also investigated respiration recovery after processing and other wounding treatments such as cutting. The results of this investigation would provide information not only on HPP effects on plant physiology but also insights on packaging requirements for storage.

4.2 Materials and methods

4.2.1 Plant material

Carrots (*Daucus carota*, cv. Stephano) were purchased at the local market (Sydney, Australia) and stored at 1°C for 24 hours. The following day carrots were taken out of storage and conditioned to room temperature for processing and analysis.

4.2.2 Respirations rate measurements

Twelve whole similar carrots were weighed and placed individually into glass jars (1050 mL) which were sealed and connected to pure air flow (BOC, Sydney Australia). Gas samples were automatically collected using in-house manufactured equipment. A software incorporating an oxygen sensor (KE–25, Japan Storage Battery Co. Ltd, Japan) and a carbon dioxide sensor (Horiba PIR–2000 Ltd, Tokyo Japan) in a range between 0-100% and 0 – 3 % V/V respectively was used for quantification. Sealed jars containing one carrot each were firstly flushed with air for 10 minutes and then left sealed, allowing the carrot to alter the gas atmosphere. Gas samples were withdrawn and O_2 and CO_2 concentrations were recorded at 30 minute intervals resulting in 3 measurements per carrot every 1.5 hours. Linear regression (Excel 2003 v.5.1.2600, Microsoft Corporation, Seattle USA) was used to calculate the rate of the gas concentration change and was converted to respiration rate taking into account the mass of the carrot and the volumetric void space in the jar using the units recommended by

Banks et al., (1995). All measurements were performed at 20-22°C. Calibrations were performed using three empty jars flushed with pure air (BOC, Sydney, Australia), and the average of the gas concentrations used to correct the gas concentration measurements prior to regression calculations.

4.2.3 High pressure and thermal processing

Carrots were either pressure treated whole in a 2 litre pressure processing unit (Flow International Corporation, USA; compression time <10 s, decompression time <5 s, 18-20°C; 2-30 min) or blanched at 90°C for 4 minutes or cooked at 100°C for 20 minutes and cooled with ice water (Table 4.1). Immediately after processing samples were dried with tissue paper and prepared for respiration measurements.

Table 4.1: Experimental treatment conditions

Treatment	**Duration (min)**
Raw	0
Pressure 100 MPa	2, 10 and 30
Pressure 300 MPa	2, 10 and 30
Pressure 600 MPa	2, 10 and 30
Steamed 90°C	4
Cooked 100°C	20

4.2.4 Headspace ethanol and acetaldehyde analysis

The presence of ethanol and acetaldehyde were investigated as indicators of anaerobic metabolism in raw and processed carrots. Whole carrots were placed into 1050 mL sealed jars and placed into a water bath at 30°C for 20 minutes (to enhance volatile release). A fiber (Supelco SPME with 2μm – 50/30 μm DVB/Carboxen/PDMS stable flex, Pennsylvania, USA) was inserted for 10 minutes through a rubber septum to allow volatile capture. Volatile quantification was done in a GC (Varian 4000, California, USA) at 250°C, 9.50 min run. Calculations were done considering each respective standard. An acetaldehyde (CH_3CHO, Fluka, Switzerland) calibration curve was constructed between 1.56-6.24 μg (R^2: 0.976) and an ethanol (CH_3CH_2OH, Fluka, Switzerland) calibration curve between 1.58-6.35 μg (R^2: 0.874).

Due to high biological variation, single carrots were labelled and used for respiration as well as for head space volatiles; following each carrot respectively before and after processing. All headspace analyses were done in triplicate.

4.2.5 Statistical analysis

Significant differences between respiration rates at different pressure levels were firstly checked for homogeneity using Levene's test (assess the equality of variances in different samples). In circumstances where variations in treatments were non-homogeneous, data was transformed for statistical analysis. All treatments were analysed for significance using one-way analysis of variance (ANOVA) and Tukey's test to determine mean differences between treatments. Significant differences and interactions between pressures and time combinations were analysed using two-way analysis of variance (MINITAB version 13.31).

4.3 Effects of high pressure processing (HPP) on carrot respiration

Oxygen consumption and carbon dioxide production were used as stress indicators in this study. Figure 4.1 presents the respiration rates observed in carrot samples processed under different pressures and treatment times (100-600 MPa, 2, 10 and 30 minutes), steamed or cooked. The results showed significant increases in carbon dioxide production at moderate pressure levels (100 MPa) compared with all other treatments. At this pressure, the carbon dioxide production rate was processing duration dependent, increasing from 3.5, 4.5 to 6.5 x 10^{-3} $mol.kg^{-1}.s^{-1}$ for 2, 10 and 30 minutes respectively (Figure 4.1A). This increase could be attributed to a stress response as a consequence of cellular damage as observed by Kato and Hayashi (1999a, b), who identified a similar response in organelle membranes of animal cells with nuclei, lysosomes and vacuoles damaged at 100 – 250 MPa.

Above 100 MPa there was a significant decrease in CO_2 production, with minimum rates observed at 600 MPa. Above 100 MPa, there was also a decrease in oxygen consumption, again reaching its minimum at 600 MPa (Figure 4.1B). This decrease may indicate cell damage, causing the metabolism to slow down with eventual cellular death. Metabolic responses of cooked carrots showed the lowest gas production/consumption rates, similar to those observed after application of high pressure at 600 MPa, while, steamed samples behaved similarly to HPP samples

processed at 300 and 600 MPa for 2 minutes. These results confirmed that tissue damage starts between 100 and 200 MPa, similarly observed in Chapter 3 when measuring electrolyte leakage.

Recent studies on the characterization of HPP and effects on fresh lettuces using chlorophyll fluorescence image analysis indicated that applying high pressures of 125 MPa and temperatures lower than 45°C would cause only minor and reversible effects on lettuces, while a decline in photochemical efficiency was observed at 150 MPa at temperatures higher than 45°C (Schlüter et al., 2009). These results lead to the conclusion that above the threshold of high pressures and heat (which is product dependent), HPP would not be applicable as a mild process for fresh produce (Schlüter et al., 2009). Similar deductions can be found in this current study, where only low pressures showed reversible changes or recovery of respiration, while at above 300 MPa the tissue was non-responsive in terms of respiration rates.

A useful indicator of product physiology is the respiratory quotient (RQ = carbon dioxide production / oxygen consumption) (Kader, 1986). In this research the RQ was found to be 2 to 3 for moderate pressure treated samples (100 MPa for 2, 10 or 30 minutes) indicating anaerobiosis; whereas unprocessed samples presented an RQ of 1.

Samples processed at 300 MPa showed a decrease in their metabolic response as time increased, presenting almost no respiration at 300 MPa processed for 30 minutes (similar to 600 MPa). It is postulated that the high RQ found in 100 MPa samples were the result of a stress response, followed by either slow oxygen diffusion into the root due to the resistance of the skin or the loss of oxygen already in the cells (air spaces) after decompression at a sudden rate. Literature indicates that anaerobic responses may be found in large roots due to low inner oxygen concentrations, large diffusion resistance through the skin or enzyme damage (Abdul-Baki and Solomos et al., 1994). Studies done on potatoes showed an increase in CO_2 concentrations without changing the oxygen concentrations, due to the resistance of the tuber flesh when placed into a sealed chamber (Banks and Kays, 1988), while Wadsö et al., (2004) stated that respiration reduction in the inner core of a root is mainly due to low oxygen pressures and not due to internal diffusion resistance.

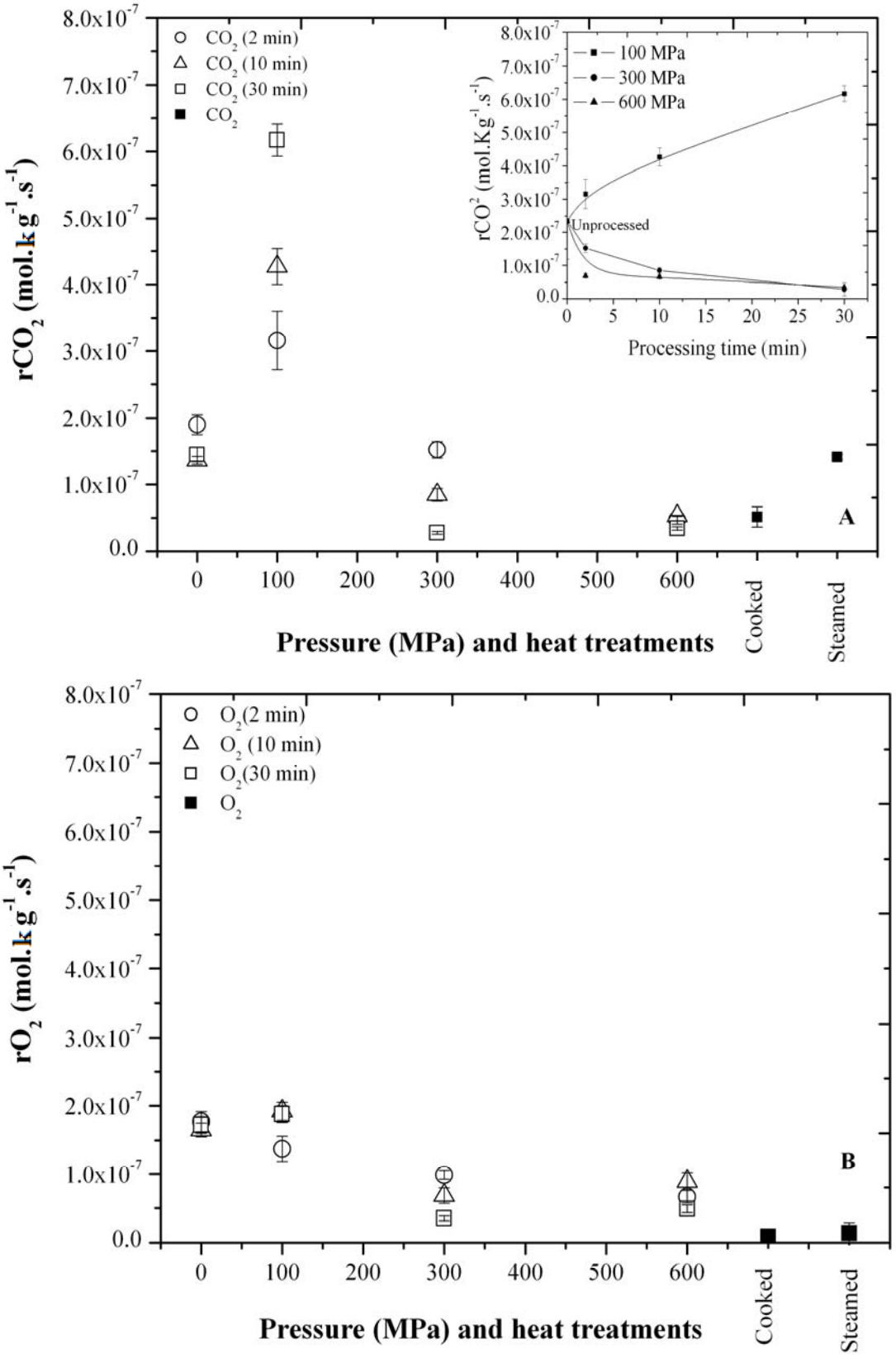

Figure 4.1: Carbon dioxide production rates (A) with processing time effects (right corner). Oxygen consumption rates (B) for different pressure and heat treatments.

Meanwhile, gas dissolution in an aqueous environment surrounding the membrane can also affect gas diffusion rates (Finean et al., (1978) cited by Marangoni et al., (1996)). This was observed in cucumber fruit in the form of change in the gas production/consumption rate and diffusion when gaseous intercellular spaces were filled with water (Kuroki et al., 2004). In this study an oxygen gradient is expected to exit from the skin to the core of the carrot (centre).

Submitting the carrot tissue to high pressures would enhance gases solubility (O_2, N_2 and CO_2), affecting respiration patterns in the cells (if viable). During compression, gases may change their inside the cells by larger driven forces resulting in higher nitrogen and oxygen levels inside the cells. While during decompression, these gases are released when reaching atmospheric pressures by escaping through cell voids or nucleation if a point is found. If nucleation happens inside the cells, then these may be damaged or disrupted and if nucleation happens outside the cells it would potentially cause cell dissociation or void enlargement causing tissue breakage.

When oxygen levels reach the lowest limit in the produce, a metabolic shift from aerobic to anaerobic can occur (Gram and Beaudry, 1993; Beaudry, 1999). In carrots, oxygen concentrations below 0.5-2% have stimulated anaerobic conditions with the evidence of lactic and ethanolic fermentations after 2 days storage at 5 and 15°C (Kato-Noguchi, 1998). Similar findings were observed by Sode and Kühn, (1998) at oxygen concentrations below 15% in modified atmosphere packed cut carrots.

The effect of high pressures on enzymes involved in plant physiological metabolism could also have promoted a metabolic shift; however there is little understanding of all the mechanisms involved at this stage in literature.

It is postulated that carrots processed at moderate pressure (100 MPa) will present a stress or wound response. This results in an increase in carbon dioxide production rates and low oxygen diffusion (root density and the skin barrier), consequently giving an abrupt shift to anaerobic metabolism. In terms of compression and decompression during a pressure cycle, it could be possible that oxygen concentrations briefly increase due to high compression in the tissue stimulating an increase in respiration rates. While after abrupt pressure removal (decompression), the tissue may be left to release the high quantities of carbon dioxide. Decompression may also promote bubble formation

breaking membranes and consequently affecting respiration rates. And if the bubble formation does not occur during decompression, it could also be possible to have an increase in cell membrane permeability due to protein denaturation (snapping). A feasible alternative hypothesis could also be an effect of high pressure on enzymes involved in the metabolic pathways, leading to anaerobiosis. This fermentative metabolism normally occurs when the oxidative electron transport system is inhibited, forcing the use of NADH and pyruvate in glycolysis to continue ATP production (Ke et al., 1995); another alternative is that high levels of accumulated carbon dioxide would reduce the pH in the cells, thereby affecting enzymes involved in metabolic pathways such as phosphofructokinase (PFK, an enzyme that regulates glycolysis) (Mathooko, 1996). A step diagram is used to describe the possible pathways of carrots treated with high pressure at 100 MPa in comparison to cut wounded carrots (Figure 4.2). According to the previous respiration results high carbon dioxide is liberated after treatments at 100 MPa, while oxygen consumption does not significantly change. This oxygen limitation in the root (which could be due to simple gas diffusion through the skin and dense root structure) will lead to the accumulation of NADH and pyruvate promoting fermentation. A slower diffusion rate due to a flooded tissue structure would disturb respiration patterns. If cells are still viable after high pressure treatments, then they may be operating at much lower oxygen levels.

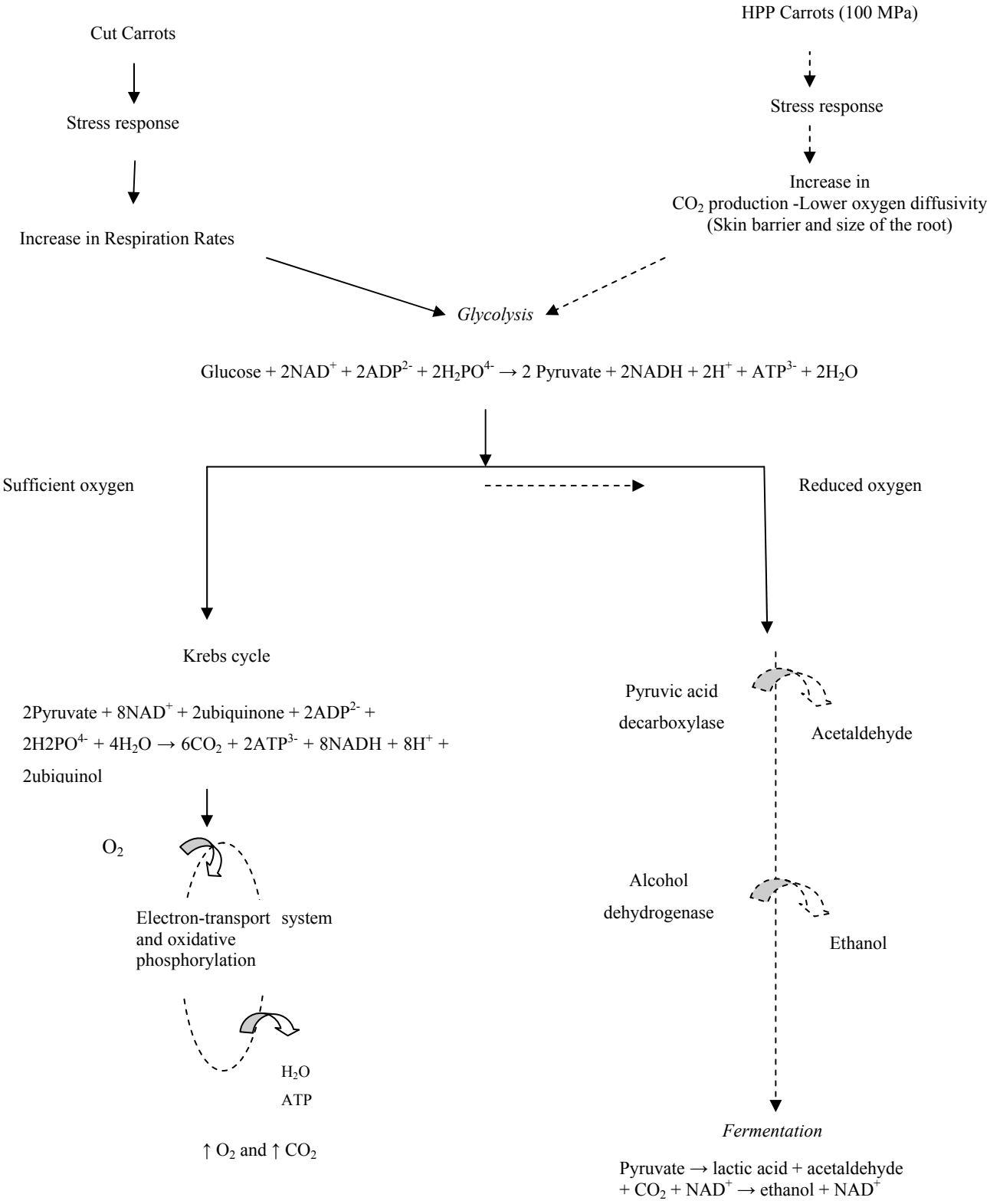

Figure 4.2: Stress mechanisms affecting carrot physiology after cutting and high pressure processing (modified from Salisbury and Ross, 1992).

4.4 Anaerobiosis measured in terms of the production of acetaldehyde and ethanol

Previous literature has shown that fermentative products such as ethanol and acetaldehyde can be produced even in aerobic conditions. Ethanol production was observed in stressed carrots when they were physically shaken under aerobic conditions in comparison to unshaken carrots (Seljåsen et al., 2001), while Valle-Guadarrama et al., (2004) found fermentative metabolites (acetaldehyde and ethanol) in avocados stored in aerobic conditions at 5 and 20 °C.

When applying high pressures to carrots, an increase in carbon dioxide production rates with higher concentrations of acetaldehyde and ethanol was found even at moderate pressures (100 MPa for 2 minutes) Figure 4.1. Although it is not precisely clear what mechanisms of the respiration pathway may be affected by pressure treatment, the results confirmed that a shift from aerobic to anaerobic metabolism occurred in pressure treated samples. Both anaerobic sub-products in samples processed at 100 MPa for 2 minutes increased more than five-fold compared with raw samples (Figure 4.3 and Figure 4.4). Interestingly this current study shows that anaerobic products did not increase as processing time increased, in contradiction to the carbon dioxide trends. It could be possible that by subjecting the carrots to a longer processing time, there could be accumulation of sub-products (ethanol and acetaldehydes) reaching such high levels that they could be blocking further production as a way of equilibrating the system. Literature explains that the accumulation of acetaldehyde and ethanol catalysed by pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) could also cause a shift from aerobic to anaerobic metabolism (Mathooko, 1996). This can occur in a low oxygen concentration environment (Kato-Noguchi, 1998). Meanwhile, an increase in CO_2 levels in aerobic conditions does not necessarily mean an increase in the production of acetaldehydes or ethanol (Mathooko, 1996). It could be possible that moderate pressures can inactivate enzymes involved in the fermentation steps such as pyruvic acid decarboxylase (PDC) and/or alcohol dehydrogenase (ADH), limiting the production of fermentative sub-products as processing time increases. The production of acetaldehyde at 600 MPa for 2 minutes was half the quantity produced in raw samples, while ethanol presented no significant differences between the raw and processed samples. This again suggests that 600 MPa stops most metabolic reactions of the cells.

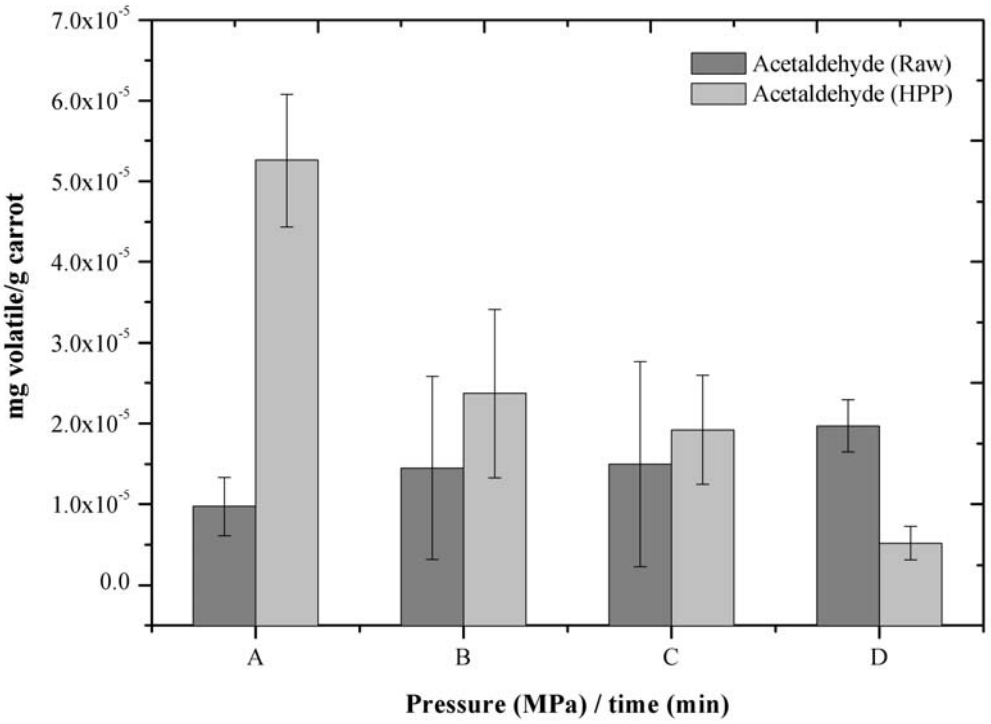

Figure 4.3: Acetaldehyde production in headspace of raw and pressure treated carrots (100 MPa A: 100 MPa /2 min; B: 100 MPa/10 min; C: 100 MPa/30 min; D: 600 MPa/2 min). Measurements were done in triplicate.

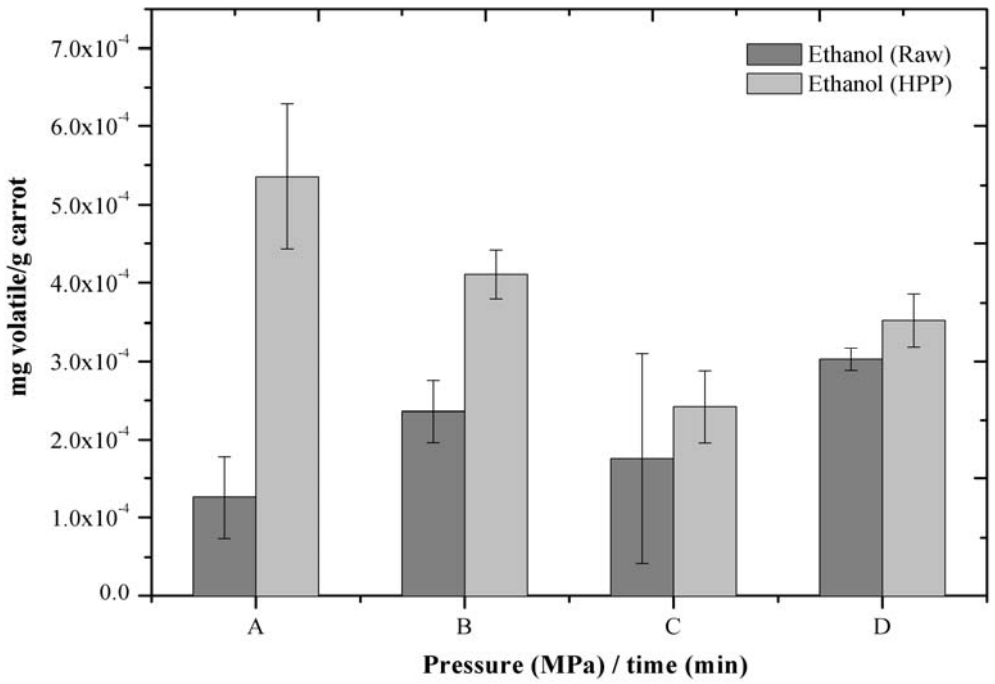

Figure 4.4: Ethanol production in headspace of raw and pressure treated carrots (100 MPa A: 100 MPa /2 min; B: 100 MPa/10 min; C: 100 MPa/30 min; D: 600 MPa/2 min). Measurements were done in triplicate.

4.5 Cut and high pressure wounding effects on carrots

From the previous section, it was observed that most anaerobic compounds are developed when low pressures (100 MPa) are applied. The question is how does the wounding response caused by HPP compare with a cut wounding response?

Interestingly, carrots wounded by cutting displayed an increased respiration rate but maintained an RQ of 1, while pressure treated carrots showed an increase in carbon dioxide production rates resulting in an RQ of ~3, indicating a shift from aerobiosis to anaerobiosis (Figure 4.5). This is an important finding as it clearly shows the different responses to wounding by reduced oxygen consumption in HPP. Previous studies on carbon dioxide production by wounded (cut) carrots showed a proportional increase to the nature of wounding, from slices to stick (Surjadinata and Cisnero-Zevallos, 2003). An immediate four to five fold increase in respiration rate was also found in sliced potatoes at aerobic conditions in comparison to whole potatoes (Laties, 1962). Furthermore, carbon dioxide production increased from approximately 4 to 7.5 and 9 ($\mu g.kg^{-1}.s^{-1}$), in fresh, sliced and shredded radish respectively (Saavedra del Aguila et al., 2006). Unfortunately most literature on wounding of commodities does not cover oxygen measurements but only carbon dioxide production. Therefore, it is assumed that most wounding responses after cutting are represented by an RQ of 1, with no anaerobic responses as observed after HPP.

Wounding responses can also be measured by heat production (Wadsö, et al., 2004). The authors found that vegetables under stress could produce up to 150% more heat in comparison with unprocessed ones. Wadsö, et al., (2004) also indicates that vegetables under stress can exhibit enhanced biochemical reactions due to cell decompartmentation and therefore more enzyme-substrate contact. Studies on wounding responses in carrots suggest that carrots have certain protective mechanisms to confront stress. This was observed when injured carrot cells became stronger by additional lignin secretion as a defensive mechanism (Satoh et al., 1992) or acclimatized when exposed to low temperatures (Gomez Galindo et al., 2004d).

These protective mechanisms could be activated after the application of high pressures, showing respiration rate tends to recover as time progress. An experiment done on respiration trends during storage is described below.

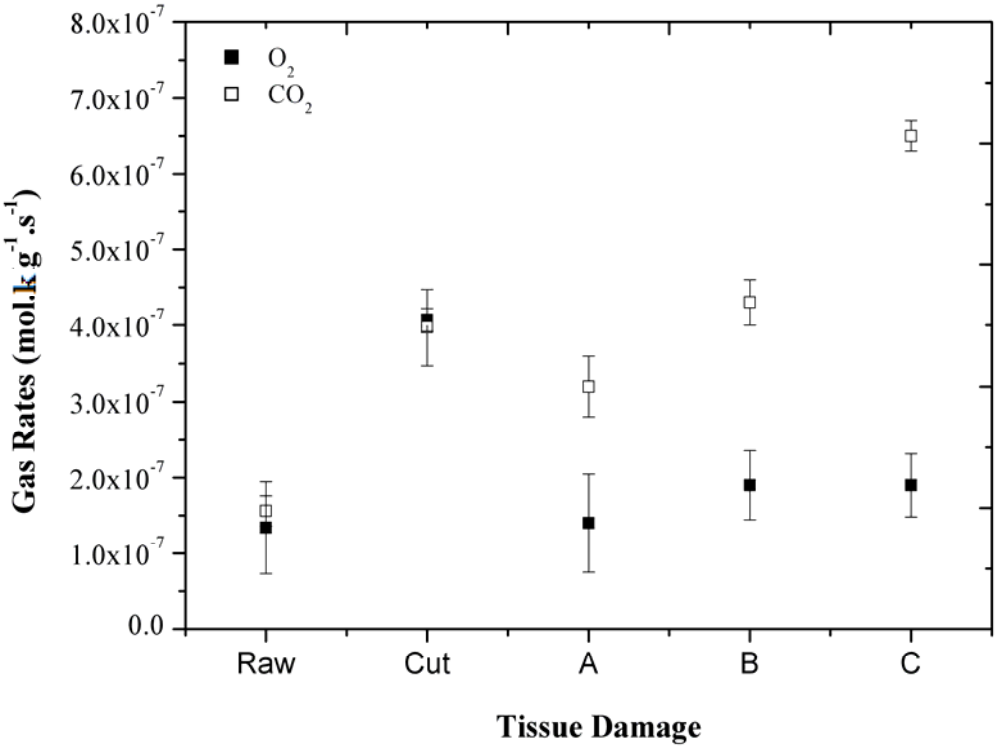

Figure 4.5: Gas consumption/production rates in fresh carrots after cutting and high pressure processing at 100 MPa for different durations (A: 2 minutes; B: 10 minutes and C: 30 minutes).

4.6 Physiological changes during storage

In this experiment, carrot respiration rates were followed before and after high pressure processing for 4 days of storage (at ambient temperatures exposed to atmospheric air conditions). The aim was to observe if respiration rates would consistently stay high as observed in the previous section or if a recovery response would take place. If so, how long would this recovery take to occur? Would the tissue continue respiration activities?

Results indicated that high levels of carbon dioxide were produced immediately after high pressure processing, decreasing to near steady state as storage time increased and reaching an RQ of ~ 1 after 4 days of storage at 20-22°C (Figure 4.6). Similar recovery patterns were found in wounded cut carrots with the maximum carbon dioxide peak

production occurring between day 1 and 2 of storage, followed by a decrease to steady state by day 9 at 10°C (Surjadinata and Cisnero-Zevallos, 2003). Studies of fresh-cut radish pointed to an uncontrolled cellular repair mechanism that promoted high respiration rates after 1 hour, settling down after 4 hours at 1, 5 and 10°C (Saavedra del Aguila et al., 2006). Tissue respiratory recovery may occur after a self-regulation mechanism where higher levels of ATP are produced to return to the initial respiratory state (Saavedra del Aguila et al., 2006).

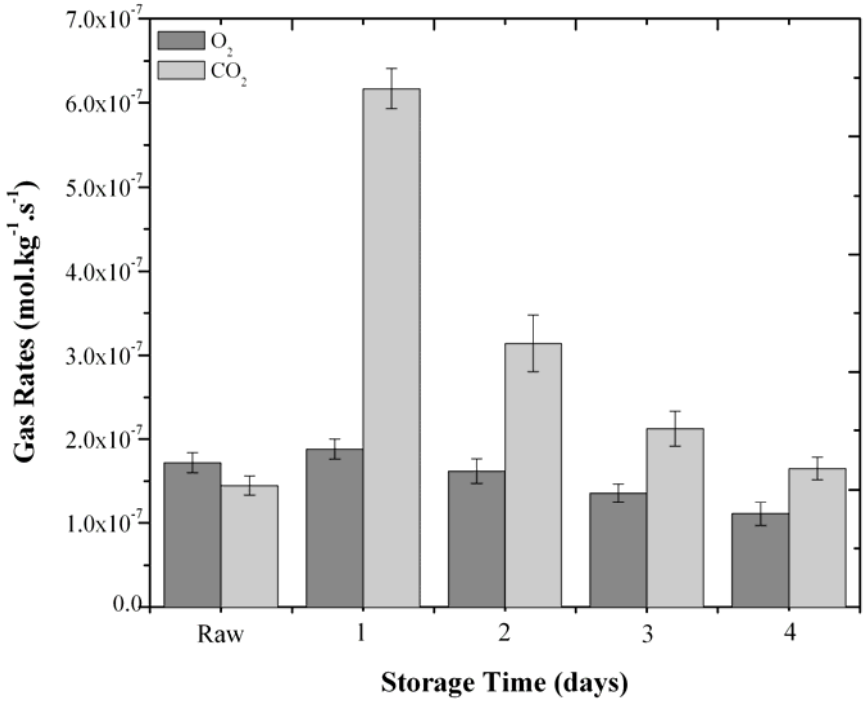

Figure 4.6: Oxygen consumption and carbon dioxide production by carrots treated with high pressure (100 MPa, 30 mins) stored for 4 days at 20-22°C.

From this current research it is confirmed that tissue under stress is able to produce significantly higher levels of carbon dioxide as a response mechanism. These high levels of carbon dioxide may trigger tissue recovery. Cell repair is most likely to occur through storage time and the rate of recovery may become lower as storage time increases, finally resulting in the initial respiration rate.

4.7 Cumulative wounding response

Figure 4.7 shows a cumulative wound response due to pressure-processing followed by cutting carrots into disks. In this experiment gas production/consumption rates were measured in raw (unprocessed), pressure treated at 100 MPa (A), 100 MPa for 2 min

followed by cutting (A'), 600 MPa for 2 min (B) and 600 MPa for 2 min followed by cutting (B'). Carrots were cut into 1 cm discs using a stainless steel blade. The results clearly showed a cumulative effect of each physical stress on carbon dioxide gas rates: $2.5x10^{-3}$, $5.5x10^{-3}$ and $7.5x10^{-3}$ ($mol.kg^{-1}.s^{-1}$) where the pressure applied was 100 MPa. Oxygen consumption rate did not change after pressure treatment; however it increased two-fold after cutting. It can be inferred from these results that cells were still 'alive' as evidenced by their ability to respond to the external stress caused by cutting.

