Impact of ice crystal development on electrical impedance characteristics and mechanical property of green asparagus stems

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2   **Impact of Ice Crystal Development on Electrical Impedance Characteristics and**

3   **Mechanical Property of Green Asparagus Stems**

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Abstract

18

19   The effects of ice crystal formation during freezing using three different

20   methods: slow freezing, air blast freezing and liquid nitrogen spray freezing on the cell

21   membrane state and mechanical property of asparagus stem samples were investigated.

22   X-ray computed tomographic analysis revealed coarser ice crystal growth with slow

23   freezing compared to the rapid freezing methods, which were characterized by fine and

24   homogeneous ice crystal growth. However, electrical impedance analysis clarified that

25   the structural damage and functional decline of cell membranes occurred regardless of

26   the freezing method. Further, the elastic property of each frozen sample declined greatly

27   after thawing and showed little improvement with rapid freezing. These results suggest

28   that the reduction in turgor pressure following changes in the cell membrane state,

29   attributable to the formation of ice crystals during freezing, is a major factor in the

30   mechanical property, and this phenomenon is independent of the freezing rate and ice

31   crystal size.

32

Nomenclature

C_m capacitance of cell membrane (F)

j imaginary unit (−)

P constant phase element exponent (−)

R real part of the impedance (Ω)

R_i intracellular fluid resistance (Ω)

R_e extracellular fluid resistance (Ω)

T constant phase element coefficient ($\mathrm{F \cdot s}^{(P-1)}$)

X imaginary part of the impedance (Ω)

Z complex impedance (Ω)

Z_CPE impedance of constant phase element (Ω)

θ phase angle (rad)

ω angular frequency (rad/s)

1. Introduction

Freezing is one of the main methods employed for long-term food preservation, which inhibits microbial, metabolic and enzymatic activities, offering convenience for consumers and superior taste retention and nutritional value compared to other methods. However, changes in the physical and chemical structures resulting from the formation and growth of ice crystals during freezing can lead to the degradation of food quality. The characteristics of ice crystals, e.g., size, shape and distribution, formed during freezing are closely related to the quality of frozen foods. Therefore, many studies have aimed to elucidate the mechanism of ice crystal formation and the characteristics of ice crystals formed in foods (Kiani & Sun, 2011; Chevalier et al., 2000).

The formation of ice crystals requires two stages: the generation of ice nuclei, followed by the growth of crystals (Delgado & Sun, 2001). Since the moisture in foods forms fine and homogeneous ice crystals when subjected to the temperature range for crystal growth over a short period, causing less deterioration in quality, (Chevalier et al., 2000; Kono et al., 2017), various rapid freezing methods have been employed in the field of practical frozen food production for quality improvement. Additionally, technology to control ice crystal formation in foods such as ultrasonic freezing, which facilitates ice nucleation (Zheng & Sun, 2006; Bhaskaracharya et al., 2009), pressure shift freezing (Otero & Sanz, 2000; LeBail et al., 2002) and supercooling application (Miyawaki et al., 1992; Kobayashi et al., 2015) to minimize and homogenize ice crystal formation have been reported. In these ongoing studies, the technique to visualize the 3D structure of ice crystals by X-ray computed tomography (X-ray CT) proposed by

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72 Mousavi et al. (2005) has been expanded for the effective evaluation of ice crystal

73 characteristics. Since then, the technique has been widely applied to understand the

74 growth behavior of ice crystals at sub-zero temperatures in food materials such as

75 soybean curd (Harnkarnsujarit et al., 2016a), polysaccharide gels (Harnkarnsujarit, et al.,

76 2016b), tuna meat (Kobayashi et al., 2015) and potato tissue (Ullah et al., 2014; Zhao &

77 Takhar, 2017).

78   During the freezing of vegetables, depolymerization of the cell wall, rupture of

79 cell membranes and alteration of osmotic pressure triggered by ice crystal formation

80 lead to losses of cell wall rigidity and cellular turgor, resulting in significant

81 modification of the mechanical property after thawing (Li et al., 2018). Several previous

82 studies have associated the changes in mechanical properties with the structural

83 destruction of the cell wall following the formation and expansion of ice crystals during

84 freezing (Paciulli et al., 2015; Van Buggenhout et al., 2006; Fuchigami et al., 1995).

