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Research note

Effect of vacuum infused cryoprotectants on the freezing tolerance of strawberry tissues

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ABSTRACT

Whole strawberries were vacuum infused with cryoprotectants to improve their freezing tolerance. The strawberries were infused with 12 g/100 g trehalose solution; 0.2 g/100 g cold-acclimated wheatgrass solution (AWWE) containing antifreeze protein (AFP) or combination of 12 g/100 g trehalose with 0.2 g/100 g AWWE under vacuum for 14 min. The fruits were frozen in liquid nitrogen and thawed at room temperature before being evaluated for cell viability, drip loss and preservation of texture. The results showed that the combined effects of both cryoprotectants significantly improved the freezing tolerance of the treated strawberries. The cryoprotection effect was influenced by the heterogeneity of the tissues in the fruits.

1. Introduction

Freezing is an extensively used method to preserve the quality of food products which may result in textural changes leading to tissue softening. Attempts have been made to improve the resistance of fruit and vegetables to freezing damage by several methods (Moraga, Martínez-Navarrete, & Chiralt, 2006; Suutarinen, Heiska, Moss, & Autio, 2000). However, loss of the fresh-like characteristics of the product due to freeze-induced damage of cell membranes, turgor loss and the consequent loss of cell viability, could not be avoided.

Innovation on freezing of fruit and vegetables should be based on knowledge of the natural cryoprotection mechanisms at the cellular level. Cold-induced stress responses comprise complex metabolic processes regulated at the genetic level (Gómez Galindo, Sjöholm, Rasmusson, Widell, & Kaack, 2007). Among these cryoprotective mechanisms, certain plant tissues accumulate osmotically active substances in the cytoplasm (e.g. sugars such as sucrose and trehalose) as well as antifreeze proteins (AFPs) in the apoplast during growth in the field in late autumn. Apart from decreasing the chemical potential of water and the freezing point in the cytosol, the hydrophilic nature of sugars stabilizes the cell membrane through hydrogen bondings. In the apoplast, accumulation of AFPs provokes minimal crystal growth forming very small, stable hexagonal bipyramids. AFPs are also strong inhibitors of recrystallization (Smallwood & Bowles, 2002).

Phoon, Gómez Galindo, Vicente, and Dejmek (2008) significantly improved the freezing tolerance of spinach leaves by vacuum infusing trehalose. Infusion was accompanied by application of pulsed electric fields aiming at distributing the trehalose in intra and extracellular spaces. This strategy successfully maintained cell vitality, turgidity and fresh-like characteristics of the leaves after thawing. Cruz, Vieira, & Silva. (2009) infused watercress leaves with AFP, improving their turgidity without a complete recovery of their fresh-like characteristics.

The present study aims at improving the freezing tolerance of whole strawberries by infusing cryoprotectants in their structure. We here report preliminary results on the use of trehalose and AFP alone or in combination for preserving cell viability and texture of strawberry fruits. Suutarinen, Änäkäinen, and Autio (1988) described the heterogeneous structure of strawberries (Fig. 1). Epidermal cells form the compact outer layer. The second layer is composed of hypodermal cells and the third layer of cortical cells with larger intercellular spaces. The other two zones are the vascular bundles and the pith. The heterogeneity of strawberries and their genetic inability for cold-induced responses challenged us to investigate their preservation during freezing/thawing cycle by infusion of cryoprotective solutions.

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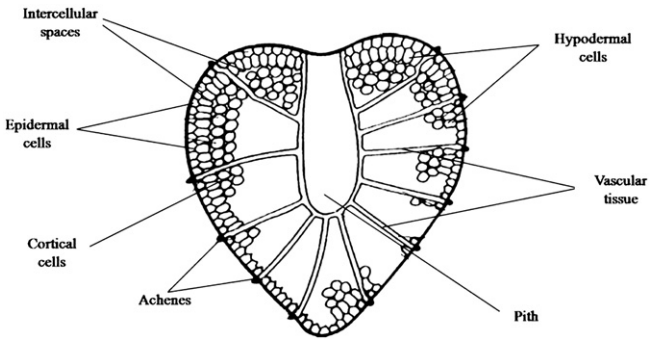

Fig. 1. Schematic illustration of strawberry tissues (adapted from Suutarinen et al., 2000).

