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Contents

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3-D Visualization of Cell Membrane of Cucumber Fruits Stored at Different Temperature

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Abstract

The 3-D visualization of cell membrane of cucumber fruits stored at 5°C (chilled) and 25°C (non chilled) for 9 days was demonstrated. The 3-D images were reconstructed from a series of 100 cross-sectional of 2-D images by utilizing 3-D visualization software. The size distribution of cell membrane was determined from the volume of cell for both storage conditions with time. The cell membrane of cucumber fruits during storage period was then observed through the change in volume of cell. The volume of cells was calculated from 70 to 125 of cells for each storage conditions. In day 1, the percentage of volume size of cell larger than 8 x 10^{-4}µm for both storage conditions of 5 and 25°C were 17.7 and 22.1% respectively. The percentage of larger volume size of cell decreased to be 1.6 and 9.7% for 5 and 25°C at storage time of 9 d. In contrast to this, the percentage of small volume size of cell increased with time from 21.5 and 23.5% at day 1 to be 56.0 and 42.4% at days 9 for 5 and 25°C. It was found that the percentage of larger size of cell for both storage conditions decreased and the percentage of small size of cell increased with time. Change in the percentage of volume size of cell with time was thought to be related

with weight loss i.e. water loss. Higher percentage of small volume size of cell for fruits stored at low temperature might indicate the higher speed of damage in cell due to impact of chilling.

Keywords: 3-D visualization, cell membrane, size distribution, chilling, weight loss

1. Introduction

Low temperature storage is an effective means of keeping horticultural products at a high post harvest quality. However, for some horticultural products, storage under low temperature may cause chilling injury. Chilling injury is a physiological defect of plants and their products that results in reduced quality and loss of product utilization following exposure to low but non-freezing temperatures (Parkin *et al.*, 1989). The injury symptoms may develop as characterized by appearance of surface injuries, such as surface pitting, discoloration, water-soaking, etc., if the period of exposure to chilling temperature becomes longer (Saltveit, 2002).

Change in cell membrane permeability as a response to chilling temperature has often been investigated as a possible cause of chilling injury (Lyons, 1973). Increase in cell membrane permeability and increase in rates of ion leakage are associated with chilling sensitive tissue (Saltveit, 2000). The cell membrane permeability regulates the water movement in cell and the dynamic state of water can be used to detect a chilling-induced permeability change in cell membrane (Naruke *et al.*, 2003; Purwanto *et al.*, 2005)). In order to better understand the mechanism of change in membrane permeability in cell structure of horticultural products during storage at low temperature, visualization and analysis of 3-D image of cell structure during storage will be essential for the studying on postharvest technology.

At present, there are a number of well-established methods for calculating cell volume changes (Kimelberg *et al.*, 1992). Methods for determining volume changes at single-cell level have been presented (Altamirano *et al.*, 1998), volume estimation by 3-D has been proposed by Allanson *et al.*, 1999). However, no data available on the 3-D visualization and analysis of the changes in cell membranes of horticultural products during storage under low temperature. The objective of this study were to visualize the 3-D of cell membrane of cucumber fruits stored under chilled and non chilled temperature and to investigate the changes in cell membrane size during low temperature storage with time.

2. Material and Method

2.1. Plant Material and Storage Condition

Freshly harvested cucumber fruits (*Cucumis sativus*, L cv. Alfa Fushinari and Flesco 100) were used as sample. One day after harvesting, the fruits were transported to the laboratory at ambient temperature. Sample of fruits were selected for the uniformity of size and weight in the range of 85 - 100g. Fruits were then put into polyethylene bags (340 x 240 x 0.08 mm) which each bag contains 4 fruits. Storage temperature was set at 5°C (chilled) and 25°C (non chilled) for 9 days. The polyethylene bags were ventilated once a day to prevent anaerobic condition.

