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CELL MEMBRANE DAMAGE BY VACUUM TREATMENT AT DIFFERENT PRESSURE REDUCTION RATES

XIAOYONG SONG[1,2] and YUNFEI LI[2,3]

[1]Institute of Refrigeration & Cryogenic Engineering, Shanghai Jiao Tong University, 800 Dongchuan, Shanghai 200240, China
[2]Department of Food Science & Technology, Shanghai Jiao Tong University, 800 Dongchuan, Shanghai 200240, China

[3]Corresponding author. TEL: +86 21 34206918; FAX: +86 21 34206918; EMAIL: yfli@sjtu.edu.cn

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ABSTRACT

To investigate the damage of cell membrane induced by vacuum treatment at different pressure reduction rates, the onion epidermis was subjected to three different pressure reduction rates in a miniaturized, visual and vernier regulation experimental setup. The results of physical tests agreed well with the result of optical microscopy, which showed that treatment at the medium pressure reduction rate resulted in minimum changes in the spaces between the cell membrane and cell wall, water loss, relative electrical conductivity, color and micromechanical properties. Experimental results showed that the pressure reduction rate had obvious effects on the cell membrane integrity of vacuum-treated onion epidermis.

PRACTICAL APPLICATIONS

This work tested the effect extent of different pressure rates on the cell membrane integrity during vacuum treatment, which has been demonstrated to provide many benefits to the food processing industry. This research could help the users to choose the appropriate operating parameters, to predict the characteristics of vacuum cooled products, to understand the mechanism of vacuum treatment and to learn the design of vacuum cooling equipment.

INTRODUCTION

Vacuum treatment is an effective method extensively used for cooling some agricultural and food products (Rennie *et al.* 2000; Wang and Sun 2002a,b, He and Li 2003; Cheng 2006; Jin and Xu 2006; Sun and Wang 2006; Tao *et al.* 2006; Zhang and Sun 2006; Jackman *et al.* 2007, Ozturk and Ozturk 2008). The performance of vacuum treatment is related to the operating parameters of a vacuum cooling system, such as the pressure reduction rate. Only few reports are available on the effects of the rates of pressure reduction on the cooling process and physicochemical characteristics of samples (Self *et al.* 1990; McDonald and Sun 2001; Rennie *et al.* 2001a,b, Brosnan and Sun 2003). There are still many inconsistencies among the viewpoints on the effects of pressure reduction rate on vacuum treatment. In addition, the above-mentioned research mostly focused on some properties at the macroscopic level, such as mass loss, cooling time, temperature distribution, etc.

Under vacuum, the balances among plant cell interior turgor pressure, the cell membrane, the cell wall and the atmosphere are broken. Water in the vacuoles will evaporate and escape into the atmosphere through the cellular membrane system, which may increase cell osmotic pressure and damage the cell structure. However, there is insufficient experimental data available to be conclusive about whether different pressure reduction rates during vacuum treatment would lead to damage of tissue structure at the cellular level.

The main aim of this work was to evaluate the influence of the pressure reduction rates on the plasma membrane of the onion epidermis. Relative electrical conductivity (REC), weight change, color and micromechanical properties were studied, which are associated with the cell membrane integrity of many plant-based foods.

MATERIALS AND METHODS

Preparation of Sample

Fresh and healthy red onion (*Allium cepa* L.) bulbs were bought at a local market in Shanghai. The outer brown papery leaf bases and underlying outermost fleshy leaf bases were removed and the second scale below the surface was selected. The epidermis was cut into rectangular pieces with a surgical knife and was carefully removed with forceps from the equatorial region of the inner (concave) surface of the tissue. In mechanical testing, epidermal strips of approximate size 30×5 mm were cut in two different orientations: one orientation was parallel to the vascular bundles (longitudinal samples) and the other was perpendicular to the vascular bundles (transverse samples).

Onion bulb epidermal tissue was used as study object for the following reasons. It is easy to separate from other tissues and can be cut into pieces of size and shape needed in the experiment. It consists of a single layer of large-size cells that is easy to describe geometrically. Its cellular arrangement can be observed using conventional optical technique.

