Textural and Ultrastructural Changes in Carrot Tissue as Affected by Blanching and Freezing

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ABSTRACT: The impact of blanching and freezing conditions on firmness retention and ultrastructural changes in the cell wall and middle lamella of carrot tissues were studied. Freezing caused extensive degradation of cell wall pectins as evident from the rapid loss in tissue firmness. High-temperature short-time blanching (100 °C, 0.58 min.; 90 °C, 2.12 min.) retained firmer texture than low temperature long time blanching (80 °C, 11.64 min.; 70 °C, 71.1 min.). Freezing at rapid rates of -4.5 °C/min and -2.4 °C/min showed less softening than slow rates of -0.19 °C/min and -0.05 °C/min. Softening was further enhanced in blanched-frozen carrots. Severe structural damage due to growing ice crystals and substantial loss of pectic material were seen at slower freezing rates.

Key Words: texture, carrot, freezing rate, transmission electron microscopy

Introduction

Although frozen foods fill important niches in the food market, their growth in sales has slowed down considerably due to competition with fresh, shelf-stable and refrigerated alternatives (Dagnoli and Erikson 1989). The reason may be that consumers have perceived deficiencies in frozen foods, which they expect to be close to a fresh product in quality (Mallikarjunan and Hung 1997). Also, freezing processes that have been designed to overcome quality deficiencies are less efficient causing an increase in the cost of the product. An optimum combination of blanching, freezing rate, storage and thawing conditions will provide maximum product quality. For frozen food categories to compete with shelf-stable and refrigerated foods, steps must be taken to improve processing efficiency and achieve a more consistent high quality product without increasing the cost substantially. In order to establish the controls needed to deliver consistently high quality frozen foods to the consumer, more specific knowledge on each of these processing steps are essential (Taylor and others 1994).

Texture in frozen fruits and vegetables are considered to be an important component of product quality. Pectic polysaccharides, which are abundantly found in the primary wall and the middle-lamella between cells are primarily responsible for most of the texture of fruits and vegetables (Jarvis 1984). Because of consumer demand for the firm, crisp and succulent textures of raw vegetables, considerable amount of research has been directed towards modifying processing techniques to retain more of the textural quality of fresh products (Bourne 1989). At a fundamental level, investigators have tried to understand the characteristics, both chemical and structural, of plant cells and tissues that are responsible for the eating texture and how they are affected by processing conditions (Greve and others 1994a). Works by Sajjaanantakul and others (1989) with extracted pectins and Greve and others (1994b) on carrot genotypes have indicated that some of these changes are due to the β-eliminative cleavage of pectic polymers. These reactions were more likely with a higher ester content in the pectins (Sajjaanantakul and others 1989). The effect of low-temperature long-time blanching (60 °C for 2 h or 74 to 79 °C for 20 to 30 min) on improvement of texture of frozen vegetables was observed in several studies on different foodstuffs preserved by different methods, such as canned vegetables (Lee and others 1979; Bourne 1989 a, b) and frozen carrots (Favier 1990; Fuchigami and others 1995 a, b). However, the time-temperature protocols used in these studies were not equivalent in terms of residual activity of blanching indicator enzymes and were either over-blanched or the thermal treatment was not sufficient for desirable reduction in enzyme activity. It is generally accepted that the rate of freezing is also critical in determining the quality of a frozen food and has a direct impact on efficiency of the process (Taylor and others 1994).

A double first order kinetic model for heat inactivation of lipoxygenase (LPO) as a blanching indicator in carrot homogenate was previously developed (Roy 1999). Equivalent thermal treatments necessary to achieve 20 percent activity of the enzyme were determined to be 71.10 min at 70 °C, 11.64 min at 80 °C, 2.12 min at 90 °C, and 0.58 min at 100 °C. The objective was to determine the effects of equivalent enzyme-activity blanching treatments, freezing rates and combined treatments of blanching and freezing on textural changes and structural alterations at the cellular level in carrot tissues. The results from the two studies were compared to determine the optimum processing conditions which best retain the textural character of the fresh raw tissues.

Materials and Methods

Raw material

Green Giant baby-cut carrots (California produce, Pillsbury Co., Minneapolis, Minn., U.S.A.) available fresh in the local market were used. Carrots were cut into 1-cm cubes, eight pieces of which were used for each set of blanching and freezing experiments. Each treatment was replicated twice and the analyses were carried out in triplicates for each replication. Tissues were cut from the cortical parenchyma region of carrot roots for TEM processing.