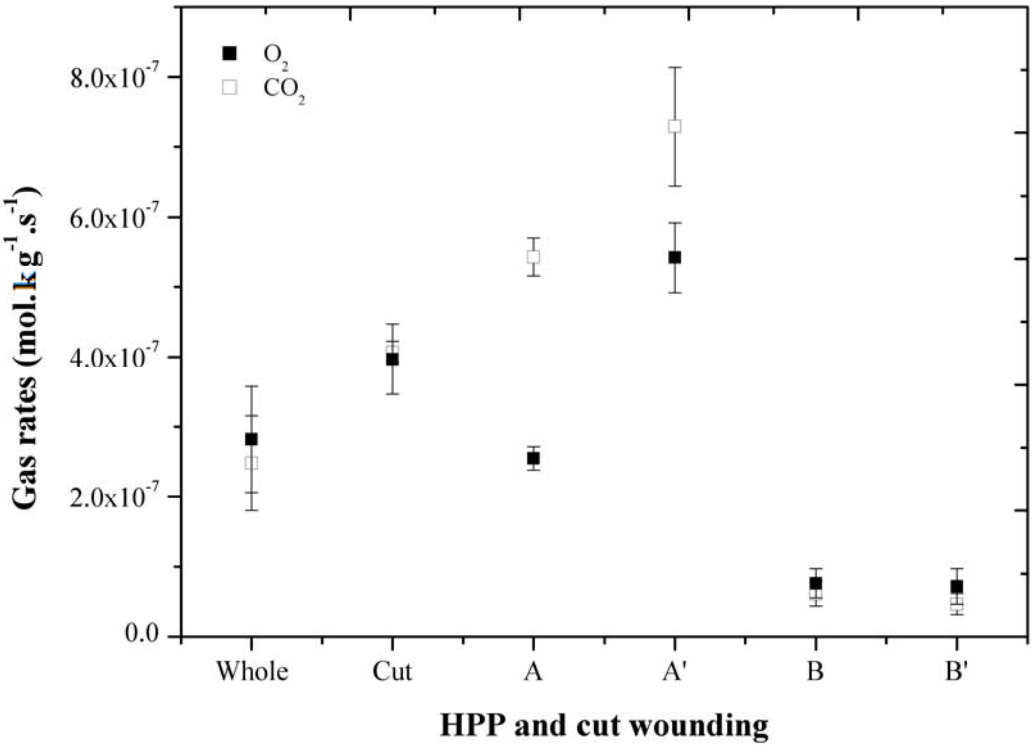

Figure 4.7: Gas production/consumption rates after physical stresses: A = 100 MPa for 2 minutes; A' = 100 MPa for 2 minutes followed by cutting; B: 600 MPa for 2 minutes; B' = 600 MPa for 2 minutes followed by cutting.

In samples treated with 600 MPa for 2 minutes, respiration rate was reduced to almost zero and no physiological responses were observed after cutting. Carrots treated at 600 MPa thus appear to be effectively 'dead', probably due to inactivation or damage of enzymes involved in the respiration pathways (phosphofructokinase (PFK), pyruvate kinase (PK) (during glycolysis) and succinate dehydrogenase (SDH) (during the TCA cycle) or simply membrane disruption.

These results may have implications when deciding if HPP is to be considered a minimal process and furthermore in choosing packaging formats. Vacuum packaging helps to prevent the growth of lactic acid bacteria (*Lactobacillus* spp., *Carnobacterium* spp. and *Leuconsotoc* spp.) (Kakiomenou et al., 1996); however, in order to package the product under a vacuum, carrots must not respire as this will cause accumulation of carbon dioxide promoting the loss of vacuum in the pack and spoilage. This study indicates that pressure treatments only greater than 300 MPa (2 minutes process duration) would avoid the development of off-flavours by stopping metabolic respiration.

4.8 Effects of packaging during processing

During high pressure processing, there is a drastic initial compression which may induce gas or liquid ingress into the tissue and an instantaneous decompression where degassing may occur. An experiment on carrots unpacked, air packed and vacuum packed in oxygen barrier pouches (Cryovac, Australia) during processing was performed firstly to investigate whether respiration patterns would be affected when samples were in direct contact with the compressible fluid in the chamber during processing for 2 minutes. Secondly, this study was performed to observe if respiration patterns would be affected by having oxygen available inside the packed bag or if removing all gases by vacuum packing would change respiration patterns. Mechanistically, carrots that are in direct contact with the compression fluid (water) would probably present tissue with air spaces flooded with water as these gases are more easily dissolved under high pressures into the tissue. On the other hand, carrots air packed would have more oxygen available and diffusing into the tissue to continue respiration, as the oxygen available is likely to pass through the skin into the tissue during compression. Higher air availability could enhance bubble formation during decompression, damaging cells. Vacuum packed carrots would have lost some of the oxygen within the tissue when vacuuming and would have limited oxygen during processing, leading to a more likely stress response and anaerobiosis. The experimental data showed that, all HPP samples were significantly different from raw samples but there were no significant differences in gas production/consumption rates ($P<0.05$) between unpacked, air and vacuum packed carrots immediately after processing (Figure 4.8). By depriving cells of oxygen, they will shift to an alternative respiration pathway, which would be anaerobic respiration with high carbon dioxide liberation.

Overall, this experiment showed that the respiration rates are more pressure than packaging dependent. This implies that anaerobic respiration may be occurring in cells located below the surface, where the oxygen level becomes limited after HPP, and is apparently unaffected by external concentrations.

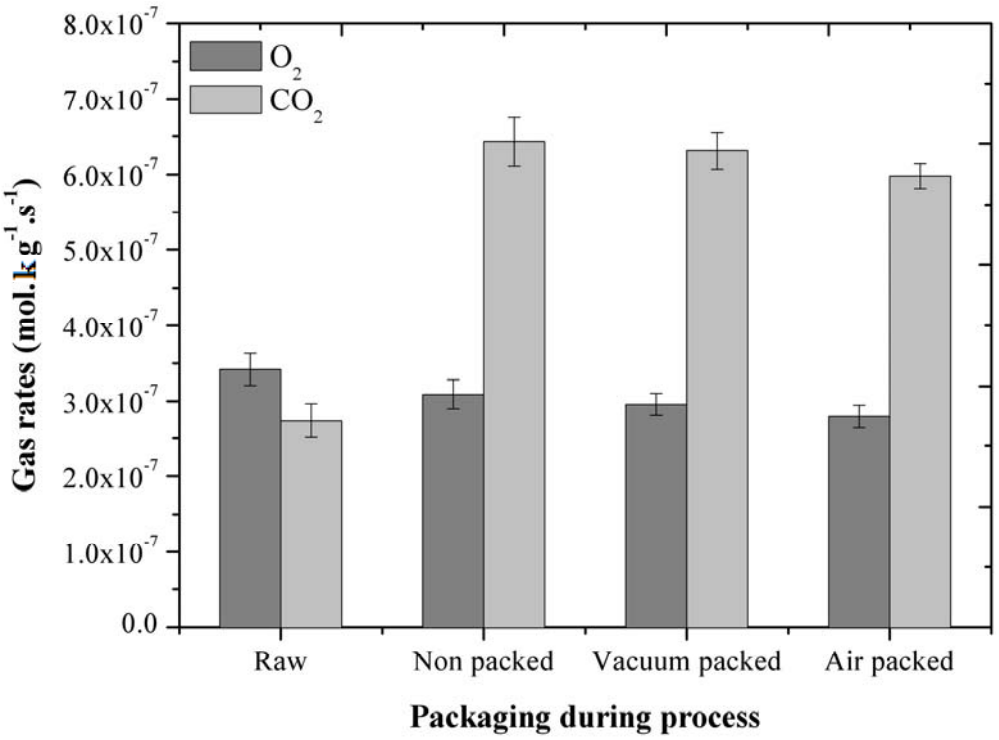

Figure 4.8: Gas production/consumption rates of non-packed, vacuum and air packed whole carrots immediately after pressure processing at 100 MPa for 2 minutes.

4.9 Conclusions

The work described in this chapter investigated how pressure treatments promoted changes in carrot respiration rates under different pressure and time combinations. Pressures of 100 MPa stimulated cells to shift from aerobic to anaerobic metabolism which recovered after 4 days. This response is similar to the stress response found in minimally processed carrots. The effects on respiration pattern were consistent with potential wound repair mechanisms, correlating with tissue damage described using relative electrolyte leakage (Chapter 3). A pressure of 300 MPa slowed down metabolic responses, especially when processed at 30 minutes where no subsequent physiological responses were found. In addition, high pressures of 600 MPa did shut down the plant

metabolism completely at all processing times. The cause for this could have been either enzymic inactivation or membrane disruption.

For commercial application of high pressure processing to carrots, it is recommended to consider that when processing at 300 MPa (≤10 min) or below carrot cells are still respiring (similarly to blanched product), and in this environment subsequent spoilage may result, while at above 300 MPa processing over 30 minutes vacuum packing would be feasible for storage-life extension.

5 Understanding texture changes of high pressure processed fresh carrots (*)

5.1 Introduction

High pressure processing (HPP) of vegetables has been described as a means of maintaining desirable textural characteristics because it causes less tissue damage than cooking treatments (Islam and Igura, 2003). There is evidence that high pressure does alter physico-chemical properties of vegetable matrices by inducing changes in their structure as observed in Chapter 3 and by other authors (Basak and Ramaswamy, 1998; Préstamo and Arroyo, 1998; Butz et al., 2002). For this reason, in this study texture (evaluated as hardness and cutting forces, cellular changes, separation and structure quantification) was evaluated in combination with a study of enzyme activation/inactivation after high pressures to provide more understanding of the tissue response to treatment. As already known during thermal or pressure processing, tissue firmness may be lost due to cell wall breakdown and loss of turgidity (Dörnenburg and Knorr, 1998; De Belie, 2002; Sila et al., 2004). The previous findings of increasing electrolyte leakage in carrot tissue after HPP (Chapter 3) indicated significant tissue damage above 200 MPa, however this damage was found to be less than in frozen samples. These results were supported by microscopy images showing changes within the tissue structure. In terms of textural changes after HPP, the effects can be characterized by an initial texture loss, also called instantaneous pressure softening (IPS), which was followed by a gradual change during the pressure hold time (Basak and Ramaswamy, 1998) as reviewed in Chapter 2, section 2.5. Texture recovery observed by measuring the firmness of the tissue, could be achieved after applying mild pressure conditions such as 100 MPa for 30 minutes (Basak and Ramaswamy, 1998).

Biochemical changes may also play an important role in texture changes. Studies of carrot tissue have indicated that pectin degradation only occurred in cooked and not in pressure treated carrots (Kato et al., 1997). Enzymatic degradation shortens demethylated pectin chains resulting in drastic tissue softening on cooking.

(*) Material from this chapter is included in the paper: Trejo Araya X. I., Hendrickx M, Verlinden B.E., Van Buggenhout S, Smale N.J., Stewart, C., and Mawson A. J. (2007). Understanding texture changes of high pressure processed fresh carrots: A microstructural and biochemical approach. *Journal of Food Engineering*, 80: 873-884.

A two-stage sequence explains this tissue softening (see Chapter 2, section 2.1.6.3): a partial demethylation of pectins and associated methanol production is the result of pectin methylesterase activity, followed by depolymerization of the lower degree of methylated pectins by polygalacturonase (Vu et al., 2004). Evidence of enzymic activity can be investigated by measuring crosslinkage by calcium ions between pectins, which increases hardness of the tissue as a result.

There is already substantial literature available on textural changes due to heat and HPP, but there is less understanding of how those changes relate to the microstructure and enzymic activity at high pressures (no external heat applied). Therefore, the aim of this part of the work was to characterise the textural changes occurring during pressure-treatment of carrots under ambient temperature conditions (where heat effects on the cell wall chemistry do not occur) by relating mechanical, microstructural and biochemical changes to the tissue properties. Improved understanding of the mechanisms of textural changes may open further opportunities for potential uses and process optimisation.

5.2 Materials and methods

5.2.1 Plant material

Carrots (*Daucus carota* L., var. 'Laguna', a large variety) were bought at a local Leuven Belgium distributor and stored at 4°C (for a maximum period of 5 days) until further use. Cylinders (12 mm diameter and 10 mm height) of core and cortex material were obtained by using a stainless-steel cork borer (10 samples / treatment) (Figure 5.1).

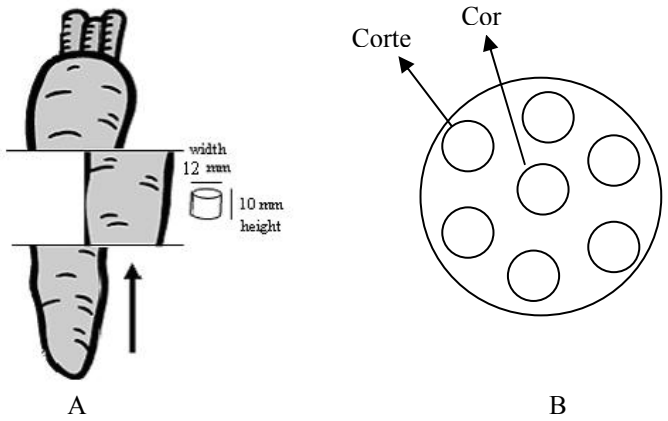

Figure 5.1: Longitudinal (A) and cross-sectional (B) diagrams of how carrot cylinders were obtained from the core and cortex tissue of carrots.

5.2.2 High pressure treatment

Carrot cylinders from both core and cortex were chosen for this study after observing considerable structural differences between these tissues in Chapter 3. Larger areas of both tissues could be found in a specific carrot variety (*Daucus carota* L., var. 'Laguna'). Samples were vacuum packed in double film polyethylene bags and then processed at pressure levels from 100 MPa to 550 MPa for 2, 10 or 30 minutes. The high pressure vessel (Engineered Pressure Systems Intl., Temse, Belgium) of 590 mL volume was temperature controlled using a helicoidal copper tube jacket in thermal contact with the outer wall of the vessel and connected to a heating/cooling unit (Cryostat TCPS, Kul Circulatiekoeler, Serie P6, Belgium). The pressure transmitting medium was propylene and glycol (60% Dowcal N, The Dow Chemical Co., Horgen, Switzerland).

Temperature data were captured using thermocouples fixed to the lid of the vessel. The maximum temperature reached during processing was 39°C at 600 MPa, 30 minutes. Pressure data were also recorded. Sensors were placed to monitor the temperature of the liquid and the centre and the inner border of the vessel together with the pressure profile at 4 s intervals (Figure 5.2). Compression and decompression rates were 16 MPa/s and 10 MPa/s, respectively. Data were captured by a SCXI system using LabVIEW (National Instruments, Zaventem, Belgium).

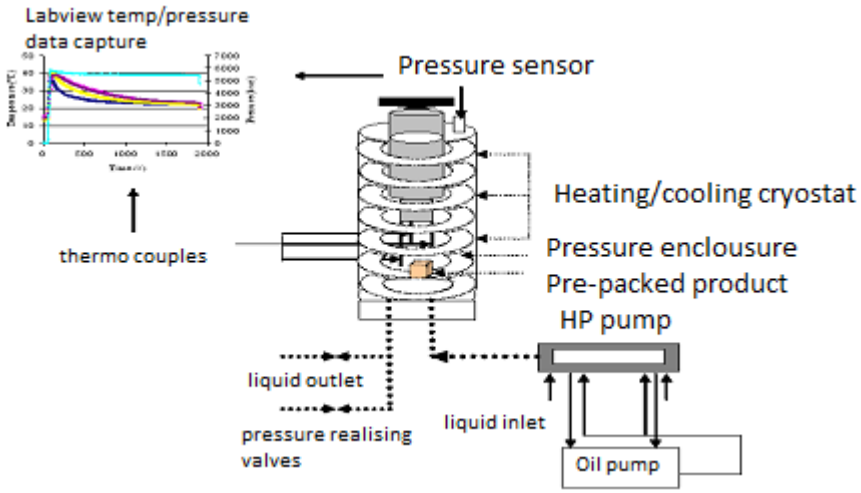

Figure 5.2: High pressure vessel and monitoring system.

5.2.3 Water loss

An experiment was conducted to compare the cutting force responses of carrot tissue that had been submitted to high pressures against tissue that had been dried to 14% w/w. The aim of this experiment was to observe if the water lost as leakage after high pressure had a similar effect to water been withdrawn out of the tissue by drying.

To produce samples with controlled water loss, carrot (cortex) cylinders were placed in a glass desiccator previously equilibrated to a relative humidity of 75.5% ± 1.1% (Greenspan, 1977) at ambient temperature using a saturated salt solution (NaCl). The sample mass was monitored as a function of the incubation time and samples were removed at varying extents of moisture loss to a maximum of approximately 14 % (based on sample mass at the initiation of desiccation).

5.2.4 Calcium soaking pre-treatment

Calcium pre-treatment was done to investigate whether pectins were available to crosslink with calcium after hydrolysis by pectin methyl esterase at different pressure treatments. If that was the case, then textural improvements such as increased hardness were expected.

Carrot cylinders (cortex) were packed in a double film polyethylene bag with a calcium chloride solution (0.5% w/v $CaCl_2$) (Sila et al., 2004). A carrot: brine ratio of 12 g: 12 mL was used. After packing, the samples were high pressure processed for 30 minutes and then left at room temperature for another 30 minutes after pressure release, resulting in a total contact time of 1 hour before texture measurements were carried out.

5.2.5 Pectinmethylesterase (PME) activity assay

Processed and raw samples were frozen with liquid N_2 and stored at -80°C until required for further analysis. The PME extraction was done by homogenizing 2.5 g of the frozen sample in a pestle followed by the addition of 0.2 M Tris-1M NaCl buffer (pH: 8.0) in the proportion of 1 (sample weight, kg) : 1.3 (volume of buffer, L) at 4°C. Residual PME activity was measured by titration of free carboxyl groups released from a pectin solution (apple pectin, 70-75% degree esterification at 22.5°C, from Fluka Chemical, Switzerland) using a pH-stat titrator (Metrohm, Switzerland) and 0.01 M NaOH.

The PME activity unit (U) was defined as the amount of enzyme required to release 1 µmol of carboxyl groups per minute. PME activity measured in $U.mL^{-1}$ is expressed by equation 5.1 (Reed, 1966; cited by Ly Nguyen, 2004):

$$\text{PME activity (U/mL)} = \frac{\text{Volume of NaOH (mL)} \times \text{Molarity of NaOH (M)} \times 10^6}{\text{Time (min)} \times \text{Volume of sample (mL)} \times 10^3} \quad (5.1)$$

5.2.6 Determination of degree of methylation

5.2.6.1 Preparation of alcohol insoluble residue (AIR)

Ten grams of samples were taken for extraction of AIR using the procedure presented by McFetters and Armstrong (1984). The procedure consisted of mixing the sample with 50 mL of 95% ethanol and homogenizing (Buchi mixer B-300, Flawil, Switzerland) three times. The suspension was filtered (Filter paper No. 595, diameter: 100mm; Schleicher and Schuell, Dassel, Germany) and the residue mixed with 25 mL of ethanol, homogenized and filtered again. The residue was mixed with 25 mL of acetone, left for 5 minutes, and then filtered. The final residue was dried in a vacuum oven at 40°C for 24 hours, and weighed to obtain the AIR.

5.2.6.2 Galacturonic acid analysis

Galacturonic acid content was determined using a colourimetric hydroxyl-phenyl-phenol method (Blumenkrantz and Asboe-Hansen, 1973). To estimate the sample's galacturonic acid content, a standard curve of galacturonic acid was made (0 to 100 µg/mL D-galacturonic acid, R^2: 0.993) using deionised water as the blank.

AIR (0.02 g) was hydrolysed drop-wise by the addition of 8 mL of 98% sulphuric acid and 2 mL of deionised water. After 5 minute, another 2 mL of deionised water was added to achieve complete dissolution and the samples were then diluted with 50 mL of deionised water. Galacturonic acid analysis was performed by mixing 0.6 mL of the hydrolysed AIR solution in a chilled test tube with 3.6 mL of chilled tetraborate reagent. The samples were heated at 100 °C for 5 minute and then cooled in ice water. Samples were mixed with 60 µL of m-hydroxyphenyl for 1 minute using a vortex mixer. Absorbance measurements were performed at 520 nm using a spectrophotometer (Ultrospec 2100 Pro, Amersham Biosciences, Uppsala, Sweden). The blank solution was prepared with 60 µl of 0.5% NaOH.

5.2.6.3 Determination of degree of methylation

Measurement of the degree of methylation consisted of taking 20 mg of AIR and mixing this with 8 mL of deionised water in a sonicator for 10 minutes. This was followed by the addition of 3.2 mL NaOH (2M) and incubation at 20 °C for 1 hour with occasional shaking. Samples were then neutralized by adding 3.2 mL of 2M HCl and equilibrated at 25°C for 15 minutes. Samples were diluted with 50 mL of phosphate buffer (pH~7.5). Methanol determination was performed by transferring 1 mL (in triplicate) of the sample into a Pyrex bottle followed by the addition of 1 mL of alcohol oxidase solution, mixing and incubating at 25°C for 15 minutes. Then 2 mL of pentadione solution was added to each Pyrex bottle (except the blank) and incubated at 58°C for 15 minutes. Samples were then cooled to measure absorbance at 412 nm at 25°C (Klavons and Bennett, 1986). A standard curve for methanol was prepared (1 to 20 µg methanol, $R^2 = 0.989$) following the above methanol determination procedure and using phosphate buffer as a blank. The degree of methylation (DM) is expressed by equation 5.2.

$$\text{Degree of methylation (\%)} = \frac{\text{Moles of methanol}}{\text{Moles of anhydrous galacturonic acid}} x100 \qquad (5.2)$$

5.2.7 Texture analysis

Texture measurements were performed with a TAXT2 texture analyser (Stable Micro Systems, Surrey, England). The compression force at 30% strain was obtained using a cylindrical flat-probe (25 mm diameter; aluminium). Samples were placed on the platform as upright cylinders and measured with a 250 N load cell at a deformation rate of 1 $mm.s^{-1}$. The hardness of the samples was defined as the peak force at 30% strain. A cutting test was performed on all samples using a stainless steel blade (supplied as a standard attachment with the TAXT2 texture analyser) with a deformation rate of 1 $mm.s^{-1}$ and 75% strain. Samples were placed as upright cylinders on the platform (i.e. in the same position as for the compression test). Displacement values at maximum force and force-deformation profiles were used to characterize the texture of the tissue. Ten cylinders were tested for each type of tissue and pressure/duration treatment.

5.2.8 Microstructural analysis

Processed and raw carrot tissue samples were fixed using glutaraldehyde, washed with ethanol (50, 70 and 95%) solutions and infiltrated using a Historesin Embedding Kit (Leica, Bensheim, Germany) for 5 days. Samples were then cut into 5 μm sections using a microtone (Microm HM355, Microm Laborgeräte GmbH, Walldorf, Germany). For observations, duplicates at the level of the treatment and at the level of the sectioning were taken resulting in a total of four images per treatment. Samples were stained with ruthenium red (0.02 %) for 2 hours, which binds to de-esterified (acidic) pectins (Sabba and Lulai, 2002). Using a light microscope (Olympus BX-50 from Olympus, Optical Co. Ltd, Tokyo), tissue images were taken in a quadrant clockwise pattern with four snapshots/sample and four continuous snapshots of the cut surface damaged samples. A magnification of 40 x was used for all analyses.

Image analysis was performed using commercial software (analySIS 5, Soft Imaging System GmbH, Bensheim, Germany). All cells, intercellular spaces and cavity areas were described as 'particles'; these particle sizes were divided into groups as described by Van Buggenhout et al., (2006). The following factors were analysed: cell proportion (%), particle elongation which measures the length-width relationship (from 1 to 20, where 1 is spherical and 20 very elongated) and particle shape factor $(4\pi \times \text{size} / \text{perimeter}^2)$. Images considered a total of over 2,700 and 4,500 particles for core and cortex samples, respectively. Samples characterized for surface cut damage were obtained from the cutting test and analysed by counting the broken-through and broken-between cells (sum = 100%) using the method described by Verlinden et al., (1996). The complement of the total count to reach 100% was denominated as error (< 6.2%).

5.2.9 Statistical analysis

Microstructure histograms were constructed using SAS (v8, Cary, USA). For hardness and cutting forces analysis, data were firstly checked for homogeneity using Levene's test. In circumstances where variations in treatments were non-homogeneous, data were transformed into their inverse for statistical analysis. All treatments were analysed for significance using one-way analysis of variance (ANOVA) and Tukey's test to determine mean differences between treatments. Significant differences and

interactions between pressures and time combinations were analysed using two-way analysis of variance (MINITAB version 13.31).

5.3 Results and discussion

5.3.1 Textural changes

5.3.1.1 Effects of HPP on tissue hardness

The hardness of both core and cortex parts of the carrot were studied on samples taken as shown in Figure 5.1. Both tissues showed similar trends in hardness loss with increased pressure. Core measurements were significantly higher than those for the cortex for all treatments (Figure 5.3). Higher hardness values observed in the core tissue are attributed to the nature of its structure consisting of lignified cell wall as observed in Chapter 3. No significant differences between samples processed at 100 MPa for 2 minutes and raw samples were observed, while longer durations (both 10 and 30 minutes) caused a significant decrease of approximately 30 N. Processing at 200 MPa or higher caused a significant drop in hardness. However significant differences were not observed between pressure levels ranging from 200 to 550 MPa or between process durations.

Significant ($P<0.05$) hardness losses were also observed in cortex samples treated at ≥200 MPa for 2 minutes compared with raw samples (Figure 5.4). Reductions of 5, 25 and 50 % in hardness were observed in the cortex for treatments at 100, 200 and 300 MPa for 2 minutes.

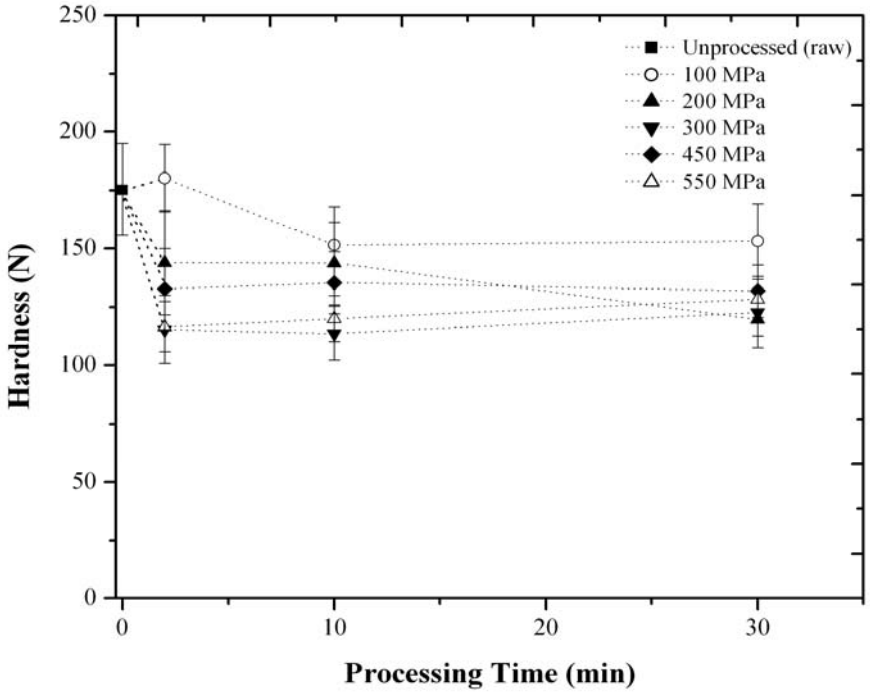

Figure 5.3: Effect of applied pressure and process time on core carrot tissue hardness (zero time represents the raw samples).

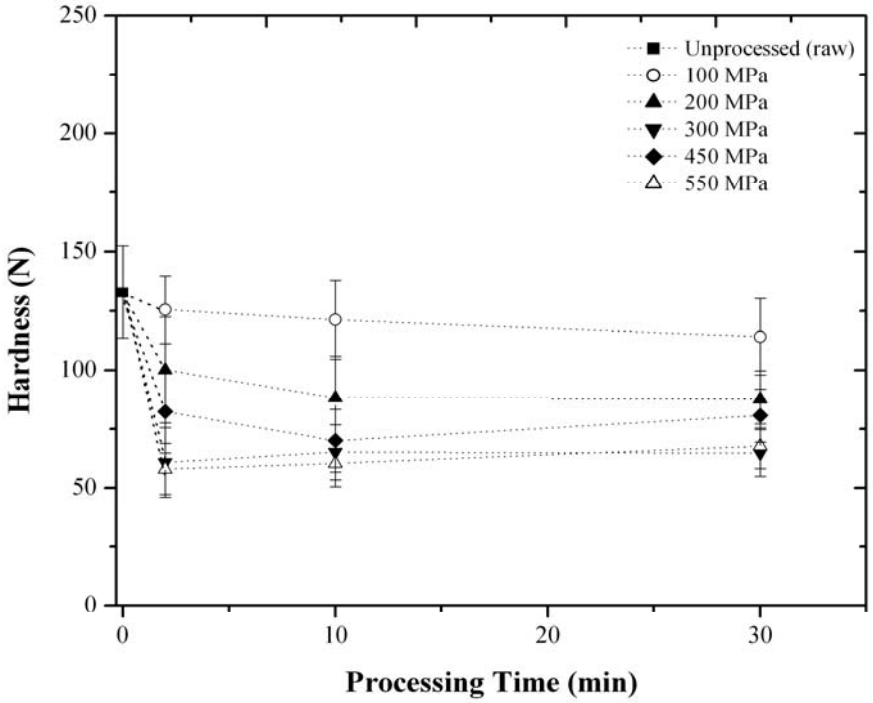

Figure 5.4: Effect of applied pressure and process time on carrot cortex tissue hardness (zero time represents the raw sample).

It is not yet clear why further hardness loss above 300 MPa was not found, but a similar firmness loss in carrots was observed by Michel and Autio (2001), processed at 300

MPa and above. It is important to keep in mind that water under pressure can be compressed by up to 15% of its initial volume at 600 MPa (22°C) (Cheftel, 1995) and air spaces will be effectively eliminated. Therefore, it is expected that above a certain pressure threshold (which will be product structure dependent) the tissue in this case might not be further compressed or be further disrupted during processing. Consequently no further texture loss would be expected. Basak and Ramaswamy, (1998) described the initial hardness loss as an instantaneous pulse softening (IPS). Their study of pressure treated carrots showed an IPS of 13.5% and 47% after 10 minutes at 100 MPa and 200 MPa, respectively, although almost 100% firmness recovery was observed after 60 minutes holding time at 100 MPa. Studies of firmness changes in carrots after heating have also reported a sudden loss of firmness; however this loss was >50% from the initial state within a few minutes of processing. This rapid firmness loss is attributed to membrane disruption, which reduces the cell turgor pressure (Greve et al., 1994b). A sudden 50% firmness loss (as measured by rupture stress) was also found in carrots after cooking between 2 and 20 minutes (Verlinden and De Baerdemaeker, 1997).

During high pressure processing turgor loss and cellular changes can take place including cell conformation changes, cell elongation, cell separation or debonding and/or cell membrane disruption showing as leakage. There are no reports on β-elimination (non-enzymatic pectin degradation reaction) while applying high pressures. This is probably because HPP does not affect covalent bonds (Cheftel, 1995). Studies done on cauliflower, using scanning electron microscopy (SEM) analysis have indicated that at pressures above 200 MPa texture losses occur due to cellular rupture, turgor loss and a disruption in the parenchyma tissue with cell collapse (Préstamo and Arroyo, 1998). Similar results were observed in this research with carrot tissue (Chapter 3). Numerical simulation of mechanical forces acting on yeast cells under high pressure confirms that there can be extensive cell wall disruption between 400 and 500 MPa caused by volume reduction of approximately 15% (Hartmann and Delgado, 2004).

The effect of processing time was also studied for each different pressure level. An increase in hardness was expected by tissue recovery as explained by Basak and Ramaswamy (1998) during the holding time. In this study, extended processing times

(30 minutes) at 300 to 500 MPa showed some increase in hardness of 6 and 10% (Figure 5.4); however, this increase was not significant. This lack of time dependency suggests that loses in hardness is likely to be a result of compression/decompression rather than due to a time dependent structural or chemical reaction, or else that at pressures above 200 MPa, the reactions in cells (e.g. protein denaturation in cell membranes) are complete within 2 minutes processing.

5.3.2 Effect of high pressure processing on texture during cutting

The cutting forces of both the core and cortex tissue of the carrot were also studied. Normally when cutting through a brittle food sample, numerous peak forces appear in the force-displacement graph. In this study, the core was expected to be the more brittle part of the sample due to its composition (lignified cells) and therefore two forces (Force 1 (first peak) and Force 2 (overall peak force)) were identified for each measurement. Figure 5.5 shows how the two identified forces converged for samples processed at 300 MPa and 200 MPa, for 2 and 30 minutes respectively. These results demonstrate an increasing loss of brittleness with increasing pressure. It is interesting to note that processing at 100 MPa caused samples to become more brittle than the unprocessed samples (24% and 12% greater than raw for 2 and 30 minutes, respectively). The cause of this increase in cutting force is not clear but could be due to a change in cellular arrangement after HPP compression without significant cellular damage.

The cortex tissue showed an increase in peak cutting force with increasing pressure. This increase is possibly due to loss of turgor, enhancing the resistance of the tissue to being cut. The tissue became ‘rubber-like’. As the cortex tissue became more ‘rubbery’ there was also a significant increase in displacement at the peak force. This increase in displacement was greater than two fold at 300 MPa compared with untreated samples (Figure 5.6). At higher pressures, no further increase in displacement was observed ($P<0.05$). These results are in agreement with the hardness losses reported in Figure 5.3 and, again processing times did not significantly influence cutting resistance. The cutting action of a wedge shaped blade can be divided into different steps: (1) before entering the specimen; (2) deflection point; (3) cutting into the specimen (force increases); (4) storage of strain energy, when the two halves are forced apart; (5) crack

propagation starts (force falls); and finally (6) crack propagation stabilization (Vincent et al., 1991).

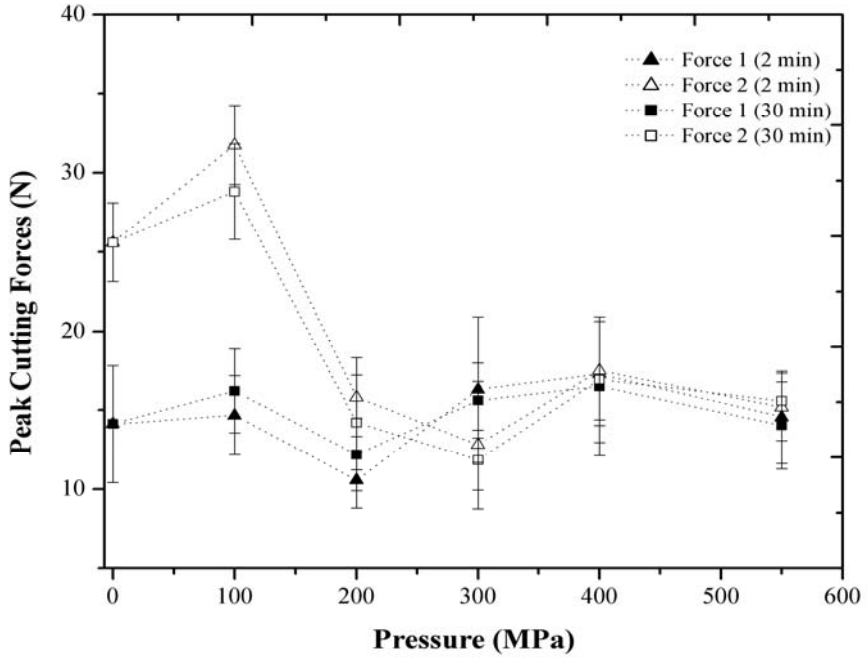

Figure 5.5: First peak (fracturabiliy) (F1) and second peak (peak force) (F2) cutting forces representing brittleness in core tissue processed at different pressure and processing times.