Experimental Setup

Figure 1 shows the experimental apparatus consisting of the miniature vacuum chamber on the stage of a microscope, vacuum pressure transducer, vacuum pumping system and data acquisition system.

The samples were observed using light microscopy (Leica DFC280, Heidelberg, Germany) during vacuum treatment processes under three different pressure reduction rates of 3.61, 1.68 and 0.95 mbar/s. Serial images were captured at 30-s intervals by a camera (Leica DM LB2) connected to the microscope. The images were processed and analyzed using an image processing software (Leica QWin, Cambridge, U.K.). A vacuum pressure transducer (NS-P-I1, TM, Shanghai, China) in the current output mode (4–20 mA) was connected to a data acquisition module (ADAM-4018, Advantech, Taiwan) and a computer. Ten samples were observed per pressure reduction rate.

Pressure Control

During the experiments, the pressure of the chamber was allowed to drop from the normal atmospheric pressure to the final value (6 mbar). The pressure reduction rate was modeled on the basis of an exponential decay function (Brosnan and Sun 2003):

$$p = p_i e^{-\beta t} \tag{1}$$

where p is the pressure in the chamber (mbar), p_i is the initial pressure (mbar), t is the time (s) at which the experiment began and β is the coefficient that characterizes the rate at which the pump reduces the pressure in the chamber (s^{-1}).

The values of β were 0.01860, 0.00867 and 0.00487 in this work, which correspond to the fast, medium and slow pressure reduction rates, respectively (Table 1). The pressure varied with the change in the β value. Once the pressure reached 6 mbar, the vacuum was broken and the sample was removed. Ten replicates were used for each treatment. The change in pressure over time for each β value is presented in Fig. 2.

Color Analysis

A color difference meter (Model WSC-S, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) was used

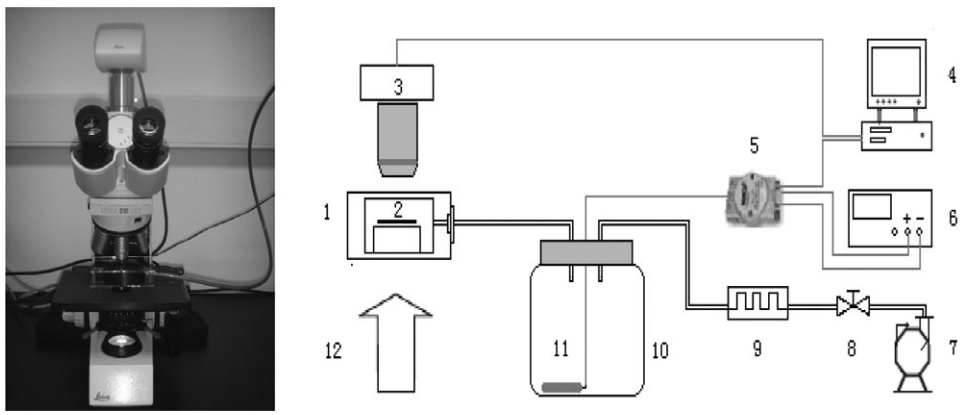

FIG. 1. MICROSCOPE SYSTEM (LEFT) AND A SCHEMATIC DIAGRAM OF THE VACUUM TREATMENT SYSTEM (RIGHT)
(1) Miniature vacuum chamber; (2) sample; (3) video camera; (4) data logging computer; (5) data acquisition module; (6) stabilized direct current supply (24 V); (7) vacuum pump; (8) high vacuum fine tuning valve; (9) condensing unit; (10) vacuum chamber; (11) vacuum pressure transducer; (12) constant light source.