Blanching process

An EX 210 constant temperature waterbath was used as the water blanching equipment. Temperature of blanching water in a beaker was kept constant at 70, 80, 90, or 100 °C and the carrot cubes heated at the desired temperature–time
condition. The cubes were rapidly cooled in ice water to minimize over-processing before texture measurement or processing for TEM was done.

**Freezing methods**

Baby-cut carrots cut into 1-cm cubes were subjected to 4 freezing conditions to create temperature profiles with freezing rates ranging from −0.05 °C/min to −4.5 °C/min. For quick freezing, the sample was contained in a wire mesh basket and exposed to liquid nitrogen vapor (−174 °C) inside a stainless steel flask and frozen under natural convection. Quick freezing was also achieved by using a conventional cryo freezer (−60 °C, Cryo-Fridge, American Scientific Products, Dublin, Ohio, U.S.A.) with forced convection. For slow freezing, a laboratory scale chest freezer (−23 °C, Kenmore, Sears, Hoffman Estates, Ill., U.S.A.) with natural convection was utilized. The slowest rate was achieved by using a chest freezer (−20 °C) wherein the sample was placed in an insulated container and frozen with natural convection. The temperature profiles at the center of the cubes were recorded with embedded Type T thermocouples. The samples were cooled to an internal temperature of −20 °C and immediately transferred to a −20 °C freezer. After 1 h of storage, they were thawed for 17 min at room temperature (−25 °C) and analyzed on the texture analyzer. Freezing time for each treatment was calculated according to Taylor and others (1994) as the time required for the center of the sample to decrease from the initial freezing temperature (point at which ice crystallization first begins to occur) to a temperature 5 °C lower. This is the plateau during which the major water in the system changes phase into ice. Therefore, the freezing time was in seconds and the freezing rate was calculated as 5 °C divided by the time to freeze (expressed as °C/min).

**Texture measurement**

Carrot cubes were analyzed on a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, U.K.) and force–time curves were obtained. The instrument was equipped with a strain gauge type load cell having a capacity of 25 kg to 1 g. A flat cylindrical probe, 2.6 cm in dia was used with 1 cm² contact area with the sample. Probe test speed was set to 0.4 mm/s and the maximum force (N/m²) to compress the samples to 35% of their original height was determined.

**LM and TEM observations**

Tissue blocks of approximately 1 mm³ were cut from the cortical parenchyma region of the processed cubes. Tissues were fixed in 2% glutaraldehyde buffered at pH 6.8 with 0.025M sodium phosphate buffer for 4 to 5 h at room temperature and post-fixed in 1% osmium tetroxide for 2 h at 4 °C. This was followed by enbloc staining with 1% aqueous uranyl acetate for 8 h at 4 °C and dehydration with gradient concentrations of ethanol and propylene oxide. The tissues were then embedded in epon-araldite resin and placed in the oven at 60 °C for 72 h for solidification. Blocks were sectioned with an ultramicrotome at 1 μm thickness and stained with toluidine blue for light microscopy. Sections of 60 to 90 nm were cut with a diamond knife (Diatome) and stained with uranyl acetate and lead citrate for observation with a transmission electron microscope (TEM) at an accelerating voltage of 100 kV.

**Statistical Analysis**

The data was analyzed using the General Linear Models Procedure (PC-SAS®, 1996). Analysis of covariance was done using sample height as a covariate. When a significant treatment effect (p ≤ 0.05) was found, data means were compared using LS means procedure.

**Results and Discussion**

**Changes in temperature during freezing**

A set of freezing curves (temperature versus time) is shown in Figure 1. Freezing times (sec) and rates attained at four different conditions were determined (Table 1). Curves obtained by freezing at −174 °C under natural convection and at −60 °C with forced convection showed rapid decrease in temperature at the center of the cubes. Supercooling was very difficult to detect at these two rates. The initial freezing points were depressed to −3.0 to −5.7 °C for forced convection freezing (−60 °C) and −7.0 to −9.5 °C for natural convection freezing (−174 °C) (Figure 1). During natural convection freezing at −23 °C, the temperature decreased to −2.3 °C after 355 sec due to supercooling and then increased to an initial freezing point of −1.4 °C due to emitted latent heat of crystallization. The freezing plateau was the zone of maximum ice crystal formation. Following this, the temperature gradually dropped to −20 °C in about 1095 sec. Insulated freezing at −20 °C showed a lower temperature of supercooling (−3.4 °C after 2520 sec) and then an increase to −1.2 °C. Thereafter, a temperature drop from −6 °C to −12.5 °C took about 2630 sec.

