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CO_2 processing and hydration of fruit and vegetable tissues by clathrate hydrate formation

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ABSTRACT

CO_2 hydrate can be used to preserve fresh fruits and vegetables, and its application could contribute to the processing of carbonated frozen food. We investigated water transformation in the frozen tissue of fresh grape samples upon CO_2 treatment at 2–3 MPa and 3 °C for up to 46 h. Frozen fresh bean, radish, eggplant and cucumber samples were also investigated for comparison. X-ray diffraction indicated that after undergoing CO_2 treatment for several hours, structure I CO_2 hydrate formed within the grape tissue. Phase-contrast X-ray imaging using the diffraction-enhanced imaging technique revealed the presence of CO_2 hydrate within the intercellular spaces of these tissues. The carbonated produce became effervescent because of the dissociation of CO_2 hydrate through the intercellular space, especially above the melting point of ice. In addition, suppressed metabolic activity resulting from CO_2 hydrate formation, which inhibits water and nutrient transport through intercellular space, can be expected.

1. Introduction

To preserve the freshness of fruits and vegetables, suppressing biological activities such as respiration, which continues after harvest, is an important consideration. The nutritional components in the bodies of fruits and vegetables decompose to extend the plant's life when supply from the roots ceases. Lowering water mobility within the living cell is a method to suppress the biological activities, resulting in the shelf life extension of fruits and vegetables. Freezing is a traditional and effective method to inhibit the mobility of water in the tissues for food preservation. However, improvements in the freezing process by means of controlling water crystallization is an important issue yet to be solved, as the formation of large ice crystals results in significant damage to the tissues because of the morphology of ice and volume expansion caused by ice formation. Using inert gases such as Ar or Xe has been suggested as a means of maintaining the quality of fresh produce for longer periods than are currently possible. It is thought that the suppression of metabolic activity by inert gases results from the formation of 'structured' water (with a clathrate-like structure), and this has been attributed to freshness longevity (Oshita, Seo, & Kawagoe, 1999). Recently, studies have reported the improved quality of apple tissues after high-pressure (150 MPa) Ar treatment, because of the presence of Ar hydrate in one case (Wu, Zhang, & Wang, 2012) and a combination of Xe hydrate formation and freezing in another (Arunyanart, Siripatrawan, Makino, & Oshita, 2014). In this respect, it is expected that CO_2 hydrate can be used to preserve fresh fruits and vegetables by suppressing the water mobility in living cells, and its application could contribute to the processing of carbonated frozen food.

Gas hydrates (or clathrate hydrates) are host–guest compounds. They are ice-like crystalline materials consisting of water molecules, which are hydrogen-bonded to form host cages (Sloan & Koh, 2008). Their equilibrium pressure and temperature conditions differ, depending on the guest. The formation of Ar hydrate requires very high pressure (10.5 MPa at 0 °C). Xe hydrate can be formed at near atmospheric pressure (0.2 MPa at 0 °C), but Xe is sufficiently expensive to preclude its use as a preservative. In

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contrast, the formation conditions of CO_2 hydrate are relatively easy to create (1.3 MPa at 0 °C), and the use of CO_2 hydrate as a carbon capture and storage (CCS) material has been investigated (Wang, Lang, & Fan, 2013). The crystal structure of CO_2 hydrate has a unit cell consisting of two dodecahedron (5^{12}) and six tetrakaidecahedron ($5^{12}6^2$) cages, which have high CO_2 storage capacity. About 150 volumes of CO_2 gas per volume of sample (v/v) is stored within crystalline CO_2 hydrate at standard temperature and pressure (STP) (Udachin, Ratcliffe, & Ripmeester, 2001; Staykova, Kuhs, Salamatin, & Hansen, 2003). The application of CO_2 hydrate to the manufacture of frozen desserts has been proposed, and it has been reported that CO_2 and CH_4 could be stored in tomatoes, eggplants and mushrooms (Peters, Smith, & Brisson, 2010; Wang, Ma, Lin, Sun, & Cooper, 2013). Mushrooms exhibited a particularly high CH_4 storage capacity of ~120 v/v (STP), which is comparable to that of crystalline CH_4 hydrate at ~170 v/v (STP). The effect of high pressure gases (<~1 MPa) such as CO_2 and CH_4 on grape tissue is not well known, though low-pressure CO_2 treatment (>~0.1 MPa) minimizes the structural damage of grapes associated with the freezing-thawing process (Goni, Fernandez-Caballero, Sanchez-Ballesta, Escribano, & Merodio, 2011). Understanding water transformation in plant tissues upon CO_2 treatment is therefore important for the CO_2 processing of fruits and vegetables. It is also necessary to reveal the occurrence of CO_2 hydrate or water phases caused by CO_2 treatment, known as clathrate like water, within plant tissues.