TABLE 1. APPLIED PRESSURE REDUCTION RATES

Treatment	P_i (mbar)	P_f (mbar)	Time (s)	Pressure reduction rate (mbar/s)	β value (s^{-1})
Fast	1000	6	275	3.61	0.01860
Medium	1000	6	590	1.68	0.00867
Slow	1000	6	1050	0.95	0.00487

P_i, the initial pressure; P_f, the final pressure.

to measure the color change in the onion epidermis. The Commission Internationale de l'Eclairage (CIE) L^*, a^*, b^* color space coordinates were used to record color measurements. The color was presented in terms of L^*, a^* and b^* values, where L^* was 0 for black and 100 for white, a^* values indicated red (+) to green (−) and b^* values indicated yellow (+) to blue (−). In addition, chroma (c^*) and hue angle ($h°$) were calculated using the following equations (Harbourne *et al.* 2009).

$$h° = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$c^* = \left[(a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad (3)$$

Ten readings were obtained for each treatment.

Weight Change

The water loss of the samples was measured by drying samples in a forced-air oven at 80C for 5 h (Yu *et al.* 2002). Mass was registered before and after vacuum treatment by using an analytical balance.

REC

Electrolyte leakage was expressed by the relative leakage rate according to the method of Deng *et al.* (2005) with slight modification. Samples weighing c. 0.4 g were rinsed with deionized water, gently blotted with tissue paper to remove excess water and then immersed in 25 mL beakers with 20 mL deionized water for 2 h. Initial electrolyte leakages from onion slices were determined using a digital conductometer (FE30, Mettler Toledo, Columbus, OH). The samples were boiled for 30 min and cooled to 25C to assess the total electrolytes. The relative leakage was expressed as a percentage of the total electrolytes. Ten replicates were analyzed per treatment.

Mechanical Properties

The tensile strength tests were performed using a TA-XT2 analyzer (Stable Microsystems, Surrey, U.K.) according to ASTM D882-00 (ASTM 2000), with a test speed of 0.05 mm/s. The samples were clamped with the tensile grips with an initial distance of 20 mm between the two grips. The tensile stress (MPa) and strain at break (%) were obtained

