Effects of pressure reduction rate on vacuum cooled lettuce quality during storage

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[1]Department of Agricultural and Biosystems Engineering, Macdonald Campus, McGill University. 21 111 Lakeshore Rd., Ste. Anne-de-Bellevue, QC, Canada H9X 3V9; [2]Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Boul. Gouin, Saint-Jean-sur-Richelieu, QC, Canada J3B 3E6; and [3]Ontario Ministry of Agriculture, Food and Rural Affaires, 4890 Victoria Ave N., Box 8000 Vineland Station, ON, Canada L0R 2E0

Rennie, T.J., Vigneault, C., Raghavan, G.S.V. and DeEll, J.R. 2001. **Effects of pressure reduction rate on vacuum cooled lettuce quality during storage**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **43**:3.39-3.43. A study was conducted 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. Lettuce was cooled at three different rates and stored for 16 days at 1°C and 85% relative humidity (RH) conditions. Mass loss, visual quality, and chlorophyll fluorescence measurements were made throughout the storage period. The results showed that the pressure reduction rate had no apparent effect on overall quality. Average mass loss in storage was 2.9%. Final quality after storage was deemed to be "fair" regardless of the rate of cooling. However, the chlorophyll fluorescence measurements indicated that the cooling process may have stressed the plant tissue, but the stress was minimal and subsided after a day or two of storage. It appears as if there is no advantage/disadvantage to cooling vacuum under different pressure reduction rates for the rates evaluated in this study as far as product quality is concerned. This is important if vacuum coolers are to be designed with slower pressure reduction times. **Keywords:** vacuum cooling, *Lactuca Sativa*, precooling, chlorophyll fluorescence.

Une étude a été menée afin de déterminer le niveau d'impact sur la qualité de la laitue pendant son entreposage lorsque pré-refroidie sous vide à différents degrés de réduction de pression. La laitue fut refroidie sous trois différents régimes de pression et entreposée pendant 16 jours à 1°C et une humidité relative (HR) de 85%. La perte massique, la qualité visuelle, et la fluorescence chlorophyllienne furent déterminées périodiquement pendant la durée de la période d'entreposage. Les résultats ont démontré que le degré de réduction de la pression, lors du pré-refroidissement sous vide, n'a eu aucun effet sur la qualité d'ensemble de la laitue. La moyenne de la perte massique durant l'entreposage était de 2.9%. La qualité finale après l'entreposage fut jugée acceptable sans égard au taux de pré-refroidissement employé. Cependant, les mesures de fluorescence chlorophyllienne ont indiqué que le procédé de pré-refroidissement pourrait avoir causé un stress au tissu végétal. Toutefois, le niveau de stress était minime et il disparut après un ou deux jours d'entreposage. Il semblerait qu'il n'y ait aucun avantage/désavantage, sur la qualité de la laitue, à pré-refroidir sous vide en utilisant différents régimes de pression, tel qu'étudiés. Ceci prend toute son importance lors de la conception de systèmes de pré-refroidissement sous vide fonctionnant à des régimes différents de réduction de pression. **Mots-Clés**: refroidissement sous vide; *Lactuca Sativa*, pré-refroidissement; fluorescence chlorophyllienne.

INTRODUCTION

Lettuce (*Lactuca Sativa*) is a perishable commodity that requires immediate precooling and refrigerated storage after harvest to prolong its shelf life. Lettuce can be stored up to 2 to 3 weeks when held at optimum conditions of 0°C and 98-100% RH (Hardenburg et al. 1986). The preferred method of precooling is vacuum cooling, which can reduce the lettuce temperature from field temperature to 1°C in less than 25 min. However, lettuce can be precooled by other methods, though not as effectively (Edeogu et al. 1997). Forced-air cooling can result in excessive moisture loss and hydrocooling can be slower and can also leave water trapped between the leaves creating conditions conducive for the development of microbial growth.