2. Materials and methods

2.1. Raw material handling

Strawberries (10.5° ± 1.6°Brix) grown in the south of Sweden during summer 2010 were purchased daily from the same supplier at the local market. The fruits were washed, hand stemmed and selected according to size, firmness and similar visual ripening.

2.2. Cryoprotectant solutions

The following cryoprotectant solutions were prepared: Trehalose (Cargill, Denmark) and spray dried, unpasteurised cold-acclimated winter wheat grass extract (AWWE) as a source of AFP (Microstar Biotech Ltd., Zhuhai, China; AWWE contained 12 g/100 g proteins). Three different solutions were prepared: 12 g/100 g trehalose solution; 0.2 g/100 g AWWE solution and a third solution containing 12 g/100 g trehalose and 0.2 g/100 g AWWE. Trehalose concentration was isotonic to the strawberry juice.

2.3. Vacuum infusion

Vacuum infusion was carried out at 20 °C in a chamber connected to a vacuum pump. Based on results of preliminary tests aiming at finding conditions for maximal mass gain in the fruits, whole strawberries (70 g) were immersed in 250 ml of one of the solutions for 14 min. This duration comprised a gradual increase of the vacuum for 4.5 min, a holding time of 5 min at −86 kPa (manometric) and a gradual release of the vacuum for 4.5 min.

2.4. Freezing and thawing

After vacuum infusion, fruits were submerged in liquid nitrogen. One fruit had a thermocouple inserted in the middle to follow the freezing temperature over time with a data logger (USB TC-08, Pico Technology, Cambridgeshire, UK). The fruit reached −18 °C at the centre after 25 s. The frozen samples were stored at −18 °C for 10 min before thawing at room temperature (20 °C) for 2 h and let to drip overnight at 4 °C. For thawing, the package of 70 g strawberries was placed in a plastic funnel and covered with parafilm to avoid evaporation. All the experiments were repeated three times with 3 packages of strawberries for each treatment, resulting in total of 9 replicates. Replications were done in different days.

2.5. Mass gain

The mass gain of the vacuum infused strawberries was calculated from the following equation, in percent:

$$m_{\text{weight gain}} = \frac{m - m_0}{m_0} \cdot 100 \quad (1)$$

Where m is the mass of the infused strawberry and m_0 is the initial mass of the fresh strawberry.

2.6. Drip loss

The strawberries were weighed after freezing and 20 h after thawing on an analytical balance (Precisa Instruments Ltd, Switzerland). The drip loss was calculated from the Eq. (2):

$$m_{\text{drip loss}} = \frac{m_1 - m_2}{m_1} \cdot 100 \quad (2)$$

Where m_1 is the mass of the frozen strawberries and m_2 is the mass of the strawberries after thawing.

2.7. Microscopic observations

The viability of the cells was evaluated with fluorescein diacetate (FDA, Sigma–Aldrich, USA). The strawberries were cut longitudinally in 2 mm thick slices using sharp razor blades. From every longitudinal slice, a rectangular piece with the following dimensions: $a = 15$ mm, $b = 5$ mm and $c = 2$ mm was transversely cut (Fig. 2) and incubated for 5 min in a 0.5 mol/L sucrose solution 10^{-6} mol/L FDA in the darkness at room temperature. Stained sections were examined under fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co, Japan) equipped with a Nikon

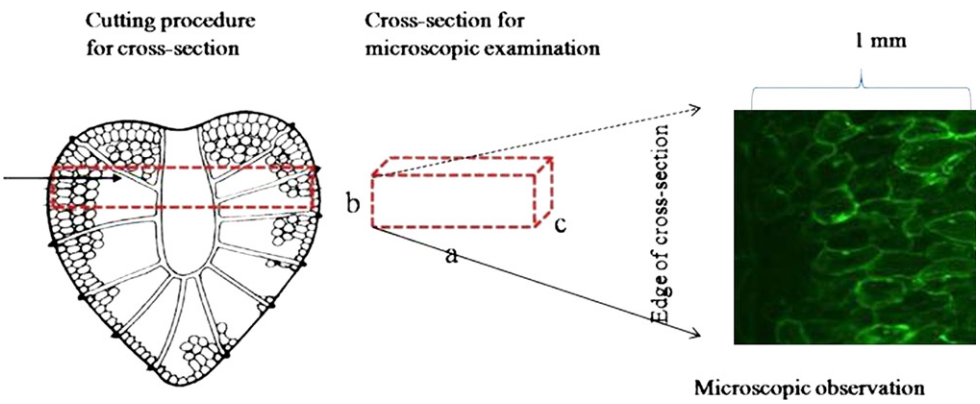

Fig. 2. Preparation of cross-section for microscopic observations.