2.2. Measurement

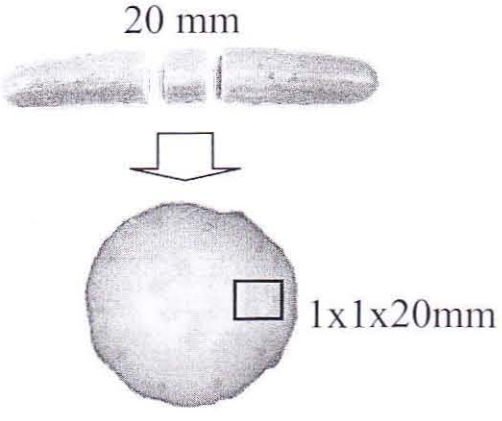
Measurements were carried out every day for weight loss and the occurrence of pitting as a result of chilling injury. Analysis of 3-D images was done at day 1, 5 and 9 for all storage conditions. Weight loss, i.e. the amount of water loss, was expressed as a percent of initial weight of sample.

2.3. 3-D Analysis

2.3.1. Image Measurement

Sarcocarp of cucumber fruits with dimension of 1 x 1 x 20 mm was used as a sample (Figure 1). After dissection, the sample was soaked for 1 h in a solution of fluorescent indicator (Rhodamine B 0.5 wt.%). The stained sample was then put into paraffin sample holder (outer diameter of 5.5mm, inner diameter of 2.5 mm and length of 30mm), which has been filled with OCT compound embedding medium. Sample was then frozen under an air temperature about -36 °C for 10 min. After that, the sample was set at MSIPS (micro-slicer image processing system) (Ueno, 2004 and Do, 2004). Image of exposed surfaces were taken with the CCD camera photometric (Cool Snapes, Roper Scientific) through fluorescence microscope at 200 Magnification. The speed of cut rotation of micro-slicer was set at 60rpm with the thickness of 5μm. The temperature condition of MSIPS during cutting was maintained at -40 °C. The images of 100 cross-sections were serially obtained per sample for both storage condition of 5 and 25°C with time.

Figure 1: Part of the sample dissected from fruits



2.3.2. Visualization of Cell Membrane

The recorded 2-D images had 256 gray level with the resolution of 512x512 pixel (1 pixel = 0.7 μm). The 3-D images was reconstructed by utilizing 3-D visualization software Slice O matic (Imagelabo Company, Japan) and displayed the cell membrane. The size distribution of cell membrane was determined from the volume of cell for both storage conditions with time.

3. Result and Discusion

3.1. 3-D of Cell Membrane

The 2-D images of cell membrane for cucumber fruits stored at 5°C (chilled) and 25°C (non chilled) storage at day 1 are shown at Figure 2. The 3-D image of cell membrane was reconstructed from a series of 100 cross-sectional 2-D images. Each image of cell was firstly marked and automatically adjusted by software Slice O matic as shown at Figure 3. The process was carried out for all cell images for 100 of 2-D images which each cell has a different color. The result of 3-D image of cell membrane of cucumber fruits at 5°C in day 5 which reconstructed from 100 images of 2-D image is shown at Figure 4. The dimension of 3-D image is 358.4x358.4x500μm.

Figure 2: 2-D images of cell membrane for cucumber fruits stored at 5°C and 25°C at day 1

