Journal of Food Engineering 108 (2012) 473–479

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

The effect of osmotic dehydrofreezing on the role of the cell membrane in carrot texture softening after freeze-thawing

Hiroko Ando [a], Kazuhito Kajiwara [a], Seiichi Oshita [b], Toru Suzuki [c,*]

[a] School of Bioscience & Biotechnology, Tokyo University of Technology, 1404-1 Katakura Hachioji, Tokyo 192-0982, Japan
[b] Department of Biological and Environmental Engineering, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[c] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-847, Japan

ARTICLE INFO

Article history:
Received 1 March 2011
Received in revised form 30 July 2011
Accepted 21 August 2011
Available online 5 September 2011

Keywords:
Texture
Water permeability
Nuclear magnetic resonance
Osmotic dehydrofreezing
Carrot tissue

ABSTRACT

To understand the protective mechanism of the osmotic dehydrofreezing technique on carrot texture after freeze-thawing, two mechanical texture parameters, fracture stress related to the cell wall and initial modulus related to the cell membrane, as well as cell membrane water permeability using PFG-NMR were evaluated. In particular, to understand the role of the cell membrane in texture alteration, tissue in which the cell membrane was exposed to chloroform vapor was used. Although dehydrofreezing protected texture from freezing damage, the effect was only observed with respect to fracture stress, with exhibited values close to those for raw tissue. However, there was no protective effect on initial modulus and water permeability, in which values did not differ from those of cell membrane-free tissue. More specifically, osmotic dehydrofreezing had no effect on the cell membrane induced by freeze-thawing.

1. Introduction

Freezing is the most suitable method for the preservation of food characteristics such as nutrition, color and flavor. However, it is well known that fresh agricultural products exhibit significant deterioration in texture (i.e., softening) after freeze-thawing due to destruction of cellular tissue (Brown, 1967; Reid, 1980; Fuchigami et al., 1995; Pearce, 2001; Yamada et al., 2002; Dejmek and Miyawaki, 2002; Ohnihisi et al., 2003). Since fresh agricultural products contain large amounts of water, it is thought that the formation of ice crystals in cellular water induces an expansion of the cellular volume, resulting in damage to cellular structures (Ohnihisi et al., 2003; Pearce, 2001).

Thus, attempts have been made to reduce the cellular water content in order to reduce changes in tissue texture resulting from freezing damage (Howard and Campbell, 1946; Talburt and Legault, 1950; Lazar et al., 1961; Garrote and Bertone, 1989; Biswal et al., 1991; Tregunno and Goff, 1996; Robbers et al., 1997; Forni et al., 1997; Spiazzi et al., 1998; Moyano et al., 2002; Talens et al., 2003; Ohnishi and Miyawaki, 2005; Wu et al., 2009; Borquez et al., 2010). Specifically, it was suggested that the osmotic dehydrofreezing technique is effective in maintaining the texture of agricultural products after freeze-thawing, with minimal damage to cellular integrity. For certain vegetables, such as carrot and broccoli, osmotic dehydrofreezing using a 50% (w/w) sucrose solution has been previously used in commercial processing (Ohnishi and Miyawaki, 2005). The protective effect of osmotic dehydrofreezing against freeze-thawing-induced texture softening was effective in carrots and broccoli. However, this effect was not observed in potatoes (Ohnishi and Miyawaki, 2005) and kiwi fruit (Spiazzi et al., 1998). It is thought that these contradictory results are due to an unclear understanding of the mechanisms of freezing and osmotic dehydration damage as it pertains to vegetable tissues. Therefore, the use of osmotic dehydrofreezing for agricultural products requires further study to elucidate the causes of its differential effects on vegetable texture after freeze-thawing.

Recently, we investigated cell membrane water permeability in fresh and freeze-thawed onion tissue using a non-destructive method, Pulsed Field Gradient Nuclear Magnetic Resonance (PFG-NMR). The PFG-NMR technique which is basically a modified simple spin–echo experiment for measurement of Spin–Spin relaxation time T_2, has been used to study the translational diffusion of water molecules in material. Furthermore, "restricted diffusion" can be investigated as an alternative application of this technique (Tanner, 1978). When the self-diffusion of water molecules in a small compartment system such as a cell is measured by PFG-NMR, the diffusion coefficient depends on the diffusion time and usually decays over time. Such diffusion time dependency on diffusion coefficient is used to be described mathematically by using a model that tissue is made of connected cells with semi-permeable membrane. In the model, cell size, a and permeability, P, are included as adjustable parameters. By using this model, several investigators (Tanner, 1978; Meerwall and Ferguson, 1981; Anisimov et al., 1998; Kinsey

* Corresponding author. Tel.: +81 03 5463 0623; fax: +81 3 5463 0585.
E-mail addresses: hiando@bs.teu.ac.jp, toru@kaiyodai.ac.jp (T. Suzuki).

0260-8774/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2011.08.013

et al., 1999; Clark and Bihan, 2000) have simultaneously determined both adjustable parameters, membrane permeability, P and cell size, a, in biological tissues from fitting to the experimental results. However, the absolute values of parameters are difficult to determine from PGF-NMR data alone since both of these parameters are characteristically alternative and adjustable in nature. On the other hand, we fixed one parameter of them, cell size from light microscopy data after freeze-thawing, so that it could be estimated more precisely the cell membrane permeability in onion tissues by PFG-NMR. The results showed that cell membrane water permeability in onion tissue dramatically increased due to the freeze-thawing treatment (Ando et al., 2009), suggesting that freeze-thawing damages vegetable tissues primarily through destruction of the cell membrane rather than the cell wall.