**Effect of blanching and freezing conditions on texture**

The effect of 4 equivalent enzyme-activity blanching treatments on firmness retention in carrots were determined and compared (Figure 2). Processing conditions like blanching and freezing resulted in substantial dissolution, depolymerization and apparently destruction of cell wall pectins, mostly by β-eliminative cleavage reactions as pointed out by Greve and others (1994b) and Fuchigami and others (1995 a, b). As a result, the pectins were broken down into smaller sized polymers, lost interpolymer associations and were either released from the primary cell walls and middle-lamella or remained loosely bound to the walls by relatively sensitive bonds. Consequently, wall strength and firmness of the tissues were rapidly lost. Results indicated that High-temperature short-time (HTST) treatments at 90 and 100 °C gave
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Firmer products retaining 45.6% and 49.5% of tissue firmness of the control (169.9 \( \times 10^4 \) N/m\(^2\)). Firmness at the two low temperature long time (LTLT) treatments at 70 and 80 °C were reduced to 33.2% and 39.2%, respectively. Both the HTST treatments were statistically different from each other (\( p < 0.01 \)) and from the two LTLT treatments (\( p < 0.001 \)). This indicated that LTLT treatments in our study were not optimum for activation of the enzyme pectin methyl esterase (PME). As a result, the higher ester content in the tissues could have promoted destruction of pectins by β-eliminative cleavage of the polymer. Previously reported blanching protocols at low and high temperatures utilized for textural or chemical studies in vegetables were not equivalent in terms of residual enzyme activity. Fuchigami and others (1995b) reported a firming effect of low temperature preheating of carrots (60 °C for 2 h) over high temperature blanching (100 °C for 3 min). However, our current model indicated that the low temperature treatment retained a much higher residual LPO activity (29.5%) than the high temperature treatment (0.83%). Comparatively firmer texture of canned carrots blanched at 74 °C for 20 to 30 min over carrots conventionally blanched at 100 °C for 4 to 5 min observed by Lee and others (1979) can be attributed to the same reason.

Freezing of raw and blanched carrots resulted in considerable softening of tissues and loss in firmness (Figure 3). However, rapid freezing gave firmer products than slow freezing rates. Freezing at a fast rate of −4.5 °C/min retained 26.8% firmness of the control (194.6 \( \times 10^4 \) N/m\(^2\)) compared to −2.4 °C/min (12.9%), −0.19 °C/min (6.9%) and −0.05 °C/min (4.4%), which showed a high degree of softening. Differences occurred between the rates of −4.5 °C/min and the other three rates (\( p < 0.01 \)) and between −2.4 °C/min and −0.05 °C/min (\( p < 0.05 \)).

The blanching treatment at 100 °C for 0.58 min was used as the optimum blanching condition for studying the combined effect of blanching with freezing on texture. Softening was further enhanced in blanched-frozen tissues. Although the fast rate of −4.5 °C/min still retained 16.8%, slower rates showed considerable reduction, in the order: −2.4 °C/min (5.4%), −0.19 °C/min (4.4%) > 0.05 °C/min (3.5%). The freezing rate of −4.5 °C/min was statistically different from the other three rates (\( p < 0.01 \)). The three slower rates were not different from one another. These results correlated well with our previous findings that HTST blanching and rapid freezing at −4.5 and −2.4 °C/min gave higher recoveries of cell wall galacturonic acid compared to LTLT blanching and slow freezing (Roy and others 1999).

Structural alterations by light and electron microscopy

Light micrographs of raw carrot parenchyma showed a well integrated tissue structure (Figure 4 a-1). Cells with clearly defined organelles and large vacuoles pressing the cytoplasm against the cell wall due to turgor pressure were visible under transmission electron microscope (a-2, a-3). The vacuoles were separated from the cytoplasm by single membrane tonoplasts, which were intact providing turgor pressure to the cells and mechanical rigidity to the plant tissues. The plasmalemma were in close contact with the cell walls. The middle lamella (ML) appeared highly dark at the junction of the primary walls formed by two or three adjacent cells. High magnification micrographs revealed a microfibrillar phase of parallel bundles of cellulose molecules and a granular matrix phase around and among the fibrous components. The granular components were identified as the pectic substances, which had a cementing role among the fibrous components.