85 However, especially in plant cells with low water permeability, the dehydration stress

86 caused by ice crystal growth in the extracellular region leads to fatal disruption of cell

87 membranes and results in significant texture deterioration (Ando et al., 2012). Although

88 the loss of turgor with cell membrane rupture has been considered to be one of the

89 major factors that affect the elastic property of tissues (Ando et al., 2012; Li et al., 2018),

90 it remains unclear how difference in the freezing rate and characteristics of ice crystals

91 formed contribute to the degree of cell membrane damage and the mechanical property.

92   Electrical impedance spectroscopy (EIS) has been employed as a method to

93 quantitatively evaluate the structural damage of cell membranes of biological tissues

94   (Pliquett, 2010). In EIS, the measured impedance frequency characteristics are generally

95   analyzed using the equivalent circuit model (Zhang et al., 1990; Yamamoto &

96   Yamamoto, 1977). Biological tissues are electrically inhomogeneous cell assemblies;

97   thus, a modified model was developed (Ando et al., 2014) by incorporating a constant

98   phase element (CPE) to the cell model proposed by Hayden et al. (1969). Since the

99   values of intra- and extracellular fluid resistance and cell membrane capacitance in

100   biological tissues can be quantified using the modified model, it has been used to

101   evaluate damage in the cell membranes of vegetables and fruits during heating

102   (Watanabe et al., 2017; Ando et al., 2017; Imaizumi et al., 2015), drying (Ando et al.,

103   2014) and cold storage (Imaizumi et al., 2018; Watanabe et al., 2018).

104   The present study aimed to clarify the influence of the various characteristics of

105   ice crystals formed at different freezing rates on cell membrane damage and mechanical

106   property. The X-ray CT technique was applied to evaluate ice crystal characteristics in

107   asparagus stems, which are commonly consumed as a frozen product throughout the

108   world, and then the relationship between ice crystal characteristics, cell membrane

109   damage as quantified by EIS analysis and the compressive elastic property after

110   freezing-thawing was investigated.

111

112   **2. Materials and methods**

113

114   *2.1   Sample preparation*

115   Stems of green asparagus (*Asparagus officinalis* L.) were obtained from a local

116 market, stored in a refrigerated chamber at 5 °C, and used for the experiments within 3

117 days. The asparagus stem (initial length of 270 mm) was cut into thirds perpendicular to

118 the fiber direction, then the central part was cut into 30 mm length (diameter of 10–12

119 mm), and used as the sample. For the mechanical test only, four samples were cut into

120 10 mm length from one stem and distributed to each test section to minimize individual

121 differences. The samples were blanched in boiling water for 1 min, then immediately

122 cooled in iced water.

123

124 *2.2   Freezing procedure*

125   The blanched samples were frozen by slow (SL) freezing, air blast (AB)

126 freezing or liquid nitrogen spray (LNS) freezing as slow, intermediate and rapid rate of

127 freezing respectively. In the SL freezing, the samples were placed on a polystyrene foam

128 board and frozen in a freezer (SF-3120F3, Nihon Freezer, Tokyo, Japan) at −30 °C. AB

129 freezing was carried out in a freezer (FMF-038F1, Fukushima Industries Corp., Osaka,

130 Japan) equipped with four fans (9GT, Sanyo Denki Co., Ltd., Tokyo, Japan) at a

131 temperature of −40 °C and an air velocity of 2.8 m/s. Samples frozen in a liquid

132 nitrogen spray freezer (EMP-10NS, Ebara Inc., Tokyo, Japan) at a temperature of

133 −60 °C and an air velocity of 1.4 m/s were used as the LNS frozen sample. After

134 freezing, the samples were immediately offered for the measurement process described

135 below. The samples were thawed at room temperature (25 °C) for 2 h, and used for the

136 experiments. The temperature of the central part of the sample during freezing was

137 measured using a T-type thermocouple with a wire diameter of 0.34 mm and retrieved

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138   in a data logger (GL220, Graphtec Corp., Kanagawa, Japan) at 1 sec intervals.