In the present study, in order to gain a more detailed understanding of the protective effect of osmotic dehydrofreezing on texture softening after freeze-thawing, cell membrane damage in carrot tissue as a result of osmotic dehydrofreezing was evaluated using water permeability, as determined by PFG-NMR measurement according to our previous study (Ando et al., 2009). Cell structure was also observed using light microscopy. In order to evaluate in detail the texture alteration due to dehydrofreezing, we analyzed texture alteration in carrot tissue using two mechanical parameters: (i) fracture stress, which is thought to correlate with cell wall strength and (ii) initial modulus, which is thought to correlate with cell membrane mediated turgor pressure. Furthermore, to understand the role of the cell membrane in maintaining carrot texture, cell membrane-free tissue, prepared using chloroform vapor, was compared to tissue subjected to osmotic dehydrofreezing.

2. Materials and methods

2.1. Materials

Fresh carrot (*Daucus carota* L.) was obtained from a local market. The carrot was cut into 1 cm^3 cubes for texture experiments. For the NMR experiment, the carrot was prepared as a $4 \times 4 \times 10$ mm block of tissue.

2.1.1. Osmotic dehydrofreezing

Osmotic dehydrofreezing was conducted according to the method described by Ohnishi and Miyawaki (2005). Freshly cut, raw carrot tissue was immersed in 100 ml of a 50% (w/w) sucrose solution at 10 °C for 5 h in order to reduce the water content of the sample. The solution was stirred with a magnetic stirrer. After 5 h, the sample was removed from the sucrose solution and placed in a 0.9% NaCl solution for 2 s in order to wash the sucrose from the tissue surface. Subsequently, the samples were pat dried using a paper towel. The sample tissues were then packed in a plastic bag and placed in a −18 °C freezer. After 1 week, the samples were thawed at room temperature (25 °C) for 1 h. A portion of these samples was used for texture measurement before and after dehydration treatment without freezing. Prior to texture measurement, each sample was rehydrated using a 0.9% NaCl solution at 10 °C for 5 h.

2.1.2. Preparation of cell membrane-free tissue using chloroform vapor

Two 50 ml beakers, one with chloroform (30 ml) and the other with distilled water (30 ml) were placed in a glass container. The glass container was sealed and kept at 25 °C overnight in order to generate saturated chloroform vapor in the container. The raw carrot samples were placed on a dish and put into the container for 3 h. Chloroform dissolves phospholipids in the cell membrane and thus the cell membrane can be selectively removed from various tissues. This has become the standard technique for removing cell membranes from animal tissues. The application of this technique results in significant changes in the rheological characteristics of carrot tissues (Ohnihisi et al., 2003). Moreover, it has been confirmed through observation of electrical properties that semi-permeability of cell membrane in vegetable tissue lost by chloroform vapor treatment for 3 h (Ohnihisi et al., 2003). After removing the samples (cell membrane-free tissue) from the container, they were frozen at −18 °C and stored for 1 week prior to being thawed at room temperature. As a control, a portion of the samples was used for texture measurement without freezing.

2.1.3. Freezing and thawing conditions

The prepared sample tissues were packed in plastic bags and placed in a −18 °C freezer for 1 week. Prior to measurement, they were thawed at room temperature (25 °C) for 1 h.

2.2. Methods

2.2.1. Determining water content

The water content of the sample tissue was measured to know the approximate degree of dehydration. Thus, instead of AOAC method, we used more simple method, that is, the tissue sample (initial weight: W_1) was dried in an oven at 105 °C to attain an equilibrium weight, W_2, then the water content was calculated using Eq.1, so that the water contents in this paper were not absolute value but relative value.

$$\text{Water content } [\%] = (W_1 - W_2) \times 100/W_1 \quad (1)$$

2.2.2. Light microscopy observation

For light microscopy observation, the carrot tissue samples were sliced to a thickness of approximately 0.5 mm and placed on a glass slide. The specimens were stained with 1% Safranin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and imaging was immediately performed at room temperature using an Olympus BX51 (Olympus Optical Co., Ltd., Tokyo, Japan). Cell size (cell diameter) was calculated using these images.

2.2.3. PFG-NMR measurement

Stimulated echo pulse sequence was carried out using a Bruker AM200WB NMR spectrometer with a 4.7T magnet equipped with a pulse field gradient accessory, as previously reported (Ando et al., 2009). Using this method, it is possible to observe the so-called restricted diffusion phenomenon of water molecules, i.e., the water diffusion coefficient decreases and approaches a constant value with increasing diffusion time, Δ.

When restricted diffusion is observed using PFG-NMR, the water permeability of the intercellular barrier, P, was estimated from the relationship between the diffusion coefficient, D and diffusion time, Δ, based on a one-dimensional structural model (Tanner, 1978; Anisimov et al., 1998). In this model, the diffusion coefficient, D_∞, when the diffusion time, Δ, is infinite, obeys Eq. (2).

$$1/D_\infty = 1/D_0 + 1/(P \times a) \quad (2)$$

where D_0 and a are diffusion coefficient at $\Delta \rightarrow$ zero and compartment size, respectively.

In this study, a modified method to accurately estimate permeability was used. More specifically, a fixed cell size parameter, a, obtained by microscopic observation was introduced to Eq. (2) with measurement diffusion coefficient value, D_∞ and D_0, by PFG-NMR (Ando et al., 2009).

2.2.4. Texture measurement

The mechanical properties, fracture stress and initial modulus, of the tissue sample were measured using a RE-3305 texture analyzer

(Yamaden Co., Ltd., Tokyo, Japan). A flat wedge-type probe was used, which had a sample contact area of 20 mm^2 (2 × 10 mm). The samples were compressed to 50% of their original height using a probe speed of 1 mm/s. All measurements were conducted at room temperature. The initial modulus (G) was calculated from the slope (initial tangent) of the linear zone of the stress–strain curve that corresponded to approximately 10% deformation. Fracture stress (F) was determined from the maximum stress peak of the curve. These measurement procedures were repeated with five samples for each tissue.

