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Effects of Blanching and Freezing on the Structure of Carrots Cells and their Implications for Food Processing

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Abstract: Blanching and freezing effects are focused on the microscopic structure, whether the softening of frozen vegetables (carrots) is related to changes in pectic substances or to firmness. Under light and electron microscopy, in the frozen raw samples, some cells were disrupted, producing cavities. The cytoplasm was destroyed and enclosed in a very thin layer inside the cell, and the organelles attached to the plasmalemma had almost disappeared. The formation of a transparent region close to the plasmalemma was observed, as a result of the extraction of some altered materials (hemicellulose, cellulose, pectin). The cell walls in the blanched samples appeared quite different from those in the frozen samples. They did not display tissue disruption as in the frozen samples, but the organelles in the cytoplasm were also altered. We also report substantial vesicle formation (swollen walls) in the cell wall, and a dark granular material inside. When the blanched samples were also frozen, compartmentalization of the material inside was observed. The pectin content was higher in the frozen and frozen blanched samples than in the raw and blanched samples. The semithin and ultrathin structure was in accordance with the objective values obtained by Kramer/Shear cell, where the greatest damage occurred to the samples in the freezing process. © 1998 SCI.

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Key words: carrot; structure; blanching; freezing; food

INTRODUCTION

Freezing is one of the best methods available in the food industry for preserving food products of high quality. However, processing operations destroy the cytoplasmic structure, producing loss of turgor, weakness of the cell wall and some degree of cell separation. All these changes have dramatic effects on the texture of the product, which is an important attribute in the quality of frozen fruit and vegetables.

The effect of processing variables on the cell structure and physical characteristics of carrots was determined by Rahman *et al* (1971); in carrots a considerable disorganization of cells occurs (Tatsumi *et al* 1991). Pichioni *et al* (1994) examined ultrastructural changes of the stored, lightly-processed carrots. Among all treatments freezing temperature is the most critical factor affecting the cell structure of the carrots (Fuchigami *et al* 1995). The rate of freezing is regarded as critical in tissue damage. On the one hand, slow freezing causes the formation of large ice crystals and irreversible damage to cell structure and texture (Goodenough and Atkin 1981). On the other hand, food immersed in liquid nitrogen may crack due to freezing.

Vegetables must also be blanched before freezing, to extend their shelf-life, and this process inactivates enzymes and reduces microbiological contamination. Both treatments produce a gradual breakdown in the

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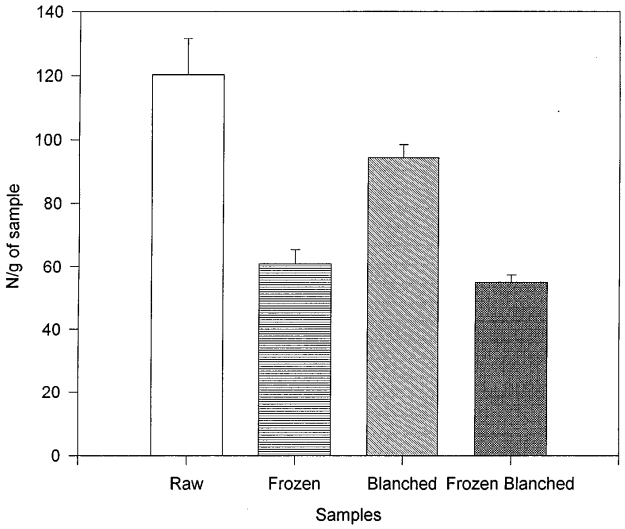

Fig 1. Kramer/shear cell force (N g^{-1} of sample) in carrots.

protoplasmic structure organisation, with subsequent loss of turgor pressure, the release of pectic substances (Fuchigami *et al* 1995) and a final softening effect.

According to Morris (1985), polysaccharides are highly hydrophylic giant molecules that can radically affect the physical properties; their water-binding capacities can be shown dramatically in gel formation (gelation). The uronic acid polymers and pectin interact in the gel network. The gels become even more complicated when they are filled with inclusions such as air bubbles, water, oil droplets, ice or fat crystals (Pomeranz 1991; Rahman 1995).

The present study, therefore focused on the changes of the microscopic structure produced in the different processing stages of blanching and freezing, and we investigated whether the softening of frozen vegetables (carrots) is related to changes in pectic substances.