139

140   *2.3   X-ray CT evaluation of ice crystals*

141   Ice crystals formed in the samples were visualized by the indirect observation

142   method using X-ray computed tomography (CT), as previously reported (Mousavi et al.,

143   2005; Kobayashi et al., 2015). Prior to evaluation using a micro focus X-ray CT system

144   (SMX-100CT, Shimadzu Corp., Kyoto, Japan), ice crystals in the sample were

145   sublimated in a freeze-dryer (FD-20BU, Nihon Techno Service Co., Ltd., Ibaraki,

146   Japan). The temperature of the dryer was gradually increased for 1 week from –40 °C to

147   15 °C at a pressure below 10 Pa. It was assumed that the sublimated ice crystals left

148   pores with minimal deformation and that these pores were of the same size as the ice

149   crystals. Both whole samples and samples cut into quarters in parallel to the fiber

150   direction with a sharp knife were scanned using the X-ray CT system at an X-ray tube

151   voltage of 45 kV and a current of 100 μA; 1800 transmission images were obtained

152   through a 360 degree rotation. Tomographic images were reconstructed using software

153   (Exfact VR, Nihon Visual Science Inc., Tokyo, Japan). Cross-sectional images with

154   20.7×20.7 μm^2 pixel size of the quartered samples were used for estimation of the size

155   distribution of ice crystals. Three samples from each freezing condition were scanned,

156   and 12 cross-sectional images randomly selected from each sample were used for the

157   analysis. The obtained gray-scale images were binarized, then watershed segmentation

158   was applied for the proper detection of ice crystal areas in reference to the literature

159   (Zhao et al., 2017; Haris et al., 1998). Feret's diameter (i.e., line segment connecting the

160 two perimeter points that are the furthest apart) of the ice crystal area was calculated

161 using software (ImageJ version 1.51j8, W. S. Rasband, U. S. National Institutes of

162 Health, Bethesda, MD, USA).

163

164 *2.4   Electrical impedance spectroscopy (EIS)*

165   Electrical impedance characteristics of the samples before and after

166 freezing-thawing were measured using an impedance analyzer (E4990A, Keysight

167 Technologies, Santa Rosa, CA, USA) equipped with stainless needle electrodes. The

168 electrodes spaced 10 mm apart were inserted from the periphery to the central axis of

169 the sample to a depth of 5 mm, and impedance magnitude $|Z|$ (Ω) and phase difference θ

170 (rad) of the sample were measured at 65 points (logarithmic frequency intervals) over

171 the frequency range of 50 to 5 MHz. Before the measurement, the sample temperature

172 was conditioned to a room temperature of 25 °C to prevent temperature differences

173 from influencing the impedance characteristics.

174   The measured impedance data were analyzed using the equivalent circuit

175 model, as shown in Fig. 1. The model by Hayden et al. (1969) shown in Fig. 1 (a),

176 comprised of cell membrane resistance, C_m, and extra- and intracellular fluid resistances,

177 R_e and R_i, respectively, was proposed as a structural model of a single cell in biological

178 tissues. The model shown in Fig. 1 (b) was modified with a constant phase element,

179 CPE (Zoltowski, 1998), and has been proposed (Ando et al., 2014) as a model of

180 biological tissues, taking into account the time constant distribution of the impedance

181 characteristics being attributed to the non-uniform electrical characteristics of cells in

182   the tissue, which has been proven to be a valid model of biological tissues in many

183   studies (Imaizumi et al., 2015; Watanabe et al., 2017). The impedance of CPE (Z_{CPE}) is

184   represented as:

185    $$Z_{CPE}=\frac{1}{(j\omega)^{P}T}\ ,$$ (1)

186   where ω denotes the angular frequency (rad/s), T denotes the CPE coefficient, and P

187   denotes the CPE exponent ($0 \leq P \leq 1$). The measured impedance data were fitted to the

188   equation of the total impedance of model (b) using complex nonlinear least squares

189   curve fitting (Macdonald, 1992), and each circuit parameter was estimated. The root

190   mean squared error, RMSE, was calculated to evaluate the goodness of fit of the model.

191   Here, the CPE constant T was converted to an apparent capacitance C using the

192   equation below (Ando et al., 2014; Hsu & Mansfeld, 2001):

196    $$C=T^{\frac{1}{P}}(R_{e}+R_{i})^{\frac{1-P}{P}}\ .$$ (2)

193   In this study, the apparent capacitance C, obtained from Eq. (2), was defined as the cell

194   membrane capacitance C_{m}. The detailed protocol of the analysis has been reported

195   previously (Ando et al., 2017).