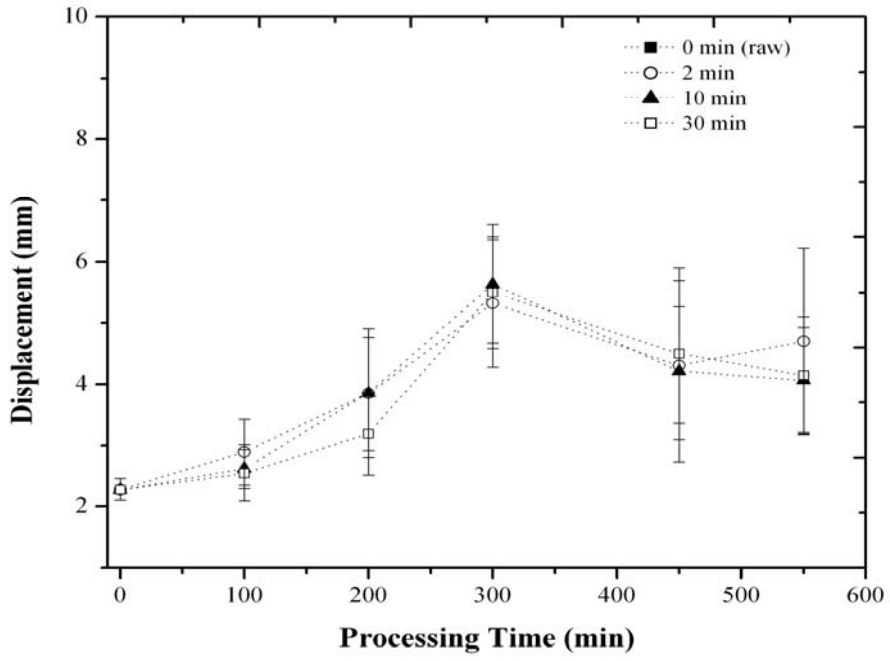

Figure 5.6: Displacement at maximum force for cutting of fresh carrot cortex tissue after high pressure processing at different pressures and holding times measured by the cutting test (zero displacement represents the raw sample).

Figure 5.7 shows the force-displacement profiles for raw and processed carrots. A shift in displacement at maximum force from 2.5 mm to 5.0 mm is evident between the raw and the 550 MPa (for 30 minutes) treated samples. The peak force required to cut through the sample increased from ~21 N for the raw sample to ~43 N for the high pressure processed sample. Statistical analysis indicated significant differences in peak cutting force between samples processed at different pressure levels, while treatment time was not influential ($P<0.05$).

It is thought that higher cutting forces may occur either when the blade has to penetrate a rigid material, or when the material has a more 'rubbery' texture, putting resistance to the penetration. Dowgiallo (2005) confirms that a more deformable material will require a higher cutting force to deform the material. All pressure treated samples exhibited a trend of increasing force with increasing displacement at maximum force, consistent with a more deformable material with less cell integrity and hence having a more rubbery-like texture. Similar force-deformation curves were found by Kato et al., (1997) for both pressure treated carrots (700 MPa, 45 minutes) and carrots cooked for 3 minutes. In contrast, a sharp upright force-displacement curve was found for potato and apple tissue with increasing turgidity after being submerged in mannitol solutions (Lin and Pitt, 1986). Overall, this provides further evidence that textural changes in fresh carrot during high pressure processing at ambient temperatures are mainly caused by membrane damage leading to turgor loss. Tong et al., (1999) explains that turgidity loss is dependent on the apoplastic solute content and water in the tissue. Cell permeability changes in high pressure treated vegetables increase the transport of solutes from the inside to the outside of the cell (and vice versa) resulting in a 'soaked' appearance after processing (Préstamo and Arroyo, 1998). A soaked carrot appearance was also observed in this research after pressure treatments.

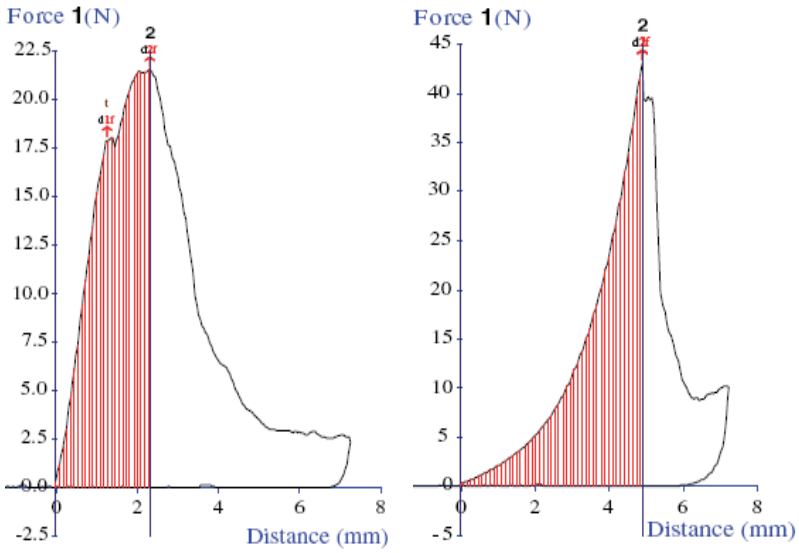

Figure 5.7: Cutting force-displacement curves for raw (left) and high pressure processed (550MPa, 30 minutes) carrots (right).

In this study, evidence of turgor loss due to cellular leakage was found in samples processed at 550 MPa for 30 minutes. This is similar to the behaviour reported in Chapter 3, where damage was indicated from the measurement of electrolyte leakage. To understand whether cellular leakage (be it intracellular or intercellular) after high pressure processing was a major contributor to texture changes, comparisons were made between the texture of partially desiccated samples (placed in a desiccator at RH = 75%, 20°C) and pressure treated samples (100 to 550 MPa, 2 minutes). Pressure treated samples lost up to 50% hardness with a maximum of only 5% mass loss (presented as leakage). The cutting test showed an 11.6% increase in displacement at maximum force for raw samples which had lost ~14% of their initial mass (by drying), while high pressure treated samples showed a 36% displacement increase after losing only approximately 5% of leaked liquid (Figure 5.8). This large increase in deformation shown as displacement for pressure treated carrots can be explained by liquid being pushed out of the cells under compression forces. This is because the more permeable membranes leak after losing membrane integrity, while dehydrated samples may have lost free available water but still contain intact cell membranes.

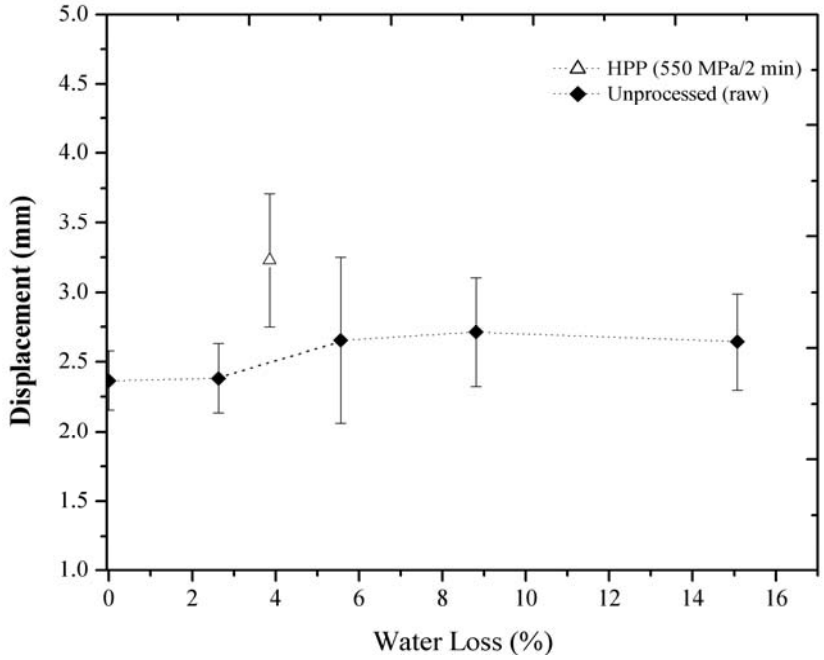

Figure 5.8: Cutting deformation changes in carrots cortex submitted to gentle drying and high pressure treatment (550 MPa/2 minutes).

To better understand the differences in textural changes between raw and processed carrots we can imagine the carrot tissue being composed of cells that are plastic ellipsoids packed together with air and water in the voids between the cells. When the system is submitted to gentle drying conditions, most free water between the cells will be removed first but not to the extent to cause cell lysis. Studies of water loss by drying demonstrated that small percentages of water loss are sufficient to damage the plasma and tonoplast membranes, but not sufficient to produce lysis (Lewicki and Pawlak, 2003). Therefore cell walls become more rigid in the initial phase of drying and slowly tend to collapse under stress during shrinkage. In contrast, cells of pressure treated samples were compressed against one another, producing instantaneous deformation and some disruption, resulting in greater relative electrolyte leakage (Chapter 3) and hence turgor loss. As a consequence, larger texture changes were associated with pressure treatment than with dehydration. The patterns of cell fracture after cutting samples with the blade correlated with their respective compression results (Figure 5.9). Images showed that damage to raw carrots consisted mainly of cleavage of individual cells (broken-through), due to their higher turgidity and strong cell adhesion (Figure 5.9A). The less turgid cells in pressure treated samples presented more evidence of cleavage between cells. This is attributed to cell-cell debonding, which is typical of a

weakened middle lamella (Figure 5.9B). Similar observations were made by Verlinden et al., (1996) for cooked carrots.

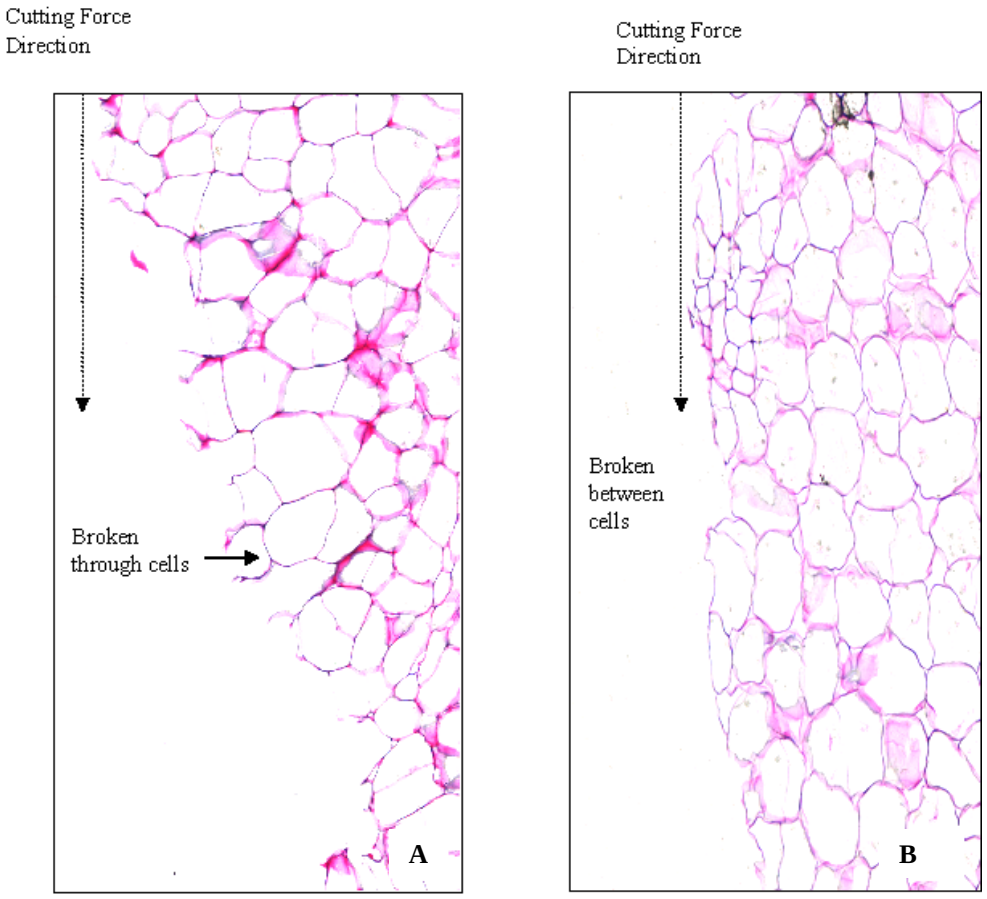

Figure 5.9: Light microscope images of tissue damage when cut with a blade during a cutting test. 'A': untreated raw carrot cortex tissue; 'B': pressure treated carrot cortex tissue (550 MPa/30 minutes).

Figure 5.10 shows a reasonable linear correlation between hardness and the percentage of broken-through cells. The inset graph shows how the percentage of these cells decreased as pressure increased, falling to 15% at 550 MPa. Interestingly, there was a major decrease in the percentage of broken-through cells at 200 MPa (Figure 5.10 left corner), which could possibly represent where the tissue break point was exceeded as observed in Chapter 3 and 4.

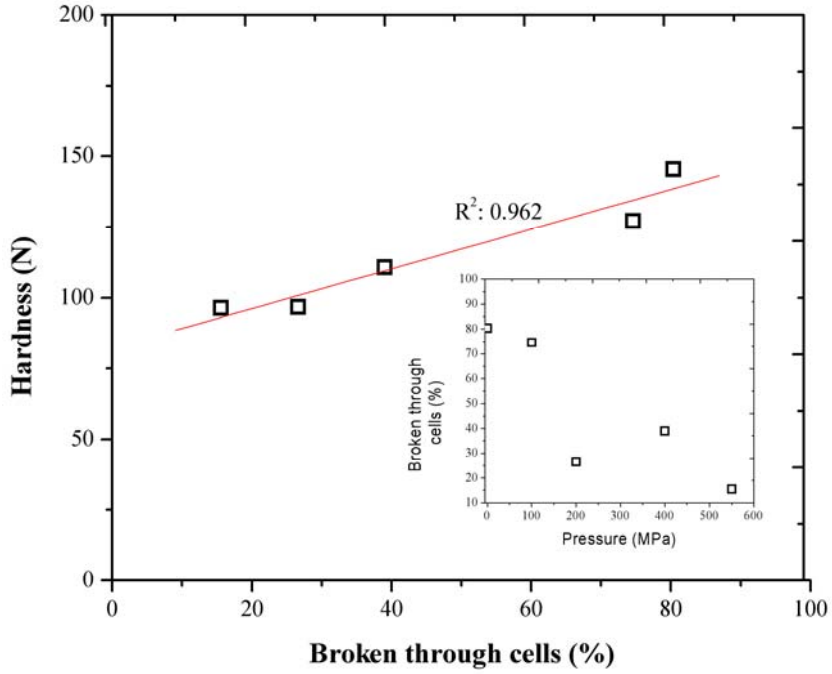

Figure 5.10: A relationship between tissue hardness and percentage of broken-through cells was observed, with the percentage of broken through cells against pressure (right corner graph).

The above results have shown significant textural changes, such as hardness losses, cutting force changes and evidence of rubberiness after the application of high pressures. These changes relate to the overall tissue structure of carrots. The results do not provide information of what is actually happening to the cells that form that tissue. The following section aims to identify the main cellular shape changes that occurred and to quantify those changes to find correlations with macro textural measurements.

5.3.3 Microstructural changes

5.3.3.1 Structural changes to cell walls

Microstructure images of raw and processed cortex tissue are presented in Figure 5.11. The raw carrot images show a more organized cell distribution and a higher degree of cell to cell contact throughout the tissue. Pressure treated samples show evidence of cell wall buckling and folding, and a reduction of cell to cell contact. A more loose and irregular matrix was obtained after processing with increased cell separation, presumably as a result of middle lamella breakdown.

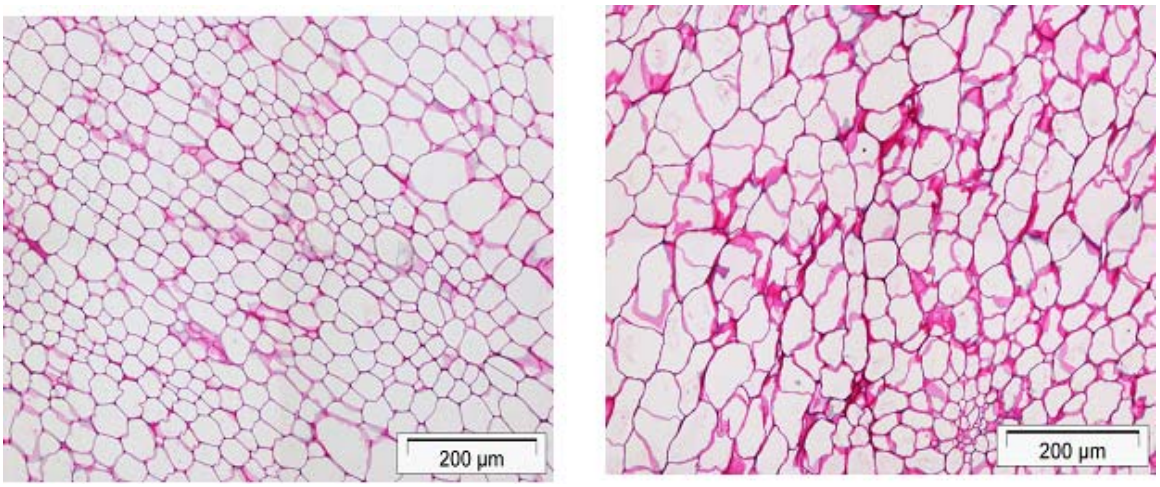

Figure 5.11: Light microscopy images of carrot cortex tissue from raw (left) and pressure treated (300 MPa/2 minutes) samples (right).

Histograms of geometric parameters (shape factor and elongation) obtained by image analysis indicated that at low pressures (100 to 200 MPa / 2 minutes) cell walls were only slightly more disrupted and disorganized compared to the raw samples, while at 300 and 400 MPa (for 2 minutes) greater differences were observed. A definite increase in cell wall thickness appeared in HPP samples. This could be explained by increased cell wall folding during compression. Extended processing times (10 and 30 minutes) resulted in further cell wall changes, suggesting that perhaps there are some chemical reactions occurring in cell walls during this holding time or that simply cells are still re-organizing or recovering during holding time.

In general, despite the evidence of considerable cell wall conformational changes due to high pressure, few broken areas were observed. Similar observations were made for steamed carrots (Kidmose and Marten, 1999), while cell damage and middle lamella separation was found in slightly cooked carrots by Fuchigami et al., (1995). Hartman and Delgado (2004) explained that most cell wall damage during high pressure processing is generated by excessive strain on membranes and stress on cell walls.

5.3.3.2 Shape factor

A shape factor of 1 indicates a spherical particle, while a value less than 1 represents a less regularly shaped particle. The shape factor analysis on core tissue showed very similar frequency distributions for raw and 100 MPa samples, while above this pressure particles shifted towards less spherical values, especially for samples treated with 500

MPa for 2 and 10 minutes (Figure 5.12). As processing time increased to 30 minutes, there was a shift for all treatments towards less spherical shapes with an average shape factor below 0.5. The more homogeneous trend observed in graph C could be the result of cells becoming more compacted with each other as processing time increased, leading to a new cellular arrangement in the tissue.

The shape factor analysis of cortex tissue showed the highest frequency percentage at 0.75 for raw samples. A clear shift in the distribution towards lower values was observed with increasing pressure during treatment, with values of 0.45-0.50 showing the highest frequency for 300 and 550 MPa treatments (Figure 5.13)

These shifts of cell shape factor are attributed to the compression and decompression effects on the tissue system during processing. Similar trends were found in frozen carrots pre-treated with high pressure (300 MPa, 15 minutes at 60°C with calcium soaking) in comparison with a fresh control (Van Buggenhout et al., 2006).

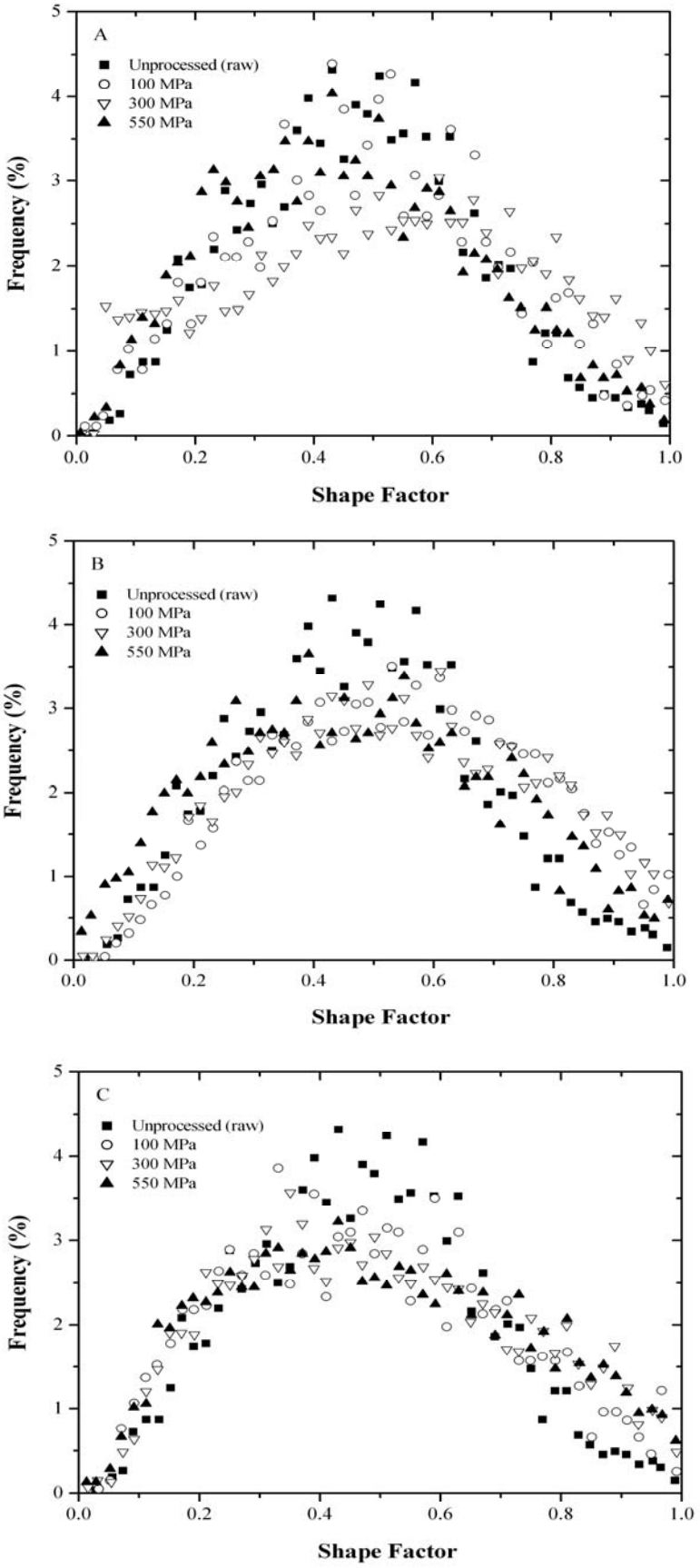

Figure 5.12: Shape factor analysis of carrot core cells treated at different pressure levels for 2 minutes (A), 10 minutes (B) and 30 minutes (C).

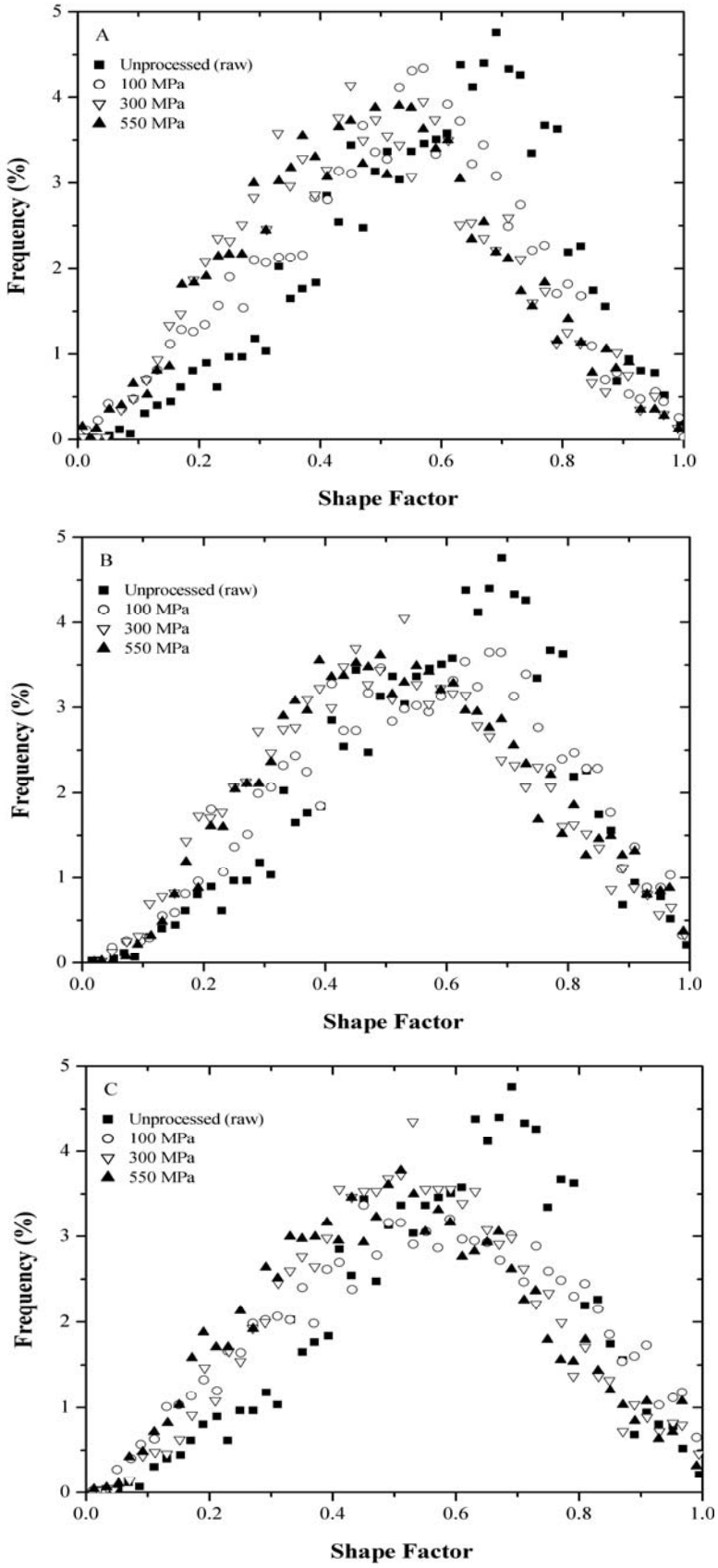

Figure 5.13: Shape factor analysis of carrot cortex cells treated at different pressure levels for 2 minutes (A), 10 minutes (B) and 30 minutes (C).

5.3.3.3 Elongation

Figure 5.14 shows the elongation index of carrot core cells treated at different pressure levels for 2 minutes (A), 10 minutes (B) and 30 minutes (C). Elongation indices ranged from 1 to 20, where 1 represents a more spherical particle, while 20 represents a highly elongated particle. Most cells had elongations between 2 and 4. The highest frequency was found to be around 40% for samples processed at 100 and 300 MPa for 2 minutes. As time increases from 2 to 30 minutes, cells started to become more elongated shifting towards the right which means higher elongation factor of 6 (A, B and C).

Raw cortex tissue showed evidence of less cellular elongation than did the core tissue, presenting a larger frequency (43%) around an elongation of factor 1 (Figure 5.15).
The evaluation of the holding time effect showed this to be similar to that observed for the core tissue. Elongation of cells in the cortex tissue increased as HPP processing time increased, presenting the biggest shift towards the right (more elongated) at 30 minutes (Figure 5.15). These observations might seem contradictory to the previous hardness data, which showed no time effect on this texture characteristic (Figure 5.3). However, this could indicate that even though hardness changes might not result from conformational changes, cells might still be reorganizing their shape during extended holding times, this not necessarily leading to tissue failure. The results also suggest that hardness may mainly be attributed to turgor pressure which is lost due to cell membrane disruption, and therefore the residual hardness is attributed to what is left in strength from the cell wall structure and junctions within the tissue.

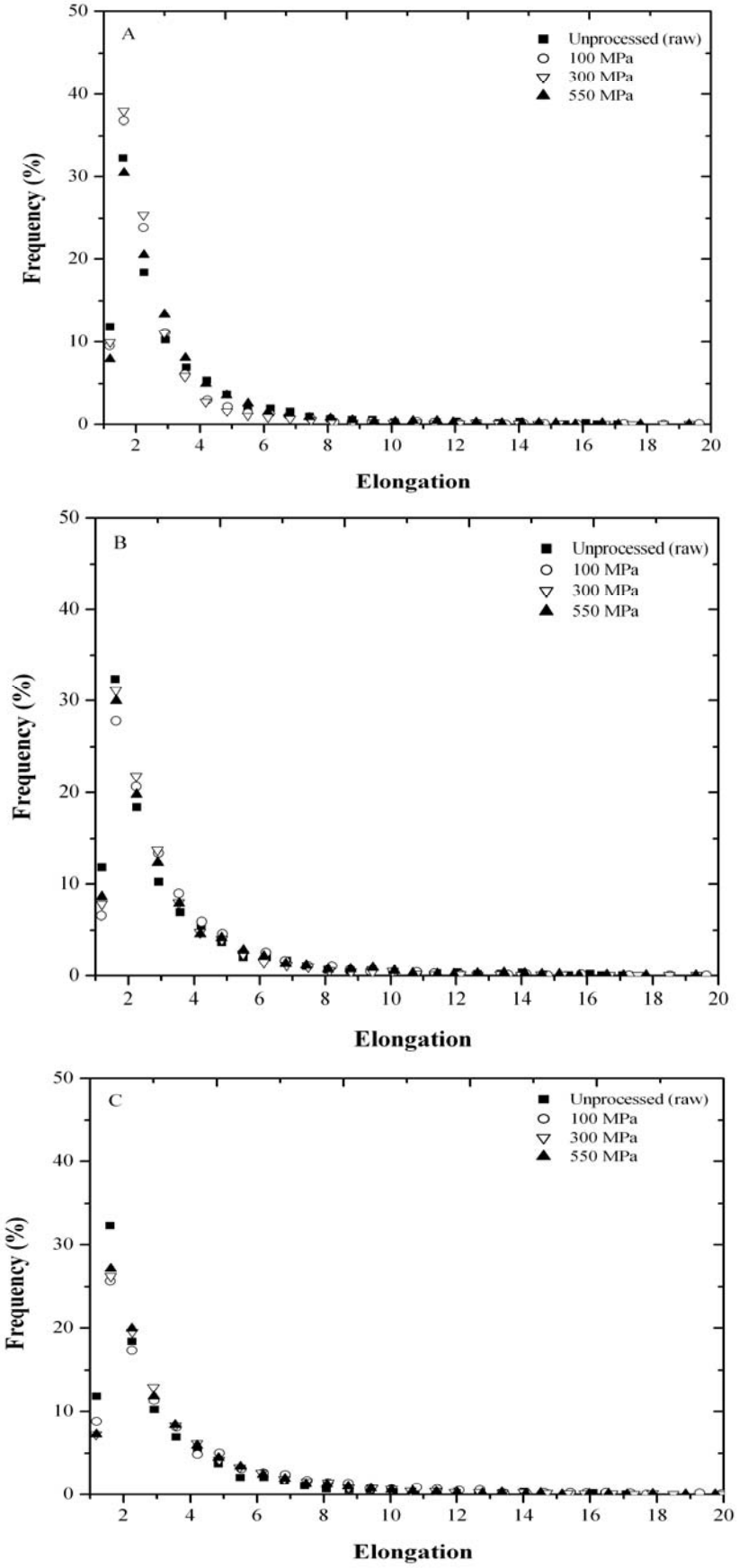

Figure 5.14: Elongation index of carrot core cells treated at different pressure levels for 2 minutes (A), 10 minutes (B) and 30 minutes (C).

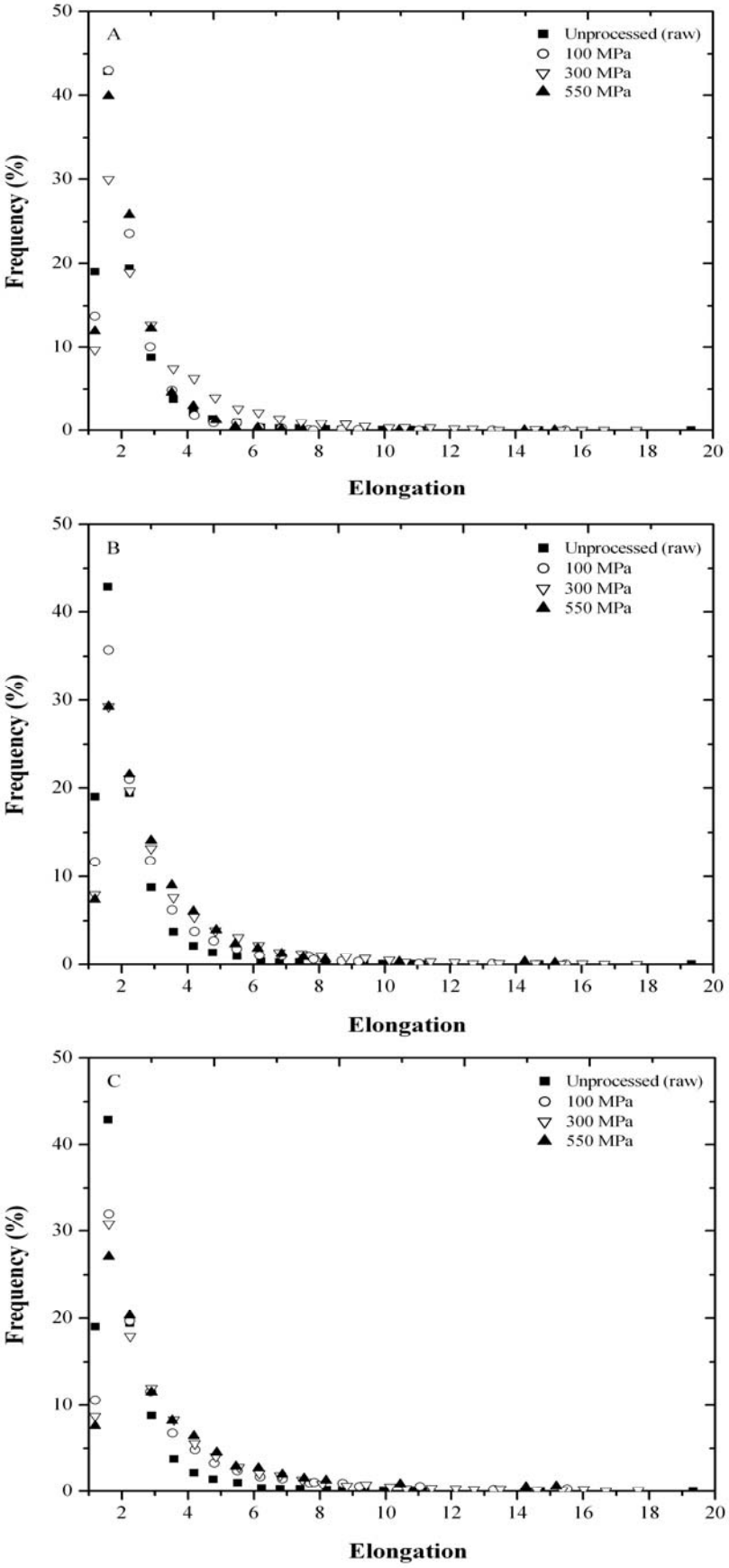

Figure 5.15: Elongation index of carrot cortex cells treated at different pressure levels for 2 minutes (A), 10 minutes (B) and 30 minutes (C).