MATERIALS AND METHODS

Plant material

Raw carrots (*Daucus carota*, Nantes variety) were grown in Toledo (Spain). The carrots were washed, and cut into (3–4 mm thick slices. The carrot slices were treated as follows: blanching for 3 min in boiling water followed by freezing in an experimental tunnel (Frigoscandia Contracting AB, Helsinborg, Sweden, at 5·5 m s^{-1} air speed and −40°C temperature). The samples were divided into four lots: (a) raw carrots; (b) frozen raw carrots; (c) blanched carrots; and (d) frozen blanched carrots.

Histological sample preparation

The carrot slices were fixed for 2 h in glutaraldehyde (30 g $litre^{-1}$) in 1 g $litre^{-1}$ cacodylate buffer, pH 7·4, then washed three times in cacodylate buffer and post-fixed in 10 g $litre^{-1}$ OsO_4 (osmium tetroxide). After alcohol dehydration, samples were embedded in Epon 812 resin (Testillano *et al* 1993). Semithin sections were observed under the light microscope by phase contrast prior to ultramicrotomy. Ultrathin sections were cut with a glass knife on an LKB ultramicrotome III (Leica Instruments GmbH, Nussloch, Germany) collected on an uncoated grid and stained with uranyl acetate (10 g $litre^{-1}$) for 10 min and lead citrate (20 g $litre^{-1}$) for 2 min (Vanable and Goggeshall 1965). The sectioned samples were examined with a Philips 300 electron microscope (Philips Elentron Optics BV, Eindhoven, The Netherlands (at 75 KV).

Firmness

The texture was determined using the Kramer/Shear cell (Johnson 1965), designed to produce the chewing motion of a human mouth and give an objective texture profile correlated with sensory texture profiling. The firmness was expressed in Newtons (N) g^{-1} of sample.

Pectin content

The pectin content was determined by the Dietz and Rouse method (1953). Pectins were extracted from AIS (alcohol-insoluble solids), in successive extractions with

TABLE 1
Pectic substances of carrots (% anhydrogalacturonic acid, AGA (mg g^{-1})). Extraction: water (PW), sodium hexametaphosphate (PH) and sodium hydroxide (PN)[a]

Samples	*PW*	*PH*	*PN*	*Total*
		(% *AGA*)		
Raw	0·81 ± 0·15	0·67 ± 0·33	2·65 ± 0·12	4·13 ± 0·6
Frozen	0·94 ± 0·17	2·05 ± 0·16	3·53 ± 0·16	6·52 ± 0·5
Blanched	0·67 ± 0·03	1·66 ± 0·20	1·92 ± 0·25	4·25 ± 0·4
Frozen blanched	1·16 ± 0·07	0·45 ± 0·45	3·16 ± 0·30	6·48 ± 0·8

[a] Mean values of triplicate determinations.

