Available online at www.sciencedirect.com
SCIENCE DIRECT®
Postharvest Biology and Technology 33 (2004) 263–273
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Effects of pressure reduction rate on quality and ultrastructure of iceberg lettuce after vacuum cooling and storage

S.Y. He [a], G.P. Feng [b], H.S. Yang [a], Y. Wu [b], Y.F. Li [b,*]

[a] *School of Power and Mechanical Engineering, Shanghai Jiao Tong University, Shanghai 200030, China*
[b] *Department of Food Science and Engineering, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai Qi Xin Road 2678, Shanghai 201101, China*

Received 6 October 2003; accepted 9 March 2004

Abstract

A study was conducted to determine if the pressure reduction rate in a vacuum cooler would have an effect on the physical and chemical quality characteristics as well as the ultrastructure of iceberg lettuce after cooling and storage. Three different pressure reduction rates were taken to cool iceberg lettuce in a vacuum cooler. Subsequently, vacuum cooled lettuce were stored at 1 °C and 85% relative humidity (RH) for 2 weeks. The changes of mass, firmness, ascorbic acid, chlorophyll, catalase, and ultrastructure were measured throughout the storage period to decide the quality variation induced by different pressure reduction rates. The results of physical and chemical tests agreed well with the result of transmission electron microscopy (TEM), which showed that the moderate pressure reduction rate achieved the maximum values of tissue firmness, ascorbic acid and catalase. Membrane systems observed by TEM under the moderate pressure reduction rate were kept intact compared to the other two pressure reduction rates. The moderate pressure reduction rate achieved the best quality and shelf-life of iceberg lettuce.

Keywords: Vacuum cooling; Pressure reduction rate; Ultrastructure; Microscopy

1. Introduction

Vacuum cooling is one of the most effective methods to remove the field-heat from fresh fruit and vegetables. The theoretical studies about vacuum cooling involved in liquid/solid mixtures, liquids, cooked meat and spherical solid foods were developed by Burfoot et al. (1989), Houska et al. (1996), Dostal et al. (1999), Wang and Sun (2002a,b), and He and Li (2003), respectively. The effect of vacuum cooling on extending the shelf-life of produce has been shown by DeEll and Vigneault (2000), Burton et al. (1987), Martinez and Artes (1999), and Turk and Celik (1993, 1994). Moreover, studies about vacuum cooling of iceberg lettuce have been described elsewhere (Haas and Gur, 1987; Rennie et al., 2000).

The effect of different pressure reduction rates on the cooling process was examined by Rennie et al. (2001a), whose conclusion was that changing the pressure reduction rate had no significant effect on mass loss, temperature reduction per percent mass loss, or temperature differences between the various locations.

* Corresponding author. Tel.: +86-21-64783085; fax: +86-21-64783085.
E-mail address: yfli@mail.sjtu.edu.cn (Y.F. Li).

0925-5214/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2004.03.006

Structural heterogeneity of biological materials, such as vegetables, fruit and meat, brings an additional complexity to the migration of water in a porous solid system. Mc Donald and Sun (2001) conducted experiments to determine the effect of evacuation rate on the vacuum cooling process of a cooked beef product. Results showed that final evacuation rate had a significant effect on chilling loss and total product yield, with a slower evacuation rate increasing total product yield and decreasing chilling loss. In addition, results indicated that the impact of pressure regulation on quality attributes, such as texture, colour and shelf-life in cooked meats needs to being investigated further. The suggestion is consistent with what James et al. (1989) and Bailey (1993) proposed: "rapid rates of cooling can be achieved after pressure-cooking by applying a vacuum to the vessel. In delicate products the rate at which the pressure is released and the vacuum applied can affect the textural qualities of the food. As the pressure is released, boiling can occur within the foodstuff and the resulting expansion would cause rupture within the tissue".

Although it is known that pressure reduction rates of vacuum cooling affect the quality of products, there has been little work on quality and shelf-life of iceberg lettuce induced by different pressure reduction rates during vacuum cooling. Rennie et al. (2001b) conducted a study to determine if changing the rate of pressure reduction in a vacuum cooler would have an effect on the quality of the lettuce after cooling and during storage at 1 °C and 85% RH. The study focused on product appearance, including such quality parameters as mass loss, visual quality and chlorophyll fluorescence, and drew a conclusion that the pressure reduction rate had no advantage/disadvantage on overall quality. In essence, consumers pay more attention to nutritional value and safety (Paull, 1999). Furthermore, ultrastructural investigations dealing with postharvest quality of fruit and vegetables after processing and storage are scarce (Nieto et al., 2001). Therefore, the techniques of light microscopy and TEM, which are widely used in studying plants under various stresses, such as excess of heavy metals (Molas, 2002), acid rain (Gabara et al., 2003), salt (Pareek et al., 1997) and drought (Farrant et al., 1999; Popova, 1998), hyperhydrocity (Olmos and Hellin, 1998), low temperature (Stefanowska et al., 1999; Aldesuquy et al., 2000) are adopted in the present paper. The aim of the current study is to further explore the effects of pressure reduction rate on physical and chemical quality characteristics as well as ultrastructure of iceberg lettuce induced by vacuum cooling and cold storage.

2. Materials and methods

2.1. Plant material

Iceberg lettuce (*Lactuca sativa L.*) were harvested on 14 April 2003 and vacuum cooled 2 h later. The outer-most leaves were removed and the lettuce were weighed.