Though vacuum cooling has relatively low operating costs, large produce quantities need to be cooled to justify the purchase due to the large capital cost of the cooler. Traditionally, vacuum coolers have been designed to drop the pressure to the operating pressure in 5 to 10 minutes. If a smaller capacity vacuum pump is installed, the peak refrigeration load can be reduced (Rennie et al. 2000). Thus two components, the refrigeration system and the vacuum pump, would have been decreased in capacity, reducing the capital cost. However, the time required to cool the produce would be increased, and the total amount of lettuce that could be cooled in a day would be decreased. For small-scale operations, the reduction in the capital cost may make the system more affordable.

The effects of slower vacuum cooling on lettuce quality are unknown. The increased time could have a detrimental effect on lettuce quality as the lettuce is exposed to ambient temperatures for a longer period of time. Changing the rate of pressure reduction changes the rate of water evaporation from the plant tissue. Slower rates of water evaporation may lead to less tissue damage.

Chlorophyll fluorescence has become an important analytical tool for analyzing many environmental and physiological aspects of plants. It is a measure of the primary processes of photosynthesis that occur in the chloroplasts, including light absorption, excitation energy transfer, and the photochemical reaction of photosystem II (PSII) (DeEll et al. 1999). Other levels of photosynthesis influence the primary level and thus chlorophyll fluorescence is affected by numerous

factors in a very complex manner (Krause and Weis 1991). Research has shown that the amount of light emitted (fluoresced) can be correlated to the stress that the plant is under (Krause and Weis 1991). Water stress and cold stress in plants affect the normal operation of photosynthesis and these can been detected by chlorophyll fluorescence measurements (Schapendonk et al. 1992; Khanizadeh et al. 2000). Mir et al. (1998) measured chlorophyll fluorescence from apples with surface defects. For apples with CO_2 injury, healthy regions had F_v/F_m of approximately 0.75 and decreased as low as 0.33 in the CO_2 damaged region. Low O_2 and high CO_2 stress have been detected in apples in cold storage by changes in F_V/F_M (variable fluorescence/maximum fluorescence) and T½ (DeEll et al. 1998). The T½ measurement is the time required for the fluorescent measurements to increase from the minimum value (F_O) to half the difference between the minimum and maximum fluorescence (F_M). An important advantage to chlorophyll fluorescence is that it has the ability to detect stress before visual symptoms occur. There is an indication that chlorophyll fluorescence has potential to be used as a measurement of storage quality of fruits and vegetables (Toivonen 1992; DeEll et al. 1995; Forney et al. 2000).

The objective of this study was to investigate the effects different pressure reduction rates had on lettuce quality in an effort to determine if vacuum coolers with slower cooling rates were feasible. Chlorophyll fluorescence was used as a measure to assess the effects of the pressure reduction rate on the health of the lettuce tissue during the subsequent storage.

MATERIALS and METHODS

Plant material

Freshly harvested 'iceberg' lettuce (*Lactuca Sativa*) was bought from a local distribution centre each morning. The lettuce had been harvested in the morning and was not precooled. The lettuce was transported to the Horticultural Research and Development Centre of Agriculture and Agri-Food Canada (St. Jean-sur-Richelieu, Quebec). The temperature of the lettuce during this time was near room temperature (23°C). Initial mass of lettuce heads were 729.5 ± 135.0 g prior to precooling.

Vacuum cooling and storage

The tests were performed using a laboratory scale vacuum cooler. The cooler was a Model Y1 series 77-003 "Lyo-Tech" freeze-dryer (Lyo-San Inc., Lachute, QC). Using only the vacuum pump and the refrigeration system allowed the freeze-dryer to perform the identical function as a vacuum cooler. The vacuum pump dropped the pressure from normal atmosphere to 26.663 kPa in an average time of 353 s.

Table 1. Visual quality evaluation scales (from Kader et al. 1973).