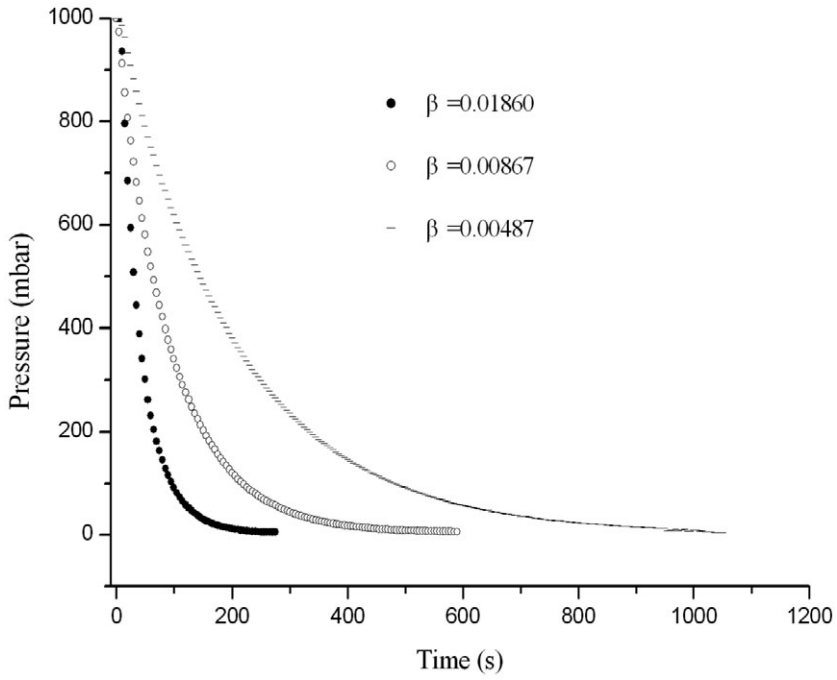

FIG. 2. PRESSURE VERSUS TIME PLOTS FOR THE THREE β VALUES USED IN THE EXPERIMENT

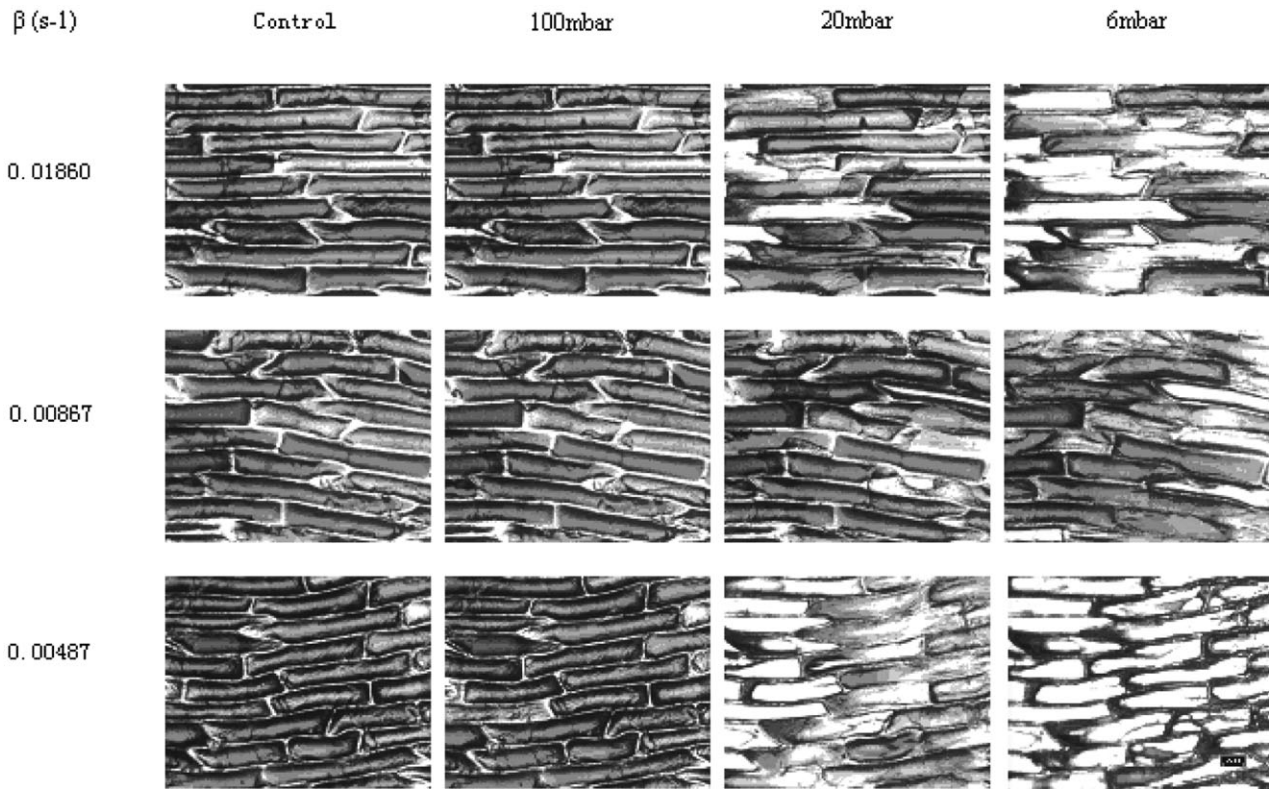

FIG. 3. RAW IMAGES OF ONION EPIDERMIS AT 1000 (CONTROL), 100, 20 AND 6 MBAR (SCALE BAR CORRESPONDS TO 500 μm)

from stress versus strain curves. Ten longitudinal and 10 transverse samples were used per pressure reduction rate.

Data Analysis

Analysis of variance was performed and the mean separation was determined by the least significant difference test ($P \leq 0.05$) using SPSS 13.0 program for windows (SPSS Inc., Chicago, IL)

RESULTS AND DISCUSSION

Raw Images

In Fig. 3, typical images of onion epidermis are presented under 1,000, 100, 20 and 6 mbar at three pressure reduction rates. At the 100 mbar level, they do not show obvious alterations compared with the fresh sample (control) under all pressure reduction rates. At 20 mbar, slight color changes of the onion epidermis were observed; the color of certain cells shifted from purplish red to white. Of the three rates, the least change was found at the moderate pressure reduction rate ($\beta = 0.00867$) and the most prominent change was observed at the slow pressure reduction rate ($\beta = 0.00487$). At 6 mbar, more individual cells shifted from purplish red to white. It was noted that the degree of destruction of the onion epidermal tissue with the slow pressure reduction rate was significantly ($P < 0.05$) different from that with the other two pressure reduction rates; most intracellular regions changed from purplish red to white with the slow pressure reduction rate, whereas with the other two rates, some intracellular regions remained red, especially for the moderate pressure reduction rate.