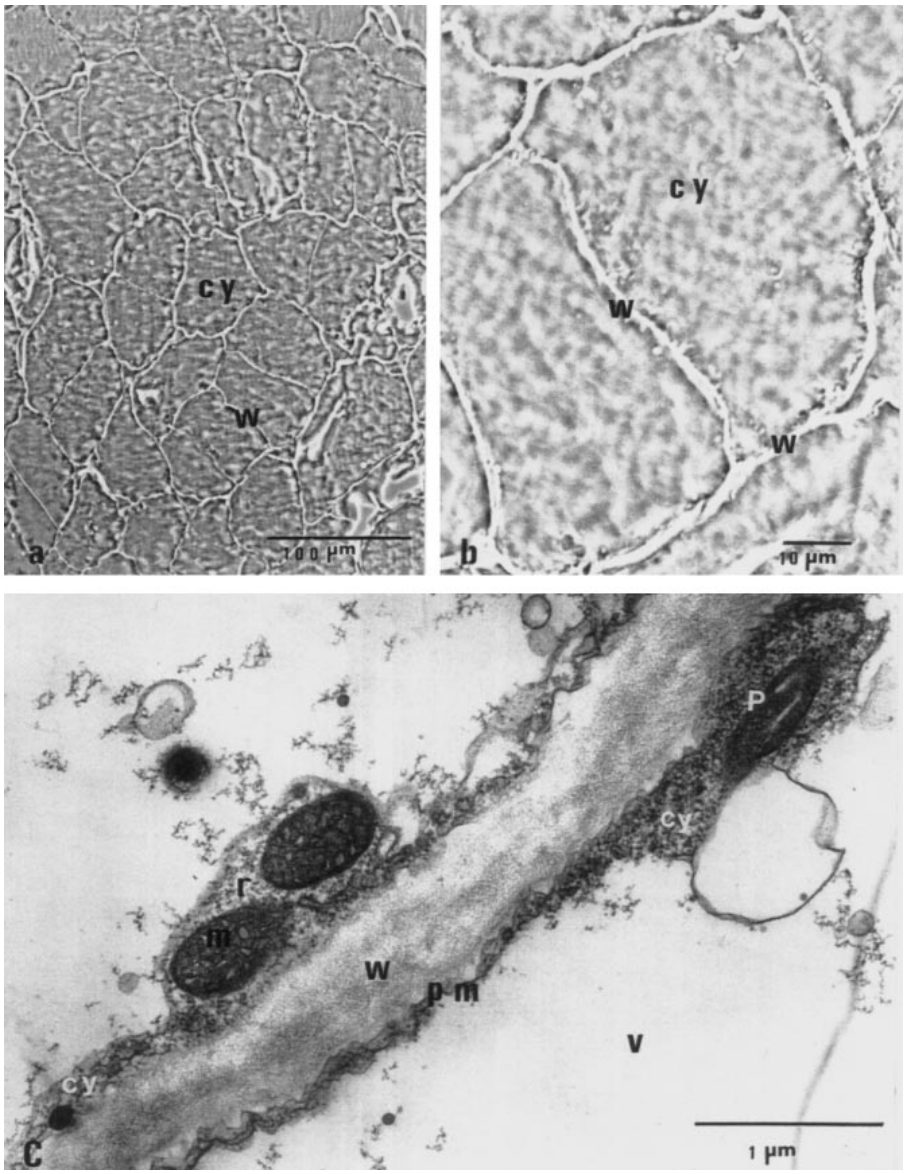

Fig 2. Raw carrot cell: (a) under light microscopy, 1 µm semithin section showing a panoramic view of the walls (w) and cytoplasm (cy), bar: 100 µm; (b) cells at greater magnification, bar: 10 µm; (c) ultrastructure of the cytoplasm attached to the plasmalemma (pm) showing ribosomes (r), plastids (P) and mitochondria (m). Large vacuoles (v) can be seen, bar: 1 µm.

distilled water, sodium hexametaphosphate 40 g litre^{-1} and 0·05 M sodium hydroxide.

All separations were made by centrifugation followed by decantation. Each fraction was analysed by the colorimetric reaction between carbazole and galacturonic acid in the pectic substance. The results were expressed as percentage of anhydrogalacturonic acid (AGA) per gram of sample.

RESULTS AND DISCUSSION

It is well-known that blanching and freezing are critical factors which affect and disrupt the cell structure. Therefore, a correlation between pectic substances, firmness (shear pressure), texture (sensory test) and histological observations (microscopy), gives us an idea of what the product is like in terms of quality.

The firmness (shear pressure, Fig 1) had impaired values after the two treatments. The firmness of the frozen samples decreased by about 50% in comparison with the raw samples. However, the blanched samples decreased by only 21.5%, as a result of the formation of a gel from the effect of the heat on the pectic substances. Both treatments together slightly reduced the firmness compared with the freezing treatment on its own, and it is clearly seen that the freezing process causes the most softness in the tissue.

In all the pectin extractions (Table 1), the pectin content was higher in the frozen and frozen blanched samples than in the raw and blanched samples. The