Score	Visual quality	Description
9	Excellent	essentially free from defects
7	Good	minor defects; not objectionable
5	Fair	slightly to moderately objectionable defects; lower limit of sales appeal
3	Poor	excessive defects; limit of salability
1	Extremely poor	not useable

Using the WorkBench for Windows™ software, a system to control the internal pressure of the chamber was devised. The pump was started and ran at the same speed for all the trials. An automated air leak was used to let air into the chamber so that the rate at which the pressure in the chamber dropped could be controlled by the amount of air going through the air leak. The air leak consisted of a tube attached to the chamber and three inlet solenoid valves of different diameters. Opening and closing the valves allowed different amounts of air in. An exponential decay equation for the pressure as a function of time was developed and used in the software to control the opening and closing of the solenoid valves. In theory, the rate at which the pressure is decreased follows an exponential decay function. In practice, there is a slight deviation, generally when low pressures are achieved (Wang and Gitlin 1964). The pressure could only be controlled when the pressure had reached 26.7 kPa as the pump removed the air faster than the air leak could supply air at pressures higher than this. Thus, the equation used in the software was:

$$P = Ae^{-B\theta} \quad (1)$$

where:

P = pressure in chamber (kPa),
A = 26.7 kPa,
θ = time (s), and
B = coefficient characterizing rate at which pump reduces pressure in chamber (s^{-1}).

The pressure control system began once the pressure was reduced to 26.7 kPa. By changing the B value, the rate at which the pressure dropped was changed. Once the pressure reached 800 Pa, the control system maintained the pressure at 800±40 Pa by opening and closing the solenoid valves.

The study was designed to use three different rates of pressure reduction, values of B were 0.00940, 0.00159, and 0.00396, corresponding to fast, medium, and slow cooling rates, respectively. The time required to drop the pressure from 26.7 kPa to 800 Pa was 515, 3045, and 12 226 s for the fast, medium, and slow cooling rates, respectively. For each rate, ten lettuce heads were selected and the outer wrapper leaves removed. Each head was weighed, visually evaluated for quality (Table 1), and placed into the vacuum chamber. Three of the ten heads were pierced with needle thermocouples (type T) to read the mass-average temperature of the lettuce as determined by the method of Smith and Bennett (1965). Each treatment was vacuum cooled until the average temperature of the three thermocouples reached 2.5°C. The lettuce were weighed again and then immediately placed into cold storage at 1°C and 85% RH. The boxes were covered with a perforated bag to avoid direct airflow on the lettuce and to maintain a high RH in the boxes. Separate boxes were used for each of the replicates and for each of the treatments. This experiment was repeated twice to give three replications.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were taken at 1°C, using a modulated fluorometer (OS-500, Opti-Sciences Ltd., Tyngsboro, MA). The unit was portable with four light sources (modulated, saturation, actinic, and far red), a photodiode detector, computer hardware and software, standard 3.5" diskette drive, user input keys, a LCD screen, a 12 V battery and

Table 2. Results of the quality evaluation.

Speed	Initial		Day 9		Final	
	Quality index*	Mass loss (%)	Quality index	Mass loss (%)	Quality index	Mass loss (%)
Fast	8.4	0	7.4	1.6	6.3	3.2
Medium	8.2	0	7.2	1.8	6.2	2.9
Slow	8.5	0	7.4	1.7	6.4	2.7
Significance	NS**	NS	NS	NS	NS	NS

* 30 lettuce heads used per treatment
** Not Significant

charger, and a 9 mm measuring probe connected to the light sources and detector through a system of fibre optic cables. The modulated light was a 655 nm solid state source with adjustable intensity (< 1.0 µmol m^{-2} s^{-1} or 0.217 W/m^2) which emitted radiation at wavelengths greater than 660 nm. Filters blocked all radiation above 700 nm. A 35 W halogen lamp provided the saturating pulse light with adjustable intensity up to 10 000 µmol m^{-2} s^{-1} for duration of 0.1 to 3.0 s. The actinic light was a solid state source whose peak emission wavelength was approximately 670 nm with a variable intensity up to 450 µmol m^{-2} s^{-1}. The PIN silicon photodiode detector is filtered to receive radiation from 710 to 760 nm.