197

198   *2.5   Mechanical property*

199   A universal testing machine (5542, Instron, Norwood, MA, USA) equipped

200   with a 500 N load cell was used for sample testing. The sample was compressed by a

201   cylindrical plunger, 1000 mm^2 in base area at a speed of 0.5 mm·s^{-1}, from the side of

202   the cylinder shape of the sample until the compressive strain reached 50 % of the initial

203   thickness. The trigger load was set to 0.05 N. The diameter of the sample was measured

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204 using a caliper. The slope of the initial linear range (strain < 0.1) in the compressive

205 curve was calculated as an index of the elastic property (Ando et al., 2012; Chiralt et al.,

206 2001; Ando et al., 2016). The test was carried out at a room temperature of 25 °C.

207

208 *2.6   Statistical analysis*

209   Statistical analyses were performed using R software version 3.3.3 (R Core

210 Team). Differences among the means were compared using Tukey's multiple range test

211 with analysis of variance at a significance level of $p < 0.05$.

212

213 **3.   Results and discussion**

214

215 *3.1   Freezing rate and ice crystal distribution*

216   Figure 2 shows the changes in sample temperature during freezing. In AB

217 freezing, approximately 12 min was needed to reach –20 °C and about 10 min for LNS

218 freezing, while SL freezing required 50 min. In each freezing condition, plateaus were

219 observed around –0.8 °C, indicating the formation and growth of ice crystals in this

220 temperature region. Figure 3 shows cross-sectional images of whole and quartered

221 samples after freeze-drying obtained by X-ray CT. In the freeze-dried SL sample,

222 relatively large voids were observed in the interior, revealing the formation of coarse ice

223 crystals during freezing. In the freeze-dried AB and LNS samples, although slightly

224 coarse voids can be observed in the surface area, finer voids are distributed in most

225 regions, indicating that ice crystals were minimized in the AB and LNS freezing. The

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226   relatively coarse ice crystals were formed in the surface area because they were

227   developed at the point of boundary which is presumed to be structurally weak between

228   the cortical tissue just inside the epidermis and the supporting collenchyma tissue. In

229   addition, as is especially pronounced in the AB and LNS samples, ice crystals tended to

230   expand in the vascular bundles interspersed within the parenchyma tissue those have

231   relatively large spaces for ice crystals to grow without being hindered by cell walls.

232   Histograms of the ice crystal diameter extracted from each quartered sample are shown

233   in Fig. 4. It should be noted that the inherent air spaces in the sample tissue and

234   unavoidable slight shrinkage and deformation during freeze-drying process might have

235   affected the evaluation of the ice crystal size. In the SL frozen sample, the histogram

236   peaks around 150–200 μm and gradually declines to over 1000 μm, indicating wide

237   variation in the diameter of ice crystals formed during freezing. In the samples frozen

238   by AB and LNS, the diameter showed a relatively sharper peak and converges to around

239   500 μm, suggesting that fine, homogenously sized ice crystals were formed compared to

240   those in SL sample. Here, the mean values of the ice crystal size of SL, AB and LNS

241   samples were 428, 337 and 317 μm, and median values of those were 380, 320 and 297

242   μm respectively. In general, the size of ice crystals depends on the rate of freezing.

243   Therefore, it was thought that fine, homogenous ice crystals were formed in AB and

244   LNS samples because ice crystal formation occurred over a shorter time period in these

245   samples, as previously reported (Kono et al., 2017; Kobayashi et al., 2015). The larger

246   size of ice crystals observed in this study compared to tuna (Kobayashi et al., 2015) and

247   mycoprotein (Mousavi et al., 2005) was attributed to the higher moisture content of

248 vegetable tissues (typically greater than 90 %). Although the ice crystal size of the LNS

249 sample was slightly finer than those of AB sample, notable differences was not observed

250 between AB and LNS, suggesting that a large decrease in ice crystal size cannot be

251 expected by excess increases in the freezing rate as reported by the previous study in

252 which the ice crystal diameter declined exponentially with increases in the freezing rate

253 (Kono et al., 2017).

254

255 *3.2   Evaluation of cell membrane damage*

256   Figure 5 shows the impedance characteristics on the complex plane (Cole-Cole

257 plot) of the fresh, blanched and each frozen-thawed samples. The impedance

258 characteristics of the fresh samples displayed as a large arc, while the impedance of the

259 blanched sample and each frozen-thawed samples showed significantly compressed arcs.