2.2. Experimental set up

Tests were performed using a laboratory-scale vacuum cooler (Shanghai PuDong Freezing Dryer Instruments Co., Ltd.), equipped with a rotary vane vacuum pump (model 2XZ-2), and pumping speed of $2\,l\,s^{-1}$, rotary speed 1400 rev min^{-1}, and power 0.37 KW. The vacuum volume was approximately 0.2 m^3. The sides and bottom were equipped with cooling coils. The coils were subjected to natural convection.

The vacuum cooler was instrumented with temperature sensors and a pressure sensor. Eight type-T thermocouples were used for temperature measurements. The type of pressure sensor was CPCA-130Z (Shanghai Zhentai Instruments Co., LTD), a capacitance membrane gauge with a measurement range 10 KPa and known for high accuracy and rapid response.

The data collection and control of signals, such as pressure and temperature were conducted by I-7000, a family of network data acquisition and control modules. The control module was connected with a software called "King of Combination" (Beijing Asia Control Automatic Software Co., Ltd.). In order to eliminate the error of the second conversion, the temperatures received by the PC were demarcated by a second scale standard mercury thermometer with a measurement range 0–50 °C.

2.2.1. Temperature measurements

Twenty iceberg lettuce (each about 0.5 kg) were cooled in each treatment in the vacuum cooler. Centre

temperature and surface temperature of lettuce were recorded based on eight iceberg lettuce. Each iceberg lettuce was instrumented with two type-T thermocouples. One type-T thermocouple was installed at the centre of the iceberg lettuce and the other was underneath the first leaf of lettuce. The temperature data at each iceberg lettuce were averaged for every 10 s to reduce variation and noise effects before analysis of the temperature distribution.

2.2.2. Pressure regulation

During the experiments, the pressure of the cooler was allowed to drop to an initial working pressure ($P_i = 10000$ Pa) at an evacuation speed of 7.2 $m^3\,h^{-1}$. Upon reaching the initial pressure, the chamber pressure was carefully regulated using a calibrated air bleed valve to simulate different rates of pressure decrease until the chamber pressure reached its final value ($P_f = 600$ Pa). The pressure was controlled to fluctuate around 600 Pa by using an air leak through a small tube. The air leak was modified by the use of a solenoid valve and a bleed valve. The diameter of the valve openings ranged from 1 to 5 mm. The pressure sensor CPCA-130Z supplied the operating pressure to the data acquisition system, which determined whether or not the leak should be opened and to what extent. The process continued until the centre temperature of the iceberg lettuce almost reached 1 °C. Once this value was obtained, the vacuum was broken and the iceberg lettuce removed.

The pressure reduction rate was modeled on an exponential decay function in the form of Eq. (1).

$$p = p_i e^{-Yt} \quad (1)$$

where p is the pressure (Pa), p_i the initial pressure (Pa), t the starting time (min) when the control began Y the process variables

Three pressure reduction rates were identified in Table 1. Three replicates were used for each treatment.

Table 1
Applied pressure reduction rates

Treatment number	Time (10,000–600 Pa) (min)	Pi (Pa)	Y-value (min^{-1})
1	15	10000	0.18756
2	30	10000	0.09378
3	60	10000	0.04689

2.3. Firmness measurement

The texture instrument TA—XT2 (Stable Micro Systems, UK) was used to determine firmness of the iceberg lettuce. The cylinder probe was 5 mm (P/5) and the load cell was 5 kg. The insertion depth was 10 mm. When starting the test, the downward speed of the probe was 1.0 mm s^{-1}. The functions of force, distance, and time were recorded automatically, and data acquisition rate was 500 pps. Test results obtained from 20 iceberg lettuce (of the same treatment) gave the typical mean maximum peak force values, while the force after the bioyield point was an indication of the firmness of the underlying lettuce.

2.4. Chlorophyll ($a + b$) measurement

One gram of fresh leaves was homogenized in 5 ml of 80% acetone and centrifuged at 10000 × g for 15 min in the centrifuge (Heraeus, Kendro Laboratory Products GmbH, D-63405 Hanau/Germany). The transparent supernatant was then filtered and brought to 15 ml with 80% acetone. Absorbance was measured at 652 nm using a 756MC spectrophotometer (Leng Guang, Shanghai Precision & Scientific Instrument Co., Ltd., China). The chlorophyll concentration was calculated according to Han (1992).

2.5. Ascorbic acid and catalase measurement

The iodine method was used to measure the catalase in lettuce leaves (Han, 1992). Decay of H_2O_2 is proportional to catalase activity in the original sample. When the reaction between catalase and H_2O_2 is over, the unliberated H_2O_2 is tested by the iodine method. The principle is that H_2O_2 is incubated with KI aided by the catalyzer of $(NH_4)_6Mo_7O_4{\cdot}4H_2O$, and liberates I_2, which is then titrated with $Na_2S_2O_3$. According to the difference between the control and tested value, the quantity of hydrogen peroxide liberated by catalase can be calculated. Measurement of ascorbic acid by direct titration with 2,6-dichlorophenolindophenol dye (Marck KGaA, 64271 Darmstadt, Germany) was used in this study because it is simple and rapid (Huang, 1989; Albrecht and Schafer, 1990). Ascorbic acid from a test sample was extracted by *meta*-phosphoric acid–acetic acid solution, followed by titration with 2,6-dichlorophenolindophenol dyestuff until a salmon

pink color was obtained. The extracting solution used was 3% *meta*-phosphoric acid in 0.01 N H_2SO_4, and the preparation of dyestuff solution was based on an official AOAC (1980) method.