Overall, the cell distribution analysis helped to capture general trends in cellular structure. The cell distribution showed that most pronounced changes occurred in samples treated at 300 MPa and 550 MPa. This correlates with previous analysis of hardness and cutting forces. Holding time during the application of high pressures does have an effect on the cellular structure, showing larger deformation of cells as time increased. As explained previously by Perrier-Cornet et al., (1995), during holding time cell volume will still decrease, allowing the transfer of water and internal solutes to exit the cell. This is an important finding, as it was not necessarily expressed as a hardness change in our study; supporting the conclusion that most hardness losses are attributed to turgor loss (due to cell membrane disruption) and less due to the actual cellular conformation at the studied conditions.

5.3.4 Biochemical changes

Softening of plant tissue during storage can be explained by pectin solubilisation and the release of low molecular weight uronides from cell walls (Fennema, 1996). As previously described in Chapter 2, pectins are formed from galacturonic acid that can be esterified to various degrees. This is called degree of esterification (DE) or degree of methylation (DM) (Baker et al., 2005). The degree of methylation will vary depending on the product, process and extraction; for example commercial pectin may have a DM ranging from 20 to 70%. In this study 55% DM was observed, while Sila et al, (2004) obtained around 75% in raw carrots.

One of the major advantages of the application of high pressures is the reduction of texture loss after subsequent heat treatment in comparison to just heating (cooking) (Ludikhuyze et al., 2002). Even though pectin can still be solubilised from cell walls when applying high pressures (Kato et al., 1999b), there is no promotion of the beta elimination reaction, which is the splitting of glycosidic bonds (covalent bond) of pectins that leads to softening in cooked vegetable products (Fennema, 1996). Studies done on the application of high pressure and cooking of carrots by Sila et al., (2004), have shown that by activating PME at 60°C, pectin will be de-esterified and calcium ions will bind to them forming a network that will consequently reduce softening. Fennema (1996) explains that at 60-70°C some vegetables can also liberate calcium previously bound to cell wall/middle lamella, facilitating interactions that would lead to improved texture.

Two possible mechanisms are expected to occur when applying high pressures: (1) to activate PME activity, reducing the degree of methylation and, if interaction with calcium ions occur, less texture loss is expected to happen. Or (2) PME will be inactivated, and the degree of methylation would not change and therefore there would be no enhancement of calcium bridges formation.

Figure 5.16A shows the measured residual PME activity in the core tissue. High levels of variability within treatment were observed, this was probably because of proportions of lignified cells within the tissue. However, the results still showed a decrease in residual PME activity of 20 % after pressure treatments of 550 MPa, 2 min. Figure 5.16B presents the residual PME activity in the cortex after several pressure-time treatments. A marked reduction of 50% was observed after 30 minutes processing at 550 MPa. Results published by Sila et al., (2004), showed an 80% reduction in PME activity at 500 MPa and ambient temperature conditions.

Other findings on PME have indicated that differences in results may depend on the product variability and on the extraction methods used. For example PME activation in orange juice was observed by Cano et al., (1997) when using high pressures between 200 and 400 MPa. Partial PME inactivation was found by Basak and Ramaswamy (1996) when pressure treating orange juice and various vegetables between 100 and 400 MPa with holding times of up to 60 minutes (Basak and Ramaswamy, 1998). PME inactivation in orange juice has been shown to be holding time dependent (600 MPa, 1 to 15 minutes) when working at low pH (3.0), but not at a higher pH (4.1) (Sellahewa, 2002).

Inactivation of PME at high pressures can be due to the rupture of the configuration of the protein molecule (Riahi and Ramaswamy, 2003). In general the results observed for Figure 5.16B indicated a clearer trend where high pressure processing effects on PME can be divided into lower pressure activation (100 to 300 MPa) and higher pressure inactivation (400 - 550 MPa). These results are consistent with the ones reported previously by Hendrickx et al., (1998).

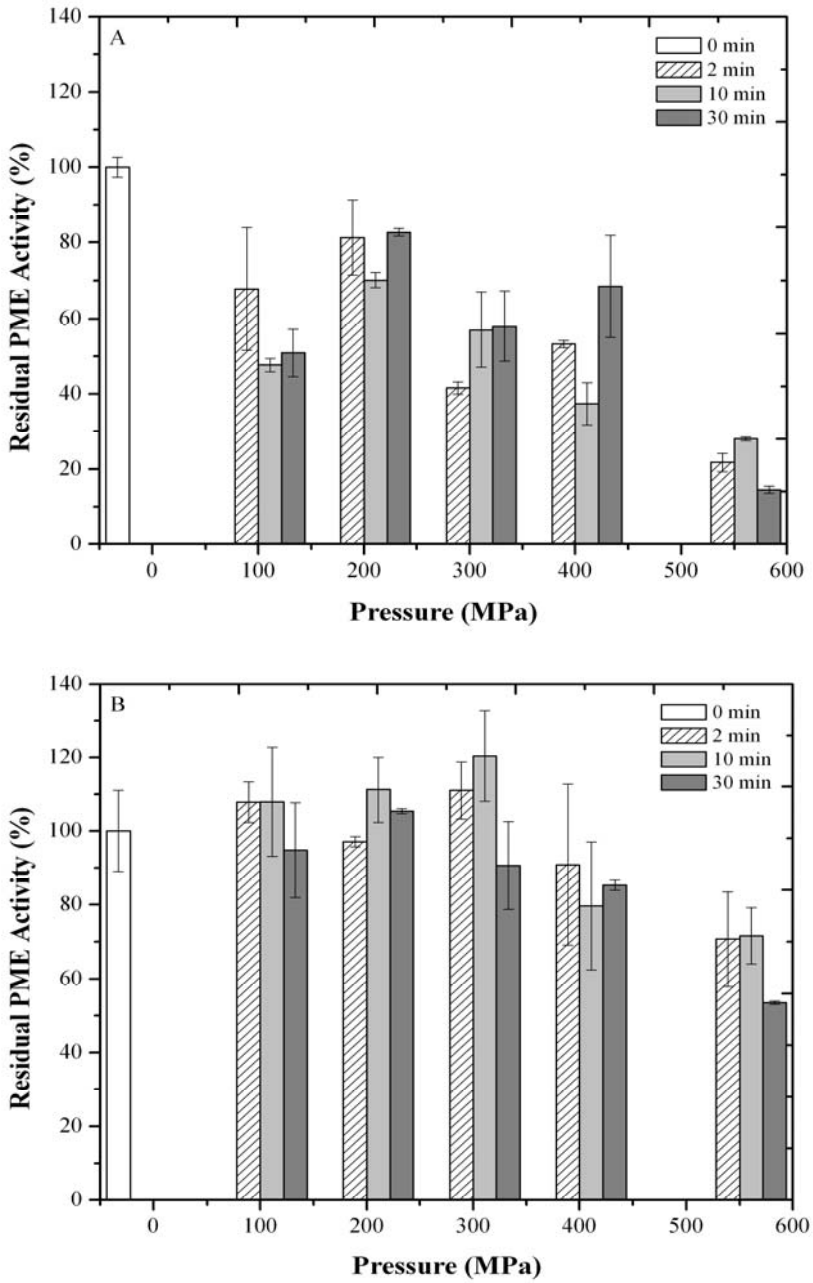

Figure 5.16: Residual PME activity of carrot core (A) and cortex (B) tissue treated at different pressures and processing times (relative to activity of corresponding raw tissue).

Despite high levels of sample to sample variability, the degree of methylation (DM) results shows in Figure 5.17A correlate with PME activity. After treatments at 400 MPa for 30 minutes, the degree of methylation or deesterification in the core was reduced by over 50% in comparison to the raw sample. This reduction could relate to an increase in activity of PME in the core, rather than a decrease in galacturonic acid extraction.

Figure 5.17B shows a decrease in the degree of methylation in the cortex tissue as pressure increases. There seems to be a relationship between PME (Figure 5.16B) activity and the degree of methylation. Cortex carrot tissue still showed some residual PME activity at 400 MPa which explains the decrease in DM. This was also observed at 550 MPa. Results observed by Ng and Waldron (1997) indicated a decrease in degree of methylation for treatments at 200 to 400 MPa compared to non-treated raw samples.

Overall, the trends in carrot enzyme activity and DM showed less effect of pressure in the core tissue than in the cortex, probably due to the lignified structure of the cells.

Verification of the limited extent of pectin-calcium binding was obtained in a separate experiment. Cortex tissue samples were immersed in calcium chloride solutions (0.5 % w/v) and processed for 30 minutes at different pressures. The results indicated that the addition of calcium in this manner did not increase hardness in samples treated above 200 MPa. However, raw and 100 MPa treated samples showed a significant increase in hardness after calcium soaking (Figure 5.18).

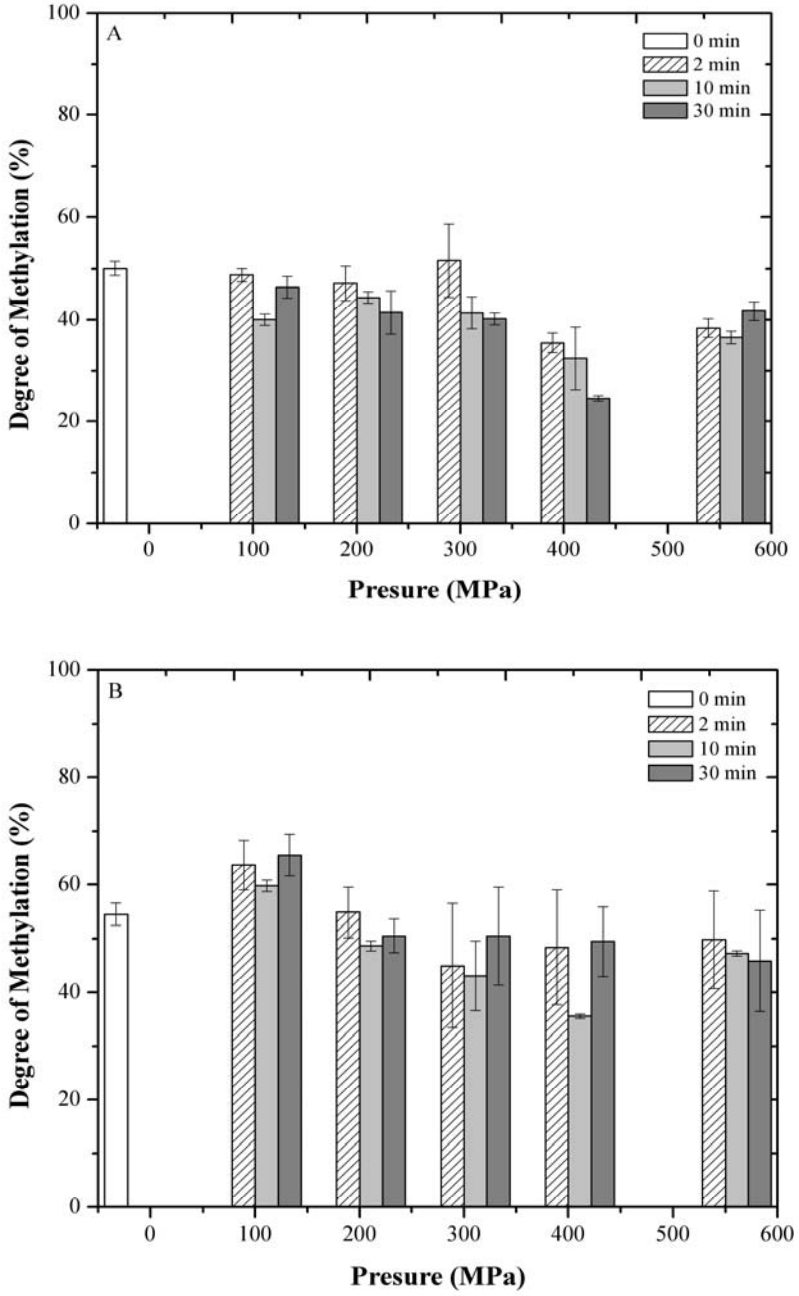

Figure 5.17: Degree of methylation of pectin for carrot core (A) and cortex (B) tissue treated at different pressures and processing times.

This increase could possibly have been due to the fact that the carrots studied had 50% of methylated pectins available to crosslink, as well as a non-damaged cellular structure providing some turgor. While at pressures of 200 MPa may still have had some degree of methylated pectins to be crosslinked by calcium, their cell membranes were now disrupted and so they were less able to hold the structure together leading to lower hardness values. Previous hardness results showed that raw cortex tissue was not significantly different from 100 MPa samples (Figure 5.4). This confirms that when

cell walls present only slight damage and some cross-linkage with calcium, hardness may be improved (similarly observed by Massey and Woodmans, 1973) while damaged cells after HPP treatments (≥200 MPa) would be less able to cross-link and present more irreversible disruption; therefore, no hardness improvements were found.

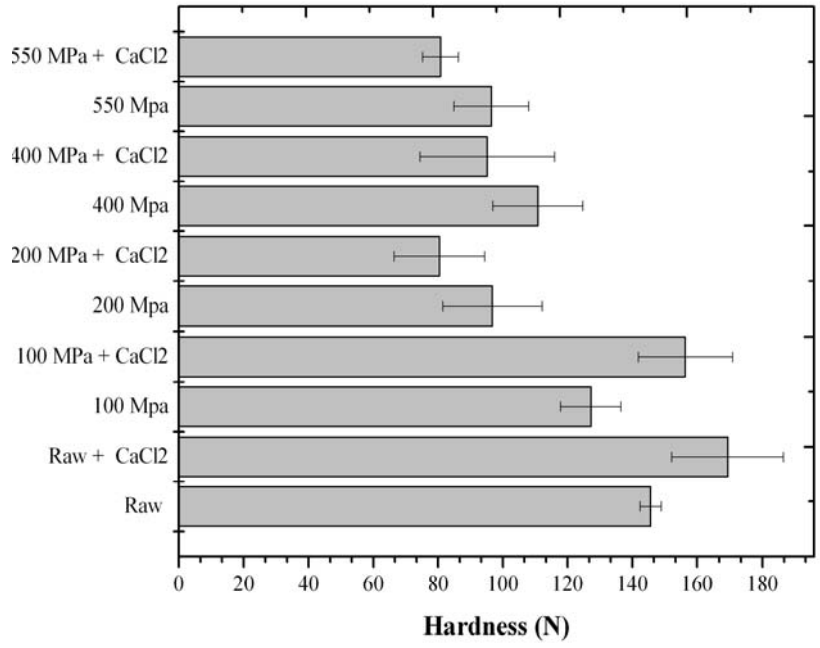

Figure 5.18: Comparison of cortex tissue hardness before and after immersion in calcium chloride solutions (0.5 % w/v) and high pressure processing (100-550 MPa, 30 minutes).

This leads to the conclusion that textural changes in fresh carrots treated with high pressure are mainly caused by direct tissue damage by cell membrane disruption with consequent turgor loss, rather than biochemical changes (under the conditions investigated in this research).

5.4 Conclusions

In this chapter, an approach to a better understanding of textural changes had been discussed by linking microstructure and biochemical changes related to texture of carrots processed over a range of pressures (at ambient conditions). Results indicated that observed textural changes are primarily caused by the very rapid changes in hydrostatic pressure (compression and/or decompression) promoting turgidity loss. This study also shows the difference in texture loss between the core and the cortex tissue, which are known to be formed by different cell types.

Some biochemical changes were observed; however, no calcium cross-linkage occurred, which confirms that primary texture losses are due to loss of turgor under the studied conditions. This conclusion was confirmed by the limited influence of pectin-calcium binding. Sudden and significant reductions in tissue hardness were found with relatively mild treatments (100 MPa, 2 minutes). There was limited evidence of tissue (cortex) recovery following 30 minutes of processing at pressures of 300 to 550 MPa. Microstructural analysis identified indices strongly correlated with hardness changes in the cortex tissue, while the core showed little cellular change. In general the data obtained allowed better understanding of the 'flexible and rubbery-like' texture resulting from application of high pressure to fresh carrots. Furthermore this research demonstrated the importance of the cellular structure of the tissue on texture parameters. In this case, the core tissue showed less cellular change due to its lignified composition resulting in a harder structure in comparison to the cortex.

From the technological point of view, changes in texture (under the conditions studied) are primarily due to the direct impact of the hydrostatic pressure application and not to biochemical reactions. Texture loss could possibly be overcome by manipulating the turgidity of the cells before pressure treatment. Alternatively, texture loss could be reduced through activation of PME during high pressure processing at elevated temperatures using an optimal combination of pressure and temperature.

There is now a strong body of experimental data showing significant textural changes resulting from high pressure processing. There is also evidence that structural changes are similar to those occurring during mild thermal treatments (or pre-treatments). Given the importance of the pressure magnitude and relative insignificance of process duration, investigation is warranted into the extent to which texture loss could be ameliorated through controlled rates of compression and decompression. Unfortunately, this is not possible on most equipment available at this time. Given the significant textural changes observed, it is also important that further studies be conducted on the responses of consumers to high pressure processed horticultural products to better identify potential commercial opportunities, or limitations of this process.

6 Structural and textural changes in carrots subjected to weight loss and osmotic manipulation before and after high pressure processing

6.1 Introduction

Alterations of fresh produce cell walls, middle lamella and cell turgor pressure by loss of membrane integrity or water can change the mechanical properties of plant tissues (Dolores and Canet, 2002). Turgor pressure (or pressure potential), which represents the hydrostatic pressure inside the cells, is the major contributing factor to tissue stiffness (Herppich et al., 2004). Its excess is the difference between the osmotic pressure of the intercellular fluid cell contents and the external environment (Falk et al., 1958; Ramana and Taylor, 1994). When tissue loses its turgor, it also loses its internal stress and therefore it becomes more flaccid. However its resistance to compression can remain constant or may even increase (Vincent, 1990). If a plant material has lower osmotic pressure than its medium it will swell due to water ingress by osmosis, while tissue with higher osmotic pressure than the medium will shrink due to plasmolysis (Escalada Pla et al., 2006).

Loss of turgidity was found to be the main cause of hardness loss when carrots were high pressure processed above 200 MPa (Chapter 5). The loss in turgidity was attributed to cellular structural changes and cell membrane disruption, as evidenced by increased relative electrolyte leakage (Chapter 3). However no evidence was gathered to demonstrate whether or not the tissue could maintain or regain its turgidity after pressure treatments or if controlled weight loss (prior to processing) would reduce the initial turgor pressure, allowing the tissue to better withstand the pressure treatment.

Studies done on carrots during storage have indicated that carrot tissue subjected to less than 5% (w/w) weight loss showed decreased compressive Young's Modulus and fracture work, while above 5 % (w/w) weight loss both parameters increased (Nielsen et al., 1998). Previous studies have also indicated that cell plasmolysis prior to cooking could help to overcome firmness losses in carrot tissue (Greve et al., 1994b).

The present study aimed to measure the effects of turgidity changes before and after HPP by two approaches: firstly, by analysis of the influence of prior weight loss on turgidity loss after high pressure processing (at ambient temperatures) in carrot tissue; secondly, by examining if tissue damage by high pressure or dehydration processing is reversible or irreversible by immersing samples in mannitol solutions before and after processing.

6.2 Materials and methods

6.2.1 Plant material

One kilogram bags of pre-packed (polyethylene) carrots (*Daucus carota* L., var. 'Stephano') were purchased at the local Sydney market and stored at 1.0 ± 0.5 °C. The following day, carrots were labelled and stored in their opened bags resulting in weight loss of circa 0.6 % day^{-1} at 5.0 ± 0.5 °C (RH: 92-97%). Daily weight monitoring of carrot bags was performed using electronic scales (Mettler Toledo PR 5002; Switzerland (±0.01g)). Two bags were removed for analysis at various storage durations providing samples with 0 (fresh), 0.7, 1.3, 4.4, 7.7 and 11.4 % (w/w) weight loss (~20 days in total) (Table 6.1).

6.2.2 High pressure treatment

The ends of each carrot were cut and discarded and the middle section used to obtain 10 mm x 10 mm cylinders with a cork borer (mainly from the cortex parenchyma tissue). Thirty two cylinders were randomly selected and vacuum packed for each treatment. Samples were subjected to various destructive measurements: 10 for compression force (Compressive Young's Modulus CYM), 10 for cutting force, 5 for juiciness assessment, 5 for volume change and 2 for microscopy. For each weight loss level, six process options were investigated: raw (unprocessed), 100, 200, 300, 450 and 600 MPa all for 2 minutes (Table 6.1). High pressure processing was performed at ambient temperatures (18-20°C), using a 2L high pressure vessel (Flow International Corporation, USA), with a compression time of less than 10 seconds and a decompression time of 5 seconds.

6.2.3 Texture measurements

Texture tests were carried out using a TA-XT2 texture analyser (Stable Micro Systems, Surrey, England). Upright carrot cylinders were placed on the texture analyser platform and compressed uniaxially direction using a flat cylinder probe (75 mm diameter) with a

compression speed of 1 mm.s^{-1} to 30% maximum strain (based on the sample height of 10 mm). The relative compression Young's modulus was estimated as the slope of the first third of the stress-strain curve and normalised relative to the raw samples. Peak force during cutting was measured using a stainless steel blade (TA-XT2 blade fitting) cutting through the sample at 1 mm.s^{-1} speed to 75% strain and was also reported relative to the raw samples.

Table 6.1: Process steps for experimental analysis on carrots.

Turgor pressure manipulation by (1): Weight loss prior to HPP.

(2): Mannitol immersion after HPP.

Experiment 1	**(1) Weight Loss / HPP**	Texture Microscopy Juiciness Cell Leakage
Raw carrot (0% - 11.4% weight loss)	No HPP / HPP (100 - 600 MPa, 2 min) →	
Experiment 2	**(2) HPP / Mannitol immersion**	
Raw carrot (with 11.4% weight loss)	No HPP / No mannitol	
Raw carrot (with 11.4% weight loss)	No HPP / 0.0* - 0.8 M mannitol →	Texture Microscopy Specific volume Juiciness
Raw carrot (with 11.4% weight loss)	HPP 100-600 MPa / No mannitol	
Raw carrot (with 11.4% weight loss)	HPP 100-600 MPa / 0.0 - 0.8 M mannitol	

* In distilled water

6.2.4 Juiciness and cellular leakage

Juiciness of carrot cylinders was determined by a method modified from Paoletti et al., (1993). Firstly, the surface of each sample was dried using tissue paper. Two filter papers (Whatman N°1, 55mm diameter) were then weighed, followed by weighing the sample (Mettler Toledo PB 303, ± 1.0 mg). The sample was then placed between the two filter papers and compressed using the TA-XT2 texture analyser at head speed of 1 mm.s^{-1} to 80% strain. The compressed samples were discharged and the filter papers (with absorbed juice) were weighed immediately (Mettler Toledo PB 303, ± 1.0 mg). The difference in mass of the filter paper before and after compression divided by the total sample mass was used as a measure of the sample's juiciness. Five replicates of each measurement were performed.

Cellular leakage (extruded out of the cells during HPP treatment) was collected from the sample bag and weighed immediately into a pre-weighed 50 mL beaker.

Leakage was expressed as percentage of the sample batch weight prior to processing. Leakage values were the result of only one measurement (after HPP processing).

6.2.5 Microscopy

Carrot cylinders were cut into halves transversally (5 mm height), fixed in 4% neutral buffered formalin for 72 hours at room temperature, then dehydrated in ethanol solutions (50-100 v/v %) using a tissue processor (Leica TP 1050, Germany) and embedded in paraffin wax using a tissue embedder (Leica E61160, Germany). For histological observations, embedded samples were cut into 5 μm sections and stained with Ruthenium Red (0.02%) for 1 minute (modified from Sabba and Lulai, 2002). Images were captured using a light microscope (x10) (Leica DMLB, Germany) with a Nikon camera (DS-5M, Japan). Duplicates of each treatment were embedded and two images per sample were photographed, giving a total of four images per treatment.

6.2.6 Specific volume change

After carrots had lost ~11.4 % of their initial weight they were tested for specific volume change in cells. Carrot cylinders were manipulated by immersion in 500 mL beakers with D-mannitol ($C_6H_{14}O_6$; Sigma Chemical Co, USA) solutions of 0.0 (i.e. in RO (reverse osmotic) water), 0.1, 0.2, 0.4, 0.5, 0.6, 0.7 and 0.8 M in a ratio of 1:3 (sample: solution w/w) to study turgidity changes (Lin and Pitt (1986); Alvarez et al., (2000); Bajema et al., (1998); Ramana and Taylor (1994)). Solutions were buffered with 0.02 M K_2HPO_4 and 0.02 M KH_2PO_4 to prevent tissue degradation during soaking (Lin and Pitt, 1986). Based on preliminary testing and the findings of Bajema et al., (1998), samples were immersed for 24 hours at ambient temperatures (Georget et al., 2004). Assuming that the volume change of the tissue is proportional to the cell volume change (Lin and Pit, 1986; Ramana and Taylor, 1994 and Alvarez et al., 2000), sample volumes were calculated using the Archimedes principle of water displacement using a scale (Mettler Toledo PB 303, ± 1.0 mg). Specific volume ($cm^3.kg^{-1}$) changes of carrot tissue samples were calculated by using the sample's volume and weight (5 replicate /treatment).

6.2.7 Statistical analysis

Significant differences between treatments were determined using analysis of variance (ANOVA) and Tukey's test for pair wise comparisons with a confidence interval of 95%. Analysis included the interaction effects of weight loss-pressure and mannitol immersion-pressure. All results are presented with standard error bars (SE), except cellular leakage, which is presented as one measurement per treatment.

6.3 Results and discussions

6.3.1 Effects of weight loss prior to HPP on tissue turgidity

Tissue stiffness, cutting force, cell leakage and juiciness were measured to analyse the effects of weight loss prior to high pressure treatments. This was based on previous literature that indicated that cells already exhibit elasticity to control turgor pressure (Greve et al., 1994b); therefore weight loss would make cells more flaccid and potentially leaving them more able to withstand the application of high pressures. This part of the study aims to investigate if cell membrane damage could be reduced by prior weight loss. Furthermore, if cells are able to regain turgor after high pressures, then this would confirm reversible damage and at what conditions this would happen.

The effects of weight loss on tissue stiffness were described by the compressive Young's modulus (CYM) in Figure 6.1. The stiffness of samples which did not undergo high pressure treatment showed reductions of almost half after losing 7.4 -11.4 % of their initial weight. This is a direct result of reduced turgor. Falk et al., (1958) explains that the decrease in stiffness with increased weight loss is a result of loss of turgidity in the tissue. Turgidity is lost when cells lose water, decreasing their volume; this volume decrease depends on the elasticity of the cell wall for each tissue (Lambers et al., 1998). Evidence of this was found by Nielsen et al., (1998) when storing carrots for 19 weeks at 1°C and 97% RH, which resulted in stiffness reductions from ~8.5 MPa to ~6 MPa after weight loses of ~5.6% w/w to 21.8% w/w respectively. Similar reductions of 1.5 MPa in stiffness was found in this study between 4 and 8 % w/w weight lost.

Analysing the effects of HPP at zero weight loss (fresh) showed that low pressure treated (100 MPa) and unprocessed (raw) carrots were not significantly different in

stiffness, but significantly higher than samples processed at ≥ 200 MPa. This is probably due to significantly increased permeability of plasma membranes and decompartmentation of cells as also observed in plant cell culture above 200 MPa by Dörnenburg and Knorr (1998). Previous studies found a 'tissue break point' between 100 and 200 MPa in carrot tissue subjected to high pressure (2 min) by a significant increase in electrolyte leakage (Chapter 3; Trejo et al., 2005). No significant differences in stiffness were found for treatments at 200 to 600 MPa at any level of weight loss. Similar trends in stiffness were observed when measuring peak forces during cutting with a blade. The cutting force for both the unprocessed and 100 MPa samples were significantly higher than the rest of the samples at 0% prior weight loss. Above 200 MPa, samples were not affected by weight losses prior to processing (Figure 6.2). The structural changes occurring in these experiments were observed using light microscopy (Figure 6.3). Images were captured for raw, 4.4% and 11.4% prior weight loss in addition to samples after HPP at 200 MPa (2 minutes). It is interesting to highlight the increase in cell shrinkage and cell wall collapse as weight loss increased (Figure 6.3 A-C). Samples with higher weight loss (11.4%; Figure 6.3 C) exhibited a loss of cell roundness. Samples pressure-processed at 200 MPa presented a less homogeneous structure and shrinkage in comparison to raw tissue (A). Comparing these structural changes to the CYM results, it can be seen that the 11.4% weight loss sample (Figure 6.1C) had significantly higher stiffness than sample D (200 MPa, 2 min) from (Figure 6.1D), even though their structures, looked similar under the microscope. The reason for this difference could be attributed to cell membrane damage (which is not observed in the images). Damage to cell membranes will allow cellular leakage to occur due to an increase in permeability. This will lead to a loss of turgor in the cell and consequently loss of stiffness of the tissue.

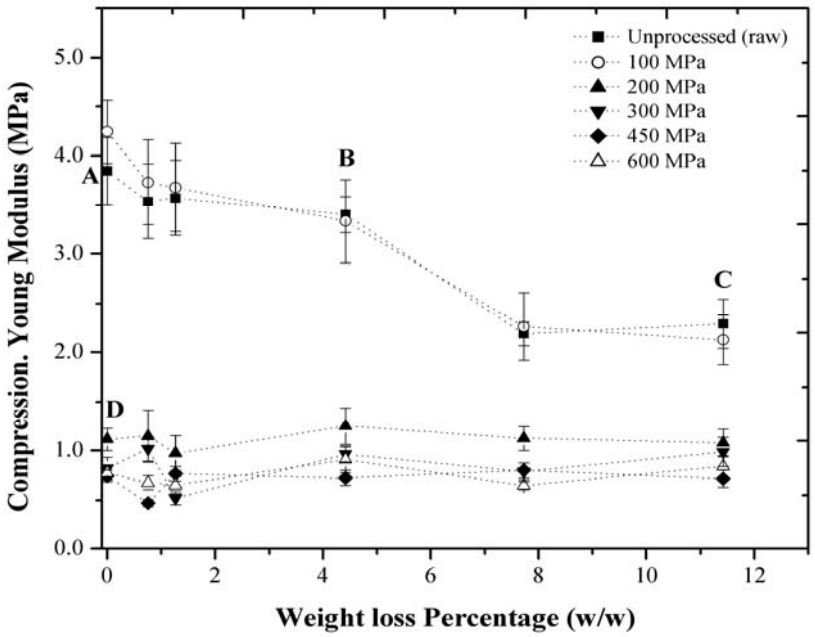

Figure 6.1: Effects of weight loss and pressure processing effects on the Compression Young's Modulus (CYM) of carrot tissue. A, B, C and D are points described with microscopic images in Figure 2. Error bars represent standard error (SE), n = 10.

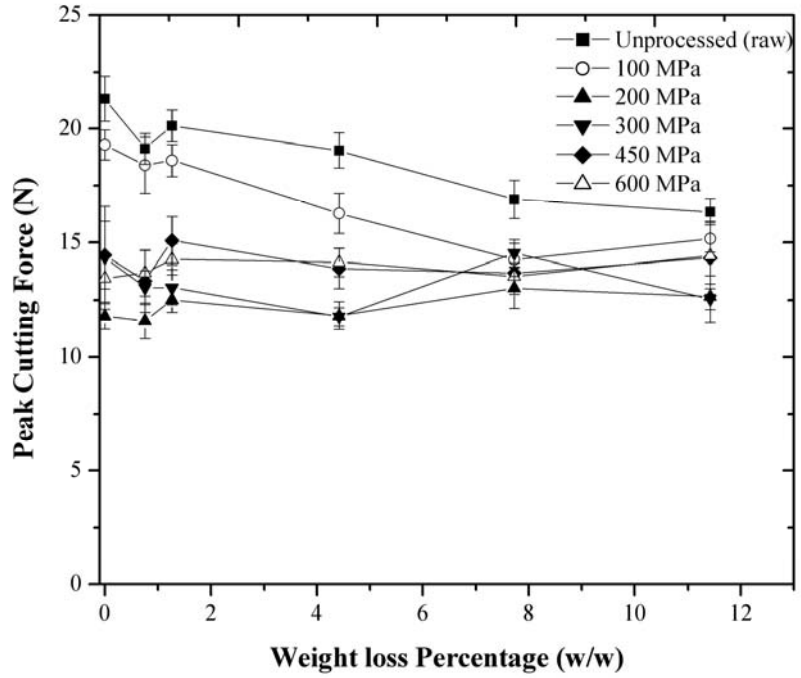

Figure 6.2: Effects of weight loss and pressure processing on peak cutting force of carrot tissue. Error bars represent standard error (SE), n = 10.

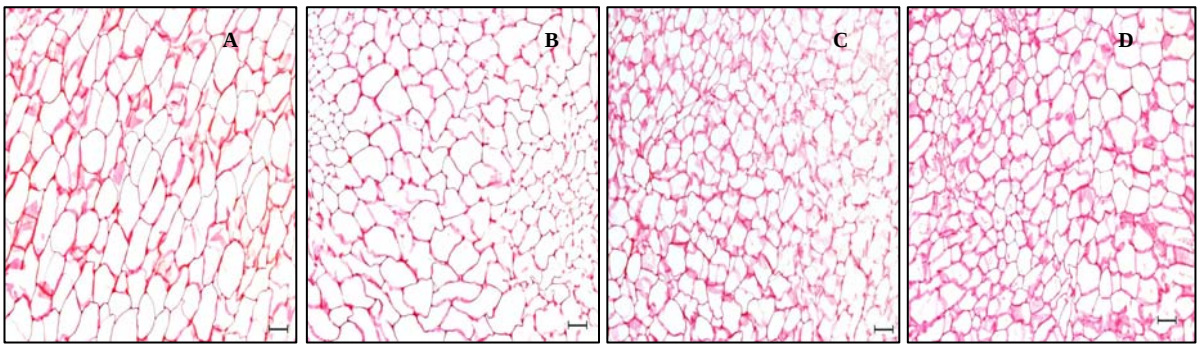

Figure 6.3: The effect of weight loss during storage and high pressure processing on carrot tissue. A: 0% weight loss, unprocessed; B: 4.4% weight loss, unprocessed; C: 11.4% weight loss, unprocessed D: 0% weight loss and pressure-processed (200 MPa/2 minutes) carrot tissue. Bar in the right bottom corner represents 50 μm.

It is expected that if cellular disruption occurs above 100 MPa, then leakage from the cells will also take place. In this part of the study, cellular leakage was measured before and after weight loss and high pressure processing (Figure 6.4). Raw and 100 MPa processed samples did not present leakage when fresh nor after weight loss while stored. At 200 MPa and above, leakage was observed. The weight loss prior to processing highly influenced the cellular leakage; with a considerable reduction in leakage (fluid losses) at weight losses above 2 % for 200-600 MPa pressure treatments. Above 2 % weight loss, leakage seemed to become essentially constant. It seems that even low water removal (of 2 %) from the tissue during storage would have a significant effect on leakage reduction.

To confirm leakage effects on juiciness, quantitative tests were performed on fresh carrots and stored carrots exposed to different weight losses as shown in Figure 6.4.