3. Results

3.1. Water content in carrot tissue after dehydration and chloroform vapor treatments

The reduction of water content in vegetable tissue has been thought to play a pivotal role in the mechanism of osmotic dehydrofreezing in preventing texture alteration (Ohnishi and Miyawaki, 2005). The water content prior to freezing for the dehydrated tissue sample, the raw tissue and the cell membrane-free tissue are compared in Fig. 1. The water content of the raw carrot tissue and the cell membrane-free tissue differed slightly; 91.2 ± 1.0% and 92.2 ± 2.9%, respectively. On the other hand, the water content of the osmotic dehydration tissue decreased to 51.9 ± 0.6%. This result indicates that osmotic dehydration significantly reduced the free water content of the tissue sample. Furthermore, it should be noted that the water content in the osmotic dehydrofrozen tissue recovered to 90.1 ± 0.6% after freeze-thawing and rehydration (data not shown).

3.2. Light microscopy observation of cell structure

The microstructure of carrot tissue after various treatments is shown in Fig. 2. Although it has been reported that the protective effect of osmotic dehydrofreezing on vegetable tissue microstructure could be visualized using light microscopy (Ohnishi and Miyawaki, 2005), significant differences between conventionally frozen and osmotic dehydrofrozen tissue was not observed in the present study. Furthermore, little change was observed in the microstructure (i.e., cell form) of all frozen-thawed samples. The cell size (diameter) of the carrot tissue was 108 ± 23.0 μm (n = 60). In the following section, water permeability was calculated using a cell diameter of 108 μm.

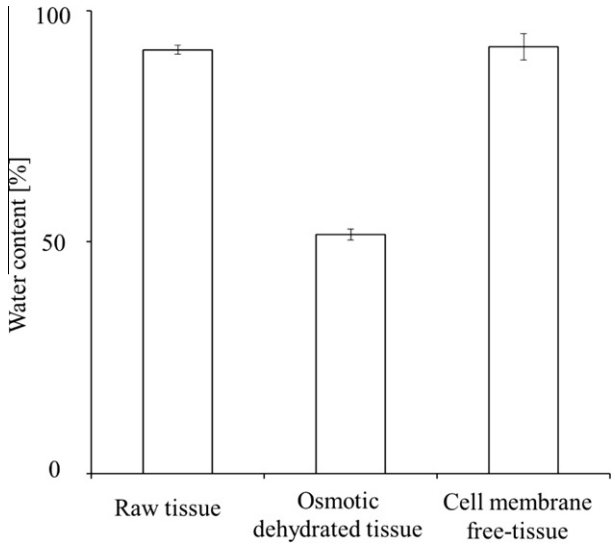

Fig. 1. Differences in the water content [w/w%] of raw, osmotic dehydrated and cell membrane-free carrot tissues before freezing.

3.3. Determination of water permeability behavior using PFG-NMR

The behavior of the apparent diffusion coefficient with diffusion time is shown in Fig. 3. In the raw carrot tissue (■), the diffusion coefficient decreased with increasing diffusion time, that is, a typical restricted diffusion phenomenon was shown. In contrast, in the case of the frozen-thawed tissue (□), the restricted diffusion weakened. This change in the diffusion restriction was also shown in the cell membrane-free tissue (●), osmotic dehydrofrozen tissue (▲) and dehydrated tissue before freezing (Δ).

In order to calculate water permeability, the diffusion coefficients (D_∞ at Δ = 1200 ms and D_0 at Δ = 10 ms from PFG-NMR measurement) and cell size (a = 108 μm from light microscopy observation), as known parameters, were introduced into Eq. (2). The water permeability in the raw carrot tissue was 1.67×10^{-5} m/s ($D_\infty = 0.82 \times 10^{-9}$ m^2/s and $D_0 = 1.52 \times 10^{-9}$ m^2/s). In the literature, the water permeability in intact cotton tissues has been reported as 1×10^{-5} m/s using the PFG-NMR method (Anisimov et al., 1998). On the other hand, the water permeability was significantly increased after freeze-thawing and chloroform vapor treatment. The water permeability for carrot tissue after freeze-thawing ($D_\infty = 1.54 \times 10^{-9}$ m^2/s and $D_0 = 1.74 \times 10^{-9}$ m^2/s) was 1.30×10^{-4} m/s. That of the osmotic dehydrofrozen tissue was 9.57×10^{-5} m/s ($D_\infty = 1.61 \times 10^{-9}$ m^2/s and $D_0 = 1.94 \times 10^{-9}$ m^2/s). This study showed that the water permeability of both the conventionally frozen and osmotic dehydrofrozen tissues increased up to 10-fold after thawing as compared to the raw tissue. Additionally, a similar significant increase in water permeability up to 1.24×10^{-4} m/s ($D_\infty = 1.17 \times 10^{-9}$ m^2/s and $D_0 = 1.30 \times 10^{-9} \times$ m^2/s) was observed for chloroform vapor treated tissue. The calculated results are shown in Fig. 4, where water permeability before and after freeze-thawing are compared. These results suggested that osmotic dehydrofreezing, as well as normal freezing, destroyed the cell membrane of the carrot tissue.

On the other hand, the osmotic dehydrated tissue before freezing (it was also rehydrated) showed a small change in water permeability, 5.74×10^{-5} m/s ($D_\infty = 1.38 \times 10^{-9}$ m^2/s and $D_0 = 1.78 \times 10^{-9}$ m^2/s). Although the degree of increase was less than in other tissues, the osmotic dehydration treatment likely caused the increase in water permeability.