Immediately after cooling, the lettuce heads were placed in the refrigerated storage and dark-adapted for 1800 s and then F_V (dark-adapted variable fluorescence), F_V/F_M (dark-adapted variable fluorescence/maximum fluorescence), and T½ (fluorescence half-life) were measured (fast actinic test) with the modulation intensity set at 50, actinic intensity at 125, and the detector gain at 110, with run time of 3 s. The dark-adapted tests were performed under a single green 40-W safe light, to provide low-level illumination during the fluorescence analysis to allow the operator to operate the instrument. The storage room lights were turned on and after 1200 s measurements of the F_V' (light-adapted variable fluorescence) and F_V'/F_M' (light-adapted variable fluorescence/maximum fluorescence) were made (yield test) with a modulation intensity of 200, saturation intensity at 255 for 3 s, and the detector gain set at 15. The procedure was repeated on days 1, 2, 6, 9, 13, and 16.

Quality evaluations

The visual quality of the lettuce was evaluated before cooling and on days 2, 6, 9, 13, and 16. The quality index scale used is shown in Table 1. The mass of the lettuce was measured before and after cooling, as well as on days 2, 6, 9, 13, and 16. The percent mass loss based on the mass just after cooling was calculated for all measurements.

Statistical analysis

Three different rates of vacuum application were used in this experiment. Three replicates were used for each treatment. Due to the length of time necessary to perform the treatments, each replicate was run on a different day. Thus, the experiment had each treatment performed on the same day, with the order of treatments being randomized within each replicate. As the characteristics of the lettuce could change from one day to the next, the replicates were treated as block factors in the statistical analysis of the data. A multivariate analysis of variance (MANOVA) was used with time as a repeated measure as the data collected were always on the same lettuce heads over time. The statistical analysis was performed using SAS 6.1 for Windows™. This allowed for the determination of significance between the treatments for each day samples were taken, as well as the effects of the replication (block effect) and interactions between replication and time, replication and treatment, and treatment and time.

RESULTS and DISCUSSION

Quality evaluation

The results from the visual quality are presented in Table 2. The product behaved in the same manner for all three treatments. The overall quality of the lettuce after 16 days of storage was classified between “fair” and “good”. The differences between the different treatments were minimal and it may be concluded that the rate of vacuum application has no overall effect on the quality of the stored lettuce. It should also be mentioned that the delay in cooling of the lettuce was not the same for all the trials. All the lettuce was bought at the same time but only one set could be cooled at a time. However, this time delay was randomized in the design. Even with this time delay, the quality of the lettuce at the end of the storage period was acceptable.

Results from the mass loss (Table 2) indicated that the rate of pressure reduction had no effect on the subsequent storage mass loss of the lettuce. The mass loss with respect to time was nearly linear with the final percent mass loss after 16 days of storage ranging between 2.7 and 3.2 %. The mass loss during cooling showed no differences for the different pressure reduction rates (data not shown).

Chlorophyll fluorescence

There were no significant differences in T½ values due to the different pressure reduction rates. However, storage time had an effect on the measurements as the lowest incidents of T½ values occurred immediately after cooling and then increased gradually over time, as shown in Fig. 1 (note that these are the averages over the three replications, with 10 heads of lettuce per replication). This indicates that the cooling process itself may stress the plant tissue, but that the tissue does recover to an extent during the subsequent storage.

The F_V (Fig. 2) values showed no significance due to the different pressure reduction rates, however, like the T½ values, it showed an increase in the value in the subsequent storage. A decrease in F_V represents a general decline in chloroplast function (Krause and Weis 1984). Thus the initial drop in the F_V reading suggests a stress from the cooling operation that subsides during storage.

The F_V/F_M values were most affected by the slowest pressure reduction rate and it took longer to recover than the other rates.