Juiciness was lower (12%) for fresh (unprocessed) and 100 MPa samples in comparison with the other HPP treated samples. These results suggest that raw and processed tissue at 100 MPa may still have a stronger cell structure which resists compression during the juiciness test, holding the liquid inside. Lower juiciness results means that cells are probably more compacted and in an ordered tissue matrix, preventing the release of exudate during compression.

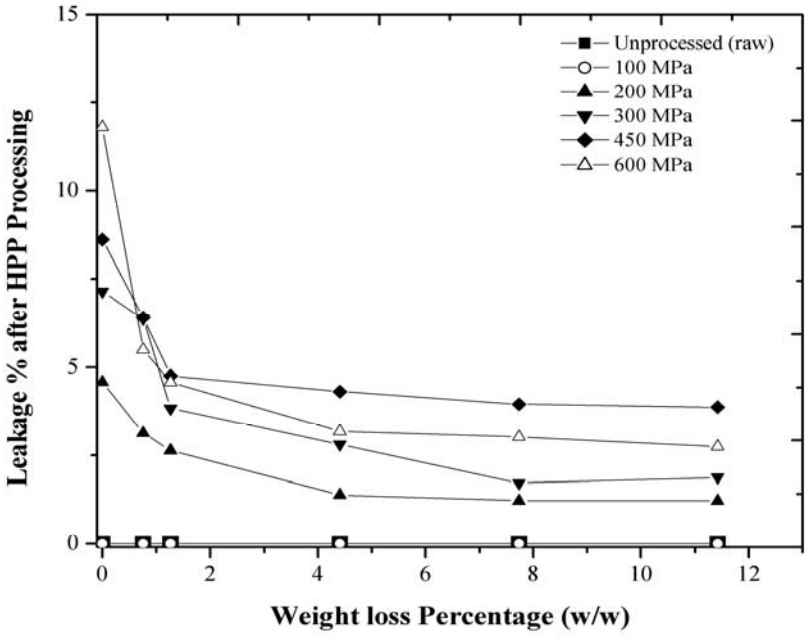

Figure 6.4: Cellular leakage percentage after high pressure processing (100 to 600 MPa, 2 minutes duration) as a function of percentage weight loss.

From the cellular leakage observations found in Figure 6.4, it is expected that if cell disruption leads to leakage, then juiciness would also be expected to be increased by high pressures, this by releasing more easily the cell contents. Samples treated with pressures above 200 MPa gave significantly higher juiciness values compared with unprocessed samples. It may be the case that the HPP process mobilizes cellular liquid from the cells into cell voids, which is then released during the compression in the juiciness test. By allowing the tissue to lose some of its initial weight prior to HPP, then less liquid would be available to exude during the juiciness test. For high pressure treated samples above 200 MPa, a significant drop in juiciness was observed at the first stage of weight loss (below 2 %). Then after that, juiciness followed a more flat trend with respect to weight loss. At 11.4% weight loss, HPP samples processed at 600 and 200 MPa dropped approximately by 8 and 4 % in juiciness, respectively, in comparison with their initial juiciness (Figure 6.5). The maximum observed juiciness values were found for the samples with 0% weight loss and pressure treated at 300 MPa (~27 % juiciness) followed by 450 and 600 MPa. It is not clear why 300 MPa would have more of an impact on the tissue structure than 600 MPa. Loss of juiciness correlates with increase in leaked fluid (Figure 6.6).

These results are consistent with the texture changes shown above 300 MPa (2 min, 20°C) where no further hardness changes (Trejo et al., 2007) were found.

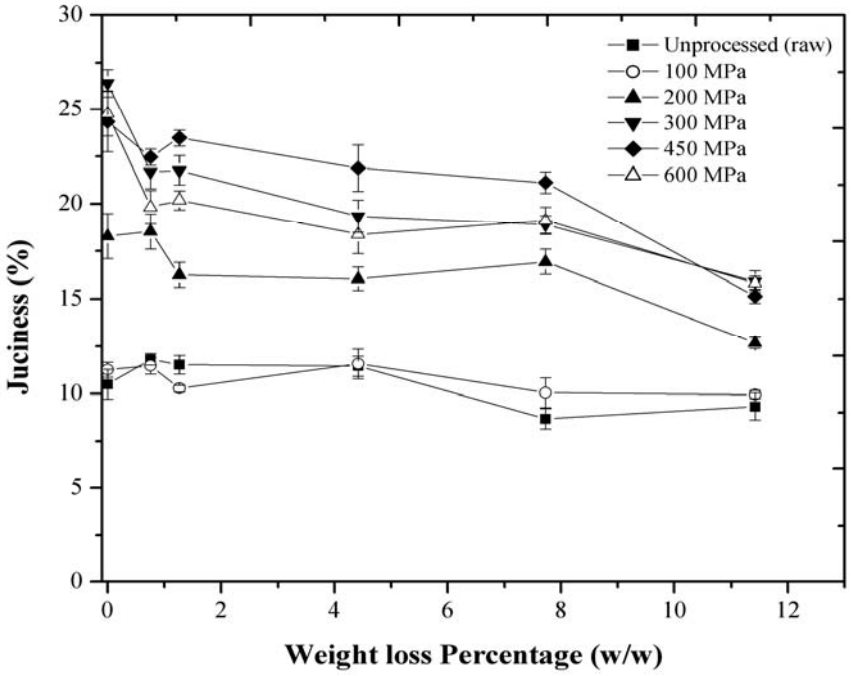

Figure 6.5: Weight loss and pressure processing effects on the juiciness of carrot tissue.

Tangwongchai et al., (2000) also found larger texture loses at 300 MPa in cherry tomatoes. The author suggest that air bubbles may be formed between 200 and 300 MPa which then may be trapped in cells and become larger during decompression, forming cavities as a consequence. At higher pressures, these bubbles were not seen, probably due to the gas escaping during decompression into voids between cells causing less damage. Furthermore, Kato and Hayashi (1999a) studied the effects of pressures on plant and animal tissues at the molecular level. The authors observed a reversible separation of the surface of proteins, membranes and lipids in contact between faces producing tunnels or holes between 100 and 250 MPa. Processing at 300 MPa showed membrane breakdown due to protein denaturation. In this study, it could be possible that above 200 MPa there was no further denaturation to escalate damage, thus making this a threshold point in the case of carrots.

Figure 6.6 presents the sum of water released due to leakage and juiciness testing at different pressures (fresh carrots). A three step trend is observed for leakage and juiciness. The first part is a flat linear trend up to 100 MPa. From 200 MPa this trend increases significantly and then flattens out again from 300 MPa onwards. This shows a

leakage increase as pressure increased. When executing the juiciness test, there was only a limited amount of water left to be released, and therefore the juiciness trend tends to slightly decrease above 300 MPa. Both trends are represented by the total curve, which represents the sum of juiciness and leakage exuded by the tissue. The trend is in agreement with previous findings on relative electrolyte leakage where a 'tissue break point' was observed between 100 and 200 MPa. Above that pressure electrolyte leakage increased but in a steadier trend. It is also possible to correlate these juiciness and cellular leakage also to textural changes, as observed in Chapter 5 where significant hardness loss was observed above 200 MPa, but little differences were evident between 300 and 600 MPa treatments.

These results also confirm that the apparent lower juiciness in >450 MPa processed tissue is the result of higher leakage in these samples during HPP processing, resulting in less water available to be expelled during juiciness test.

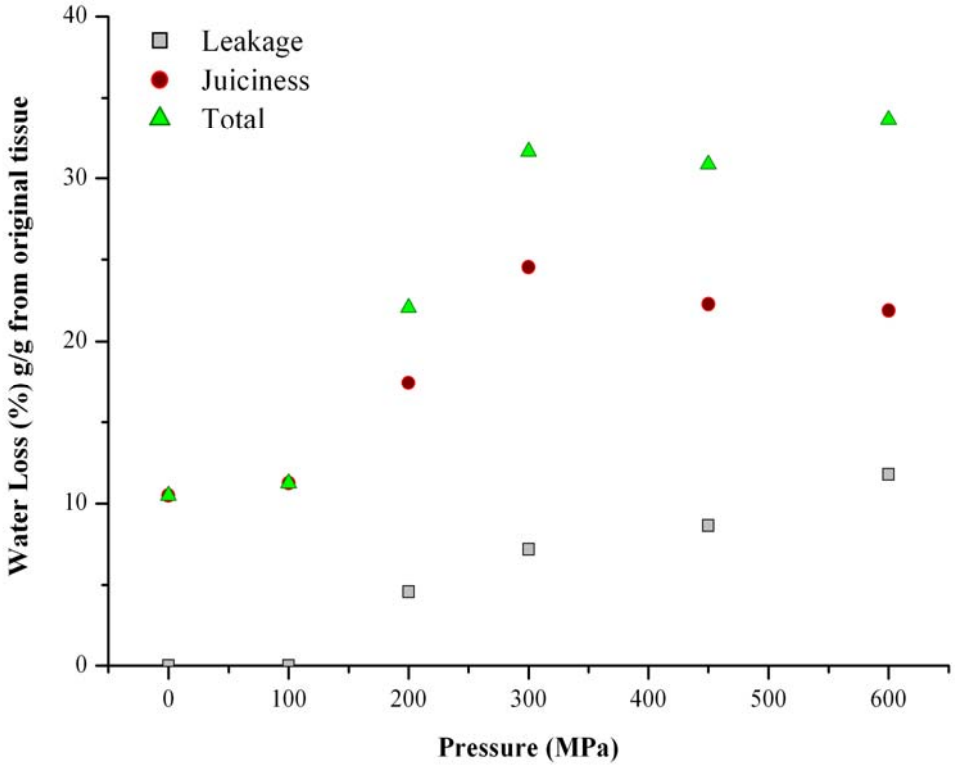

Figure 6.6: Sum of water release on leakage and on juiciness testing at different pressures (0% weight loss (w.l)).

6.3.2 Changes in turgidity when using mannitol solutions on raw samples and after HPP

Tissue stiffness, juiciness and volume changes were analysed using mannitol as an osmotic solution. The aim of this part of the research was to investigate if carrot tissue could regain its stiffness after HPP. In other words, is the cellular damage caused by HPP reversible or irreversible and under what conditions?

Raw carrot samples immersed in 0.1 and 0.2 M mannitol or water (0.0M) showed considerable increases in stiffness of 76, 62 and 55% respectively, compared with unsoaked (raw) samples. Figure 6.7 showed that raw samples (weight loss 11.4%) may regain their stiffness if immersed into mannitol solutions up to 0.4 M. The stiffness trend also suggests that the samples' isotonic point is between 0.4-0.5 M.

By using light microscopy it was possible to correlate the increase in stiffness found at 0.1 and 0.2 M mannitol representing swollenness. By reaching the isotonic point (between 0.4-0.5 M), cells started to collapse and a hypertonic point was observed at 0.8M represented by cell shrinkage (Figure 6.8).

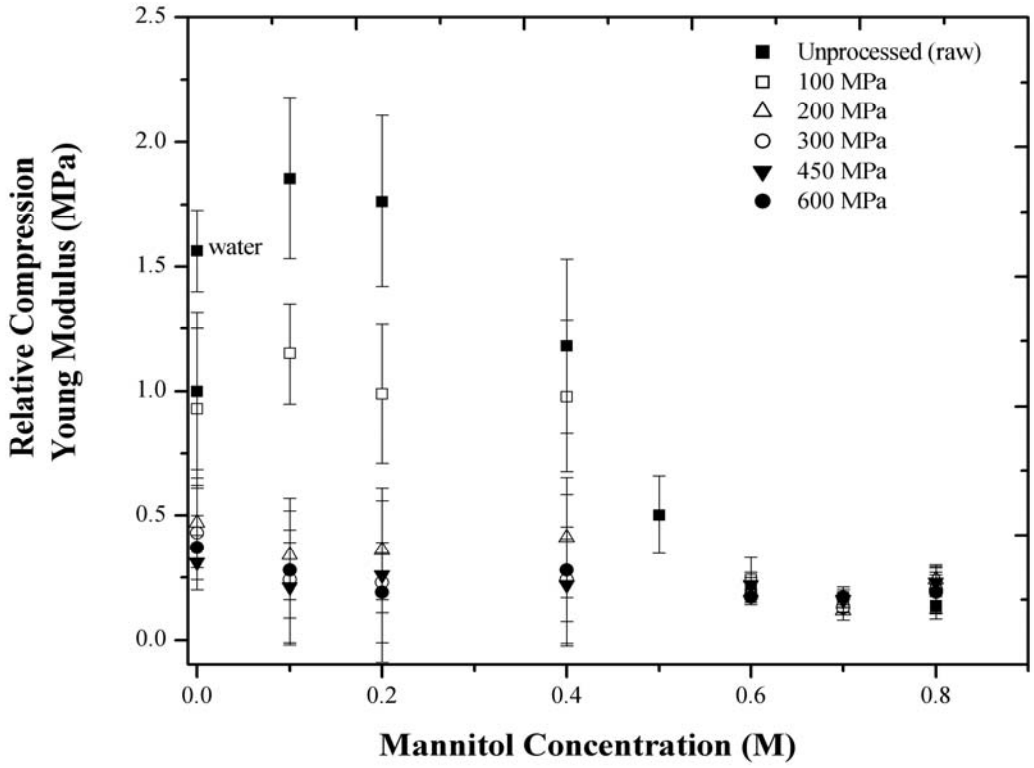

Figure 6.7: Effect of mannitol concentration on carrot tissue stiffness in relation to the raw samples before and after high pressure processing (Bars: standard error).

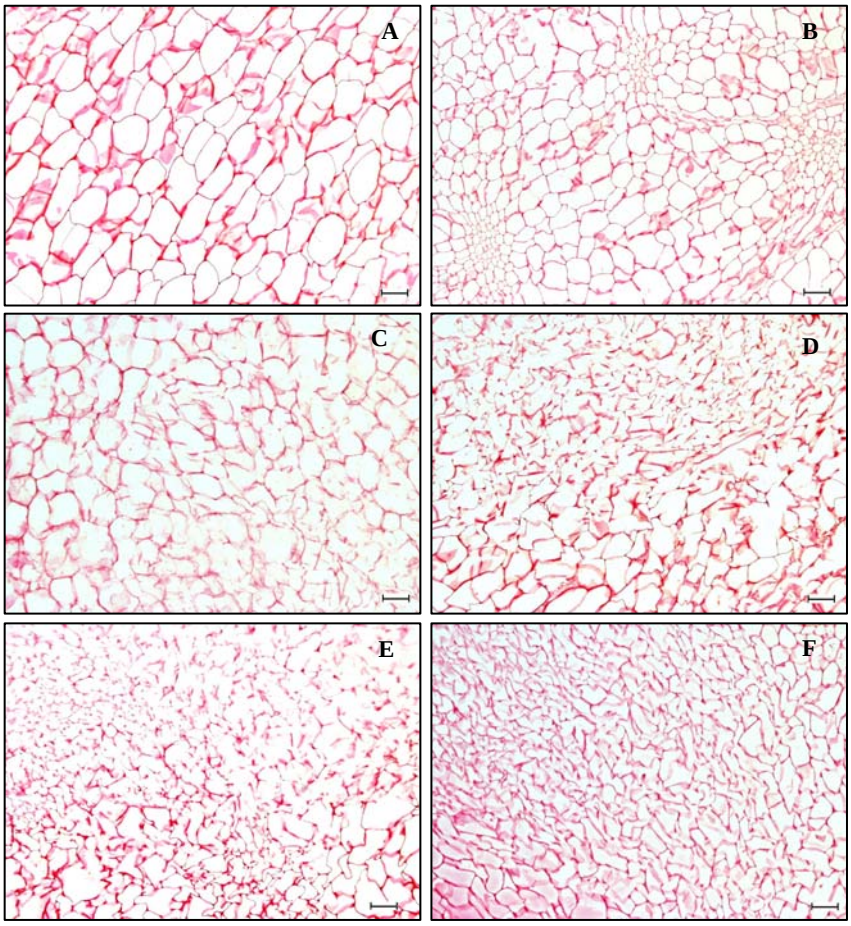

Figure 6.8: Mannitol effect on fresh carrot tissue. A: Fresh unsoaked; B: immersed in water (0.0 M mannitol); C: immersed in 0.1 M mannitol; D: immersed in 0.2 M mannitol, E: immersed in 0.4 M mannitol and F: immersed in 0.8 M mannitol. Bar in the right bottom corner represents 50 μm.

Studies performed on potato tissue showed a significant increase in Young's modulus at 0.5 M mannitol concentration (Dolores and Canet, 2002), and as shown in carrots, a hypertonic point may be reached above 0.5 M mannitol. In practical terms these results suggested that by moving above a certain mannitol concentration threshold, the tissue will release solutes out of the cells promoting shrinkage and consequently turgor and texture loss.

Data from this current research showed stiffness reductions of more than 50% when raw carrots were immersed in > 0.6 M mannitol. For carrots treated with high pressures followed by mannitol immersion, it was observed that treatments at 100 MPa would still allow carrot cells to take up mannitol solutions at 0.1 to 0.4 M with some stiffness

increase as a result (~20% at 0.1 M), while above this pressure, there was no stiffness regain at any mannitol concentration.

These results correlate with previous observations of increase in electrolyte leakage (Chapter 3), and hardness loses (Chapter 5) from 200 MPa upwards. It is important to note that plant tissues can still regulate their turgor pressure when an external disturbance is applied to cell walls (Braam, 1999). Zimmermann et al., (1980) described that plants can have a biphasic osmotic regulatory response towards turgor pressure. This is explained by a first phase where tissue can swell or shrink rapidly according to the exposed solution, followed by a second phase where the cells can adjust their osmotic pressure in response to stress; this can be by an osmoregulation mechanism in unprocessed plant tissue. In the case of HPP, cells may have lost their functionality or ability to readjust to the conditions above 100 MPa.

Research done by Georget et al., (2004) on the role of mannitol in the thermal transition in carrot tissue indicated that stiffness could not be regained after freezing, heating or drying followed by mannitol immersion, confirming irreversible damage to the cell membranes of the processed tissue, possibly due to cellular disruption. Results from Chapter 3 (Trejo et al., 2005), showed how freezing and cooking treatments had a significant impact on the cellular disruption in comparison to HPP. This was measured by electrolyte leakage with up to 14, 8 and 1 times higher levels of leakage for freezing, steamed and HPP at 100 MPa processing conditions in comparison to raw samples. Other studies done on carrot heating and cellular turgidity suggest that promoting cell plasmolysis (using mannitol at 1M), reduced firmness in comparison to fresh (unplasmolysed) disks (Greve et al., 1994b). Furthermore, plasmolyzed cooked disks presented some cell turgidity after rehydration (immersion in water), while fresh-cooked carrot disks showed no turgor pressure after cooking (3 minutes). The study of Greve's et al., (1994b) suggests that firmness loss due to heating could be overcome by prior osmotic treatments. It is important to note that in this current study, carrot samples had previously undergone 11.4% weight loss; this was done to reduce the initial turgidity as Greve et al., (1994b) achieved using mannitol. However this current work showed that cells were not able to regain stiffness after HPP above 100 MPa.

6.3.3 Specific volume changes of carrot tissue affected by high pressures

Specific volume measurement was included in this study to provide more information on the tissue response to HPP at a micro scale, based on the assumption that the tissue changes were indicative of cellular changes.

Specific volume measurements were obtained by submerging samples in water to measure their volume displacement. This was related to their initial weight giving a density measurement for each sample which was converted into specific volume. Figure 6.9 shows how samples shrank on processing at different levels of pressure.

The results show the highest specific volume for raw tissue samples as expected. Treatments at 100 MPa showed a large variation between replicate samples. This variation could be because at low pressures the tissue may be going through various structural arrangements to adapt to conditions which were not severe enough to result in considerable cellular disruption. Samples processed at 200 MPa or higher showed a decreased specific volume, which reached the lowest value at 450 and 600 MPa. This reduction in specific volume is the result of high compression forces, which in decompression will probably relax back promoting extracellular spaces being flooded with cell contents.

From these data, it would appear that 100 MPa is a transition stage as shown by the larger variability between samples. Studies on volume change on a single cell organism such as yeast, presented 15% volume shrinkage at 250 MPa (Hartmann and Delgado, 2004). In this present study, it was observed that carrot specific volume decreased by approximately 6% and 7% after the application of 300 and 600 MPa respectively.

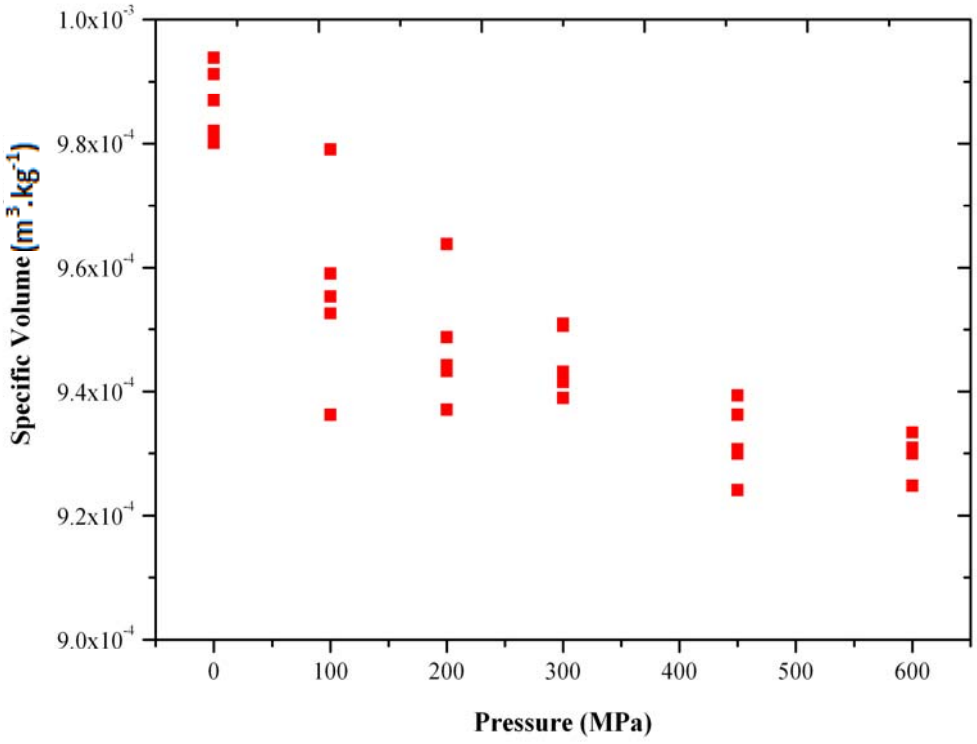

Figure 6.9: Specific volume changes of raw carrot tissue after 11.4% w.l. and after different levels of high pressure.

Under compression, gas filled voids would be compressed and potentially dissolved. In addition as shown in Figure 2.14 (Chapter 2), water and liquid foods are compressed from 10-15% at 600 MPa (Cheftel, 1992; Aparicio et al., 2011 and Hartmann and Delgado, 2004). Because the specific volume measurements carried out here on carrots are after decompression, the liquid components volume change would be relaxed but the lower specific volume measured shows that voids are not restored. This suggest that during the compression phase of the pressure cycle, the total volume reduction for carrot tissue would be of the order of 17 to 20% (at least 7% void volume reduction at 600 MPa as shown in Figure 6.9 plus 10 -15% fluid compression). This volume change affected cell shape conformation as observed in Chapter 5 (shape factor and elongation) and consequently texture.

6.3.4 Specific volume changes in carrot tissue as related to leakage and juiciness

The effects of pressure application on leakage and juiciness were related to specific volume changes of the tissue (micro-level). It was observed that even though at 100

MPa samples exhibited no leakage after HPP (Figure 6.4), there was some reduction in specific volume (Figure 6.10).

Specific volumes were similar for 200 and 300 MPa with a difference in leakage of less than 1%. Carrots treated at 450 and 600 MPa showed the lowest specific volume and higher leakage.

Overall, the relationship between leakage and specific volume change was classified into three stages. Stage 1 represented the raw and 100 MPa treated carrots where no leakage was observed but structural changes took place at low pressures. Stage 2 is where leakage appears, probably due to increased cellular permeability or some disruption resulting from HPP (200 – 300 MPa). Finally, stage 3 almost doubled leakage values compared with stage 1. This last stage shows the smallest specific volume or most shrinkage.

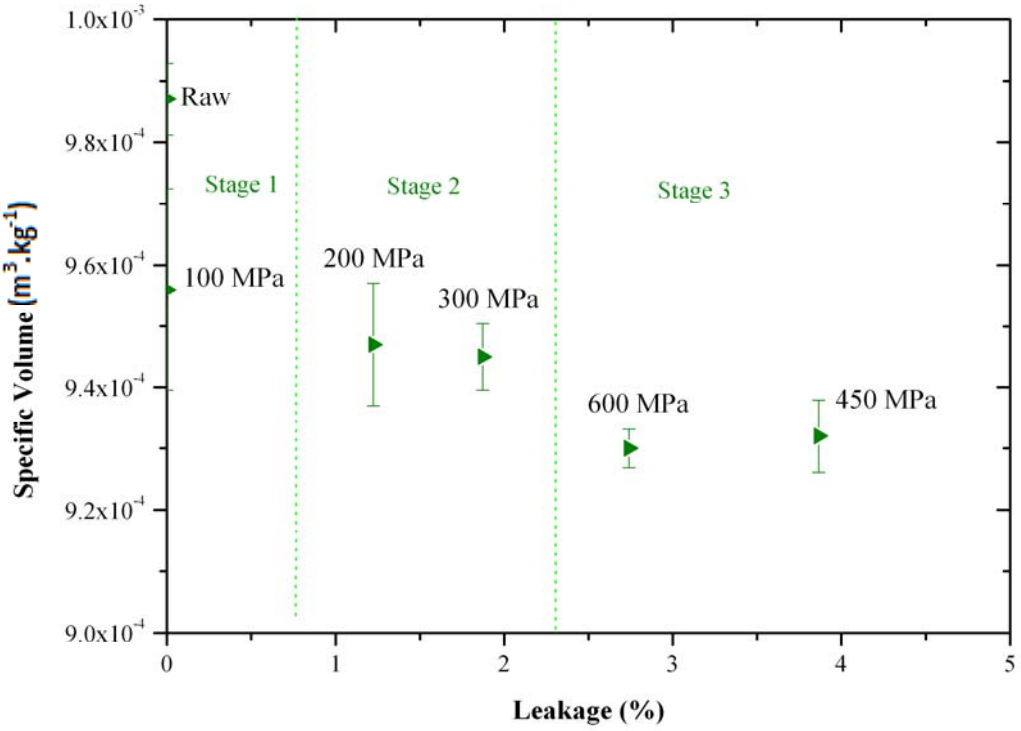

Figure 6.10: Specific volume change and leakage percentages at different pressures (11.4% w.l)

Studies of internal leakage substances in yeast cells submitted to high pressures have indicated an increase in ion and amino acid concentrations as pressure increased above 300 MPa (Shimada et al., 1993). The researchers used transmission electron

microscopy to observe nuclear membrane disruption when applying 100 MPa at room temperature for 10 minutes. Severe inner structure damage was observed at 200 MPa and alterations of cytoplasm and mitochondria at 400-600 MPa. These findings for a single cell model correlates with the trends found in this current research.
It is known that leakage of cellular content will have a direct effect on tissue juiciness. In this work samples with the largest volume shrinkage (450 and 600 MPa), also presented the highest juiciness values (Figure 6.11).

With this result it is confirmed that by promoting cell disruption, the whole matrix becomes looser and therefore more juices are available to be squeezed out during the juiciness test.

Samples treated at 300, 450 and 600 MPa showed similar juiciness, but lower specific volume. It could be possible that 300 MPa may be a threshold pressure that the carrot tissue can tolerate. By applying pressures above 300 MPa, cell contents are released (leakage) and what is left can be further expressed with the juiciness test (compressing the sample to 80% strain). Previous studies on HPP in carrot tissue have shown hardness losses of 5, 25 and 50% for treatments at 100, 200 and 300 MPa (all at an initial temperature of 20°C). No further increase in hardness loss was found above these pressures (Chapter 4; Trejo et al., 2007).

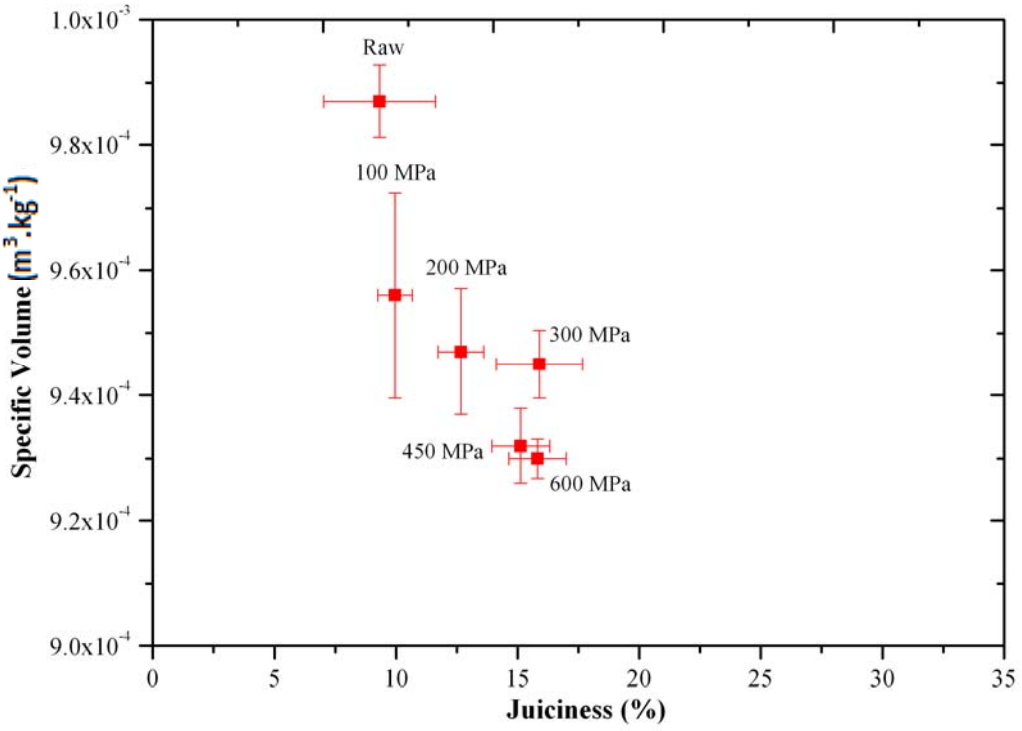

Figure 6.11: Specific volume change and juiciness percentages at different pressures (11.4% w.l).

6.3.5 Specific volume changes in carrot tissue as affected by mannitol as osmoticum pre and post high pressure treatment

This section aims to evaluate whether cells can be made to regain turgor or their volume after high pressure treatments by using mannitol solutions at various concentrations. The results showed that raw tissue was sensitive to various mannitol concentrations, exhibiting swelling between 0.1 and 0.4 M, and shrinkage between 0.6 and 0.8M (Figure 6.12). The trend was very similar to that for stiffness (Figure 6.7), showing an isotonic point between 0.4 and 0.5 M and a hypertonic effect above this concentration. Previous results have also showed a reduction in relative specific volume between 0.5 and 0.6 M mannitol relative to the raw control in potato tissue using mannitol (Alvarez et al., 2000). A reduction in relative specific volume between 0.5 and 0.6 M mannitol was also observed in Ida Red apple variety (Lin and Pitt, 1986); kiwifruit and melons (Sajnin et al., 1999).

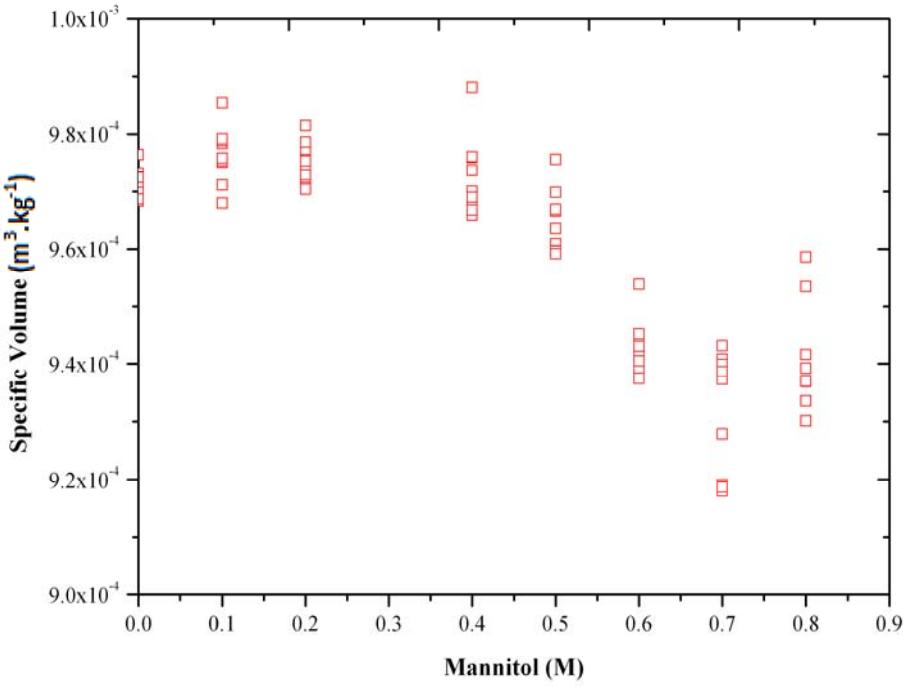

Figure 6.12: Specific volume changes at different mannitol concentrations in raw carrot tissue with 11.4% w.l before high pressure treatments.

In the current study carrots had previously gone through a 11.4% weight loss during storage. It is interesting to observe that even though these cells had presented significant shrivelling and deformation, they responded to the osmoticum solution at various concentrations.

Figure 6.13 shows specific volume as a function of different mannitol concentrations and pressures (samples were high pressure processed and then immersed into mannitol solutions). Two factors need to be considered in the specific volume changes. Firstly the weight loss factor (11.4%) shown by the unprocessed sample or zero pressure was followed by the high pressure effects. Secondly the pressure effect can be observed at zero mannitol concentration, where unprocessed samples showed a high specific volume, decreasing as pressure increased. When the unprocessed samples were then submerged into 0.1 M mannitol solution, an uptake of that solution was observed increasing the specific volume. As the mannitol concentration increased further, the specific volume decreased. High pressure treated samples also seemed to take up some of the solution, but their specific volumes became constant above 0.1 M mannitol. It is likely that HPP treated tissue could promote cells to 'relax' when placed into the medium, rather than responding to it as an osmoticum. Confirmation of this is observed

at the highest mannitol solutions (0.8 M) where unprocessed carrots responded to the osmoticum by reducing their specific volume while high pressure treated samples did not respond to the osmoticum. This may be because cell membrane damage could have increased permeability allowing solutes to transfer in and out of the cells easily, no matter the external conditions.

It is therefore concluded that the pressure effects are larger than the mannitol effects on cells and that the tissue has largely lost the ability to respond to an osmoticum after HPP treatment.