3.4. Effect of osmotic dehydrofreezing on carrot texture

Typical stress–strain curves for raw, frozen-thawed, osmotic dehydrofrozen and cell membrane-free tissues are shown in Fig. 5. From these curves, fracture stress, initial modulus and the rupture strain for the tissue samples were calculated and the results are shown in Table 1.

Raw tissue exhibited a rupture strain of 10% of initial thickness, while rupture strain in the frozen-thawed tissue increased to 40% of initial thickness. Both rupture strains in osmotic dehydrofrozen tissue and cell membrane-free tissue were approximately 20%.

Fracture stress and initial modulus for raw carrot tissue were 91 (±4.0) × 10^5 N/m^2 and 10 (±6.5) × 10^5 N/m^2, respectively; whereas after freeze-thawing, they decreased significantly to 27 (±15) × 10^5 N/m^2 and 2.4 (±1.5) × 10^4 N/m^2, respectively. The decrease in carrot tissue texture after freeze-thawing has been reported in the literature (Fuchigami et al., 1995).

Osmotic dehydrofrozen tissue, however, exhibited anomalous textural characteristics; the fracture stress result (90 (±1.9) × 10^5 N/m^2) is consistent with almost no structural damage, while the significant decrease in initial modulus (9.4 (±3.4) × 10^4 N/m^2) is similar to the result with conventional freezing. Frozen-thawed cell membrane-free tissue exhibited similar properties to those of osmotic dehydrofrozen tissue, with a fracture stress

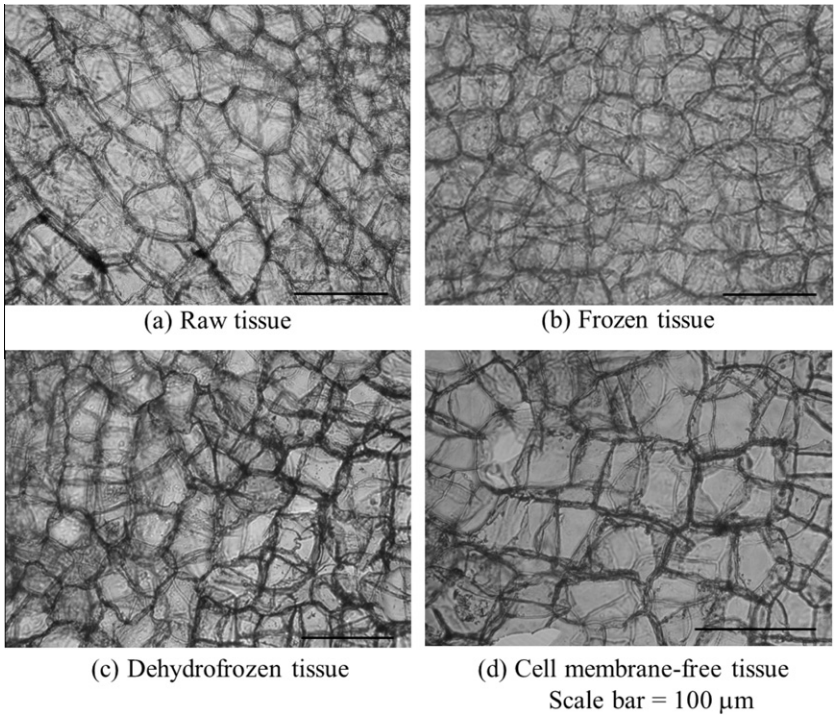

Fig. 2. Changes in the cell structure of raw, frozen, osmotic dehydrofrozen and cell membrane-free carrot tissues. Tissues were observed using light microscopy at room temperature. Samples (b)–(c) were observed after freeze-thawing.

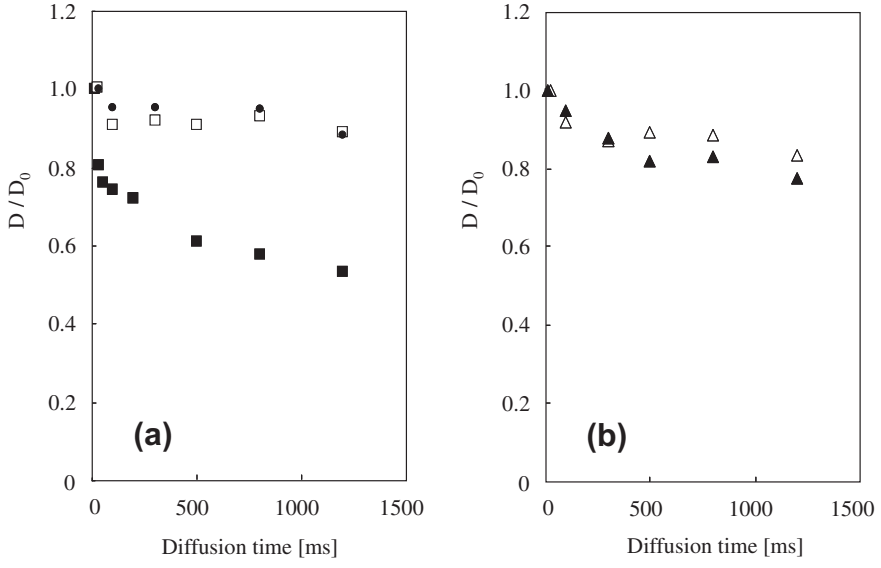

Fig. 3. Determination of diffusion coefficient behavior with different diffusion times in carrot tissue before and after freeze-thawing using PFG-NMR. Raw tissue ■, frozen-thawed tissue □ and cell membrane-free tissue ● is shown in (a) and osmotic dehydrated and rehydrated tissue ▲ and osmotic dehydrofrozen tissue Δ is shown in (b).

of 96 (±1.5) × 10^5 N/m^2 and initial modulus of 2.1 (±0.30) × 10^4 N/m^2.