2.6. Ultrastructure evaluation

For transmission electron microscopy (TEM), samples of the outer and inner leaves from the middle part were hand-cut (approximately 4 mm^2) and fixed in 3% glutaraldehyde solution buffered in 0.05 M sodium cocadylate (pH, 6.9) for 8 h at room temperature. The samples were postfixed for 1 h in 1% OsO_4, similarly buffered, dehydrated in graded ethanol and embedded in Epon 812 epoxy resin (Spurr, 1969). Ultrothin (about 80 nm) sections were cut on a LKB-Nova ultracut microtome (LKB Co., Sweden), stained with uranyl acetate and lead citrate (Reynolds, 1963) and viewed using a JEM-100cx II(JEOL LTD., Japan). Photographs were taken of at least three random sites in three different sections and representative pictures were presented.

The use of microscopy interfaced with photography similarly could provide information at the microstructural level. Microscopic techniques should be more widely utilized in texture evaluation (Jackman and Stanley, 1995). For light microscopy (LM), half-thin sections (1–2 μm thick) of the Spurr-embedded tissue were cut on the LKB-Nova ultracut microtome and stained with dye. Sodium dihydrogen phosphate (0.5 g), basic fuchsin (0.25 g) and methylene blue (0.2 g) were dissolved in 15 ml 0.5% boric acid solution, and then 70 ml distilled water was added and the pH value of the dye solution was adjusted to 6.8. When staining, the original solution needed to be diluted 1:1 by distilled water, and tissue was observed by a light microscope.

3. Results and discussion

The changes of mass, firmness, ascorbic acid, chlorophyll, catalase, and ultrastructure were measured throughout the storage period to decide the quality variation induced by different pressure reduction rates. Table 2 summarizes the data on physical and chemical evaluations (intermediate data not shown) as well as the TEM observations of lettuce after vacuum cooling at different pressure reduction rates and subsequent 2 weeks cold storage. The Statistical Analysis System computer package (SAS Institute, Inc., 1987) was used for analysis of the data in these experiments.

3.1. Physical and chemical quality

3.1.1. Firmness

As one of the textural traits, firmness is very important in determining consumer acceptability (Sams, 1999). The plateau of force after the bioyield point was an indication of lettuce firmness. The peak force

Table 2
Chemical and physical properties and results of TEM and LM of lettuce before and after vacuum cooling and storage

Treatment number and time	Postharvest testing (14 April 2003)	After vacuum cooling and cold storage for 2 weeks (28 April 2003)			
		No. 1	No. 2	No. 3	No. 4
Ascorbic acid (mg 100 g^{-1})[a]	3.556	3.229b[c]	3.410a	3.140c	2.900d
Chlorophyll (mg 100 g^{-1})	0.165	0.057a	0.045a	0.055a	0.060a
CAT activity[b]	1.596	1.546b	1.684a	1.669a	1.688a
Mass loss (%)		3.529a	3.614a	3.189b	0.381c
Firmness (*N*)	51.349	44.139ab	46.060a	45.090a	42.484b
TEM image (degree of damage)	Control	Severe	Light	Severe	
Results of LM (cell wall)	Intact	Intact	Intact	Intact	

[a] The results of chemical quality parameters (ascorbic acid, chlorophyll, catalase activity) were the average value of 10 individual lettuce replicates of the same treatment; the results of mass loss and firmness were 20 individual lettuce replicates of the same treatment.

[b] Catalase activity in mg hydrogen peroxide liberated g^{-1} sample (lettuce) per minute.

[c] Mean separation in rows (within three different pressure reduction rates and the situation (No. 4) of direct cold storage) by Duncan's multiple range test, $P = 0.05$.

of the lettuce tested was 51.349 N. After 2 weeks of cold storage at 1 °C and 85% relative humidity (RH), the peak force under treatment 1 (achieving the pressure reduction within 15 min) was 44.139 N, the peak force under treatment 2 (achieving the pressure reduction within 30 min) was 46.060 N, and the peak force under treatment 3 (achieving the pressure reduction within 1 h) was 45.648 N, while the tissue firmness value of treatment 4 (direct cold storage without vacuum precooling) was the lowest, 42.484 N. Results revealed that the firmness of lettuce reduced with storage time and that vacuum cooling aids in maintaining the firmness of lettuce. Comparison of peak forces under different treatments showed that treatment 2 was the best in maintaining lettuce firmness.

3.1.2. Mass loss

After vacuum cooling and 2 weeks of cold storage, the mass loss of lettuce from the four treatments was 3.529, 3.614, 3.189 and 0.381%, respectively. Although the differences between mass loss in treatments 1 and 2 were small, the overall trend was that the faster the pressure reduction, the greater the mass loss. The result was consistent with the conclusion of Mc Donald and Sun (2001).

3.1.3. Ascorbic acid

The ascorbic acid content of the lettuce was $3.556\,\text{mg} \times 100\,\text{g}^{-1}$. After vacuum cooling and 2 weeks of cold storage, the ascorbic acid content of lettuce treated by the three pressure reduction rates were $3.229\,\text{mg}\,100\,\text{g}^{-1}$, $3.410\,\text{mg}\,100\,\text{g}^{-1}$, $3.140\,\text{mg}\,100\,\text{g}^{-1}$, respectively. Accordingly, the ascorbic acid of treatment four was $2.900\,\text{mg}\,100\,\text{g}^{-1}$. The results indicate a positive effect of vacuum cooling on the ascorbic acid content of lettuce, and that treatment two maintained greater ascorbic acid content of the lettuce.

