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A new approach for the preservation of apple tissue by using a combined method of xenon hydrate formation and freezing

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ABSTRACT

Freezing usually causes cell and tissue damage in frozen fruits. This study attempted to use a combined method of xenon hydrate formation and freezing (CXF) for the preservation of apple parenchyma tissue and to compare it with the freezing alone process (FAP). CXF included two steps. The first step was to initiate a certain amount of xenon hydrate by introducing the apple parenchyma tissue to the xenon gas at 1.0 MPa and 1 °C for 0, 1, 2, 3, 4, 5, 6 and 7 d. It was found that the amount of xenon hydrate in apple parenchyma tissue increased with storage time and 2 d was optimum to obtain the certain amount of xenon hydrate. In the second step, the sample with optimum xenon hydrate formation was frozen at −20 °C. The results showed that CXF was more effective in maintaining firmness, turgor pressure, and cell membrane integrity of the apple parenchyma tissue than FAP. A typical restricted diffusion phenomenon which indicates that water molecules are maintained in the apple parenchyma cells was found in the CXF samples, while the FAP samples showed an unrestricted diffusion phenomenon. In addition, firmness, turgor pressure, cell membrane integrity, and restricted diffusion phenomenon of the CXF samples were similar to those of the fresh samples. The CXF could preserve the apple parenchyma tissue because of the bulk water inside the cells and the water surrounding the cells which transformed to ice crystals is limited. Thus, cell and tissue damage due to the formation of ice crystals was reduced. The obtained results indicated that the CXF is effective for the preservation of the apple parenchyma tissue.

Industrial relevance: There has been an attempt to improve the quality of frozen fruit by using innovative techniques, in opposition to simply freezing. This present work proposed xenon hydrate formation for the reduction of bulk water before freezing in order to reduce freezing damage due to a large amount of ice crystal formation. The combined method of xenon hydrate formation and freezing has been proved to be able to reduce cell membrane damage usually occurring in frozen fruit. Thus this new technique has potential to be used for improving the quality of frozen fruit. The xenon hydrate formation is considered as an innovative technique for the preservation of fruit, which is expected to be useful for the frozen food industry.

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1. Introduction

Freezing is an excellent method for preservation and storage of fruits in the food industry because it preserves color, flavor and nutrition values of fruits. During freezing process, bulk water in fruit tissue is transformed to ice crystals. The ice crystals expand the tissue matrix leading to cell volume changes, resulting in cellular structural damage (De Ancos, Sánchez-Moreno, De Pascual-Teresa, & Cano, 2012; Silva, Gonçalves, & Brandoão, 2008). Freezing damage of cellular structure due to ice crystals usually occurs in plant tissues because of the semi-rigid nature of cells which leads to loss of tissue firmness, and a subsequent loss of textural quality in frozen fruits (Chassagne-Berces et al., 2009; Khan & Vincent, 1996; Zaritzky, 2012).

An attempt to improve quality of frozen fruits using novel preservation technique has been investigated. Osmotic dehydrofreezing technique has been successfully used in freezing of fruits with minimal damage to cellular integrity and texture. This technique aims to reduce the water content in food materials by immersion in aqueous solution before freezing (Ando, Kajiwara, Oshita, & Suzuki, 2012; Li & Sun, 2002; Silva et al., 2008). The disadvantage of an osmotic dehydrofreezing technique is that it is a type of an aqueous solution that affects the sensory characteristics of products (Dixon & Jen, 1977). Furthermore, leaching of food materials in the aqueous solution can result in loss of the nutritional content of products (Blanda et al., 2009). Recently, xenon hydrate formation has been introduced as an innovative technique for the preservation of agricultural products. Xenon gas is used for the preservation of agricultural products due to its non-polar nature and no reaction with biological materials (Purwanto, Oshita, Seo, & Kawagoe, 2001). Gas hydrates, composed of water and gas, are

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crystalline solids or ice-like crystals of which gas molecules are trapped in water lattice that are composed of hydrogen-bonded network of water molecules. Gas hydrate formation is a time-dependent process. The process of gas hydrate formation from water and gas comprises four steps. In the first step, labile ring structures of water pentamer $(H_2O)_5$ and water hexamer $(H_2O)_6$ are formed from water molecules. The labile ring structure can be formed when the water becomes supercooled water of which optimally at low temperature and under pressure. In the next step, structured water is initiated when labile clusters (hydrogen bond network) of water molecules form around the dissolved gas molecules. After that, the labile clusters agglomerate. In the final step, the labile clusters grow and form rigid gas hydrate (Sloan & Koh, 2008). Wang, Ando, Kawagoe, Makino, and Oshita (2009) found that barley coleoptile cells preserved by xenon hydrate formation at low temperature showed significantly higher viability than those preserved by freezing. However, the viability of barley coleoptile cells under xenon hydrate formation decreased with storage time. Ando et al. (2011) reported that texture characteristics such as elasticity of the onion tissue after xenon hydrate formation-decomposition decreased with the amount of xenon hydrate. They hypothesized that the cell membrane of onion tissue was damaged due to the growth of xenon hydrate with storage time. The results of using xenon hydrate formation for the preservation of agricultural products had a limitation because increasing the amount of xenon hydrate with storage time leads to cell and tissue damage.