The significant decrease in both fracture stress and initial modulus in osmotic dehydrofrozen tissue and frozen-thawed cell membrane-free tissue were not observed before freezing. In the absence of freezing, i.e., for only pretreated samples, significant changes in fracture stress were not observed in either the osmotic dehydro-rehydrated tissue or the cell membrane-free tissue; the fracture stress was 88 (±4.0) × 10^5 N/m^2 and 90 (±1.9) × 10^5 N/m^2, while the initial modulus decreased considerably to 3.4 (±0.14) × 10^5 N/m^2 and 5.6 (±3.5) × 10^4 N/m^2, respectively. These results indicate that the initial modulus of the osmotic dehydrated tissue and the cell membrane-free tissue had decreased prior to freezing. From these results, it was confirmed that osmotic dehydrofreezing is a superior freezing method for preventing texture alteration in carrot tissue. However, the effect of osmotic dehydrofreezing was shown in the fracture stress of carrot tissue. Moreover, the change in the texture of osmotic dehydrofrozen tissue was shown to be a similar phenomenon to that of the cell membrane-free tissue.

4. Discussion

As described in the Section 3, it was confirmed that osmotic dehydrofreezing did not completely protect against texture alter-

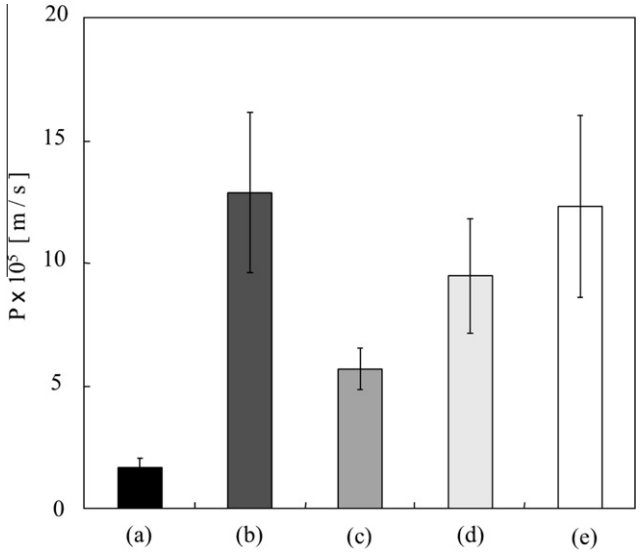

Fig. 4. Estimated *in situ* carrot cell membrane water permeability in raw tissue (a), freeze-thawed tissue (b), osmotic dehydrated and rehydrated tissue (c), osmotic dehydrofrozen tissue (d) and cell membrane-free tissue (e).

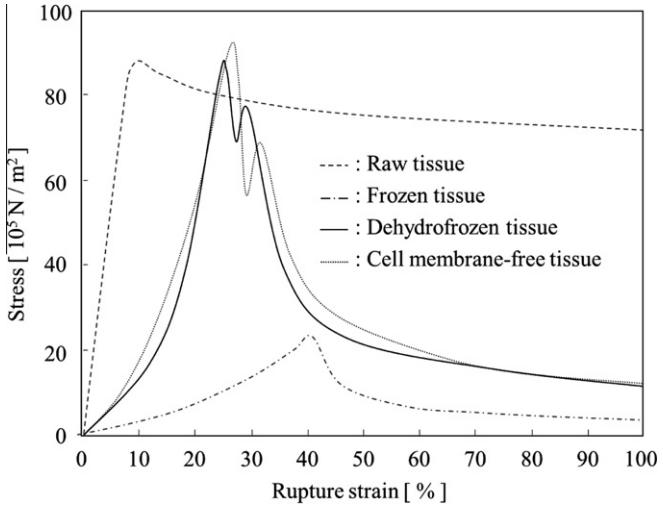

Fig. 5. Typical force–time curves of raw, frozen, osmotic dehydrofrozen and cell membrane-free tissues. Samples (b)–(d) were measured after freeze-thawing. In particular, the texture of osmotic dehydrofrozen tissue was measured after rehydration using a 0.9% NaCl solution.

ation in carrot tissue after freeze-thawing. Specifically, significant alteration in initial modulus was observed after dehydrofreeze-thawing. However, several studies have suggested that osmotic dehydrofreezing could protect vegetable and fruit texture after freeze-thawing (Biswal et al., 1991; Torreggiani, 1993; Tregunno and Goff, 1996; Robbers et al., 1997; Spiazzi et al., 1998; Moyano et al., 2002). Differences in results between this study and previous studies are thought to be due to differences in the texture evaluation methods used.

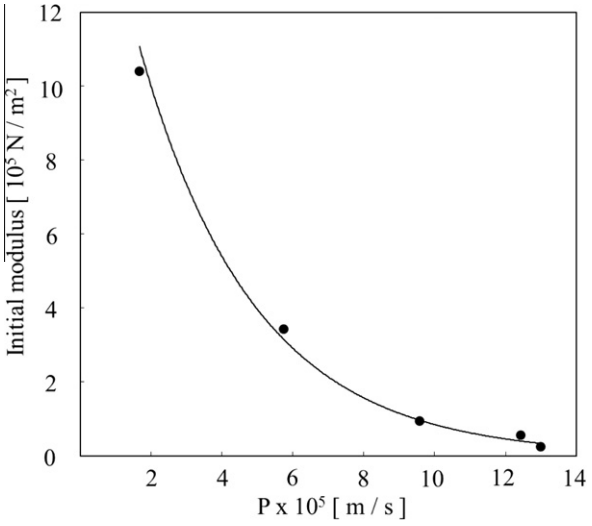

Fig. 6. Relationship between the initial modulus (G) and water permeability of carrot tissue cell membrane.