The color in the cells is largely determined by anthocyanin in the vacuole. Anthocyanin is an organic macromolecule and is not membrane permeable. In our preliminary tests, onion epidermis was selected to verify the membrane permeability of anthocyanin during osmotic dehydration. Results showed that water was drawn out of the cells, shrinking the vacuole and the cytoplasm. Plasmolysis happened and the red areas became much smaller, and anthocyanin accumulated and remained in the vacuoles. It was deduced that, in this study, the color shift possibly took place when the vacuolar and the cell membrane disruption happened. Vacuum treatments, with the continuous reduction of vacuum chamber pressure, cause the changes in spatial distribution of pressure gradients in the tissue. Onion epidermis has a monolayer structure in which the epidermal cells are directly exposed to vacuum condition, resulting in cell disruption and releasing turgor pressure. In addition, the microstresses due to water transfer

TABLE 2. EFFECTS OF PRESSURE REDUCTION RATES ON THE COLOR OF ONION EPIDERMIS

		After vacuum treatment		
Color	Before vacuum treatment	$\beta = 0.01860$	$\beta = 0.00867$	$\beta = 0.00487$
L^*	20.57 ± 1.3350^a	44.70 ± 0.0862^b	38.97 ± 0.0000^c	48.54 ± 0.0252^d
a^*	25.75 ± 1.0013^a	16.67 ± 0.2868^b	17.43 ± 0.0458^c	13.24 ± 0.3306^d
b^*	5.64 ± 0.2150^a	1.29 ± 0.1815^b	1.84 ± 0.1572^c	0.53 ± 0.0808^d
c^*	26.37 ± 0.9381^a	16.72 ± 0.2738^b	17.52 ± 0.0370^c	13.25 ± 0.3334^d
$h°$	12.38 ± 0.9019^a	4.42 ± 0.6854^b	6.03 ± 0.5211^c	2.27 ± 0.2922^d

Values are means of 10 replications ± SD. Significant differences (at $P < 0.05$) within the same row are shown by different letters.

are one of the main reasons leading to cell rupture (Mayor *et al.* 2008). The present results showed that the cell membrane integrity of onion epidermis was affected significantly ($P < 0.05$) by different pressure reduction rates during vacuum treatments.

Color Analysis

Color changes in onion epidermis under the three pressure reduction rates are listed in Table 2. The samples were lighter after vacuum treatment than before vacuum treatment (i.e., lower L^* value). The fresh onion epidermis had a higher c^* value (26.37), and the samples after treatment had lower c^* values, which were 16.72, 17.52 and 13.25 for the fast, medium and slow pressure reduction rates, respectively. Chroma is a measure of the purity or saturation of the color; therefore, low chroma would be desirable compared with the optical microscope photomicrographs of onion epidermis surface morphology (Fig. 4). Table 2 also shows that the color values for a^*, b^* and $h°$ were greatly decreased compared with the fresh samples. In addition, among the three pressure reductions rates, the moderate rate led to the highest a^* and c^* values and the lowest L^*, b^* and $h°$ values of the vacuum-treated samples. On the contrary, the slow rate resulted in the lowest a^* and c^* values and the highest L^*, b^* and $h°$ values. This suggested that the pressure reduction rate had a significant effect ($P < 0.05$) on the color of the onion epidermis. The maximum variation was observed at the slow pressure reduction rate and a minimal change was found at the moderate pressure reduction rate.