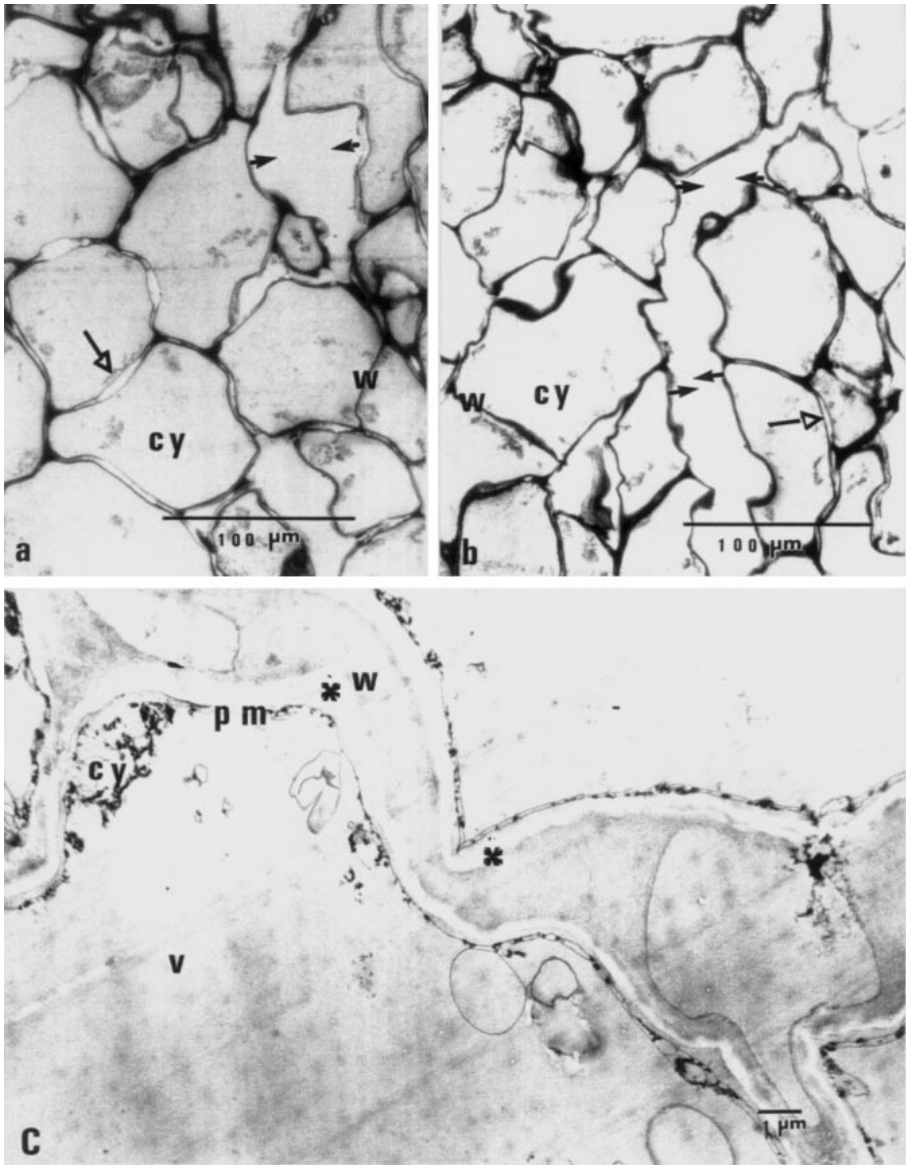

Fig 3. Frozen carrot cell: (a) and (b) under light microscopy, 1 µm semithin section showing a panoramic view of the walls (w) and cytoplasm (cy), disrupted walls (→) in certain regions of the cells are observed. In some cases this alteration affects several cells (⊢► ◄⊢) bars: 100 µm; (c) ultrastructure of the cytoplasm which is destroyed, vacuoles (v) are seen most of the cells. The cell walls have a transparent layer (∗) close to the plasmalemma (pm). Separations between plasmalemmas are observed, bar: 1 µm.

values of pectic substances extracted with water, were around 1 mg g^{-1} GAA. In the sodium hexametaphosphate extraction the values were about 50% higher than in the water extract.

As for sodium hydroxide extracts, the frozen and frozen blanched samples had the highest values. The formation of a gel in the blanched samples decreased pectin extraction. This gives us an idea of how cells are damaged in the middle lamella, the main damage being due to freezing rather than blanching.

The pectic substances are structurally composed of a linear chain of 1–4-D-galacturonic acid units and they form two types of gels depending upon their degree of esterification (high and low methoxyl pectins). The extraction procedure determines the pectin substances as (a) water/soluble pectic substances of high methoxyl content; (b) sodiumhexametaphosphate-soluble pectic substances, which are the low-methoxyl pectinates of the polyvalent cations calcium and magnesium. The polyphosphates have a sequestering effect on the calcium and magnesium ions, solubilising the low-methoxyl pectinates; (c) pectic substances soluble in sodium hydroxide, which include protopectin and the calcium and magnesium pectates not removed by the sodium hexametaphosphate extractions.

Fuchigami *et al* (1995) observed cell separation of the middle lamella when carrots were boiled for 6 min, frozen at − 35°C and thawed; the cell damage was visible. Our results for total pectic substances are, generally speaking, in accordance with theirs.