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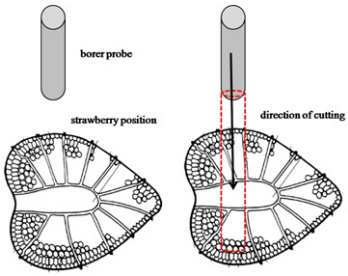

Fig. 3. Cutting test for texture analysis.

digital camera (digital sight DS-Qi1Mc, Nikon Co, Japan) at a magnification of 4×. A graduated metre was placed along the microscopic plate and slid slowly, mm by mm, to evaluate the viability of the cells along the whole length (15 mm) of the rectangular piece. Undamaged, viable cells could be easily identified by a bright fluorescence.

2.8. Texture analysis

Texture was measured at 20 °C using a Universal Instron testing machine (series 442H1004, UK) with a 100 N load cell. Crosshead speed was set at 60 mm/s and the penetration depth was 20 mm. A single, whole, strawberry (height: 30 ± 2 mm; width: 27 ± 3 mm) was placed on a flat platform and a sharpened 5 mm diameter cork borer adapted to fit the Instron was used to cut through the strawberry tissue from one side to another, penetrating to 80% of the total fruit width (Fig. 3). The textural parameter measured on the resulting force-distance curves was firmness and the mean values of six replicates, expressed in N, were reported.

2.9. Statistical analysis

Significant difference between the treatments was assessed by one-way analysis of variance (ANOVA, 95% significance level) with Tukey's comparison test using STATISTICA 6.0 (Statsoft Inc., Tulsa, UK).

3. Results and discussion

3.1. Mass gain and drip loss

The mass gain of strawberries infused with AWWE alone was significantly lower ($p < 0.05$) than that infused with trehalose alone and with the combination of both (Table 1).

The drip loss of strawberries infused with trehalose and AWWE separately was significantly lower ($p < 0.05$) compared to untreated samples. The combination of trehalose and AWWE resulted in further significant reduction ($p < 0.05$) of the drip loss than samples infused with trehalose or AWWE alone (Table 1). Water starts freezing in the extracellular space causing tissue damage and the role of AFPs accumulated in the extracellular space for tissue protection is widely documented (Griffith & Antikainen, 1996). Fruits accumulate sucrose and reducing sugars in the cytoplasm which may have also contributed to cryoprotection.

Table 1
Mass gain and drip loss of untreated and vacuum infused samples after freezing/thawing cycle.

Treatment	Mass gain (g/100 g)	Drip loss (g/100 g)
Untreated	–	$32.2^{a} \pm 3.2$
VI (Tre)	$14.8^{a} \pm 0.3$	$25.1^{b} \pm 2.5$
VI (AWWE)	$9.0^{b} \pm 1.2$	$23.6^{b} \pm 1.0$
VI (Tre + AWWE)	$19.0^{a} \pm 3.4$	$12.1^{c} \pm 1.0$

VI: Vacuum infusion.
Tre: Trehalose.
AWWE: cold-acclimated winter wheat grass extract.
Reported are average values and standard deviations.
Values with different letter in a given column are significantly different ($p < 0.05$).

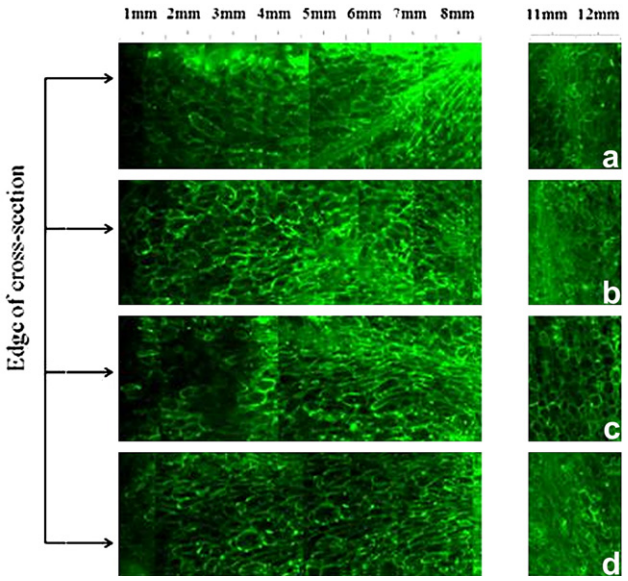

Fig. 4. Cell viability tests for (a) fresh fruit and vacuum infused fruits with (b) trehalose, (c) AWWE, (d) combination of trehalose and AWWE.