Color intensity is positively correlated with anthocyanin concentration (Nørbæk *et al.* 1998). In this work, the cell membrane system may be damaged and resulted in color shrifts. The results revealed that the cell membrane integrity of the onion epidermis was affected by vacuum treatment, and the degree of the effect depended on the pressure reduction rate. Of the three pressure reduction rates, both the slow and fast vacuum treatment led to prominent destruction,

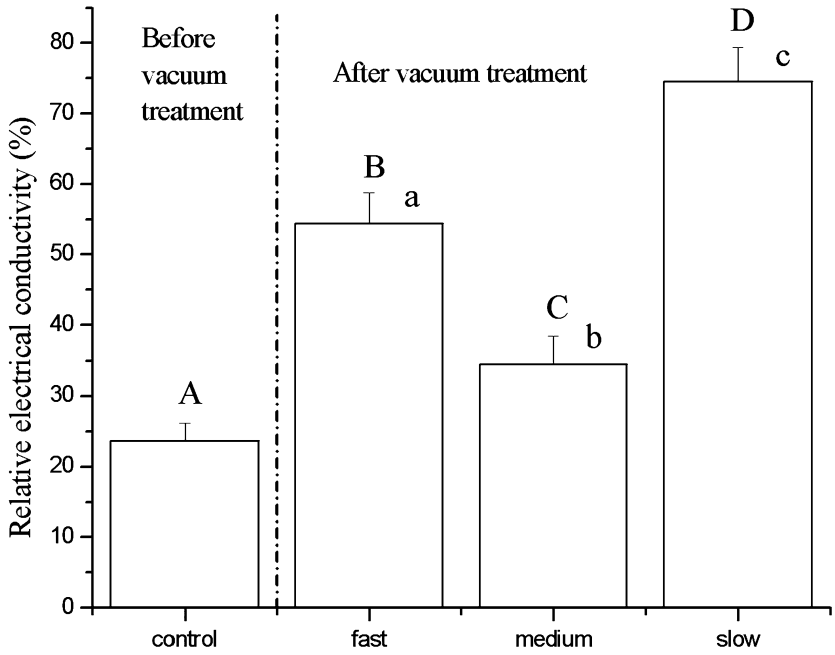

FIG. 4. EFFECT OF THE PRESSURE REDUCTION RATES ON THE RELATIVE ELECTRICAL CONDUCTIVITY (%) OF ONION EPIDERMIS. THE FAST, MEDIUM, AND SLOW PRESSURE REDUCTION Rates Corresponded To β VALUES OF 0.01860, 0.00867, AND 0.00487, RESPECTIVELY. FOR EACH PRESSURE REDUCTION RATE, VALUES FOLLOWED BY DIFFERENT LETTERS (A–D) and (a–c) INDICATE SIGNIFICANT DIFFERENCES ($P < 0.05$, $n = 10$)

TABLE 3. EFFECT OF PRESSURE REDUCTION RATE ON THE SPACES BETWEEN THE CELL MEMBRANE AND CELL WALL

	Changes in the spaces between the cell membrane and cell wall (%)			
β value (s^{-1})	Control*	100 mbar	20 mbar	6 mbar
0.01860	100	97.76 ± 2.1425[a]	50.37 ± 4.1987[a]	40.46 ± 3.8354[a]
0.00867	100	95.64 ± 2.5510[a]	55.87 ± 5.5124[b]	54.11 ± 4.8875[b]
0.00487	100	98.23 ± 2.1002[a]	32.23 ± 6.3107[c]	14.57 ± 5.5268[c]

* Spaces between the cell membrane and the cell wall before vacuum treatment (taken as 100%). Values are means of 10 replications ± SD. Significant differences (at $P < 0.05$) within the same column are shown by different letters.

while the medium treatment caused less damage. It can be assumed that treatment with the moderate pressure reduction rate yields better results.