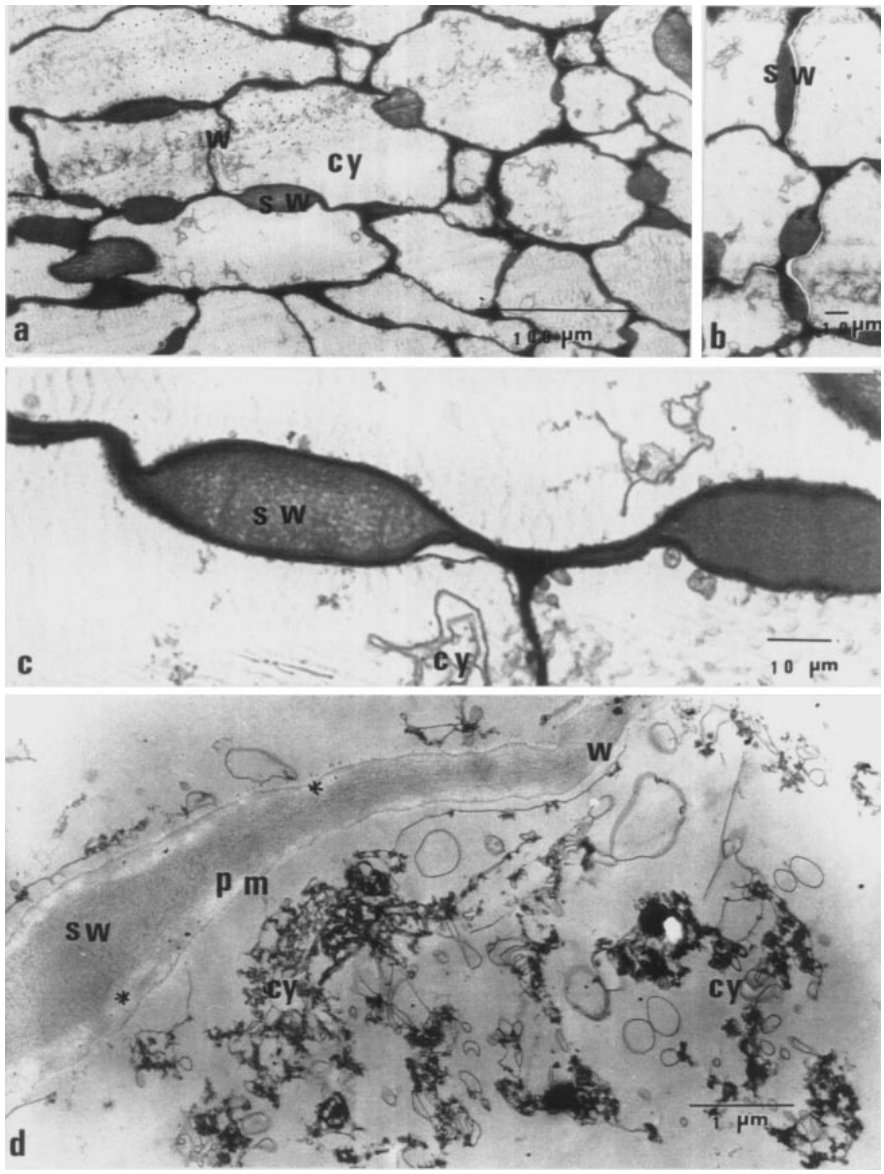

Fig 4. Blanched carrot cell: (a) and (b) under light microscopy, 1 μm semithin section showing a panoramic view of the walls (w) and cytoplasm (cy); several cells had very dense and swollen cell walls (sw); (b) bar: 100 μm; (b) bar: 10 μm. (c) Magnification of the swollen walls showing a dark granular material inside, bar: 10 μm; (d) under the EM the ultrastructure of the cell wall is seen with an opaque material. The cytoplasm organelles are altered. Transparent regions (∗) near the plasmalemma (pm) are not continuous, bar: 1 μm.

Detection of changes in structure requires the use of the microscope and vast differences in experimental samples in fine structure were revealed by optical and electron microscopy.