Reduction of freezing damage caused by ice crystal is a key factor in the preservation of frozen fruits because fruits contain a large amount of water (Silva et al., 2008), their cellular structure could be destroyed by ice crystal during freezing. A procedure to reduce the amount of bulk water before transformation to ice crystal could reduce freezing damage. This present work proposed xenon hydrate formation in the reduction of bulk water before freezing in order to reduce freezing damage due to ice crystal formation. There has been no research using xenon hydrate formation for the preservation of fruit tissue. The xenon hydrate formation is considered as an innovative technique for the preservation of fruit, which is expected to be useful for the frozen food industry. Therefore, the objective of this research was to use the combined method of xenon hydrate formation and freezing (CXF) for the preservation of apple parenchyma tissue in comparison with the conventional freezing alone process (FAP).

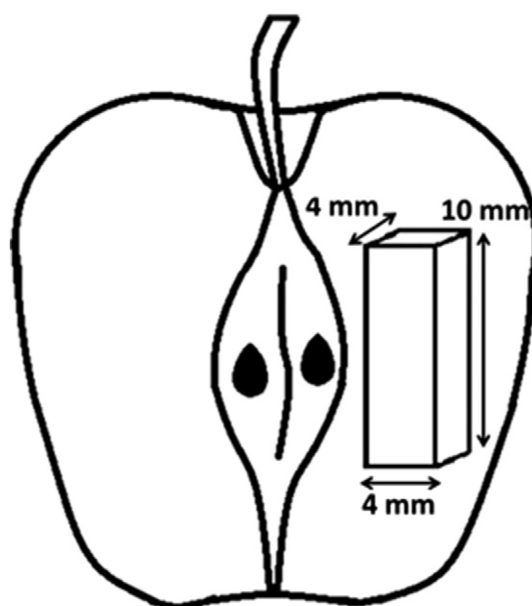

Fig. 1. Apple parenchyma tissue block.

2. Materials and methods

2.1. Sample preparation

The apple parenchyma tissue was used as studying structural cell components because of its low metabolic activity (Snaar & Van As, 1992) and its macroscopic flesh homogeneity. Fresh apples (*Malus pumila* cv. Fuji) at commercial maturity (total soluble solids at 20 °C were in the range of 13–14 °Brix) were purchased from a local supermarket in Tokyo, Japan. The apple was cut into a 4 mm × 4 mm × 10 mm tissue block, which was taken equidistantly from the outer cortex and the core in the parenchyma region (Fig. 1) and dipped into 1.0% citric acid solution for 10 min in order to prevent enzymatic browning (De Ancos et al., 2012).

2.2. Combined method of xenon hydrate formation and freezing vs freezing alone process

In this study, two methods including CXF and FAP were compared. The fresh sample was used as reference for each measurement. The schematic of the experimental setup was shown in Fig. 2. For the CXF method, the optimal xenon hydrate formation was first determined and followed by freezing the xenon hydrate processed sample at −20 °C. Xenon hydrate formation was conducted by placing an apple tissue block into a nuclear magnetic resonance (NMR) glass tube, having an outer diameter of 10 mm, and xenon gas (99.995%, Iwatani Co., Tokyo, Japan) was introduced under pressure of 1.0 MPa at 1 °C for 2 d using a pressurizing unit, as shown in Fig. 3. The tube was then kept in a medical chest freezer (SANYO, MDF-435, Tokyo, Japan) at −20 °C for 7 d. From the preliminary studies, xenon hydrate formation was conducted by introducing xenon gas under pressure of 1.0 MPa at 1 °C for 0, 1, 2, 3, 4, 5, 6 and 7 d and the formation of xenon hydrate was monitored daily by using a NMR measurement of solid echo. It was found that the optimal xenon hydrate formation in apple parenchyma tissue is 2 d. When stored longer than 2 d, cell membrane damage caused by a larger amount of xenon hydrate was evidenced as measured by using a self-diffusion coefficient of water molecules. After the optimal xenon hydrate formation was obtained, the sample was stored at −20 °C for 7 d. For FAP, the apple tissue block was placed in a NMR glass tube and was frozen in a medical chest freezer at −20 °C for 7 d. After storage, both CXF and FAP samples were thawed at 20 °C for 1 h before the measurements of texture, water content in cellular structure, and self-diffusion coefficient. For the CXF samples, xenon hydrate was decomposed by releasing xenon gas from the NMR glass tube together with thawing of ice crystal.

2.3. NMR measurement of the amount of xenon hydrate

Solid echo NMR was used to measure the amount of xenon hydrate in apple parenchyma tissue sample. A 25-MHz pulsed NMR spectrometer (JNM-MU25A, JEOL Ltd., Tokyo, Japan) with an attached temperature control unit (NM-VT/MU25, JEOL Ltd., Tokyo, Japan) was used. In order to measure the free induction decay (FID) by NMR spectrometer, dimension of apple tissue block of 4 mm × 4 mm × 10 mm was required to get stable NMR signals. To measure the spin–spin relaxation time of protons (T_2) in the solid component of the sample, a solid echo pulse sequence was applied following the method of Ando, Suzuki, Kawagoe, Makino, and Oshita (2009) and Gribnau (1992). The sequence of a solid echo pulse consists of 90°–τ–90°–τ–echo, where 90° is the flip angle induced by the radio frequency pulse and τ is time between pulses in a pulse sequence. The signal intensity was recorded as free induction decay. The pulse interval for eliminating the background effect from the empty sample tube was determined to be 8 μs. The repetition time and scan number were 30 s and 16 times, respectively. The amount of xenon hydrate in apple parenchyma tissue was determined from the FID as the solid ratio (the number of molecules of solid components to the

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