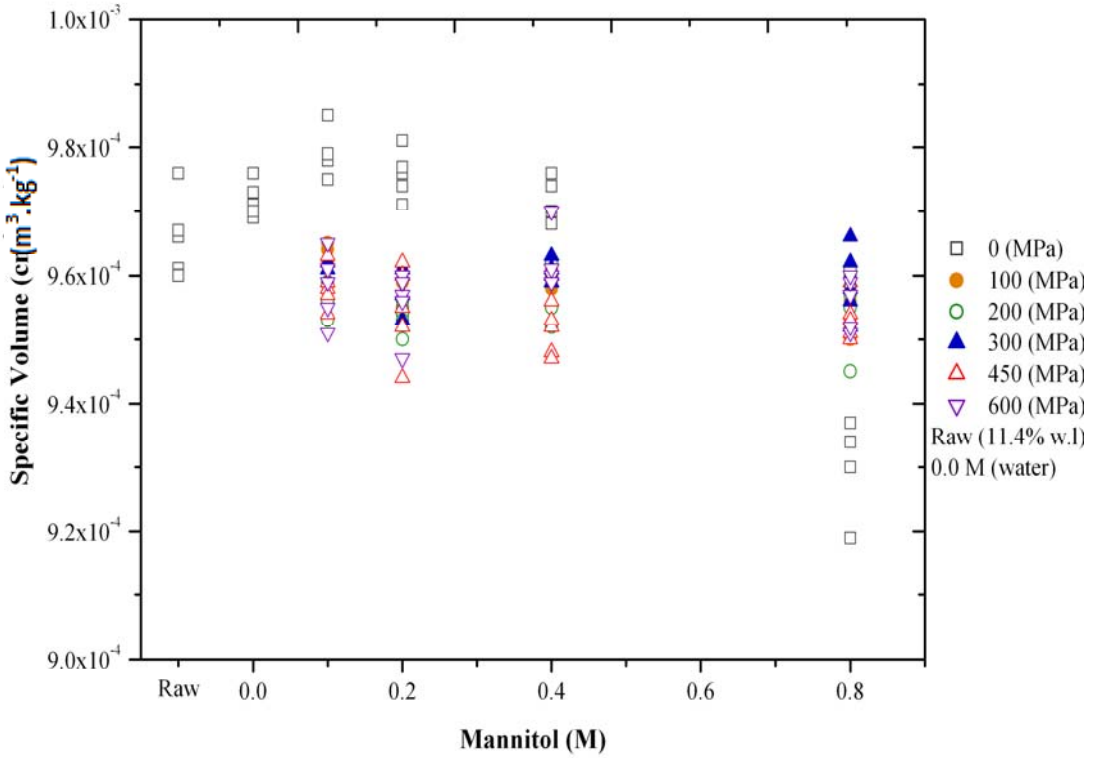

Figure 6.13: Specific volume changes at different mannitol concentrations before and after high pressure treatments.

6.4 Conclusions

Weight loss during storage (5°C), had a significant influence on carrot stiffness and cutting forces for raw (unprocessed) and HPP samples. Low pressure treatment (100 MPa, 2 minutes) presented a very similar trend to raw samples. This is in line with previous indications that mild HPP treatment causes only limited cell damage. Above 200 MPa, the effects of weight loss prior to HPP became insignificant in comparison to

the effects of the high pressure per se. All samples treated at above 200 MPa showed no further texture losses at any weight loss percentage.

Leakage from cells after HPP increased as pressure increased. Juiciness of carrots was zero for raw and 100 MPa samples. At ≥ 200 MPa, juiciness increased reaching a peak at 300 MPa, above which there were no further changes. Correlations between juiciness and specific volume at the micro-level were found, showing higher volume shrinkage of cells at higher pressures.

Manipulating the tissue with mannitol (0.0 to 0.4M), increased the stiffness of raw carrots. Above 0.4 M mannitol there was shrinkage of the tissue and a loss of turgor. Shrinkage was observed by using light microscopy and specific volume analysis, which correlated with stiffness values. Mannitol did not affect specific volume for pressure processed samples. In other words, specific volume could not be regained and no significant differences were found between samples processed from 100 to 600 MPa.

From this study, it is concluded that reducing tissue turgidity by weight loss prior to HPP would not reduce texture changes caused by HPP. However it was possible to improve stiffness losses due to HPP when applying low pressures by tissue turgidity manipulation with mannitol.

In this research it was possible to analyse and correlate most of the HPP effects at the micro cellular level by measuring specific volume changes and comparisons then with quality attributes. This is an important approach to optimizing new applications and processes.

7 Sensory perception and quality attributes of high pressure processed carrots in comparison to raw, sous vide and cooked carrots (*)

7.1 Introduction

Research on consumer perceptions of emerging technologies has indicated that high pressure processing (HPP) is rated positively, and preferred by consumers over genetic modification, irradiation and other emerging non-thermal technologies (Cardello et al., 2007). Indeed, high-pressure 'pasteurization' has become a commercial reality with most fruit- and, to a lesser extent, vegetable-based refrigerated food products such as juices and pastes. Currently the international market includes a range of fruit smoothies, jams, juices, apple sauce, fruit preparations as ingredients for yogurts, fruit blends, guacamole and other avocado products and tomato-based salsa. Later HPP applications moved to meal kits containing acidified sliced bell peppers and onions; additionally meal kits containing heat-and-serve beef or chicken slices, ready-to-eat meat products, and seafood, including oysters and lobsters, are in the market in the USA, Europe and Australia (Stewart et al., 2007). Oey et al., (2008) conducted a broad review on the effects of HPP on various quality attributes of fruit and vegetable based food products, concluding that sensory analysis was required for better understanding of the effects of HPP processing, which may be product dependent. To date, findings are a little inconclusive, indicating the loss of flavour compounds in HPP (800 MPa/ambient temperatures/20 min) strawberry purée for example (Lambert et al., 1999). The authors also suggested that further research is required on the correlation of analytical data with sensory analysis over time to provide more understanding of the HPP effects (Lambert et al., 1999).

Based on the literature recommendations indicating that further research needs to be done on sensory perception correlating to analytical analysis, the current research aimed to evaluate whether structural changes caused by pressure application to whole carrot pieces (used as a model of turgid cells) would have an effect on sensory perception and the presence chemical compounds.

(*) Material from this chapter is included in the paper: Trejo Araya X. I., Smale N.J., Zabaras, D., Winley, E., Forde, C., Stewart, C. and Mawson, A. J. (2009). Sensory, chemical and physical attributes of high pressure processed carrots in comparison to raw, sous vide and cooked treatments. *Journal of Innovative and Emerging Technologies*, 10 (4): 420 – 433.

In the previous chapters (5 and 6), the structural changes occurring due to the application of HPP to carrots were explored, along with the influence of these changes on texture. Cell membrane damage and cell wall modification are therefore likely to influence the sensory properties of the product. Flooded intercellular spaces can influence colour perception. Structural changes could also influence the release of volatiles from the cell compartments and availability of pigments (e.g. carotenoids).

This study aimed to investigate if sensory panellists could notice any changes in high pressure processed carrots and to correlate sensory perception to analytical methods to assess quality. This will help to compare high pressure processing effects to the effects of other traditional processes such as cooking and sous vide. In addition, a shelf life study was to be done over 14 days of storage at 4°C, to complete a full picture of information on whole vegetable pieces as part of a processed-chilled product.

7.2 Materials and methods

7.2.1 Plant material

Carrots (*Daucus carota* L., var. 'Stephano') were purchased from a local Sydney market and stored at 1°C for a maximum of three weeks. Whole carrots were washed, peeled and cut into sticks (7 mm width x 7 mm height x 100 mm length) using a potato chip cutter.

7.2.2 Processing procedures

Carrot sticks were mixed and 500 g samples were randomly vacuum packed into high oxygen barrier pouches (Cryovac, Australia). Packaged samples were then divided into: raw (unprocessed); sous vide (90 - 95°C for 5 min in contact with water; Ohlsson, 2002b); cooked (boiled at 100°C for 20 min) or pressure treated at 600 MPa for 2 min. After processing, all bags were immersed in ice water to promote rapid cooling. After cooling, some samples were assessed for sensory quality, headspace volatiles and physical assessment, while others were stored at 4°C for monitoring of physical and chemical changes for up to 14 days.

HPP was conducted in two different units due to accreditation requirements for human consumption followed by Food Science Australia – Sensory protocols and the large number of samples. Samples to be used for sensory and chemical analyses were processed in Werribee, Victoria, Australia, using a 35 L unit (UHP QFP 35L-600-S, Avure Technologies, Inc., Kent, WA, USA) at pressures of 550-600 MPa for 2 min, using water as the pressure transmission medium. The come-up time to reach target pressure was < 10 s and the depressurization time was <5 s. Carrots used for quality assessment of texture, colour and microscopic analysis were processed in Sydney, NSW, Australia, using a 2 L pressure unit (Avure Technologies, Inc., Kent, WA, USA) at pressures of 550-600 MPa for 2 min using water as the pressurizing medium. The come-up time to reach pressure was <10 s and the depressurization time was <5 s. As all HPP was conducted at ambient temperature (no application of external heating), differences due to the vessel volume and other unit dependent variables were considered unlikely to introduce significant differences between the samples used in the investigated conditions. However it is important to keep in mind that adiabatic heating and temperature distribution can differ within vessels (volumes), especially when applying external heating into the process. Processed samples were then transported and stored for 16 h in total at temperatures below 2°C with temperature data being logged using I-buttons (± 0.5°C, 1 min frequency; manufactured by Dallas Semiconductors, USA). Samples of 500g were sent for microbiological analysis to an external laboratory (EML consulting services, accredited by the National Association of Testing Authorities NATA, Sydney Australia) that used conventional methodology. Samples were conditioned to room temperature prior to sensory and chemical analyses. Heat treatments (sous vide and cooking) were performed using a temperature controlled water bath (Black and Decker, Australia).

7.2.3 Sensory analysis training and assessment

An experienced group of 10 trained panellists participated in the sensory analysis. The sensory panel underwent 16 h of training (done by a sensory expert and the author) during which they developed and defined a descriptive vocabulary of 19 attributes to quantify differences in odour, appearance, texture, flavour and aftertaste properties (Table 7.1). The vocabulary was developed through repeated exposure to raw, sous vide, pressure treated (600 MPa) and cooked carrots. To maximize the panellists' exposure to probable variability within the carrots, each panellist received different

carrot samples several times during training. The sensory attributes were further defined with the aid of reference standards by the panellists. Samples were served as carrot sticks (7 mm width x 7 mm height x 100 mm length), which were placed in a cup and equilibrated to room temperature before assessment.
Assessments were conducted one week after training and samples were again presented as sticks, placed in a cup and equilibrated to room temperature before assessment.

Each panellist received 12 blind-coded samples comprising all 4 carrot treatments in triplicate. The session was conducted in one day with 10 min breaks between replicates to reduce sensory fatigue. During the assessment, water and unsalted breadsticks were also provided as palate cleansers. All sensory attributes were rated on 100 mm unstructured line scales anchored at 0 (“Low”) to 100 (”High”), for each descriptive term. Data was recorded and stored using the Compusense Five sensory data acquisition software (Compusense Inc., Guelph, Ontario, Canada). The experimental design used for this research was obtained from package – CycDesigN, Version 2 (Whitaker et al., 2002). Assessment results are found in Table 7.2 - Table 7.5).

Table 7.1: Sensory attributes of carrots generated by trained panellists.

ATTRIBUTE	DEFINITION	ANCHORS
Odour		
Odour impact	The intensity of the initial impact of the odour.	Low to High
Green	The intensity of crushed carrot leaves odour.	Low to High
Processed	The intensity of over boiled mixed vegetables (potatoes/ beans/ carrots)	Raw to Over steamed
Appearance		
Intensity of Orange	The depth of the colour	Yellow to Dark orange
Visual firmness	How the carrots stand in the cup – assess on the whole cup.	Limp to Hard
Brightness	The degree of fluorescence	Dull to Bright
Surface moisture	The amount of moisture left on the stick	Dry to Wet
Flexible	The degree to which sample flexes by hand Scale from 'not at all' (breaks easily) to 'very flexible' (bends without fracturing).	Very flexible or bends without fracturing
Texture		
Fibrous	The degree of woodiness on the initial bite – clean break = soft, woody=fibrous.	Soft to Woody
Crunchy	The initial sound and feel of the bites	Silent to Loud
Moisture in mouth	The amount of moisture expelled from the sample into your mouth while chewing.	Dry to moist
Chewing time	Amount of chewing required to breakdown the sample. Ranged from low: just disappears in the mouth to high: more than 20 chews.	Low to High
Flavour		
Flavour impact	The initial impression of the intensity of the overall flavour of the sample	Low to High
Sweetness	The intensity of sweet – as defined by the basic taste solution for sweetness	Low to High
Bitterness	The intensity of bitter – as defined by basic taste solution for bitterness	Low to High
Processed	The intensity of over boiled mixed vegetables (potatoes/ beans/ carrots).	Raw to Over steamed
Green	The intensity of crushed carrot leaves flavour.	Low to High
Aftertaste/After feel		
Aftertaste impact	The residual flavour intensity that remains after the sample has been swallowed.	Low to High
Bitterness	The chemical, bitter taste sensation that remains in the mouth after swallowing the sample.	Low to High

7.3 Gas chromatography / mass spectrometry and olfactory gas detection analysis

Chemical analysis of carrot samples consisted of a headspace volatiles quantification using GC/MS and GC/MS-O for olfactory detection and description. Headspace analysis was performed for each treatment in triplicate. Approximately 100 g of carrot sticks were placed into a beaker, sealed and placed in a water bath at 30°C for 1 h for capture of volatiles using a Supelco SPME fibre (2 μm – 50/30 μm DVB/Carboxen/PDMS stable flex; Pennsylvania, USA). A preliminary experiment was performed to optimize the time required for volatile absorption through the fibre without saturation but enough to allow quantification of volatiles using GC-MS. The results indicated differences between exposure times (10 min, 20 min, 30 min, 1hr and 2hrs). One hour of exposure was found to be enough time for absorption without saturation, as applied by Zabaras and Wyllie (2001). After each experiment the fibre was left to equilibrate inside the GC equipment at 240°C for 1hr to ensure that residual volatile compounds were removed before the next sampling.

A known volume (20 μl) of an internal standard (4 methyl-1-pentenol; 39.57 $\mu g.ml^{-1}$) was placed onto a piece of filter paper (glass microfibre filters GF/C, Whatman, England) and placed in the flask with the carrots for each volatile capture. The fibre was then inserted into a GC-MS (4000 W/ODO II, Varian USA) set at a flow rate of 5.0 ml min^{-1}. The injector was set at 280 °C and operated in the split less mode for 2 min. Volatile components were resolved using an Innowax capillary (30 m x 0.32 mm, 0.5 μm film thickness; Hewlett-Packard, Palo Alto, USA.) with a temperature program that started at 40 ºC (held for 1 min) and then heated at 5 ºC per min to 240 ºC (held for 5 min); the total run time was 45 min. The identification of compounds was achieved using identification software (AMDIS, Version 2.1) and the National Institute of Standards Technology mass spectral search program (NIST, Version 2.0, 2002; NIST USA). For calculation purposes, each volatile peak area was divided by the internal standard area for each sample and standardized to the initial sample weight (Zabaras and Wyllie, 2001). Volatile capture was done in triplicate.

For olfactometry gas chromatographic analysis, the GC/MS was connected to an odour port (ODO-II, SGE; Tingwood, Australia). The sample was then split between the MS

detector and the odour port in the ratio of 1:3. Three assessors were asked to sniff each treatment for 30 min and verbally describe the perceived volatiles over time (maximum one session per assessor per day). For each olfactory assessment, the collected sample was injected into the GC-MS at the same time as a microphone was fitted to the panellist. This was connected to a personal computer for verbal odour description and intensity perception based on a scale from 0 (weak) to 100 (strong) using an intensity detector provided by Compusense Five sensory data acquisition software (v. 3.0, Compusense Inc., Guelph, Ontario Canada). The audio data was captured using the GoldWave digital audio editor (Version 5.6; accessed from www.goldwave.com). The sound profile was then matched with the odour intensity peaks of the GC/MS data as described by Frank et al., (2006). Each treatment was assessed in triplicate (by three assessors), which gave a list of descriptors for the perceived volatiles for each treatment. Nine main descriptors given by the olfactory test were chosen to compare treatments, these were: fruity, alcohol, woody, herb, carrot, sulphur, cooked, pine and earthy.

7.4 Physical measurements

7.4.1 Texture measurements

Texture measurements (hardness, cutting and bending forces) were performed using a TA-XT2 texture analyser (Stable Micro Systems, Surrey, England), with 20 replicates assessed for each treatment. The compression force at 30% strain was obtained using a cylindrical flat-probe (25 mm diameter; aluminium). The sample was placed on the platform as a flat stick or 'block' (7 mm width x 7 mm height x 100 mm length) along the platform and measured with a 250 N load cell at a deformation rate of 1 $mm.s^{-1}$. The hardness of the samples was defined as the peak force at 30% strain. A cutting test was also performed on all stick samples using a stainless steel blade (supplied with the TA-XT2) with a deformation rate of 1 $mm.s^{-1}$ and 75% strain. Samples were placed as flat beams on the platform and the peak force was evaluated. A three point bending test was performed to analyse the carrot snapping action, using carrot sticks with 7 mm width x 7 mm height x 60 mm length dimensions. Prior to assessment, a notch (3.5 mm) was made in the middle of the sample. Each sample was placed between the two bars at a distance of 40 mm and bent until breakage with a third bar moving perpendicular to the midpoint of the sample at a speed of 1 $mm.s^{-1}$ (Alvarez et al.,

2000). The results were expressed as the maximum bending force required to break the sample and the displacement at the point of breaking.

7.4.2 Juiciness measurements

Juiciness of carrot sticks were determined by a method modified from Paoletti et al., (1993). First, the surface of each sample was dried using tissue paper. Then two filter papers (Whatman N°1, 55mm diameter) were weighed, followed by weighing the sample (Mettler Toledo PB 303, ± 1.0 mg). The samples were then placed between the two filter papers and compressed using the TA-XT2 texture analyser at 1 $mm.s^{-1}$ to 80% strain. The compressed samples were discarded and the filter papers (with absorbed juice) were weighed immediately (Mettler Toledo PB 303, ± 1.0 mg). The difference in mass of the filter paper before and after compression divided by the total sample mass was used as a measure of sample juiciness. Five replicate measurements were performed for each treatment.

7.4.3 Colour measurements

Colour was measured using a tristimulus colourimeter (Chroma Meter, CR-400 Minolta, Japan). The device was calibrated against a white plate (Y: 94.0, X: 0.3130, Y: 0.3193). For measurement, thirty flat sticks were placed side by side on a white surface and 30 measurements per treatment were recorded. The results were analysed using CIE L*a*b* uniform colour space (Lab), where L* indicates luminosity, a* correspond to colouration from green (-) to red (+) and b* to colouration from blue (-) to yellow (+).

7.5 Cryo-scanning electron microscopy (Cryo-SEM)

Carrot samples were prepared immediately for microscopy after processing. The method followed was suggested by Romeo (2006), where samples were cut into (1 x 1 x 3 mm) sticks with a sharp razor blade and mounted in a brass block (10 mm x 25 mm diameter). The block was submerged in liquid nitrogen for 45 s and the carrot then fractured by running a razor blade across the surface. The cooled brass block containing the remainder of the sample was placed into the scanning electron microscope (Philips SEM 505, Holland) for observation. Images were taken with an image capture system (Meeco products, Sydney, Australia) and image scale represented by a 0.1 mm bar scale.

7.5.1 Data analysis

Sensory descriptive data analysis was performed using analysis of variance (ANOVA; SPSS v. 14.0) with treatment (N = 4) and assessor (N = 10) as the main treatment factors. The interactions between sample and replicate (sample×replicate) and sample and assessor (sample×assessor) were also investigated to evaluate the integrity of the data set. Significant differences are indicated in Table 7.2 – 7.5) using different letters.

Significant interactions between treatment and assessor (treatment×assessor) were observed for some attributes. The treatment trends have been compared between the processes and significant differences were based on a comparison of the relative size of the F-statistic for the treatment×assessor interaction and for the product.

For two of the attributes, "bitter taste" and "bitter aftertaste", the interaction F value (treatment*assessor) was equivalent to the treatment effect F value. Therefore, significant differences for these attributes were the result of differences in the way assessors rated the attributes and not purely as a result of differences between treatments. Based on this finding, both bitterness attributes were excluded from further analysis. For the remaining attributes, the size of the F value for perception was greater than for the product effect as such; therefore differences in these attributes could were more dependent on panellist perception differences as individuals than the actual treatment effects perception.

Statistical analysis of quality measurements and headspace volatiles was performed using ANOVA and Tukey's test for pairwise comparison with a confidence interval of 95%. Analysis also included the interaction effect of storage treatment×time. All graphically presented results are shown with standard error bars (SE).

7.6 Results and discussion

7.6.1 Sensory description

The estimated means for significant main effects ($P < 0.05$) were produced along with standard errors of difference (SED). Twice the SED (SED x 2) was equivalent to a Least Significant Difference (LSD) based on the number of samples.

Most of the measured attributes were found to have significant differences ($P<0.05$) across the four carrot treatments.

7.6.2 Appearance of carrots

Carrot appearance was classified by the descriptors: *intensity of orange*, *visual firmness*, *brightness*, *surface moisture* and *flexibility* (judged using finger touch). The results indicated significant differences ($P<0.05$) in *intensity of orange*, with the lowest scores for the raw, judged as a 'dull' colour by panellists, and the cooked sample as less intense of orange colour, while the sous vide and pressure treated (600 MPa) samples had the highest orange colour scores (Table 7.2). Visual *firmness* was significantly higher for raw samples in comparison to all other treatments, in which firmness decreased in the order: sous vide > 600 MPa > cooked. The *brightest* samples were the cooked and pressure treated ones (600 MPa), which were not significantly different from one another and, which were perceived to have an almost fluorescent orange colour in comparison with the other samples. This was probably due to pigments being released into the surface after membrane disruption. The observed *surface moisture* was rated higher in the processed samples, with the highest score for cooked samples and the lowest in raw carrots. This result is probably due to cellular leakage, confirming greater damage to the cell membranes in cooked samples. The pressure treated samples exhibited greater *flexibility* in comparison with the rest of the samples, while raw carrots were judged as 'rigid' and cooked samples fell apart when examined between the fingers.

Table 7.2: Influence of different processing methods on carrot appearance, as judged by a trained sensory panel.

Sample	Orange	Visual Firmness	Brightness	Surface Moisture	Flexibility
Raw	51.95 ^{c}	86.68 ^{a}	47.12 ^{c}	28.17 ^{d}	26.83 ^{c}
HPP*	73.53 ^{a}	44.08 ^{c}	70.72 ^{a}	79.62 ^{b}	85.90 ^{a}
Sous Vide	66.02 ^{b}	52.13 ^{b}	59.77 ^{b}	64.63 ^{c}	78.45 ^{b}
Cooked	53.58 ^{c}	10.02 ^{d}	73.33 ^{a}	92.23 ^{a}	8.87 ^{d}
F-value	2.32	2.07	3.00	2.06	2.38
SED	2.38	2.54	1.68	2.59	1.95

*HPP: 600 MPa. Significant difference ($P<0.05$) are presented with different letters.

7.6.3 Texture perception

The trained sensory panel evaluated the following textural attributes *fibrous, crunchy, moisture in the mouth* and *chewing time* (Table 7.3). Panellists described the pressure treated carrots as the most *fibrous* followed by sous vide treatment, which were not significantly different from the raw samples. Cooked samples were perceived to be much lower in fibrous intensity. Raw carrots were judged as the *crunchiest* with the longest *chewing time*, while pressure treated and sous vide samples were less crunchy than raw carrots, but not significantly different from each other. The cooked samples were perceived as very low in *crunchiness*. Simon and Lindsay (1983) reported that carrots can lose up to 30 times their shear force (using a Kramer shear press) after canning in comparison to fresh carrots. This research shows that raw carrots, when cooked, lose their *fibrousness* and *crunchiness* (measured as texture attribute*)* 93 and 97 %, respectively, in comparison to cooked samples.

The level of *moisture* release perceived in the mouth was inversely related to the *crunchiness* and *chewing time*, suggesting that a greater water release during mastication may lower the perceived *crunchiness* and shorten the *chewing time* of the samples. The sensory analysis indicated that even though pressure treated carrots appeared to be more '*flexible*' (by using the fingers), they were more *fibrous* in texture (Table 7.3). This combination of attributes was considered to be unique by the panel. The perceived results are in agreement with previous findings of hardness profile, where HPP carrots showed a more curved trend showing more flexible texture, while raw carrots showed a texture profile with a more upright trend and presenting a more rigid texture (Chapter 5, Figure 5.7). *Moisture in mouth* perception could also be related to the surface moisture on the samples due to processing. The results showed that cooked carrots were perceived as highest in moisture, followed by the pressure treated and sous vide carrots (which were not significantly different), and raw samples.

Table 7.3: Influence of different processing methods on carrot texture, as judged by a trained sensory panel.

Sample	Fibrous	Crunchy	Moisture	Chewing time
Raw	56.78 [b]	83.42 [a]	45.70 [c]	80.83 [a]
HPP*	68.38 [a]	59.75 [b]	67.33 [b]	70.82 [b]
Sous Vide	60.88 [b]	58.15 [b]	62.27 [b]	65.93 [b]
Cooked	3.73 [c]	2.22 [c]	91.45 [a]	4.38 [c]
F-value	2.32	418.68	53.45	2.06
SED	2.38	1.68	2.59	2.59

*HPP: 600 MPa. Significant difference ($P<0.05$) are presented with different letters.

7.6.4 Odour perception

The odour of the carrot samples was described by *odour impact*, *green odour* and *processed odour* (low to high; Table 7.4). Panellists perceived raw and pressure treated carrots to be highest in *green odour*, followed by the sous vide and cooked samples. This result suggests that raw samples maintained their natural *green odour* best and that in comparative terms, HPP had a less damaging effect than other treatments on the carrots' *green* character. Cooked samples had the highest *odour impact*, followed by pressure treated, raw and sous vide. All samples differed significantly from each other in perceived *processed odour*, with cooked samples significantly higher in intensity followed by the sous vide samples, and then pressure treated followed by raw carrots. Overall, the pressure treated carrots were more similar to raw than the other treatments. These results highlight the less destructive nature of HPP and its ability to maintain the intrinsic character of the carrot compared to thermal processes. This is in agreement with what has been previously reported in the literature, which reports that there are high losses of aroma in cooked carrots in comparison to carrots processed by sous vide (Werlein, 1998). Employing less destructive processing allows carrots to better maintain their inherent character, while cooking increases the odour impact due to a release of many low molecular weight volatile compounds.

Table 7.4: Influence of different processing methods on carrot odour, as judged by a trained sensory panel.

Sample	Odour impact	Green	Processed
Raw	57.65 [c]	66.38 [a]	4.83 [d]
HPP*	63.05 [b]	65.73 [a]	22.33 [c]
Sous Vide	53.48 [c]	53.35 [b]	32.08 [b]
Cooked	69.75 [a]	5.32 [c]	89.80 [a]
F-value	10.52	63.41	125.35
SED	2.17	3.63	3.29

*HPP: 600 MPa. Significant difference (P<0.05) are presented with different letters.

7.6.5 Flavour and aftertaste perception

The flavour of carrot samples was described by the following attributes *flavour impact, sweetness*, *bitterness*, *processed*, *green*, *aftertaste impact* and *bitter* (Table 7.5). In general, the results showed lower intensity ratings for *flavour impact*, *aftertaste impact* and *sweetness* in cooked samples when compared with the raw, sous vide and pressure treated samples. Cooked carrots were lower in perceived *sweetness* intensity when compared with all of the other samples. This may be due to a reduction in sugar content, which has been previously observed in canned carrots (between 30 and 50% relative to raw samples; Simon and Lindsay, 1983). Reductions in sugar content are caused by leaching during cooking (Varming et al., 2004). Sweetness in strained carrot has been previously correlated with total sugar content, while cooked flavour and aftertaste have been correlated with high terpinolene content (Howard et al., 1995). Furthermore, cooked carrots had the highest intensity of *processed flavour*, followed by the sous vide and pressure treated samples, which again were not significantly different from one another. The highest perceived *green flavour* was in the raw samples, followed by pressure treated and sous vide carrots, which were not significantly different from one another.

In this study, *bitterness* and *aftertaste* analysis showed significant interactions between products and also assessors; this could indicate a lack of agreement between panellists on the bitterness within samples or it can point to sources of disagreement between assessors in terms of product ranking and/or scale use. For this reason, significant differences between treatments were not able to be determined. It is important to

highlight the fact that, in general, not all carrots present bitterness. Therefore it is difficult to account for this parameter using random samples. As previously mentioned, bitterness in carrots is often associated with the presence of 6-methoxymellein, which is promoted by stress reactions (Seljåsen et al., 2004). This increases the variation within samples with regards to bitterness.

Table 7.5: Influence of different processing methods on carrot flavour and aftertaste, as judged by a trained sensory panel.

Sample	Flavour Impact	Sweetness	Bitterness	Processed	Green	Aftertaste Impact	Bitter aftertaste
Raw	58.80 [a]	50.05 [a]	14.55	3.42 [c]	58.05 [a]	50.18	13.72
HPP*	57.78 [a]	48.67 [a]	21.37	23.48 [b]	49.35 [b]	45.55	19.57
Sous Vide	60.20 [a]	53.93 [a]	11.75	21.87 [b]	45.22 [b]	50.45	9.60
Cooked	34.27 [b]	11.90 [b]	17.20	90.03 [a]	5.07 [c]	30.12	15.10
F-value	33.69	61.50	6.83	460.22	92.23	16.74	5.61
SED	2.13	2.50	1.57	1.77	2.45	2.34	1.74

*HPP: 600 MPa. Significant difference ($P<0.05$) are presented with different letters.

7.7 Physical measurement

7.7.1 The effects of different treatments and storage time on carrot juiciness

Juiciness is a measure of the juice released from tissue during compression and is dependent on the cellular structure, turgidity, integrity and cell wall strength. Cooked carrots were significantly juicier than any other samples (Figure 7.1). High pressure processed samples were significantly juicier than raw samples. In a separate experiment, approximately the same juiciness percentage for raw (~10 %) and pressure treated (~25%) samples were observed (Chapter 6, Figure 6.5), indicating repeatability in the method used even though samples had a different shape (cylinders). Sous vide samples were significantly ($P<0.05$) less juicy than the cooked and pressure treated samples. A significant increase in juiciness was observed in sous vide samples in comparison with raw samples for day 7 of storage (Figure 7.1). The lowest juiciness was observed in raw carrots, due to their intact microstructure and stronger cell wall, resulting in less release of the cell contents when compressed.

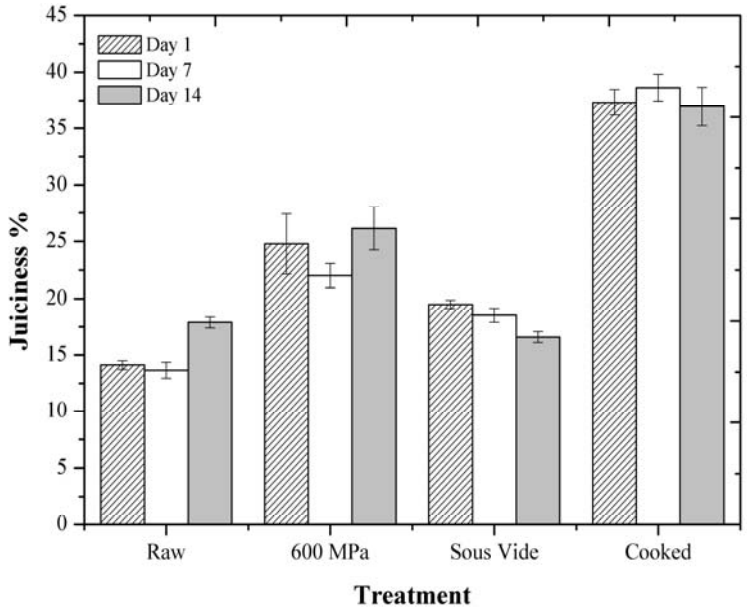

Figure 7.1: Instrumental juiciness of different carrot treatments during 14 days storage at 4°C.

Szczesniak and Ilker (1988) indicated that raw carrots were perceived as much less juicy (ranked 1 on a 0-10 scale) in comparison with other vegetables. This can be explained by carrots having tissue with high cell wall reinforcement components such as lignin (Davis and Gordon, 1980).

Statistical analysis showed significant differences ($P<0.05$) between treatments but no significant changes during storage, except for the raw samples, which showed an increase in juiciness as storage time increased. However, what was perceived as 'juiciness increase' could be due to the sample's quality degradation, caused by their anaerobic storage conditions and consequent bacterial growth. Trends in moisture perception and juiciness for raw, HPP, sous vide and cooked samples are shown in Figure 7.2A. A strong correlation between perceived moisture and juiciness was found with an R^2 of 0.98 (Figure 7.2B).

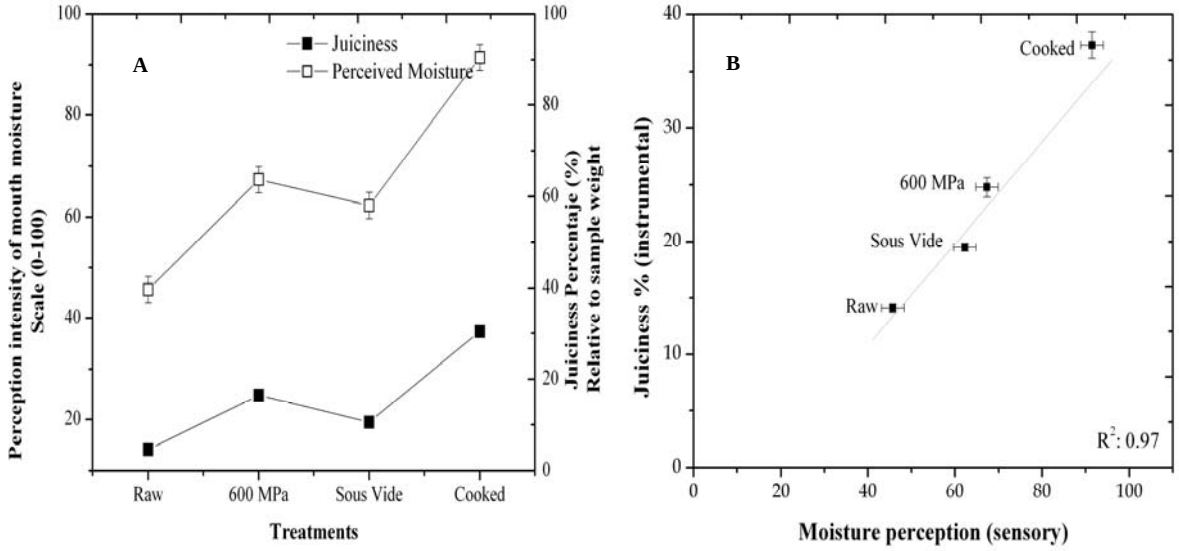

Figure 7.2: Trends of moisture perception and juiciness for different treatments (A). Linear correlation between juiciness and moisture perception (B).

7.7.2 The effects of different treatments and storage time on carrot hardness

Tissue hardness measured by compression indicated significant differences between treatments ($P<0.05$). The decrease in hardness compared with the raw sample at day 1 was 29% for sous vide 44% for pressure treated and 96% for cooked samples, respectively (Figure 7.3). Similar decreases in hardness were observed by De Roeck et al., (2008) in cooked and processed carrots using HPP combined with heat relative to their respective raw control samples. It was explained that cooked carrots lost their texture mainly due to beta-eliminative depolymerisation of pectin, while HPP combined with heat could preserve texture better due to a possible fortification of networks between low methoxylated pectins by calcium ions (De Roeck et al., 2008).