260 Generally, the Cole-Cole plot of biological tissues that have a cellular structure is

261 described as a circular arc (Cole, 1932); therefore, the large arc of the impedance

262 characteristics of the fresh sample indicates that the cell membrane structure was

263 normally maintained. Compression of a Cole-Cole plot was reported in the drying of

264 potatoes (Ando et al., 2014), freezing of potatoes (Zhang & Willison, 1992) and heating

265 of spinaches (Watanabe et al., 2016), and the phenomenon was thought to be a result of

266 cell membrane injury. Therefore, the results obtained in this study suggest that cell

267 membranes in the sample tissues were injured during blanching and the

268 freezing-thawing process, and the smaller size of the arcs of the frozen-thawed samples

269 suggests that the freezing treatment caused greater cell membrane damage compared to

13

270　blanching treatment.

271　　　　In this study, the obtained impedance characteristics were analyzed with the

272　modified Hayden model, shown as model (b) in Fig. 1. Solid lines in Fig. 5 represent

273　approximations given by the model fitted using the complex nonlinear least squares

274　method. Here, only the arc region of the impedance spectra was used for the fitting,

275　since the other region is not related to the cellular structure (Ando et al., 2014). The

276　measured impedance spectra and solid lines were well matched for all samples (the ratio

277　of RMSE to the average of measured impedance value was less than 0.8 %), which

278　confirms that the present model is reasonable for application to the samples.

279　　　　The estimated values of the model parameters: cell membrane capacitance, C_m,

280　extracellular fluid resistance, R_e, and intracellular resistance, R_i, are shown in Table 1.

281　The value of C_m, which depends on the lipid bilayer structure of the membrane

282　(Ashrafuzzaman & Tuszynski, 2013) of the fresh sample, was the highest, indicating

283　that the structure was substantially maintained. However, in the blanched sample, C_m

284　was reduced to one-quarter or less of that of the fresh sample, suggesting structural

285　damage of cell membranes by heat treatment. The same phenomenon has been reported

286　for spinach (Watanabe et al., 2017) and Japanese radish (Ando et al., 2017), and is

287　presumed to be attributable to thermal denaturation of phospholipids contained in the

288　cell membrane. The C_m values of each frozen-thawed sample were greatly decreased to

289　1.0–1.2 % of the fresh sample, and there was no significant difference between each

290　freezing method. Cell membrane injury of plant tissues during freezing has been

291　reported in many studies, which propose that the formation of ice crystals during

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292 freezing is responsible for cell membrane damage (Ando et al., 2012; Delgado &

293 Rubiolo, 2005). Although the results obtained in this study support this viewpoint, the

294 level of cell membrane damage was comparable with all freezing methods despite the

295 difference in the size of ice crystals, which suggests that the phenomenon of cell

296 membrane damage during freezing is independent of ice crystal size.

297   The high values of R_e and the low value of R_i of the fresh sample were due to

298 the low electrolyte concentration of the extracellular fluid and the high electrolyte

299 concentration of the intracellular fluid, indicating that the permselectivity of the cell

300 membrane is functioning normally. In the blanched sample, R_e was decreased and R_i

301 was increased because the difference in the electrolyte concentration between the intra-

302 and extra-cellular fluids could not be maintained due to the decline in cell membrane

303 function. In the frozen-thawed sample, R_e decreased markedly and in contrast to the

304 fresh and blanched samples, R_i had a larger value than R_e. As in the case of C_m, there

305 was no significant difference in R_e and R_i between each freezing method. In the

306 freezing process, the cell interior is rapidly dehydrated concomitant with ice crystal

307 growth in the extracellular region (Palta, 1990), and results in a cell volume decrease

308 due to plasmolysis. Further decreases in the R_e of frozen-thawed samples could be

309 explained by the increase in the amount of extracellular fluid due to the above

310 phenomenon, which resulted in the increase of the electrical flow path and decrease in

311 electrical resistance. Conversely, the increase in R_i value was attributed to limitation of

312 the electrical flow path from the decrease in intracellular volume. These changes in

313 model parameters were also observed in the fresh-frozen samples (not blanched) by

15

314 each freezing method, and there was no significant difference due to the presence or

315 absence of blanching (data not shown), indicating that freezing-thawing has a greater

316 influence on cell membrane damage than blanching.