3.1.4. Chlorophyll

The chlorophyll content of the lettuce was $0.165\,\text{mg}\,100\,\text{g}^{-1}$. After vacuum cooling and 2 weeks of cold storage, the chlorophyll content of lettuce treated by the three pressure reduction rates were $0.057\,\text{mg}\,100\,\text{g}^{-1}$, $0.045\,\text{mg}\,100\,\text{g}^{-1}$ and $0.055\,\text{mg}\,100\,\text{g}^{-1}$, respectively. The chlorophyll of lettuce from treatment 4 was $0.060\,\text{mg}\,100\,\text{g}^{-1}$. After 2 weeks of cold storage, the chlorophyll content under all treatments declined very quickly, and the mean loss of chlorophyll was approximately 66% of the initial value. The loss of chlorophyll might be incurred by low temperature and weak light during storage. The detailed mechanism of chlorophyll degradation in fruit and vegetables has been discussed by Heaton and Marangoni (1996).

3.1.5. Catalase

Catalase, as an antioxidant, can minimize free radical damage (Sherwin and Farrant, 1998) to ensure tissue survival from stress. The catalase activity of lettuce was $1.596\,\text{mg}\,\text{g}^{-1}\,\text{min}^{-1}$. After vacuum cooling and 2 weeks of cold storage, the catalase activity values of lettuce induced by the three treatments were 1.546, 1.684 and $1.669\,\text{mg}\,\text{g}^{-1}\,\text{min}^{-1}$, respectively. The catalase activity of lettuce from treatment 4 was $1.688\,\text{mg}\,\text{g}^{-1}\,\text{min}^{-1}$. Catalase activity of lettuce increased in all but treatment 1 after vacuum cooling and cold storage. However, the impact of processing on the antioxidant capacity of fruits and vegetables seems to be a neglected area and little information is available (Kaur and Kapoor, 2001).

3.2. Morphological and structural characteristics

Light microscopy and TEM images of lettuce from all treatments were carried out after vacuum cooling and after 2 weeks of cold storage. Light microscopy observations revealed that the morphology of lettuce tissue under all treatments, remained intact. For example, tissue from treatment 1 and subsequent 2 weeks storage showed intact cell walls, although cell separation and formation of large intercellular spaces occurred (Fig. 1A, arrowheads). Fresh lettuce (control, Fig. 1B) had cells that were full and arranged closely. Fig. 1C also showed no destruction of cells walls under treatment 3, in which pressure reduced within 1 h during vacuum cooling.

In order to clarify the effect of pressure reduction rate on the ultrastructure of iceberg lettuce tissue, many experimental observations using TEM were performed. The results of TEM after vacuum cooling showed that the cooling process led to variation of ultrastructure, such as plasmolysis, irregular membrane structure, discontinuity of plasmalemma and tonoplast, compared with the control (Fig. 2A), which showed many membrane structures and clear

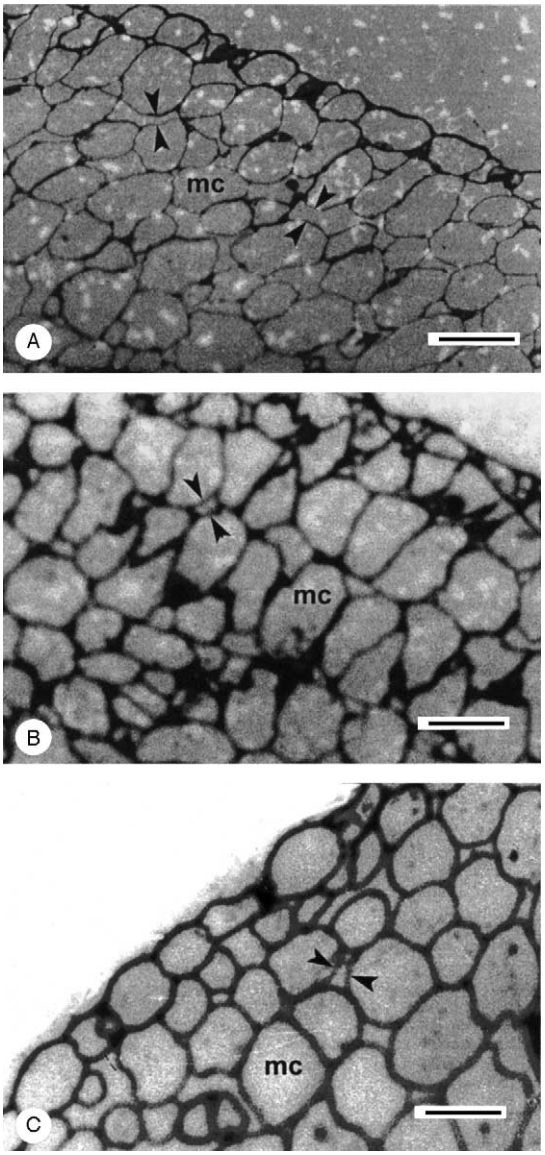

Fig. 1. Light microscope micrographs of leaf tissue. Bar = 100 μm (A, B, C). Abbreviation: mc, mesophyll cell: (A) outer leaf tissue treated by vacuum cooling with pressure reduction within 15 min and after cold storage at 1 °C and 85% RH for 2 weeks; (B) inner leaf tissue of fresh lettuce (control); (C) outer leaf tissue treated by vacuum cooling with pressure reduction within 1 h.