Significant differences in hardness were also observed between storage durations. The pressure treated and sous vide samples increased significantly in hardness (40% and 50%, respectively) at day 14 in comparison with day 1, while cooked samples did not show such an increase over time. It is not clear why these samples showed an increase in hardness. One possibility is that this hardening over time could be due to an initial significant deterioration of the tissue due to processing, which then recovers during storage via cellular rearrangement. The other possibility is that PME demethylated pectins, enhancing calcium bridging. Werlein (1998) reported an increase of 27% in

shear force in sous vide carrots after storage (3-7 days, 2°C) (measured by a Kramer shear press).

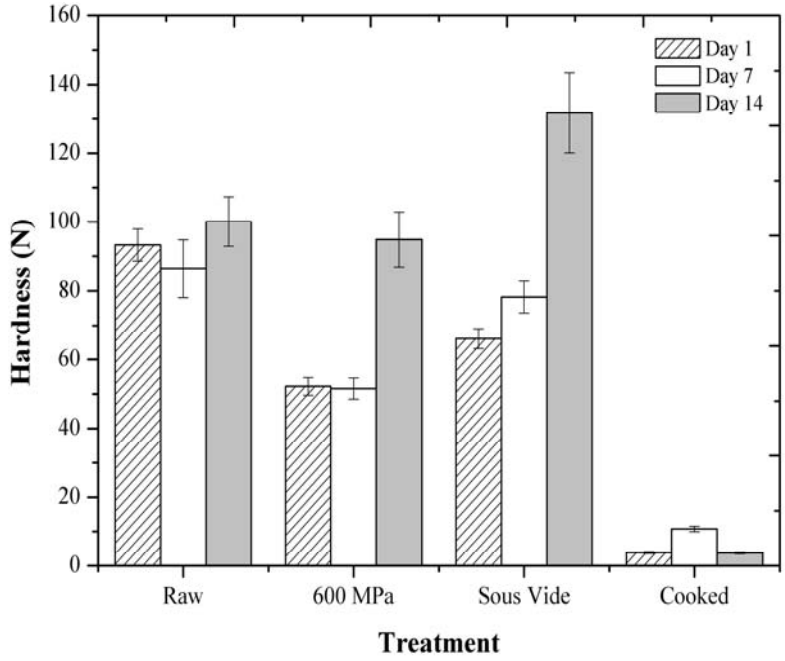

Figure 7.3: Tissue hardness for different carrot treatments during 14 days storage at 4°C.

The mean hardness at day 1 presented similar trends with treatments to sensory perception of crunchiness (Figure 7.4A). The perception of crunchiness of pressure treated and sous vide carrots was very similar (~ 60 on a 100 point scale). Hardness measured instrumentally was significantly different between the processing treatments. A linear relationship was formed between instrumental measurement of hardness and sensory perception of crunchiness for different treatments (Figure 7.4B).

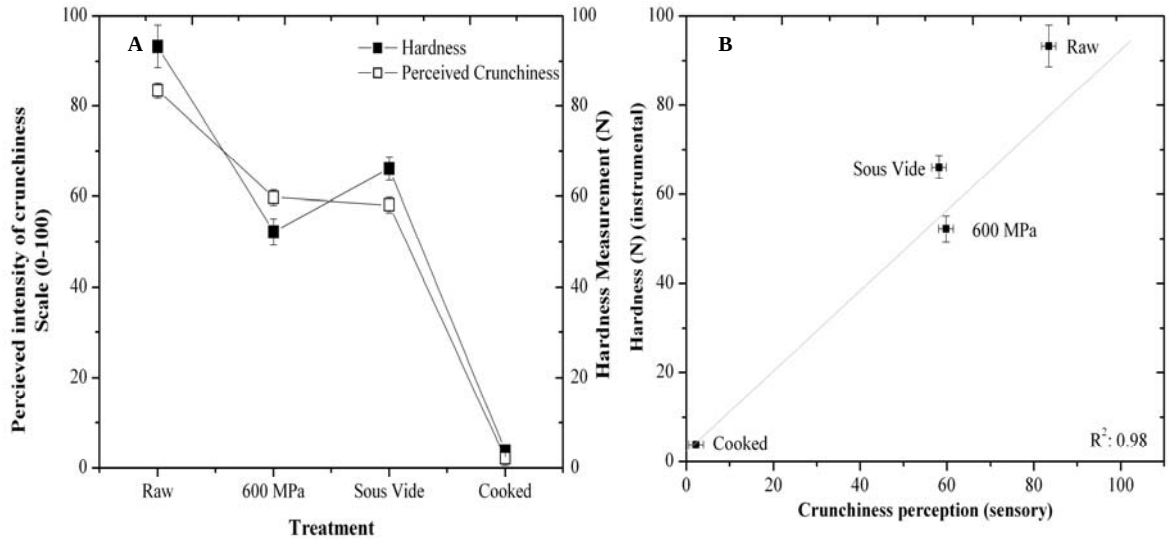

Figure 7.4: Trends of perceived crunchiness and hardness for different treatments (A). Linear correlation between perceived crunchiness and hardness (B).

7.7.3 The effects of different treatments and storage durations on carrot peak cutting force

The cutting force action applied by a blade to the carrot sample can indicate either how resistant the tissue is to fracture (using the maximum force during the cutting cycle) or it can indicate how 'rubbery' the tissue is, as indicated by an increase in both displacement and cutting force. Low cutting forces were associated with a softer product and in this case cooked samples were very soft. Results showed slightly higher cutting forces in pressure treated samples in comparison with raw (Figure 7.5). Previous experiments also showed that greater forces were required to cut through pressure treated samples in comparison to raw carrots (Chapter 5 and Trejo et al., 2007). Sous vide and cooked samples required significantly less force to be cut than raw and pressure treated samples. During storage, the peak cutting force for raw and sous vide carrots decreased slightly over time, while there insignificant changes were observed in the pressure treated samples.

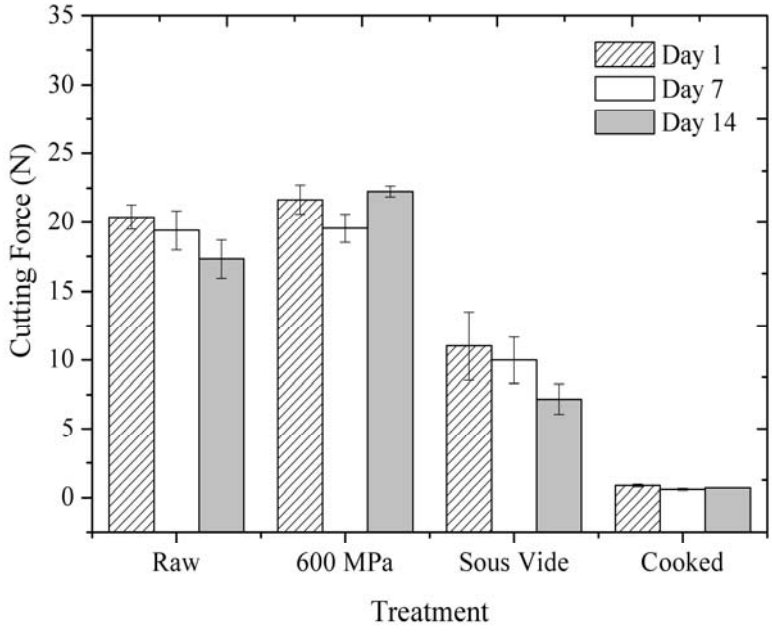

Figure 7.5: Peak cutting force for different carrot treatments during 14 days storage at 4°C.

7.7.4 The effects of different treatments and storage time on carrot bending force

The three point bending test was performed to measure the force required to snap the carrot sample in half. Cooked carrot was not suitable for this test due to their extremely soft texture. Raw samples required considerably less displacement before snapping as compared with processed samples (Figure 7.6). Significant differences in bending forces were found between sous vide and pressure treated carrots. This is an interesting finding, considering previous work (Trejo et al., 2007) that reported significant texture losses in pressure treated carrots, as compared with raw carrots; furthermore HPP samples were more flexible as shown by the increased displacement during the cutting action. Pressure treated carrots presented the highest displacement before snapping (Figure 7.6), which correlates with flexibility results as described by the sensory panel (Table 7.2).

The bending peak force increased with storage time for processed samples; however the trend was more heterogeneous, with larger variations between samples. Stored raw carrots exhibited an increase in displacement before snapping, while pressure treated and sous vide carrots required less force (0.5-1.8 N) with greater displacements (4 to 7

mm). The bending peak force results are in agreement with previous sensory perception results, where pressure treated carrots (600 MPa) was found to be more flexible than other samples (Section 7.6.2 Table 7.2).

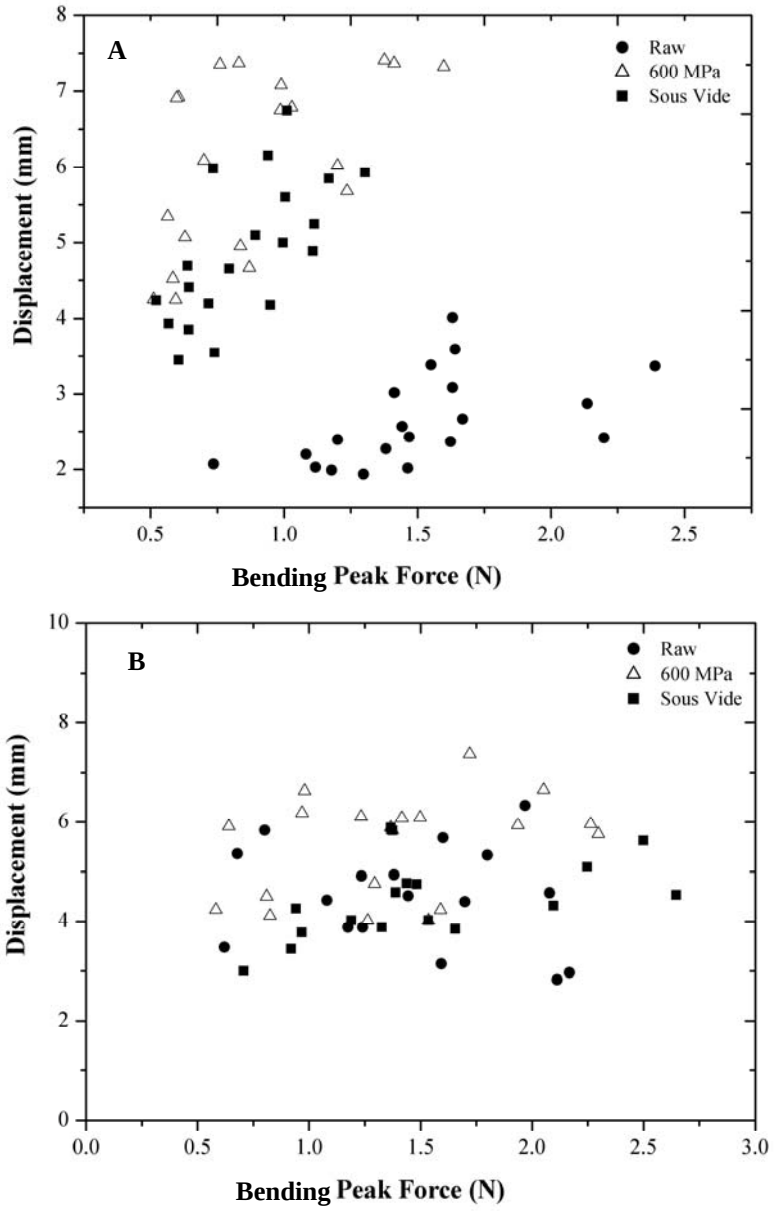

Figure 7.6: Displacement versus bending peak force for different carrot treatments, A = day 1 and B = day 14 at 4°C.

It is not clear why processed samples would increase in snapping force as time increased, apart from a possible pectin-calcium interaction that have occurred through time as a consequence of cell disruption; this could have led to some changes in peak forces.

An increase in displacement is consistent with structural changes leading to a loss in turgidity and resulting in a more flexible matrix. After 14 d of storage, a shift in displacement of raw samples indicates greater sample flexibility. This could possibly be due to microbial growth (which will be analysed later in the chapter) and/or fermentation under the anaerobic packaging conditions, modifying perhaps the pH and consequently causing membrane damage (turgor loss).

7.7.5 The effects of different treatments and storage times on carrot colour

Colour measurements were collected at day 1, 7 and 14 of storage. Raw samples exhibited significantly higher b* values (more yellow) in comparison with the other treatments at day 1 (Figure 7.7). b* values for pressure treated, sous vide and cooked samples showed no significant differences between each other. Werlein (1998) observed differences between sous vide and cooked samples where sous vide were less yellow (lower b* value) than cooked samples at day 1. The effects of storage time for colour parameter b* indicated that most changes occurred at the beginning of the first week of storage rather than later (14 days) for all samples.

Significant differences were observed in a* values between different treatments at day 1. At day 1, raw carrots presented the highest a* value (more red). Pressure treated and sous vide samples presented significantly lower a* scores in comparison with raw, while cooked carrots presented the lowest a* value out of all samples indicating less red colour (Figure 7.7). It is possible that the loss of redness in cooked samples is the result of the leaching of pigments through cooking.

Analysing both parameters a* and b* together, it is observed that raw samples were less orange by presenting the highest a* and b*, while a* values were predominant for sous vide and HPP samples, indicating a more intense orange colour. These results are in agreement with the findings of Eshtiaghi et al., (1994), where redness retention of pressure treated carrots was shown to be higher than in raw and water blanched carrots.

It is interesting to mention that the highest orange colour scored by the trained panel was found in HPP and sous vide carrots (Table 7.2). The storage trial showed that samples did lose some of the yellow colour (lowering their b* value) as time progressed.

No significant differences were observed in redness with time for most samples except for sous vide which showed a slight decrease as storage time increased.

The third colour parameter analysed was lightness (L*). In general the results showed no significant differences in L* values between HPP and sous vide treatments, but differences were observed between the raw and cooked samples. Lightness was significantly affected by storage, decreasing in the first week but not further in the second week for raw and HPP samples. Sous vide sample L* values did not change over time, while in cooked samples L* diminished during storage. In terms of whitening development while stored, mentioned previously in section 2.2.3 and Tatsumi et al., (1991), samples in this experiment did not showed whitening or a dry surface due to vacuum packaging.

It is important to mention that as storage duration increased, some samples showed microbiological deterioration, which will be further discussed in Section 7.8.3. The effects of which (e.g. sliminess) may have affected the colour measurements.

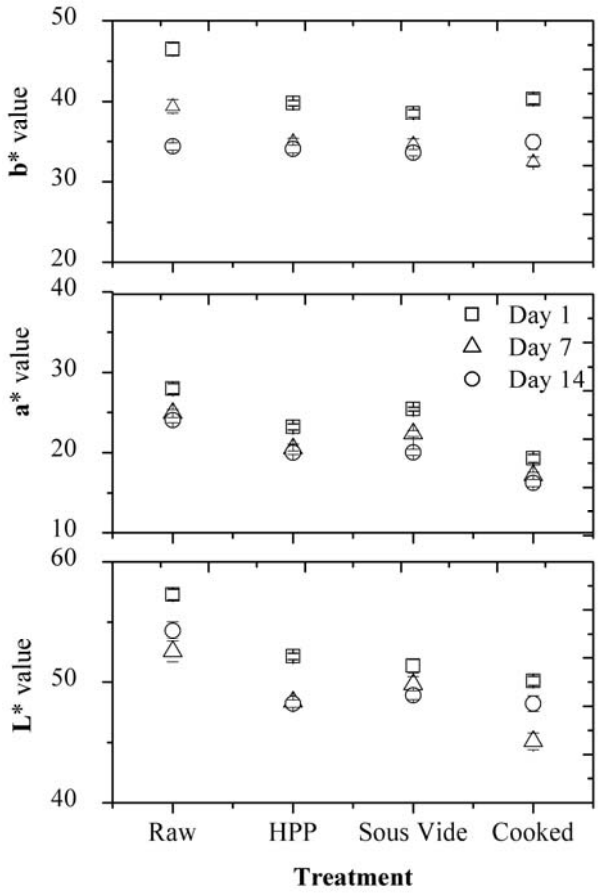

Figure 7.7: Colour changes in carrot tissue after processing and storage at 4°C.

7.8 Chemical analysis

7.8.1 Headspace volatiles

The headspace volatiles of carrots were analysed by GC-MS at day 1 and day 14 of storage at 4°C. Carrot volatiles are divided into monoterpenes (including a-pinene, sabinene, b-myrcenen, limonene, y-terpinene, p-cymene, terpinolene), terpenoids (including b-caryophellene) and sesquiterpenes (including copaenen, cuparene, b-bisabolene), while irregular monoterpenes such as 6-methyl-5 hepeten2-one and b-ionone mostly come from carotenoids (Kjeldsen et al., 2003). The main carrot volatiles found in the four treatments and determined by GC-MS-O are given in Table 7.6.

Representative examples of the GC-MS chromatograms of raw and pressure treated carrot samples are presented in Figure 7.8. In this study, concentrations of α and β-pinene (which represent the green-carrot notes) were significantly higher ($P<0.05$) in raw carrots in comparison with processed carrots (Figure 7.9A). This may have an

effect on the sensory perception of these compounds represented by *'carrot top'* and '*fresh green'* notes. Sensory results from this work, such as those for green flavour, were significantly higher for raw carrots as compared with the rest of the samples, which correlates well with the analytical results. Furthermore, there were significant differences in the '*green*' odour perception by the panellists between the raw, sous vide and cooked carrots, but no significant differences were observed between the raw and pressure treated carrots. Published reports indicate that terpinolene and caryophellene contribute significantly to carrot flavour intensity (Simon and Lindsay, 1983).

According to the results, all monoterpenes and terpinolenes were still present after all treatments, meaning that the intrinsic carrot compounds were not lost and, in some cases, were even increased in processed carrots relative to the raw samples.

Previous studies of the application of HPP to strawberry aroma have indicated that raw and pressure treated (200 to 500 MPa, 20 minutes, 20°C) samples presented no significant differences in volatiles, while some new compounds were formed in those processed at higher pressures (800 MPa; Lambert et al., 1999). Other studies showed preservation of most peach volatiles after HPP, but some enzymatic formation of aldehydes and alcohols occurred due to cell disruption (Sumitani et al., 1994).

Table 7.6: Retention time and odour descriptor for the main fresh carrot volatiles measured by GC-MS and GC-MSO.

Compound	Retention time (minutes)	Odour Descriptor
α-Pinene	4.4	Carrot top
Camphene	5.3	-
Sabinene	6.5	-
β-Pinene	7.7	Fresh green
γ-Phelandrene	7.7	Pine
Myrcene	7.9	Green
α-Terpinene	8.2	Citrus
Limonene	8.7	Lemon
P-Cymene	10.9	Carrot
Terpinolene	11.1	Grass
6-Methyl-5 hepten 2-one	12.9	Bitter/spicy
α -Caryophellene	21.6	Sweet/grassy
α - Longapinene	22.1	Fresh/green
α –Farnesene	23.7	Floral
Curcumene	24.2	Carrot

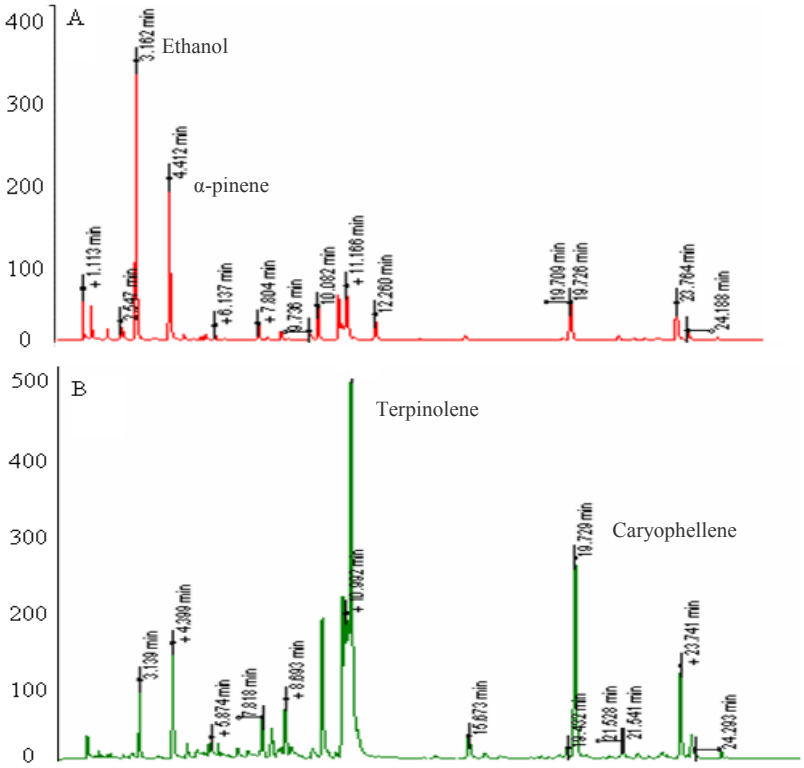

Figure 7.8: A single GC-MS chromatogram example of raw (A) and pressure treated (B) carrot tissues at day 1. Axis X: Abundance, axis Y: time (min).

Other studies of carrot volatiles and their odour description have indicated that acetaldehyde contributes to the "sweet" note, while myrcene contributes to the "green", "earthy" and "carrot top" notes (Heatherbell et al., 1971). Caryophyllene can be "perfumy", while a,p-dimethyl styrene had a strong 'woody green' note (Heatherbell et al., 1971). P-cymene is also a compound that has been described as contributing to a 'carrot top' note. This was high in raw and pressure treated carrots on day 1, while for sous vide and cooked samples it only reached proportions of 0.66 and 0.33, respectively, of the level in the raw sample (Figure 7.9A).

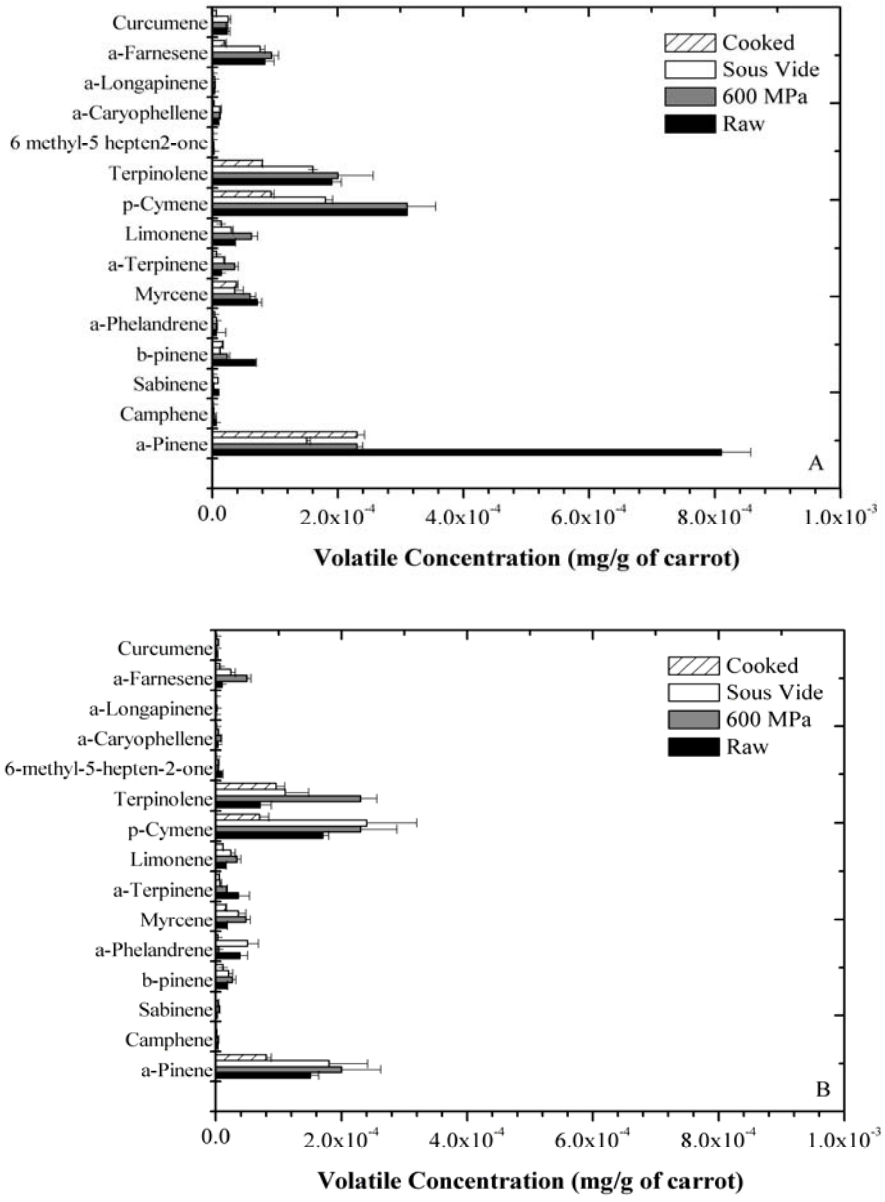

Figure 7.9: Main volatile compounds of raw, sous vide, HPP and cooked carrots (A = day 1; B = day 14 at 4°C).

It has been reported that canning carrots can transform compounds, such as styrene, to produce dimethyl sulphides and ethanethiol compounds, giving the typical canned carrot flavour (Heatherbell et al., 1971). α- and β-pinene, myrcene and p-cymene concentrations decreased significantly in raw samples during storage, while concentrations were stable in pressure treated samples (Figure 7.9 A and B). These findings demonstrate how sensitive some compounds can be to heat but not to HPP.

Terpinolene concentrations increased during storage of pressure treated samples in comparison to the rest of the treatments (Figure 7.9B). There is no clear explanation for this increase apart from product deterioration and release of compounds to outside the cells making them more available. Some compounds, including a-terpinene and a-phelandrene, increased in raw carrots after 14 d storage. This increase is more likely to be due to sample variation than due to a chemical reaction or product. Heatherbell et al., (1971) indicated that p-cymene, octanal, 2 decenal and a,p-dimethyl styrene increased during storage after canning, while Alasalvar et al., (1999) suggest that microbial contamination will influence the changes in volatiles, and that appropriate volatiles could therefore be used as quality indicators.

In contrast to these results, studies of volatiles during the storage of fresh carrot (cut into sticks) have indicated there were no changes in volatiles during 28 days of storage, except for an exponential increase propanol as storage temperature increased from 25 to 35°C, which was attributed to microbial growth (Alasalvar et al., 1999).

Vacuum packaged carrots studied in this research showed fermentative products such as ethanol and acetic acid during storage of raw, sous vide and cooked carrots, while pressure treated samples showed some production of ethanol but no production of acetic acid (Figure 7.10). This is in agreement with Seljåsen et al., (2004) who reported that fermentative products were observed in raw carrot samples stored between 2 and 20°C using different types of ventilated packaging. In the current research, the GC-MS-O analysis described '*alcohol*' odours at 3.16 min for most samples (equivalent to ethanol) and '*vinegar*' odours at 16.8 min (equivalent to acetic acid) in all samples except those that were pressure treated, correlating well with the GC-MS results. A significant concentration of ethanol was found in raw and sous vide samples on day 1 in comparison to pressure treated or cooked samples (Figure 7.10). However, there was

no evidence of acetic acid in either of these samples at that time. As storage time increased, acetic acid increased significantly in most samples except those that were pressure treated. Raw samples also showed a three-fold increase in ethanol production after 14 days (Figure 7.10). This is a result of conditions induced by the vacuum packaging employed for storage. Previous studies conducted with vacuum packed carrots slices stored at 4°C for 8 d showed maintenance of colour and appearance but no results of microbial or anaerobic spoilage were reported (Buick and Damoglou, 1987). While this research showed some significant changes in the carrots' colour parameters, it also showed the formation of sub-products due to spoilage such as acetic acid in raw, sous vide and cooked carrots after 14 days of storage. Results showed no evidence of the formation of acetic acid in pressure treated samples or an increase in ethanol in these samples. A reason for this could be that high pressures can stop the carrot's respiration metabolism resulting in a 'dead product' as shown in Chapter 4.

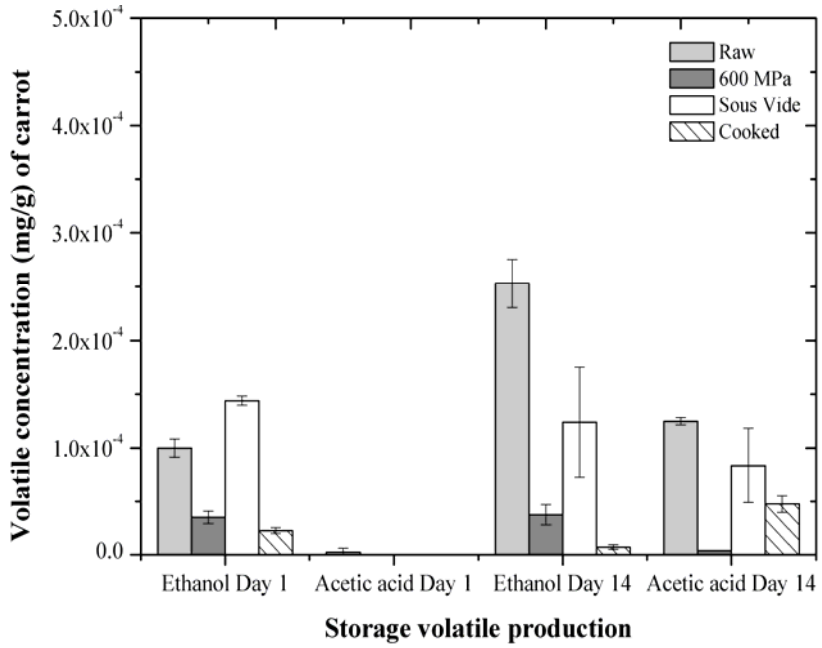

Figure 7.10: Ethanol and acetic acid production in vacuum packed carrots at day 1 and after 14 days of storage at 4°C.

7.8.2 GC/MS-O perception of headspace carrot volatiles

Three assessors described the olfactometry perception of samples passing through the GC/MS-O column. The trends indicated that raw samples had higher '*carrot*' and '*green*' odour intensity in comparison to the rest of the treatments; while more earthy

notes were perceived in the sous vide carrots. Cooked carrots presented less earthy, woody and herb notes (Figure 7.11).

Similar results were reported by Shamaila et al., (1996) in their study of headspace volatiles in water blanched carrots, and they concluded that volatiles could be affected by heat-promoted degradation, evaporation and leaching of compounds. Sulphur derivatives were perceived in cooked carrots on day 1 (Figure 7.11A).

The storage effects on the volatile perception showed that most samples, including the raw carrots, developed a sulphur smell '*rotten rubbish*' (as described by the assessors) odour, except for the pressure treated carrots, after 14 d storage at 4°C (Figure 7.11B).

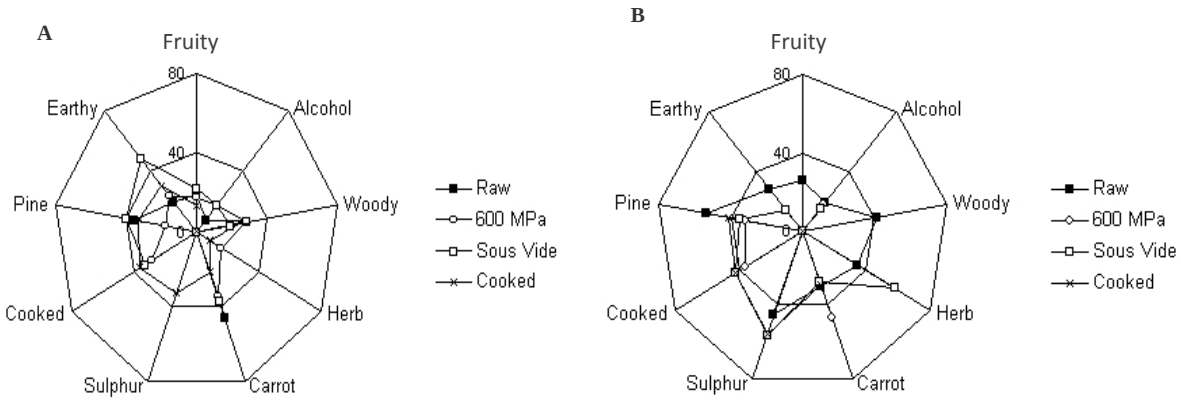

Figure 7.11: Intensity of volatiles from carrot samples assessed using olfactometry and presented using panellist descriptors for day 1 (A) and day 14 (B) of storage at 4°C.

The olfactometry test showed that most perceived odours in raw samples had increased by day 14. This outcome did not correlate with the previous GC/MS trends as most of the volatile concentrations quantified decreased during storage. A reason for this could be that some compounds are more potent to human nose than others, and in some cases volatiles were detected only by two assessors, making correlations between instrumental and olfactory perceptions difficult.

The olfactory results through storage not only showed that most basic carrot notes were maintained in raw, sous vide and pressure treated samples, but also that there was the formation of new compounds due to fermentation and microbiological spoilage in all samples, except for the pressure treated samples. This was in agreement with the

fermentation products results (Figure 7.10) and could explain why raw samples were perceived to have a more intense odour after storage.

Overall, agreement between the GC/MS and GC/MS-O analysis was found at day 1, especially in the identification of characteristic carrot compounds and their perception.

7.8.3 Microbiological quality contamination

Yeasts, moulds and lactic acid bacteria are the most common microbial populations in handled and processed vegetables (Zagory, 1999); *Clostridium perfringens* can also be found in vacuum packed vegetables products. Therefore, these species were enumerated for all carrot treatments on days 1 and 14. Previous studies on aerobic mesophiles in vegetables after HPP showed a decrease of 1 log cycle ($cfu.ml^{-1}$) after processing at 350 to 400 MPa and 20°C for 10 minutes (Arroyo et al., 1997). The results from this research showed lower standard plate counts in HPP samples in comparison with the rest of the treatments at day 1 (Table 7.7). There was evidence of the presence of yeast and moulds at day 1 in sous vide samples, whilst no evidence of these microorganisms was found for the rest of the treatments. *Clostridium perfringens* counts were $<10.g^{-1}$ for all treatments. After 14 days of storage at 4°C, no increase in counts of *Clostridium perfringens* was observed (Table 7.7).

It is important to highlight that carrots were pre-washed but not sanitised with chlorine before processing, to avoid changes in sensory perception. This may have contributed to the high initial microbial load. However literature suggests that sanitizing does not necessarily prevent growth during storage (Nguyen-the and Carlin, 1994; Zagory, 1999).

From a safety perspective, literature suggest that the application of high pressures above 350 MPa for at least 5 minutes at 36°C are necessary to obtain a product such as carrots to be microbiologically acceptable (Arroyo et al., 1997). Overall, HPP samples had considerably lower SPC ($cfu.g^{-1}$) than raw and sous vide samples, while very similar to cooked samples immediately after processing (day 1). After 14 days of storage, cooked and HPP samples again presented the lowest and similar SPC counts, followed by sous vide then raw. In terms of lactic acid bacteria, raw samples appeared with the highest count after 14 days of storage, followed by cooked sous vide and HPP. These results

are similar to those found for acetic acid production (Figure 7.10), where the highest concentration was found in raw samples. While, less correlation was found in LAB and acetic acid concentration for cooked and sous vide samples, high pressure samples did show correlations between LAB and acetic acid concentrations proving to be the lowest in both cases. This result may contribute to further storage research for commercialization.