317   From the above results, it was shown that the cell membrane was structurally

318 and functionally damaged during the freezing-thawing process, and that this

319 phenomenon was not dependent on ice crystal size. As noted above, intracellular

320 moisture is rapidly dehydrated in response to ice crystal formation and growth during

321 freezing. In addition, during freezing-thawing, cells are subjected to a multitude of

322 stresses including chilling stress, and mechanical and chemical stresses resulting from

323 ice crystal formation (Steponkus, 1984). However, the results obtained suggest that the

324 cell membrane damage resulting from the above causes cannot be prevented by

325 minimizing ice crystal size with a rapid rate of freezing.

326

327 *3.3   Mechanical properties after thawing*

328   Figure 6 shows the representative compressive curve of the blanched and each

329 frozen-thawed samples. In the blanched sample before freezing, the force increased with

330 compression and reached over 30 N, and fracture occurred in the sample around a strain

331 of 0.5. The increase in force was suppressed in the frozen-thawed samples, suggesting a

332 decrease in tissue rigidity, and fracture was not observed in each freezing method.

333 Although the force in the LNS and AB frozen samples seemed to be slightly higher than

334 that of the SL frozen sample, the difference between the freezing methods was quite

335 small and the forces were significantly decreased compared to the blanched sample. The

336   initial slope from the origin to the force at a strain of 0.1 in the force-strain curve is

337   shown in Table 2 as an index of the elastic property of the samples. The values after

338   freezing were greatly decreased to 22–32 % of the blanched sample. In a comparison of

339   each freezing method, the initial slopes of the rapid freezing methods (AB and LNS)

340   were slightly higher than that of SL. However, the ability to improve the change in the

341   mechanical property was limited even by rapid freezing, and the substantial change

342   during freezing-thawing could not be inhibited. Decline in the mechanical property after

343   freezing-thawing of vegetables has been reported previously (Paciulli et al., 2015; Van

344   Buggenhout et al., 2006; Fuchigami et al., 1995), and this phenomenon was considered

345   as the result of the destruction of the tissue structure, e.g., the cell wall, following ice

346   crystal formation. However, although smaller ice crystals were formed by rapid freezing

347   in this study, substantial improvement in the mechanical property was not observed

348   compared to slow freezing, in which coarse ice crystals were obviously formed. As

349   shown by the impedance analysis, the structural damage and functional decline of the

350   cell membrane during freezing-thawing occurred equally among each freezing condition.

351   These changes in the cell membrane state cause the loss of turgor pressure, which has a

352   large impact on the mechanical properties, especially the elastic property of vegetables

353   (Gonzalez, Anthon, & Barrett, 2010; Greve et al., 1994). Therefore, as suggested by

354   Chassagne-Berces et al. (2009) and Ando et al. (2012), it was assumed that a reduction

355   in turgor pressure following changes in the cell membrane state, resulting from ice

356   crystal formation during freezing, is a major factor in the mechanical property. Further,

357   the results obtained indicate that this phenomenon is independent of freezing rate and

358   ice crystal size.

359

360   **4. Conclusions**

361

362   In the present study, the influence of the size of ice crystals formed in

363   asparagus stem tissue on the cell membrane state and mechanical property was

364   investigated. Although finer ice crystals were observed in the rapid freezing methods,

365   the structural damage and functional decline as estimated by electrical impedance

366   analysis were at the same level as those of the slow-frozen sample, in which coarse ice

367   crystals were clearly observed. Also, the elastic property of the samples after thawing

368   was greatly reduced as compared with those before freezing, and improvements by

369   rapid freezing were limited. These results suggest that the decline in the mechanical

370   property is mainly attributed to the structural damage and functional decline of cell

371   membranes following ice crystal formation during freezing, rather than the size of ice

372   crystals. Although it has been believed that the finer ice crystals formed at faster rate of

373   freezing leads to the improvement of quality after thawing, the present study showed

374   that the fine ice crystals do not necessarily bring about texture improvement in

375   vegetable tissue. Therefore, inhibition of changes in the cell membrane state that occur

376   during freezing and maintenance of turgor pressure are important factors that should be

377   addressed in order to further improve the textural quality of frozen vegetables.