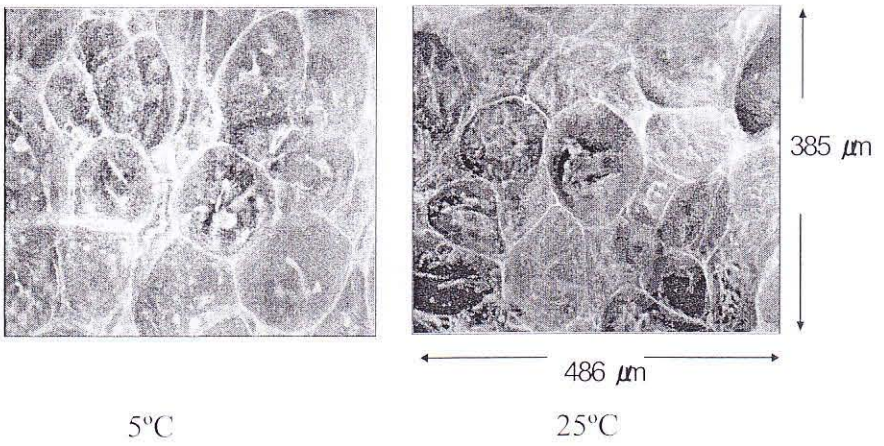


Figure 3: Reconstruction process of 2-D images of cell membrane

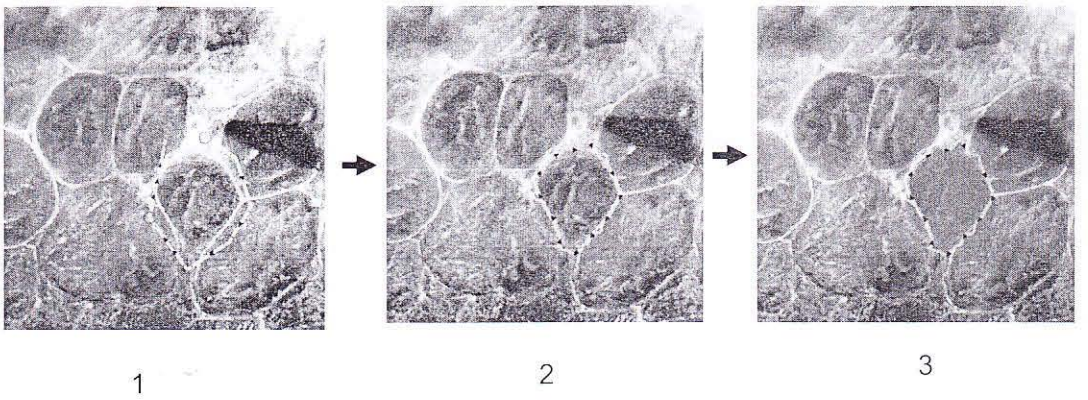
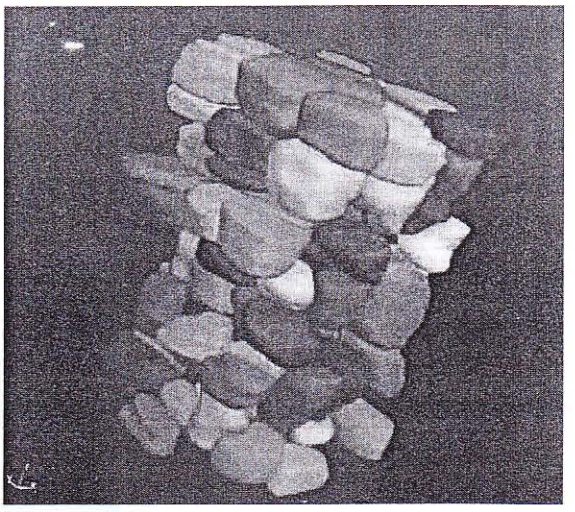


Figure 4: 3-D image of cell membrane of cucumber fruits 5°C at day 5



3.2. Cell Membrane Size

Figure 5 and 6 show the change in distribution of volume of cells with time for both storage conditions of 5 and 25°C. The volume of cells was calculated from 70 to 125 of cells for each storage conditions and only full sizes of cells were considered. In day 1, the percentage of volume size of cell larger than 8×10^{-4}μm for both storage conditions of 5 and 25°C were 17.7 and 22.1% respectively. The

percentage of larger volume size of cell decreased to be 1.6 and 9.7% for 5 and 25°C at storage time of 9 d. In contrast to this, the percentage of small volume size of cell increased with time from 21.5 and 23.5% at day 1 to be 56.0 and 42.4% at days 9 for 5 and 25°C. Change in the percentage of volume size of cell with time for both storage conditions was thought to be related with weight loss i.e. water loss (Figure 7). Loss of water in cucumber fruits during storage period caused the volume size of cell becomes smaller.