In the raw samples (Fig. 2(a) and 2(b)), under the light microscope, 1 μm semithin sections gave a panoramic view of the cell walls, where the longitudinal cell walls were quite homogeneous along the wall. However, the transverse cell walls were thinner and both walls had a translucent appearance. The ultrastructure showed (Fig 2(c)) the cytoplasm reduced by the enormous vacuole to a thin layer containing many organelles such as ribosomes, plastids an mitochondria. The large vacuole acts as a storage organelle and as a regulator of turgor pressure, where the osmotic pressure pushes the cell wall and keeps the cell from wilting. The cells are bound within rigid cell walls consisting of an extracellular matrix outside the plasma membrane rich in cellulose. The cell wall is composed of tough cellulose microfibrils embedded in a highly cross-linked matrix of polysaccharides (mainly pectins and hemicellulose) and glycoproteins.

As regards the frozen raw samples, Fig 3(a) shows physical changes in the tissue; the cell wall is heterogeneous, very dense and black, and irregular in shape. It is truly remarkable how the cells are disrupted (hallow arrow) in certain regions of the cells, producing cavities, some of them wider than others. In some cases

this alteration affects at several cells (Fig 3(b), solid arrow), producing much more profound damage. This is explained by the ice crystal effect, which causes separation among the cell layers. Much greater changes were observed (Fig 3(c)) in the ultrastructure of the cell wall, where the cytoplasm was destroyed and enclosed in a very thin layer and the vacuole occupied most of the cell. The organelles attached to the plasmalemma had almost disappeared. The cell walls had a transparent layer close to the plasmalemma and separations between both plasmalemmas were observed.

Blanching causes visible changes in the cell components. The blanched samples (Fig 4(a) and 4(b)) showed very dense and swollen walls in several cells. These dark vesicles occupied the whole or part of the cell wall. Magnification of the swollen walls revealed that they were filled with a dark granular substance (Fig 4(c)). The cell walls were quite different from those in the frozen samples. They do not have tissue disruption as in the frozen samples. The EM revealed (Fig 4(d)) how the initial cytoplasm organelles were altered. The transparent region near the plasmalemma is discontinuous and less apparent than in the frozen samples. In the frozen blanched samples (Fig 5(a)), under light microscopy, the tissue section showed great disruption among the cells (arrows) in the panoramic view of the walls and cytoplasms. The disrupted cell walls and vesicles were more apparent than in the blanched samples. The swollen wall was divided into visible compartments (Fig 5(b)), as if the substance it contained had been dis-