378

379   **Acknowledgement**

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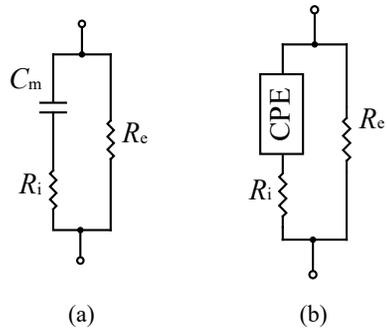
Fig. 1　　Equivalent circuit models of biological tissues.
(a) Hayden model, (b) modified Hayden model. C_m: capacitance of cell membrane,
R_i: intracellular fluid resistance, R_e: extracellular fluid resistance, CPE: constant phase element.
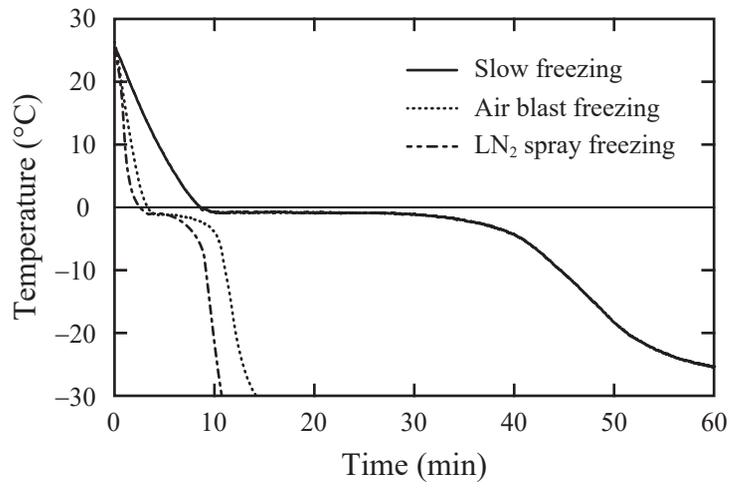
Fig. 2   Changes in the temperature of central part of asparagus samples during freezing.
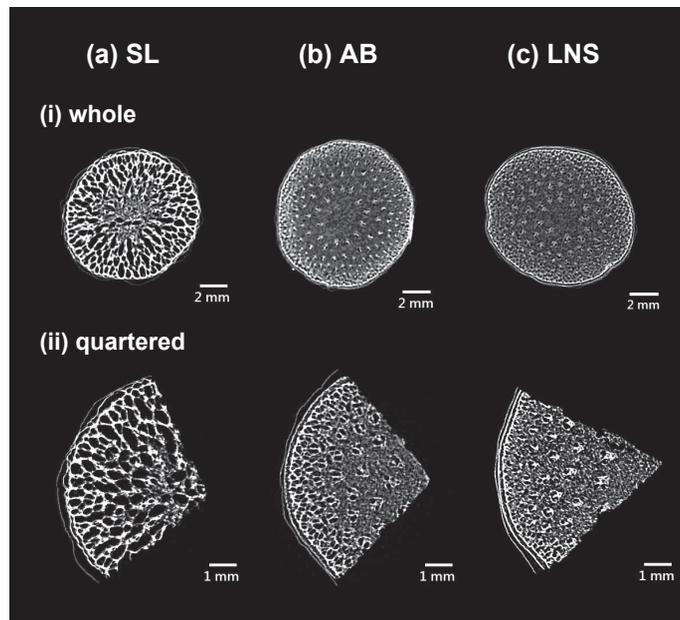
Fig. 3 Representative reconstructed cross-sectional images of whole (i) and quartered
(ii) frozen asparagus samples. (a) SL: slow freezing, (b) AB: air blast freezing, (c) LNS: liquid
nitrogen spray freezing. Higher brightness part represents higher density part.
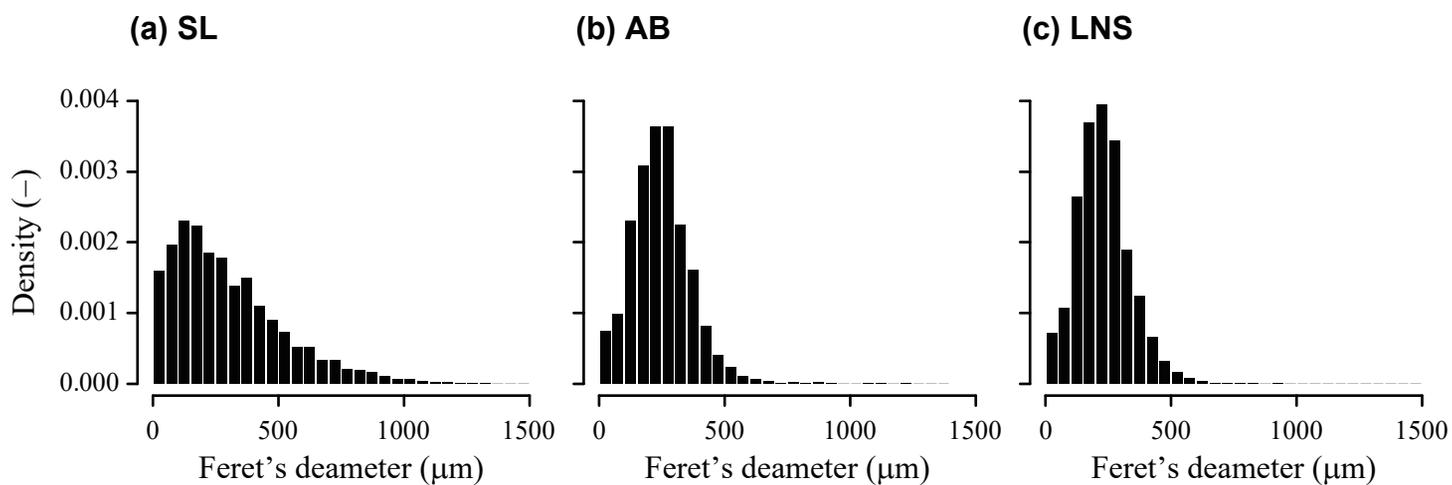
Fig. 4   Ice crystal diameter distribution of frozen asparagus samples. Class interval width of the histograms is 50 um.
(a) SL: slow freezing, (b) AB: air blast freezing, (c) LNS: liquid nitrogen spray freezing. Feret's diameter: line segment connecting the two perimeter points that are the furthest apart.