3.2. Microscopic observations

Fig. 4 shows the results from microscopic observations using fluorescein diacetate (FDA) to identify cell viability of fresh and vacuum infused samples. The pictures demonstrate that cell viability in all tissues is preserved after the vacuum treatment. In

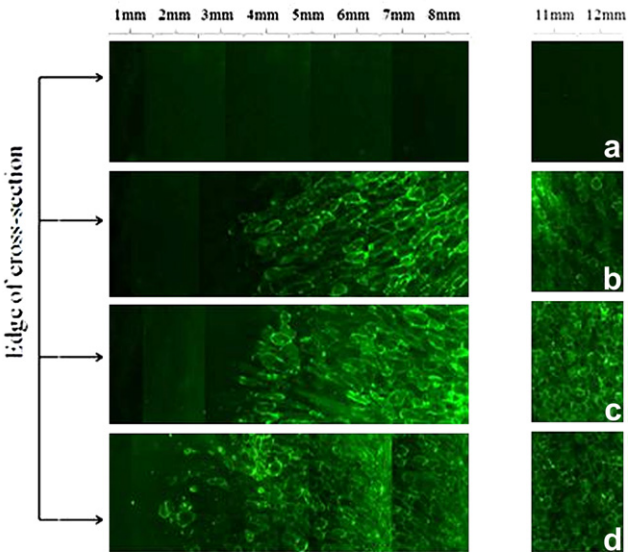

Fig. 5. Cell viability test of strawberries after one freezing/thawing cycle for (a) untreated strawberry and vacuum infused with (b) trahalose, (c) AWWE, (d) trahalose and AWWE.

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Fig. 5, the micrographs of frozen/thawed samples are presented. As expected, freezing and thawing untreated strawberries caused the loss of cell viability in all tissues of the fruit (Fig. 5a). When trehalose and AWWE were infused alone into the samples prior to freezing, cell survival was observed in the cortical tissue and the pith. When the combination of trehalose and AWWE was used, viable cells appeared from the 2nd mm from the surface, confirming better cryoprotection of the cortical and vascular tissue, right bellow the hypodermal layer, and the pith.

The results presented here provided evidence that cell viability can be preserved in strawberries after one freezing/thawing cycle. However, the cryoprotection effect was influenced by the heterogeneity of the tissues in the fruits, and the viability of cells of the more compact tissues close to the surface (epidermal and probably part of hypodermal) could not be preserved.