Spaces between the Cell Membrane and Cell Wall

Table 3 shows the effects of the pressure reduction rates on the spaces between the cell membrane and the cell wall, which were analyzed using an image processing software (Leica QWin). Ten samples were used per pressure reduction rate. The spaces decreased slightly when the pressure decreased from the standard atmosphere pressure (control) to 100 mbar, and there were no significant differences ($P > 0.05$) among the different pressure reduction rates. The space decreases were very significant when the pressure decreased from 100 mbar to 20 mbar. For the three β values 0.01860, 0.00867 and 0.00487, the spaces decreased from 97.35, 94.63 and 98.11% to 51.52, 56.32 and 31.66% ($P < 0.05$), respectively. At 6 mbar, the spaces induced by the three pressure reduction rates reached their final values, which were 39.95, 54.54 and 12.26% ($P < 0.05$), respectively.

When the pressure in the vacuum chamber was reduced through a vacuum pump, the difference between the surrounding atmosphere and the turgor pressure in the sample placed in the chamber gradually increased, which led to an increase in the force acting on the plasma membrane. Consequently, the driving force of the turgor pressure gradient induced cell swelling, which resulted in a decrease in the spaces between the cell membrane and the cell wall. The current results indicated that the degree of decrease in the spaces between the cell membrane and the cell wall depended on the pressure reduction rate; the most significant change was observed at the slow pressure reduction rate and the least change was found at the moderate pressure reduction rate.

Weight Change

It can be seen that the rates of pressure reduction had remarkable effects ($P < 0.05$) on water loss of the treated onion epidermis (Table 4).

For the most slow vacuum treatment ($\beta = 0.00487$), the greatest mass and water loss were encountered, with values of 81.47 and 97.90%, respectively. The minimums of mass and water loss were found under the pressure reduction rate with the β value of 0.00867, which were approximately 16.65 and 20.53%, respectively. Compared with the fast and slow pressure reduction rates, mass and water loss under the medium pressure reduction rate decreased obviously. This large reduction in mass and water loss may be of benefit in maintaining greater freshness and quality. The results showed that the mass loss can be controlled by modulating the vacuum.

The major disadvantage of vacuum cooling is the loss of weight due to moisture removal, which can be now explained by the cell rupture in this study. Water diffusion within the tissue became easier when the cytoplasm and vacuole membranes were broken down.

REC

Figure 4 shows the variations in the REC under different pressure reduction rates. The REC differed significantly ($P < 0.05$) before and after the vacuum treatment. For example, the initial REC value of onion epidermis was 23.67%; after vacuum treatment, the REC values at fast, moderate and slow pressure reduction rates were 54.47, 32.45 and

TABLE 4. EFFECTS OF PRESSURE REDUCTION RATES ON THE WEIGHT CHANGES OF ONION EPIDERMIS

β-value (s^{-1})	W_0 (10^{-3} g)	W_v (10^{-3} g)	W_{vb} (10^{-3} g)	ΔW_m (%)	ΔW_w (%)
0.01860	124.13 ± 2.442[a]	56.09 ± 6.167[a]	22.16 ± 3.134[a]	54.86 ± 4.162[a]	66.74 ± 4.194[a]
0.00867	127.83 ± 2.120[a]	106.53 ± 0.642[b]	24.13 ± 1.253[a]	16.65 ± 1.631[b]	20.53 ± 2.182[b]
0.00487	125.30 ± 0.700[a]	23.23 ± 2.334[c]	21.04 ± 2.060[a]	81.47 ± 1.772[c]	97.90 ± 0.956[c]

W_0, original mass of sample; W_v, mass of sample after vacuum treatment; W_{vb}, mass of sample after vacuum treatment followed by baking in a hot air oven at 80C for 5 h; $\Delta W_m = (W_0 - W_v) \times 100\%/W_0$, percentage of mass loss of sample; $\Delta W_w = (W_0 - W_v) \times 100\%/(W_0 - W_{vb})$, percentage of mass loss of sample. Values are means of 10 replications ± SD. Significant differences (at $P < 0.05$) within the same column are shown by different letters. ($n = 10$).