One major difficulty is detecting the changes in water phases (water, ice and clathrate-like water) within fruits and vegetables. Nuclear magnetic resonance imaging (MRI) is typically used to visualize water or solutions as a liquid phase within tissues (As & Duynhoven, 2013; Taglienti, Massantini, Botondi, Mencarelli, & Valentini, 2009; Herremans et al., 2014). MRI is also used for frozen samples to visualize unfrozen water (Koizumi et al., 2006), but is not applicable to visualize the difference between ice and gas hydrate as a solid phase. Recently, X-ray micro-computed tomography (CT) by means of the absorption contrast X-ray imaging technique has been applied in the imaging of gas filled pores within plant tissues using in–house equipment (Kuroki, Oshita, Sotome, Kawagoe, & Seo, 2004) and synchrotron radiation (Verboven et al., 2008). The X-ray micro-CT using synchrotron radiation is useful to visualize smaller sized samples, such as an entire organism or single cells, with high special resolutions of 1 μm or higher (Attwood, 2006; Dhondt, Vanhaeren, Loo, Cnudde, & Inze, 2010). However, whole produce, comparable sizes of samples, or *in vivo* observations are not applicable to this method because sample size is limited (<~1 mm) for high-resolution imaging, thus magnification and measured volume is a trade-off relation. In addition, because of low density and small density differences in wet plant tissues or food materials, the quality of the imaging dataset is not high enough to visualize multi-phase materials without a contrast agent (Arunyanart et al., 2015; Mousavi, Miri, Cox, & Fryer, 2007). Another X-ray imaging method is phase-contrast X-ray imaging, which has higher density resolution than absorption contrast X-ray imaging because the light elements have approximately 1000 times larger phase shift cross sections than their absorption cross sections (Momose, 2005). To date, there are few reports on the application of phase-contrast X-ray imaging to plant imaging, and these measurements have been limited to only room temperature conditions (Cloetens, Mache, Schlenker, & Lerbs-Mache, 2006; Young, Parham, Zhong, Chapman, & Reaney, 2007; Mayo, Chen, & Evans, 2010).

In this study, the powder X-ray diffraction (PXRD) technique was used to confirm the presence of crystalline CO_2 hydrate which can occur in fruit and vegetables tissues. The diffraction enhanced imaging (DEI) technique (Takeya et al., 2012) as a phase-contrast X-ray imaging method was employed to detect phase shifts caused by the multicomponent system inside the whole body of fresh produce at a temperature below the melting point of ice (0 °C). Internal observations of frozen fresh produce after CO_2 processing by the phase-contrast X-ray imaging are reported. The water reaction with CO_2 within the tissues of the fresh produce will also be discussed.

2. Materials and methods

2.1. Preparation of CO_2 treated fresh produce

Whole grape and bean samples, freshly cut radishes and eggplants about $20 \times 15 \times 15$ mm in size and cucumbers $\varnothing 17.5 \times 20$ mm in size were prepared for the experiment. A sequence of treatment processes, referring to a general CO_2–H_2O phase change (Fig. 1), were performed separately for each sample.

Approximately 10 g of sample was put into a 755-ml high-pressure vessel (Fig. 1, point A), and placed in an incubator set at 3 °C. High purity CO_2 gas was then injected into the vessel, and the pressure was kept at 3 MPa for 24 h in order to form CO_2 hydrate (Fig. 1, point B). Next, the vessel was moved into a freezer set at −30 °C and stored for >2 h. At this condition, the CO_2 pressure was reduced to 1.7 MPa and the remaining liquid water within the tissue of the sample was frozen (Fig. 1, point B′). This is because gas hydrate dissociation can be delayed at temperatures below the melting point of ice because of ice formation, which is known as the self-preservation of gas hydrates (Takeya & Ripmeester, 2010; Falenty & Kuhs, 2011). The pressure was then slowly decreased to atmospheric pressure at −30 °C to collect samples from the high-pressure vessel (Fig. 1, point C). The sample without CO_2 treatment was frozen under atmospheric pressure through the melting point of ice (Fig. 1, point A–D). Throughout the treatment process, the applied pressure was set below the liquefaction pressure of CO_2.

CO_2 hydrate is stable below −55 °C at an atmospheric pressure, so samples after the CO_2 treatment above were quickly soaked in liquid nitrogen to avoid dissociation of the CO_2 hydrate formed within the samples. Then, the samples were transferred to a container kept at <−170 °C for further analyses.

2.2. PXRD measurements

Powder X-ray diffraction (PXRD) was employed to identify the crystalline phases of water after high-pressure CO_2 treatment, and to estimate the gas capacity of plant tissues. Characterization and quantitative analysis was performed using PXRD. Samples were ground into fine powders under a N_2 atmosphere of vaporized liquid N_2 at <−170 °C. The powder was loaded into a Cu holder, which was placed in a cryogenic cell attached to the X-ray diffractometer. PXRD measurements were performed using Cu K_α radiation and parallel beam optics (40 kV, 40 mA, Rigaku model Ultima III, Tokyo, Japan) at −150 °C to avoid dissociation of the CO_2 hydrate.

2.3. DEI measurements

The DEI method can visualize gas hydrates coexisting with ice and gas bubbles, to a density resolution of ~0.01 g/cm^3. X-ray images were collected using 35 keV monochromatic synchrotron X-ray radiation, supplied by the vertical wiggler beam line (BL-14C) at the Photon Factory, Tsukuba, Japan. The DEI system had a field of view of 25×35 mm, and spatial resolution of 40 μm. To obtain three-dimensional (3D) X-ray CT images, samples with a diameter of <15 mm and length of 20 mm were rotated 180° in 0.72° steps, with a total measurement time of ~60 min. During each DEI measurement, the sample was immersed in liquid methyl acetate (99.5%, Kishida Chemical Co., Osaka, Japan) to prevent