Fracture stress is considered to reflect the mechanical strength of cell walls in vegetable tissue (Warner et al., 2000). Furthermore, it is well known that turgor pressure, resultant from the low water permeability of the cell membrane, plays an important role in determining vegetable texture (Abbott, 1999). Although several complicated factors, such as cell wall strength and pectin-mediated cellular connections, are involved in determining vegetable texture (Abbott, 1999), the cell wall and membrane may be the major determining factors of vegetable texture. In the present study, the effect of osmotic dehydration on texture was demonstrated using both cell membrane water permeability and texture in carrot tissue. Results indicated that the cell membrane water permeability of osmotic dehydrofrozen tissue had increased prior to freezing, meaning that the cell membrane had incurred damage as a result of osmotic dehydration. In order to assess the relationship between water permeability and texture, we plotted the relationship between cell membrane water permeability and initial modulus. As shown in Fig. 6, PFG-NMR revealed that initial modulus decreased as a function of cell membrane water permeability. Characteristic on texture between small carrot tissue for NMR measurement and large sample for texture measurement was almost same. So, in this paper, the texture of 1 cm cube tissue was shown to be compared with the results for water permeability.

Thus, it was confirmed that initial modulus is a mechanical parameter that reflects cell membrane permeability. From the present observations, it was found that while osmotic dehydrofreezing did not completely protect cell membranes after

Table 1
Fracture stress (F), initial modulus (G) and rupture strain of carrot tissues before and after freeze-thawing ($n = 5$).

Sample	F [10^5 N/m^2]	G [10^5 N/m^2]	Rupture strain [%]
Raw tissue	91 ± 4.0	10 ± 6.5	10 ± 1.8
Frozen tissue*	27 ± 15	0.24 ± 0.15	43 ± 2.2
Dehydrated tissue**	88 ± 4.0	3.4 ± 0.14	7.2 ± 8.9
Dehydrofrozen tissue**	90 ± 1.9	0.94 ± 0.34	24 ± 3.9
Cell membrane-free tissue	90 ± 1.9	0.56 ± 0.35	24 ± 3.8
Cell membrane-free and frozen tissue*	96 ± 1.5	0.21 ± 0.030	21 ± 1.4

* Before texture experiment, the tissue was thawed at room temperature.
** Before texture experiment, the tissue was rehydrated by using 0.9% Nacl solution.

freeze-thawing, it can protect tissue texture, as indicated by fracture stress measurements that correlate to cell wall strength. Regarding the softening of vegetable texture due to freezing, the traditional explanation is that vegetable cell walls are broken by stress attributable to the expansion of cellular volume due to the formation of ice crystals (Fuchigami et al., 1995). On this basis, the protective mechanism of osmotic dehydrofreezing is considered as the following. When the water content in vegetable tissue is reduced by osmotic dehydration, the cell wall would be protected from freezing damage because of decreased expansion stress, resulting in texture preservation after freeze-thawing (Ohnihisi et al., 2003). From our study, it was also confirmed that reducing water content in carrot tissues, using osmotic dehydration (Fig. 1), induced a protective effect through alleviation of fracture stress relating to cell wall strength after freeze-thawing. However, such protective effects are also observed in the cell membrane-free tissue, despite the tissue retaining the free water; that is, the water content was approximately 90%, the same as that of the raw tissue. These contradictory results suggest that the model of the mechanism for conventional freezing-induced texture softening, due to cell wall damage, is incorrect (Fig. 7a). Therefore, reconsideration of the mechanism is required.

The chloroform vapor treatment, used to prepare the cell membrane-free tissue, is considered a mild treatment for selectively eliminating cell membranes in vegetable tissues. This treatment has been frequently used to investigate the function of the cell membrane in vegetable tissues (Ohnishi and Miyawaki, 2005; Naruke et al., 2003). We have previously reported that low cell membrane water permeability in vegetable tissues is increased by chloroform vapor treatment (Ando et al., 2009). Furthermore, it is known that raw vegetable tissue cell membranes characteristically have very low water diffusivity in comparison to animal cells, which are relatively permissive towards water mobility (Ando et al., 2006; Garrote and Bertone, 1989). The lower water permeability of vegetable cell membranes might cause significant texture softening after freeze-thawing by the following mechanism. When raw vegetables are frozen, particularly during the initial stage of the freezing process, ice crystals would be randomly generated in only a few cells of the