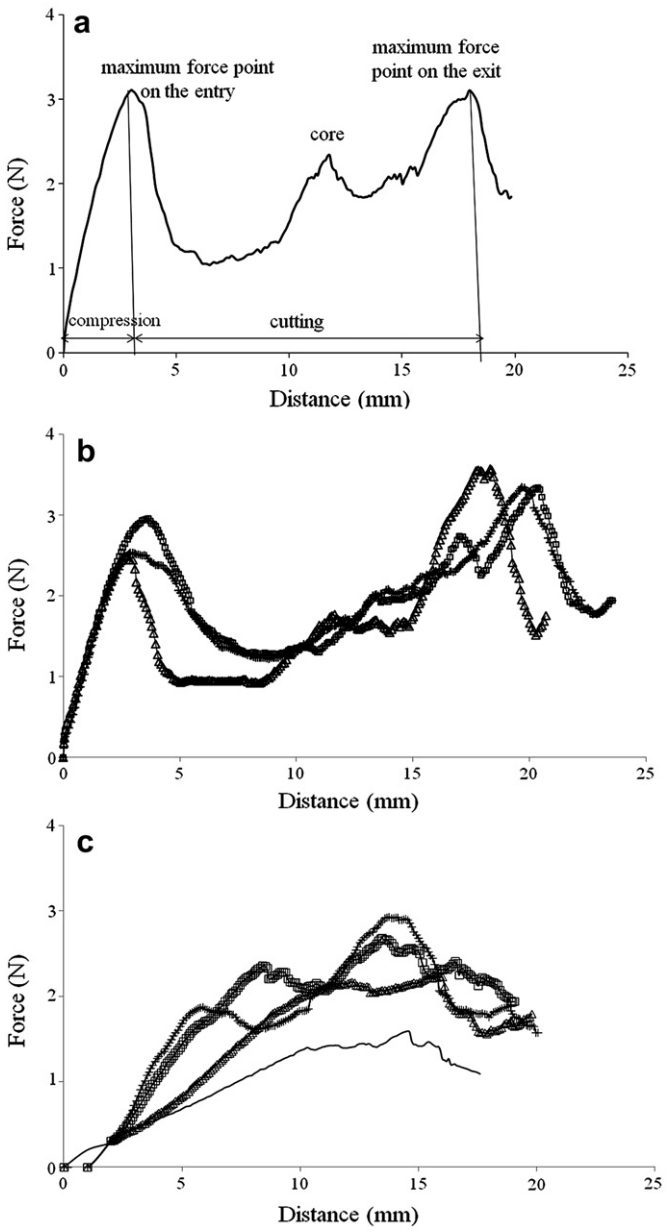

Fig. 6. Force-distance curves obtained during cutting tests. (a) fresh, (b) vacuum infused, (c) frozen and thawed fruits. Δ VI (trehalose), □ VI (AWWE), + VI (trehalose + AWWE), - untreated.

3.3. Texture analysis

Fig. 6 shows the force-distance values of the texture measurements. In the first part of the curve (approximately 3 mm, Fig. 6a), there is a rapid increase of the force up to a yielding point (compression). Here, the samples are deformed and compressed without cutting the tissue. The end of the first peak indicates the point of internal fracture and was taken as the parameter to measure flesh firmness. The force at this point was around 3.0–3.5 N with slope (firmness) of 1062 ± 32 N/m, for the fresh strawberry samples. After the first peak, the borer continued cutting through the tissue reaching the second, firmer layer of the flesh, the core (pith), which produced the second peak on the curve. The force necessary to cut this tissue was around 2.0–2.5 N, corresponding to slope of 562 ± 17 N/m. As the cutting test continued to 80% of penetration in the strawberry width, the last peak showed that the force necessary to cut the cortical tissue was around 3.0–3.5 N. The slope of the third peak was 484 ± 15 N/m.

Fig. 6b reports curves showing that vacuum infused strawberries with different cryoprotectants follow the same pattern than those of fresh fruits, confirming that vacuum infusion did not affect the texture of the fruits. The corresponding slope values were in the range between 980 and 990 ± 30 N/m, 400–645 N/m and 400–540 ± 16 N/m for the first, second and third peak, respectively.

In Fig. 6c, the force-distance curves showed that the compression part for the frozen and thawed samples was greater, causing visible bending of the strawberry surface before the borer started to cut through the tissue. However, after cutting through the surface (epidermal layer), further penetration of the probe detected a second peak with slope of 280 ± 8 and 340 ± 10 N/m for the strawberries infused with only trehalose or AWWE. The third peak for these samples also showed lower values in comparison to the fresh fruit, but still providing partial preservation of the flesh firmness in the pith and cortical tissue. The best preservation of the tissue was attained by the infusion of the mixture of cryoprotective solutions which can be seen from the texture curve with slope values of 546 ± 16 N/m for the second and 455 ± 14 N/m for the third peak, similar to those obtained for the fresh fruits. The preservation of cell vitality shown above (Fig. 5) and the preservation of texture, strongly suggest that vital cells keep their turgor pressure.

4. Concluding remarks

The combined effects of trehalose and AFP significantly improved the freezing tolerance of strawberry fruits. The cryoprotection effect was influenced by the heterogeneity of the tissues. Tissues close to the surface of the fruits did not survive thawing. Cortical tissue, vascular tissue and pith survived the freezing/thawing cycle.

The protective role of trehalose in combination with AFP when located in the extracellular space is not obvious, neither the fact that some cells survived thawing only with the combination of the cryoprotectants. This is an attractive issue, as the potential industrial success of our results will depend on a deeper understanding of the relations between microstructure, fruit physiology and cryoprotection mechanisms.

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