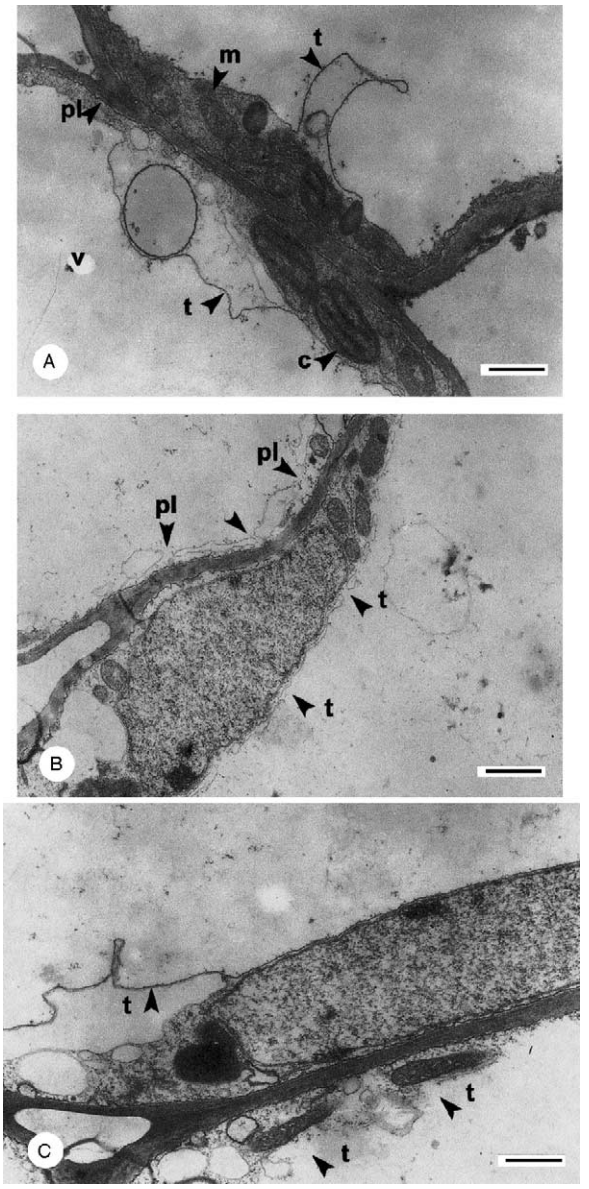

Fig. 2. Ultrastructure (TEM) of leaf tissue affected by vacuum cooling. Bar = 1 μm (A, B, C). Abbreviations: m, mitochondria; t, tonoplast; c, chloroplast; v, vacuole; pl, plasmalemma; (A) fresh inner leaf tissue (Control); (B) inner leaf tissue as treated by vacuum cooling with pressure reduction within 1 h; (C) inner leaf tissue treated by vacuum cooling with pressure reduction within 30 min.

organelles. Furthermore, the degree of damage of outer leaf tissue was more severe than that of inner leaf tissue. After vacuum cooling with treatment 3, severe ultrastructural injuries were observed (Fig. 2B), as the plasmalemma was withdrawn from the cell wall and the tonoplast was torn. The ultrastructure of the membrane system under treatments 1 and 2 was very similar and seemed less damaged than for treatment

3 (Fig. 2C), in which the plasmalemma was intact but tonoplasts were either withdrawn inward in the upper cell or showed discontinuity in the lower one.

With regards to the TEM results induced by vacuum cooling and subsequent 2 weeks of cold storage, treatment 3 showed that considerable plasma membrane withdrawal from the cell wall had occurred. In addition, the plasmalemma was ruptured in places and vacuoles were no longer visible, with vesicle formation, and organelles which were originally close to the cell wall moved inward due to lysis of the tonoplast (Fig. 3A). Mitochondria in these cells were much damaged, with distinct outer membranes ruptured (Fig. 3B). The TEM results of treatment 2 (Fig. 4A and B) showed only slight withdrawal of the plasma membrane from the wall in some places and a clearly visible tonoplast. Defective chloroplasts and lysis of the tonoplast (Fig. 5A) were found in treatment 1. Furthermore, Fig. 5B showed partially discontinuous cytoplasm and apparent plasmolysis.