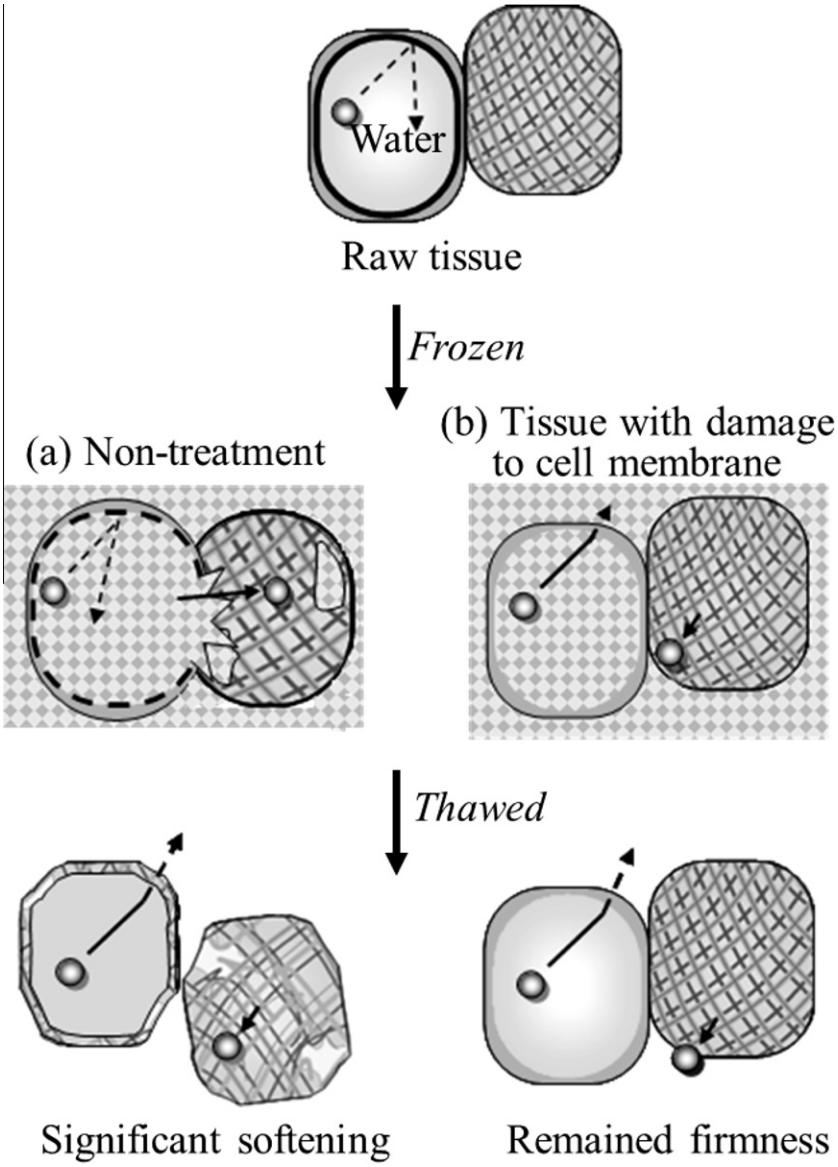

Fig. 7. Schematic of the vegetable softening process due to freezing. (a) Freezing of raw tissue. The cell structure, including the cell wall, ruptures in response to cell volume expansion due to the lower water permeability of the cell membrane. (b) Cell membrane-free tissue. The cell wall structure does not sustain damage, as cellular water diffuses freely in the absence of the cell membrane. The spherical shape represents a water molecule, the heavy black line represents the cell membrane and the heavy gray area outside of the cell membrane and the mesh area represents the cell wall.

tissue or outside the cell. It is unlikely that ice crystals are generated in all cells or outside cells simultaneously. When ice crystals develop in only a few cells of the tissue or outside the cell, unfrozen cellular components become highly concentrated. On the other hand, cellular component concentrations in unfrozen cells, adjacent to the frozen cells, remain unconcentrated. Therefore, spatial extremes in disproportionate concentrations are generated in the tissue. Thus, water in unfrozen cells next to the frozen cells is strongly absorbed into the frozen cells due to the high concentration gradient, i.e., osmotic pressure. However, with an intact cell membrane, it is difficult for cellular water to diffuse into the intercellular space, due to low water permeability (Fig. 7a), as indicated by the results of our NMR experiments. Therefore, the extreme osmotic pressure differences between cells cause plasmolysis, resulting in fatal disruption of the cell membrane, as well as irreversible distention or stretching of the cell wall. These concomitant injures to cell membranes and the cell wall would not only cause vegetable withering, due to decreased turgor pressure, but also affect vegetable firmness due to decreased cell wall strength. In contrast, when the cell membranes of vegetable tissues have been injured or eliminated before freezing, by extreme osmotic dehydration or chloroform vapor treatment, cellular water easily diffuses between the cells, resulting in distension of the cell wall lost by the absence of resistance from the cell membrane was due to the capacity existed of water is tempering and easing. Therefore, vegetable firmness, which reflects cell wall structure, is better maintained after freeze-thawing (Fig. 7b).

Recently, although many researchers also showed the effect of dehydrofreezing for texture of agricultural product (Wu et al., 2009; Borquez et al., 2010), revolutionary technique of dehydrofreezing has not been developed until now. Wu et al. (2009) investigated the effect of dehydrofreezing technique with vacuum drying for eggplant. The damage of cell membrane in eggplant after freeze-thawing was measured by using electrical properties.

The result showed that the cell membrane suffers significant damage even when the dehydration technique with vacuum drying was used. On the other hand, this paper allowed osmotic dehydrofreezing technique to step up a new stage by making clear the mechanism of damage by osmotic dehydrofreezing in vegetable tissue. In order to reach the successful of preservation of texture in vegetable tissue after freeze-thawing, it need to study both techniques dehydration and rehydration of water from the tissue through investigation of the most suitable solution, concentration for vegetable tissue in future.

5. Conclusions

We agree that osmotic dehydrofreezing is an useful technique for protecting against texture softening following freeze-thawing. However, the protective effect can only be realized in firmness or fracture stress associated with cell wall characteristics. Moreover, by a comparison with results from cell membrane-free tissues, we propose a new hypothesis for the protective mechanism of osmotic dehydrofreezing towards vegetable texture. Specifically, vegetables exhibiting cell membrane damage, due to the dehydration process, can better maintain their firmness even after freeze-thawing because elastically deformations of the cell membrane by freezing do not act to draw or stretch the cell wall. This effect is independent of the bulk water content in vegetable tissues. In order to understand this new hypothesis in more detail, further chemical and biological experimentation is necessary. However, the findings presented herein are key points in the development of new freezing techniques for fresh vegetables and aid in rethinking the classic explanation of the texture softening phenomena due to the freezing of fresh vegetables and fruits.

References

Abbott, J.A., 1999. Quality measurement of fruits and vegetables. Postharvest Biology and Technology 15, 207–225.

Ando, H., Fukuok, M., Miyawaki, O., Suzuki, T., 2006. Damage evaluation on freeze-thawing process of food by using NMR. Trans. of the JSRAE. 23, 305–312, in Japanese.