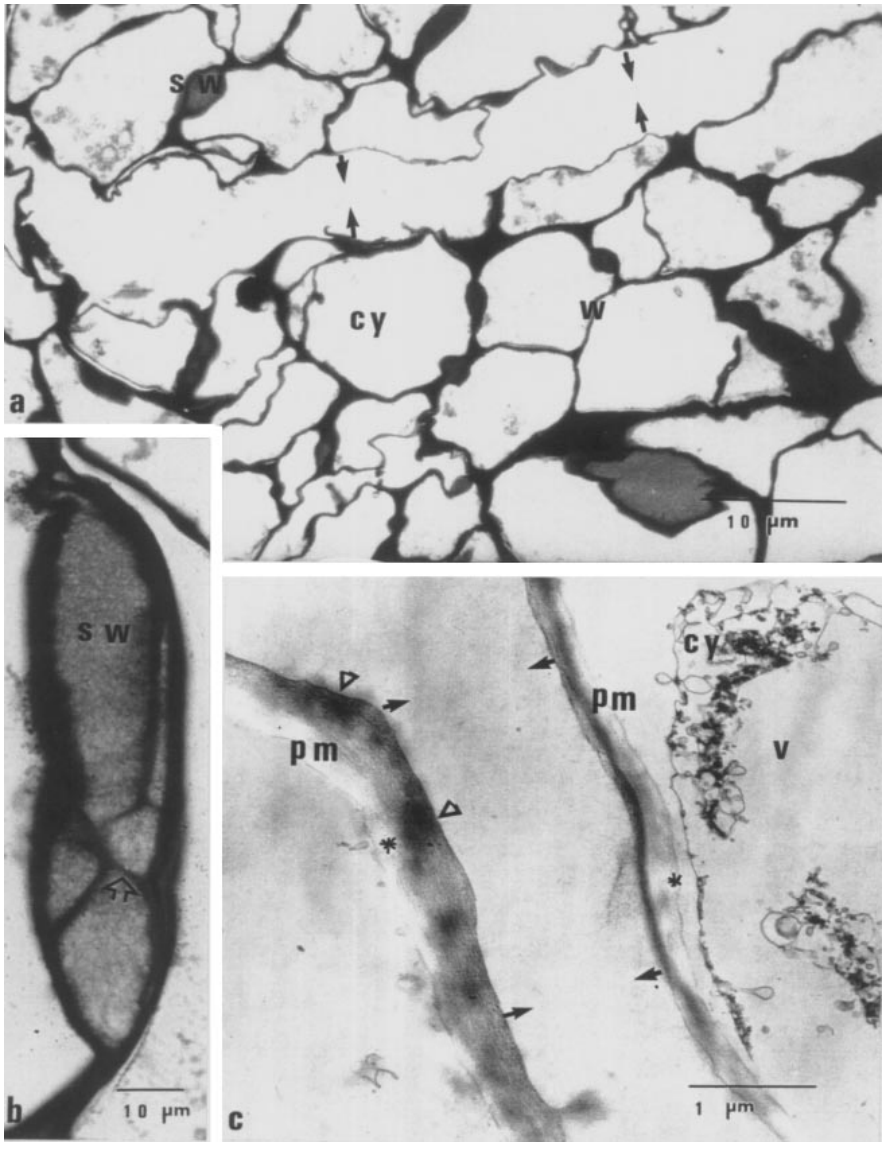

Fig 5. Frozen blanched carrot cell: (a) under light microscopy, 1 µm semithin section showing a panoramic view of the walls (w) and cytoplasm (cy) with greater disruption (–►◄–) among cells. Walls containing very dark material can be observed, bar: 10 µm; (b) portion of the swollen wall (sw) with compartmentalisation (⇨) inside, bar: 10 µm; (c) ultrastructure at the cell wall showing the cavities (–►◄–) between cells. Opaque material (△) and transparent (∗) regions close to the plasmalemma (pm) are remarkable; the cytoplasm has changed dramatically and considerable retraction has occurred, bar: 1 µm.

rupted by the freezing process. The EM showed almost no organelles near the plasmalemma, and the cytoplasm had changed dramatically with considerable retraction (Fig 5(c)). The ultrastructure at the cell wall showed a cavity between the cells. Opaque material and transparent regions were detected. Both structures give good information about the changes in the carrot structure as a result of the different treatments assayed.

The transparent layer observed after freezing could be responsible for the change in permeability and could cause changes in the membrane potential. The Na^+, K^+ ATPase maintains the osmotic balance across the cell membrane by keeping the intracellular concentration of Na^+ low.

The formation of the black vesicles after blanching is explained as a gelation process of the network of cellulose microfibriles, hemicellulose and polysaccharides based on pectins. The pectins particularly abundant in the middle lamella are negatively charged, highly hydrated and accompanied by a cloud of cations, such as calcium. Calcium cross-links help hold cell wall components together and there are also proteins.

The semithin and ultrastructure were in accordance with the objective values obtained by Kramer/Shear cell, where the greatest damage occurred to the samples in the freezing process. Structural observation is also a good tool for showing the high damage inflicted by the blanching process. This effect was not detected with the Kramer/Shear cell, where the values of the blanched samples were higher than those of the frozen samples. This could be explained, because of the formation of a gel, a higher shear force being required in the firmness assay.

Manzoni and Crivelli (1974) reported that heat treatment is highly damaging, affecting the pectic substances and producing cell separation. Schwimmer (1972) also observed the disruption of the internal organelles and the membrane. However our study shows substantial vesicles formation (swollen walls) in the cell wall and a dark granular material inside, due to the blanching process. When the blanched samples were also frozen compartmentalisation of the material inside was observed. It is evident that the combination of blanching and freezing causes considerably greater disruption in the cellular structure than freezing alone. In this work we report how the loss of texture and turgor relates to the cell structure. Figures 2–5 clearly show several alterations in the cellular structure.

We also report on the formation of a transparent region close to the plasmalemma in Fig 3(c), formed as a result of the extraction of some altered materials (eg hemicellulose, cellulose, pectin content). This extraction is homogeneous along the cell walls of frozen carrots and it is less apparent and scattered in the case of the cell walls in blanched carrots (Fig. 4(d)).

CONCLUSIONS

The Kramer/Shear cell test showed that the blanching treatment is better for firmness than the others, due to gel formation, but the structure observed under the electron microscope revealed that this treatment induced more disruption of the cell. The use of the electron microscope is an important tool to analyse the effects of the different treatments in the processed vegetable products.

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