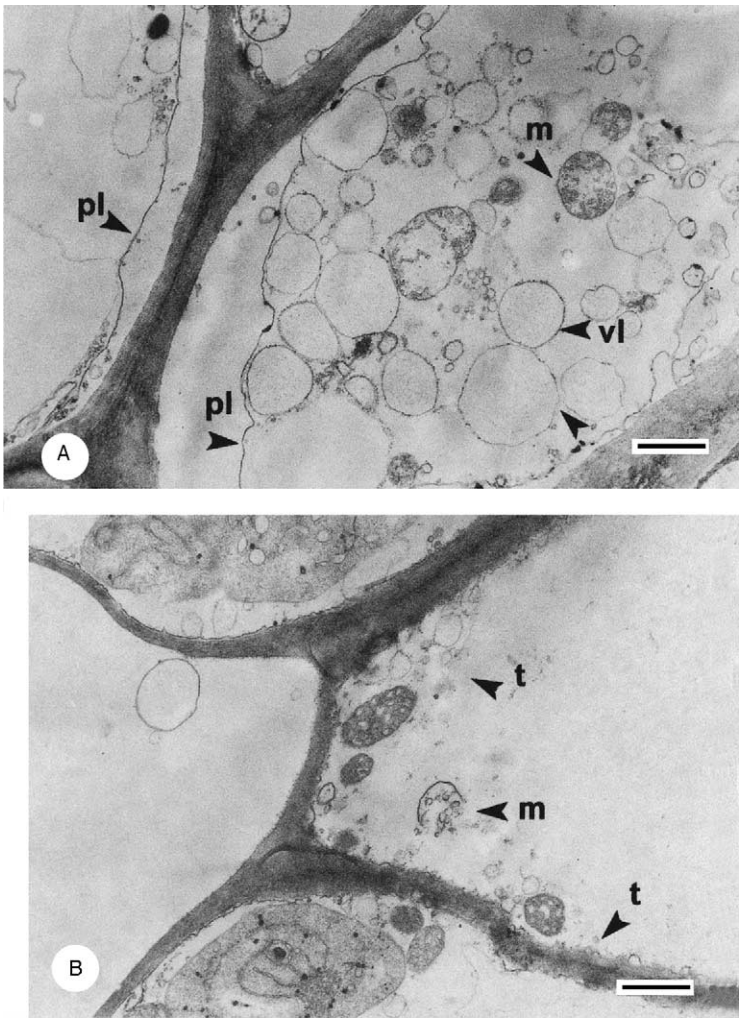

Fig. 3. Ultrastructure (TEM) of inner leaf tissue treated by vacuum cooling with pressure reduction within 1 h and storage at 1 °C and 85% RH for 2 weeks. Bar = 1 μm (A, B). Abbreviations: m, mitochondria; t, tonoplast; pl, plasmalemma; vl, vesicles; (A) ruptured tonoplast with vesicles formation as well as organelles moved inward; (B) damaged mitochondrion and unclear tonoplast.

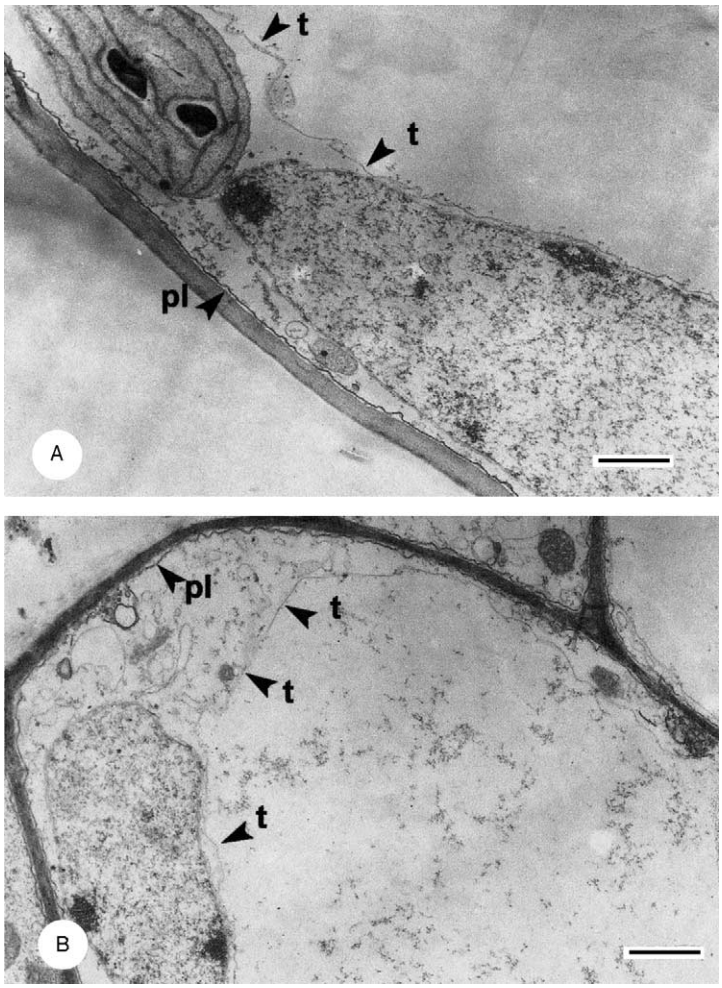

Fig. 4. Ultrastructure (TEM) of outer leaf tissue treated by vacuum cooling with pressure reduction within 30 min and storage at 1 °C and 85% RH for 2 weeks. Bar = 1 μm (A, B). Abbreviations for TEM: t, tonoplast; pl, plasmalemma; (A) clear tonoplast, light plasmolysis; (B) intact tonoplast and membrane system.

The results revealed that the ultrastructure of lettuce became degraded after storage. In the same storage conditions, the degree of destruction of the internal lettuce structure depended on the pressure reduction rate under which the lettuce were vacuum cooled. Among the three pressure reduction rates, both the slow pressure reduction rate of treatment 3 and the fast pressure reduction rate of treatment 1 led to damaged organelles, while treatment 2 caused no damage. In short, TEM results of lettuce tissue after vacuum cooling and storage induced by both slow pressure reduction rate of treatment 3 and fast pressure reduction rate of treatment 1 showed prominent destruction both in the internal membrane system and in the organelles. The moderate pressure reduction rate of treatment 2 achieved better lettuce quality and shelf-life.