Tissue structure was intact after HTST blanching at and 100 °C for 0.58 min (Figure 4 b1-3) and 90 °C for 2.12 min (c1-
3), with dense cell wall and middle lamella and very little textural alterations. Rupture of tonoplasts and PL with slight loss of pectic material was seen at 90 °C. However, LIIT blanching of carrots at 80 °C for 11.64 min (d1-3) and 70 °C for 71.10 min (e1-3) showed cell separation and rupture at some areas of the tissue, loss of pectin from the periphery of the cell wall together with cell shrinkage and wilting. Cell organelles, plasmalemma (PL) and tonoplasts were mostly ruptured.

Freezing caused considerable damage to the cellular structure. Rapid freezing rates of −4.5 °C/min (Figure 5 a-1) and −2.4 °C/min (b-1) showed well defined tissue structure with cells closely pressed against each other. Freezing at −4.5 °C/min showed no cell separation and high retention of cellulose and pectin (a-2, a-3). A freezing rate of −2.4 °C/min revealed cell separation under TEM with some loss of pectin and broken tonoplasts, but PL was mostly intact (b-2, b-3). Freezing at slower rates of −0.19 °C/min (c-1) and −0.05 °C/min (d-1) showed mechanical damage to the tissue due to growing ice crystals together with cell separation. Damage of tonoplasts and PL caused loss of cellular turgor and cell shrinkage. Loosening of cellular network resulted from loss of pectic material from the cell wall and ML (c-2, c-3 and d-2, d-3).

Structural damage was further enhanced when tissues...
were blanched and then frozen, but a rapid rate of \(-4.5^\circ C/\) min still maintained considerable structural integrity (Figure 6 a-1). The cell wall and ML were dense though ML indicated a small degree of cell separation (a-2, a-3). Cellulose fibrils were well oriented. Rupture of tonoplasts and PL caused minor cell shrinkage. Rupture of cell wall and ML coupled with more evident as freezing rate was lowered to \(-2.4^\circ C/\min \) (b-1). Loss of pectin and disorientation of cellulose fibrils were seen (b-2, b-3). The two slower rates showed high degree of cellular damage as seen from severe tissue disruptions (c-1, d-1). The most notable feature was considerable cell shrinkage causing wilting of the walls (c-2, c-3 and d-2, d-3). Loss of pectic material and consequent loosening of the cellulose network was more pronounced at the slowest rate of \(-0.05^\circ C/\min \). This caused a high degree of tissue softening and loss of wall strength. The maceration of tissues and decrease in pectic polymers due to blanching might have caused a decrease in subsequent freezing tolerance thus enhancing structural damage. Light microscopy and TEM confirmed that HTST blanching and rapid freezing at \(-4.5^\circ C/\min \) were optimum for better structural integrity in frozen carrots. These results were consistent with the reported cryo-SEM observations on frozen carrots by Fuchigami and others (1995a). The authors observed minimum cell damage when tissues were frozen at a rapid rate using a programmed freezer as compared to slow rates of freezing using a conventional freezer.

**Conclusions**

Substantial depolymerization and destruction of cell wall pectins during processing of frozen carrots severely affected the firmness and textural quality in such foods. Carrots blanched to equivalent remaining enzyme levels at four different time–temperature protocols differed in their textural and structural character. Tissue exposure to high temperature for a few seconds showed greater retention of firmness. While rapid freezing rates retained better texture and high degree of cellular integrity, considerable softening and structural damage was seen at slower rates. Susceptibility of tissues to freezing damage was further enhanced in blanched-frozen carrots, but a rapid freezing rate of \(-4.5^\circ C/\min \) still showed much less softening and comparatively slighter damage than the slower rates. Therefore, on the basis of this study, HTST blanching and rapid freezing at \(-4.5^\circ C/\min \) can be recommended as optimum thermal processing conditions for improvement of textural quality in frozen carrots.

**References**


MS 19990326

Thanks are due to Mr. Preston Stogdill and Ms. Cheryl Jensen of the EM core facility, MU for technical assistance. This investigation was part of the work for the M.S. thesis of co-author Siddhartha S. Roy. This work was supported in part by a grant from the United States Department of Agriculture National Research Initiative (96-35500-3321).

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