TABLE 5. EFFECT OF PRESSURE REDUCTION RATES ON THE MICROMECHANICAL PROPERTIES OF ONION EPIDERMIS

			After vacuum treatment		
Variable	Orientation	Before vacuum treatment	$\beta = 0.01860$	$\beta = 0.00867$	$\beta = 0.00487$
Stress (M Pa)	Longitudinal	2.490 ± 0.1890[a]	1.870 ± 0.2447[b]	2.353 ± 0.1481[a]	2.291 ± 0.1354[a]
	Transverse	1.725 ± 0.2694[a]	0.856 ± 0.0821[b]	1.547 ± 1.5470[a]	1.467 ± 0.3427[a]
Strain (%)	Longitudinal	26.278 ± 0.2517[a]	7.378 ± 0.4042[b]	2.271 ± 0.2918[c]	2.412 ± 0.1528[cd]
	Transverse	27.345 ± 2.3000[a]	11.944 ± 1.1540[b]	4.359 ± 0.2254[c]	4.578 ± 0.4509[cd]

Values are means of 10 replications ± SD. Significant differences (at $P < 0.05$) within the same row are shown by different letters. The thickness of samples is 0.10 ± 0.00 mm. ($n = 10$).

68.54%, respectively. This suggested that the cellular membrane system was seriously damaged. In addition, the degree of destruction of the onion epidermis structure was associated with the pressure reduction rate. Of the three pressure reduction rates, both the slow ($\beta = 0.00487$) and the fast pressure reduction rates ($\beta = 0.01860$) led to severe damage; the damage was especially severe at the slow pressure reduction rate, while the moderate pressure reduction rate ($\beta = 0.00867$) resulted in slight damage.

Change in electrolyte leakage is an important indicator of plasma membrane integrity of cells (Deng *et al.* 2005). Disruption and damage to cell membranes altered permeability, resulting in a loss of electrolytes. This conductivity behavior could well reflect the color changes (Fig. 3) during vacuum treatment.

Mechanical Properties

The mechanical property is an important quality attribute of many fruits and vegetables (Vanstreels *et al.* 2005). Changes in the mechanical properties of the samples treated with different pressure reduction rates are shown in Table 5. The stress in either orientation at the fast pressure reduction rate was smaller after vacuum treatment than before vacuum treatment ($P < 0.05$); however, no significant differences were observed before and after vacuum treatment for the medium and slow pressure reduction rates ($P > 0.05$). As for strain in both longitudinal and transverse orientations, significant differences were observed before and after vacuum treatment with the three different pressure reduction rates ($P < 0.05$).

The relationship between the mechanical properties and the cell structural parameters (cell membrane, cell wall and turgor pressure) has been investigated by a number of workers (Zhu and Melrose 2003; Vanstreels *et al.* 2005; Oey *et al.* 2007). However, no reports are available on the effect of pressure reduction rates on the micromechanical properties of vacuum-treated onion epidermal tissue. The current study showed that of the three pressure reduction rates, both stress and strain in either orientation at the fast pressure reduction rate differed remarkably from those at the other two pressure reduction rates ($P < 0.05$), and no distinct differences were observed between the medium and slow vacuum treatments. It can be seen from Table 5 that the mechanical behavior of onion epidermis was affected by different pressure reduction rates during vacuum treatment.

CONCLUSIONS

Changing the pressure reduction rate had obvious effects on the surface morphology, spaces between the cell membrane and cell wall, mass, REC, color and mechanical properties. All of these indices could basically reflect the damage to cellular membrane. Of the three pressure reduction rates, treatments at the slow and fast pressure reduction rates led to remarkable effects on the cell membrane integrity of the onion epidermis, while treatment at the medium pressure reduction rate caused less damage. It can be assumed that treatment with the moderate pressure reduction rate resulted in relatively good quality.

Onion epidermis has a monolayer structure in which the epidermal cells are directly exposed to the vacuum condition. Different rates of pressure reduction resulted in changes in the rate of water evaporation and distribution of pressure in the tissues. Consequently, the microstress acting on the tissue structure varied due to water transfer and pressure gradients, resulting in different degrees of cell membrane damage. For an in-depth understanding of the behaviors of tissues at different pressure reduction rates, a more detailed micromechanical model is needed in our future work.

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