Table 2 summarizes the data on physical and chemical results along with the LM and TEM observations. The trend in the macroscopic results agreed well with that of the ultrastructural results. For example, the lowest value for catalase under treatment 1 corresponded with severe injury of the membrane system. Similarly, the more severe the destruction of the membrane system was, the lower the tissue firmness. Similar results by Shomer et al. (1998) showed

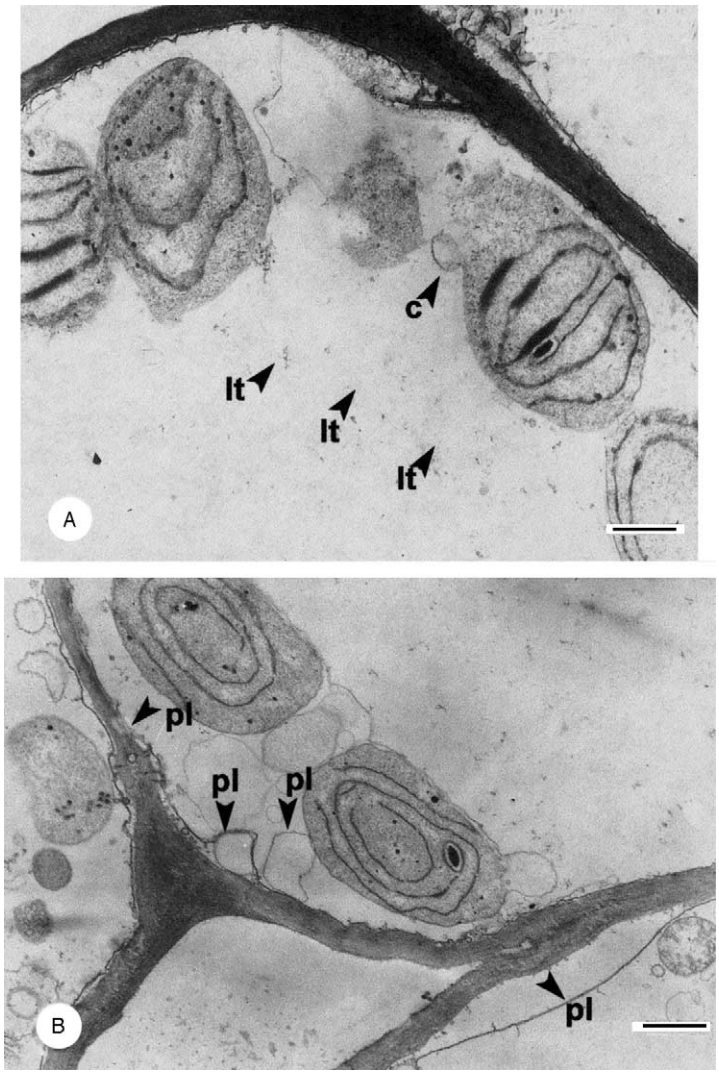

Fig. 5. Ultrastructure (TEM) of outer leaf treated by vacuum cooling with pressure reduction within 15 min and storage at 1 °C and 85% RH for 2 weeks. Bar = 1 μm (A, B). c, chloroplast; pl, plasmalemma; lt, lysis of tonoplast; (A) defective chloroplast and lysis of tonoplast; (B) interrupted membranes and plasmolyzed cytoplasm.

that possible reasons for the faster deterioration of date fruit frozen at high temperatures was in damage to cell membranes and cell walls.

4. Conclusions

In summary, experiments were performed to correlate the pressure reduction rate of vacuum cooling to microstructure, ultrastructure, physical and chemical quality of iceberg lettuce for a better understanding of the mechanisms induced by vacuum cooling and cold storage. The results demonstrated that treatment 2 had achieved the maximum value of ascorbic acid, greater catalase activity and maximum firmness. The results of the present study indicate that the moderate pressure rate during vacuum cooling was superior in terms of lettuce quality. However, further study should

be carried out on structural heterogeneity of biological materials.

References

Albrecht, J.A., Schafer, H.W., 1990. Comparison of two methods of ascorbic acid determination in vegetables. J. Liquid Chromat. 13, 2633–2641.

Aldesuquy, H.S., Abdel-Fattah, G.M., Baka, Z.A., 2000. Changes in chlorophyll, polyamines and chloroplast ultrastructure of *Puccinia striiformis* induced 'Green islands' on detached leaves of *Triticum aestivum*. Plant Physiol. Biochem. 38, 613–620.

AOAC, 1980. Official Methods of Analysis, 13. Association of Official Analytical Chemists, Washington, DC.

Bailey, C., 1993. Food refrigeration-current technology and future options. Food Sci. Technol. Today 8, 24–31.

Burfoot, D., Hayden, R., Badran, R., 1989. Simulation of a pressure cook/water and vacuum cooled processing system. In: Field, R.W., Howell, J.A., Process Engineering in the Food Industry Developments and Opportunities, Elsevier, London, pp. 27–41.

Burton, K.S., Frost, C.E., Atkey, P.T., 1987. Effect of vacuum cooling on mushroom browning. Int. J. Food Sci. Technol. 22, 599–606.

DeEll, J.R., Vigneault, C., 2000. Vacuum cooling and storage temperature influence the quality of stored mung bean sprouts. HortScience 35, 891–893.

Dostal, M., Kyhos, K., Houska, M., Gree, R., 1999. Vacuum cooling of solid foods. Czech J. Food Sci. 17, 103–112.

Farrant, J.M., Cooper, K., Kruger, L.A., Sherwin, H.W., 1999. The effect of drying rate on the survial of three desiccation-tolerant angiosperm species. Ann. Bot. 84, 371–379.

Gabara, B., Sklodowska, M., Wyrwicka, A., Glinska, S., Gapinska, M., 2003. Changes in the ultrastructure of chloroplast and mitochondria and antioxidant enzyme activity in *Lycopersicon esculentum* Mill, leaves sprayed with acid rain. Plant Sci. 164, 507–516.