Fig. 5.　　Representative Cole-Cole plots for the fresh, blanched (a) and frozen-thawed (b) samples.
SL: slow freezing, AB: air blast freezing, LNS: liquid nitrogen spray freezing.
Solid lines represent approximations given by the modified Hayden model in Fig. 1b.

Fig. 6.   Representative compressive curves of the blanched and frozen-thawed samples.

Figure captions

Fig. 1.   Equivalent circuit models of biological tissues. (a) Hayden model, (b) modified Hayden model. C_m: capacitance of cell membrane, R_i: intracellular fluid resistance, R_e: extracellular fluid resistance, CPE: constant phase element.

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Fig. 6.   Representative compressive curves of the blanched and frozen-thawed samples.

Table 1   Equivalent circuit parameters obtained from the model fitting.

Condition	C_m (pF)	R_e (Ω)	R_i (Ω)	RMSE (Ω)
Fresh	1550[a] ± 219	5460[a] ± 498	540[a] ± 24.4	9.7–31.9
Blanched	355[b] ± 35.6	970[b] ± 80.9	880[b] ± 57.4	3.3–6.0
Blanched and frozen-thawed				
Slow freezing	18.6[c] ± 7.4	587[c] ± 35.1	2190[c] ± 316	0.39–0.78
Air blast freezing	16.2[c] ± 9.6	585[c] ± 42.2	2140[c] ± 336	0.52–0.75
Liquid nitrogen spray freezing	18.4[c] ± 9.6	581[c] ± 52.5	2250[c] ± 142	0.36–0.74

C_m: capacitance of cell membrane, R_e: resistance of extracellular fluid, R_i: resistance of intracellular fluid, RMSE: root mean squared error. The data are mean values of 11–12 replicates (± standard deviation). Different superscripts denote significant differences ($p < 0.05$).

Table 2　Elastic parameter of the samples determined using the compressive test.

Condition	Initial slope (N)
Blanched	$6.2^{a} \pm 1.2$
Blanched and frozen-thawed	
Slow freezing	$1.4^{c} \pm 0.2$
Air blast freezing	$1.8^{b} \pm 0.3$
Liquid nitrogen spray freezing	$2.0^{b} \pm 0.4$

Initial slope: slope from the origin to the force at strain of 0.1 in the force-strain curve. The data are mean values of 52 replicates ($\pm$ standard deviation). Different superscripts denote significant differences ($p < 0.05$).

Highlights

► Ice crystals formed in asparagus stems during freezing were evaluated by X-ray CT.

► Fine, homogenous ice crystals were observed with the rapid freezing methods.

► Structural damage and functional loss of cell membranes occurred with each freezing.

► The decline in elasticity was slightly improved by rapid freezing methods.

► The change in cell membrane state is a main factor in texture loss with freezing.