Figure 5: Change in volume size of cell with time at 5 °C

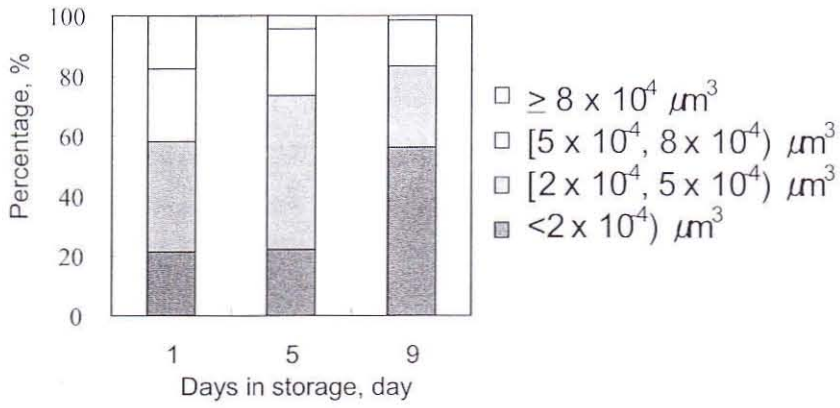


Figure 6: Change in volume size of cell with time at 25 °C

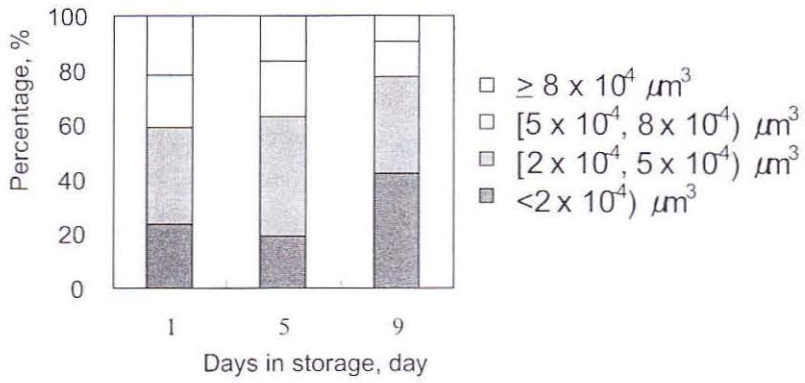
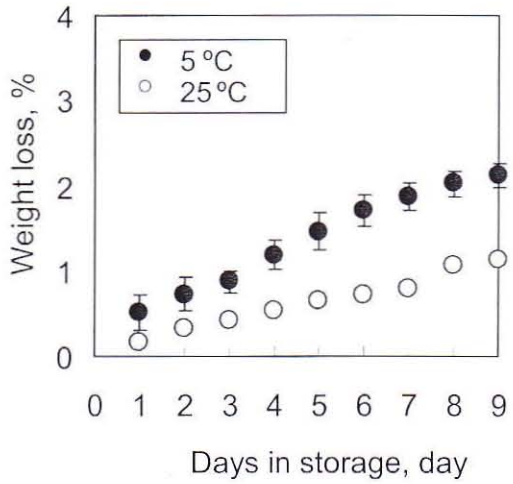