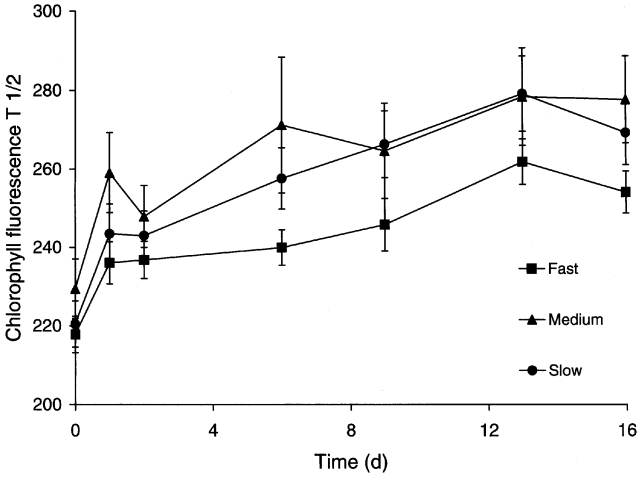

Fig. 1. Chlorophyll fluorescence T½ values for lettuce vacuum cooled at different rates and held at 1°C for 16 days (average of three replicates).

The initial F_V/F_M measurements for the slowest pressure reduction rate taken after cooling were significantly different from the other two, with a value near 0.805 whereas the other two were between 0.820 and 0.825. However, the differences had subsided by day 2 and no significance was detected the remainder of the storage period (Fig. 3). It is possible that for a slower pressure reduction rate, the longer exposure to low pressure could cause a stress, whereas with faster rates the duration of the stressful situation is not long enough to affect the plant tissue. With the three different dark-adapted measurements, no conclusive deductions can be made as to the magnitude of the stress due to different pressure reduction rates.

For the light-adapted condition, F_V' (Fig. 4) and F_V'/F_M' (Fig. 5) measurements were made after exposing the lettuce to light for at least 1200 s. For both the F_V'/F_M' and the F_V' measurements, significant differences for the treatment main effects were not detected. Both showed effects due to time, with

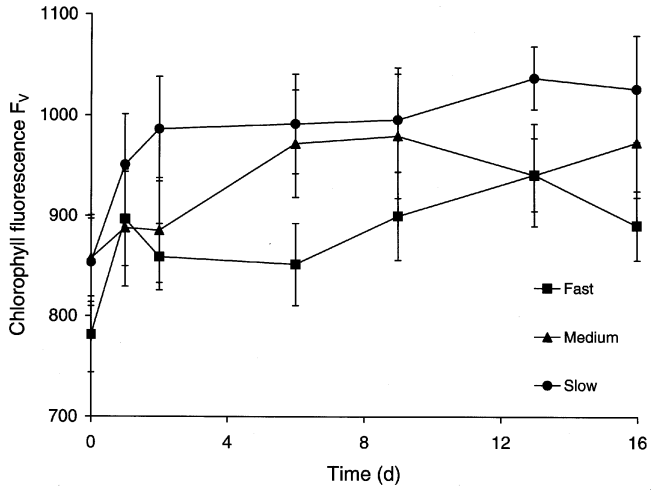

Fig. 2. Chlorophyll fluorescence F_V values for lettuce vacuum cooled at different rates and held at 1°C for 16 days (average of three replicates).

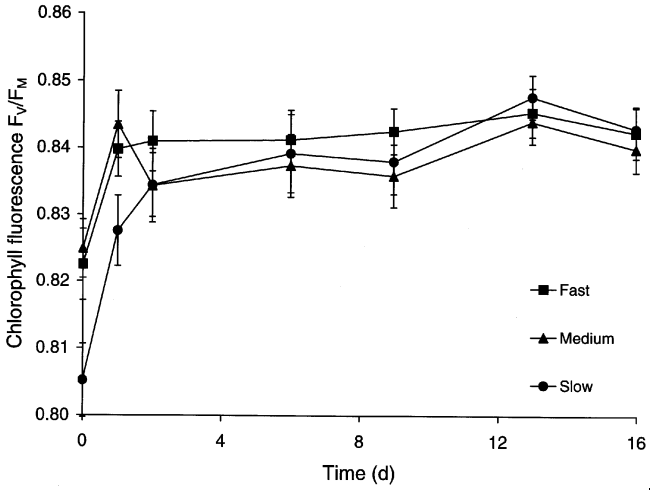

Fig. 3. Chlorophyll fluorescence F_V/F_M values for lettuce vacuum cooled at different rates and held at 1°C for 16 days (average of three replicates).

the F_V' increasing and the F_V'/F_M' decreasing during the first couple of days of storage.