Ando, H., Fukuoka, M., Miyawaki, O., Watanabe, M., Suzuki, T., 2009. PFG-NMR study for evaluation of freezing damage in onion tissue. Bioscience, Biotechnology, and Biochemistry 73, 1257–1261.

Anisimov, A.V., Dautova, N.Y., Sorokina, N.R., 1998. Water diffusion in biological porous systems: a NMR approach. Magn. Res. Imaging. 16, 565–568.

Biswal, R.N., Bozorgmehr, K., Tompkins, F.D., Liu, X., 1991. Osmotic concentration of green beans prior to freezing. Journal of Food Science 56, 1008–1011.

Borquez, R.M., Canales, E.R., Redon, J.P., 2010. Osmotic dehydration of raspberries with vacuum pretreatment followed by microwave-vacuum drying. Journal of Food Engineering 99, 121–127.

Brown, M.S., 1967. Texture of frozen vegetables: effect of freezing rate on green beans. Journal of the Science of Food and Agriculture 18, 77–81.

Clark, C.A., Bihan, D.L., 2000. Water diffusion compartmentation and anisotropy at high b values in the human brain. Magnetic Resonance in Medicine 44, 852–859.

Dejmek, P., Miyawaki, O., 2002. Relationship between the electrical and rheological properties of potato tuber tissue after various forms of processing. Bioscience, Biotechnology, and Biochemistry 6, 1218–1223.

Forni, E., Sormani, A., Scalise, S., Torreggiani, D., 1997. The influence of sugar composition on the colour stability of osmodehydrofrozen intermediate moisture apricots. Food Research International 30, 87–94.

Fuchigami, M., Hyakumoto, N., Miyazaki, K., 1995. Programmed freezing affects texture. Pectin composition and electron microscopic structures of carrots. Journal of Food Science 60, 137–141.

Garrote, R.L., Bertone, R.A., 1989. Osmotic concentration at low temperature of frozen strawberry halves. Effect of glycerol, glucose and sucrose solutions on exudate loss during thawing. Food Science Technology 22, 264–267.

Howard, L.B., Campbell, H., 1946. Dehydrofreezing-a new way of preserving food. Food Inds. 18, 674–676.

Kinsey, S.T., Locke, B.R., Penke, B., Moerland, T.S., 1999. Diffusional anisotropy is induced by subcellular barriers in skeletal muscle. NMR in Biomedicine 12, 1–7.

Lazar, M.E., Chapin, E.O., Smith, G.S., 1961. Dehydrofrozen apples: recent developments in processing methods. Food Technology 14, 32–36.

Meerwall, V.E., Ferguson, R.D., 1981. Interpreting pulsed gradient spin-echo diffusion experiments with permeable membranes. Journal of Chemical Physics 74, 6956–6959.

Moyano, P.C., Vega, R.E., Bunger, A., Garretón, J., Osorio, F.A., 2002. Effects of combined processes of osmotic dehydration and freezing on papaya preservation. Food Science Technology Internal 8, 295–301.

Naruke, T., Oshita, S., Walton, J.H., Kuroki, S., Seo, Y., Kawagoe, Y., 2003. T_1 relaxation time and other properties of cucumber in relation to chilling injury. Acta Horticulturae 599, 265–271.

Ohnihisi, S., Fujii, T., Miyawaki, O., 2003. Freezing injury and rheological properties of agricultural products. Food Science Technology Research 9, 367–371.

Ohnishi, S., Miyawaki, O., 2005. Osmotic dehydrofreezing for projection of rheological properties of agricultural products from freezing-injury. Food Science Technology Research 11, 52–58.

Pearce, R.S., 2001. Plant freezing and damage. Annals of Botany 87, 417–424.

Reid, D.S., 1980. Cryomicroscope studies of the freezing process in tissue and in model systems. Internal Journal of Refrigeration 3, 226–228.

Robbers, M., Singh, R.P., Cunha, L.M., 1997. Osmotic-convective dehydrofreezing process for drying kiwifruit. Journal of Food Science 62, 1039–1042.

Spiazzi, E.A., Raggio, Z.I., Bignone, K.A., Mascheroni, R.H., 1998. Experiments on dehydrofreezing of fruits and vegetables: mass transfer and quality factors. International Institute of Refrigeration Proceeding Series 6, 401–408.

Talburt, W.F., Legault, R.R., 1950. Dehydrofrozen Peas. Food Technology 4, 286–291.

Talens, P., Escriche, I., Martínez-Navarrete, N., Chiralt, A., 2003. Influence of osmotic dehydration and freezing on the volatile profile of kiwi fruit. Food Research International 36, 635–642.

Tanner, J.E., 1978. Transient diffusion in a system partitioned by permeable barrier. Application to NMR measurements with a pulsed field gradient. Journal of Chemical Physics 69, 1748–1754.

Torreggiani, D., 1993. Osmotic dehydration in fruit and vegetable processing. Food Research International 26, 59–68.

Tregunno, N.B., Goff, H.D., 1996. Osmodehydrofreezing of apples: structural and textural effects. Food Research International 29, 471–479.

Warner, M., Thiel, B.L., Donald, A.M., 2000. The elasticity and failure of fluid-filled cellular solids: Theory and experiment. PNAS. 97, 1370–1375.

Wu, L., Orikasa, T., Tokuyasu, K., Shiina, T., Tagawa, A., 2009. Applicability of vacuum-dehydrofreezing technique for the long-term preservation of fresh-cut eggplant: effects of process conditions on the quality attributes of the samples. Journal of Food Engineering 91, 560–565.

Yamada, T., Kuroda, K., Jituyama, Y., Takezawa, D., Arakawa, K., Fujikawa, S., 2002. Role of the plasma membrane and the cell wall in the responses of plant cells to freezing. Planta 215, 770–778.