Table 7.7: Microbiological assessment for raw and processed samples at day 1 and 14 of storage.

Microbiology test	**Raw**	**600 MPa**	**Sous Vide**	**Cooked**
DAY 1				
SPC (cfu/g)	5.1E+03	<10	5.0E+04	1.5E+02
Yeast / (g)	<100	<100	600	<100
Mould / (g)	<100	<100	1000	<100
LAB (cfu/g)	<100	<100	<100	<100
Clostridium perfringens / (g)	<10	<10	<10	<10
DAY 14				
SPC (cfu/g)	1.2E+08	8.5E+06	3.40+07	5.4E+06
Yeast / (g)	<100	<100	<100	<100
Mould / (g)	<100	<100	<100	<100
LAB (cfu/g)	8.00+07	2.80+06	2.2E+07	5.7E+07
Clostridium perfringens / (g)	<10	<10	<10	<10

SPC: Standard plate count; LAB: Lactic acid bacteria

Photographs of raw and processed carrots after 14 d of storage at 4°C are shown in (Figure 7.12). Images show how raw carrots (Figure 7.12A) have lost their colour and the vacuum packages have swollen during storage in comparison to the processed samples, which could be due to respiration and/or microbial fermentation, this confirms the loss in texture previously found in Figure 7.6, shown as a displacement increment, which in other words could describe tissue deterioration.

Pressure treated carrots (Figure 7.12B) showed the best colour preservation and maintained vacuum, while sous vide carrots showed a slightly poorer orange colour (Figure 7.12C). Cooked packed carrots were slimy after storage. This was probably because samples were firstly cooked and then dried and vacuum packed. This probably led to cross contamination giving high LAB after 14 days of storage.

7.9 Cryo-scanning electron microscopy of carrot tissue

Carrot tissue was observed using Cryo-SEM to show the cut surface. Images clearly showed a more compacted and structured character for the raw tissue. This tissue exhibited little evidence of cell separation or disruption, while pressure treated and sous vide carrot images presented a less organized arrangement (Figure 7.13). Samples processed at 600 MPa showed a very similar structure to sous vide tissue, which corresponds with the similarities observed between most of the quality changes observed for these two processes (Figure 7.13).

Figure 7.12: Vacuum packed, processed and raw carrots after 14 days of storage at 4°C; A: raw; B: pressure treated (600 MPa, 2 min); C: sous vide; D: cooked.

Frozen water inside the cells was present in all samples due to the technique applied for analysis. It is interesting to observe that for the pressure treated samples, less tissue damage resulted in smaller gaps between ice crystals inside the cells and less cell separation (Figure 7.13B). Similar ice patterns have been previously observed in

cauliflower tissue before and after HPP (Préstamo and Arroyo, 1998). Images of cooked cells showed disruption as characterised by larger gaps between ice crystals inside the cells. Davis and Gordon (1980) supported this finding reporting observation in less damage of steamed carrots as compared with cooked carrots via SEM images. Overall, the cryo-SEM images provided more insight into the tissue structural changes that occurred due to different processing techniques and can help in understand differences and similarities within the quality changes observed throughout this research for all treatments.

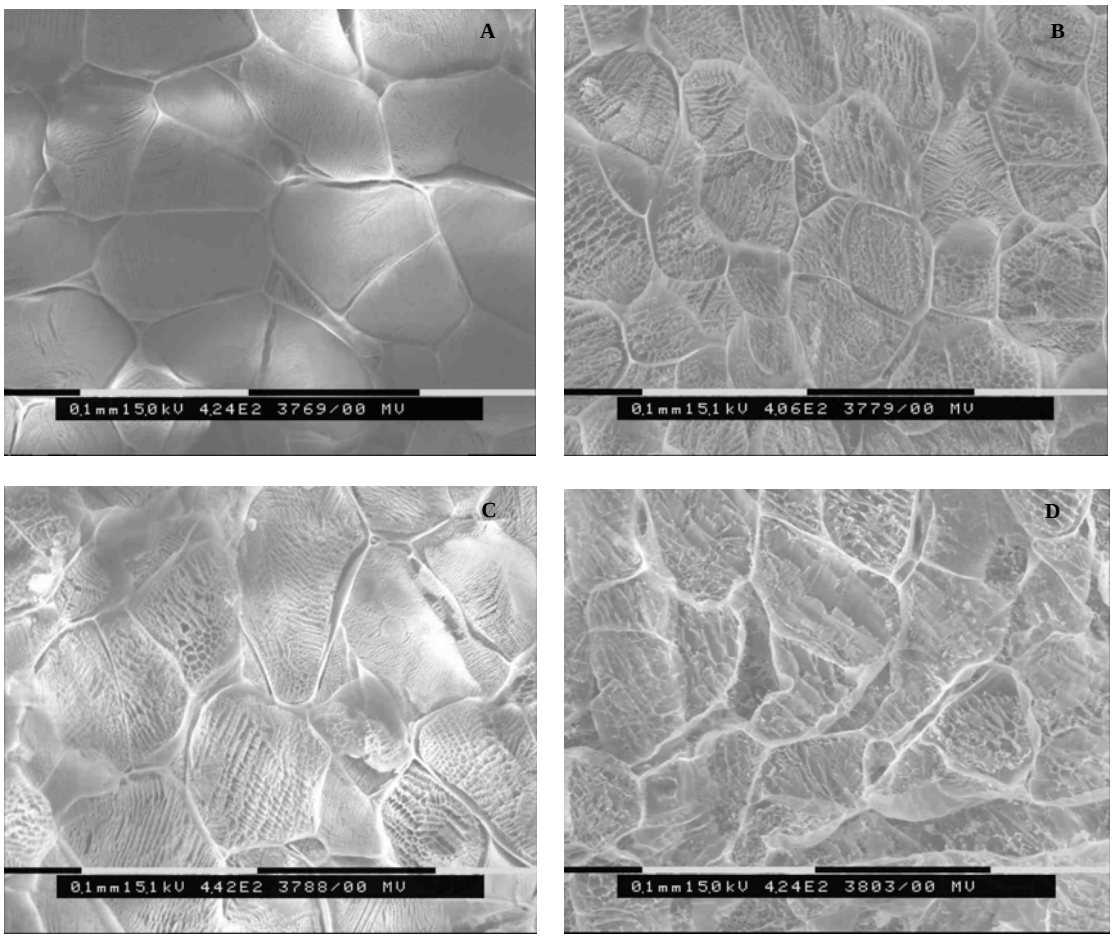

Figure 7.13: Scanning electron microscopy of carrot tissue at day 1; A: raw; B: pressure treated (600 MPa, 2 min); C: sous vide; D: cooked.

7.10 Conclusions

Correlations between sensory and physical attributes were observed across all treatments in this study. The perceptual characteristics of pressure treated (600 MPa) carrots were not significantly different to those of sous vide samples in most cases

(sweetness and green flavour; crunchy texture). However pressure treated samples were perceived as more *orange, brighter, moister*, more *flexible,* more *fibrous* and had greener *odour* tones than sous vide. The sensory appearance showed that even though carrots appeared to be more '*flexible*' (by using the fingers), they were more *fibrous* in texture than the rest of the treatments. This was described as a unique positive attribute of the high pressure processed samples. Raw carrots were perceived as having more '*green*' flavour and '*firm*' texture, as expected, but with less orange colour; opposite perceptions were found in cooked carrots. These results are consistent with observations of the cellular microstructure, and specifically structural differences between carrot samples observed in cryo-SEM images.

Headspace volatile analyses showed significant differences between raw and processed carrots at day 1. Volatile concentrations diminished significantly with storage time except for pressure treated (600 MPa) samples, which better maintained their volatile levels during storage. This study observed some correlations between GC/MS and GC/MS-O; these correlations may have been stronger if not for the unavoidable large variation between individuals, the numerous carrot samples and further variations between different perceptions of the odorant compounds. With regard to undesirable volatile production, pressure treated samples did not show an increase in ethanol during storage, whilst this was observed in both sous vide and raw carrots. Furthermore, pressure treated carrots were the only samples that did not produce acetic acid after 14 days.

It has been widely mentioned that HPP is an accepted alternative food processing technology due to its benefits of preserving most food quality attributes (Cardello et al., 2007). No evidence was found to suggest that the applications of HPP to carrots caused quality deterioration substantially different from that caused by mild heat treatments such as sous vide. Furthermore, HPP carrots were better preserved during 14 days of storage by not producing acetic acid and presenting less presence of lactic acid bacteria. The detrimental effects of cooking were not perceived in pressure treated carrots. Moreover, although the texture of pressure treated carrots was perceived as more *flexible,* it also had a more fibrous texture not observed in any other samples. Previous reports on HPP for food applications indicated that this technology will only be successful if added value is achieved or if the product characteristic can be retained at a

higher level as compared to thermally/traditionally processed foods. This work provides more correlations between sensorial perception and textural and chemical (volatile) changes in carrots that have undergone high pressure processing, as well as how those changes compare to the quality of both raw and thermally processed carrots through a period of 14 day of storage. These results may be generally applicable to what could be predicted to happen to other 'hard' tissue vegetable products subjected to high pressure processing.

8 Overall discussion

Unfortunately, it is not yet possible directly to observe structural changes happening in food systems during a high pressure process cycle (compression, holding time and decompression). At the moment we can only understand changes through observations of properties of food systems and structures after the high pressure batch process has finished (like any black box system). This leaves researchers to postulate possible mechanisms of action occurring in food systems under pressure by: (a) using basic science concepts and (b) observations of changes in properties and structure caused by high pressure processing. In this work, experimental measurements such as hardness, cutting force, juiciness, respiration rate and sensory evaluation were employed to assess alterations of quality attributes of carrots subjected to high pressure treatments.

Applications of high pressures were shown to alter various quality attributes of carrots (used as a model system). Pressures from 100 to 600 MPa resulted in decreases in textural parameters such as hardness, cutting force and stiffness. Product juiciness increased at 300 MPa and the aroma composition was slightly altered. As a consequence the sensory attributes of the carrots were modified for some attributes. Furthermore, physiological properties like respiration rates were also disturbed as a result of pressure application, respiration shifting from aerobic to anaerobic. By interpreting these observations in terms of the key mechanisms affecting carrot tissue, an increased understanding of HPP processing of carrots can be developed. Such understanding could lead to optimisation of HPP to obtain processed materials of required properties, and prediction of how HPP would affect other whole fruit and vegetable products. Figure 9.1 shows a possible mechanism from the structural level that occurs in carrot tissue subjected to high pressures. This diagram will help to understand further discussion given in the chapter on HPP effects in carrot tissue.

The mechanisms of textural changes occurring during high pressure processing

One of the main effects observed when applying HPP to whole carrot pieces was a reduction in textural parameters such as hardness, peak cutting forces and stiffness. Hardness of carrot tissue processed at lower pressure (100 MPa) was not different from raw samples but significant reductions occurred from 200 MPa onwards. The

mechanisms involved in hardness loss, for example, can be related to (1) the turgor loss of cells and (2) shape alterations of the cell walls. Turgor loss is the result of an increase in cell membrane permeability or disruption, promoting leakage of interior cell liquids.

In this study, turgor loss shown as leakage was found to occur progressively above 100 MPa and as the pressure increased, cellular leakage reached its maximum in this studied conditions at 600 MPa for fresh samples processed for two minutes (Chapter 6, Figure 6.4).

(1) Turgor loss and cell membrane disruption mechanisms

In this study, it was observed that after high pressure treatments (600 MPa) the tissue volume was reduced by 6% relative to unprocessed samples (Chapter 6). This volume reduction may be related to the partial collapse of intercellular spaces and a reduction in the thickness of cell walls in the tissue, which comprise a loose meshwork of polymers. It is thought that, during the application of high pressures, the air contained in intercellular voids will begin to dissolve into the tissue fluids as the voids collapse. Because gas solubility (O_2, N_2 and CO_2) increases with increasing pressure, the gas will readily dissolve into free water within the tissue. Concentration differences across the cell membranes will make the gas redistribute resulting in much higher concentrations of nitrogen and oxygen inside the cells. During decompression the gas that has dissolved and diffused throughout the tissue will reform into bubbles because at atmospheric pressure these gases will now be highly supersaturated in the liquid phase. The formation of gas bubbles requires nucleation sites which may or may not be present inside the cells. If bubbles form inside the cells they may cause disruption of membranes and breakage of cell walls. If intracellular nucleation does not occur, then the dissolved gas may form large bubbles in the re-expanding extracellular spaces. Tangwongchai et al., (2000) observed that pressures of 300 MPa result in air bubbles being trapped in the cell voids during decompression and forming cavities. Disrupting of cells will result in extracellular spaces being flooded with cell contents reducing the ease of gas diffusion and increasing the risk of anaerobiosis.

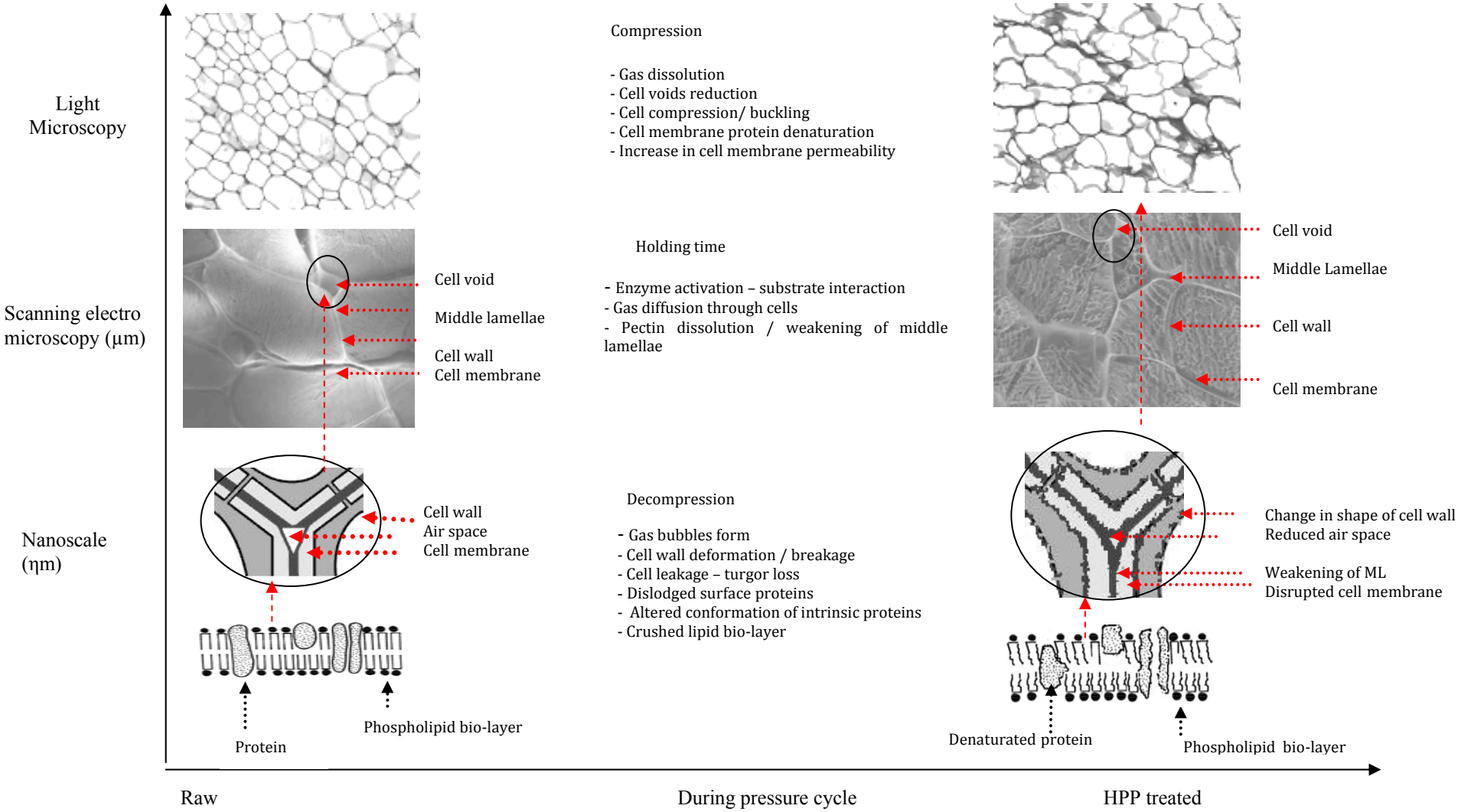

Figure 9.1 Factors affecting quality attributes on carrot cells before, during and after high pressure processing.

It is also known that high pressures of 600 MPa can compress liquid water by up to 15% (Fenemma, 1996), which in the case of carrot would equate to 15% of the 90% (water in carrot); this is equivalent to approximately 14% of the carrot volume. This volume reduction will mean cells and intercellular spaces being rearranged, with cell wall conformation changes such as elongation, affecting the functionality and structure of the original tissue structure.

The stresses put on membranes as a result of compression can cause cell deformation and protein denaturation (Cheftel, 1992 and 1995). Damage to the cell membrane components will increase membrane permeability and decompartmentation. Decompartmentation is caused by the creation of pores or tunnels through the membranes, connecting the vacuole to the cytoplasm or the cytoplasm to the outside of the cell; these changes can lead to enzyme-substrate interaction and cell leakage (Kato et al., 2002).

Cell membrane disruption may be worse if bubble formation occurs during decompression and widespread membrane breakage occurs. Even if bubble nucleation within cells does not occur, apparently intact cell membranes may still increase in permeability as a result of denaturation of membrane-spanning proteins.

Evidence of cell membrane disruption was observed when carrot tissue was immersed in a dye called Trypan blue, which is only taken up by non-viable (damaged) cells (Chapter 3). Image analysis could be used to estimate the proportion of damaged cells present after each treatment. As expected frozen carrot tissue presented the highest percentage of damaged cells of all the samples (58.6%). This was due to ice crystals formed within cells piercing the cell membrane in carrots as has been reported for other products (Van Buggenhout, 2005). Light microscopy images of frozen carrots showed damaged areas following the xylem rays through the core tissue. Based on the carrot's structure, the inner part, also known as xylem or core, contains rays composed of parenchyma and lignified cells radially-arranged along the vessels. It seemed that the parenchyma rays were more susceptible to freezing damage than others. Raw carrots presented 31% damage. Steamed samples presented 42.4 % damage and high pressure processed carrots (600 MPa, 2 min) showed 24.5 % damage, respectively. The apparent differences between the raw and HPP samples' damage percentages can be attributed to

the cutting technique which was applied after processing, where HPP samples were perhaps less brittle than the raw carrots, and with weaker cell-to-cell adhesion, which may explain why less cell rupture was seen in sections cut from HPP treated carrots. This difference in cutting failure can be seen visually in the light microscopy section in Figure 3.6 (Chapter 3).

The apparently low level of damaged cells observed in the core area of HPP carrots suggests that large numbers of cell membranes were still intact after the treatment. This is evidence against widespread membrane rupture that might be caused by gas bubbles forming within cells. This suggests that, instead, the changes are due to an increase in permeability causing turgor loss. Therefore it is important to know if this cellular damage is reversible or irreversible under the studied conditions. Increased cell permeability resulting from membrane protein denaturation may result in cell leakage but still intact and functioning membranes. A possible way to study membrane reversibility is to increase resistance to high pressures by losing some of the initial turgor pressure of cells (as weight loss), so they could potentially overcome the high compression forces by giving them more flexibility. This was investigated in Chapter 6 by measuring texture parameters such as stiffness, juiciness and leakage of high pressure processing of partially dehydrated carrot samples. Results indicated that weight loss (0-11.4%) prior to high pressure treatments did not protect the tissue from losing hardness and stiffness above 200 MPa. This implies that postharvest water loss will not affect HPP "processing-ability". Cell disruption and turgor loss appears to be irreversible above 200 MPa.

In such circumstances it may be possible to restore turgor if the water loss can be reversed. If turgor pressure cannot be restored, it follows that membrane damage is more likely to be the cause of cell leakage. To investigate this, a physical approach using turgor manipulation (mannitol immersion) was applied before and after HPP (Chapter 6). Non-pressure treated samples showed an increase in specific volume when immersed in up to 0.4 M mannitol, which led to an increase in stiffness measurements. An isotonic point was found between 0.4-0.5 M. More concentrated solutions (0.8 M) led to considerable tissue shrinkage. To investigate whether HPP had provoked reversible damage, carrot samples were tested with mannitol immersion after HPP treatment. The results showed no changes in specific volume for all pressure treated

samples immersed in mannitol. These results demonstrated that HPP had caused irreversible damage, even at low pressures (100 MPa) (Chapter 6, Figure 6.13). Therefore cells could not further take up the solution.

In this study it was observed that weight loss during storage of carrots did cause cell turgor pressure to decrease. As weight loss increased, cell shrinkage, folding and buckling were observed, leading to losses in stiffness of the tissue and an increase in strain forces during compression. Cell shrinkage and buckling were reversible when samples were immersed into water or mannitol solutions, which indicate that cells had not been permanently damaged. Therefore the mechanisms involved in high pressure damage to cell membranes can be divided in: changes in conformation of proteins, membrane protein denaturation or dislodging of surface-associated proteins, changes in the arrangement of the lipid bi-layer, the formation of tunnels and major disruption of the membrane (Cheftel, 1992; Kato et al., 2002). Progressively more serious damage may occur during HPP to the point where no reversibility is possible. It is therefore concluded from this work that there is not much left to do "physically" to help prevent cell membrane damage, particularly under high pressures (from 200 MPa upwards), and consequent loss of turgor in the product.

(2) Mechanisms for structural changes in cell walls during HPP

The residual texture left after the loss of turgor due to cell membrane damage can be attributed to the cell wall network in the tissue. During the application of high pressures, forces will put cell walls under stress, causing them to lose their roundness as the intercellular spaces are compressed. Microscopy analysis showed that the cells deformed particularly in the radial direction causing elongation. Once the pressure is suddenly released, weakened cell walls may buckle and collapse or break. Furthermore, if the intercellular cement (middle lamella) is weakened, then high pressure will affect cell to cell junctions enhancing debonding or separation.

Literature search on deformation of wood cells under compression and shearing forces suggests that cells deformed in an S-shape mode when compression forces were applied and a brick-shape mode when compression and shear forces acted (De Magistris and Sálmen, 2008). From the images obtained in this study, HPP carrot cells deformed similarly to the brick-shape mode. This is probably because when applying high

pressures, not only compression forces act on the tissue but shear forces between cells also occur. De Magistris and Sálmen (2008) were able to model the different cell deformations under different forces applied; however they suggested that the most important factor to follow deformation patterns is the network and structure of the tissue itself. In the case of carrots, the inner part (core) is known for having lignified cells along the vessels. Lignification gives cells more strength and therefore less deformation and elongation was observed in this region. Greater cell breakage and deformation were observed in the cortex area, which is composed of parenchyma cells with thinner cell walls. Consequently, as expected, significantly greater hardness losses (approx. 50% difference) were found in the cortex tissue than in the core at various pressure levels.

Presence of pectin methylesterase in carrot tissue under high pressures

Cell walls are mainly composed of cellulose, hemicellulose and pectins. During heat treatments, pectins from carrot tissue can be hydrolysed, weakening cell walls and middle lamella and promoting cell separation and consequently a softer texture. Recent studies have also shown that high pressures (600 MPa) can increase pectin gelation in the middle lamella of carrot phloem cells, which could lead to increased gelation during compression, reducing cell separation (Furfaro et al., 2009). In this study light microscopy showed images of how pressures above 550 MPa promoted cell-cell debonding when applying cutting forces using a blade. This was probably due to weakening of the middle lamella as a result of pectin hydrolysis (Chapter 5). In contrast, fresh carrot cells were firmly jointed to each other, resulting in breakage through cells when cutting forces were applied. At higher pressures (550 MPa), significant reductions in PME residual activity were observed. Verification of the limited extent of pectin-calcium binding by immersion in $CaCl_2$ solutions showed why the cortex tissue did not increase in hardness above 200 MPa (Chapter 5). This led to the conclusion that texture related enzymes such as pectin methylesterase (PME) were not the main cause of hardness losses at the studied conditions; turgor loss due to membrane disruption.

HPP effects on physiology: respiration rates and mechanisms related to changes

Physiological responses such as respiration rate also showed evidence of membrane damage (Chapter 4). This was observed when respiration patterns shifted towards anaerobiosis at low pressure levels (100 MPa). It seemed that as the pressure increased,

cells were not able to cope with stress, reaching a point where cells lost viability (> 300 MPa, 30 min). A number of factors may explain the observed disturbance of aerobic respiration. First, cell voids becoming filled with water after high pressure treatment will result in less oxygen being available for respiration. Secondly, high pressure could be damaging membranes of the mitochondria affecting electron transport. Respiratory data showed how carrots responded to stress and cell reorganization at low pressures (100-200 MPa) by increasing the liberation of carbon dioxide significantly (RQ>1), while pressures above 300 MPa promoted cellular "death", with similar patterns of reduced oxygen consumption and carbon dioxide liberation seen in 600 MPa-treated tissue as in steamed or cooked samples. At low pressures, cellular leakage led to stress responses, increasing respiration rates and metabolic changes. Depending on the level of pressure applied, damage can be reversible or irreversible. Schlüter et al., (2009) found that the application of low pressures (125 MPa) in combination with temperatures below 45 °C would provoke a fully reversible effect on the photochemical efficiency of lettuces; this was determined by measuring chlorophyll fluorescence. The reversibility became less as pressure increased to 150 MPa. In the current study, function reversibility was observed as respiration recovery at 100 MPa; no recovery was observed from 300 MPa upwards. This finding could be commercially applied when wanting to chill-store vacuum packed HPP processed vegetables.

Structural cell changes affecting sensory perception of HPP carrots

Structural changes within cells and tissue, such as cell deformation, cell membrane disruption and cell separation, will have an effect on sensory quality. Cell separation could affect flavour perception due to the lack of juices released from the cells, unlike in fresh tissues where cells are ruptured during chewing. Cell separation will also affect texture (stiffness or hardness) measurements. Cell disruption means leakage and potential changes in the product's sensory attributes.

In this study sensory perception of raw and processed carrots was evaluated (Chapter 7; Trejo et al., 2009). Sensory perception showed that the sweetness characteristic of carrots after HPP was not significantly different from raw carrots, even though some leakage had occurred above 200 MPa. It is possible that even though sugars may have leaked from the cells, they were still present at high concentration within the tissue; as

the HPP process is done in vacuum packed packaging, these sugars were still perceived in the sample when tasted.

Contrasting results were found in cooked carrots which lost their sweetness significantly due to leakage of sugars out of the cells and diffusion into the cooking water. Leakage may also cause changes in flavour perception as compounds are driven out of the cells. If flavour compounds are exposed to oxygen or other chemical reactions such as no enzymatic reactions, they can turn rancid. Acid release may lower change the pH of the cells and lead to the development of unpleasant flavours (Fenemma, 1996). In this study the flavour perceptions such as *flavour impact* and *sweetness* were similar to raw and the *green* tones and *processed flavour* perceptions to sous vide. In terms of colour perception, cell leakage was thought to be the cause of increase in orange intensity on HPP, probably due to the exposure of carotenoids released from the cells to intercellular spaces.

Cell membrane alterations may affect the strain resistance under high pressures, and textural characteristics such as bendiness and crunchiness. Analysis of sensory perception of HPP carrots showed higher fibrousnesses and a rubbery-crunchy type of texture. This was not observed in other processed samples when heat was applied, giving HPP carrots a unique texture (Trejo et al., 2009). This opens the opportunity to create novel products with different and acceptable sensory attributes.

HPP applications in other products

From these results, it is expected that most root vegetables with similar cellular structures to carrots, such as parsnip and beetroot, would respond similarly to HPP.

Previous firmness measurements in various HPP vegetable and fruit products have shown that tissues with a stronger cell wall structure are less likely to lose their firmness, as expected. When applying high pressures to products with stronger cell walls such as carrots, apples and celery, these seem products to lose most of their firmness above 200 MPa; while pears and red peppers lose their texture at 0.1 to 100 MPa (Michel and Autio, 2001). This confirms that after cell membranes have been damaged and turgor is lost, cell walls can still hold some of the product's firmness. In contrast, products with a weaker cell wall structure or more air spaces between cells will lose their firmness at lower pressures with weaker cells to hold the structure thereafter.

In this study hardness and stiffness in carrots showed signs of reduction above 200 MPa. However HPP carrots were still more highly rated for hardness than cooked carrots and similarly rated to those that had been sous vide processed. HPP carrots had the new texture feel of a novel rubbery-crunchy texture. This new texture sensation could open other product opportunities for consumer sensations that may be desirable.

It is thought that product with lignified tissue will in some respects be more protected from high pressure loss of firmness due to the rigidity of the cell wall framework, as observed in the core tissue of carrots. Therefore, fibrous tissue structures with more lignified cells like those found in asparagus could result in a better preserved product after HPP. Furthermore high pressures could protect the softer tissues found in the top of the stem of asparagus and help to soften the middle and base part, resulting in a more even 'fresh type' of texture compared with a cooked product. This study has shown how HPP differs from freezing and cooking processes in terms of cell damage and texture; and how HPP products may have attributes similar to raw in many ways. Asparagus could therefore be a potential HPP treated whole vegetable product that could gain added value.

Furthermore, considering that texture is one of the most important quality parameter for fresh vegetables and that HPP has been proven to better preserve this quality; then another possible application could be a vacuum packed – HPP chilled vegetable mix used for stir fry ingredients as an alternative to frozen vegetables.

Other applications of high pressures could be to increase diffusion rates by using the tissue break point (or increase in membrane permeability) during pickling or candying operations. In this study it was observed that membrane disruption started to occur between 100 and 200 MPa in carrots. Under these conditions, cells are becoming more permeable and are starting to become disrupted. Furthermore, having some disruption in the cells could promote more health benefits by letting compounds such as antioxidants and vitamins become more available for absorption after HPP treatments. Studies have shown that vitamins such as vitamin C found in orange juice can be preserved and made more bioavailable when high pressures are applied in combination with moderate temperatures (400 MPa, 40 °C, 1 min) (Indrawati et al., 2004).

Furthermore, Sánchez-Moreno et al., (2003) found that high pressure treatment at 400 MPa/25°C/15 min could increase lycopene and β-carotene extraction by 77% and 35% respectively in tomato purée compared with the raw product. In general, high pressures could increase the extraction of bioactive compounds from fruit and vegetables and probably improve their bioavailability. This could be used as a benefit over traditional technologies by better preserving the sensorial and nutritional characteristics thus delivering improved functional foods (Sánchez-Moreno et al., 2009).

For future applications, it is recommended firstly to identify the conditions where the tissue 'break point' occurs for each product. This point (pressure/time combination) will allow either speeding up or slowing down processes or reactions. Furthermore, it will help to 'tailor' the product's final quality attributes such as texture in whole vegetable pieces.

Most studies done on high pressure applications have being applied to liquids or semi-liquid products. It is clearly a challenge to apply HPP to whole vegetable pieces (Guerrero-Beltrán et al., 2005). Although this research has confirmed that indeed there are significant texture losses (hardness and stiffness) in the tissue, it was interesting to discover that those losses were not perceived negatively by a trained sensory panel, especially in comparison to raw samples. Furthermore, literature indicated that vacuum packing of vegetables does not always provide prolonged shelf life for two main reasons: firstly because of risk of micro-organism growth and secondly because most commodities produce ethanol and acetaldehyde under anaerobic conditions, causing browning and off odours (Buick and Damoglou, 1987). The results from this research showed that high pressure processing was able to preserve vacuum packed carrots very similarly to sous vide. In addition HPP vacuum packed carrots showed evidence of neither ethanol nor acetic acid and less lactic acid bacteria development even after 14 days of storage at 4°C in comparison with raw, cooked or sous vide processed carrots.

This provides an opportunity for application of high pressure to chilled vegetables for the food service industry, without the quality deterioration found in fresh vacuum packed, cooked or frozen vegetables. Furthermore, high pressure processing has been shown to be the alternative technology most acceptable to the public compared to others such as irradiation or the addition of natural antimicrobials, which is an important

advantage if wanting to further commercialize HPP products (Cardello, 2003). In terms of advantages of using HPP, Earnshaw (1996) concluded in a discussion paper on HPP on foods, that even though this process may not replace freezing or canning, it could open opportunities in products that have a shorter shelf life, high value and functional properties such as vitamins and flavours that would otherwise be lost because they are sensitive to heat.

9 Conclusions and recommendations

Overall, this research suggested possible mechanisms involved in response at a cellular level to high pressure processing in carrots. Applying high pressures from 0.1 to 100 MPa disturbed the cell wall arrangement with some shape changes observed. However, cell membranes were not disrupted with any turgor loss (leakage) as a result. Between 100 and 200 MPa pressure could affect cell metabolism with the tissue showing signs of stress such as increasing respiration rates shifting to anaerobiosis. A tissue break point was observed between 100 and 200 MPa, where leakage started to occur with consequent turgor and textural changes. Above 200 MPa, leakage increased and texture (hardness) continued to decrease but at a lower rate. The pressure effects above 200 MPa promoted irreversible cellular changes that could not be ameliorated by prior turgor reductions in the form of weight loss.

Sensory perception confirmed that even though high pressure (600 MPa) did decrease texture (i.e. hardness), the trained sensory panel had a positive response to most attributes of high pressure processed carrots, giving ratings very similar to those for raw and sous vide processed carrots. In addition, HPP carrots had less undesirable compound formation while stored for 14 days at refrigerating temperatures. This confirmed that high pressure processing has the potential to allow product preservation while keeping most of the products' sensory attributes.

Based on literature and experimental work it was possible to identify and postulate what may be happening in cell membranes, walls and air spaces that will define the final texture and other quality attributes of the product. By identifying key mechanisms, it is possible to understand the benefits of high pressure processing in whole vegetable pieces and identify potential applications such as:

- Opportunities to tailor new products or new sensory experiences.
- Opportunities for processing more 'fresh' vegetables with added value alternatively to traditional techniques.
- Low pressures could be used to increase other process rates such as diffusion rates.

- Pressure below the break point could be used to stress the tissue's metabolism. This could create a protective mechanism that would allow the product to extend its shelf life such as done in apples by applying heat shocks before storage.
- High pressures applied to vegetables could lead to the retention of a higher concentration of health-beneficial compounds which could also be more available for body absorption.

This study has identified opportunities for high pressure processing of whole vegetable pieces. However, there are still many questions not yet answered about the process as such that may provide more opportunities or help to improve the current processes. In terms of the effects of the whole pressure cycle (compression, holding time and decompression), how each individual step would affect the tissue is as yet unknown. For example, are cells disrupted by high compression forces or actually by the instantaneous decompression step? If so, could this decompression step perhaps be controlled by dropping the pressure in stages to allow the tissue to respond to changes? If compression or decompression is the main cell damage contributor, then the holding time becomes a less significant variable and therefore multiple cycles could be applied to increase microbial damage and secure food safety with the absence of heat. Fruits and vegetables have a complex structural matrix composed of living cells, air spaces, and water. Flooding air spaces before HPP could help to prevent cellular disruption. However, the way tissue structures are affected by high pressures will also depend on their composition and cellular arrangements.

It is always important to first understand the structure of the product under study before conducting any processing, to be able to identify mechanisms affecting quality changes. This will lead to achievement of the desirable final product more successfully, and the optimization of processes as such.

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