In all the measurements for chlorophyll fluorescence, the main interest is a difference in the values between treatments rather than indication of stress alone. Vacuum cooling is known as the best method to pre-cool lettuce, therefore any stress that may be detected is of no interest for this study. The absence of difference in chlorophyll fluorescence readings suggests that different rates of pressure reduction will not stress the lettuce any differently then in regular vacuum cooling.

CONCLUSIONS

The results from the mass loss and visual quality evaluation suggest that there is no difference in the overall quality when cooled with the different pressure reduction rates used in this study. In all cases the lettuce remained in the range of “fair” to

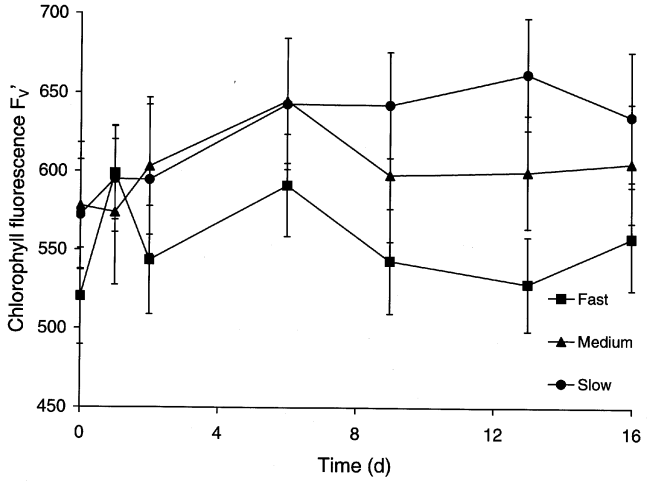

Fig. 4. Chlorophyll fluorescence F_V' values for lettuce vacuum cooled at different rates and held at 1°C for 16 days (average of three replicates).

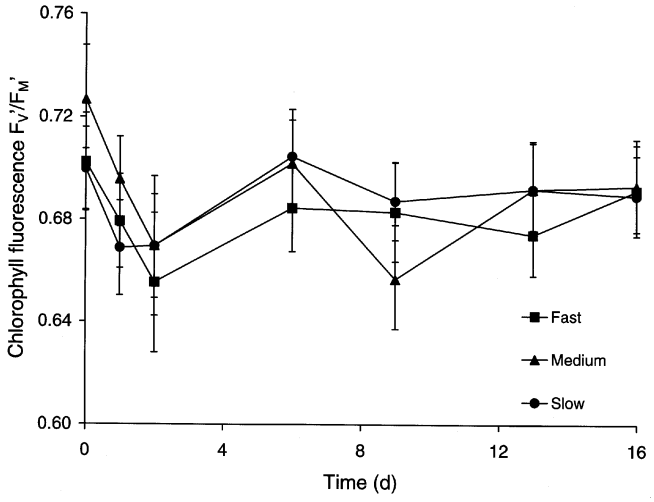

Fig. 5. Chlorophyll fluorescence F_V'/F_M' values for lettuce vacuum cooled at different rates and held at 1°C for 16 days (average of three replicates).

good" throughout the storage period and the final mass loss was on average 2.9%. There was no significant difference in chlorophyll fluorescence measurements due to the different pressure reduction rates. The chlorophyll fluorescence measurements suggested that the cooling process may stress the plant tissue but that the tissue does recover. In all cases the lettuce tissue appeared to remain healthy, the level of stress was minimal, and there was no effect on overall lettuce quality. It appears as if there is no advantage/disadvantage to cooling vacuum under different pressure reduction rates for the rates evaluated in this study as far as product quality is concerned. This is important if vacuum coolers are to be designed with slower pressure reduction times.

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