Haas, E., Gur, G., 1987. Factors affecting the cooling rate of lettuce in vacuum cooling installation. Int. J. Refrig. 10, 82–86.

Han, Y.S., 1992. Experimental Guidance of Food Chemistry, Beijing Agriculture University Press, Beijing (in Chinese).

He, S.Y., Li, Y.F., 2003. Theoretical simulation of vacuum cooling of spherical foods. Appl. Therm. Eng. 23, 1489–1501.

Heaton, J.W., Marangoni, A.G., 1996. Chlorophyll degradation in processed foods and senescent plant tissues. Trends Food Sci. Technol. 7, 8–15.

Houska, M., Podloucky, S., Zitny, R., Gree, R., Sestak, J., Dostal, M., Burfoot, D., 1996. Mathematical model of the vacuum cooling of liquids. J. Food Eng. 29, 339–348.

Huang, W.K., 1989. Test and Analysis of Foods, Light Industry Press, Beijing (in Chinese).

Jackman, R.L., Stanley, D.W., 1995. Perspectives in the textural evaluation of plant foods. Trends Food Sci. Technol. 6, 187–194.

James, S.J., Burfoot, D., Bailey, C., 1989. The engineering aspects of ready meal production. In: Field, R.W., Howell, J.A., Process Engineering in the Food Industry Developments and Opportunities, Elsevier, London, pp. 43–58.

Kaur, C., Kapoor, H.C., 2001. Antioxidants in fruits and vegetables—the millennium's health. Int. J. Food Sci. Technol. 36, 703–725.

Martinez, J.A., Artes, F., 1999. Effect of packaging treatments and vacuum-cooling on quality of winter harvested iceberg lettuce. Food Res. Int. 32, 621–627.

Mc Donald, K., Sun, D.W., 2001. Effect of evacuation rate on the vacuum cooling process of a cooked beef product. J. Food Eng. 48, 195–202.

Molas, J., 2002. Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni(II) complexes. Environ. Exp. Bot. 47, 115–126.

Nieto, A., Castro, M.A., Alzamora, S.M., 2001. Kinetics of moisture transfer during air drying of blanched and/or osmotically dehydrated mango. J. Food Eng. 50, 175–185.

Olmos, E., Hellin, E., 1998. Ultrastructural differences of hyperhydric and normal leaves from regenerated carnation plants. Scientia Hortic. 75, 91–101.

Pareek, A., Lata Singla, S., Grover, A., 1997. Short-term salinity and high temperature stress-associated ultrastructural alterations in young leaf cells of *Oryza sativa* L. Ann. Bot. 80, 629–639.

Paull, R.E., 1999. Effect of temperature and relative humidity on fresh commodity quality. Postharvest Biol. Technol. 15, 263–277.

Popova, L.P., 1998. Fluridone- and light-affected chloroplast ultrastructure and ABA accumulation in drought-stressed barley. Plant Physiol. Biochem. 36, 313–319.

Rennie, T.J., Raghavan, G.S.V., Vigneault, C., 2000. Determination of the product refrigeration load in vacuum cooling of lettuce. Appl. Eng. Agric. 16, 459–464.

Rennie, T.J., Raghavan, G.S.V., Vigneault, C., Gariepy, Y., 2001a. Vacuum cooling of lettuce with various rates of pressure reduction. Trans. ASAE 44, 89–93.

Rennie, T.J., Vigneault, C., Raghavan, G.S.U., DeEll, J.R., 2001b. Effects of pressure reduction rate on vacuum cooled lettuce quality during storage. Canad. Biosyst. Eng. 43, 339–344.

Reynolds, E.S., 1963. Use of lead citrate at high pH as an electron opaque stain for electron microscopy. J. Cell Biol. 17, 208–212.

Sams, C.E., 1999. Preharvest factors affecting postharvest texture. Postharvest Biol. Technol. 15, 249–254.

Sherwin, H.W., Farrant, J.M., 1998. Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii and Xerophyta viscose*. Plant Growth Regulat. 24, 201–203.

Shomer, I., Borochov-Neori, H., Luzki, B., Merin, U., 1998. Morphological, structural and membrane changes in frozen tissues of Madjhoul date (*Phoenix dactylifera L.*) fruits. Postharvest Biol. Technol. 14, 207–215.

Spurr, A.R., 1969. A low viscosity epoxy resin embedding medium for eletron microscopy. J. Ultras. Res. 26, 31–46.

Stefanowska, M., Kuras, M., Kubacka-Zebalska, M., Kacperska, A., 1999. Low temperature affects pattern of leaf growth and structure of cell walls in winter oilseed rape. Ann. Bot. 84, 313–319.

Turk, R., Celik, E., 1993. The effects of vacuum precooling on the half cooling period and quality characteristic of iceberg lettuce. Acta Hort. 343, 321–324.

Turk, R., Celik, E., 1994. The effects of vacuum cooling on the quality criteria of some vegetables. Acta Hort. 368, 825–829.

Wang, L.J., Sun, D.W., 2002a. Modelling vacuum cooling process of cooked meat. Part 1. Analysis of vacuum cooling system. Int. J. Ref. 25, 854–861.

Wang, L.J., Sun, D.W., 2002b. Modelling vacuum cooling process of cooked meat. Part 2. Mass and heat transfer of cooked meat under vacuum pressure. Int. J. Ref. 25, 862–871.