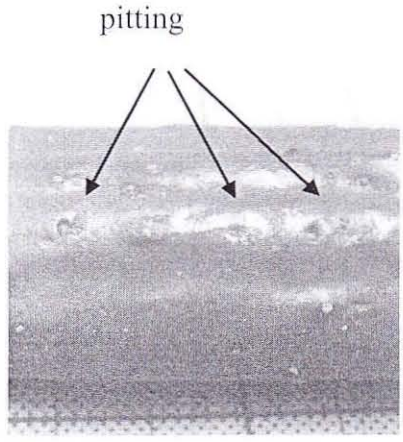
Figure 7: Weight loss at 5 and 25 °C



3.3. Pitting and Other Chilling Injury Symptoms

Pitting was observed clearly at 6 d in cucumber fruits stored at 5°C (Figure 8). However, no pitting was found in fruits stored at 25°C until period of storage although discoloration at the tip of fruits was observed in all samples. The weight loss after 9 d for cucumber fruits stored at 5 and 25°C was 1.5% and 2.5%, respectively. Similar result was obtained by Naruke *et al.*, (2003) for cucumber fruits stored at 2 and 15°C. The weight loss for both conditions was almost the same. Water loss, i.e. weight loss, has a close relationship to membrane water permeability. The relationship between the weight loss and the occurrence of pitting was explained by Morris and Platenius (1938). From the 3-D images, the effect of chilled temperature storage condition on the damage of cells was not clearly observed. However, higher percentage of small volume size of cell for fruits stored at low temperature might indicate the higher speed of damage in cell due to impact of chilling.

Figure 8: Pitting in fruits stored at 5°C



4. Summary and Concluding Remarks

3-D visualization of cell structure was demonstrated from a series of 100 cross-sectional 2-D images. The percentage of larger volume size of cell for both storage conditions decreased with time. In contrast to this, the percentage of small volume size of cell increased with time. Change in the percentage of volume size of cell with time for both storage conditions was thought to be related with weight loss i.e. water loss. From this study, the damage of cells due to the chilled temperature condition was not clearly observed in 3-D images during period of storage.

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References

[1] Allanson, L., Khatibi, S., Gustavsson, T., Blomstrand, F., Olsson, T. and Hansson, E. 1999. Single-cell volume estimation by three-domensional wide-field microscopy applied to astroglial primary cultures. *Journal of Neuroscience Methods*, 93, 1-11.

[2] Altamirano, J., Brodwick, MS., Alvarez-Leefmans, FJ. 1998. Regulatory volume decrease and intercellular Ca^{2+} in murine neuroblastama cells studied with fluorescent probes. *J Gen Physiol*, 112, 145-160.

[3] Do, G.S., Y. Sagara, M. Tabata, K. Kudoh, and T. Higuchi. 2004. Three-dimensional measurement of ice crystals in frozen beef with a micro-slicer image processing system. International Journal of Refrigeration 27 (2004) 184–190.

[4] Kimelberg, HK., O'Connor, ER., Sankar, P. and Keese, C. Methods for determination of cell volume in tissue culture. *Can J Physiol Pharm*, 70, S323-333.

[5] Lyons, J.M., 1973: Chilling injury in plants. *Ann. Rev. Plant. Physiol.*, 24, 445-466.

[6] Morris, L.L. and Platenius H., 1938: Low temperature injury to certain vegetables after harvest. *Proc. Amer.Soc. Hort.Sci.*, **36**, 609-613.

[7] Naruke, T., Oshita S., Kuroki S., Seo Y. and Kawagoe Y., 2003: T_1 relaxation time and other properties of cucumber in relation to chilling injury. *Acta Hort.*, **599**, 265-271.

[8] Parkin, K.L., Marangoni A., Jackman R.L., Yada, R.Y. and Stanley D.W., 1989: Chilling injury: a review of possible mechanism. *Journal of Food Biochemistry*, **13**, 127-153.

[9] Purwanto, Y.A., H. Tsuchiya, S. Oshita, Y. Kawagoe, Y, Makino. 2005. Determination of chilling injury index in cucumber fruits trhough proton NMR analysis. Proceeding of IC-RVEA, September 12-15, 2005, Kanazawa, Japan.

[10] Saltveit, M.E., 2000: Discovery of chilling injury. In: *Discoveries in Plant Biology* (eds Kung, S.D. and Yang S.F.). World Scientific Publishing, Singapore, pp. 423-448.

[11] Saltveit, M.E., 2002: The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. *Postharvest Biology and Technology*, **26**, 295-304.

[12] Ueno, S, G.S. Do, Y. Sagara, K. Kudoh and T. Higuchi. 2004. Three-dimensional measurement of ice crystals in frozen dilute solution. *International Journal of Refrigeration* **27**: 302-308.